# GLIMPSES OF CCRAS CONTRIBUTIONS
## (50 GLORIOUS YEARS)

## VOLUME-VI

# PHARMACOLOGY RESEARCH AND SAFETY STUDIES

**CENTRAL COUNCIL FOR RESEARCH IN AYURVEDIC SCIENCES**
Ministry of AYUSH, Government of India
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# PREFACE

The history of use of Ayurveda medicines dates back to thousands of years and it sustained owing to their safety and therapeutic potential. However, it becomes imperative to generate the evidence to demonstrate the safety and efficacy of these medicines. Pharmacological profile and safety /toxicity studies are the major aspects of drug research. Pharmacological studies encompass biological effect, efficacious dose range and overall potency of the optimized lead. It is very important to perform all pharmacological studies in relevant in-vitro and in-vivo test system, which has closest resemblance to human disease condition. These studies give a further understanding into the mechanism of action of the medicine. The Council has undertaken different biological screening on 389 Ayurvedic formulations/medicinal plants.

Safety/toxicity of Ayurvedic formulations/Medicinal plants are carried out to find out the safest dose range, lethal dose, to generate Safety/Toxicity data. The preclinical toxicity testing on various biological systems reveals the species-, organ- and dose- specific toxic effects of an investigational product. So far, Council has screened 160 drugs for various toxicity studies

The present document is an effort to exhibit the pharmacological research undertaken by the Council since inception which may be used as a ready reference.

**Prof. Vaidya K.S. Dhiman**
**Director General**
**Central Council for Research in Ayurvedic Sciences**

# PROLOGUE

The Central Council for Research in Ayurvedic Sciences was established in 1969, since then it has been engaged in the drug research. Pharmacology contributes immensely in the drug research to exhibit the safety and the therapeutic potential of a drug through experimental studies. The classical Ayurvedic medicines, new combinations and formulations based on the leads from Local Health traditions/ folk claims are studied in experimental models using different animal species to establish efficacy and safety profile.

CCRAS has initiated programmes for screening of biological activity and safety/toxicity study of Ayurvedic formulations/ Plants. CCRAS has developed the experimental (in-vivo and in-vitro) laboratory for screening of biological activity and safety profile of Ayurvedic formulation and explore the effectiveness of drugs with safety through its five institutes, namely Central Ayurveda Research Institute for Drug Development (CARIDD), Kolkata; Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute (CSMRADDI), Chennai; Regional Ayurveda Research Institute for Drug Development (RARIDD), Gwalior; National Ayurveda Research Institute for Panchakarma (NARIP), Cheruthuruthy and Regional Ayurveda Institute for Fundamental Research (RAIFR), Pune . Further, Council also collaborates/outsources through institutes of national repute to carry out such studies.

The Council brings out a comprehensive document providing the glimpse of the research work done by the council since inception in the area of Pharmacology research and safety studies. This book may serve as a ready reference for the readers giving a quintessence of Pharmacology & Safety/Toxicity research of the Council.

**Dr. N. Srikanth**
**Deputy Director General**
**Central Council for Research in Ayurvedic Sciences**

# EXECUTIVE SUMMARY

Ayurveda, the science of life, evolved as a comprehensive system of healthcare systematically through scientific experimentations of high order backed by sound and reproducible evidence base and stood the test of the time. Several strategies and road maps are being drawn to carry forward merits of this science so as to meet the current day health needs and mainstream its core strengths alongside through research & development in this country and across the globe.

The core component of pharmacology research comprise of validation of the Ayurvedic formulations/medicinal plants by different biological screening methods and safety/toxicity studies, find out effective dose range and dose dependent effect and generation biological screening data for clinical implementation, contemporary scientific and pharmacological leads for important diseases of National importance based on strength of Ayurveda and from the folklore collected from various parts of India by the Council.

The Council undertakes validation of classical Ayurvedic formulations through pharmacological studies to generate evidence on efficacy and safety and scientific validation of new combinations (coded drugs) collected through folklore claims from various parts of the country, under drug development. The classical Ayurvedic formulations are already in the healthcare system and coded Ayurvedic drugs are made available into the healthcare system through systematic process of drug development viz. drug standardization and quality control, preclinical safety/toxicity studies and biological activity studies (as appropriate) and clinical trials as per requirement.

The Council is working toward validation through pharmacological screening and safety/toxicity study of these drugs in the diseases of National importance. The council is also working hard to revive the traditional systems and knowledge prevailing among tribes and in villages through collection of their folklore claims for various ailments and is working towards validation of these claims so the knowledge can be used for masses.

CCRAS has developed the experimental (in-vivo and in-vitro) laboratory for screening of biological activity and safety profile of Ayurvedic formulation and exploring the effectiveness of drugs with safety through its five peripheral Institutes, namely Central Ayurveda Research Institute for Drug Development (CARIDD), Kolkata; Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute (CSMRADDI), Chennai; Regional Ayurveda Research Institute for Drug Development (RARIDD), Gwalior; National Ayurveda Research Institute for Panchakarma (NARIP), Cheruthuruthy; Regional Ayurveda Institute for Fundamental Research (RAIFR), Pune and the Council collaborates/ outsources through institutes of national repute. However, Council has started pharmacology/toxicology research in 13 centers/units viz its various institutes/centers/units viz. Pharmacological Research Unit, Calcutta (PhRUC), Pharmacological Research Unit, Varanasi (PhRUV), Pharmacological Research Unit, Lucknow (PhRUL), Pharmacological Research Unit, Trivendrum (PhRUT), Pharmacological Research Unit, Jamnagar (PhRUJ), Toxicity Research Unit, Jhansi (TRUJh), Indian Institute of Phanchkarma,

Cheruthuruthy (IIPC), Central Research Institute, Mumbai (CRIM), Pharmacological Research Unit, Delhi (PhRUD), Pharmacological Research Unit, Jaipur (PhRUJ), Pharmacological Research Unit, Jodhpur ((PhRUJ), Pharmacological Research Unit, Patiala (PhRUP), Toxicity Research Unit, Patiala (TRUP) in India in which 8 centres/unites were merged with currently working unites/centres/ institute and remaining was closed.

Council has generated scientific evidence on efficacy of 389 Ayurvedic formulation/medicinal plants on different diseases/conditions viz. Diabetes Mellitus, Analgesic, Anti-Inflammatory, Anti-Cancer, Asthma, Wound Healing, Hepatoprotective, Anti-Hyperlipidemia, Urolithiasis, Rheumatoid Arthritis, Anti-Atherosclerotic, Immunomodulators, Ulcerative Colitis, Antipsychotic etc.

Council has generated scientific evidence on safety/toxicity of 160 Ayurvedic formulation/ medicinal planst/bhasma/rasakalpa. In the recent past the Ayurvedic herbo-mineral preparations are repeatedly being targeted for their levels of lead, mercury, arsenic and copper, thus challenges the safety and efficacy of Ayurvedic formulations. This led to a great need to study and screen the herbo-mineral/metallic preparations meticulously to give confidence and benefit to both the practitioners and the public as well. Considerable efforts have been put in by the Central Council of Research in Ayurvedic Sciences (CCRAS) and completed a multi-centric preclinical toxicity/safety study of bhasmas/rasakalpas and generate the scientific evidences on safety of bhasmas/rasakalpas i.e. Naga bhasma, Swarna Bhasma, Tamra Bhasma, Hridyarnava Rasa, Rasamanikya, Trivanga Bhasma, Kasisa Bhasma, Makaradhwaja, Rasa Sindoora, Mahalaxmi Vilasa Rasa, Yograja Guggulu, Mahayogaraja Guggulu, Arogya vardhini Vati, Vasanta Kusumakara Rasa etc.

Further, pharmacological validation of approximately 27 classical Ayurvedic formulations is continuing for generation of scientific evidence on safety and efficacy on different diseases/ conditions viz. hypothyroidism, Ulcerative colitis, arthritis, Genotoxicity, middle cerebral artery occlusion ischemic-reperfusion injury, Atherosclerosis, nephroprotective, Diabetic Nephropathy, Gout, diabetes, Gastro Intestinal Illness, Dengue, Migraine, Anti-inflammatory, Analgesic, hepatoprotective, neurotoxicity and reproduction toxicity in various institutes of the Council engaged in pharmacological research.The Council carried out safety studies of certain coded drugs under WHO Biennium programme.

The research outcomes of these studies are being published in journals especially in council's official publication JRAS (Journal of Research in Ayurvedic Sciences) and JDRAS (Journal of Drug Research in Ayurvedic Sciences) for wider dissemination. The evidence of the safety of Ayurvedi medicines will strengthen integration of Ayurveda with other systems of medicine and also help in convincing scientific community across the world which may also improve its market in the country and world at large.

The Council has also laid its vision document 2030 with short term and long term goals in for achieving its objectives and further strengthening of scientifically validated Ayurveda for achieving the ultimate goal of 'Health for all' The council is dedicated in dissemination of its research finding through monographs and book publications.

# CONTENTS

# CHAPTER 1

# GENESIS AND OVERVIEW

## Background

The science of Ayurveda has been in vogue in this country from the earliest times and serving the medical needs of most of our people. The systems was developed by ancient scholars on the basis of their own philosophy, oriental methodologies and practices prevalent in that era and have popularised and almost completed it in all aspects as a system of medicine. The advent of foreign invasions and cross cultural interaction had definite impact on the system. The beginning of twentieth century saw efforts to revive it. The members of the Imperial Legislative Council got the resolution of investigations and recognition of the system was accepted in the year 1916. The Indian National Congress also passed similar resolution in 1920. This led to establishment to number of colleges of Ayurveda.

In the post independence era, the efforts to develop research gained momentum. As per recommendation of the various Committees, grant-in-aid projects were sanctioned to selected colleges. The Central Council for Ayurvedic Research as an Advisory body was established in 1962 and finally the Central Council for Research in Indian medicine & Homoeopathy (CCRIM&H) was established in 1969. This Council initiated research programmes in the Indian systems of Medicine & Homoeopathy in different parts of the country and started coordination at the National level for the first time.

The Central Council for Research in Ayurveda & Siddha (CCRAS), an apex body for the formulation, coordination and development of research in Ayurveda & Siddha on scientific lines was established in March 1978 after reorganization of CCRIM&H. The Minister of Health & Family Welfare is the President of the Governing Body of the Council while the Joint Secretary chairs the Standing Finance Committee. The Scientific /Research Programmes are supervised by the respective Scientific Advisory Committee chaired by eminent scholars of the system.

The Central Council for Research in Ayurvedic Sciences is a Registered Society under Societies Registration Act XXI of 1860 on 29.07.2011 (Formerly Registered as Central Council for Research in Ayurveda and Siddha on 30th March, 1978).

## Research areas

The Central Council for Research in Ayurvedic sciences (CCRAS), an autonomous body under Ministry of AYUSH, Govt. of India is apex body in India for undertaking, coordinating, formulating, developing and promoting research on scientific lines in Ayurvedic sciences. The activities are carried out through its 30 Institutes/Centres/Units located all over India and also through collaborative studies with various Universities, Hospitals and Institutes. The research activities of the Council include

Medicinal Plant Research (Medico-ethno Botanical Survey, Pharmacognosy and Tissue Culture), Drug Standardization, Pharmacological Research, Clinical Research, Literary Research & Documentation. Besides this, Council is conducting outreach activities viz. Tribal Health Care Research Programme, Ayurveda Mobile Health Care programme, Swasthya Rakshan Programme and National Programme for Prevention and control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS).

## Objectives

1. The formulation of aims and patterns of research on scientific lines in Ayurvedic sciences.
2. To undertake any research or other programs in Ayurvedic sciences.
3. The prosecution of and assistance in research, the propagation of knowledge and experimental measures generally in connection with the causation, mode of spread and prevention of diseases.
4. To initiate, aid, develop and co-ordinate scientific research in different aspects, fundamental and applied of Ayurvedic sciences and to promote and assist institutions of research for the study of diseases, their prevention, causation and remedy.
5. To finance enquiries and researches for the furtherance of objects of the Central Council.
6. To exchange information with other institutions, associations and societies interested in the objects similar to those of the Central Council and especially in observation and study of diseases in East and in India in particular.
7. To prepare, print, publish and exhibit any papers, posters, pamphlets, periodicals and books for furtherance of the objects of the Central Council and contribute to such literature.
8. To issue appeals and make applications for money and funds in furtherance of the objects of the Central Council and to accept for the aforesaid purpose gifts, donations and subscriptions of cash and securities and of any property whether movable or immovable.
9. To borrow or raise monies with or without security or on security mortgage charge, hypothecation or pledge of all or any of the immovable or movable properties belonging to the Central Council or in any other manner whatsoever.
10. To invest and deal with the funds and monies of the Central Council or entrusted to the Central Council not immediately required in such manner as may from time to time be determined by the Governing Body of the Central Council.
11. To permit the funds of the Central Council to be held by the Government of India.
12. To acquire and hold, whether temporarily or permanently any movable or immovable property necessary or convenient for the furtherance of the objects of the Central Council.

13. To sell, lease, mortgage and exchange, and otherwise transfer any of the properties movable or immovable of the Central Council provided prior approval of the Central Government is obtained for the transfer of immovable property.

14. To purchase, construct, maintain and alter any buildings or works necessary or convenient for the purpose of the Central Council.

15. To undertake and accept the management of any endowment or trust fund for donation, the undertaking or acceptance whereof may seem desirable.

16. To offer prizes and grant of scholarships, including travelling scholarships in furtherance of the objects of the Central Council.

17. To create administrative, technical and ministerial and other posts under the Society and to make appointments thereto in accordance with the rules and regulations of the Society.

18. To establish a provident fund and/or pension fund for the benefit of the Central Council's employees and/or their family members.

19. To do all such other lawful things either alone or in conjunction with others as the Central Council may consider necessary or as being incidental or conducive to the attainment of the above objects.

20. To undertake R & D Consultancy projects and transfer of patents on drugs and process to industry.

21. To undertake R & D projects sponsored by industries in public/private sector.

22. To undertake international and interagency collaboration.

23. Utilization of results of research conducted and payment of share of royalties/consultancy fees to those who has contributed towards pursuit of such research.

24. To enter into arrangements with scientific agencies of other countries for exchange of scientists, study tours, training in specialized areas, conducting joint projects etc.

25. To provide technical assistance to Govt./Private agencies in matters consistent with the activities of the Council.

26. To assist Medicinal Plants Board, Government of India in achieving its objectives.

27. To constitute small Management Committees consisting of eminent Scientists/ Physicians of local areas to monitor the R & D activities and suggest remedial measures for the improvement of activities of all Central as well as Research Institutes of the Council.

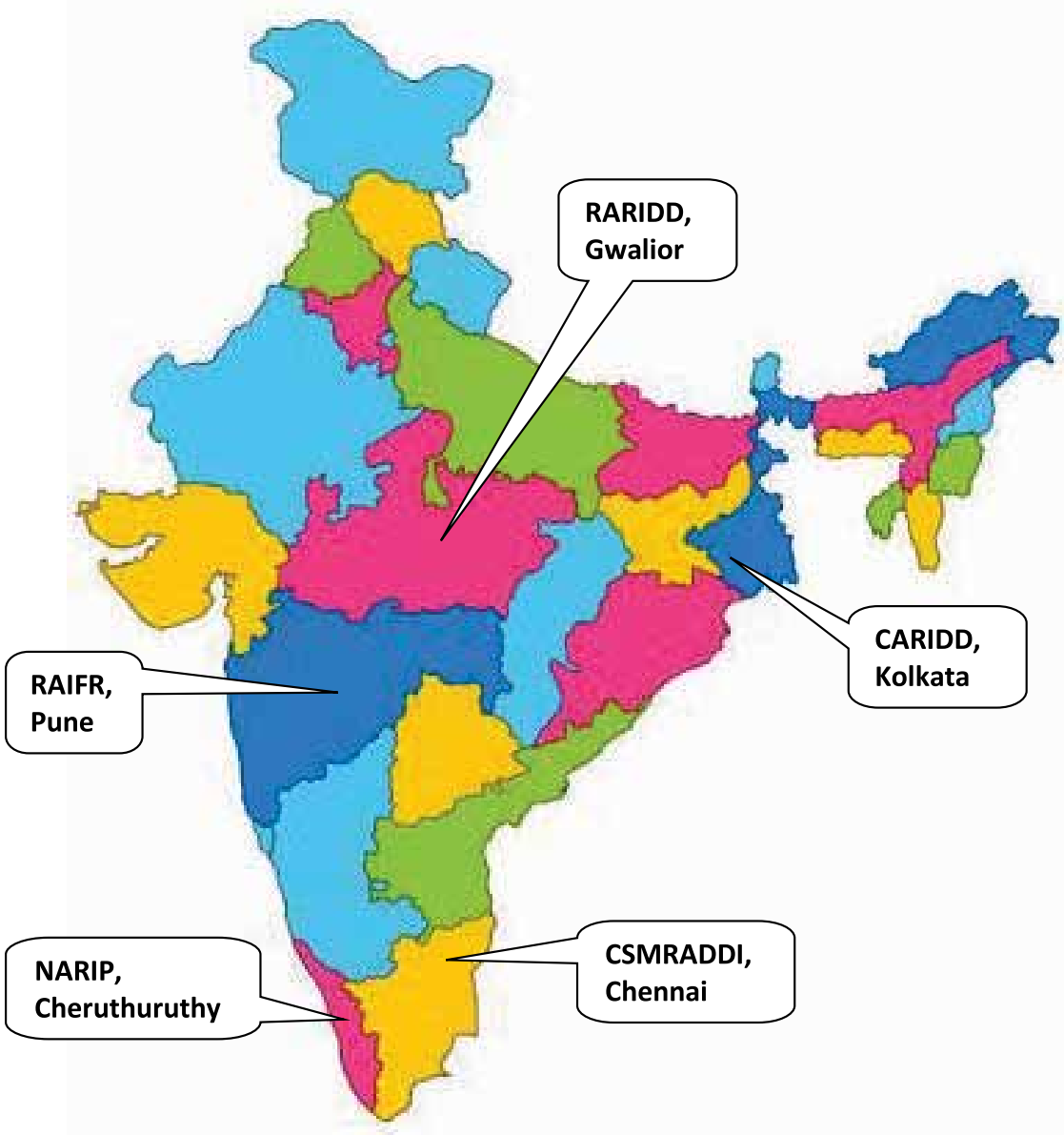
## Reorganization of Various Pharmacology Research Institute/Centres/Units Functioning Under CCRAS

| S. No. | Institutes/units engaged in Pharmacology/Toxicology research | Status of Organization |
|---|---|---|
| 1. | Pharmacological Research Unit, Calcutta (PhRUC) | Merged with CARIDD, Kolkata |
| 2. | Pharmacological Research Unit, Varanasi (PhRUV) | |
| 3. | Pharmacological Research Unit, Lucknow (PhRUL) | |
| 4. | Pharmacological Research Unit, Trivendrum (PhRUT) | Merged with CSMRADDI, Chennai |
| 5. | Pharmacological Research Unit, Jamnagar (PhRUJ) | |
| 6. | Toxicity Research Unit, Jhansi (TRUJh) | Merged with RARIDD, Gwalior |
| 7. | Indian Institute of Phanchkarma, Cheruthuruthy (IIPC) | Merged with NARIP, Cheruthuruthy |
| 8. | Central Research Institute, Mumbai (CRIM) | Merged with RAIFR, Pune |
| 9. | Pharmacological Research Unit, Delhi (PhRUD) | Closed |
| 10. | Pharmacological Research Unit, Jaipur (PhRUJ) | Closed |
| 11. | Pharmacological Research Unit, Jodhpur ((PhRUJ) | Closed |
| 12. | Pharmacological Research Unit, Patiala (PhRUP) | Closed |
| 13. | Toxicity Research Unit, Patiala (TRUP) | Closed |

## List of Present CCRAS Institutes engaged in Pharmacology Research

| S. No. | Name of the peripheral institute |
|---|---|
| 1. | Central Ayurveda Research Institute for Drug Development (CARIDD), Kolkata, West Bengal |
| 2. | Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute (CSMRADDI), Chennai, Tamil Nadu |
| 3. | Regional Ayurveda Research Institute for Drug Development (RARIDD), Gwalior, Madhya Pradesh |
| 4. | National Ayurveda Research Institute for Panchakarma (NARIP), Cheruthuruthy, Kerala |
| 5. | Regional Ayurveda Institute for Fundamental Research (RAIFR), Pune, Maharashtra |

## Location of Map showing Different Pharmacology & Toxicology Centres of CCRAS



## GLIMPSES OF PHARMACOLOGY RESEARCH AND SAFETY STUDY FACILITIES

### A. *In-vitro* Pharmacology/toxicology Lab

Cell observation room with Epifluroscence Inverted Microscope

Instrumentation Room with Cryopreservator, Cell analyser and Plate reader

Instrumentation Room with Multiplate Fluorescent Reader and Cell counter reader

Instrumentation Room with Water Purification Unit, Auto fumigation Unit

Aseptic Room with Biosafety cabinet

LCMS

## B. *In-vivo* Pharmacology/toxicology Lab

Animal house

Experimental animals & animal cages

Biological screening lab

Biochemical lab

Biochemical lab

Biochemical lab

## C. Animal and Experimental Pathology lab

Histopathology lab

Tissue archival area

Upright fluoroscent microscope

Histology microscope

# CHAPTER 2

## VALIDATION OF AYURVEDIC SINGLE DRUGS AND COMPOUND FORMULATIONS

The Council since inception is dedicated in the validation of Ayurvedic formulations/medicinal plants through pharmacology and safety/toxicity studies to consolidate the use of Ayurvedic formulations/ medicinal plants backed by proper scientific evidences.

The Central Council for Research in Ayurvedic Sciences has undertaken pharmacology and safety/ toxicity screening of different Ayurvedic formulations/medicinal plants in certain identified diseases/ conditions of National importance. The Council has undertaken pharmacological screening on different diseases/conditions like diabetes, inflammation, pain, hepatoprotection, thrombocytopenia, cancer, urolithiasis, rheumatoid arthritis, Dislipidimea, atherosclerosis, wound, diabetic wound besides carrying out antioxidant and antimicrobial, immunomodulatory, anti-asthmatic, adaptogenic, antiulcerative, antifertility and abortifaceint, antipsychotic, anxiyolytic and anti-spasmodic, antidepressant-antipsychotic, anti-diarroheal, anti-convulsent, antipyretic, hypnotic, vasoconstrictor, oestrogenic, anti-hypertensive, bronchodilator & anaphylactic, anti-oxytocic, cardiac stimulant, anti-spermatogenic, antiovulatory, antiimplantation, and diuretic biological activity.

The Council has also undertaken diffent biological and safety/toxicity screening on formulation/ medicinal plants/bhasmas/rasakalpas. The preclinical toxicity testing on various biological systems reveals the species, organ and dose specific toxic effects of an investigational product. The toxicity of substances can be observed by (a) studying the accidental exposures to a substance (b) *in-vitro* studies using cells/ cell lines (c) *in-vivo* exposure on experimental animals. The specific guideline instructs that the maximum selected dose should be sufficient to identify the target organ toxicity. From the toxicological evaluation, the no observed effect level (NOEL) or NOAEL, which may be useful for human studies, may be established. The low dose, intermediate dose, and high dose used in the toxicity test provide the NOEL, dose–response relationship, and target organ toxicity in animals, respectively. The Council has undertaken safety/toxicity of different bhasma and Rasakalpa like Naga bhasma, Swarna Bhasma, Tamra Bhasma, Hridyarnava Rasa, Rasamanikya, Trivanga Bhasma, Kasisa Bhasma, Makaradhwaja, Rasa Sindoora, Mahalaxmi Vilasa Rasa, Yograja Guggulu, Mahayogaraja Guggulu, Arogya vardhini Vati and Vasanta Kusumakara Rasa.

The Council has also screened 389 Ayurvedic formulations/medicinal plants for pharmacological/ biological activity and 160 Ayurvedic formulations/ Bhasmas/ Rasakalpas/ medicinal plants for safety/ toxicity study. At present, 19 Ayurvedic formulations/medicinal plants are in progress and many more are under pipeline at various stages. Following are the categorisation of various studies for biological/ pharmacological screening and toxicity/safety study done by the Council since inception for validation of Ayurvedic formulations/medicinal plants.

## 2.1. BIOLOGICAL SCREENING OF AYURVEDIC SINGLE DRUGS AND COMPOUND FORMULATIONS

### BACKGROUND

Considering the importance to generate tangible evidence of Ayurveda formulations and single drugs for their therapeutic potential, 389 studies have been carried out for biological activities since inception. Besides conducting studies through its own institutes viz. Central Ayurveda Research Institute for Drug Development, Kolkata; Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute, Chennai; Regional Ayurveda Research Institute for Drug Development, Gwalior; National Ayurveda Research Institute for Panchakarma, Cheruthuruthy; Regional Ayurveda Institute for Fundamental Research, Pune , the Council collaborates /outsources through institutes of national repute.

16 Intra mural research projects have been completed, 10 Intra mural research projects are ongoing and 12 IMR project are under pipeline on biological activity of Ayurvedic medicine/Medicinal plants. Under pharmacology research, Ayurvedic drugs were studied for their biological activity in various areas such as diabetes mellitus, bronchial asthma, anti-cancer, immunomodulation, wound healing, anti-urolithiatic, anti-arthritic, migraine, dengue, anti-inflammatory, analgesic, adoptogen, hepatoprotective, anti-dyslipidemic activity.

### CORE OBJECTIVES

1. Evaluation of the pharmacological activity/biological screening of Ayurvedic formulation/ Medicinal plants
2. Find out effective dose range and dose dependent effect
3. Generate biological screening data for clinical implementation

### MATERIAL AND METHODS

The gross physical achievements including the biological activity of Ayurvedic formulations/ Medicinal plants since inception were compiled summarized and presented based on the information available in the published monographs, technical reports and annual reports of CCRAS.

### INTRODUCTION

Pharmacological studies gauge biological effect, efficacious dose range and overall potency of the optimized lead. It is very important to perform all pharmacological studies in relevant in- vitro and in-vivo test system, which has closest resemblance to human disease condition. These studies give a further understanding into the mechanism of action of the medicine. Based on the results obtained from preclinical assessments viz. pharmacological safety, efficacy, ADME and toxicological profile of the

drug, clinical phase studies are designed and Investigational New Drug (IND) filing is done as per the regulatory requirements . It is imperative to have safety and efficacy evaluation completed for a test compound before proceeding to translate for clinical trials. Preclinical pharmacology studies play an essential role for providing particulars to design clinical studies to determine whether the test compound is as efficacious and safe in humans as it was observed in animal studies. Study performed in preclinical setting includes determination of safety, efficacy, tolerability and toxicity for the test compound. These studies help to propose a safe and efficacious startup dose for human studies. Furthermore, it won't be wrong to state that without these preclinical pharmacological studies, it is not possible to strategize and design clinical trial in humans.

Ayurvedic medicine is time-tested traditional system of medicine, originated about 5000-years back in India. Ayurvedic medicines can be a potential and effective alternative for the treatment of diseases like cancer, diabetes, renal impairment, obesity, immunosuppression or autoimmunity, heart diseases, neurological disorders, etc. in which modern medicines have limited or no success rate or have serious adverse-effects. Plants have always been an important source of drugs. A large number of the world's populations, especially in developing countries, depend upon medicinal plants as an alternative and complimentary drugs therapy for various ailments. Some of the most common practices involve the use of crude plant extracts, which may contain a broad diversity of molecules with often unknown biological effects. Since the medicinal plants are being used indiscriminately without notifying to their efficacy and possible unhealthy or toxic effects, the World Health Organization has recommended that traditional plants used for the treatment of diseases need further scientific investigation on their efficacy and toxic side effects. Plants produce bioactive compounds which act as defense mechanisms against any disease, and at the same time, may be toxic in nature. However, the general acceptability of herbal medicines has been limited by a lack of defined chemical characterization, efficacy, dose regimen, and adequate toxicity data to evaluate their safety. Therefore, it has become essential to assess the efficacy and safety of plants used for medicinal purposes for biological efficacy and possible toxicity.

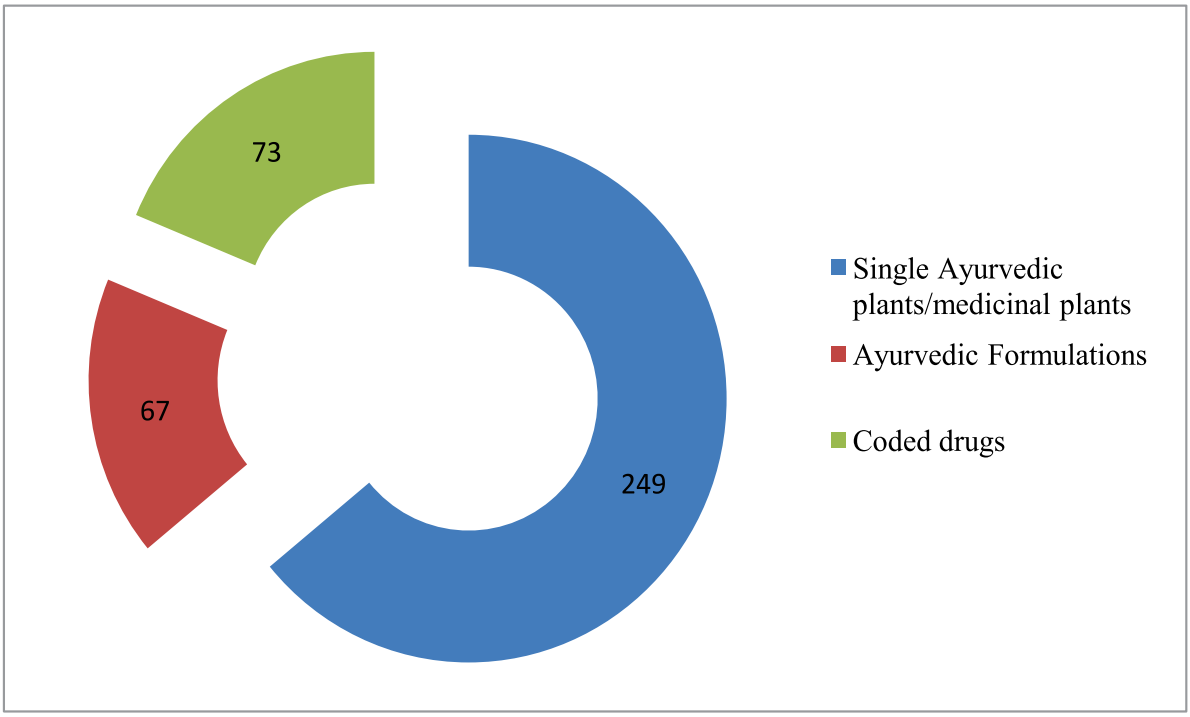
The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings. This past decade has obviously witnessed a tremendous surge in acceptance and public interest in natural therapies both in developing and developed countries, with these herbal remedies being available not only in drug stores, but now also in food stores and supermarkets. It is estimated that up to four billion people (representing 80% of the world's population) living in the developing world rely on herbal medicinal products as a primary source of healthcare and traditional medical practice which involves the use of herbs is viewed as an integral part of the culture in those communities.

Although scientific research and the number of publications on Ayurvedic drugs are increasing

day by day, considering the therapeutic significance of Ayurvedic medicines, numbers of preparations and parameters to be evaluated, those reports are considered to be insufficient. Therapeutic assessment of many preparations and their different safety reports are still unexamined and unreported. In this modern age, scientific evidence is very much essential for better acceptability of a therapeutic agent along with the history of traditional use of that medicine. Insufficient scientific evidences and only few published reports in support of the therapeutic efficacy and safety of Ayurvedic medicines may be one of the several reasons for this. Hence, extensive researches on Ayurvedic medicines and their reporting in reputed international journals are very much important and time-demanding. Extensive in vitro, in vivo, and clinical trials and observational study reports are badly needed for the evaluation of each of Ayurvedic polyherbal preparations and publications of those investigated results in international journals. Traditionally, Ayurvedic medicines are very effective for the treatment of diseases without or with minimum side-effects compared to Allopathic medicines, however there is a need to generate scientific evidences in support of their therapeutic effectiveness and safety. If this can be done, Ayurvedic medicines may be a potential source of alternative treatment in case of the failure or less effectiveness of modern medicines. Ayurvedic medicines have glorious historical background for the treatment of all diseases with minimization/without side-effects. To keep faith on Ayurvedic medicines and to spread its popularity all over the world, extensive preclinical, clinical and observational studies on animal and human for the efficacy and safety of those medicines as well as publications of those results are badly needed. Positive results of the investigations may be highly accepted to the international community including physicians, scientists, practitioners, and other people. In case of negative findings for therapeutic efficacy, toxicity, adverse-effects, etc. the effective steps should be taken with the modification of the formulation, manufacturing, administration and doses of that specific preparation. All the above steps may be taken for the betterment of Ayurvedic medicines and welfare of global health.

## Biological screening

The Council has undertaken different biological screening on formulations/medicinal plants. Total 389 drugs were screened for various pharmacological activities in which 249 single medicinal plants, 67 Ayurvedic formulations and 73 coded (new combination) drugs have been screened since inception. Following are the diseases wise categorisation of various studies for efficacy and safety done by the council for validation of classical drugs.




Numbers of studies on single Ayurvedic drugs, Ayurvedic formulation and coded drugs

**Council has done following major studies since inception for biological screening of Ayurvedic single drugs/compound formulations:**

## 2.1.1. ANTI-DIABETIC ACTIVITY

### BACKGROUND

Diabetes mellitus *(Madhumeha)* is a group of metabolic diseases characterized by hyperglycaemia which is caused due to reduced insulin secretion, decreased glucose utilization and increased glucose production. The secondary pathophysiologic changes occur in multiple organ systems due to metabolic dysregulation associated with Diabetes mellitus. The two categories of diabetes are type I or Insulin Dependent Diabetes Mellitus (IDDM) & type II or Non Insulin Dependent Diabetes Mellitus (NIDDM). Complete or near total insulin deficiency is found in type I. Type II diabetes mellitus is characterized by variable degree of insulin resistance, impaired insulin secretion and increased glucose production. The classical symptoms of diabetes mellitus are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

Diabetes mellitus is one of the major global health problems with an increase in worldwide prevalence from about 30 million cases in 1985 to 177 million cases in 2000 and world wide estimates project that more than 360 million individuals will have diabetes by the year 2030. The prevalence of the disease increases with the age and affects men and women similarly but is slightly greater in men > 60 years. Types II diabetes mellitus is increasing more rapidly due to obesity caused by sedentary life habits and changed life style.

Insulin is the only treatment for type I diabetes and conventional modem medicine provides a number of drugs for controlling the blood sugar level in the patients of type II diabetes mellitus. However, with the prolonged treatment doses of the drugs often needs to be increased to control the blood sugar level and a time comes when patient has to be switched over to insulin. Such patients become cases of insulin dependent diabetes mellitus. With a view to help the suffering community there is a need to find a safer drug, which can be used to control the blood sugar level to use safely for longer periods.

*Madhumeha* has been vividly described in classical Ayurvedic Texts. A number of predisposing and etiological factors and complications of this disease are described in great detail. Acording to Ayurveda, *Madhumeha* is a type of *Prameha.* All types of *Prameha* ultimately lead to *Madhumeha.* The food items and life styles which vitiate *kapha* may predispose *Prameha.* Sedentary life style, excessive sleep and over consumption of curd, meat soups, fat rich food, milk, milk products, jaggery preparations etc. causes *Prameha.* Ayurveda emphasizes on the elimination of the etiological factors as the main principle of treatment of the disease. Accordingly regulation of diet and exercise has been laid special emphasis. The use of herbal and herbo-mineral preparations for the treatment of the disease is also

mentioned. Different Ayurvedic formulations were used in this study series for clinical evaluation.

**Council has done following studies since inception for validation of classical drugs in the management of '*Madhumeha'*.**

**Study 1:** A study with *Ficus gibbosa Blume* (leaves and stem bark) has benn completed. All the animals except Group I (control) were treated with Streptozotocin + Nicotinamide which selectively injures the cells of islets of langerhan in the endocrine pancreas. This leads to decrease /no synthesis of insulin from the endocrine pancreas leading to hyperglycaemia similar to tlpe-2 diabetes. If the injured cells can recover, they may further produce insulin to overcome the hyperglycaemia. In the present study, during the histopathology evaluation of the pancreas, injury to the islets of langerhans was recorded as degeneration/necrosis and graded. The primary target organ of present model of type-2 diabetes is pancreas. Similarly vacuolar changes in the tubules of kidney and liver were also recorded and graded. Liver and kidney changes/ lesions were formed due to persistent hyperglycaemia and considered as secondary lesions. Majority of the animals from the Group 3 (Low Dose L) and 6 (Average Dose SB) showed comparable change as in pancreas as in disease control group 2. However group 7 (High Dose L) and group 8 (High Dose SB) clearly showed no pancreatic lesion as compared to Group 2 (disease control) which may be due to recovery of the injured endocrine pancreatic cells due to the treatment. Ficus gibbosa stem bark hydro-alcoholic extract when administered by oral route in High dose group animals to the streptozotocin+ Nicotinamide induced type 2 diabetes in SD rat, treatment related histopathological changes of reversal of injury to islets of pancreas were observed in the animals as compared to the disease control (Group 2) animals.

**Study 2:** A coded Ayurvedic formulation 'AYUSH-D" was studied for antidiabetic activity in experimental animals. Effect of AYUSH D was studied against Type II diabetes which was induced by administration of STZ (60 mg/kg, i.p.) followed by nicotinamde administration (230mg/kg, i.p.) after 15 minutes. Diabetes was then confirmed by measuring blood glucose on 3$^{rd}$ and 7$^{th}$ days and thereafter weekly up to 4 weeks. AYUSH D was found to decrease hyperglycemia in type II diabetic rats.

**Study 3:** *Mimosa pudica* root powder aqueous and hydroalchoholic extract was studies for its *in-vitro* anti-diabetic activity. Its Cytotoxicity assays have been completed and it is found to be non cytotoxic in liver (Chang Liver), pancreas (RIN5F) and adipocyte (3T3L1) cell lines. Further, its activity was studied in Streptozotocin induced cellular damage in RIN5F cell line. Aqueous and hydroalchoholic extracts showed prevention of cellular damage and increased proliferation of the cells. Mechanism of action of anti-diabetic drugs is by two ways; first it may increase insulin secretion and secondly it may increase the glucose uptake by the cells. Hence, insulin secretion activity was studies in RIN 5 F cells. However, the insulin secretion by the cell line was not observed after repeated assays. Hence, it was concluded that the test system (RIN5F) may not be suitable for such study. ATP Assay was performed which indicated

reversal of cellular damage caused by Streptozotocin by both the aqueous and hydroalchoholic extracts. The later, showed more effective ATP release.

**Study 4:** The anti-diabetic potential of Katakakhadiradi kashayam was assessed by using both aqueous and hydroalcoholic extracts of formulation on four cell lines (3T3L1, Chang Liver, C2C12 and RIN5F); each cell line serving as *in vitro* model for the respective organs. (3T3L1: Adipocyte; Chang Liver: Liver; C2C12: Skeletal Muscle and RIN5F: Pancreas).

The *in vitro* biochemical assays- Alpha Amylase and Alpha Glucosidase inhibition form preliminary assays in identifying the probable mechanism involved in prevention of pathogenesis of diabetes. Natural alpha amylase and glucosidase inhibitors of herbal origin are an effective therapeutic approach to control Diabetes. Established inhibitor of both enzymes: Acarbose was used as positive control. Both aqueous and hydroalcoholic extracts of *Katakakhadiradi* formulation are able to inhibit the activity of alpha glucosidase. The major ingredients of this kasayam are predominantly of *Tikta* and *Kashaya rasa. Hence, Kleda shoshana* karma is pronounced. Most of the starchy foods that we consume fall in the *Madhura* category as per Ayurveda which is the major etiology behind development of *Prameha*. Thus, as per *Vishesha siddhanta,* all the *tikta* and *kashaya rasa* drugs which are *Akash, Vayu and Prithvi mahabhuta pradhan* are inhibitory to the *Jala mahabhuta.* Besides, both are *medoshoshana* which renders them useful in *Medovaha srotasa vyadhi*. Highest extent (100%) was observed with hydroalcoholic extract at 100µg/mL and for aqueous extract at 3mg/mL. Strong inhibition of alpha-glucosidase and low inhibition of alpha amylase could be potentially used as an effective complementary therapy for postprandial hyperglycemia with minimal side effects.

This was followed with *in vitro* cell line studies: Cytotoxicity assay (MTT) so as to determine the effect of treatment on viability of each cell line over time is performed. This allowed for determination of concentration of each extract that is non-cytotoxic to specific cell line and thereby use it in further experimentation. Insulin, Metformin and Glibenclamide due to their anti-diabetic effect were used as positive controls. Drug concentrations tested for the extracts ranged from (5µg/mL to 600µg/mL). The Proliferation and $IC_{50}$ concentration determined for each cell line (Represented in table format on page. no.78).

Adipose tissue dysfunction constitutes a primary defect in obesity and is ultimately linked with diabetes and other chronic health issues. The study of differentiation of preadipocytes to mature adipocytes and testing effect of extracts as potential anti-adipogenic agent is essential and gives indications regarding therapeutic interest of formulation as anti-obesity candidate. Results of current study demonstrates that adipogenesis was substantially reduced when incubated with extracts both aqueous and hydroalcoholic in a dose dependent manner, which suggests a significant increase in lipolysis activity. The results suggest that extracts may be useful in treatment of diabetes associated with obesity.

This was followed by studying glucose uptake in presence and absence of insulin. Results of current study indicate both the extracts showed an increase in glucose uptake by 3T3L1 matured adipocyte cell line. Here, the development of fibroblast (~ mamsa) to adipocyte (~component of meda) is corrected by inhibiting *abaddha meda* (~dyslipidemia) production thereby arresting further progress of the disease. However, when the data in presence and absence of insulin is compared, in absence of insulin both the extracts showed enhanced glucose uptake which indicates that they might be useful in the treatment of Type I diabetes.

In order to study the molecular mechanism, proteome analysis was the next step. Proteomics indicated both aqueous and hydroalcoholic extracts of *Katakakhadiradi* formulation regulated proteins and hence pathways related to insulin secretion, glucose uptake and insulin resistance. Proteins associated with Wnt signaling, Angiogenesis and Akt signaling all reported to be associated with pathogenesis of diabetes (type 2) were identified.

**Study 5:** Hamsapadi (*Adiantum lunulatum* Burm.) was studies on experimental animals and no significant activity on hypoglycaemic was observed.

**Study 6:** Whole plant decoction of Hamsapadi Bheda (*Adiantum caudatum* Linn.) (2000mg/kg) was studies on rats and found hypoglycaemic activity.

**Study 7:** Kaju (*Anacardium occidentale* Linn.) was studied on laboratory animals and found significant hypoglycaemic activity.

**Study 8:** Trivanga Bhasma was studies on experimental animals and found **s**ignificant hypoglycaemic activity.

**Study 9:** Yasada Bhasma was studies on experimental animals and found **s**ignificant hypoglycaemic activity.

**Study 10:** The LC-MS based qualitative bottom-up proteomic approach has incredible applicability in characterization at organismal proteome level. In this study, antidiabetic action of hydroalcoholic extract of an ayurvedic formulation was traced through proteomics profiling in an animal experimentation model. The study groups were composed of healthy control, vehicle control, Glibenclamide standard treated, extract 250mg/kg and 500mg/kg body weight orally treated categories. The blood samples were collected after 28 days of treatment and used to obtain serum. The serum proteins from rats were digested in-solution and subjected to mass spectrometric analysis. The multivariate statistical analysis for pre-post treatment comparison yielded 19 significant proteins. While, within above mentioned five groups comparison yielded 37 significant proteins. Highly confident $R^2$ and $Q^2$ values were observed in Partial Least square discriminant analysis (PLS-DA). The drug was found to impact Insulin based carbohydrate metabolism, oxidative stress, blood coagulation, haemoglobin metabolism, nervous and blood vessels development related proteins and pathways. DENN domain-containing protein 4C, 6-phosphofructo-

2-kinase/fructose-2,6-bisphosphatase 2, MAX gene-associated protein, probable E3 ubiquitin-protein ligase MYCBP2, receptor-type tyrosine-protein phosphatase F, nuclear receptor coactivator 6, alpha-2-HS-glycoprotein precursor and alpha-2-HS-glycoprotein precursor proteins which are closely associated with GLUT-4 based glucose transportation and insulin metabolism were observed. Serine-protein kinase ATM, polyribonucleotide nucleotidyltransferase 1, mitochondrial and CLIP-associating protein 1that are associated with oxidative-nitrosative stress and cellular senescence, were identified. Microtubule-actin cross-linking factor 1, Serotransferrin precursor, porphobilinogen deaminase, Phosphorylase b kinase regulatory subunit beta, A-kinase anchor protein 13, glutamine-fructose-6-phosphate transaminase 2 and axin-2 were some of other expressed proteins involved in significant functions.

**Study 11:** Proteomic analysis of Streptozotocininducedcell based model of Diabetes was evaluated. RIN5F (Pancreatic cell line) was treated with 25 mM Streptozotocin (dose decided after cytotoxicity assay) for one hour and later suspended in normoglycemic media for 24 hours. After 24 hrs, cells were harvested and were processed for protein digestion. Samples were subjected to LC- MS analysis and results obtained were analyzed with the help of Uniprot Data base. The results obtained suggested Streptozotocin treatment related up-regulation of Serine/threonine-protein kinase D1 protein, and 3-phosphoinositide-dependent protein kinase 1; and down regulation of Phosphatidylinositol 3, 4, 5-trisphosphate 5-phosphatase 2 (Ptd $(3,4,5)P_3$ ). Ptd $(3,4,5)P_3$ plays a crucial role in regulation of PI3K-dependent insulin signaling. Conclusion: The study indicates Streptozotocin treatment at sub-lethal dose (25 mM) to pancreatic cell lines (RIN5F) leads to down regulation of PI3K mediated disturbance in Akt pathway which is crucial in cell glucose metabolism.

**Study 12:** *Chandraprabha vati* is a classical Ayurvedic formulation, markedly used for mitigation of *Prameha*, which correlates in many ways with obesity, metabolic syndrome, and diabetes mellitus. The study was aimed to investigate effect of *Chandraprabha vati* in experimentally induced hyperglycemia and lipid profile alterations. Antidiabetic effect of *Chandraprabha vati* was studied in fifty five Wistar rats. Graded doses of *Chandraprabha vati* (50, 100 and 200 mg/kg) were administered orally for 7 days to normal and alloxan-hyperglycemic rats (65 mg/kg, intravenously), and to glucose loaded normal rats for oral glucose tolerance test (OGTT). Fasting plasma glucose levels were assessed on different time intervals along with plasma cholesterol and triglycerides. Metformin (500 mg/kg, orally) was used as standard drug. *Chandraprabha vati* did not cause any significant reduction in blood glucose levels of normal rats ($p>0.05$) but normalized the impaired glucose tolerance at 60 and 120 ($p<0.05$-$p<0.001$) min in OGTT when compared to vehicle control.In alloxan-hyperglycemic rats, administration of Chandraprabha vati (200 mg/kg) significantly reduced blood glucose at 3h, 12h, 3$^{rd}$ day and 7$^{th}$ day ($p<0.01$-$p<0.001$) along with reduction in cholesterol and triglycerides levels ($p<0.01$-$p<0.001$) when compared to diabetic control group. The effects were comparable with standard drug metformin. In conclusion, *Chandraprabha vati* exhibited antihyperglycemic effect and attenuated alterations in lipid

profile. This further validates the use of *Chandraprabha vati* for correction of *prameha* in clinical practice.

**Study 13:** *Bhasmas* are unique Ayurvedic metallic preparations used for medicinal purposes since ancient times. Vanga bhasma, an Ayurvedic preparation of tin is used in traditionally for treatment of diabetes. Vanga bhasma was subjected to evaluate the antidiabetic activity in alloxan-induced diabetic rats. Graded doses of vanga bhasma (25 and 50 mg/kg) were administered intragastrically to normal and experimental diabetic rats. Normoglycemic study and oral glucose tolerance test were evaluated in normal rats while antidiabetic effect was evaluated in alloxan-induced hyperglycemic rats. Metformin was used as reference standard. Vanga bhasma treatment did not influence the blood glucose in normal rats but normalized the impaired glucose tolerance and alloxan-induced hyperglycemia on long term treatment. In conclusion, vanga bhasma, on prolong administration exhibits antihyperglycemic effect.

**Conclusion:** The studies have shown very promising results of Ayurveda intervention for the treatment of diabetes, though it cannot be completely cured but can be successfully managed with different modalities of Ayurveda. The Ayurvedic intervention is also free from any adverse events thus can provide a safe and effective option for the management of diabetes.

## 2.1.2. ANALGESIC ACTIVITY

### BACKGROUND

Pain has been defined by The International Association for the Study of Pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. This process enables an individual to take protective measures, by providing with rapid awareness about threatening or potentially threatening injury. However, if the painful sensation remains after removal of the detectable stimulus, it calls for a regimen for pain management.

An analgesic is any member of the group of drugs used to achieve analgesia, relief from pain. Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which temporarily affect, and in some instances completely eliminate, sensation.

**Council has done following studies since inception for validation of classical drugs in the management of Pain.**

**Study 1:** Madala (*Ailanthus excelsa* Roxb.) (Stem bark) was evaluated for analgesic activity on isolated guinea pig ileum and it showed dose dependent relaxant activity. Significant activity on exploratory behavior, chemical stimulus and no analgesic effect and toxic at high doses by i/p.

**Study 2:** Formalin test on rats with drug Idivallathi Mezhagu showed significantly inhibited only early phase of formalin induced nociception in the dose of 1000 mg/kg, body weight. Formalin test on rats with drug VSI (alco.ext.) showed significantly inhibited only early phase of formalin induced nociception in the dose of 300 mg/kg body weight.

**Study 3:** Formalin test on rats with drug Thalaga Mathirai showed significantly inhibited only early phase of formalin induced nociception in the dose of 100 mg/kg body weight.

**Study 4:** Formalin test on rats with drug Eswarmooli (alco.ext.) showed significantly inhibited both the phases of formalin induced nonciception in the dose of 500 mg/kg body weight. Eddy's hot plate thermal stimulus on albino mice with drug Eswarmooli (alco.ext.) showed 311 maximum analgesic score at 150 minute and found to be significant.

**Study 5:** Formalin test on rats with drug Avuri 10% ointment (chlo.ext.) showed significantly inhibited only early phase of formalin induced nociception. Formalin test on rats with drug Avuri Choornam showed significantly inhibited early phase of formalin induced nociception in the dose of 500 mg/kg body weight.

**Study 6:** Eddy's hot plate thermal stimulus on albino mice with drug VS1 in the doses of 100, 300,500 and 1000 mg./kg. body weight and with the drug Idivallathi mezhugu in the doses of 100,300,500 and

1000 mg/kg body weight were carried out. Eddy's hot plate thermal stimulus on albino mice with drug VSI (alco.ext.) showed 321 maximum analgesic score at 180 minute and found to be significant. Eddy's hot plate thermal stimulus on albino mice with drug VS3 showed 311 maximum analgesic score at 90 minute and found to be significant. Writhing test in mice with drugs VS1 in Choornam form and alco. extract form showed significant analgesic activity.

**Study 7:** Formalin test on rats with drug Phyllanthus anarus showed significantly inhibited early phase of formalin induced nociception in the dose of 500 mg/kg body weight.

**Study 8:** Formalin test on rats with drug Emblica officinalis showed significantly inhibited early phase of formalin induced nociception in the dose of 500 mg/kg, body weight.

**Study 9:** Eddy's hot plate thermal stimulus on albino mice with drug Nellivatral showed 321 maximum analgesic score at 180 minute and found to be significant.

**Study 10:** Hot plate analgesic test in mice with the coded drug $OP_2$ and $OP_3$ showed significant inhibition at the dose of 300 mg/kg body weight.

**Study 11:** The test drugs (SG 1 and SG 2) were screened for analgesic activity by radiant heat method in SD rats. Analgesic activity in Swiss mice was screened through Eddy's hot plate method and Acetic acid induced writhing method. No sign of toxicity and mortality was observed in the animals which received the test drugs at dose of 2000 mg/kg body weight. The test drugs showed significant analgesic activity in SD rats and Swiss mice by radiant heat method and eddy's hot plate method respectively. The test drugs could not significantly reduce the number of writhings induced by I/P administration of acetic acid in Swiss mice.

**Study 12:** A coded drug Ayush-64 showed relaxant activity on isolated guinea big ileum. No activity on exploratory behaviour and analgesic activity and showed significant writhing effect in rats.

**Study 13:** GVK Compound (a Coded drug) did not showed effect on exploratory behaviour, analgesic activity but showed significant writhing activity on chemical induction in rats.

**Conclusison:** The studies show that the Ayurvedic interventions are effective against urolitiasis in experimental animal model and authenticated its uses in Ayurveda.

## 2.1.3. ANTI-INFLAMMATORY ACTIVITY

**BACKGROUND**

Inflammation is a reaction of living tissues towards injury, and it comprises systemic and local responses. Inflammation is the body's immediate response of the immune system to infection and irritation. It is like a double-edged sword because although inflammation eliminates invading pathogens and initiates the healing process, uncontrolled inflammation can lead to injury of tissues and cells, chronic inflammation, chronic diseases and neoplastic transformation.

**Council has done following studies since inception for validation of classical drugs in the management of inflammation.**

**Study 1:** Whole plant decoction (1000mg/kg) of Hamsapadi Bheda (*Adiantum caudatum* Linn.) showed significant anti-inflammatory activity in acute condition in rats.

**Study 2:** Carrageen induced paw odema study on albino rats with the drug Idivallathi mezhugu in the doses of 50, 100, 250, 500 and 1000 mg./kg. It showed maximum 52.12% anti-inflammatory activity in the does of 500 mg/kg.

**Study 3:** *Cardiospermum halicacabum* in choornam form showed 73.01% anti inflammatory activity in the doses of 500 mg/kg in experimental animals.

**Study 4:** Thaazhampoo maathirai in the dose of 200 mg/kg body weight showed 54.24% antiinflammatory activity in experimental animals.

**Study 5:** *Pergularia extensa* in Thailam form in the doses of 7.5 ml/kg body weight showed maximum 82.89% anti-inflammatory activity in rats.

**Study 6:** Neermulli Kudineer in the doses of 20 ml and 30 ml/kg body weight showed 62.68% anti-inflammatory activity in experimental animals.

**Study 7:** Carrageenin induced paw edema study on albino rats with the drug VSI (alcoholic extract) in the dose of 100 mg/kg body weight showed 66.10% anti-inflammatory activity.

**Study 8:** Eswarmooli (alcoholic extract) showed maximum 44.06% anti-inflammatory activity in the dose of 50 mg/kg, body weight. Avuri (chloroform extract) showed maximum 13.51% topical anti-inflammatory activity in the dose of 10% ointment.

**Study 9:** The drug $OP_2$ with the dose of 100 mg/kg, body weight and $OP_3$ (300 mg/kg. body weight) showed significant anti-inflammatory activity in animal models.

**Study 10:** The anti-inflammatory activity of K-1 extract (a coded formulation) were evaluated in experimental animals.

a) **Evaluation of acute anti-inflammatory activity of K-1 extract on Carrageenan induced rat paw oedema:** Rats were treated with K-1 extract (90, 450 & 900 mg/kg, p.o.) suspended in 1% SCMC on the day of experiment. Diclofenac sodium (10 mg/kg p.o.) was taken as a standard drug to compare the efficacy of the test compound. 1 hour after above treatment, 0.1 ml of 1% Carrageenan was injected subcutaneously into the plantar region of right hind paw to induce oedema. The paw volume was measured initially and at 1, 2, 3 and 4$^{th}$ hour after carrageenan injection using plythesmographic method and Percentage inflammation was calculated.

b) **Evaluation of chronic anti-inflammatory activity of K-1 extract on Cotton pellet granuloma in rats:** Sterilized cotton pellets each weighing 10 mg were implanted in to the groin of rats under light ether anesthesia. Animals were treated daily with vehicle, K-1 extract and Diclofenac sodium for seven days. On 8$^{th}$ day the animals were sacrificed by cervical decapitation and the cotton pellets were removed surgically, freed from extraneous tissue, wet and dry weight of the granuloma was determined.

Results showed that the K-1 extract at the dose of 450 and 900 mg/kg exhibited significant reduction in oedema and granuloma weight (both dry & wet), when compared with control group. Where as, K-1 at dose of 90 mg/kg treated animals did not exhibited any significant anti inflammatory activity. The K-1 extract being considered an anti-inflammatory agent.

**Study 11:** Anti-inflammatory activity of C-1 oil upon topical application was evaluated against Carrageenan induced rat paw oedema (acute) and Complete Freund Adjuvant induced arthritis (chronic) in Wistar Rats. C-1 oil was applied topically once and once daily up to 21 days for acute & chronic inflammatory activity studies respectively. Paw size and joint thickness were measured using plethysmometer and vernier calliper daily up to 21 days in case of chronic inflammation, where as, paw size alone was measured in case of acute inflammation. Results showed that there was a moderate decrease in the paw volume of rats treated with C-1 oil. The coded drug C-1 oil has been found to have a moderate anti-inflammatory activity.

**Study 12:** Shunthi Guggulu (Triphalashodhit and Gomutrashodhit) was evaluated for its anti-inflammatory activity in rats. As a preliminary study, the test drugs were screened for safety and anti inflammatory activities. The test drugs were safe up to 2000 mg/kg in female rats. During preliminary studies, the test drugs were found to possess significant anti inflammatory activity at doses 250 and 500 mg per kg body weight in rats.\

**Study 13: *In-vitro* study:** *Trayodashang guggulu* (TG) is an important Ayurvedic polyherbal formulation which has been used for treatment of various inflammatory conditions like arthritis and associated pain

in the Ayurvedic system of medicine. *Trayodashang guggulu* was evaluated for *in-vitro* antioxidant and anti-inflammatory activities. *Trayodashang guggulu* (TG) was standardized as per standard procedures and TLC profile was carried as per Ayurvedic Pharmacopoeia of India. The *in-vitro* antioxidant effect of its aqueous extract (AqTG) (in different concentrations) was evaluated by various *in-vitro* methods viz. DPPH (1, 1-diphenyl-2-picryl-hydrazil) and hydroxyl radical scavenging and reducing power assay and total phenolic and flavonoids contents. *In-vitro* anti-inflammatory activity was evaluated by assaying inhibition of albumin denaturation, membrane stabilization (hypotonicity-induced haemolysis), anti-lipoxygenase and antiproteinase activities. TG was found as per pharmacopoeial standards. *In-vitro* results showed that TG has high antioxidant, membrane stabilizing, protein denaturation inhibitory, anti-lipoxygenase and antiproteinase activities along with presence of fare amount of flavonoids and phenolics. *Trayodashang guggulu* exhibited *in-vitro* antioxidant and anti-inflammatory activities. This preliminary study supports the therapeutic claim of the formulation as anti-inflammatory and antioxidant drug in Ayurvedic system of medicine and advocates its use inflammatory conditions.

***In-vivo* study:** *Trayodashang guggulu* (TG) was also evaluated for analgesic and anti-inflammatory activities in experimental animals with different models. TG was administered orally with acute dose of 270 and 540 mg/kg of rats for evaluating pain and inflammatory models. Evaluation of analgesic activities was done by Eddy's hot plate, tail immersion and formalin induced pain models whereas anti-inflammatory activities were screened by carrageenan and formalin induced inflammations in rats. TG was found to be as per pharamacopoeial standards. TG (270 and 540 mg/kg) significantly decreased the inflammatory pain i.e. flinching (early and late phase) and licking (early and late phase) associated with formalin pain model whereas it did not show any significant reduction of centrally acting pain models i.e. Eddy's hot plate, tail immersion. Further, oral administration of the TG at doses of 270 and 540 mg/kg and indomethacin (10 mg/kg) showed significant and time-dependent reduction of inflammation in both of the models. Results showed that TG caused significant reduction in inflammations and inflammatory pain as in formalin model without any significant changes in neurogenic pain associated with hot plate and tail immersion model.In conclusion, *Trayodosang guggulu* inhibited the inflammatory pain induced by formalin in rats as well as showed anti-inflammatory activity in formalin and carrageenan induced paw edema in rats. It further validates its traditional uses in various inflammatory diseases.

**Conclusison:** The studies show that the Ayurvedic interventions are effective against urolitiasis in experimental animal model and authenticated its uses in Ayurveda.

## 2.1.4. ANTI-CANCER ACTIVITY

### BACKGROUND

Cancer is a disease in which cells in the body display uncontrolled division/multiplication. Cancer is also holistic term for a large group of diseases that can affect any part of the body. Other terms used are tumour and neoplasm. One defining feature of cancer is the rapid multiplication of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs, the latter process is referred to as metastasis. Metastasis is a major cause of death from cancer. Cancers are classified in two ways: by the type of tissue in which the cancer originates (histological type) and by primary site, or the location in the body where the cancer first developed. Here are some common examples of site of origin classification:

- Adenocarcinoma–originates in glandular tissue
- Blastoma–originates in embryonic tissue of organs
- Carcinoma–originates in epithelial tissue (i.e., tissue that lines organs and tubes)
- Leukemia–originates in tissues that form blood cells
- Lymphoma–originates in lymphatic tissue
- Myeloma–originates in bone marrow
- Sarcoma–originates in connective or supportive tissue (e.g., bone, cartilage, muscle)

Today, cancer is a common household word, with each of us closely associated with at least one near and dear one, a family member or a friend, a neighbor or a colleague, diagnosed with cancer. Globally, the five most common cancers considered in both gender are; cancers of the lung (1,824,701; 13%), breast (1,676,633; 11.9%), colorectal (1,360,602; 9.7%), prostate (1,111,689; 7.9%), and cervix uteri (527,624; 3.7%), comprising 46.2% of the 28 cancers reported.

According to the International Agency for Research on Cancer (IARC), a World Health Organization entity, India had 1.8 million people living with cancer (within five years of diagnosis) in 2012. During that year, about a million new cases were recorded, while about 6,83,000 deaths due to cancer were registered. A large number of cases aren't detected (undiagnosed), under-diagnosed or deaths due to cancer are not even registered, making the situation more alarming. Moreover, the early and reliable/specific cancer diagnosis is essential for adequate and effective treatment because every cancer type requires a specific treatment regimen that encompasses one or more modalities such as surgery, radiotherapy, chemotherapy, immunotherapy, and targeted therapy. But most of these therapies are like a hiltless double-edged sword, i.e. you are bound to be wounded, either you attack or defend.

By 2032, researchers estimate there will be 22 million cancer diagnoses and 13 million deaths worldwide each year. But this burden is not-and will not-be shared equally, according to World Cancer Report 2014, published by the International Agency for Research on Cancer (IARC), the specialized cancer agency of the World Health Organization (WHO).

Ayurveda acknowledges that cancer cells are always present in the body, but when the body is in a state of *Dosha* balance (or homeostasis), this is not a problem. *"According to Ayurveda, unbalanced physiology (Doshas) leads to faulty inherent intelligence leading to malfunctioning of genes and gene behavior leading to diseases like cancer"*. According to Ayurveda, *Arbuda* (Karkata) is produced due to many causes; most common cause of cancer is diet/nutrition, study states that about 70% of cancers are due to lifestyles errors and unhealthy dietary habits. Many medicinal plants are known to have anticancer effects according to ancient Ayurvedic text. They reduce the proliferation of cells and there is reduction in the size of tumor after treatment.

**Council has done following studies since inception for validation of classical drugs in the management of cancer.**

**Study 1:** Therapeutic and chemo preventive efficacy study of Nandimezhugu (oral) was tested against 7, 12-dimethylbenzanthracene (DMBA) induced mammary tumours in Sprague-Dawley rats. The study revealed that the Nandimezhugu at therapeutic (90 & 135 mg/kg BW) and chemo preventive (90 mg/kg BW) doses significantly reduced the mammary tumour incidence, latency, frequency, rate of growth and malignancy when compared to the DMBA control group.

**Study 2:** Therapeutic and chemo preventive efficacy study of Cancer Gazkesari (oral) was tested against 7, 12-dimethylbenzanthracene (DMBA) induced mammary tumours in Sprague-Dawley rats. The study revealed that the Cancer Gazkesari at therapeutic (22.5 and 45 mg/kg BW) and chemo preventive (22.5 mg/kg BW) doses significantly reduced the mammary tumour incidence, latency, frequency, rate of growth and malignancy when compared to the DMBA control group.

**Study 3:** *In-vitro* Anticancer potential of ten selected plants, *Holoptelea integrifolia* (Roxb.) (Chirbilwa CH); *Albizzia lebbeck* Benth (Shirisha SH); *Ficus lacor* Buch.Ham (Plaksha PL); *Cissampelos pareira* (L.) Poir. (Patha PT); *Balanites aegyptiaca* (L.) Del, (Ingudi HI); *Sesbania grandiflora* L. (Hathaga HA); *Thespesia populnea* Soland ex Corr (Parish PA); *Ficus religiosa* L.(Pimpal PM); *Ficus bengalensis* (Vad V); *Callicarpa macrophylla* (Priyangu PR) was assessed on four cancer cell lines (HeLa, RIN-5f, Chang Liver and MCF-7). Two plants have displayed anticancer potential; (1) *Balanites aegyptiaca* (L.) Del, (Ingudi HI) has shown activity against all cell lines, (2) *Cissampelos pareira* (L.) Poir. (Patha PT) is effective against RIN-5f cell line only. To ensure that the cytotoxic activity displayed by both plant extracts leads to cell death via apoptotic pathway, two methods were utilized a) DNA fragmentation assay and b) Multi-caspase analysis. Both methods resulted in affirmation of mode of action of plant extracts

is via apoptosis induction. In order to decrypt the molecular mechanism of action of plant extracts on cancerous cell line, proteome level analysis was mandatory. Thus, proteome extraction was carried out for untreated and treated samples, protein estimation was done using Bradford assay and quantified samples (100µg) were subjected to in-solution digestion prior to MS analysis. Data was acquired on Agilent LC MS machine and data analysis was done using Spectrum mill software. The accession list of identified proteins was rectified using NCBI database, i.e. latest/modified accessions were obtained. Thus, obtained accession list was further scrutinized (as NCBI is a redundant database, i.e. it contains repetitive entries of proteins) using UniProt database, which is a manually curated and non-redundant database. Also proteins associated with apoptosis were refined using UniProt database. It is observed that proteins associated with apoptosis pathway have been triggered in treated samples. Also p53 protein is observed to be present in treated samples (at higher concentration of plant extract i.e. concentration close to IC50). Few proteins that promote tumor growth and proliferation have also been identified, but it is very obvious that an immortal cell line would express them. It can be concluded that both the plant extracts HI and PT that have been found to be effective, induced apoptotic pathway mediated via p53 protein activation which is usually suppressed or continuously degraded (by Mdm2, which is negative regulator of the p53).

**Study 4:** The *in-vitro* anti-acncer activity of fifteen herbal extract i.e. Kantakari (*Solanum xanthocarpum*), Yashtimadhu (*Glyceriza glabra*), Daruharidra (*Berberis aristata*), Pippali (*Piper longum*), Sunthi (*Zingiber officinalis*), Katuki (*Picrorhiza kurrao*), Guduchi (*Tinospora cordifolia*), Vidanga (*Embelia ribes*), Devadaru (*Cedrus deodara*), Ashwagandha (*Withania somnifera*), Amalaki (*Phyllanthus amarus*), Vacha (*Acorus calamus*), Kanchanar (*Bauhinia variegate*), Haritaki (*Terminalia chebula*) was conducted against fourteen different cell lines i.e. Colo205 (colon), Hop62 (lung), HT29 (colon), SiHa (cervix), MIAPACA2 (pancrease), DWD (oral), T24 (bladder), PC3 (prostate), A549 (lung), ZR-75-1 (breast), A2780 (ovary), DU145 (prostate), MCF7 (breast), K562 (leukemia). Out of which nine plants extract have shown the anti-cancer activity against eight different cell lines. Cedrus deodara has shown activity against three different cell lines i.e. PC3, A2780 and MCF-7. However, none of the plants exhibited anti-cancer activity against HT29, SiHa, MIAPACA2, T24 and ZR-75-1.

**Study 5:** *Nothapodytes nimmoniana (J. Graham)* (Icacinaceae), also known as Amruta or Narkya is an important medicinal plant. It has wide spectrum of pharmacological activities like anticancer, anti-HIV, anti-malarial, antibacterial, anti-oxidant, anti-inflammatory, anti-fungal and anti-anaemic activity. Hela cell lines were treated with *N. nimmoniana* extracts to understand its anticancer activity. The processed samples were analysed for differentially expressed proteins and metabolites on RRLC-ESI-QTOFMS. The metabolites were analysed using multivariate statistical tools in Mass Profiler Professional (MPP) and MetPA, while Proteins were analysed with tools in MPP and also with STRING and PANTHER. Therefore, the present study was aimed to analyze the proteome and metabolome of HeLa cell line in

response to *N. nimmoniana* extracts treatment.The unidentified proteins have a great chance of becoming signature molecules of cervical cancer. The multi-omics approach has greatest metabolites and proteins profiling potential hence was applied here to find signatures of cervical cancer.

**Study 6:** *Flacourtia indica* (Flacourtiaceae) is an Ayurvedic plant used for the treatment of various diseases. Cytotoxicity of the hydro-alcoholic (50%) extract of stem branches of *Flacourtia indica* (HAFI) was evaluated by using in-vitro models (Allium assay, brine shrimp lethality) and antiproliferative effect was studied in yeast proliferation assay. HAFI showed significant brine shrimp lethality at 24 h, significant decrease in root growth and mitotic index and marked reduction of cell viability of yeast. In conclusion, the hydroalcoholic extract of branches of *Flacourtia indica* exhibited cytotoxic and antiproliferative activity.

**Study 7:** Kanchnar guggulu (KG), an important Ayurvedic formulation containing anticancerous plants, is used clinically for treatment of cancer (Arbuda) and tumour of both benign and malignant nature. The aim of the present study is to investigate the cytotoxic and antiproliferative activity of Kanchnar guggulu. The hydro-alcoholic (50%) extract of KG (HAKG) was prepared and used in present study. The standardization of the formulation was performed as mentioned in pharmacopoeia. Antimitotic activity was assessed in *Allium cepa* assay while antiproliferative effect was studied in yeast proliferation model. Methotrexate (MTX) was used as standard anticancer agent. The physicochemical studies viz. ash content, extractive value, moisture content, pH indicated that the formulation- Kanchnar Guggulu intended for study was of pharamacopoeial standard and up to the mark. In *Allium* assay, HAKG (1, 2 and 3 mg/ml) and MTX (0.02 mg/ml) showed significant concentration dependant inhibitory influence against the dividing cells of *Allium* roots and decreased root growth and mitotic index as compared to control. Antiproliferative studies showed that HAKG (1, 5 and 10 mg/ml) and MTX (0.025, 0.05 and 0.1 mg/ml) exhibited interruption in cell proliferation of *Saccharomyces cerevisiae* as observed by marked reduction in number of dividing cells and inhibition of cell viability compared to control. The results indicated the cytotoxic potential of HAKG due to its antimitotic and antiproliferative effects. The effect may be attributed to the presence of flavonoids and phenolics. In conclusion, the preliminary investigations on the formulation of Kanchnar guggulushowed that formulation exhibited cytotoxic effect by inhibition of cell division (antimitotic) and antiproliferative action and substantiates its potential for the treatment of cancer. It further validates its traditional use in the treatment of cancer.

**Study 8:** *Oroxylum indicum* (Family- Bignoniaceae), commonly known as Shyonak, is a plant of Ayurvedic system of medicine known to be used ethnomedicinally for treatment of cancer in North-East region of India. The study was aimed to evaluate antimitotic and antiproliferative potential of its stem bark. The hydro-alcoholic (50%) extract of stem bark of *Oroxylum indicum* was prepared and used in present study. Antimitotic activity was assessed in *Allium cepa* assay while antiproliferative effect was studied in yeast proliferation model. Methotrexate was used as standard anticancer agent. In *Allium* assay,

hydroalcoholic extract of *Oroxylum indicum* (4, 5 and 6 mg/ml) and methotrexate (0.02 mg/ml) showed significant concentration dependant inhibitory influence against the dividing cells of *Allium* roots and decreased root growth and mitotic index as compared to control. Antiproliferative studies showed that hydroalcoholic extract of *Oroxylum indicum* (4, 5 and 6 mg/ml) and methotrexate (50 and 100 µg/ml) exhibited interruption in cell proliferation of *Saccharomyces cerevisiae* as observed by marked reduction in number of dividing cells and inhibition of cell viability compared to control. The results indicated the cytotoxic potential of extract due to antimitotic and antiproliferative effects. The effect may be attributed to the presence of flavonoids, alkaloids and phenolic compounds like baicalein, oroxylum A, chrysin. In conclusion, the hydroalcoholic extract of stem bark of *Oroxylum indicum* exhibited antimitotic and antiproliferative activity and indicates cytotoxic potential of the plant extract against abnormal cell growth as like in cancer. The study substantiates its ethnomedicinal use in the treatment of cancer.

**Conclusison:** The studies show that the Ayurvedic interventions are effective against different cancer cell lines and authenticated its uses in Ayurveda.

## 2.1.5. BRONCHODILATOR ACTIVITY

**BACKGROUND**

Bronchial asthma (*Tamaka Shwasa*) is prevalent all over the world. It is characterized by chronic airway inflammation and increased airway responsiveness resulting in symptoms of wheeze, cough, chest tightness and dyspnoea. It is also functionally characterized by the airflow limitations usually reverses spontaneously or with treatment. The available treatment in modem medical science like bronchodilators, steroids even in the form of inhalers and leukotrienes modifiers have made tremendous success in providing instant or symptomatic relief in Bronchial asthma. But there is recurrent acute exacerbation and remissions and treatment has many side effects like nausea, vomiting, tremor, huskiness of voice, disturbance of hypothalamus - pituitary -adrenal axis.

Chronic bronchitis is a well-defined clinical condition in contemporary medical science classified under the broader heading of chronic obstructive pulmonary diseases (COPD) that is a progressive preventable condition, without cure. In modem medicine; antibiotics, antihistaminic, bronchodilators, expectorants etc., are commonly used for the management of chronic bronchitis. Although, they are effective in reducing the severity of the disease and suppressing the symptoms; none of these modalities of treatment provide a permanent cure and have limitations owing to their unwanted effects. In Ayurveda, the concept, etio-pathogenesis and treatment of *Shwas* and *Tamaka Shwasa* have been described in detail. Many single and compound herbal and herbo-mineral preparations are mentioned in Ayurveda classical text books.

**Council has done following studies since inception for validation of classical drugs in the management of Asthma:**

**Study 1:** The anti-asthmatic activity of Ayush-A (a coded Ayurvedic preparation targeted for asthma) was evaluated in experimental animals.

**a. Effect of Ayush - A on histamine induced bronchospasm in guinea pigs**

Guinea pigs were exposed to histamine aerosol [micro aerosol of histamine acid phosphate (1% w/v)] prior to drug treatment (0 day). Ayush-A suspended in 1% SCMC was administered for 7 consecutive days at the dose of 435 and 930 mg/kg/ p.o. Chlorpheniramine melate (2 mg/kg p.o.) was taken as a standard drug to compare the efficacy of the test compound. On day 7, two hours after the last dose, the time for the onset of pre-convulsive dyspnoea (PCD) & recovery time of the all the animals were recorded. The animals, which withstood exposure to histamine aerosol for 10 min, were considered to be completely protected. The Percentage protection offered by the treatment was calculated.

Ayush-A (435 & 930 mg/kg/p.o) showed a significant delay in the onset of PCD against histamine

induced aerosol and the recovery time was also decreased significantly after drug treatment.

**b. Effect of Ayush-A on rat mesentery mast cell degranulation using compound 48/80.**

Rats were treated with Ayush – A suspended in 1% SCMC daily for 7 days at the dose levels of 540 mg/kg & 1080 mg/kg, p.o. Prednisolone (5 mg/kg p.o.) was taken as a standard drug. Mast cell degranulation was elicited by Compound 48/80 which is a potent secretagogue. On day 7, two hours after the last dose, all the animals were sacrificed. Mast cells were isolated from the mesentery tissue and stained with toludine blue (1% w/v). The percentage protection was calculated.

Ayush-A showed a significant protection against compound 48/80 induced mast cell degranulation. And the protection against degranulation may be due to the suppression of antibody production.

**Conclusison:** The studies show that the Ayurvedic interventions are effective against asthma in experimental animal model and authenticated its uses in Ayurveda.

## 2.1.6. WOUND HEALING ACTIVITY

**BACKGROUND**

A wound can be defined as any disruption in the normal architecture and function of the skin (Lazarus et al., 1994). Wounds could be due to inherent pathological processes like ischemia or due to any external interventions like cuts, bruises, thermal insults like burns, or chemical insults due to acids etc. Since skin is the major protective barrier of the body, any injury to it should be rapidly and efficiently repaired. Under normal conditions, wounds heal fast, without much external intervention, but with healing compromised conditions like pressure ulcer, diabetes and ischemic ulcers, and any agent that accelerates healing becomes necessary. Wounds can be Acute or Chronic. Wounds cause pain, bleeding, disability and death. The aim of wound treatment has always been to reduce the risks caused by the wound itself and to minimize potential complications. Pain, haemorrhage, loss of skin continuity and tissue substance in a wound has tested man's ingenuity throughout the ages.

Worldwide wound prevalence by etiology is given in Table 1. There are estimated to be 1.6 million cases of traumatic wounds every year worldwide. Lacerations occur frequently (approximately 20 million cases a year), as a result of cuts and grazes. There are approximately 8.5 million pressure ulcers in the world that require treatment every year (Medmarket diligence, 2012). In fact, chronic wounds and their treatment pose a serious problem to the healthcare system. The incidence of chronic wound is 0.78% worldwide and the prevalence ranges from 0.18 to 0.32% (Crovetti et al., 2004). Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide.

Proper healing of wounds is essential for the restoration of disrupted anatomical stability and disturbed functional status of the skin. Repair of injured tissues occurs as a sequence of events, which includes inflammation, proliferation, and migration of different cell types. The inflammation stage begins immediately after injury, first with vasoconstriction that favors homeostasis and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process.The remodeling stage is characterized by reformulations and improvement in the components of the collagen fibre that increases the tensile strength. Factors that contribute to causation and perpetuation of the chronicity of wounds include repeated trauma, poor perfusion or oxygenation, and excessive inflammation. Tissue repair is a simple linear process in which the growth factors cause cell proliferation, thus leading to an integration of dynamic changes that involve soluble mediators, blood cells, the production of the extracellular matrix, and the proliferation of parenchymal cells.

In recent years, diabetic wounds have become a global health concern with the increase in the incidence of diabetes. Diabetic wounds are a kind of chronic and refractory ulcer. It is generally due to the microcirculatory disturbances and the reduced levels of endogenous growth factors. Delayed cutaneous

wound healing is a chronic complication in diabetic patients and is caused primarily by hyperglycemia, oxidative stress, vascular insufficiency and microbial infections.

**Council has done following studies since inception for validation of classical drugs in the management of Wound.**

**Study 1:** An Ayurvedic drug *Jatyadi Ghrita* was studied for its wound healing activity diabetic rats. Would healing property of the test drug was evaluated based on gross and histopathological findings and the test drug was found to show wound healing effect against chronic excision wound in diabetes induced rats. *Jatyadi Ghrita* improves the healing score for dryness of the wound, decreases the inflammation and helps in enhancement of crust formation. Histopathological observations indicated re-epithelialization rate from day 5 onwards in *Jatyadi Ghrita* treated rats was significantly higher as compared to other groups. *Jatyadi Ghrita* also indicated faster maturation of granulation tissue, lesser inflammatory cells and early angiogenesis.

**Study 2:** Wound healing activity of C-1 oil upon topical application was evaluated on 3 different types of wounds (excision wound, incision wound and burn wound model) in Wistar Rats. C-1 oil was applied topically once daily for 18, 09 and 21days in excision, incision and burn wound models respectively. The parameters like wound closure and epithelialisation time were studied in excision wound, tensile strength of the wound was measured in incision wound and percentage of wound contraction, epithelialization period were measured in burn wound model. Results showed that the coded drug C-1 oil has a significant effect on wound healing in all the three models studied.

**Conclusison:** The studies show that the Ayurvedic interventions are effective against wound in experimental animal model and authenticated its uses in Ayurveda.

## 2.1.7. HEPATOPROTECTIVE ACTIVITY

**BACKGROUND**

The liver is one of the most important organs of the body. It performs a fundamental role in the regulation of diverse physiological processes, and its activity is related to different vital functions, such as metabolism, secretion, and storage. Its capacity to detoxify endogenous (waste metabolites) and/or exogenous (toxic compounds) substances of organisms, as well as for synthesize useful agents, has been analyzed since the 1970s by many researchers.

The liver is also involved in the biochemical processes of growing, providing nutrients, supplying energy, and reproducing. In addition, it aids in the metabolism of carbohydrates and fats, in the secretion of bile, and in the storage of vitamins.

Because of all of these functions, hepatic diseases continue to among the principal threats to public health, and they are a problem worldwide. Hepatic disease is a term that indicates damage to the cells, tissues, structure, or liver function, and this damage can be induced by biological factors (bacteria, virus, and parasites) and autoimmune diseases (immune hepatitis, primary biliary cirrhosis), as well as by the action of different chemicals, toxic compounds [carbon tetrachloride ($CCl_4$), thioacetamide, dimethylnitrosamine (DMN), *D*-galactosamine/ lipopolysaccharide (GalN/LPS)], and unquestionably, excessive consumption of alcohol.

**Council has done following studies since inception for validation of classical drugs in the management of hepatic discorders.**

**Study 1:** Aarogyavardhini Vati was evaluated for its hepatoprotective activity in rats. No effect of Aarogyavardhini Vati and Silymarin was seen on reducing or controlling $CCl_4$ induced hepatic injury in rats. Hence alternative hepatotoxicity model should be considered for assessing the efficacy of Aarogyavardhini Vati in preclinical studies. Mercury distribution in tissues was significantly lesser from that of earlier reported study conducted in normal rats. Copper tissue distribution was first time assessed in this study which should provide baseline data for further studies.

**Study 2:** Hepatoprotective potential of *Premna Tomentosa* L. was evaluated in albino wistar rats. Hepatoprotective activity of alcoholic extract of aerial parts *Premna tomentosa* L. by inducing hepatic injury with D-galactosamine hydrochloride in rats was carried out. The lyophilized alcoholic extract of *Premna tomentosa* ranging from concentration of 1000µg/ml to 10µg/ml was used to assess the antioxidant activity by various methods. For hepatoprotective activity, the alcoholic extract of aerial parts of *Premna tomentosa* at the dose of 300 mg/kg and 500 mg/kg were administered orally for 10 days. Silymarin (100 mg/kg), a known hepatoprotective drug was used as a standard drug for comparison. The blood samples were estimated for Alanine aminotransaminase (ALT) and Aspartate aminotransaminase

(AST), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Total Protein (TP) and Albumin. The liver samples were collected, weighed and processed for histopathology. The results showed good antioxidant activity and significant (P<0.001) reduction in the biochemical parameters in rats treated with *Premna tomentosa*. Histo-pathological section showed similar trend in all the groups. The alcoholic extract of aerial parts of *Premna tomentosa* L. exerts hepatoprotective activity and it can be attributed to the antioxidant principles which scavenge the free radicals responsible for pathological severity.

**Study 3:** Hepatoprotective potential of *Azima tetracantha* l. was evaluated in Albino Wistar rats by inducing hepatic injury with D-galactosamine hydrochloride in rats. The lyophilized alcoholic extract of *Azima tetracantha* ranging from concentration of 1000µg/ml to 10µg/ml was used to assess the antioxidant activity by various methods. For hepatoprotective activity, the alcoholic extract of *Azima tetracantha* root at the dose of 300 mg/kg and 500 mg/kg were administered orally for 10 days. Silymarin (100 mg/kg), a known hepatoprotective drug was used as a standard drug for comparison. The blood samples were estimated for Alanine aminotransaminase (ALT) and Aspartate aminotransaminase (AST), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Total Protein (TP) and Albumin. The liver samples were collected, weighed and processed for histopathology. The results showed good antioxidant activity and significant (P<0.001) reduction in the biochemical parameters in rats treated with *Azima tetracantha*. Histo-pathological section showed similar trend in all the groups. The alcoholic extract of *Azima tetracantha* L. root exerts hepatoprotective activity and it can be attributed to the antioxidant principles which scavenge the free radicals responsible for pathological severity.

**Study 4:** In view of traditional use of *Bombax ceiba* flowers in the treatment of jaundice and splenic enlargement, it was proposed to evaluate hepatoprotective activity of aqueous extract of flowers of *Bombax ceiba* in $CCl_4$-induced hepatotoxicity in rats. Treatment with aqueous extract of the flowers of *Bombax ceiba* (BCAE) caused significant reduction in some of the elevated plasma liver function markers while others remained unaffected. It has also prevented necrosis of the tissue and did not offer much significant prevention of damage. The effect of BCAE differs in some respect with sylimarin, standard hepatoprotective agent which showed significant protection on $CCl_4$-induced hepatotoxicity. The study revealed that BCAE exhibited moderate kind of hepatoprotective effect. The hepatoprotection offered by BCAE may be due to presence of antioxidant phytochemicals viz. flavonoids and terpenopids compounds as $CCl_4$ induces hepatotoxicity through free radicals.

**Study 4:** The leaves of *Chenopodium album Linn.* are traditionally used in treatment of jaundice and spleen enlargement. The research work investigated the effect of aqueous extract of leaves of *Chenopodium album* on experimentally induced hepatotoxicity in rats to substantiate the traditional use in liver diseases. The hepatotoxicity was induced in rats by carbon tetrachloride (CCl4) treatment and in addition, vehicle or aqueous extract of the leaves of *Chenopodium album* (CAE) (100, 200 and 400 mg/kg) or silymarin (25 mg/kg) were administered daily orally for seven days. The hepatotoxicity was

assessed by estimating the activities of marker enzymes and by histological studies. The oxidative stress was assessed by measuring lipid peroxidation and activities of superoxide dismutase and catalase in liver. The CAE treatment significantly prevented the CCl4-induced elevations the serum levels of glutmate oxaloacatate transaminase, pyruvate oxaloacatate transaminase, alkaline phosphatase, bilirubin, lactate dehydrogenase and triglycerides while decreased the total protein. Treatment with CAE attenuated the CCl4-induced fibrosis in liver tissue and also decreased elevated lipid peroxidation levels and inhibited decrease in activities of superoxide dismutase and catalase enzymes in liver. The effects of the CAE were comparable to that of standard antioxidant hepatoprotective agent, silymarin. These findings indicate the CAE exhibited hepatoprotective effect against CCl4- induced liver damage which may be attributed to antioxidant action of CAE due to presence of antioxidant phytochemicals like flavonoids and other phenolic compounds. It indicates the therapeutic potential of *Chenopodium album* leaves and validates its traditional medicinal use in liver disorders.

**Conclusison:** The studies show that the Ayurvedic interventions are effective against hepatotoxicity in experimental animal model and authenticated its uses in Ayurveda.

## 2.1.8. ANTI-HYPERLIPIDEMIC ACTIVITY

**BACKGROUND**

Hyperlipidemia is a heterogeneous disorder commonly characterized by elevated serum total cholesterol, low density and very low-density lipoprotein cholesterol, triglycerides, and decreased high-density lipoprotein levels. Hyperlipidemia is one of the greatest risk factors contributing to the prevalence and severity of atherosclerosis and subsequent coronary heart disease. Liver synthesizes two-third of the total cholesterol made in the body. The rate limiting enzyme is 3-hydroxy-3-methylglutaryl (HMG)-Co A reductase and provides feedback regulation by controlling the cholesterol concentrations in cells. Treatment of hyperlipidemia involves diet control, exercise, and the use of lipid-lowering diets and drugs. The most commonly employed drugs for treatment of hyperlipidemia include hydroxymethylglutarate coenzyme A (HMG-CoA) reductase inhibitors, also called as statins. Other drugs employed for treatment of hyperlipidemia include bile acid sequestrants (anion-exchange resins) such as cholestyramine and colestipol; fibrates such as clofibrate, gemfibrozil, fenofibrate, ciprofibrate, and bezafibrate; niacin; cholesterol absorption inhibitors such as ezetimibe; and omega-3-fatty acids.

**Council has done following studies since inception for validation of classical drugs in the management of lipid discorder.**

**Study 1:** *Cardiospermum halicacabum* Linn was evaluated for anti-hyperlipidemic activity in rats. The study showed that Hydro-alcoholic extract of Cardiospermum halicacabum Linn Leaf has significant Hypolipidemic Activity in the atherodiet induced hyperlipidemia in Wistar Albino rats. The study conducted in blood and tissues samples of experimental animals has demonstrated that *C. halicacabum* has significant antioxidant potential (SOD, GSH, GPx and Catalase).

**Study 2:** The leaves of *Holoptelea integrifolia* are indicated for diabetes and lipid disorders. Aqueous extract of leaves of *Holoptelea integrifolia* (HIAE) was evaluated for lipid profile in normal and Tyloxapol induced hyperlipidemic rats. The aqueous extract of leaves of *Holoptelea integrifolia* (250 and 500 mg/kg) was administered orally for 7 days. Fenofibrate (65 mg/kg, orally for 7 days) was used as reference standard. The lipid profile was assessed by estimating the plasma levels of total cholesterol, triglycerides, HDL-c, LDL-c, VLDL-c and corresponding atherogenic index and LDL-c/HDL-c ratio. Administration ofthe HIAEneithercaused any significant effect on lipid profile in normoglycemic rats nor attenuated the tyloxapol-induced hyperlipidemia. The results of the present investigations reveals that aqueous extract of leaves of *Holoptelea integrifolia* did not exhibit hypolipidemic effect and does not substantiates its traditional use in lipid disorders and obesity.

**Conclusison:** The studies show that the Ayurvedic interventions are effective against hyperlipidemia in experimental animal model and authenticated its uses in Ayurveda.

## 2.1.9. LITHOTRIPTIC ACTIVITY

Urolithiasis is defined as the presence of one or more calculi in any location within the urinary tract. It has been discussed elaborately in ancient Ayurvedic texts. Susruta, the father of surgery was of the view that the dietary factors are much more responsible for the calculus formation the urinary tract (*Mutrasmari*). drug which can correct crystalloid colloid imbalance and relieves the binding mucin of calculi, antiseptic, antispasmodic and diuretic, can relax the muscles of urinary bladder and prevent the supersaturation of crystalloids and possessing anti inflammatory properties on renal tissue may have a possible role in the management of urolithiasis. Majority of the stones are calcium-containing stones, especially calcium oxalate (80%) and others are 20%. The medical management of urolithiasis is done by drug treatment and extracorporeal shock wave lithotripsy (ESWL). The recurrence of urolithiasis represents a serious problem as patients who have formed one stone are more likely to form another. Thus, medical management of urolithiasis is either costly or poses serious side effects. There is growing interest of public in Ayurvedic herbal medicine, particularly in the treatment of urolithiasis as either an alternative or an adjunctive therapy in the management of urolithiasis. The Council, since its inception has been engaged in finding out effective, inexpensive, non invasive and safe treatment modalities.

**Council has done following studies since inception for validation of classical drugs in the management of *Mutrasmari*:**

**Study 1:** An Ayurvedic formulation Sveta Parpati was studies for urolithiatic activity in rats. In acute oral toxicity study the drug *Sveta parpati* found to be non toxic. 90 days repeated oral dose toxicity studies of the drug *Sveta Parpati* was found to be non toxic. Heavy metal analysis (lead) for the drug *Sveta parpati* by Atomic Absorption Spectroscopy was carried out (found to comply within the limit, lesser than 10ppm). *In vivo* evaluation of anti urolithic activity of *Sveta parpati* by calcium oxalate induced urolithiasis in rats" has been carried out. 0.75% ethylene glycol in drinking water *ad libitum* was administered for the curative study. Qualitative and biochemical analysis of serum and urine (creatinine, calcium, phosphorous, magnesium, urea and uric acid) were estimated. 0.75% ethylene glycol in drinking water *ad libitum* for 28 days was administered for the preventive study and the drug *Sveta parpati* administration along with stone induction for 28 days for preventive study was carried out. The urinary crystals of the urolithic rats were observed under electron microscope under 10X and 20X magnifications and photographs recorded. At the end of preventive and curative study, individual urine samples of each group were collected and biochemical analysis of serum (Calcium, Magnesium, Urea, Uric acid, Creatinine and Phosphorous) and urine (Calcium, Magnesium, Urea, Uric acid, Creatinine and Phosphorous) were estimated. Individual urine samples of each group were collected and the urinary crystals were observed under electron microscope under 10X and 20X magnifications and photographs recorded. Standard curve estimation of oxalate standards at the visible spectrum at an absorbance of 570nm using UV Visible spectroscopy was completed. Biochemical estimation of oxalate in kidney for

the preventive and curative groups was estimated at the visible spectrum at an absorbance of 570nm using UV Visible spectroscopy and interpretation with oxalate standards were interpreted. Statistical analysis of plasma biochemical parameters on 30$^{th}$ day, 60$^{th}$ day and 90$^{th}$ day of 90 days repeated dose toxicity of *Sveta parpati* were interpreted. The aggregation assay in presence of the reference drug, cystone extract was carried out by recording the absorbance at 620nm at 30, 60, 90, 180 and 360 mins and the rate of aggregation was estimated. The percentage inhibition of aggregation was calculated for both the drug *sveta parpati* and the reference drug cystone. *Sveta Parpati* was found to be effective against ethylene glycol induced urolithiasis.

**Study 2:** Gokshuradi guggulu was studied to find out various physiochemical parameters like pH, loss on drying, ash values, extractive values and thin layer chromatography as per standard methods and also tested for heavy metals, microbial load and test for specific pathogens, aflotoxins (B1, B2 , G1, G2) and pesticide residue. In addition, quantitative estimation of phytoconstituents viz. total flavonoid, total phenolic and total saponin contents was also carried out. All the standardization parameters of gokshuradi guggulu were found within pharmacopoeial limits. Based on the results, it can be concluded that the test item (gokshuradi guggulu) is quality complaint product. In acute oral toxicity, the test substance is administered orally in graduated doses to several groups of experimental animals, one dose being used per group. In sub-acute oral toxicity study, the test substance is administered daily orally in graduated doses to several groups of experimental animals, one dose per group, for a period of 28 days. No mortality or moribund stage was observed. After reviewing the data of weekly body weights of animals, it was found that there was no significant change in body weights. No treatment related gross pathological changes were noted in comparison to the vehicle control group. Further, there were no histopathological changes observed due to gokshuradi guggulu treatment as compared to vehicle control group. The effect of gokshuradi guggulu was studied in in vitro models of urolithiasis. In in vitro calcium oxalate crystallization assay, the process of nucleation and aggregation was studied in sodium acetate buffer of pH 5.7 to simulate the conditions of urine so as to favor the above processes. In vitro calcium oxalate crystal growth assay was carried out to evaluate the inhibitory activity of gokshuradi guggulu in different concentration (50-2000µg/ml) and cystone against CaOx crystal growth. In calcium oxalate crystallization study, the turbidity increased linearly up to 5 min and then decreased linearly up to 15 min after the addition of calcium chloride dihydrate. Earlier increase in the turbidity was suggestive of the nucleation phenomenon, while the decrease in the later part indicated the aggregation. in vitro study, the effect of gokshuradi guggulu was studied in in vivo model of ethylene glycol induced urolithiasis in rats. Ethylene glycol (0.75% in drinking water for 28 days) was used to induce calculi. Gokshuradi guggulu (250, 500 and 1000 mg/kg orally) was administered in two regimens to study its preventive or curative role in ameliorating the urolithiasis. Gokshuradi guggulu treatement in both preventive and curative regimens attenuated the elevation of calcium and phosphorus in plasma. Gokshuradi guggulu in both preventive and curative regimen showed amelioration of ethylene glycol induced urolithiasis possibly

due to its effect on inhibition of crystal growth and dissolution.

**Study 3:** The leaves of *Elytraria acaulis* are traditionally used for correction of urinary stones. Aqueous extract of *Elytraria acaulis* (AqAE) was evaluated for its antioxidant and antilithiatic activity by in-vitro models. Antioxidant activity was screened using DDPH and H2O2 scavenging and reducing power assay. The antilithiatic activity was studied by using nucleation, aggregation assay of CaOx crystals and growth assay of brushite crystals. *Elytraria acaulis* (AqAE) significantly inhibited CaOx crystallization and inhibited growth of brushite crystals. The study revealed that leaves of *Elytraria acaulis* were found effective in prevention of the experimentally induced urinary stones and substantiate the traditional claim.

**Study 4:** In view of traditional use of *Aerva pseudotomentosa* in antilithiasis, effect of aqueous extract of *Aerva pseudotomentosa* (AEAP) on in-vitro crystallization was evaluated in calcium oxalate and brushite crystals. AEAP showed inhibition of nucleation of CaOx crystallization, growth of brushite crystals effect. The effects were comparable to standard drug, cystone, AEAP significant results against CHPD crystals ($P<0.001$). In conclusion, the preliminary investigations on leafs of *Aerva pseudo-tomentosa* have been shows effectivein prevention of the urinary stones and urinary tract infection. This investigation shows the therapeutic potential of the plant to be developed as alternative herbal anti-urolithiatic drug.

**Conclusison:** The studies show that the Ayurvedic interventions are effective against urolitiasis in experimental animal model and authenticated its uses in Ayurveda.

## 2.1.10. ANTI-ARTHRITIS ACTIVITY

**BACKGROUND**

Rheumatoid arthritis (*Amavata*) is an autoimmune inflammatory disease that causes pain, swelling, stiffness, joint destruction & its functional disability. It is defined as a chronic multisystem disease characterized by persistent inflammatory synovitis, usually involving peripheral joints in a symmetric distribution with a potential to cause cartilage destruction and bone erosions. As the etio-pathogenesis of Rheumatoid arthritis (*Amavata*) is unknown, there is no specific treatment. The Ayurvedic treatment of *Amavata* (Rheumatoid arthritis) is being increasingly recognized as an alternative approach to its treatment.

According to Ayurveda, *Amavata* (Rheumatoid arthritis) is caused due to malfunctioning of the gastro intestinal system. It is a very painful disease and causes great discomfort during its aggravation period. The main cause of this disease is formation of *Ama* (a toxic substance) due to *Agnimandya.* The etiological factors such as *viruddhahara* (improper and irregular dietary habits), *Viruddhachesta* (improper physical and psychological activities), *mandagni* (improper digestion and metabolism), sedentary habits and exercise immediately after food lead to the formation of *Ama* which gets circulated by *vyan vayu* to various *kapha sthana* especially joints and causes inflammation which leads to disease *Amavata* and it may be correlated with Rheumatoid arthritis due to its similar symptomatology. The cardinal features of *Amavata* are swelling and severe pain that seems to be of scorpion bite over the joints like hands and legs (especially knee, ankle wrist, metacarpals and metatarsals). The other symptoms are body pain, loss of appetite, excessive thirst, laziness, heaviness of the body and fever. Based on the cardinal feature and other associated features, many effective regimens are described in Ayurvedic classics.

**Council has done following studies since inception for validation of classical drugs in the management of Rheumatoid arthritis:**

**Study 1:** *In-vitro* antioxidant activity of *Vaisvanara churna* was carried out by different methods like DPPH scavenging, superoxide anion radical scavenging, hydroxyl radical scavenging, iron chelating activity, nitric oxide scavenging and determination of reducing power. In all the methods *Vaisvanara churna* exhibited significant free radical scavenging activity. *In-vivo* anti-inflammatory activity was evaluated by carrageenan induced paw edema and cotton pellet induced granuloma model. In both the methods *Vaisvanara churna* exhibited significant anti-inflammatory activity by the reduction in paw edema and inhibition of dry weight granuloma respectively when compared with vehicle control. The *in-vivo* analgesic activity was evaluated by acetic acid induced writhing model and tail immersion test. *Vaisvanara churna* exhibited significant analgesic activity by reducing the number of writhes in writhing test when compared with vehicle control. There was no significant effect observed in tail immersion test.

The *in-vivo* anti-pyretic activity was studied by baker's yeast induced pyrexia model and it was found that *Vaisvanara churna* exhibited the significant reduction in rectal temperature when compared with vehicle control. Anti-arthritic activity of *Vaisvanara churna* was evaluated by complete freund's adjuvant model, collagen induced arthritis model and by pain application measurement. In above methods *Vaisvanara churna* exhibited significant anti-arthritic activity when compared with arthritic control. The *in-vitro* anti-inflammatory activity was studied by membrane stabilization action using heat and hypotonicity induced hemolysis. The *churna* showed significant activity. *Ex-vivo* antioxidant activity was evaluated by the enzymatic and non enzymatic estimation methods. *Vaisvanara churna* treated groups showed significant alteration of enzyme levels when compared with arthritic control. The cytokines assay is carried out as per the instructions specified in the kit. There is no detectable level of cytokines produced in this measurement when compared with different concentrations of standard specified in the kit. *In-vitro* cylco-oxygensae assay for screening anti-inflammatory activity of *Vaisvanara churna* was performed and it possess anti-inflammatory activity which may be due to its inhibitory effect on cyclo-oxygenase pathway by blocking prostaglandin synthesis. In the light of the above experiments, supported by biochemical, histopathological, *in-vivo, in-vitro* anti-oxidant data, *in-vivo* efficacy studies, *ex-vivo* anti-oxidant assays this ayurvedic drug *Vaisvanara churna* found to possess potential benefits as claimed by ancient literatures.

**Study 2:** The anti-arthritic activity of *Laghu Vishagarbha Taila* (LVT) was evaluated in complete Freund's adjuvant (CFA)-induced arthritis model in rats. Arthritis was induced by intra-articular injection of 150µl CFA in knee joint. Rats were pretreated topically with vehicle (sesame oil) or LVT (0.5 ml) or piroxicam gel (0.5 g) one day before- and consecutive 28 days post-CFA injection. The animal's body weight, knee diameter, limb withdrawal threshold (LWT), weight distribution ratio on hind limb and mobility (grid cross assay, rearing and gait) were measured 2-3 times in a week. On day 28 the blood sample was collected for assessment of the hematological parameters, erythrocyte sedimentation rate (ESR) and biochemical parameters viz., rheumatoid factor (RF), C-reactive protein (CRP), tumor necrosis factor-α (TNF-ϑ), interleukins (IL-1αand IL-6) and intercellular adhesion molecule (ICAM-1). After collection of blood the animals were sacrificed and knee joint was harvested for histopathological studies. The knee diameter was significantly reduced while LWT and weight distribution ratio was significantly increased in LVT or piroxicam gel treated rats. Grid cross and gait was significantly improved but rearing was unaffected in LVT treated rats as compared to vehicle treated arthritic rats. The hematological parameters were unaffected in LVT or piroxicam treated rats compared vehicle treated arthritic rats. ESR was significantly reduced in LVT treated rats compared to vehicle control rats. LVT treatment also significantly reduced the serum level of RF and CRP. LVT or piroxicam gel treatment significantly attenuated the increased in plasma level of TNF-ϑ, IL-1ϑ, IL-6 and ICAM-1 as compared to vehicle treated arthritic rats. In histopathological evaluation, LVT significantly ameliorated the CFA induced synovitis, mononuclear cells infiltration, hyperplasia of synovium and pannus formation. It indicates that

LVT exhibited significant antiarthritic activity against CFA-induced arthritis in rats. In conclusion, laghu vishagarbha taila exhibited analgesic, anti-inflammatory and anti-arthritic action without any noticeable toxicity. The study validates the therapeutic use of laghu vishagarbha taila in Ayurvedic system of medicine for treatment of painful inflammatory condition like arthritis and other painful conditions like sciatica, lumbago, etc.

**Conclusion:** Studies have shown that the ayurvedic drug mentioned in the Ayurveda text found to possess potential anti-arthritis activity.

## 2.1.11. ANTI-ATHEROSCLEROTIC ACTIVITY

**BACKGROUND**

Atherosclerosis is one of the major risk factors for coronary artery disease. It is a complex, multifactorial inflammatory disease, characterized by the presence of lesions due to the accumulation of lipids in the walls of arteries. There are a number of genetic, metabolic, and environmental factors involved in the formation and evolution of the atherosclerotic plaque. A well-known risk factor in humans is hypercholesterolemia, i.e., elevated total cholesterol and low-density lipoprotein cholesterol (LDLc), and other important contributors to this disease includes inflammation, oxidative stress and insulin resistance. Foods rich in saturated fat and cholesterol have been linked to elevations in circulating cholesterol levels. Lipid-enriched diets are often used to induce or accelerate the rate of atherosclerotic lesion in murine models of atherosclerosis. Therefore, using high fat diet for promoting atherosclerosis is a valuable tool for understanding the disease and treatment effect. Lipoprotein oxidation and oxidative processes in general play an important role in the pathogenesis of atherosclerosis. Disorders of lipid metabolism are manifested by elevation of plasma lipids and lipoprotein fractions, which in turn results in cardiovascular diseases.

**Council has done following studies since inception for validation of classical drugs in the management of Atherosclerosis:**

**Study 1:** Premna integrifolia Linn. (Agnimantha) was studied for anti-atherosclerotic activity in experimental animals. Hydroalchoholic extract of root bark of *P. integrifolia* showed significant antioxidant activity in both in vivo and in vitro assays. The rate limiting factors HMG CoA/Mevalonate ratio, collagen, calcium and other cardiac marker enzymes were significantly controlled by hydro alcoholic extract. The Hydroalchoholic extract of root bark of P. integrifolia was found to be safe upto the dose of 2000mg/kg in rats.

**Study 2:** Parijaat (*Nyctanthes arbor-tristis* Linn.) flower extract was evaluated for anti-dyslipidemic activity rats. Parijaat flower extract showed significant effect @ 1000 and 1500mg/kg on reducing TG and VLDL levels after 4wks of treatment in High Fatty Diet induced dyslipidaemia in rats.

**Conclusion:** Studies have shown that the ayurvedic drug mentioned in the Ayurveda text found to possess potential anti-arthritis activity.

## 2.1.12. IMMUNOMODULATORY ACTIVITY

### BACKGROUND

Immunity is the body's natural defense system against various infectious diseases. The factors which trigger immunity include previous infection, immunization, and various external stimuli. Besides, immunity is capable of discriminating among body's own proteins/cells and foreign entities. As soon as the foreign particle is identified, the collective and coordinated response of specific cells and mediators against strange substances constitutes the immune response. Based on the function, immune system has been categorized in two broad categories, i.e., innate immune system (non-specific immune system) and adaptive immune system (specific or acquired immune system. The microbiological, chemical and physical barriers are also sometimes included in innate immunity; however, the main mediators of immune system which deliver instant defense include cytokines, acute phase proteins, macrophages, monocytes, complement, and neutrophils. Various distinct moieties expressed by pathogens, known as pathogen-associated molecular patterns (PAMPs), are recognized by host to detect presence of a pathogen. The germline-encoded and evolutionarily conserved host sensors known as pattern recognition receptors (PRRs) recognize the PAMPs. Once the PRRs recognize the PAMPs, an array of immune responses are quickly triggered via induction of different type I interferons, chemokines, and cytokines. An important role in host's defense is played by PRRs families such as DNA receptors (cytosolic sensors for DNA), NOD-like receptors, RIG-I-like receptors and toll-like receptors. All phases of non-specific immunity include antigen-presenting cells and macrophages which play pivotal roles in antibody-dependent cell-mediated cytotoxicity, secretion of cytokines, nitric oxide (NO) production and antigen presentation, processing and phagocytosis. Dendritic cells are responsible for the activation of naïve and memory B and naïve T cells. During various phases of dendritic cells' differentiation, the effectors of innate immunity including natural killer (NK) cells are regulated, which govern specific and natural immune responses by producing tumor necrosis factor-α (TNF-α), interferon-α (IFN-α) and granulocyte–macrophage colony-stimulating factor (GM-CSF). Complement system is the tertiary relevant component of innate immunity. The complement system is the humoral immunity's main effector among all the physiological systems of host defense. C3a and C3b (complement system's components) are activated by C9, and amplify and mediate immune response.

Adaptive immunity is acquired by generating pathogen (antigen)-specific B and T lymphocytes through a gene rearrangement process. The exposure of body to antigen with aim to produce adaptive immune reaction that develops in weeks/months but may last through whole life is called active immunity. The active immunity may either be acquired or natural. The immune system of higher animals is equipped with adaptive immunity. The antigen specific reactions (via B and T lymphoctyes) are involved in adaptive immunity. The strong phagocytic action of myeloid cells and cytotoxic T lymphocytes is enhanced by Th1 lymphocytes which produce TNF-α, IFN-α and IL-2. The IL-4, IL-5, and IL-10 are produced by Th2

lymphocytes (which are the mediators of humoral immunity), categorized by B lymphocytes-mediated production of antibodies. The toxins or microorganisms are neutralized after binding with the antibodies.

In healthy organism, the immune system maintains homeostasis within the body. The function and efficiency of the immune system are influenced by various exogenous and endogenous factors resulting in either immunosuppression or immunostimulation. Several agents possessing an activity to normalize or modulate pathophysiological processes are called immunomodulators. The biomolecules of synthetic or biological origin capable of modulating, suppressing and stimulating any components of adaptive or innate immunity are known as immunomodulators, immunorestoratives, immunoaugmentors, or biological response modifiers. Immunomodulators are generally categorized into immunoadjuvants, immunostimulants, and immunosuppressants in clinical practice. Immunoadjuvants are specific immune stimulators which enhance the efficacy of vaccine. Agents that activate or induce the mediators or components of immune system are called as immunostimulants. The resistance against autoimmunity, cancer, allergy, and infection is enhanced by immunostimulants. Additionally, these agents can also be used in the treatment of infection-associated immunopathology, hypersensitivity reactions, and autoimmune diseases.

**Study 1:** Panchgavya was evaluated for immunomodulatory activity in rats. Immunomodulatory activity of Panchgavya was evaluated by humoral and cell mediated immune responses against cyclophosphamide induced immunosuppression. Cyclophosphamide was administered at the dose rate of 5 mg/kg b. wt. i.p. on day one. Panchgavya was administered at the dose rate of 50mg per/rat, orally for 30 consecutive days. The efficacy of Panchgavya was also evaluated by anoxia tolerance test and swimming induced stress. In addition gross observations haematological and biochemical parameters were determined to observe the effects of Panchgavya on haemato-boichemical profiles in rats.

The immunomodulatory indices such as serum gamma globulin, neutrophil adhesion count and absolute lymphocyte count were determined wherein findings indicated that oral administration of Panchgavya for 30 consecutive days caused significant increase in serum gamma globulin concentration and absolute lymphocyte count as compared to control treated with cyclophosphamide alone. Panchgavya also caused significant increase in neutrophil adhesion percent on day 14 (before challenge) as compared to control group in albino rats.

The immunomodulatory activity of Panchgavya was also evaluated by anoxia tolerance and swimming induced stress. The results indicated that oral administration of Panchgavya for 30 consecutive days caused significant increase in anoxia tolerance time and swimming time suggesting immunostimulant activity of Panchgavya.

**Study 2:** Narasimha churna at a dose of 270 mg/kg produced significant increase ($P<0.05$) in antibody titre value. Similarly at dose of 135 mg/kg, there was also increase in antibody titre value but it is found

to be statically non-significant. There are also increased in WBC count and lymphocyte count and weight of spleen in Narasimha churna (270 mg/kg) treated group but values did not reached at significant level. A histopathological study shows that there is peripheral lymphocytolysis in spleen and mild lymphoid cell depletion and congestion in control group. Narasimha churna (270 mg/kg) reversed the changes in spleen and lymphnode and showed normal cytoarchitecture. Cyclophosphamide showed deleterious effect on spleen as evident by highly significant decrease in spleen weight and severe lymphoid depletion in spleen as seen in histopathological study. It severely decrease the antibody titre at level of $P<0.001$. It also decrease WBC count, lymphocyte count significantly and shows s severe lymphocytolyis and fibrosis in lymphnode.

**Study 3:** Immunomodulatory activity of Ayush AIDS was evaluated in rats. Ayush AIDS non-significantly enhance the immunological paw oedema induced by suspension of triple antigen in rats. Ayush AIDS also reversed the suppressant effect of cyclophosphamide on cell mediated immune response in rats but the values were non-significant. The standard drug produced significant cell mediated immune response in rats. The Levamisole also significantly reversed the suppressant effect of cyclophosphamide on cell mediated immune response in rats.

**Study 4:** The immunomodulatory activity and safety/toxicity of Panchgavya Ghrita was evaluated in experimental animals. The quality assured Panchagavya ghrita was subjected to immunomodulatory activities on experimental albino rats. The study revealed significant immunomodulatory activities in terms of increasing serum gamma globulin, neutrophil and adhesion count and absolute lymphocyte count. Further, chronic toxicity studies (90 days) on albino rats revealed the formulations panchagavya ghrita found safe.

**Conclusion:** Studies have shown that the ayurvedic drug mentioned in the Ayurveda text found to possess potential immunomodulatory activity.

## 2.1.13. ANTI-COLITIS ACTIVITY

**BACKGROUND**

Ulcerative colitis (UC) is a chronic inflammatory disorder involving the mucosa and sub-mucosa of the colon. Ulcerative colitis and Crohn's disease (CD) represent the two major forms of irritable bowel syndrome (IBS). It is largely a disease of the industrialized world, and is more common in urban areas and northern climates. It occurs at the rate of approximately five cases per 100,000 people1. The disease pattern is one of the remission and exacerbation and occurs most commonly among people between ages of 15-40 years. Though the exact etiology and pathophysiology is not known with certainty but genetic, immunological, reactive oxygen species (ROS) and environmental factors play a crucial role in the development of UC2-4. ROS leads to the formation of reactive peroxides and hydroxyl radicals which can cause lipid peroxidation by impairing cell membrane function and tissue damage.

The symptomatic analysis of IBS, points to *Grahani, Kaphaja Pravahika, Shokaja Atisara, Bhayaja Atisara* etc. mentioned in *Ayurveda* classics which are characterized by altered bowel habits and other gastrointestinal symptoms.

The pathogenesis of *Grahani* begins with the vitiation of *Agni* (digestive fire) in terms of its quality, quantity and function. All metabolic physiological transformations in the body are carried out under the influence of *Agni. Mandagni* (quantitative, qualitative and functional decrease of *Agni)* is the root cause of *Ama Dosha* and it is a crucial factor for manifestation of most of the diseases. *Ama Dosha,* resulting from *mandagni,* plays a pivotal role in the pathogenesis of gastro intestinal disorders such as *Grahani Roga. Bhayaj Atisara, Shokaj Atisara etc.* Vitiation of *Samana* and *Apana Vayu,* affects the enteric nervous system, alters the GI motility and hormone activity producing the symptoms of *Grahani.* All these diseases have psychological factors such as fear and anxiety as aetiology and IBS also has psychological factors responsible for its origin.

The modem IBS therapies include bulk forming agents, anti- diarrhoeal, antispasmodic and anti - depressants etc which lack demonstrable efficacy. While considering the cost and potential risks (severe constipation, severe diarrhoea, ischemic colitis) against potential benefits, potential risks outweigh the possible benefits. Therefore, exploring alternative medicines for therapeutic options, which are effective, economical and safe, are needed.

**Study 1:** In view of the use of tuber of *Amorphophallus paeoniifolius* in traditional practices for treatment of different gastric disorders and its anti-inflammatory potential, the *Amorphophallus paeoniifolius* Tuber extract (methanolic & aqueous) was evaluated for ulcerative colitis activity on acetic acid induced model. The studies revelaed that treatment with APME and APAE showed anticolitic effect in acetic acid induced ulcerative colitis as indicated by restoration of body weight and stool consistency, decreased

ulceration and inflammation of bowel and ALP and MPO activity. The changes were corroborated with histopathological findings. The effects were comparable to standard drug, prednisolone. The anti-colitic effect of the extract might be due to presence of phenolic compound, steroids or flavonoids. In conclusion, the tuber of Amorphophallus paeoniifolius have been found effective in prevention of the experimentally induced ulcerative colitis and substantiate the traditional claim. This investigation projects the therapeutic potential of the plant to be developed as alternative herbal anti-colitic drug.

**Study 2:** Jatiphaladya churna was evaluated for colitis activity experimental animals. Jatiphaladya churna at the dose level of 270 mg/kg, significantly attenuated the increased level of myeloperoxidase, nitric oxide and lipid peroxidation at a significant level of $P<0.05$ in comparision to colitis control group. Histopathological studies of colon tissue from drug treated group did not showed any remarkable changes, when compared with colitis control group. Jatiphaladya churna produced effect mainly due to attenuation of the oxidant levl viz. myeloperoxidase, nitric oxide and lipid peroxidation.

**Conclusion:** Studies have shown that the ayurvedic drug mentioned in the Ayurveda text found to possess potential anti-colitis activity.

## 2.1.14. PSYCHOTROPIC ACTIVITY

**BACKGROUND**

Psychosis (schizophrenic, schizoaffective and affective illnesses) is a group of serious illnesses that affect the mind. It is a major debilitating, complex and costly illness that strikes 1% of the world's population. It is characterized by three general types of symptoms: Positive symptoms, Negative symptoms and Cognitive symptoms (Parle and Kadian, 2013). Positive symptoms refer to a loss of contact with reality and comprise of hallucinations, delusions and positive formal thought disorders. Negative symptoms refer to a diminution in or absence of normal behaviors and include flat affect, alogia, avolition and anhedonia (Parle and Sharma, 2013). Cognitive symptoms manifest as deficits in attention, learning and memory. Hyperactivation of mesolimbic pathway and dysfunction of mesocortical pathway generates imbalance in the serotonergic, dopaminergic, GABAergic and glutamatergic neurotransmission in certain region of brain, are major reason of psychosis. Other reasons of psychosis can be attributed to heredity, stress, oxidative stress, NMDA receptor antagonists, drug abuse and traumatic injury. Antipsychotics are used for the management of psychosis are typical and atypical (Yadav et al., 2015). Adverse effects due to the use of typical antipsychotics is extra-pyramidal side effects whereas, atypical antipsychotic possess lesser extra-pyramidal side effects. In spite of the availability of a number of drugs for treatment of psychosis, however, at present there is no satisfactory remedy available for prevention and management of psychosis.

**Study 1:** Manasamitra Vataka was tested for intellect promoting activity. The test drug showed significant intellect promoting activity as indicated by antipsychotic activity, antidepressant activity, Hebb Williams Maze method, Elevated Plus Maze method and actophotometer method in rats.

**Study 2:** Ayush Manas evaluated for its central nervous system (CNS) activity by using different models in mice and rats. Ayush Manas studied at two dose levels (therapeutic dose and twice of the therapeutic dose) in mice and rats. The dose was fixed from the human dose on basis of body surface area ratio. The drug was dissolved in distilled water and administered orally to animals as per the selected dose.In gross behaviour study the drug did not produce any significant changes in behaviour of mice. Ayush Manas at dose of 300 mg/kg significantly increased the immobility time in behaviour despair test and locomotor activity in actophotometer. The drug also potentiates the pentobarbitone induced sleep in mice. Further Ayush Manas did not affect the muscle grip of the animals. The effect indicates the depressant activity of Ayush Manas without affecting the normal behaviour and muscle coordination. In anti-anxiety study, Ayush Manas at 300 mg/kg produced significant activity in elevated plus maze, hole board test and open behaviour in mice. The drugs significantly increased the time spent on open arm in plus maze, decreased the head dipping in hole board and number of square crossed and number faecal matters in open field which attributed to the anti-anxiety activity of Ayush Manas. Ayush Manas have little or no effects on MES induced convulsion in rats, reserpine induced catatonia in mice and analgesic activity

in rats. Ayush Manas at both dose levels attenuated the immobility duration as on 6th and on 7th day in chronic fatigue syndrome induced behavioural despair test in mice and significantly reversed the stress induced hypothermia in rats, which indicates adaptogenic activity of Ayush Manas. In memory learning behaviour model, Ayush Manas decreased the transfer latency in plus maze on 2nd day in comparison to the initial values which indicates retention/consolidation of memory. On third day after clozapine, though prolongation of transfer latency was observed it was much less in comparison to control indicating that the test drug at both the dose level produced antagonism of clozapine induced amnesia.From the present study it is concluded that Ayush Manas produced depressant, anti-anxiety, memory enhancing and adaptogenic activity which may be due to its "medhya" effects. Though, human physiology differs from the lower animals like rats, effect seen in some parameters at higher dose of Ayush Manas should be taken into consideration while administering it for therapeutic purpose. However, it would be prudent to watch for the above changes in clinical settings especially when administered for long duration.

**Conclusion:** Studies have shown that the ayurvedic drug mentioned in the Ayurveda text found to possess potential antipsychotic activity.

## 2.2. SAFETY/TOXICITY STUDY OF AYURVEDIC SINGLE DRUGS AND COMPOUND FORMULATIONS

### BACKGROUND

The preclinical toxicity testing on various biological systems reveals the species-, organ- and dose- specific toxic effects of an investigational product. The toxicity of substances can be observed by (a) studying the accidental exposures to a substance (b) *in vitro* studies using cells/ cell lines (c) *in vivo* exposure on experimental animals. The specific guideline instructs that the maximum selected dose should be sufficient to identify the target organ toxicity. From the toxicological evaluation, the no observed effect level (NOEL) or NOAEL, which may be useful for human studies, may be established. The low dose, intermediate dose, and high dose used in the toxicity test provide the NOEL, dose–response relationship, and target organ toxicity in animals, respectively.

### CORE OBJECTIVES

a. Safety/toxicity of Ayurvedic formulations/Medicinal plants

b. Find out the safest dose range and lethal dose

c. Generate Safety/Toxicity data for clinical implementation

d. Specific guidelines are prescribed for the use of apparently toxic medicinal plants/herbomineral formulation with certain detoxification processing

### INTRODUCTION

The herbal and natural products of folk medicine have been used for the benefit of mankind since ancient times. However, the general acceptability of herbal medicines has been limited by a lack of dose regimen and adequate toxicity data to evaluate their safety. The indiscriminate increase in the use of plant extract is further aggravated by the belief that herbs are safe simply because they are natural in origin. Plants produce bioactive compounds which act as defense mechanisms against predators and at the same time may be toxic in nature. Therefore, it has become imperative to assess the safety of plants used for medicinal purposes for possible toxicity. Toxicity studies support toxicity profiling and safety evaluation for the drug candidate, which includes a battery of *in-vivo* and *in-vitro* mutagenicity studies; animal toxicity studies in two species. Single dose acute toxicity studies could help determine the drug wash out period and its correlation with signs of toxicity, if any. Whereas repeated dose toxicity studies helps determining drug tolerability, dose range from area under curve (AUC) of drug plasma level. These results eventually help to determine no-adverse effect-level (NOAEL) and maximum tolerated dose (MTD) for the drug which ultimately helps in calculation for a safer and potentially effective start up dose regimen for human studies. Depending on indication, drug target population, duration and frequency of the drug consumption, toxicity evaluation can be further extended to other repeated dose

studies like chronic or sub-chronic repeated dose studies. If drug usage covers people of reproductive age group then animal reproductive segment studies needs to be conducted.

Ayurvedic medicines are prepared from different parts of plants such as, leaves, roots, barks, etc. or whole plants with/without the incorporation of different types of metallic and non-metallic substances called as 'Bhasma'. In spite of long history of traditional usage of Ayurvedic medicines, their scientific pedestal is time-demanding with the advancement of medical, biological and pharmaceutical sciences due to the several reasons. Firstly, in order to introduce and spread the effectiveness of Ayurvedic medicines for the treatment of diseases globally so that people all over the world can keep faith on it on the basis of scientific evidences. The concept that natural medicines are devoid of toxicity or free from all sorts of side-effects is not widely acceptable all over the world, especially to the people of developed countries, without clinical and biological evidences of therapeutic efficacy and clinical safety reports. Secondly, recent debate on heavy metal toxicity in Ayurvedic medicines such as, the presence of harmful levels of lead, mercury, arsenic and other metals demanding the extensive research on the safety and efficacy issues of Ayurvedic preparations. It is important to mention here that metals (bhasma) are included in Ayurvedic medicines as active ingredient(s) of the preparation. Ayurvedic system also warned about the possible toxicity of the heavy metals which included in its formulation and also properly indicated to detoxify the used heavy metals by special procedures such as heating. Thus, the use of metals and heavy metals in the formulation of Ayurvedic medicines is not unethical but it should be within the limit and must be ensured about its non-toxicity in the final preparation by adopting special techniques or methods. Therefore, the level of heavy metal should be strictly controlled during the manufacturing and quality control of Ayurvedic medicines. Besides, preclinical and clinical metallic toxicity studies of each of Ayurvedic preparations should be scientifically reported. Thirdly, Asava and Arista are two popular forms for the preparation of Ayurvedic medicines in which fermentation technique is applied with polyherbal materials. Several enzymes may be produced by fermentation in the manufacturing process of Ayurvedic medicines which also ultimately contribute their pharmacological activities along with other chemicals derived from the formulation. In addition, there is a possibility of bacterial contamination in the preparation of Ayurvedic medicines. Therefore, each of the Ayurvedic preparation must be tested for microbial contamination in the quality control procedure. The therapeutic efficacy and safety of each of Ayurvedic drug does not only depend on the individual properties of each added ingredients, rather it comes from the collective properties of all the ingredients when homogenously mixed them in a preparation.

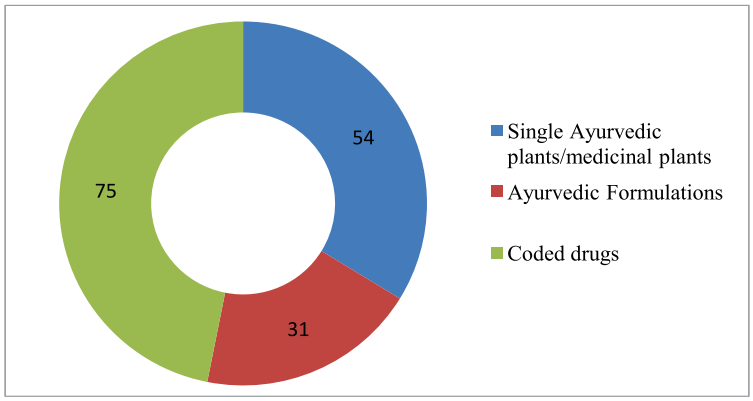
Many supporters of herbal medicines disagree that products witha long history of popular use are generally safe when usedproperly at common therapeutic doses. A crucialquestion underlying this statement is the extent to which theabsence of evidence of toxicity could be taken as evidence ofthe absence of toxicity or safety of herbal medicines. Whetherthe absence of records of adverse effects is an indication of lackof toxicity depends on the type of toxic effect and the likelihoodof observing

such an adverse outcome under the conditionsprevailing in the traditional usage. Acute symptoms and shorttermtoxic effects, such as gastro-intestinal disturbances and dermatological effects, are likely to be recognized andassociated to herbal medicine. Therefore, the absence of such observation provides some evidence of safety in theseparticular endpoints. Long-term adverse outcomes, such ascancer, liver and kidney damage, reproductive dysfunctions,birth defects and several morbidities that are more difficult todetect, however, are unlikely to be associated with the popularuse of a medicine, unless an adequately designed epidemiologystudy (preferably, a prospective cohort study) is undertaken.Thus, the absence of evidence of these adverse effects withinthe context of traditional usage of herbal medicines is notevidence of the absence of potential to cause them. As far asdrugs are concerned, safety is assumed only when the nullhypothesis (absence of toxicity) has not been disproved afterbeing challenged by properly designed and comprehensive setof pre-clinical and clinical studies that had enough statisticalpower to reject it if it were false.

It is imperative to have safety and efficacy evaluation completed for a test compound before proceeding to translate for clinical trials. Toxicological studies play an essential role for providing particulars to design clinical studies to determine whether the test compound is as safe or non-toxic in humans as it was observed in animal studies. Study performed in preclinical setting includes determination of safety, efficacy, tolerability and toxicity for the test compound. These studies help to propose a safe and efficacious startup dose for human studies. Furthermore, it won't be wrong to state that without toxicological studies it is not possible to strategize and design clinical trial in humans.

## Safety/Toxicity studies

Toxicological studies constitute a very vital part in Drug Research Programme. The Ayurvedic medicines are studied in experimental models using different animal species to establish safety profile. The studies are being carried out at Council's various institutions. 160 drugs were screened for various toxicity studies in which 54 single Ayurvedic plants/medicinal plants, 31 Ayurvedic formulations and 75 coded drugs screened during the period from 1978 to 2018 adopting prevalent guidelines.




Numbers of studies on single Ayurvedic drugs, Ayurvedic formulation, coded drugs

**Council has done following major studies since inception for safety/toxicity of Ayurvedic single drugs and compound formulations:**

**Study 1:** Acute toxicity study of Yogaraja Guggulu in albino rats was carried out. No mortality and clinical signs of toxicity were found in rats upto 10 times of test dose.

**Study 2:** Acute dermal toxicity study of Mahanarayana Taila in albino rats were carried out.No mortality and clinical signs of toxicity, were found rat's upto 10 times of test dose.

**Study 3:** Drakshavaleha and Nimbadi Churna were screened for acute toxicity study in experimental animals. Both the drugs did not produce any mortality upto the dose level of 2000mg/kg body weight.

**Study 4:** Acute toxicity study of alcoholic extract of *C. occidentalis* seeds were carried out in Wistar rats. The study indicated that alcoholic extract of *C. occidentalis* is safe upto 5000 mg/kg body weight when given in single dose and it falls in category 5 of acute toxic class. Sub acute toxicity study of alcoholic extract of *C. occidentalis* seeds @ 106, 530 and 1060 mg/kg BW revealed pharmacological activities like laxative, antidiabetic and anticholesterol activity. Dose dependent reduction in daily feed consumption, significant increase in Hb, PCV and TEC, decrease in blood glucose and serum cholesterol and dose dependant pathological changes such as vascular, degenerative and necrobiotic lesions were noticed. kidney, liver, brain, heart, testis, skeletal muscle, intestine, spleen, stomach, lung and ovary were found as the most affected target organs.

**Study 5:** Acute & Sub acute Toxicity studies of Ayush-Card (Coded formulation) at different dose levels i.e. 130, 650 & 1,300 mg/kg body weight were conducted in mice. No mortality, no significant treatment related effect on clinical signs or behavioral activity, food intake, body weight, hematological parameters and clinical chemistry parameters, histopathology were observed in all the groups of animals during the experimental period. It indicates that Ayush - Card is safe up to 1300 mg/kg, which is ten times of therapeutic dose. Chronic toxicity studies of Ayush-Card were carried out in rats. No Pre-terminal deaths, no significant treatment related effect on clinical signs or behavioral activity, food intake, body weight, hematological parameters and clinical chemistry parameters, histopathology were observed in all the groups of animals during the experimental period.

**Study 6:** Acute Toxicity studies of Ayush-Carctol (Coded formulation) at different dose levels i.e. 260, 1300 & 2,600 mg/kg body weight were conducted in mice. No mortality, no significant treatment related effect on clinical signs or behavioral activity etc were observed in the experimental period, indicating that Ayush-Carctol is safe up to 2600 mg/kg, which is ten times of therapeutic dose. Sub acute toxicity studies of Ayush-Carctol at different dose levels i.e. 90, 450 & 900 mg/kg body weight were conducted in rats. No Pre-terminal deaths, no significant treatment related effect on clinical signs or behavioral activity were observed during the experimental period. Analysis of data pertaining to food intake, body weight,

hematological parameters and clinical chemistry parameters is awaited. Organ (Liver, kidney, heart, spleen, lung, stomach, intestine, testis and ovary) samples have been sent for histopathological analysis and report is awaited.

**Study 7:** Acute oral toxicity study of Ayush-A (Coded formulation) in albino mice was found to be apparently non toxic in mice. Sub-acute toxicity studies of Ayush - A were carried out in Wistar rats. The drug was administered along with the 1% SCMC (vehicle) once daily for a period of 28 days. No significant changes were observed in food intake, urine volume, organ weight, biochemical (liver and kidney function tests) and haemotological parameters. Histopathological examination of different organs including bone marrow did not show any remarkable change in the cytoarchitecture. Administration of Ayush -A at various dose levels are considered to be safe as it did not caused any mortality, morbidity or adverse changes in the general behaviors, no alterations were observed in hematological, biochemical parameters and histopathological findings in 28 days repeated oral dose toxicity studies.

**Study 8:** Chronic toxicity studies of K-1 extract (Coded drug) were carried out in Wistar albino rats. The drug was administered along with the 1% SCMC (vehicle) once daily for a period of 90 consecutive days. No significant changes were observed in the body weight, organ weight, feed intake, biochemical (liver and kidney function tests) and haemotological parameters, urine volume and urine analysis. The histopathological study showed normal architecture suggesting no detrimental changes and morphological disturbances were caused by the daily oral administration of the K-1 extract at the dose level of 90 mg/kg, 450 mg/kg and 900 mg/kg/p.o, for 90 days in rats. As K-1 Extract at the dose of 90, 450 and 900 mg/kg did not cause any adverse effects in 90 days repeated dose oral toxicity studies it can be considered as safe and non toxic.

**Study 9:** Acute oral toxicity studies of AYUSH - D (a coded Ayurvedic formulation for diabetes) were carried out in wistar rats. The drug was diluted with honey and administered only once orally. No significant changes were observed in fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system, and somatomotor activity in AYUSH- D treated group of animals compared to vehicle control group. Further no tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were observed in the AYUSH- D treated group. The coded drug AYUSH - D at the dose of 5400 mg/kg was found to be non-toxic and safe upon acute oral administration.

Sub acute oral toxicity studies of AYUSH - D were carried out in Wistar rats. The drug was diluted with honey and given once daily for a period of 28 days. AYUSH – D at the dose level of 540, 2700 and 5,400 mg/kg, body weight showed normal behavior and no mortality was observed during the experimentation. The body weight, feed intake and water intake remained unaffected with the treatment of AYUSH-D. Urine analysis also did not reveal any significant change compared to control group. No significant alteration in behavioral, hematological and biochemical parameters of both males and females were

observed. The coded drug AYUSH-D was found to be safe and non-toxic on sub-acute oral administration in wistar rats. Histopathological studies are under progress.

**Study 10:** Acute toxicity study of Ayush- AMF (Coded formulation) was carried out in Swiss Albino mice & Wistar rats. The drug was administered once and animals were observed for 14 days. There is no significant effect on body weight, feed consumption and no clinical sign of toxicity was observed at the dose level of 2000 mg/kg body weight during 14 days observation period.\

90 days repeated dose oral toxicity study of AYUSH AMF in wistar rats were carried out at the dose level of 750, 450 and 150 mg/kg. Treatment with AYUSH AMF did not cause any change in behavioral pattern, body weight, food consumption, haematological, biochemical parameters and histopathological section at the dose level of 750 mg/kg. The dose of 750 mg/kg/day of AYUSH-AMF was found as NOEL (No Observed Effect Level).

**Study 11:** The acute oral toxicity of *Sanjivani Vati* (an ayurvedic formulation) was carried out in mice. The drug did not produce any significant toxicity in mice at a dose level of 650 mg/kg body weight.

90 days repeated dose oral toxicity study of sanjivani vati in wistar rats were carried out at at the dose of 45 mg/kg, 225 mg/kg and 450 mg/kg. The drug did not cause any change in behavioral pattern, biochemical and haematological parameter in 90 days oral toxicity study.

**Study 12:** *Cardiospermum halicacabum* Linn Leaf extract was evaluated for acute and sub-acute toxicity study in mice and rats. The drug is found safe upto the dose level of 2000mg/kg/po in rats and mice in acute toxicity study. The 28 days repeated toxicity does not show any adverse effect up to the dose of 1000mg/kg/po in rats.

**Study 13:** Cannabis Leaves churna was evaluated for acute and repeated dose toxicity in rats**.** In case of acute toxicity study, there was no mortality and no abnormality in clinical signs of the experimental animals at 325mg/kg body weight, orally. In case of sub acute toxicity study, no animals showed any external and internal gross pathological findings at various dose levels 22.5mg/kg body wt., 112.5mg/kg body weight and 225mg/kg body weight (i.e. Human Equivalent Therapeutic Dose (TD), 5 times Human Equivalent TD and 10 times Human Equivalent TD) upto 28 days in Rats. No significant changes have been observed during the evaluation of blood biochemical (SGOT, SGPT, Alkaline Phosphatase, total protein, total bilirubin, albumin), creatinine, cholesterol, triglyceride, glucose, electrolytes (sodium, potassium, chloride) and hematological parameters like RBC, WBC, HGB, MCH, MCHC, MPV, HCT, PCT, PDW, RDW, platelets at the administered doses. In acute toxicity study, no toxicity was observed @ 32.5 and 325mg/kg in mice. In sub acute toxicity study, NOEL was found to be 225mg/kg in rats.

**Study 14:** The acute oral toxicity study was carried out for *Vaisvanara churna* formulation and it was found that *Vaisvanara churna* was non toxic up to the dose level of 6500 mg/kg. The chronic toxicity

study (90 days repeated oral dose toxicity) of *Vaisvanara churna* was also carried out and it was observed that *Vaisvanara churna* at the dose level of 450 mg/kg, 2250 mg/kg and 4500 mg/kg were considered to be safe. No alterations were observed in hematological, biochemical parameters in 90 days repeated oral dose toxicity study in rats.

**Study 15:** In Acute toxicity study, *Dhatri lauha* @1000 and 2000mg/kg showed dullness, writhing and 10% mortality within 96hrs in mice. In Sub acute toxicity study, *Dhatri lauha* was safe @250mg/kg but showed 33% mortality in 500mg/kg in rats.

**Study 16:** Ethanol extract of Apamarga (*Achyranthes aspera Linn*.) was evaluated for acute toxicity study. In acute toxicity studies mortality observed above 600mg/kg (i.p.).

**Study 17:** A coded drug Ayush-64 was screened for its safety/toxicity in experimental animals. No mortality in highest dose of 5 mg/kg in acute toxicity studies. Ayush-64 apparently showed no significant change in body weight on oral administration for 15 days. There is no mortality rate in chronic studies. In Acute toxicity study, AYUSH-64 was found to be safe upto 5000mg/kg in mice.

**Study 18:** In acute toxicity study, Bimbi (*Coccinia indica W. & A.*) extract showed LD 50 value more than 2.0 gm/kg in mice.

**Study 19:** In Acute toxicity study in mice, Coded drug GVK @195, 975 and 1950mg/kg showed normal behavior upto 7$^{th}$ day. All doses of GVK showed insignificant spontaneous locomotor activity in comparison to control. In sub acute toxicity study, GVK @ 1350, 675 and 135mg/kg showed normal behavior, no significant effect in body weight, food intake, water intake, blood biochemistry, haematology and histological investigations in rats.

**Study 20:** Aqueous extract of Hingu *(Ferula asafoetida* Linn.) has no significant effect on implantation and non-toxic in acute toxicity study.

**Study 21:** Kachnar (*Bauhinia racemosa* Lam.) (Stem) showed no mortality in acute toxicity studies upto 2-0 gm/kg both in rats and mice.

**Study 22:** Kutaja (*Holarrhena antidysentrica* Wall.) Stem bark extract showed no mortality on acute toxicity studies.

**Study 23:** Madala (*Ailanthus excelsa* Roxb.) Stem bark extract showed no significant toxicity upto 1400 mg./kg. in sub-acute toxicity studies for 6 weeks.

**Study 24:** Nirgundi (*Vitex negundo* Linn.)- Non-toxic in acute toxicity study.

**Study 25:** Sati, Kachura (*Curcuma zedoaria* Rose.)- Non-toxic in acute toxicity study.

**Study 26:** Vanaraja (*Bauhinia purpurea* Linn.) (Stem and Stem bark)- LD 50 value in mice in more than 2.0 gm/kg.

**Study 27:** Thumbai (*Leucas aspera*), Pidangunari (*Permna tomentosa) and* Avuri (*Indigofera tinctoria)*- Acute toxicity study with the alcoholic and chloroform extract of the drugs Thumbai (*Leucas aspera*), Pidangunari (*Permna tomentosa) and* Avuri (*Indigofera tinctoria)* in olive oil suspension in graded doses on albino mice and rats found to be nontoxic up to 10000 mg/kg body weight.

**Study 28:** Avuri, Uthamani and Nayuruvi- The drug Avuri (*Indigofera tinctoria)* and Uthamani (*Pergularia extensa)* in oil form was found to be non toxic up to 50 ml/kg body weight in graded doses(8 doses) . The drugs Uthamani (*Pergularia extensa)* and Nayuruvi (*Achyranthes aspera)* in Choornam form found to be non toxic up to 1000 mg/kg and 500 mg/kg body weight respectively in graded doses.

**Study 29:** D2- A coded antidiabetic drug found to be nontoxic upto 3000 mg/kg body weight.

**Study 30:** Neermulli Kudineer- The drug Neermulli Kudineer in aquous extract form (Khasayam) found to be non toxic up to 50 ml/kg body weight in graded doses.

**Study 31:** *Glycyrrhiza glabra*- Acute toxicity study with the drug *Glycyrrhiza glabra* in the dose of 10,000 mg/kg body weight was found to be nontoxic on albino rats and albino mice.

**Study 32:** Idivallathi Choornam- The drug Idivallathi Choornam was found to be nontoxic up to 10,000 mg/kg body weight in mice and 5000 mg./kg. body weight was found to be nontoxic on albino mice and 10,000 mg./kg. body weight on albino rats.

**Study 33:** Sivappukukil Thailam- The drug Sivappukukil Thailam in oil form was found to be non toxic up to 40 ml/kg body weight.

**Study 34:** Thalaga maathirai and Thaazhampoo maathirai- In the drugs Thalaga maathirai, and Thaazhampoo maathirai 33.33% and 66.66% mortality were observed in the dose of 10,000 mg/kg body weight respectively.

**Study 35:** *Pergularia extensa*- The drugs *Pergularia extensa* in Choornam form and Thailam form were found to be nontoxic up to maximum doses i.e. 10,000 mg/kg and 50 ml/kg body weight, respectivelty.

**Study 36:** Vathakesari Thailam- The drug Vathakesari Thailam was found to be nontoxic up to the dose of 40 ml/kg, body weight.

**Study 37:** *Indigofera tinctoria*- The drug *Indigofera tinctoria* (Chloroform extract) in graded doses on albino rats and mice was found to be nontoxic up to 10,000 mg/kg body weight.

**Study 39:** The drug *Phyllanthus anarus* was also found to be nontoxic upto maximum dose i.e. 10,000 mg/kg body weight in rat and mice.

**Study 39:** *Aristolochia anarus* (Alcoholic ext.) was found to be nontoxic upto the dose of 3000 mg/kg, body weight in rat and mice.

**Study 40:** The drug Ayush EA was found to be nontoxic upto the dose of 10,000 mg/kg body weight in rat and mice. Ayush EA (decoction) was also found to be nontoxic upto the dose of 50 ml/kg body weight in rat.

**Study 41:** The drug Ayush VAC in Choornam form was also found to be nontoxic upto the dose of 10,000 mg/kg body weight in rat and mice.

**Study 42:** A coded drug OP1 was found to be nontoxic upto the dose of 8000 mg/kg body weight in rat.

**Study 43:** The drug ABKM and APTT (alcoholic ext.) in graded doses on albino rats was found to be nontoxic up the dose of 10,000 mg/kg body weight in rat.

**Study 44:** The drug Ayush ME was also found to be nontoxic upto maximum dose i.e. 10,000 mg/kg body weight in rat.

**Study 45:** Ayush TCT was found to be nontoxic in the dose of 10,000 mg/kg body weight in rat and 500 mg/kg in mice.

**Study 46:** Ayush P3 was found to be nontoxic in the dose of 10,000 mg/kg, body weight in rat.

**Study 47:** Ayush TCC was found to be nontoxic in the dose of 10,000 mg/kg, body weight in rats.

**Study 48:** Ayush 77 syp was also found to be nontoxic upto the dose of 50 ml/kg body weight in rat.

**Study 49:** The drug ABKM was also found to be nontoxic upto the dose of 5000 mg/kg, body weight in mice.

**Study 50:** APTT was also found to be nontoxic upto the dose of 3000 mg/kg, body weight in mice.

**Study 51:** The drug Ayush RP was also found to be nontoxic upto the dose of 3058.6 mg/kg body weight in mice.

**Study 52:** The drug Ayush OSTO was also found to be nontoxic upto the dose of 2600 mg/kg body weight in mice.

**Study 53:** The drug Ayush LIV was also found to be nontoxic upto the dose of 3360 mg/kg body weight in mice.

**Study 54:** The drug Ayush Rasayan-A was also found to be nontoxic upto the dose of 10,000 mg/kg body weight in mice. The drug Ayush Rasayan-B was also found to be nontoxic upto the dose of 2290 mg/kg body weight in mice.

**Study 55:** The drug *Annabhedhi Chenduram* was also found to be nontoxic upto the dose of 520 mg/kg body weight in mice.

**Study 56:** *Ayush-OSTO*- In chronic toxicity study, no specific abnormalities in physical and pathological profiles were observed on administration of the test compound Ayush –OSTO at various dose levels under the experimental condition.

**Study 57:** *Ayush-LIV*- In chronic toxicity study, no specific abnormalities in physical and pathological profiles were observed on administration of the test compound Ayush –LIV at various dose levels under the experimental condition.

**Study 58:** *Ayush-TAT*- In sub-acute toxicity study, no specific abnormalities in physical and pathological profiles were observed on administration of the test compound Ayush–TAT at various dose levels under the experimental condition.

**Study 59:** *Ayush - P3 (compound)*- Sub acute toxicity Study in albino rats in the doses of 900, 450, 90 mg/kg were carried out. Histological pictures depict normal architecture in the Ayush - P3 (900mg/kg) treated albino rat in comparison to control animal. No abnormal features have been seen in the tissue section of the above said dose studied.

**Study 60:** *Ayush-MM (syp)*- Sub acute toxicity study in albino rats in the doses of 1.8, 0.9, 0.18 ml/kg were carried out. Histological pictures depict normal architecture in the Ayush–MM syp (1.8ml/kg) treated albino rats in comparison to control animals. No abnormal features have been seen in the tissue section of the above said dose studied.

**Study 61:** *Ayush-TCT (compound)*- Sub acute toxicity study in albino rats in the doses of 150, 75, 15 mg/kg were carried out. The studies showed no significant changes in comparison to control group in the parameters studied except serum glucose level enhanced significantly. While formulating the drug, it may be noted that, it should control the serum glucose level.

**Study 62:** *Ayush-ME (compound)*- Sub acute toxicity study in albino rat**s** in the doses of 900, 450, 90 mg/kg were carried out. Histological pictures depict normal architecture in the Ayush - ME (900mg/kg) treated albino rat in comparison to control animals. Sub-acute toxicity studies showed no significant changes in comparison to control in all the parameters studied.

**Study 63:** *Ayush–EA*- Sub acute toxicity study in albino rats in the doses of 54.0, 27.0 and 5.4 ml/kg were carried out. Ayush–EA shows no significant effect in body weight, food intake, water intake, and weight of vital organs, blood biochemistry and hematology.

**Study 64:** *Bala Taila (Liquid)*- Acute dermal toxicity study in albino rat was carried out. The Bala Taila single application showed normal behavior, no erythema and edema seen in comparison to its own

control animal till 7 days. The drug Bala Taila showed no sign of dermal irritation, drug will be pre-clinically safe for clinical trial.

**Study 65:** *GVK (Compound)*- Acute toxicity study in albino mice in the doses of 1950, 975, 195 mg/kg were carried out. No mortality was recorded up to 7$^{th}$ day of observation. It is safe up to the higher dose level of 1950 mg/kg.

**Study 66:** *Gajkesari*- In Acute toxicity study, *Gajkesari* was found to be safe @ 2000mg/kg in mice.

**Study 67:** *Safed musli*- In Chronic toxicity study, NOEL of *Safed musli* was found to be 1000mg/kg in rats.

**Study 68**: *Coded drug LQ14***-** In Acute toxicity study, NOEL of Coded drug LQ14 was found to be 2000mg/kg in rats. In Sub acute toxicity in rats, NOEL of Coded drug LQ14 was found to be 1500mg/kg.

**Study 60:** *Coded drug PKT*- In acute toxicity study, no toxicity was observed and found safe upto 2000mg/kg in rats. In sub acute toxicity study, NOAEL was found to be 1000mg/kg in rats.

**Study 70:** *Ark Pudina*- In acute toxicity study, no toxicity was safe upto 52ml/kg or 7436mg/kg in mice. In sub acute toxicity study in rats, *Ark Pudina* @3.6, 1.8 and 0.36ml/kg showed normal behavior and 8.3% mortality was recorded at all dose level. *Ark Pudina* showed significant increase in body weight weekly.

**Study 71:** *Laghu Vishagarbha Taila*- Laghu vishgarbha taila (LVT) is an Ayurvedic medicated oil formulation. Laghu vishagarbha taila is useful in treatment of vata roga (inflammatory disorder), paralysis/hemiplegia, lock jaw, neck rigidity, stiffness and tightness of all limbs, stiffness in lumbosacral region, tremors and shaking of head. The ingredients like *Datura metel* (Leaf juice and seeds) and *Aconitum ferox* (roots) present in the formulation are considered to be poisonous substances. Due to these substances, the oil may exhibit toxic or untoward reactions on long term use. Hence, it was thought very imperative to investigate the toxicological profile of the formulations on long term dermal application. The dermal toxicity was evaluated on acute, sub-acute and sub-chronic dermal application of laghu vishagarbha taila while effect on pain and inflammation was studied in formalin- and carrageenan-induced pain and inflammation and adjuvant-induced arthritis model. No treatment related changes were noted in body weight on acuteapplication. Further, no treatment related adverse findings were seen in the clinical signs, body weight, feed consumption, water consumption, hematological, biochemical parameters, organ weights, and histopathological evaluation of animals treated with LVT for 28 or 90 days in Wistar rats. Hence, it is concluded that the LVT has no observable dermal toxicity on acute or repeated dose administration for 28 or 90 days.

**Study 72:** *Dermal Toxicity of Coded Drug-C1 oil-* The acute dermal toxicity study was performed as per OECD 402 while subacute dermal toxicity was done as OECD 410 guideline. In acute and sub acute study, total 20 rats were selected based on the body weight and randomly divided into two groups. Group I was a vehicle control group which received sesame oil treatment while group II was a test group that received C1 oil. Each group consisted of 10 animals (5 males and 5 females). In acute study, a limit test at one dose level of 2000 mg/kg bodyweight was carried out in a control and test group while sub-acute study, limit test at one dose level of at least 1000 mg/kg bodyweight was carried out in a control and test group. The oil was applied uniformly as thin and uniform a film over an area which is approximately 10 per cent of the total body surface area either single application (acute) or repeated application for 28 days (Subacute). Observations were made up to 14 days or 28 days. Cage side observations included changes in fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Body weights of each animal were recorded at the start of study and thereafter at weekly intervals. The weekly feed consumption of rats was recorded. The hematological parameters viz. haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, and a measure of clotting potential such as clotting time, prothrombin time, platelet count was investigated at the end of the test period. The determinations of calcium, phosphorus, chloride, sodium, potassium, fasting glucose, serum glutamic-pyruvic transaminase, serum glutamic-oxaloacetic transaminase, gamma glutamyl transpeptidase, urea nitrogen, albumin, blood creatinine, total bilirubin and total serum protein were made. Animals were sacrificed and subjected to a detailed post-mortem examination. Organs viz. brain, heart, liver, kidney, spleen, ovaries/testis and skin were subjected to histopathological evaluation. Further, no treatment related adverse findings were seen in the clinical signs, body weight, feed consumption, water consumption, hematological, biochemical parameters, organ weights, and histopathological evaluation of animals treated with C1 oil for 28 in Wistar rats. Hence, it is concluded that the C1 oil has no observable dermal toxicity on acute or repeated dose administration for 28 days.

**Study 73:** *AYUSH PJ7-* Acute toxicity study of coded drug, AYUSH PJ7was carried out as per OECD guideline No. 423 while Sub-acute (28 days repeated dose) toxicity study was carried out as per OECD guideline No. 407 and their modifications as per ICH guidelines and Schedule Y under Drugs and Cosmetic Act 1945. In acute oral toxicity study, total 20 rats were selected based on the age and uniform body weight range, and randomly divided into two groups. Group I was a vehicle control group which received suspension of gum acacia while group II was a test group that received AYUSH PJ7 in high dose (2000 mg/kg). Each group consisted of 6 animals (3 males and 3 females). Dose range finding study was performed to determine maximum tolerated dose for sub-acute toxicity study. Cage side observations were recorded daily while body weights of each animal were recorded at the start of study and thereafter at weekly intervals. In sub-acute oral toxicity study, total 48 rats were selected based on the body weight and randomly divided into four groups. Group I was a vehicle control group which received

gum acacia suspension while group II, III and IV were test groups that received AYUSH PJ7 in 500, 1000 and 1500 mg/kg, respectively. Each group consisted of 12 animals (6 males and 6 females). The vehicle and AYUSH PJ7 were administered orally for 28 days to individual animals of control and test group respectively. Cage side observations were recorded daily while body weights of each animal were recorded at the start of study and thereafter at weekly intervals. The weekly feed consumption and water consumption of rats were recorded. All important hematological, biochemical and urinary parameters were estimated. Animals were sacrificed and subjected to a detailed post-mortem examination. Vital organs were collected, weighed and subjected to histopathological evaluation. The cage side observation showed no signs of alterations in any of the parameters observed compared to vehicle control group in both acute and sub-acute study. Further, no mortality or moribund stage was observed throughout the study period. After reviewing the data of weekly body weights of male and female animals, it was found that there was non-significant increase in body weights after single and repeated administration of AYUSH PJ7 for 28 days. After reviewing the data no treatment related adverse findings were seen in the clinical signs, body weight, feed consumption, water consumption, hematological, biochemical parameters, organ weights, and histopathological evaluation of animals treated with AYUSH PJ7. No mortality or moribund stage was observed throughout the study period. Hence, it is concluded that the AYUSH PJ7 has no observable oral toxicity.

**Study 74:** Apamarga *(Achyranthes aspera* Linn.)- Oral LD50 was 297.52mg/kg.

**Study 75:** Arjuna (*Terminalia arjuria* W. & A.)- LD50 of aq. Extract was 250mg/kg.

**Study 76:** Bakula (*Mimusops elengi* Linn.)- Nontoxic in doses from 500 to 1000mg/k orally.

**Study 77:** Bharangi (*Clerodendrum serratum* Linn.)- Acute LD50 of saponin was 307.7mg/kg in rats & 288.2mg/kg in mice. Chronic toxicity on rats showed 23.5% mortility but no evidence of tissue damage of saponin treated guineapig for 20 days.

**Study 78:** Bhumyamalaki (*Phyllanthus niruri* Hook. f.)- Nontoxic in 2gm/kg p.o. in in acute toxicity studies.

**Study 79:** Cangeri (*Oxalis corniculata* Linn.)- Toxicological studies on petroleum ether, chloroform, benzene, methanol & aqueous extract of the plant exhibited no toxic effectin 500 & 100 mg/kg p.o.

**Study 80:** Citraka (*Plumbago rosea* Linn.)- LD50 of 50% ethanolic extract of whole plant in albino mice was calculated to be 562mg/kg i.p.

**Study 81:** Daru Haridra (*Berberis aristata* DC.)- The LD50 value of berberine sulphate in mice was found to be 24.3mg/kg i.p.

**Study 82:** Granjana (*Daucus carota* Linn.)- LD50 value was found 188.54mg/kg p.o. & no teratogenecity was shown.

**Study 83:** Guduci (*Tinospora cordifolia* (Willd.) Miers)- The LD50 of glycoside was 1132mg/kg p.o. and 428.7mg/kg i.p.

**Study 84:** Guggulu (*Commiphora mghtii* (Am.) Bhand.)- Fractions A, B, C, D (pet-ether soluble, neutral, chloroform soluble & chloroform insoluble) fractions of gum guggulu produced no toxicity on chicks in doses of 5 to 20mg/kg p.o.

**Study 85:** Gwara (*Cyamopsis tetragonoloba* Taub.)- No systemic toxicity was observed during subacute toxicity study.

**Study 86:** Haridra (*Curcuma longa* Linn.)- LD50 value of aq. Suspension was found to be 3.25ml/kg while LD50 of volatile oil emulsified in twin 80 was found 0.533ml/kg.

**Study 87:** Kamala (*Nelumbo nucifera* Gaertn.)- Nontoxic (acute administration) in mice administered in doses ranging from 100 to 800mg/kg i.p.

**Study 88:** Kapikacchu (*Mucuna pruriens* Linn.)- The LD50 value of sterol extract was 600mg/kg i.p. the drug administered 20mg/kg/rat for more than 5 days produced necrosis and sloughing of the muscles.

**Study 89:** Karanja (*Derris indica* (Lank) Bennet.)- The toxicity test performed on mice which could well tolerated upto 5g/kg (nontoxic).

**Study 90:** Karavira (*Nerium indicum* Mill.)- The acute LD50 was 86mg/kg i.p.

**Study 91:** Karvellaka (*Momordica oharantia* Linn.)- Fruit juice (15-40mg/kg) was found toxic

**Study 92:** Kiratatikta (*Swertia chirayita* Roxb ex Flem)- Dose up to 200mg/kg/op in mice did not show any toxicity.

**Study 93:** Kukundara (*Blumea lacera* DC.)- LD50 was found between 750-800mg/kg.

**Study 94:** Nagakesara (*Mesuaferrea* Linn.)- LD50 in mice being 400mg/kg i.v. and 800mg/kg i.p. and non-toxic upto 1600mg/kg p.o.

**Study 95:** *Kajal and Surma*- No toxic effect was iduced by the preparations containing 18% of lead on the eyes of rabbit after regular application for 4 months, nontoxic upto 10g/kg p.o.

**Study 96:** *Swasa Kesari*- Daily administration of the drug for 4 week did not produced any toxicity.

**Study 97:** *Ayush-9*- Administration of 100mg/kg dose did not shoed any mortility within 24 hour but higher doses of 7.5g/kg produced 33% mortility within half hour.

**Study 98:** *Ayush-11*- The LD50 value in mice was 0.34g/kg i.p. at fiducial limits between 0.212g/kg & 0.544g/kg at 95% confidence level

**Study 99:** *Ayush AC-IV*- LD50 value in mive & rats was found to be more than 2g/kg and 4g/kg respectively. AC-IV in a dose of 500mg/kg orally for 12 weeks was found nontoxic

**Study 100:** *Ocular safety and toxicity studies of an Ayurvedic herbal eye drop for dry eye syndrome*- Acute Eye Irritation study of an Ayurvedic herbal eye drop containing herbal ingredients viz. *Berberis aristata* and *Glyeyrrhiza glabra* formulated for dry eye syndrome (DES) complying the standards of Indian Pharmacopeia (IP.) was performed in New Zealand White rabbit as per the OECD guidelines for testing of chemicals (acute eye Irritation/corrosion) after fulfilling ethical requirements. 0.1 ml of the drop was placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control. Eye drop did not cause irritation to mucous membrane of eyes of rabbits, no evident signs of toxicity and clinical changes were observed. The eye drop in New Zealand White rabbits was found to be nonirritant to the ocular mucous membrane.

## 2.2.1. SAFETY OF RASAKALPA (METAL & MINERAL BASED AYURVEDIC FORMULATIONS)

Rasa Shastra- the speciality of Ayurvedic pharmaceuticals deals with preparation and therapeutic use of metal and mineral based formulations (*Bhasma and Rasakalpa*). Even though these practices are in vogue since *Vedic* period, much emphasis has been made during medieval period. As these drugs require lesser doses, cause no distaste unlike herbal drugs and faster in action, these practices became popular and widely accepted and safely used since long. Ayurvedic has specified different methods of preparation and Standard Operative Procedures (SOPs) right from the collection of raw ingredients, their purification, processing of intermediates, method of use, dosage forms etc.

The Safety of metal and mineral based Ayurvedic formulations to a large extent is evident by its long history of clinical use and endorsed by recent scientific evidences. Further the incredible expansion in the use of Ayurveda worldwide, safety and efficacies as well as quality control have become important concerns for both health authorities and the public, calls for the scientific presentation of facts. Adding to this, the information published through scientific and general print media also generated misconception regarding the quality and safety of metal and mineral based Ayurvedic formulation across the globe.

To take full advantage of the usage of potential sources of traditional healthcare, there is need to draw attention towards a number of issues related to policy on safety, efficacy, quality, accessibility and rational use. Considerable efforts have been put in by the Central Council of Research in Ayurvedic Sciences under Ministry of AYUSH in generating tangible evidence on safety of these interventions. In one such effort, the Council has completed a multi-centric preclinical toxicity/safety study of different rasakalpa/bhasma to find out the target organ of toxicity and to establish safety level after oral administration.

**Council has done following studies since inception for validation of safety of different rasakalpa/bhasma.**

**Study 1:** *Punarnava Mandura*- In acute toxicity study in mice, *Punarnava Mandoor* @ 50, 150, 500, 1000, 1500 and 3000mg/kg showed no mortality and no abnormal behavior. In Sub acute toxicity study in rats, *Punarnava Mandoor* @ 900, 450 and 90mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Punarnava Mandoor* @ 900mg/kg treated rat in comparison to control animals. NOAEL of *Punarnava Mandoor* in rats was found to be 900mg/kg. In chronic toxicity study in rats, *Punarnava Mandoor* @ 450 and 90mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats but showed significant increase in liver weight of females @900mg/kg. Histological pictures depicted normal architecture in *Punarnava Mandoor* @ 900mg/kg treated rat in comparison to control animals. NOAEL of *Punarnava Mandoor* in

rats was found to be 450mg/kg.

**Study 2:** *Kasisa Bhasma*- In sub acute toxicity studyin mice, *Kasisa Bhasma* @ 112.50 and 22.50mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in mice. *Kasisa Bhasma* @ 225mg/kg showed significant decrease in weight of liver, heart and kidney in female in mice and significant decrease in total serum protein in males of high dose. In female mice, *Kasisa Bhasma* @ 225mg/kg produced significant decrease in prothrombin time. Histological pictures depicted normal architecture in *Kasisa Bhasma* @ 225mg/kg treated mice in comparison to control animals. NOEL of *Kasisa Bhasma* in mice was found to be 112.50mg/kg. In chronic toxicity study, *Kasisa Bhasma* @ 225, 112.50 and 22.50mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Kasisa Bhasma* @ 225mg/kg treated rat in comparison to control animals. NOAEL of *Kasisa Bhasma* in rats was found to be 225mg/kg.

**Study 3:** *Swarna Bhsama*- Sub acute and chronic toxicity studies of coded drug Swarna Bhsama were carried out in Swiss albino mice and wistar rats. The drug was diluted with honey and administered once daily for period of 28 days and 90 days for sub acute and chronic toxicity respectively. Bhasma of heavy metal prepared as per SOP mentioned in Ayurvedic texts. The drug was diluted with honey and given once daily for a period of 28 days and 90 days respectively. No clinical signs of toxicity were observed in any of the test groups upon administration of the test drug in different doses. There was no significant change on behavioral pattern, body weight organ weight, hematological and biochemical parameters in mice and rats when compared to vehicle treated group. In sub-acute toxicity studies, no abnormal features were observed in the histopathological sections at dose levels of 19.5 mg/kg and 13.5 mg/kg body weight in mice and rats respectively. In chronic toxicity studies, no drug related change was observed in the histopathological evaluation at dose levels of 19.5 mg/kg and 13.5 mg/kg body weight in mice and rats respectively. The dose levels of 19.5 mg/kg and 13.5 mg/kg body weight of Drug '*Swarna Bhsama'* were found as NOEL (No Observed Effect Level) in mice and rats respectively.

**Study 4:** *Trivanga Bhasma***-** Toxicity studies of *Trivanga Bhasma* (Bhasma of heavy metal prepared as per SOP mentioned in Ayurvedic texts) were carried out in both Wistar rats and Swiss albino Mice. Sub acute and chronic toxicity studies of the drug were carried out in both rats and mice. The drug was diluted with honey and given once daily for a period of 28 and 90 days respectively. In sub acute toxicity study, *Trivang Bhasma* @ 54.0, 27.0 and 5.4mg/kg showed normal behaviour and no mortality in rats. *Trivang Bhasma* @ 54.0, 27.0 and 5.4mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Trivang Bhasma* @ 54.0mg/kg treated rat in comparison to control animals. In sub acute toxicity study in mice, *Trivang Bhasma* @ 78.0, 39.0 and 7.8mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in

mice. Histological pictures depicted normal architecture in *Trivang Bhasma* @ 78.0mg/kg treated mice in comparison to control animals. NOAEL of *Trivang Bhasma* in mice was found to be 78.0mg/kg. In chronic toxicity study, *Trivang Bhasma* @ 54.0, 27.0 and 5.4mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Trivang Bhasma* @ 54.0mg/kg treated rat in comparison to control animals. In chronic toxicity studyin mice, *Trivang Bhasma* @ 78.0, 39.0 and 7.8mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in mice. Histological pictures depicted normal architecture in *Trivang Bhasma* @ 78.0mg/kg treated mice in comparison to control animals. NOEL of *Trivang Bhasma* in mice was found to be 78.0mg/kg. It can be suggested on the basis of the analysis of all the parameters that the test drug '*Trivang Bhasma*' has no serious toxicological potential on sub-acute and chronic administration in both rats & mice. *Trivang Bhasma* has no serious toxicological implications even at doses which are equivalent to ten times the therapeutic dose in animals. Though the *Trivang Bhasma* contains lead (Pb) it did not get absorbed to a significant extent as reflected by its concentration levels in serum and different organs. Though the elevation of Zinc was observed in serum, liver and kidney it does not seems to have any serious toxicity implications.

**Study 5:** *Makardhwaja*- Sub acute and chronic toxicity studies of the drug were carried out in both rats and mice. In sub acute toxicity study, *Makardhwaja* @ 27.0, 13.5 and 2.70mg/kg showed normal behaviour and no mortality in rats *Makardhwaja* @ 27.0, 13.5 and 2.70mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Makardhwaja* @ 27.0mg/kg treated rat in comparison to control animals. NOEL of *Makardhwaja* in rats was found to be 27.0mg/kg. In sub acute toxicity studyin mice, *Makardhwaja* @ 39.0, 19.50 and 3.90mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in mice. Histological pictures depicted normal architecture in *Makardhwaja* @ 39.0mg/kg treated mice in comparison to control animals. NOEL of *Makardhwaja* in mice was found to be 39.0mg/kg. In chronic toxicity study, *Makardhwaja* @ 27.0, 13.5 and 2.70mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Makardhwaja* @ 27.0mg/kg treated rat in comparison to control animals. NOEL of *Bhasma* Xj in rats was found to be 27.0mg/kg.In chronic toxicity studyin mice, *Makardhwaja* @ 39.0, 19.5 and 3.9mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in mice. Histological pictures depicted normal architecture in *Makardhwaja* @ 39.0mg/kg treated mice in comparison to control animals. NOEL of *Makardhwaja* in mice was found to be 39.0mg/kg.

**Study 6:** *Tamra Bhsma*- Sub- acute toxicity study of *Tamra Bhsma* was carried out in Swiss Albino Mice. The drug was diluted with honey and given once daily for 28 days. *Tamra Bhsma* at the dose levels

of 2, 5, 20 mg/kg body weight did not exhibit any significant change on feed consumption, behavioral pattern, body weight, organ weight, hematological and biochemical parameters in mice when compared with control group. The drug was diluted with honey and administered once daily for period of 28 days and 91 days for sub acute and chronic toxicity respectively. In 28 days repeated dose study, total 48 rats or mice were selected based on the body weight and randomly divided into four groups. Group I was a vehicle control group which received 4% gum acacia suspension while group II, III and IV were test groups that received AJ Bhasma in 1.5, 7.5 and 15 mg/kg in case of rats or 2,10 and 20 mg/kg in mice, respectively. Each group consisted of 12 animals (6 males and 6 females). The vehicle and *Tamra Bhsma* were administered orally for 28 days to individual animals of control and test group respectively. In 90 days study, total 80 rats or mice were selected based on the body weight and randomly divided into four groups. Group I was a vehicle control group which received 4% gum acacia suspension while group II, III and IV were test groups that received *Tamra Bhsma* in 1.5, 7.5 and 15 mg/kg in case of rats or 2,10 and 20 mg/kg in mice, respectively. Each group consisted of 20 animals (10 males and 10 females). The vehicle and *Tamra Bhsma* were administered orally for 90 days to individual animals of control and test group, respectively. In both the studies, cage side observations included changes in fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Body weights of each animal were recorded at the start of study and thereafter at weekly intervals. The weekly feed consumption and water consumption of rats were recorded. The hematological parameters viz. haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, and platelet count were investigated at the end of the test period. The determinations of calcium, phosphorus, chloride, sodium, potassium, fasting glucose, transaminase glutamic-oxaloacetate, glutamic-pyruvate transaminase, Alkaline phosphatase, gamma glutamyl transpeptidase, urea, albumin, creatinine, total bilirubin and total protein were made in the plasma. The urine volume, color, visual appearance of the clarity and other urine parameters viz. specific gravity, glucose, bilirubin, ketones, occult blood, pH, protein, urobilinogen, nitrite and leukocytes were estimated. Animals were sacrificed and subjected to a detailed post-mortem examination. Organs viz. brain, heart, liver, kidney, spleen, thymus, ovaries/testis, lungs, adrenals, epididymis, oesophagus, stomach, duodenum, ileum, cecum, colon, rectum were subjected to histopathological evaluation. In both the studies, no treatment related adverse findings were seen in the clinical signs, body weight, feed consumption, water consumption, hematological, biochemical parameters, organ weights, and histopathological evaluation of animals treated with *Tamra Bhsma* for 28 days in Wistar rats or Swiss mice. No mortality or moribund stage was observed throughout the study period. Hence, it was concluded that the *Tamra Bhsma* has no observable toxicity on 28 and 90 days repeated dose oral administration.

**Study 7:** *Hridayarnava Rasa***-** Sub acute and chronic toxicity studies of the drug were carried out in both rats and mice. In sub acute toxicity study, *Bhasma* Gj @ 27.0, 13.5 and 2.70mg/kg showed normal behaviour and no mortality in rats *Bhasma* Gj @ 27.0, 13.5 and 2.70mg/kg showed no significant

effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Bhasma* Gj @ 27.0mg/kg treated rat in comparison to control animals. NOEL of *Bhasma* Gj in rats was found to be 27.0mg/kg. In sub acute toxicity studyin mice, *Bhasma* Gj @ 39.0, 19.50 and 3.90mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in mice. Histological pictures depicted normal architecture in *Bhasma* Gj @ 39.0mg/kg treated mice in comparison to control animals. NOEL of *Bhasma* Gj in mice was found to be 39.0mg/kg. In chronic toxicity study, *Bhasma* Gj @ 27.0, 13.5 and 2.70mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Bhasma* Gj @ 27.0mg/kg treated rat in comparison to control animals. NOEL of *Bhasma* Gj in rats was found to be 27.0mg/kg.In chronic toxicity studyin mice, *Bhasma* Gj @ 39.0, 19.5 and 3.9mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in mice. Histological pictures depicted normal architecture in *Bhasma* Gj @ 39.0mg/kg treated mice in comparison to control animals. NOEL of *Bhasma* Gj in mice was found to be 39.0mg/kg. Considering the various factors together including hematological, biochemical (clinical chemistry), body weight, food intake, cage side activities, neurological examinations and histopathological investigations in experimental rats in chronic toxicity study of '*Hridayarnava rasa*' administered orally did not alter any of the parameters and were found safe in rats. Moreover, *Hridayarnava rasa* causes slight toxicity to testis and spleen and heart in rats upon continuous administration for 90 days at dose range equivalent to human therapeutic dose to 10 times therapeutic dose. However, the effects were very mild which were not able to induce any clinical signs, haematological or biochemical changes.

**Study 8:** *Naga Bhasma*- Sub acute and chronic toxicity studies of the drug were carried out in both rats and mice. In sub acute toxicity study, *Naga Bhasma* @ 27.0, 13.5 and 2.7mg/kg showed normal behaviour and no mortality in rats. *Naga Bhasma* @ 27.0, 13.5 and 2.7mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Naga Bhasma* @ 27.0mg/kg treated rat in comparison to control animals. NOAEL of *Naga Bhasma* in rats was found to be 225mg/kg. The chronic toxicity study was carried out for a period of 90 days in Wistar Albino Rats. The test substance was applied to the tongue once daily for 90 days in various concentrations. All the animals were observed for pre-terminal morbidity and mortality, live phase of animals, cage side observation, physical and neurological examination at regular intervals. Blood samples for evaluation of serum chemistry and haematology were collected from the animals of each group prior to the initial dosing (0 day), day 30, day 60 and on termination day (day 91 ). The animals were fasted overnight (food withheld) prior to the blood sampling. *Naga Bhasma* @ 27.0, 13.5 and 2.7mg/kg showed no significant effect on body weight, food intake, haematology, blood biochemistry and weight of vital organs in rats. Histological pictures depicted normal architecture in *Naga Bhasma* @ 27.0mg/kg treated rat in comparison to control

animals. All the animals were observed for general behaviour, mortality and neurological reflexes during the study period. No abnormal behavior was observed in animals used for the study. None of the animals died during the study period. The animals were normal and healthy. None of the toxic symptoms related to CNS, ANS, CVS and GIT were observed in the animals. Histopathology study reveals no abnormalities as related to test compound's administration. Hematological, biochemical (clinical chemistry), body weight, food intake, cage side activities, neurological examinations and histopathological investigations in chronic toxicity study for 90 days of 'Naga bhasma' administered orally did not alter any of the parameters and were found safe at therapeutic dose (TD), five times the therapeutic dose (5TD) and ten times the therapeutic dose (10TD).

**Study 9:** *Rasamanikya*- In acute toxicity study, a total of 10 Swiss albino mice (5 Males + 5 Females) weighing 18-20 gm and 4-6 weeks old were selected for administration of 10 times of the intended therapeutic dose of test compound i.e. 78 mg/kg, dissolved in diluted honey, 1:1.5 ml. The animals were observed for any lethality every hour initially for $6^{th}$, $12^{th}$ and $48^{th}$ hour followed by daily observation of activity along with body weight recording (bi-weekly) for fourteen days. No toxic signs and abnormal behaviour in the animals were observed. The dose was tolerated at 10 times of intended therapeutic dose i.e. 78mg/kg. In repeated 15 days oral toxicity in mice and rats, A total of 48 Swiss albino mice (22-26 gm) and 48 Wistar rat (150-180 gm) of both sexes (24 males + 24 females) were divided into four groups. viz. Vehicle Control (VC, Diluted Honey 1:1.15ml), Therapeutic Dose (7.8 mg/kg in mice and 5.4 mg/kg in rats), Average Dose (39 mg/kg in mice and 27 mg/kg in rats) and High Dose(78 mg/kg in mice and 54 mg/kg in rats). All the animals were subjected to biochemical and hematological tests followed by necropsy and histopathological examination of all vital organs at midterm i.e. 50% of the animals after 48 hrs of last exposure to test compound and remaining 50% of animals from each group served as recovery group were subjected to necropsy on 15th day after last exposure to test compound. No significant treatment related effect on food intake, body weight gain, cage side observations, clinical examination, neurological examination, alleregnicity, hematological parameters, clinical chemistry parameters, organ weight and no significant changes in genotoxicological parameters except for rise in frequency of MnPCE in hign dose group animals were observed in mice and rats. In 90 days oral toxicity study, A total of 80 Swiss Albino Mice (17-19 gm) and 80 rats (110-150 gm) of both sexes (40 Males + 40 Females) were divided equally into four groups viz., Vehicle Control (VC, Diluted Honey 1:1.15ml), Therapeutic Dose (7.8 mg/kg in mice and 5.4 mg/kg in rats), Average Dose (39 mg/kg in mice and 27 mg/kg in rats) and High Dose(78 mg/kg in mice and 54 mg/kg in rats). There was no lethality on single exposure (Acute study) of the intended therapeutic dose in mice. 10-15% mortality was recorded in mice which received test compound in therapeutic, average & high dose in both repeated dose and long term toxicity. The survived animals (both rats and mice) did not show any abnormal findings in body weight gains, live phase and physical activity, biochemical and hematological profiles at all doses. The gross pathology of all organs was normal. No histopathological alteration related to dose and duration of

exposure to test compound was observed. The mild rise in frequency of micronucleated polychromatic erythrocytes (MnPCE), in those animals which received 10 times of TD, suggested possibility of potential genotoxicity.

**Study 10:** *Rasa sindoora*- In chronic toxicity (90 days) study, A total of 180 young, adult albino rats of CF strain (174 - 180 gm) of both sexes (90 males and 90 females) were divided equally into six groups viz. Vehicle control (30 % Honey (10ml/kg b.wt).), Therapeutic Dose (30 mg kg b.wt.), Average Dose (60 mg /kg b.wt.), high dose (120 mg /kg b.wt.), Vehicle control reversal (30 % Honey (10ml/kg b.wt) and high dose reversal (120 mg /kg b.wt.) consisting of fifteen male and fifteen female animals each. The blood samples were subjected for analysis of Laboratory parameters (biochemistry and hematology profiles) at last day of exposure. Mortality was observed in all the groups control as well as treated. Total 20 animals died during the course of the entire study among all the groups. No significant treatment related effect on food intake, body weight gain, clinical signs, behavioural activity, haematological profile, clinical chemistry parameters, pathological changes etc. was observed. There was mortality in all the groups. Mortality may not be related with *Rasa Sindoora* treatment because it was seen in control group also. The only biochemical changes were decreased in glucose and triglycerides which were completely reversible within 28 days. The changes observed in other parameters in the study were within normal range and therefore the study indicates that *Rasa Sindoor* in the doses administered for 90 days may not cause significant toxicity in CF rat.

**Study 11:** *Arogyavardhini vati*- In chronic toxicity (90 days) study, a total of 100 Healthy Wistar rats (120 - 150 gm) of both sexes (50 males and 50 females) were divided into six groups viz. Vehicle control (33% honey solution), Therapeutic Dose (58.31mg/kg/day), Average Dose (291.55 mg/kg/day), high dose (583.10 mg /kg/day), consisting of 10 male and 10 female animals each and Vehicle control Recovery (33% honey solution) and high dose reversal (583.10 mg /kg/day) consisting of 5 male and 5 female animals. The blood samples were subjected for analysis of Laboratory parameters (biochemistry and hematology profiles) and Estimtation of heavy metals was carried out (copper and iron) in various tissues of male and female rats at last day of exposure. *Arogya Vardhini Vati* at dose of 58.31, 291.55, and 583.10mg/kg/day produced no sign of toxicity or death of animal in repeated administration for 90 days. Autopsy of surviving animals at the end of observation period did not indicate any gross pathological change in their vital organs. Biochemical and haematological parameters also did not show any significant alteration in male and female rats and values were found to be comparable to control. No histopathological changes in selected tissues at ten times of therapeutic dose were observed. The long term oral toxicity studies in male and female rats revealed that *Arogya Vardhini Vati* is non toxic and well tolerated up to a dose of 583.10mg/kg/day in rats.

**Study 12:** *Mahalaxmi Vilasa Rasa*- In chronic toxicity (90 days) study, a total of 100 Healthy Wistar rats (120 - 150 gm) of both sexes (50 males and 50 females) were divided into six groups viz.: Vehicle

control (33% honey solution), Therapeutic Dose (28.70 mg /kg/day), Average Dose (143.50 mg /kg/day), high dose (287.00 mg /kg/day), consisting of 10 male and 10 female animals each and Vehicle control Recovery (33% honey solution) and high dose reversal (287.00 mg /kg/day) consisting of 5 male and 5 female animals. The blood samples were subjected for biochemical & haematological analysis and Estimtation of heavy metals (copper and mercury) in various tissues of male and female rats, was carried out after 90 days oral administration. Mahalaxmi Vilasa Rasa at dose of 28.70, 143.50, and 287.40 mg/kg/day produced no sign of toxicity or death of animal in repeated administration for 90 days. Autopsy of surviving animals at the end of observation period did not indicate any gross pathological change in their vital organs. Biochemical and haematological parameters also did not show any significant alteration in male and female rats and values were found to be comparable to control group. No histopathological changes in selected tissues at ten times therapeutic dose were observed. The long term oral toxicity studies in male and female rats revealed that Mahalaxmi Vilasa Rasa is non toxic and well tolerated up to a dose of 287.40mg/kg/day in rats.

**Study 13:** *Vasanta kusumakara rasa*- In chronic toxicity (90 days) study, a total of 180 young adult albino rats of CF strain (121-120 gm) of both sexes (90 males and 90 females) were divided equally into six groups viz., Vehicle control (30% Honey), Therapeutic Dose (30 mg/kg b.wt.), Average Dose (60 mg/kg b.wt), high dose (120 mg/kg b.wt), Vehicle control Recovery (30 % Honey) and high dose reversal (120 mg/kg b.wt) consisting of 15 male and 15 female animals in each group. Initial, weekly and final body weights and food/water consumption of the animals were recorded. Terminal recordings of haematological and blood biochemical parameters necropsied at the end of the study were done. There was some mortality in all groups. But mortality may not be related with *Vasanta Kusumakara Rasa* treatment as it was seen in control group also. The changes observed in various parameters in the study were within normal range. In reversal study, the isolated significant changes observed during the test substance administration period were reversed and therefore the study indicates that *Vasanta Kusumakara Rasa* in the doses administered for 90 days may not cause significant toxicity in CF rat.

**Study 14:** *Kajjali Yoga*- Healthy, young adult albino rats of SD strain, selected after initial screening of their general health and body weights, were employed in the study. They were randomly assigned to four treatment group (Low dose, Mid Dose, High Dose and High dose reversal), each consisting of 15 male and 15 female animals. Two additional groups comprising of an equal number of animals served as control/reversal control. The test substance suspended in 30% honey was administered orally by gavage in graded doses (60, 120, 240 mg/kg body weight), once every day. There was some mortality in all groups. But mortality may not be related with *Kajjali Yoga* treatment as it was seen in control group also. The changes observed in various parameters in the study were within normal range. In reversal study the isolated significant changes observed during the test substance administration period were reversed and therefore the study indicates that *Kajjali Yoga* in the doses administered for 90 days may not cause significant toxicity in SD rat.

**Study 15:** *Yogaraja Guggulu*- In acute oral toxicity study, the animals (5 male and 5 female) of the test group (HD) received a single dose of the test compound @ 3900 mg per kg. body weight on first day of the experiment. The animals were observed for morbidity, mortality and clinical signs of toxicity during next 10 days. No mortality, morbidity were recorded. Weight loss and abnormal behaviour was recorded after single exposure of test compound in 10XTD. The dose was tolerated at 10 times of intended therapeutic dose i.e. 3900 mg/kg body weight. In chronic toxicity (90 days) study, a total of 48 Wistar rats (110- 150 gm) of both sexes (24 males and 24 females) were divided equally into four groups viz.,: Vehicle control (1ml distilled water, depending upon body weight), Therapeutic Dose (270 mg /kg b.wt.), Average Dose (1350 mg /kg b.wt), high dose (2700 mg /kg b.wt), consisting of 6 male and 6 female animals in each group. No abnormal behavioral activity and no pre-terminal deaths were recorded in the mice exposed once to the test compound up to10 times of intended therapeutic dose. No major abnormalities with respect to physical, behavioural, haematological and histopathological changes except for some changes in biochemical parameters were observed on administration (oral) of test compound at various doses under the experimental conditions. The test drug was found to be non toxic up to 10 times of intended therapeutic dose in the species during the course of experiment.

**Study 16:** *Mahayogaraja Guggulu*- In sub-acute toxicity (30 days) study, A total of 40 male Wistar rats (120±20 gm), 2-3 months old were divided equally into five groups viz. Normal Control (Potable drinking water), Vehicle Control (4ml/kg, Honey : Deionised Water (2:3)), Therapeutic Dose (10mg/kg), Medium Dose (25mg/kg) and High Dose(50mg/kg). The test drug did not produce any changes in clinical signs, hematological parameters, liver function test, kidney function test and metal concentrations in blood, liver, kidney and brain of all the treated animals were comparable with control groups and very much in range of permissible limits. In 60 days repeated oral dose toxicity study, A total of 40 male Wistar rats (120±20 gm), 2-3 months old were divided equally into five groups viz. Normal Control (Potable drinking water), Vehicle Control (4ml/kg, Honey : Deionised Water (2:3)), Therapeutic Dose (10mg/kg), Medium Dose (25mg/kg) and High Dose(50mg/kg). the oral administration of Mahayogaraja Guggulu to male Wistar rats at 10 mg/kg, 25 mg/kg and 50 mg/kg dose daily for 30 and 60 days, had no effect on general health, clinical toxic signs and pre terminal deaths. The treatment did not affect growth, body weight, haematological and biochemical parameters. Mild to moderate toxicity observed during histopathological examination suggest initiation of toxic manifestations at the cellular level in liver and kidney. In view of the results observed, it was evaluated that at up to 50 mg/kg body weight of Mahayogaraja Guggulu administered for 30 and 60 days in male rats do not show any observable toxic effect except mild signs of oxidative stress and microscopic changes in cellular morphology. Further, despite the high concentrations of lead and mercury present in the drug powder, vital organs and tissues of experimental rats does not seem to accumulate these metals.

# CHAPTER 3

# SUMMARY OF PHARMACOLOGY RESEARCH AND SAFETY STUDIES

## 3.1. Summary of Pharmacology Research on Biological Screening

### A. Biological Screening of Single drugs

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 1. | Ajmoda | i) *Trachyspermum roxburghianum* (DC.)<br>ii) *Apium graveolens* L.<br>iii) *Apium leplophyllum* F. Muell. ex Benth. | Anti-hypertensive effect |
| 2. | Anantamula | *Hemidesmus indicus* R. Br. | Hypotensive effect due to central beta receptor blockade & antifungal activity |
| 3. | Ankola | *Alangium lamarckii* Thwaites | Anti-inflammatory effect |
| 4. | Antamula | *Tylophora indica* (Burm.f.) Merr. | Bronchodilator & anaphylactic activity |
| 5. | Apamarga | *Achyranthes aspera* L. | Potentiated Ach induced spasm & hyportensive effects |
| 6. | Aparajita | *Clitoria ternatea* L. | Anti-oxytocic activity |
| 7. | Aragvadha | *Cassia fistula* L. | Antifungal & antibacterial effect |
| 8. | Arishtaka | i) *Sapindus mukorossi* Gaertn.<br>ii) *Sapindus trifoliatus* L. | Anti-inflammatory & anti-implantation activity |
| 9. | Arjuna | *Terminalia arjuria* W. & A. | Hypotensive & bradycardia |
| 10. | Abhi Narikela | *Lodoicea maldivica* Pers. | Anti-diabetic effect |
| 11. | Ashoka | *Saraca asoca* (Roxb.) De Wilde | Cardiac stimulant & spasmodic action |
| 12. | Asthiaamharit | *Cissus quadrangularis* L. | Analgesic activity |
| 13. | Ashvagandha | *Withania somnifera* (L.) Dunal. | Anti-inflammatory, analgesic, antipyretic, anti-convulsant, depressant, hypotension, bradycardia and respiratory stimulant, anti-spasmodic and adaptogenic effect |
| 14. | Ashvaghna | *Thevetia neriifolia* Juss. | Cardiotonic & antifungal |
| 15. | Ativisha | *Aconitum heterophyllum* Wall. | Hypotensive effect |
| 16. | Bakula | *Mimusops elengi* L. | Antipyretic effect |
| 17. | Bhallataka | *Semecarpus anacardium* L. f. | Anti-inflammatory effect |

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 18. | Bhandira | *Clerodendrum infortunatum* L. | Inhibitory effect on granulation of mast cells |
| 19. | Bhanga/Vijaya | *Cannabis sativa* L. | Anti-stress activity in small doses |
| 20. | Bharangi | *Clerodendrum serratum* (L.) Moon | Antihistaminic effect |
| 21. | Bhringraja | *Eclipta alba* Hassk. | Hypnotic activity |
| 22. | Bhumyamalaki | *Phyllanthus niruri* Hook. f. | Hepatoprotective effect |
| 23. | Bilva | *Aegle marmelos L. Correa.* | Anthelmintic, hypoglycaemic, anti-diarrhoeal activity |
| 24. | Brihatgokshura | *Pedalium murex* L. | Spasmogenic action |
| 25. | Changeri | *Oxalis corniculata* L. | Analgesic & anti-inflammatory and adjuvant arthritis effect |
| 26. | Chitraka (Rakta) | *i) Plumbago rosea* L.<br>*ii) Plumbago zeylanica* L. | Antibacterial, antifungal and anti-fertility activity |
| 27. | Dadima | *Punica granatum* L. | Analgesic and anti-diarrhoeal |
| 28. | Danti | *Baliospermum montanum* Muell.-Arg. | Hypnotic & hypotension effect |
| 29. | Daruharidra | *Berberis aristata* DC. | Anti-inflammatory, produced depressant effect on histamine, hypotension activity |
| 30. | Devadali | *Luffa echinata* Roxb. | Hepatoprotective and decrease spontaneous motor activity |
| 31. | Dronapushpi | *Leucas cephalotes* Spreng. | Darkening of skin on local application |
| 32. | Durva | *Cynodon dactylon* (L.) Pers | Hypoglycaemic and reduce bleeding and clotting time. Hypotention and antiviral effect against vaccinia virus |
| 33. | Eranda | *Ricinus communis* L. | Anti-inflammatory activity |
| 34. | Ghritakumari | *Aloe barbadensis* Mill.<br>syn. *A.vera* Toum.ex L. | Anti-spasmodic, improve menstrual function, wound healing & anti-ulcer activity |
| 35. | Gokshura | *Tribulus terrestris* L. | Antibacterial & antifungal property |
| 36. | Grinjana | *Daucus carota* L. | Anti-estrogenic & anti-fertility effect |
| 37. | Guduchi | *Tinospora cordifolia* (Willd.) Miers | Hypoglycaemic, analgesic & anti-inflammatory activity |

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 38. | Guggulu | *Commiphora mghtii* (Am.) Bhand. syn. C. *mukul* (Hook, ex Stocks) Engl. | Cholesterol lowering property, hypolipidemic and anti-atherosclerosis activity |
| 39. | Gunja | *Abrus precatorius* L. | Anti-implantation activity, hypnotic, CNS depressant effect & anti-fertility effect |
| 40. | Gwara | *Cyamopsis tetragonoloba* Taub. syn. C. *psoraliodes* DC. | Hindered absorption of glucose taken alongwith food, hypolgycemic effect and ant- atherosclerosis activity |
| 41. | Haridra | *Curcuma longa* L. | Anti-inflammatory and anti-histaminic effects |
| 42. | Iridis | *Iris florentina* Hort. | Neuromuscular bloking activity & anti-spasmodic activity |
| 43. | Jambu | *Syzygium cumini* (L.) Skeels syn. *Eugenia jambolana* Lam. | Hypoglycaemic effect |
| 44. | Japa | *Hibiscus rosa-sinensis* L. | Hypotensive, anti-fertility, antispermatogenic, antiovulatory, anti-implantation & abortifacient activity |
| 45. | Jatamansi | N. jatamansi DC. Syn Nardostachys grandiflora DC. | Anti-epileptic, hypotensive, alpha & beta adrenergic blocking activity, anti-implantation activity, enhance the learning process, analgesic and anti-inflammatory effect |
| 46. | Jyotishmati | *Celastrus paniculatus* Willd. | Central muscles relaxant, anti-convulsant & antihistaminic effect |
| 47. | Kakajangha | *Leea aequata* L. | Antihistaminic, antipyretic and blood vascular disorder activity |
| 48. | Kakatundi | *Asclepias curassavica* L. | Hypertensive property, it produced increase in heart rate followed by arrhythmias |
| 49. | Kakodumbarika | *Ficus hispida* L.f. | Anti-inflammatory, antihistaminic and anticholagogue effect |
| 50. | Kalahara (Nilo-far) | *Nymphaea alba* L. | Anti-acetylcholine effect |

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 51. | Karnasphota | *Cardiospermum halicacabum* L. | Analgesic, anti-inflammatory and antifertility effect |
| 52. | Kamala | *Nelumbo nucifera* Gaertn. | Hypotension effect |
| 53. | Kampillaka | *Mallotus philippinensis* Muell. -Arg. | Purgative, anti-helmenthic, anti-lithotropic, wound healing, anti-inflammatory, antispasmodic and cardiac depressant activity |
| 54. | Kanchanara | *Bauhinia variegata* L. | Anti-inflammatory effect |
| 55. | Kantakari | *Solanum surattense*Bunn. f. syn. *S. xanthocarpum* Sch. & Wendl. | Antispasmodic & cardiotonic effect |
| 56. | Kapikacchu | *Mucunapruriens* (Linn.) DC. syn. ***M.****prurita* Hook. | Anti-inflammatory effect |
| 57. | Karanja | *Pongamia pinnata (L.)* Pierre syn. *P. glabra* Vent. | Antipyretic, cardiac depressant, anticholinergic and histaminic effect |
| 58. | Karavira | *Nerium indicum* Mill. | Cardiotonic & anti-stress effect |
| 59. | Karchura | *Curcuma zedoaria* Rose. | Antibacterial and antifungal effect |
| 60. | Karvellaka | *Momordica charantia* L. | Antispasmodic and antioxytocic effect |
| 61. | Karpasi | *Gossypium arboreum* L. | Uterotonic, hypotension & galactagogue effect |
| 62. | Kasamarda | *Cassia occidentalis L.* | Antianaphylactic effect |
| 63. | Kaseruka | *Scirpus kysoor* Roxb. | Hypotension antispasmic effect |
| 64. | Katuki | *Picrorhiza kurroa* Royle ex. Benth. | Antipyretic, anti-inflammatory, antiviral, hepatoprotective, antistress, muscle relaxtant, antispasmodic, laxative and bronchoconstrictor effect |
| 65. | Ketaki | *Pandanus tectorius* Sol. | Depressant & anticholinergic effects and 5mg/kg dose reduced urinary flow |
| 66. | Kiratatikta | *Swertia chirayita* (Roxb ex Flem) Karst syn. *S. chirata* Wall. | Neuromuscular bloking, anthelmintic, antispasmodic, anti-inflammatory, antifungal & antimalarial effect |
| 67. | Kokilaksha | *Hygrophila auriculata* (Schum.) Heine syn. *Asteracantha longifolia* Nees. | Antifungal, antispasmodic, antibacterial, anti-inflammatory, antipyretic and diuretic effect |
| 68. | Kukundara | *Blumea lacera* DC. | Anti-inflammatory & increase gastric secretion and acidity |

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 69. | Kulattha | *Dolichos biflorus* L. | Hypocholesterolemic & diuretic effect |
| 70. | Kusha | *Desmostachya bipinnata (L.) Stapf.* | Hypnotic activity |
| 71. | Kushmanda | *Benincasa hispida* (Thunb.) Cogn. | Antipyretic & hypoglycaemic effect |
| 72. | Lata Kasturi | *Hibiscus abelmoschus* L. syn. *Abelmoschus moschatus* Medic | Antioxytocic effect |
| 73. | Lashuna | *Allium sativum* L. | Hypocholesterolemic & anti-inflammatory effect |
| 74. | Lodhra | *Symplocos cochinchinensis* (Lour.) Moore subsp. *laurina* (Ritz.) Nooteboom syn. *S. spicata* Roxb. | Anti-inflammatory & anti-ulcerogenic effect |
| 75. | Madanaphala | *Catunaregam spinosa* (Thunb.) Tiruv. syn. *Randia dumetorum* (Retz.) Poir | Cardiac depressant & bronchodialator effect |
| 76. | Madayantika | *Lawsoni inermis* L. | Analgesic, anti-inflammatory & hepatoprotective effect |
| 77. | Mandukaparni | *Centella asiatica* (Linn.) Urban syn. *Hydrocotyle asiatica* L. | Drug caused impairment of muscular coordination & anticonvulsive effect |
| 78. | Manjistha | *Rubia cordifolia* L. | Not produce any effect on respiration & blood pressure |
| 79. | Matsyakshi | *Alternanthera sessilis* DC. | Spasmogenic effect |
| 80. | Mayaphala | *Quercus infectoria* Oliv. | Hypotensive & vasodilator effect |
| 81. | Masha | *Phaseolus mungo* L. | Antipyretic & sialogogue effect |
| 82. | Musta | *Cyperus rotundus* L. | Tranquillising, anti-inflammatory, analgesic & anti-arthritic effect |
| 83. | Nagakeshara | *Mesua ferrea* L. | Bronchodilator, anti-anaphylactic & anti-spasmodic effect |

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 84. | Nagajivha | *Enicoslemma hyssopifohum* (Willd.) Verdoom syn. E. *littorale* B. Jume | Hypoglycaemic effect |
| 85. | Nichula | *Barringtonia acutangula* Gaertn. | Produced potentiation of pentobarbital hypnosis |
| 86. | Nimba | *Azadirachta indica* A. Juss. syn. *Melia azadirachta* L. | Anti-inflammatory, analgesic, antipyretic, hypoglycaemic, anti-ulcer, anticholinergic, antihistaminic, antinocotinic & anti-mitotic effect |
| 87. | Nirgundi | *Vitex negundo* L. | Anti-inflammatory, analgesic, antibacterial & CNS depressant effect |
| 88. | Palandu | *Allium cepa* L. | Hypocholesterolemic effect |
| 89. | Palasha | *Butea monosperma* (Lam.) Taub. Syn *H. frondosa* Kocn ex. Roxb. | Anti-spasmodic, anti-implantation & anticonvulsant effect |
| 90. | – | *Panax ginseng* Mey. | Antistress & antiviral effect |
| 91. | Parijata | *Nyctanthes arbor-tristis L.* | Antispasmodic & hypotensive effect |
| 92. | Parpata | *Fumaria indica* Pugsley syn. *F. parviflora* auct. Non. Lam. | Muscles relaxant, anti-spasmodic & dyspepsia effect |
| 93. | Pashanabheda | i. *Bergenia ligulata Engl.*<br>ii. *Didymocarpus pedicellata R.Br.* | i. CNS depressant, analgesic, anti-inflammatory, diuretic, hypotensive & antilithic effects<br>ii. inotropic, hypotensive & intestinal relaxation effect |
| 94. | Patola | *Trichosanthes cucumerina* L. | Spasmodic & spasmolytic, analgesic effect |
| 95. | Paribhadra | *Erythrina variegata* L, var. *Orientalis* (L.) Merrill syn. *E. indica* Lam. | Neuromuscular blocking effect |
| 96. | Pippali | *Piper longum* L. | Antimalarial, CNS stimulant, analeptic, antinarcotic & anti-inflammatory effect |
| 97. | Pita Bala | *Sida rhombifolia* L. | CNS depressant, Antiarthritic, anti-inflammatory, hypotensive & bronchodilator effect |
| 98. | Pitabhantaka | *Solatium khasianum* C.B. Clarke | Neuromuscular blocking, anti-ovulatory & anti-epileptic effect |

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 99. | Punarnava | *Boerhaavia diffusa* L, syn. *B. repens* L. | Anti-inflammatory, diuretic & myocardial stimulation |
| 100. | Pushkaramula | *Inula racemosa* Hook. f. | Antiallergic, anti-histaminic and bronchodilator effect |
| 101. | Putiha | *Mentha arvensis* L. | Anti-spermatogenic, antiovulatory, antiimplantation & abprtifacient effect |
| 102. | Putikaranja | *Caesalpinia crista* L., syn. *C. bonducella* Flem. | Anti-inflammatory & anti-malarial effect |
| 103. | Rajakoshataki | *Luffa acutangula* (L.) Roxb. | Antifertility, abotifacient & uterotonic effect |
| 104. | Raja Patha | *Stephania hemandifolia* Walp. | Vasoconstrictor & adrenergic neuron blocking effect |
| 105. | Rakta Chandana | *Pterocarpus santalinus* L. f. | Oxytocic, mild anti-inflammatory, hypoglycaemic and antidiabetic effect |
| 106. | Rasna | *Acampe papillosa* Lindl. | Anti-inflammatory and anti-arthritic effect |
| 107. | Rohitaka | *Tecomella undulata* (Sm.) Seem syn. *Tecoma undulata* G.Don. | Spasmolytic & anti-inflammatory effects |
| 108. | Saireyaka | *Barleria prionitis* L. | Diuretic & spasmogenic effect |
| 109. | Shaka | *Tectona grandis* L. f. | Antiulcer effect |
| 110. | Shakhotaka | *Streblus asper* Lour. | Cardiac stimulant & cardiotonic effect |
| 111. | Shalaparni | *Desmodium gangeticum* DC. | Cardiac stimulant, anti-inflammatory, analgesic and anti-arthritic effect |
| 112. | Saptachakra | *Salacia chinensis* L. syn. *S. latifolia* Wall; *S. prinoides* DC. | Hypoglycaemic effect |
| 113. | Saptala | *Euphorbia dracunculoides* Lam. | Cholinergic effect |
| 114. | Saptaranga | *Salacia fruticosa* Heyne | Hypoglycaemic effect |
| 115. | Sharapunkha | *Tephrosia purpurea* Pers. | Poor antipyretic, analgesic and anti-inflammatory effect |
| 116. | Sharipha | *Annona squamosa* L. | Did not posses antifertility effect |
| 117. | Shatavari | *Asparagus racemosus* Willd. | Mild diuretic, mild hypoglycaemic & galactagogue, anticancer effect |
| 118. | Utangan | *Blepharis edulis* Pers. | Anti-inflammatory & diuretic effect |

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 119. | Shilarasa | *Altingia excelsa* Noronha | Anti-stress, anti-inflammatory, antipyretic & immumomodulator effect |
| 120. | Sirisa | *Albizia iebbek* Benth. | Intestinal relaxation, hypotension, anti-anaphylactic effect |
| 121. | Shobhanjana | *Moringa oleifera* Lam. syn. *M. pterygosperma* Gaertn. | Hypotensive effect |
| 122. | Sunishannaka | *Marsilea quadrifolia* L. | Hypotensive, cardiac depressant & anti-histaminic activity |
| 123. | Shweta Dhataki | *Calycopteris floribunda* Lam. | Antibacterial & anthelmintic effect |
| 124. | Strobilanthes heyneanus | *Strobilanthes heyneanus* Nees. | Analgesic, anti-inflammatory, CNS depressant & immunosuppressant effect |
| 125. | Tagara | *Valeriana wallichii* DC. | Antibiotic, analgesic, mild CNS depressant & antipyretic effect and inhibited growth of Ehrlich carcinoma |
| 126. | Tambula | *Piper betle* L. | Spermicidal effect |
| 127. | Thanaila | *i) Gardenia latifolia* Ait.<br>*ii) Gardenia turgida* Roxb. | Cardiac stimulant |
| 128. | Tinduka | *Diospyros peregrina* Gurke. | Anti-inflammatory, analgesic & anti-arthritic effect |
| 129. | Trivrita | *Operculina turpethum* (L.) Silva Manso | Cardiac depressant & purgative effect |
| 130. | Tulasi | *Ocimum sanctum* L. | Antistress, antiasthmatic & antirheumatic effect |
| 131. | Ulatakambala | *Abroma augusta* L. | Spasmogenic effect |
| 132. | Uttamarini | *Pergularia daemia* (Forsk.) Chiov. syn.<br>*Daemia extensa* R. Br. | Spasmolytic, CNS depressant & purgative effect |
| 133. | Utpala | *Nymphaea stellata* Willd. | Hypotensive, analgesic, antipyretic & anti-inflammatory effect |
| 134. | Vacha | *Acorus calamus* L. | Hypotensive, analgesic & antipyretic effect LD50 (i.p.) was found in between 200-250mg/kg |
| 135. | Vana Niwara Patti | *Buddleja asiatica* Lour. | Anti-ovulatory & hypotension effect |
| 136. | Varuna | *Crataeva nurvala* Buch.- Ham. | Anti-inflammatory & antipyretic effect |

| Sr. No. | Name of Single drug | Botanical name/ English name | Pharmacological activity |
|---|---|---|---|
| 137. | Vasa | *Adhatoda vasica* Nees. | Bronchodilator, cardiac stimulant & antianaphylactic effect |
| 138. | Vibhitaka | *Terminalia bellirica* Roxb. | Antihistaminic & CNS stimulant effect |
| 139. | Vidanga | *Embelia ribes* Burm.f. | Anti-implantation & antiestrogenic effect |
| 140. | Vidari | *Pueraria tuberosa* DC. | Hypotension, spasmogenic & oestrogenic effect |
| 141. | Vriddhadaru | *Argyreia speciosa* Sweet. | Not produced anti-inflammatory & diuretic effect |
| 142. | Musk | Moschus moschiferus | Antispasmodic, increase amplitude of heart, produced inconsistent rise in blood pressure but lower the BP at higher dose (6mg/kg) & caused sudden fall in BP leading to death, diuretic, analgesic, increase the rate of respiration & piloerectia |

## B. Biological Screening of Ayurvedic Compound Formulations

| Sr. No. | Ayurvedic formulations | Biological effect |
|---|---|---|
| 1. | Amruthotharam Yoga Choornam | Antifungal effect |
| 2. | Dhanwantara Gutika | Anti-inflammatory, rheumatoid arthritis, respiratory stimulant, hemiplegia, anti-anxiety & hypotensive effect |
| 3. | Jasad Bhasma | Antiulcer and antidiabetic effect |
| 4. | Kajal and Surma | No anti-inflammatory action |
| 5. | Lodhrasava | No antipsychotic, antiparkinsonism, antidepressant, anti-inflammatory & failed to protect against electro-convulsion |
| 6. | Mandura Vataka | Mild CNS stimulant, analgesic, mild antipyretic & antispasmodic effect |
| 7. | Nardiya Laxmivilas Rasa Misrana | Antidiuretic effect |
| 8. | Shilajita | Alangesic, anti-inflammatory & anti-ulcer effect |
| 9. | Tamra Bhasma | Anti-ulcerogenic effect |
| 10. | Taramanduram | Anti-ulcerogenic effect |

| Sr. No. | Ayurvedic formulations | Biological effect |
|---|---|---|
| 11. | Vettumarana Gutika | CNS depressant & antispasmodic effect |
| 12. | Vidangadi Yoga | Insignificant effect on blood pressure |
| 13. | Bilvadi Gutika | Mild CNS depressant, cholinolytic & antispasmodic effect |
| 14. | Ayush-9 | Hypotensive & anti-convulsant effect |
| 15. | Ayush-11 | Hypertensive, reduction of respiration rate |
| 16. | Ayush-12 | Hypotensive, increase in amplitude of respiration, |
| 17. | Ayush-13 | Hypertensive (dose dependence) & reduction in respiration amplitude |
| 18. | Ayush-14 | Hypotensive with respiratory stimulation in amplitude, exhibited laboured respiration & gasping in higher dose |
| 19. | Ayush-15 | 4g/kg onward showed depressant action on blood pressure & produce slight apnoea, increase in rate & amplitude of respiration |
| 20. | Ayush-16 | Failed to lower plasma cholesterol & anaesthetic activity |
| 21. | Ayush-17 | Hypoglycaemic effect |
| 22. | Ayush-49 | Antagonistic effect againt Ach, histamine, barium chloride & carbachol, higher dose caused increase in the rate &amplitude of respiration, bronchodilator effect |
| 23. | Ayush-50 | Hypotensive (dose dependent) |
| 24. | Ayush-56 | Anti-inflammatory effect |
| 25. | Ayush-61 | Hypotensive (dose dependent) & no anti-inflammatory effect |
| 26. | Ayush-62 | Hypotensive effect & Caused congestion in intestine |
| 27. | Ayush-64 | Increased gastric secretion, reduced BP, mild laxative, diuretic, hepatoprotective, antibacterial, useful in hepatitis and urinary tract infection, anti-tumour & antispasmodic effect |
| 28. | Ayush AC-II | Hypotensive effect |
| 29. | Ayush AC-IV | Oestrogenic effect |
| 30. | LA/P/TNR/l, LA/C/ TNR/2, LA/M/TNR/3 | All three drug exhibited Antispasmodic effect, LA/C/TNR/2 only produced analgesic effect |
| 31. | AP/P/TNR71 ,AP/C/ TNR/2, AP/M/TNR | Neuromuscular blocking effect |
| 32. | Neuromuscular blocking activity of some natural and synthetic heterocycles | Rotenone in 10mg/ml caused complete neuromuscular block, neostigmine 1mcg/ml partially antagonised the effct of rotenone. |
| 33. | FHL/TNR/1, FHL/ Chloroform/TNR 3 and FHL/ Acetone/TNR 5 | Fraction 1 exhibited weak & fraction 2 produced significant anticholinergic effect while fraction 3 was found inactive. Fraction 1 & 3 showed hypotensive effect |

## 3.2. Summary of Pharmacology Research on Safety Studies

### A. Safety studies on Single drugs

| S. No. | Single drug name | Botanical name/English Name | Safety/Toxicity |
|---|---|---|---|
| 1. | Apamarga | *Achyranthes aspera* L. | Oral LD50 was 297.52mg/kg |
| 2. | Arishtaka | i) *Sapindus mukorossi Gaertn.* *ii) Sapindus trifoliatus L.* | No significant untoward effect was noted by acute & subacute toxicity studies with extract by only gastric mucosal irritation was observed |
| 3. | Arjuna | *Terminalia arjuna* W.&.A. | LD50 of aq. Extract was 250mg/kg |
| 4. | Ashoka | *Saraca asoca* (Roxb.) De Wilde | Pure phenolic glycoside was nontoxic |
| 5. | Asvaha | *Thevetia neriifolia* Juss. | The toxicity pattern is identical to that of other cardiac glycosides |
| 6. | Bakula | *Mimusops elengi* L. | Nontoxic in doses from 500 to 1000mg/k orally |
| 7. | Bharangi | *Clerodendrum serratum* (L.) Moon | Acute LD50 of saponin was 307.7mg/kg in rats & 288.2mg/kg in mice. Chronic toxicity on rats showed 23.5% mortility but no evidence of tissue damage of saponin treated guineapig for 20 days |
| 8. | Bhumyamalaki | *Phyllanthus niruri* Hook. f. | Nontoxic in 2gm/kg p.o. in in acute toxicity studies |
| 9. | Changeri | *Oxalis corniculata* L. | Toxicological studies on petroleum ether, chloroform, benzene, methanol & aqueous extract of the plant exhibited no toxic effectin 500 & 100 mg/kg p.o. |
| 10. | Chitraka (Rakta) | *a) Plumbago rosea* L. *b) Plumbago zeylanica* L. | LD50 of 50% ethanolic extract of whole plant in albino mice was calculated to be 562mg/ kg i.p. |
| 11. | Daruharidra | *Berberis aristata* DC. | The LD50 value of berberine sulphate in mice was found to be 24.3mg/kg i.p. |
| 12. | Grinjana | *Daucus carota* L. | LD50 value was found 188.54mg/kg p.o. & no teratogenecity was shown |
| 13. | Guduchi | *Tinospora cordifolia* (Willd.) Miers | The LD50 of glycoside was 1132mg/kg p.o. and 428.7mg/kg i.p. |

| S. No. | Single drug name | Botanical name/English Name | Safety/Toxicity |
|---|---|---|---|
| 14. | Guggulu | *Commiphora mghtii* (Am.) Bhand. syn. C. *mukul* (Hook, ex Stocks) Engl. | Fractions A, B, C, D (pet-ether soluble, neutral, chloroform soluble & chloroform insoluble) fractions of gum guggulu produced no toxicity on chicks in doses of 5 to 20mg/kg p.o. |
| 15. | Gwara | *Cyamopsis tetragonoloba* Taub. syn. *C. psoraliodes* DC. | No systemic toxicity was observed during subacute toxicity study |
| 16. | Haridra | *Curcuma longa* L. | LD50 value of aq. Suspension was found to be 3.25ml/kg while LD50 of volatile oil emulsified in twin 80 was found 0.533ml/kg |
| 17. | Kakajangha | *Leea aequata* L. | Antihistaminic, antipyretic and blood vascular disorder activity<br>LD50 was more than 1000mg/kg p.o. |
| 18. | Kakatundi | *Asclepias curassavica* L. | Highly toxic, limit its use with great precaution |
| 19. | Karnasphota | *Cardiospermum halicacabum L.* | No toxicity upto 30g/kg p.o. |
| 20. | Kamala | *Nelumbo nucifera* Gaertn. | Nontoxic (acute administration) in mice administered in doses ranging from 100 to 800mg/kg i.p. |
| 21. | Kapikacchu | *Mucunapruriens* (L.) DC. syn. ***M.*** *prurita* Hook. | The LD50 value of sterol extract was 600mg/kg i.p. the drug administered 20mg/kg/rat for more than 5 days produced necrosis and sloughing of the muscles |
| 22. | Karanja | *Pongamia pirmata Pierre* syn.<br>*P. glabra* Vent. | The toxicity test performed on mice which could well tolerated upto 5g/kg (nontoxic) |
| 23. | Karavira | *Nerium indicum* Mill. | The acute LD50 was 86mg/kg i.p. |
| 24. | Karchura | *Curcuma zedoaria* Rose. | Found nontoxic |
| 25. | Karvellaka | *Momordica oharantia* L. | Fruit juice (15-40mg/kg) was found toxic |
| 26. | Karpasi | *Gossypium arboreum* L. | Toxicity upto 2g/100g bw |
| 27. | Kiratatikta | *Swertia chirayita* (Roxb ex Flem)Karst syn. *S. chirata* Wall. | Dose up to 200mg/kg/op in mice did not show any toxicity |
| 28. | Kukundara | *Blumea lacera* DC. | LD50 was found between 750-800mg/kg |
| 29. | Kulattha | *Dolichos biflorus* L. | Non-toxic upto a dose of 2g/kg/op |

| S. No. | Single drug name | Botanical name/English Name | Safety/Toxicity |
|---|---|---|---|
| 30. | Lodhra | *Symplocos cochinchinensis* (Lour.) Moore subsp. *laurina* (Ritz.) Nooteboom syn. *S. spicata* Roxb. | Found nontoxic upto dose of 800mg/kg i.p. |
| 31. | Madayantika | *Lawsonia inermis* L. | Found non-toxic |
| 32. | Manjistha | *Rubia cordifolia* L. | Non toxic upto 1g/kg po |
| 33. | Nagakeshara | *Mesuaferrea* L. | LD50 in mice being 400mg/kg i.v. and 800mg/kg i.p. and non-toxic upto 1600mg/kg p.o. |
| 34. | Nichula | *Barringtonia acutangula* Gaertn. | Dose upto 1g/kg p.o. did not produced toxicity |
| 35. | Nimba | *Azadirachta indica* A. syn. Juss.*Melia azadirachta* Linn. | Non-toxic upto dose of 100mg/kg/day |
| 36. | Patola | *Trichosanthes cucumerina* L. | LD50 was found to be 3g/kg approx |
| 37. | Pippali | *Piper longum* L. | LD50 value was 750-800mg/kg p.o. |
| 38 | Punarnava | *Boerhaavia diffusa* L, syn. *B. repens* L. | Did not show any toxic effect dose upto 1600mg/kg |
| 39. | Putikaranja | *Caesalpinia crista* Linn, syn. *C. bonducella* Flem. | Non toxic dose upto 2500mg/kg/oral |
| 40. | Saireyaka | *Barleria prionitis* L. | Non toxic at 4gm/kg |
| 41. | Shaka | *Tectona grandis* L. f. | Fraction-B indicated low toxicity |
| 42. | Shalaparni | *Desmodium gangeticum* DC. | The isolated compound is nontoxic upto a dose of 7g/kg |
| 43. | Sharapunkha | *Tephrosia purpurea* Pers. | Found to be nontoxic upto 1g/kg p.o. |
| 44. | Tambula | *Piper betle* L. | Nontoxic upto 1g/100g |
| 45. | Thanaila | *i) Gardenia latifolia* Ait. *ii) Gardenia turgid* Roxb. | LD50 of G. Latifolia was 250.3mg/kg & G. Turgid was 150.8mg/kg |
| 46. | Ulatakambala | *Abroma augusta* L. | Non-toxic |
| 47. | Vanakadali | *Ensete superbum* Cheesman | In Acute & subacute toxicity at the dose 490mg/kg did not show any toxicity |
| 48. | Vasa | *Adhatoda vasica* Nees. | Acute & chronic toxicity studies proved it to be comparatively safe. |
| 49. | Vidari | *Pueraria tuberosa* DC. | No toxicity upto 1g/kg i.p. |
| 50. | Vriddhadaru | *Argyreia speciosa* Sweet. | Nontoxic in the dose range of 100-750mg/kg i.p. |
| 51. | Musk | *Moschus moschiferus* | LD50 was 331.1mg/kg i.p. |

## B. Safety studies on Ayurvedic Compound Formulation

| S. No. | Ayurvedic formulations | Safety/toxicity |
|---|---|---|
| 1. | Kajal and Surma | Nontoxic upto 10g/kg *p.o.* after application on rabbit eyes for 4 months |
| 2. | Shilajita | No toxicity upto a dose 1gm/kg *i.p.* |
| 3. | Shwasa Kesari | Daily administration of the drug for 4 week did not produced any toxicity |
| 4. | Ayush-9 | Administration of 100mg/kg dose cause no mortility within 24 hour but higher doses of 7.5g/kg produced 33% mortility within half hour |
| 5. | Ayush-11 | The LD50 value in mice was 0.34g/kg *i.p.* at fiducial limits between 0.212g/kg & 0.544g/kg at 95% confidence level |
| 6. | Ayush-12 | Higher dose cause death. LD50 in mice was 0.520g/kg *i.p.*& 4.9g/kg *p.o.* |
| 7. | Ayush-13 | Higher dose cause death. LD50 was 0.058g/kg *p.o.* |
| 8. | Ayush-14 | LD50 was 0.985g/kg *i.p.* and 3.1g/kg *p.o.* The doses of 0.250 & 0.500g/kg were tolerated without any toxicity in subacute toxicity study. |
| 9. | Ayush-15 | LD50 was found 0.720g/kg *i.p.*& 3.5g/kg *p.o.* |
| 10. | Ayush-17 | LD50 was more than 2g/kg *p.o.* |
| 11. | Ayush-49 | LD50 was found between 600-650mg/kg |
| 12. | Ayush-50 | Oral LD50 was 0.150g/kg |
| 13. | Ayush-56 | Notoxicity with more than 12g/kg *p.o*. LD50 in rats was more than 4g/kg |
| 14. | Ayush-61 | LD50 was 0.680g/kg *i.p.* & 1.7g/kg *p.o.* |
| 15. | Ayush-62 | LD50 was 680mg/kg |
| 16. | Ayush-64 | LD50 was more than 2gm/kg, no acute & sub acute toxicity |
| 17. | Ayush AC-II | It produced no mortility upto 400mg/kg & nontoxic in acute & subacute toxicity study |
| 18. | Ayush AC-IV | LD50 value in mive & rats was found to be more than 2g/kg and 4g/kg respectively. AC-IV in a dose of 500mg/kg orally for 12 weeks was found nontoxic |

## C. Safety studies on Ayurvedic Compound Formulations in collaborating with World Health Organization (WHO)

The Council has been collaborating with WHO India Office since 2002 and WHO has extended support under WHO Biennium Programmes for Research studies and developing Training Technical Documents. The following are the details of support extended by WHO to CCRAS for Safety/toxicity studies.

| Sl. No. | Year | Details of the Project supported by WHO | Outcome |
|---|---|---|---|
| 1. | 2002-03 | **Project**<br>Toxicity and Standardisation of Drug –A for Chronic Stable Angina<br>**Executing Institute-** Captain Srinivasa Murthy Research Institute for Ayurveda and Siddha Drug Development, Chennai | **Objectives:** Preclinical drug development of selected Ayurvedic formulations focusing on standardization, quality assurance and preclinical safety studies.<br><br>**Outcome:**<br>• Developed SoPs for 05 formulations.<br>• Standardized the product as per pharma-copeial requirement for Quality assurance.<br>• The acute and sub acute toxicity studies revealed safety of these formulations. |
| 2. | 2002-03 | **Project**<br>Toxicity and Standardisation of Drug – B for Healthy Ageing<br>**Executing Institute-** Captain Srinivasa Murthy Research Institute for Ayurveda and Siddha Drug Development, Chennai | |
| 3. | 2002-03 | **Project**<br>Toxicity and Standardisation of Drug –C for Menopausal Syndrome<br>**Executing Institute-** Captain Srinivasa Murthy Research Institute for Ayurveda and Siddha Drug Development, Chennai | |
| 4. | 2002-03 | **Project**<br>Toxicity and Standardisation of Drug – D for Urolithiasis<br>**Executing Institute-** Captain Srinivasa Murthy Research Institute for Ayurveda and Siddha Drug Development, Chennai | |
| 5. | 2002-03 | **Project**<br>Toxicity and Standardisation of Drug–E for Diabetes Mellitus<br>**Executing Institute-** Captain Srinivasa Murthy Research Institute for Ayurveda and Siddha Drug Development, Chennai | |

# CHAPTER 4

## RESEARCH ACTIVITIES IN NEW DRUG DEVELOPMENT

In order to disseminate the knowledge of newly developed drugs the councils is working towards commercialization of these drugs.

### List of Technologies Transferred to Industry by CCRAS

| Sl. No. | Product Name | Process |
|---|---|---|
| 1. | AYUSH-64 | A process for the preparation of a therapeutically active anti-malarial preparation. |
| 2. | AYUSH Ghutti | Aherbo-mineral formulation for cough and cold |
| 3. | AYUSH-SS granules | A process for preparation of an Ayurvedic herbal compound preparation for post-natal care ( to enhance the quality and quantity of breast milk in mother having deficient lactation) |
| 4. | AYUSH AG Tablet | A process for preparation of an Ayurvedic herbal compound preparation of AYUSH AG Tablet *(Shatamuli Mandura*) for Ante natal care |
| 5. | AYUSH PK Avleha | A process for preparation of an Ayurvedic herbal compound preparation of AYUSH *Panchkola Avleha* for post-natal care (to enhance the process of recovery after delivery and other complications of puerperal period) |
| 6. | AYUSH PG Tablet | A process for preparation of an Ayurvedic herbal compound preparation of AYUSH PG Tablet for Ante natal care |
| 7. | AYUSH B R Leham | A process for preparation of an Ayurvedic herbal compound preparation AYUSH *Bala Rakshak Leham* for pediatric care |
| 8. | AYUSH KVM Syrup | A preparation for the treatment of running and stuffy nose, productive or non-productive cough with or without fever and to a process for the preparation thereof for Paediatric care |
| 9. | AYUSH 82 | An Anti-Diabetic Ayurvedic Formulation |
| 10. | AYUSH SG | An Anti-Rheumatoid Arthritis preparation |
| 11. | BAL RASAYAN | A preparation for general resistance in children |

## 4.1. DRUG DEVELOPMENT STUDIES IN PROGRESS

CCRAS has undertaken the development of the various coded formulations for different disease conditions

**Dengue:** ***Study 1:*** CCRAS is working on a collaborative research work for the assessment of safety and efficacy of a coded drug AYUSH PJ-7 for the management of Dengue.

***Study 2:*** CCRAS is evaluating the study of *Carica papaya* Leaf, *Adhatoda vasaca* Leaf and *Tinospora cordifolia* stem in Experimental Thrombocytopenia as model of Dengue.

**Analgesic & Anti-iflammatory:** CCRAS is working on the effect of coded formulation SG1, SG2, SG3, SG4 and SG5 for its analgesic and anti-inflammatory activity.

**Chronic Kidney Diseases:** CCRAS is working on a collaborative research work for the assessment of safety and efficacy study of coded formulation AYUSH-K1.

**Reproductive toxicity:** CCRAS is working on a collaborative research work for the assessment of safety and reproductive toxicity study of coded formulation AYUSH-GG and AYUSH-AG tablets.

**Hepatoprotective activity:** CCRAS is working on a collaborative research work for the assessment of hepatoprotective efficacy and underlying molecular mechanism of Ayurvedic Coded formulations, AYUSH-PKT & AYUSH-GMH in experimental animal models of alcoholic and non-alcoholic fatty liver diseases.

**Diabetes:** CCRAS is working on *in-vitro* anti-diabetic activity of AYUSH-82 using proteomic and metabolomic tools and also working on in-vivo antidiabetic activity of coded drugs AYUSH-82.

**Gastro Intestinal Illness:** CCRAS is working on the efficacy of AYUSH V-24 (a coded Ayurvedic formulation) in the management of selected Gastro Intestinal Illness in ruminants.

**Safety of herbo-metallic preparations:** CCRAS is working on the safety toxicity studies of Ayurvedic herbo mineral/metallic Formulations.

## 4.2. ONGOING INTRA MURAL PHARMACOLOGY RESEARCH PROJECTS

i. Evaluation of *Kanchanar Guggulu* against chemically induced hypothyroidism in rats.

ii. Pharmacological Evaluation of *Kutajghan Vati* in Experimentally-induced Colitis in Rats.

iii. Evaluation of *Vatari guggulu* for anti-arthritic activity through different arthritis models in experimental animals.

iv. Evaluation of Anti-arthritic activity of *Trayodashang guggulu* in Complete Freund's Adjuvant induced Arthritic Rat Model.

v. Evaluation of *in-vitro* safety study of some Ayurvedic drugs/plant extracts with special reference to Genotoxicity.

vi. Effect of *Haritkadi yoga* on middle cerebral artery occlusion ischemic-reperfusion injury and its safety in rats.

vii. Anti-Atherosclerotic Activity of *Hridayarnava Rasa* against Atherosclerosis In Animal Model

*viii.* *In-vivo* and *in-vitro* nephroprotective evaluation of AYUSH K1 extract.

viii. Effect of *Gokshuradi Guggulu* on Experimental Diabetic Nephropathy: In vitro and In vivo Studies.

ix. Biochemical and Pharmacological Evaluation of *Kaishore Guggulu* in Experimental Models of Gout (*Vatarakta*).

*x.* *In-vitro* evaluation of anti-diabetic activity of AYUSH-82 using proteomic and metabolomic tools.

*xi.* *In-vivo* antidiabetic activity of AYUSH-82.

xii. Development of medicinal plant garden for veterinary Ayurveda research at GADVASU, Ludhiana, Punjab.

xiii. Clinical evaluation of efficacy of Ayurvedic formulation for augmentation of Milk production in ruminants.

xiv. Clinical evaluation of efficacy of AYUSH V-24 (a coded Ayurvedic formulation) in the management of selected Gastro Intestinal Illness in ruminants.

xv. Study of *Carica papaya* Leaf, *Adhatoda vasaca* Leaf and *Tinospora cordifolia* Stem in Experimental Thrombocytopenia as model of Dengue.

xvi. Development of Coded drug AYUSH M-3 for the management of Migraine patients

xvii. Anti-inflammatory activity of *Shunthi Guggulu.*

xviii. Analgesic activity of SG 1, SG 2, SG 3, SG 4 & SG 5.

xix. Preclinical safety/toxicity and hepatoprotective activity of Ayush Phalatrikadi Kwath.

xx. Evaluation of Gomutra Haritki in fatty liver degeneration.

xxi. Safety toxicity studies of Ayurvedic Herbo mineral/metallic Formulations at GLP-complied Laboratories.

xxii. Exploration of Medhya properties of Yastimadhu, Gudhuchi and Vacha in animal model of neurotoxicity.

xxiii. Exploration of safety and reproduction toxicity profile of Ayush-GG Tablets.

xxiv. Exploration of safety and reproduction toxicity profile of Ayush-AG Tablets.

xxv. Evaluation of acute toxicity of some Ayurvedic drugs/plants extracts in-vivo model of Zebrafish.

xxvi. Evaluation of hepatoprotective efficacy and underlying molecular mechanism of Ayurvedic formulations, AYUSH-PKT & AYUSH-GMH in experimental animal models of alcoholic and non-alcoholic fatty liver diseases: A pre-clinical study.

# CHAPTER 5

## BOOKS AND MONOGRAPHS

**Background:** The council is dedicated in dissemination of its research finding through monographs and book publications. Since inception the council has published more than 266 books and monographs. Among them 9 books were published based on Pharmacology Research and Safety Studies. Council has published 5089 research publications. Among them 274 research work are done in pharmacology research.

### LIST OF PUBLISHED BOOKS

| S. No. | Book title | Year of publication |
|---|---|---|
| 1. | Quality and Safety of Select Rasakalpa (Metal & Mineral Based Ayurvedic Formulations): Hridayarnava Rasa | 2019 |
| 2. | Quality and Safety of Select Rasakalpa (Metal & Mineral Based Ayurvedic Formulations): Naga Bhsama | 2019 |
| 3. | Quality and Safety of Select Rasakalpa (Metal & Mineral Based Ayurvedic Formulations): Tamra Bhasma | 2019 |
| 4. | Evidence Based Safety of Ayurvedic Medicines | 2016 |
| 5. | Report of Pharmacological Profile and Safety/Toxicity of *Yograj Guggulu* and *Mahanarayana Taila* (Classical Formulations) | 2014 |
| 6. | Safety/Toxicity Study Report of Some Ayurvedic Drugs | 2009 |
| 7. | Report on Screening of Single Herbal Drug Extracts for Potential Anti-cancer Activity | 2009 |
| 8. | Research Study Profile of Rasamanikya (Classical Preparation, Physico-Chemical Characterisation, Quality Control and Safety/Toxicity Studies of Rasamanikya) | 2009 |
| 9. | Pharmacological Investigations of Certain Medicinal Plants and Compound Formulations used in Ayurveda and Siddha | 1996 |

## CCRAS VISION DOCUMENT 2030

Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India, has formulated and projected "CCRAS Vision Document 2030" with a strategy of research and development for research outcomes in next 15 years considering the strength of Ayurveda and current unmet medical needs. The core components of the document comprise sustainable development goals (SDGs) of CCRAS for vision 2030 for 15 years, 7 years strategy (long-term vision), and 3 years action document, fundamentally harmonized with the goals and recommendations of major national and international health policy documents.

CCRAS has laid its vision document 2030 To develop scientific evidence in Ayurvedic Principles, drug therapies by way of integrating ancient wisdom with modem technology and to bring Ayurveda to the people through innovations related diagnostics, preventive, promotive as well as treatment methods and also introduce scientific research for sustained availability of quality natural resources, to translate them into products and processes and in synergy with concerned organizations to introduce these innovations into public health systems.

The National Population Policy 2000, 6 National Health Policy 2002, and the National Commission on Macroeconomic and Health—2005 of the Ministry of Health and Family Welfare, Government of India, emphasized on reorientation and prioritization of research in Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) and to validate therapy and drugs in chronic and lifestyle-related diseases, mainstreaming from Indian systems of medicine and homoeopathy (ISM&H). Further, the recent three major documents related to health policy, viz., National Health Policy (NHP) 2017; Situation Analyses—Backdrop to NHP 2017, Ministry of Health and Family Welfare, Government of India; and Three-Year Action Agenda 2017-2020 (draft), 10 NITI Aayog, Government of India, highlighted on prevention through lifestyle advocacy, health care delivery through integration, co-location, and medical pluralism.

In the same way, considering the strength of Ayurveda in current unmet medical needs, the council has proposed a strategy of research and development with focused research outcomes in next 15 years emphasizing on development of new drugs based on leads from classical Ayurveda texts for diseases of national importance and systematic validation of classical formulations and therapies with a vision statement "To develop scientific evidence in Ayurvedic Principles, drugs, therapies by way of integrating ancient wisdom with modem technology and to bring Ayurveda to the people through innovations related to diagnostics, preventive, promotive as well as treatment methods and also introduce scientific research for sustained availability of quality natural resources, to translate them into products and processes and in synergy with concerned organizations to introduce these innovations into public health systems." Principally analogous with the larger goals and strategies of important health-related policies, core

strength of Ayurveda, and current health needs, the document is framed with core components, viz., SDGs of CCRAS for vision 2030 (15 years), 7 years strategy (long-term vision), and 3 years action document.

*15 years strategy:* SDGs of CCRAS for vision 2030 (15 years) emphasizes on broader goals set for 15 years, such as translation of research outcomes into practice and making them accessible to health care providers and public, mainstreaming of Ayurveda therapies through integration, generation of evidence on safety and efficacy of classical Ayurveda approaches, dissemination of research outcomes, and infrastructure development for research and development.

*7-years strategy:* From 2017-2018 to 2023-2024 (long-term vision) to convert the long-term vision into implementable policy and action as a part of the National Development Agenda with a mid-term review after 3 years, i.e., the year ending March 2020, focuses on development and validation of Ayurvedic drugs and regimen for inclusion in the important national programs, such as add-on and adjunct therapies for multidrug-resistant tuberculosis; generation of evidence for prevention and management of disorders of vision, reproductive and child health, human immunodeficiency virus/acquired immunodeficiency syndrome, cancer; important communicable diseases, viz., malaria, dengue, filariasis, and noncommunicable diseases like diabetes, osteoarthritis, anemia; improvement of memory and cognitive function other psychiatric diseases, such as anxiety neurosis, dementia, etc.; scientific evidence on safety of selected Ayurveda herbo-mineral drugs, etc.

*3-years strategy:* A 3-years action document from 2017-2018 to 2019-2020 aligned to the predictability of financial resources during the 14th Finance Commission Award period. This is also to help translate into action the goals of the government to be achieved by 2019 highlights upon development of the directives addressing different research needs; validation of fundamental principles of Ayurveda including Ayurveda biology; development of standard Ayurvedic terminologies, modules on behavioral change communication focusing on Ayurveda-based lifestyle interventions for prevention, health promotion, formats for clinical diagnosis, and clinical examination based on Ayurveda principles: Clinical decision support systems and hospital information management system (HIMS); projects on occupational health; drug development and commercialization of research products for cancer, wound healing, dengue, diabetes; dosage forms of hepato-protective agents; validation of classical Ayurveda formulations or classical Ayurveda drugs for chronic and refractory diseases, rheumatoid arthritis, osteoarthritis, hypertension, gout, urolithiasis, polycystic ovary syndrome, bronchial asthma, and chronic bronchitis; and dissemination of research outcomes.