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ACUTE AND LONGTERM TOXICITY STUDY OF A SIDDHA POLYHERBAL DURG ADATHODAI CHOORANAM

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ABSTRACT

BACKGROUND:Adathodai Chooranam is a Siddha Poly herbal formulation which is specially indicated for Bronchial Asthma in Siddha Sastric text. The toxicological profile of the drug was not reported. OBJECTIVE: The present study was undertaken as per WHO Guidelines to establish the toxicity profile of Adathodai Chooranam in an experimental animals which will render strong evidence for its safety in clinical use of Bronchial Asthma. METHOD:In acute toxicity study a single oral dose (10× Therapeutic dose) of Adathodai chooranam 2.6 gm/kg body weight was administered in Swiss albino mice and the animals were observed for 14 days. Gross pathology was performed at the end of the study. In long term toxicity study Adathodai Chooranam was administered in wistar albino rats at 270 mg/Kg body weight, 1, 350 mg/kg body weight, 2,700 mg/kg body weight once daily for 28 days. RESULT: In acute toxicity study no treatment related death or toxic signs were observed. The Long term toxicity study did not show any major toxic signs in the therapeutic dose level, when compared with the control group. CONCLUSION: The trial drug Adathodai Chooranam is safe in the dose of 1.5 g for human adult mentioned in the siddha literature.

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INTRODUCTION

Among all the system of medicine, practiced all over the world the siddha system is undoubtedly, the ancient and transcending system in many centuries. Siddha system of medicine is a gift of mankind by the Siddhars who were the greatest scientists in ancient times. In this system of Medicine the drug sources are mainly obtained from plants, animal products, minerals and metals. Herbal medicines have attained greater importance as an alternative to conventional therapy. To optimize the safe in use of a plant based medicine, one should take into account their historical applications on humans and animals as well as toxicity evaluation of the medicinal herbs and their active components. The drug Aadhathodai chooranam consists of 17 ingredients such as *Alpinia galangal wild* (Rhizome), *Alpinia officinarum Hance* (Rhizome), *Justicia adhatoda Linn* (Root bark and inflorescense), *Tragia involucrata Linn (Root*), *piperlongum Linn (fruit*), *styrax benzoin dryand* (Resin) *Curcuma longa Linn* (Finger Rhizome), *Costus Speciosus* (koen.) Sm (Root), *Embelia ribes Burm.f,* (fruit), *clerodendrum serratum (Linn) moon* (Root), *cyperus rotundus Linn* (Rhizome), *Ficus tsiela Roxb* (stem bark), *woodfordia fruticosa kurz* (flowers), *solanum trilobatum Linn*(Leaf), *Solanum Surattence Burm.f* (whole plant), *piper nigrum Linn* (Fruit). The aim of this study was to evaluate the safety profile of the poly herbal antiasthmatic siddha preparation Aadhathodai chooranam.

A survey by the National Asthma Campaign found that 60% of people with moderate Asthma and 70% with severe Asthma have used complementary and alternative medicine to treat their condition. Herbal Medicine is the third most popular choice of both adults (11%) and Children (6%) suffering from Asthma. [1] Most people think of herbs as being natural and therefore safe to take for Asthma. Although Allopathic Medicines provide immediate relief to most patients, effective low risk, non-drug strategies would provide a valuable adjunct or alternative treatment in asthma Management. Even though efforts are being persistently made for a safe and effective treatment for Asthma. In modern system of medicine, there is no definite drug without adverse reaction could not be established [2]. But Siddha System of Medicines are meant for the preventive and long term therapy with minimal side effects. However, because there have been limited research Studies on Siddha treatment for Asthma, the effectiveness and safety of many are unknown. The general public, patients and consumers are primarily interested in fact access to safe and efficient drugs, as well as in animal welfare. The aim of this study was to evaluate the safety profile of the poly herbal antiasthmatic siddha preparation Aadhathodai chooranam for further clinical evaluation .

MATERIALS AND METHODS

Procurement and Authentication of Raw Drugs:

The raw drugs were purchased from the Indian Medical Practitioners' Co-operative pharmacy and Stores Ltd., X-185, Thiruvanmiyur, Chennai. The solanum trilobatum leaf and Justicia adhathoda inflorescense and stem bark were collected from Herbal garden, National Institute of Siddha, Chennai. All the herbs were authenticated by Assistant Professor of Medicinal Botany, National Institute of Siddha, Chennai. Voucher Specimen (NIS/MB/37/2012) was deposited in the Medicinal Botany Laboratory of National Institute of Siddha, Chennai.

Preparation of Aadhathodai Chooranam:

The raw drugs were purified as per the standard operating procedure mentioned in Siddha Literature.[3,4] The Aadhathodai Chooranam was prepared as per the method of preparation described in Siddha Classical Text. The test drug dose is 1.5 gram and administered orally, twice daily with Cow's Milk for 30 days.

Animal Care and husbandry:

The acute and long term toxicity study protocol was approved by Institutional Animal Ethical Committee, National Institute of Siddha, Chennai with the IAEC Certificate Number NIS/IAEC/04/2011/11 dated on June 14, 2011. Healthy Swiss Albino Mice of either Sex with an average body weight of 20-30 g and wistar albino rats of either sex with an average body weight of 130-180 gram were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai for acute and long term toxicity studies respectively and were housed in the Animal house of National Institute of Siddha, Chennai. Each group of animals were separately housed in polypropylene cages in a ventilated room (air cycles 15/Min : 70:30 exchange ratio) under an ambient temperature of 22 $\pm$2°C and 40-65% relative humidity with 12 hours dark / 12 hours light photoperiod. They were provided with food (Nutri Lab Rodent Feed - Provimi Animal Nutrition India Pvt. Ltd., Bangalore) and purified water ad libitum. All the animals were acclimatized to the laboratory conditions for 7 days prior to study. The experiments were carried out as per the guidelines prescribed by the Committee for the purpose of conduct and supervisions of experiments on animals (CPCSEA) Ministry of Environment and Forest, Government of India and Institutional Animal Ethical Committee after obtaining its permission.

Acute oral Toxicity Study:

The acute oral toxicity study was performed as per WHO Research Guidelines for evaluating the Safety and Efficacy of herbal Medicines (1993:94 Pages) . [5]

Dose calculation:

The recommended human dose of Adathodai chooranam is 3gram/day was converted to the dose for mice using conversion table. This dose was found to be 0.26 gram/kg for mice. This dose group was designated as therapeutic dose group. 10 times the therapeutic dose was 2.6 gram /kg.

Group of animals: Table no 1:

S. No	Group	No of rats
1	Vehicle control (1ml Milk/100gbw)	10 (5 male, 5 female)
2	Toxic dose (10X therapeutic dose) (Single dose) 2.6gm/kg bw	10 (5 male, 5 female)

Overnight fasted 10 male and 10 female mice were used for the test. Each group was constituted with 5 animals per sex.A vehicle control group and experimental group were maintained. Vehicle control group received 1ml/100 gram body weight of cow's milk. The experimental group received single oral dose (10x Therapeutic Dose) of Aadhathodai Chooranam 2.6 gm/Kg body weight with cow's milk as a vehicle.

Study details:

For oral administration of test drug in mice, Aadhathodai Chooranam was mixed with Cow's milk with the help of mortar and pestle to get suspension. After the test drug administration, food was stopped for 3 hours in mice. The animals were monitored for morbidity and mortality for first 30 minutes and one hour once on the day of dosing and once a day. Daily observation includes changes in skin color (balancing, cyanosis, vasodilatation) fur, mucous membrane, abdominal distention, condition of teeth, salivation, respiration(depression, stimulation ,failure), motor activity-increased(tremors , chronic convulsions, tonic extension, piloerection, muscle spasm, spasticity, opisthotonos, hyperesthesia, loss of rigidity, reflex), motor activity decreased (ataxia, sedation, muscle relaxation, hypnosis, analgesia, anesthesia, arching & rolling, ptosis, lacrimation, exopthalmos, diarrhea writhing, moribund status/death),feed and water intake, signs of toxicity and mortality upto 14 days. The body weight was recorded individually on 0^{th}, 7^{th} and 14^{th} days. All animals were weighed and sacrificed on the 15^{th} day, after drug administration and then the vital organs including heart, lungs, livers, kidneys, sex organs and brain were grossly examined.

Long Term Toxicity Study:

The 28 days long term toxicity study was performed according to the WHO Guideline (1993; 94 pages).

Dose calculation:

To extrapolate the human toxicity profile in rats, three doses of Aadhathodai Chooranam were chosen for this animal toxicity study from clinically used human dose (3g/day in human) by using body surface area dosing table. They were; Therapeutic dose (270 mg/kg body weight), 5 times the therapeutic dose (1, 350mg/ Kg body weight) and 10 times the therapeutic dose (2,700 mg/kg body weight).

Groups of animals: Table no:2:

S.No	Group	No of Rats
1.	Vehicle control (2ml Milk/kgbw)	10 (5 male, 5 female)
2.	1XTherapeutic dose (270mg/kg bw)	10 (5 male, 5 female)
3.	5XTherapeutic dose (1350mg/kgbw)	10 (5 male, 5 female)
4.	10XTherapeuticdose(2700mg/kgbw)	10 (5 male, 5 female)

Animals were divided into four groups consisting of 10 animals in each group (5 male and 5 female). First group was kept as vehicle control whereas the second to fourth group were administered test drug Aadhathodai Chooranam at the dose of (270 mg/kg bw, 1,350 mg/Kg bw, 2,700 mg/Kg body weight) respectively. The control group received cow's milk (2 ml/kg body weight). The test drug administered orally by respective treatment daily for 28 days in the 2^{nd}, 3^{rd}, 4^{th} groups.

Observation:

Body weight, food intake and water intake were recorded before starting, drug administrative and at weekly intervals with simultaneous observation for toxic manifestation or behavioral alterations and mortality.

Collection and analyses of blood:

At the end of the study period, the overnight fasted animals were anaesthetized and blood samples were collected through retro orbital puncture into tubes with and without EDTA for hematological and biochemical analysis respectively. Hematological analysis was done by Mindraj, BC-2800 Vet auto hematology analyzer and the biochemical analysis was done by Bayer, RA-50 chemistry analyzer and Serum electrolytes was determined by Easylyte plus Na/K/Cl analyzer. The blood without the anticoagulant was allowed to clot before centrifugation (1610g at 4℃ for 10 min) to obtain serum. The serum was separated and the levels of glucose, cholesterol, triglycerides, urea, creatinine, SGOT, SGPT, total bilirubin, total protein, Albumin, Globulin, Sodium (Na+), Pottassium (K+) and chloride (Cl-) were estimated. Blood samples collected in heparinised tubes were used for full blood Count, which included TLC (Total leucocyte count),TEC(Total erythrocyte count), Platelet count, PCV, MCV, MCH, Hgb, MCHC.

Histopathology:

After collecting the blood, the rats were dissected and organs like Heart, Lungs, Liver, Stomach, Kidney, Sex organs and Brain were removed and kept in normal Saline (0.09%) carefully and then weighed on a monoplane balance. All organs were examined and then fixed in 10% buffered formaldehyde solution for histopathological observations.

Statistical Analysis:

The mean changes in body weight, daily food and water intake, organ weight relative to body weight and biochemical and hematological parameters were statistically analyzed and significant differences within groups were calculated using the paired t test to compare mean differences of control and test drug treated groups. $p \leq 0.05$ was considered statistically significant The results were expressed as the mean $\pm SD$.

RESULTS:

ACUTE TOXICITY STUDY RESULTS

In acute toxicity study the test drug Adhathodai Chooranam upto single oral dose (10x therapeutic dose) of 2.6 gm / Kg body weight did not reveal any abnormal clinical signs in all the animals. All animals survived and no treatment related morbidity occurred during the period of 14 days. Gross necroscopy did not reveal any abnormal pathology in all animals. Weight loss was not observed in control and test group animals.

Table 4.11: Cage side observation for the effect of Adathodai Chooranam at 2.6 mg / kg.b.wt.

Observation	AC M1	AC M2	AC M3	AC M4	AC M5	AC F1	AC F2	AC F3	AC F4	AC F5
General behaviour	+	+	+	+	+	+	+	+	+	+
Respiration	+	+	+	+	+	+	+	+	+	+
Cardio vascular signs	+	+	+	+	+	+	+	+	+	+
Motor activities	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+
Fur	+	+	+	+	+	+	+	+	+	+
Eyes	+	+	+	+	+	+	+	+	+	+
Mucous membrane	+	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+	+
Lethargy	-	-	-	-	-	-	-	-	-	-
Sleep	+	+	+	+	+	+	+	+	+	+
Coma	-	-	-	-	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-
Morbidity	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-

\+ Normal - Nil

LONG TERM TOXICITY STUDY RESULTS

In long term toxicity study all the animals were well oriented and active during the trial period and survived until 28 days treatment period. No signs of clinical toxicity attributable to Adhathodai Chooranam were observed throughout the study.

Table 1. There is moderate significant difference among all groups of animals on water intake . But there is no difference between therapeutic dose and control group.

Table 1: Effects of different dose level of Adathodai Chooranam on water consumption in Albino rats during 28 days treatment.

Parameter	Control	1x Therapeutic dose	5x Therapeutic dose	10x Therapeutic dose
Water in Take(ml)	133.2 ± 12.1	126.3 ± 14.5	131.4 ± 11.9	137.0 ± 16.3

Values are expressed as mean±SD from0 animals in each group.*p<0.05;**p<0.01.

Table 2. In food intake, there is significant difference among all groups of animals and moderate significant differences between therapeutic dose and control group.

Table 2 : Effects of different dose level of Adathodai Chooranam on food consumption in Albino rats during 28 days treatment.

Parameter	Control	1x Therapeutic dose	5x Therapeutic dose	10x Therapeutic dose
Food in Take(g)	61.3 ± 1.5	67.4 ± 1.3	67.8 ± 8.6	71.3 ± 5.8

Values are expressed as mean±SD from 10 animals in each group.*p<0.05;**p<0.01.

Table 3. No difference in body weight was observed on 7th day, 14th day, 21st day, 28th day between control and drug treated groups.

Table 3: Effects of different dose level of Adathodai Chooranam on Body Weight in Albino rats during 28 days treatment.

Days	Control	1x Therapeutic dose	5x Therapeutic dose	10x Therapeutic dose
0 Day	137.8 ± 21.1	163.6 ± 16.8	150.2 ± 17.9	146.3 ± 11.3
7th Day	145.7 ± 22.3	168.9 ± 16.7	152.9 ± 17.6	154.9 ± 14.4
14th Day	153.6 ± 24.9	175.8 ± 19.4	159.8 ± 21.4	159.4 ± 16.6
21st Day	160.8 ± 28.9	182 ± 22.2	165.9 ± 25.1	162.9 ± 18.8
28th Day	169.8 ± 31.8	190.3 ± 25.8	173.2 ± 30.6	169.6 ± 22.3

Values are expressed as mean±SD from 10 animals in each roup.*p<0.05;**p<0.01.

Table 4. There is moderate significant differences, among all groups of animals on RBC Count.However there is no difference between therapeutic dose and control group. No difference was observed in other hematological parameters between control and drug treated groups.

Table 4 : Effects of different dose level of Adathodai Chooranam on Hematological parameters in Albino rats after 28 days treatment.

Days	Control	1x Therapeutic dose	5x Therapeutic dose	10x Therapeutic dose
Total WBC 10^9/L)	4.07 ± 1.8	5.7 ± 3.3	4.3 ± 3.4	4.6 ± 4.2
Lymphocytes (%)	64.6 ± 11.6	58.1 ± 12.1	65.7 ± 9.9	68.0 ± 12.5
Monocytes (%)	3.0 ± 1.1	6.6 ± 7.7	3.3 ± 0.9	2.7 ± 0.8
Granulocytes (%)	32.2 ± 10.7	37.9 ± 11.3	30.9 ± 9.0	29.3 ± 12.2
Total RBC (10^{12}/L)	6.6 ± 3.3	3.9 ± 2.1	5.2 ± 1.2	4.3 ± 1.9
Hb (gm/dl)	11.0 ± 5.2	6.7 ± 3.5	8.5 ± 2.2	7.4 ± 3.2
HCT (%)	32.9 ± 16.7	18.9 ± 11.0	25.7 ± 5.9	21.3 ± 9.7
MCV (Fl)	49.7± 1.9	47.4 ± 3.7	49.7 ± 2.0	49.8 ± 2.1
MCH (Pg)	16.8 ± 0.6	17.5 ± 2.9	16.4 ± 2.1	17.4 ± 0.8
MCHC (g/dl)	34.1 ± 1.6	37.5 ± 9.3	33.1 ± 3.9	35.3 ±1.8
Platelets (109/L)	77.6 ± 37.4	120.5 ± 121.6	101 ± 81.3	60.6 ± 66.7

Values are expressed as mean±SD from 10 animals in each group.*p<0.05;**p<0.01.

Table 5. There is significant difference among all group of experimental animals on Blood Sugar level, total Cholesterol and triglycerides at the end of treatment . But there is no difference between therapeutic dose and control groups. Moderate significant difference was observed in urea level between therapeutic dose and control groups,but the difference was within the normal expected range for the rat species used in this study. No difference in Creatinine level and Total Protein was observed among all groups. Moderate significant difference among all group of experimental animals on Albumin level. But there is no difference between low and control groups. There is difference among all groups of experimental animals on globulin and No difference between therapeutic dose and control groups. In serum bilirubin and SGOT, there is no difference in all groups of animals. There is difference among all groups of experimental animals on SGPT. But there is no difference between control and therapeutic dose groups.

Table 5: Effects of different dose level of Adathodai Chooranam on Biochemical parameters in albino rats after 28 days treatment.

Days	Control	1x Therapeutic dose	5x Therapeutic dose	10x Therapeutic dose
Sugar (mg/dl)	69.5 ± 10.0	73 ± 15.0	93.5 ± 13.4	115.2 ± 16.7
Total Cholesterol (mg/dl)	118.8 ± 36.1	132.3 ± 23.3	130.6 ± 13.0	**87.3 ± 11.8
Triglycerides (mg/dl)	145 ± 64.9	129.2 ± 34.8	160.6 ± 45.5	194.5 ± 64.1
Urea (mg/dl)	53.4 ± 9.3	*41.9 ± 7.1	**36.8 ± 2.8	**35.52 ± 10.04
Creatinine (mg/dl)	0.7 ± 0.1	0.75 ± 0.1	0.84 ± 0.18	0.7 ± 0.0
Total Protein (g/dl)	7.6 ± 0.6	7.78 ± 0.3	7.9 ± 0.1	7.91 ± 0.1
Albumin (g/dl)	4 ± 0.4	3.8 ± 0.2	3.61 ± 0.2	3.88 ± 0.1
Globulin (g/dl)	3.6 ± 0.7	3.8 ± 0.3	*4.2 ± 0.2	4.0 ± 0.2
Bilirubin (mg/dl)	0.6 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.0
AST (U/L)	126.2 ± 50.6	146.8 ± 34.5	124.8 ± 30.1	133.5 ± 22.9
ALT (U/L)	56.2 ± 13.8	47 ± 8.7	65.5 ± 8.8	61.4 ± 9.2

Values are expressed as mean±SD from 10 animals in each group.*p<0.05;**p<0.01.

Table 6. There is no difference in weight of Heart, Lungs, Kidney and Brain of all groups of animals. There is significant difference among different groups of experimental animals on weight of Liver, Spleen, Stomach and male sex organs. However there is no difference between therapeutic dose and control groups. There is difference found in female sex organ weight among different groups of experimental animals but no difference between therapeutic and control groups.

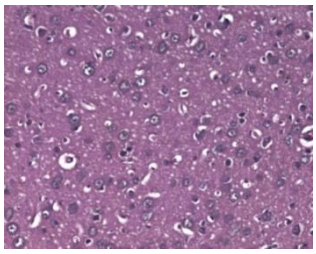
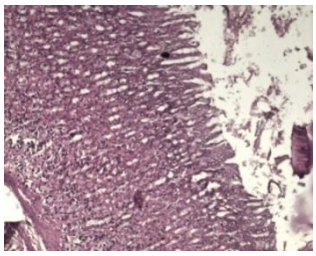
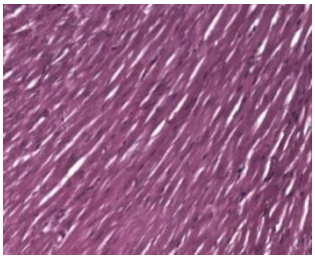
Table 6 : Effects of different dose level of Adathodai Chooranam on organs weight(g) in albino rats during 28 days treatment.

Organs	Control	1x Therapeutic dose	5x Therapeutic dose	10x Therapeutic dose
Heart	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1
Lungs	1.4 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
Liver	5.5 ± 1.4	6.6 ± 0.7	*7.3 ± 1.4	*8.1 ± 1.2
Spleen	0.5 ± 0.1	0.5 ± 0.1	*0.7 ± 0.1	0.6 ± 0.1
Stomach	1.6 ± 0.3	2.1 ± 1.4	*1.0 ± 0.5	2.5 ± 1.3
Brain	1.6 ± 0.2	1.3 ± 0.2	1.6 ± 0.4	1.4 ± 0.2
Male Sex Organ	4.5 ± 1.0	5.0 ± 0.6	*1.1 ± 0.3	*7.1 ± 1.7
Kidney	1.6 ± 0.3	1.8 ± 0.2	1.9 ± 0.3	1.9 ± 0.2
Female Sex Organ	1.41 ± 0.8	1.3 ± 0.4	*5.2 ± 1.1	2.3 ± 0.8

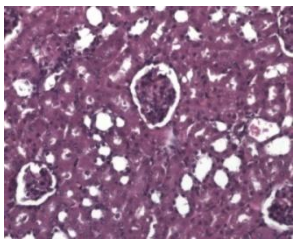
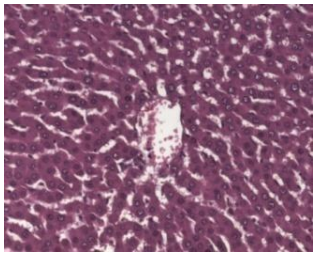
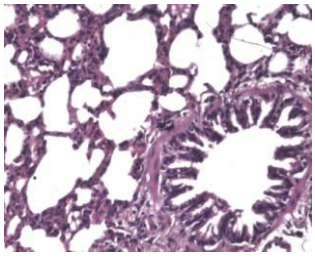
Values are expressed as mean±SD from10animals in each group.*p<0.05;**p<0.01.

Histopathological studies shown normal architecture in therapeutic dose and control groups. Liver has shown congestion and spleen has shown slightly hyperplastic.(Figure 1,2,3,4)

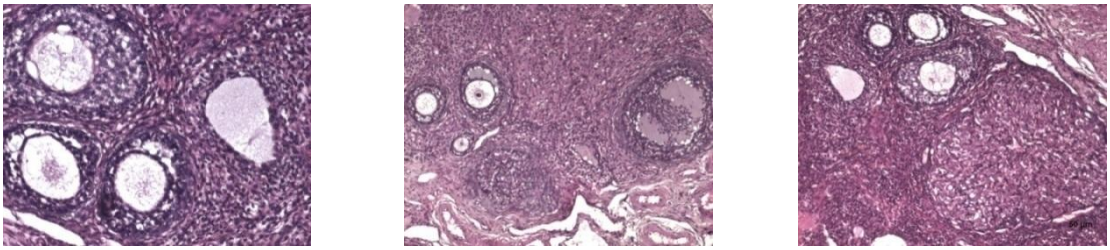
Brain **Stomach** **Heart**

Kidney **Liver** **Lung.**

Ovary Ovary Ovary



Spleen Spleen

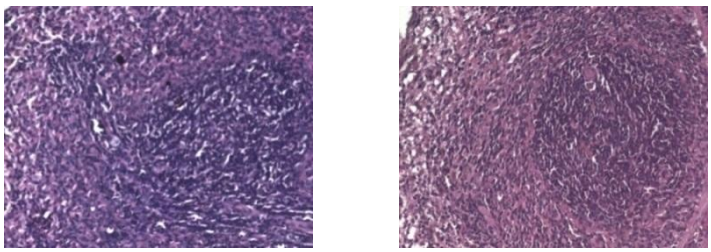
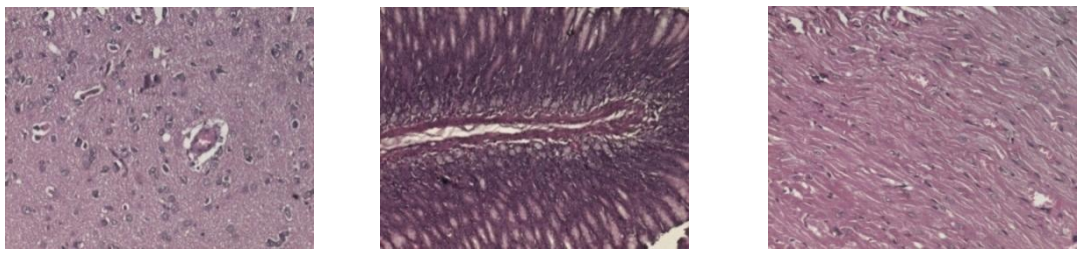
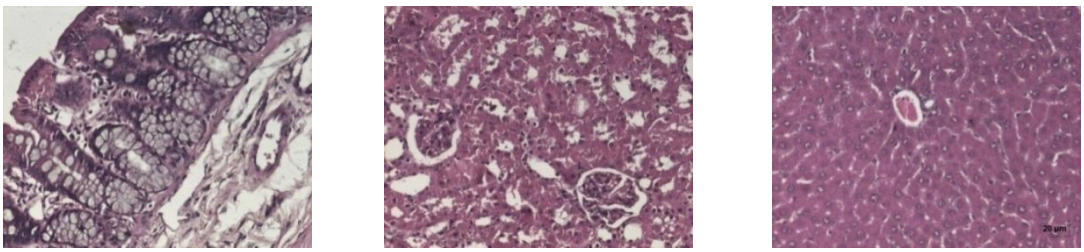


FIGURE 1: HISTOPATHOLOGY OF VEHICLE CONTROL FEMALE GROUP.

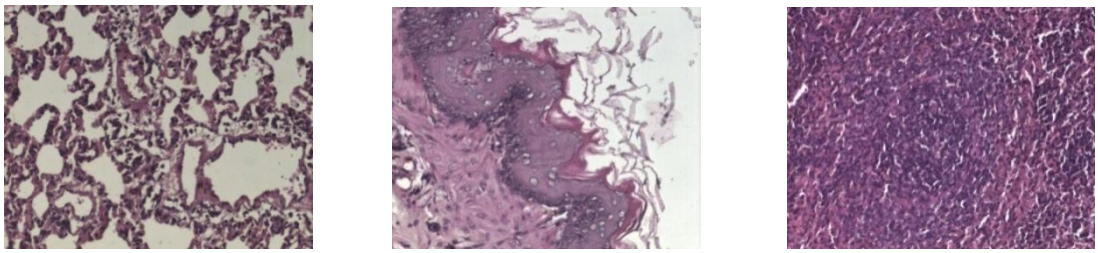
Brain Glandular Stomach Heart.



Intestine Kidney Liver



Lung Non glandular Stomach Spleen



Testicle Testicles

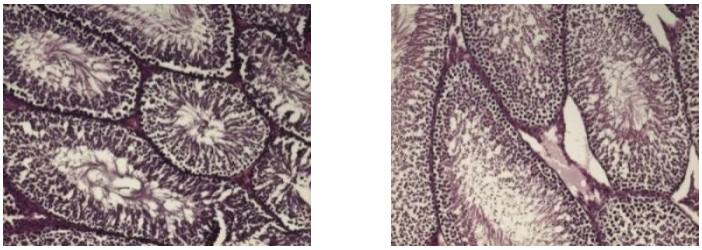
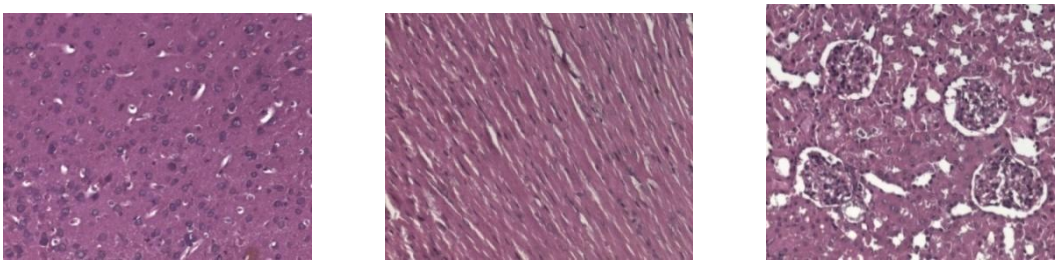
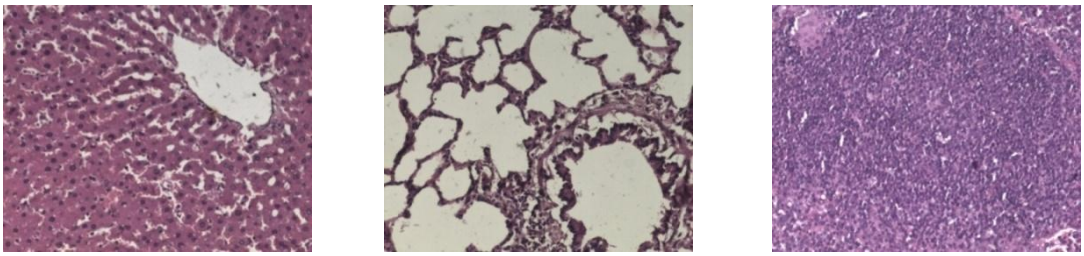


FIGURE 2: HISTOPATHOLOGY OF VEHICLE CONTROL MALE GROUP.

Brain **Heart** **Kidney**



Liver **Lung** **Spleen**



Stomach **Uterus**

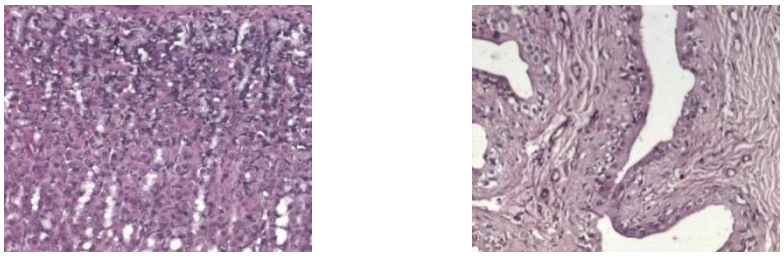
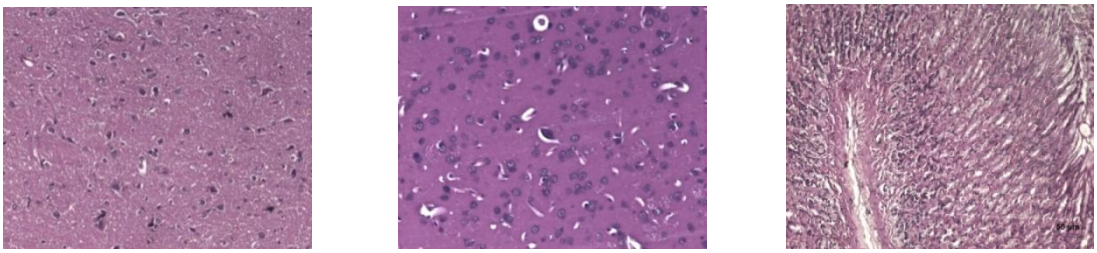


FIGURE 3: HISTOPATHOLOGY OF 1 X THERAPEUTIC DOSE FEMALE GROUP.

Brain **Brain** **Glandular Stomach**



Heart **Kidney** **Liver**

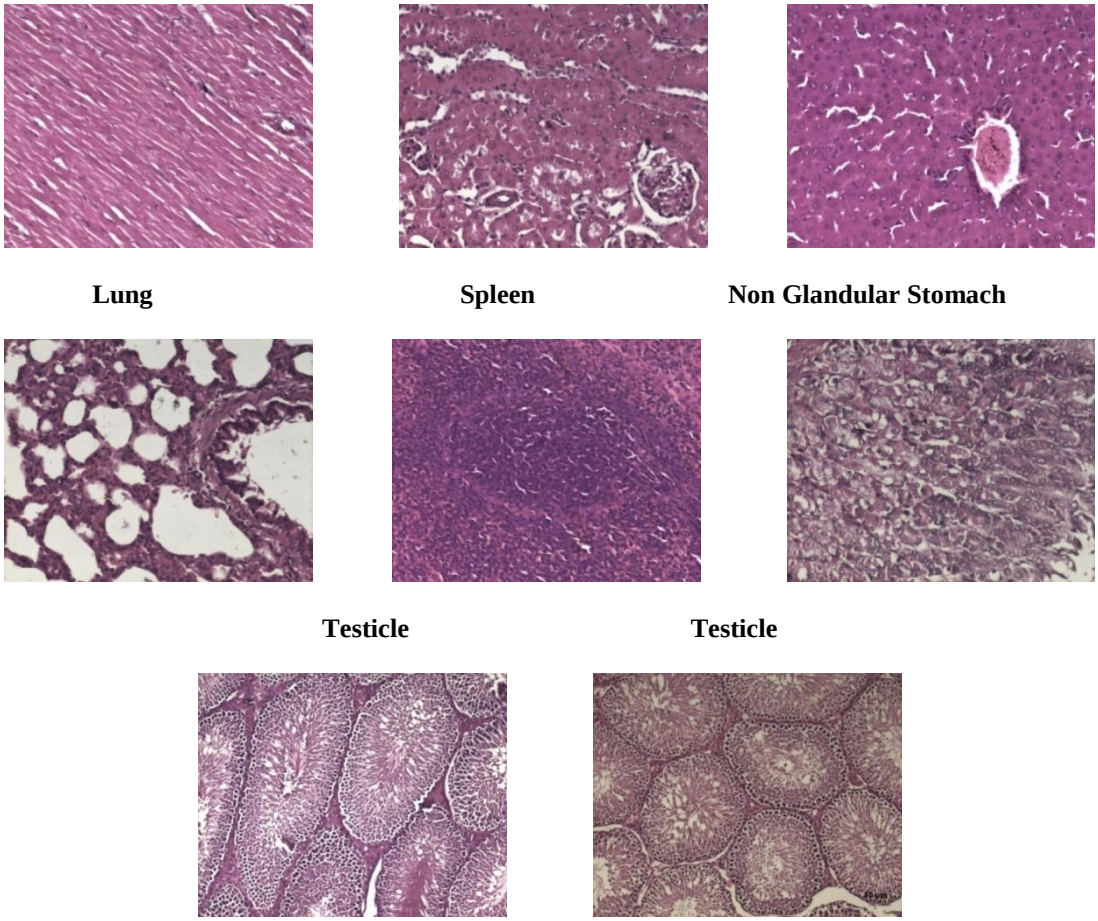
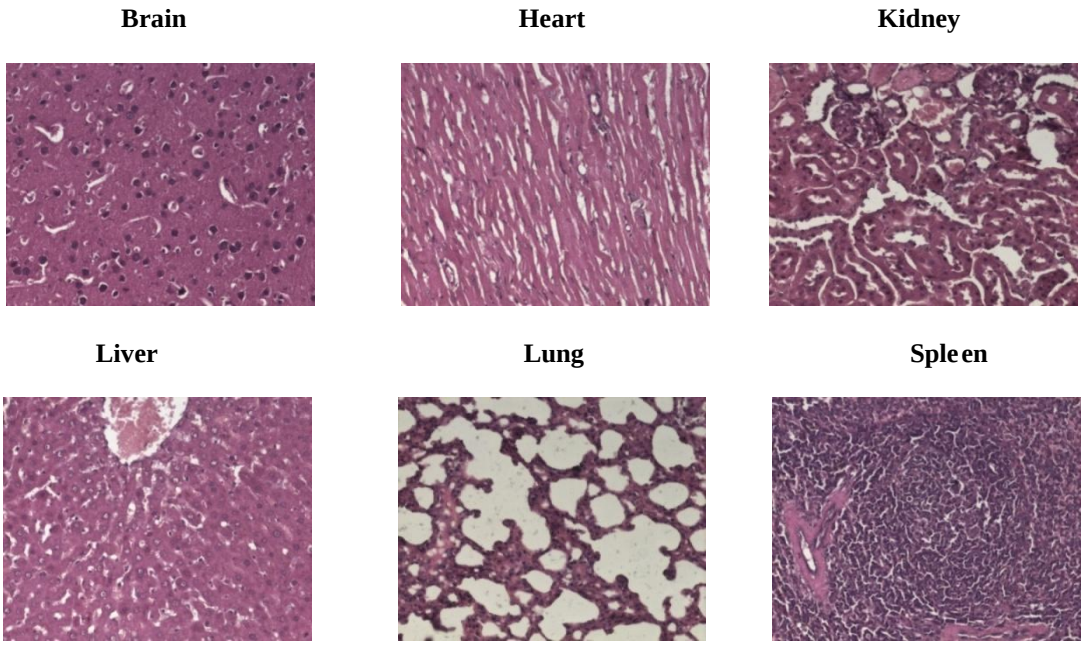


FIGURE 4: HISTOPATHOLOGY OF 1 X THERAPEUTIC DOSE MALE GROUP.

5x therapeutic dose male group exhibits focal hyperplasia in non glandular area of stomach. (Figure 5).



Stomach **Testicle**

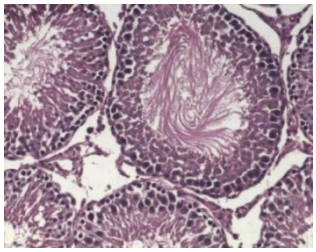
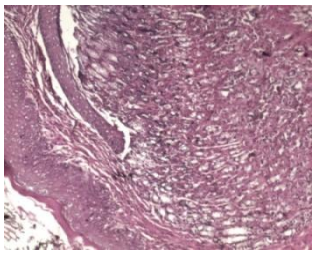
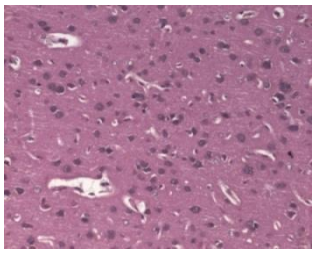
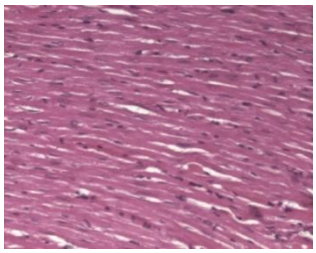
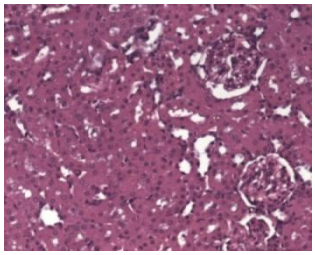



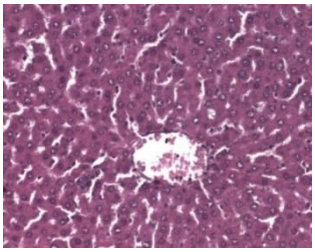
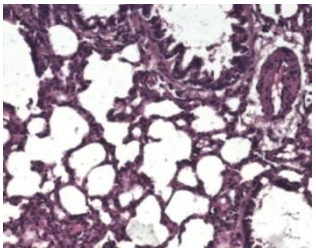
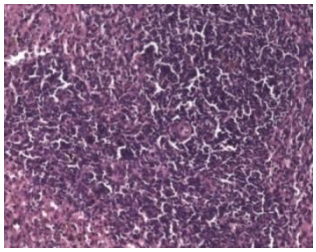
FIGURE 5: HISTOPATHOLOGY OF 5 X THERAPEUTIC DOSE MALE GROUP.

5x therapeutic dose female group exhibits squamous metaplasia in uterus.(Figure 6).

Brain **Heart** **Kidney**

Liver **Lung** **Spleen**

Uterus

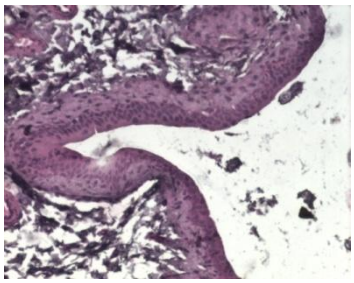


FIGURE 6: HISTOPATHOLOGY OF 5 X THERAPEUTIC DOSE FEMALE GROUP.

10x therapeutic female group exhibits hyperplasia and Squamous melaplasia in uterus. No abnormalities detected in other organs. (Figure 7).

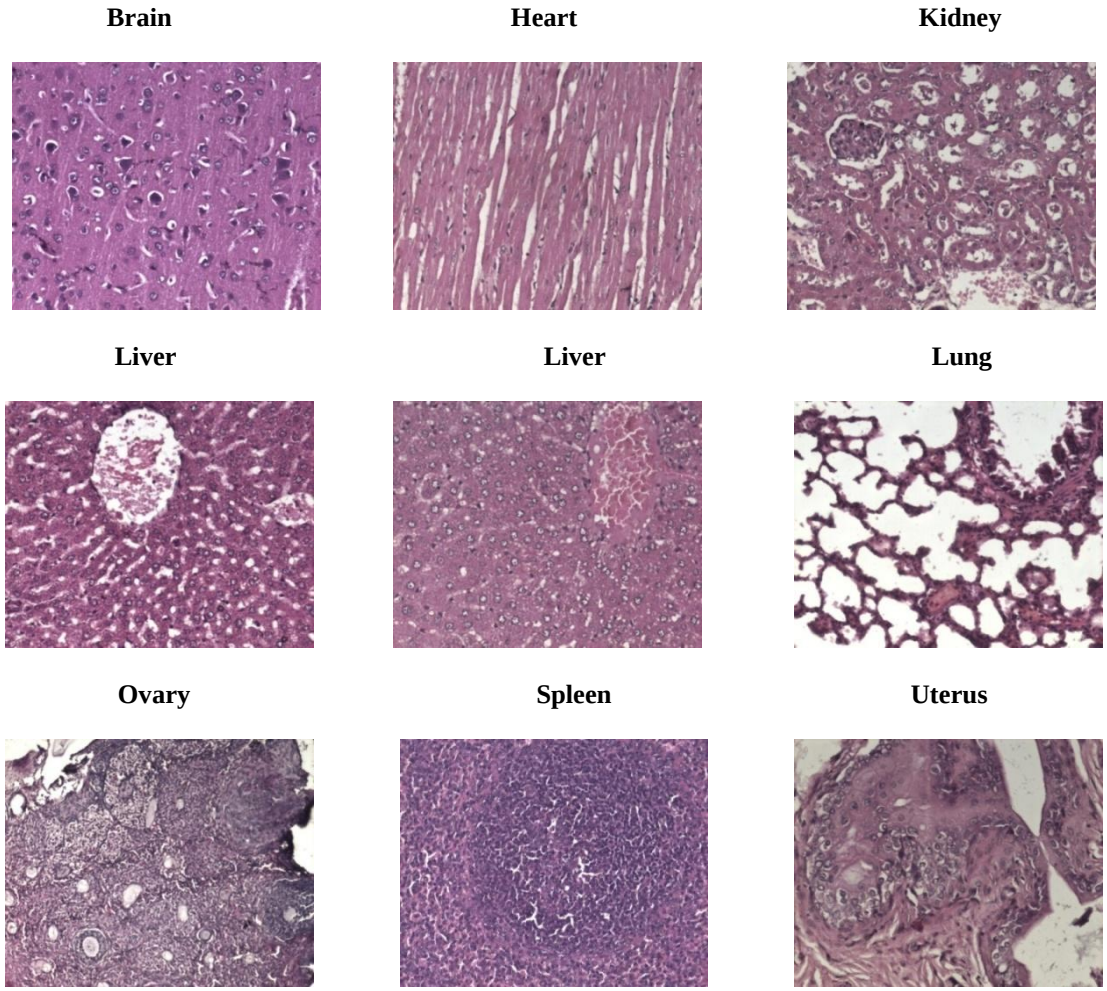
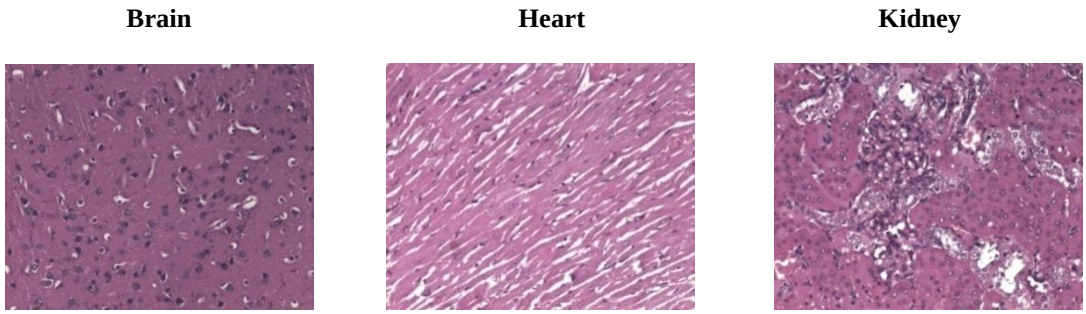


FIGURE 7: HISTOPATHOLOGY OF 10 X THERAPEUTIC DOSE FEMALE GROUP.

10x therapeutic dose male group exhibits mild tubular epithelial cell degeneration in kidney and mild vacuolar degeneration in liver.(Figure 8)



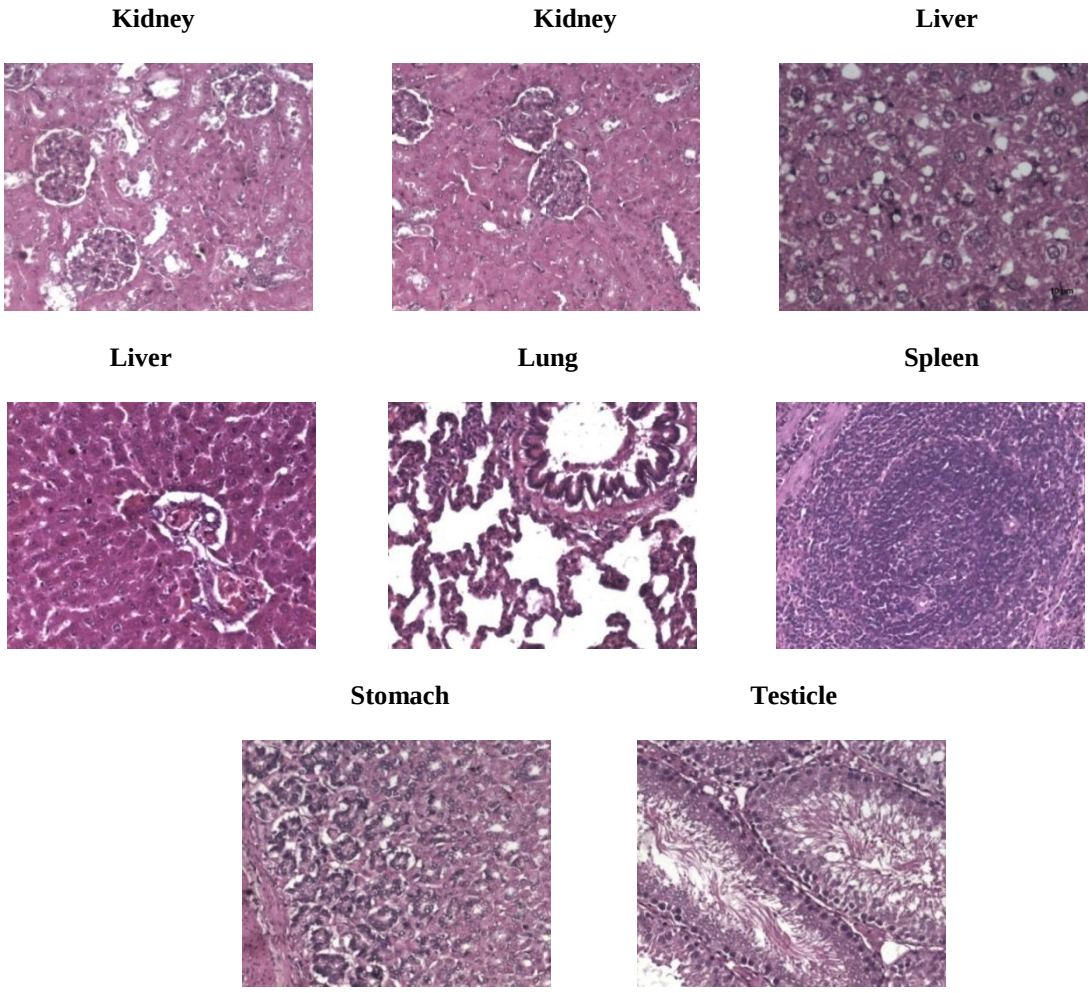


FIGURE 8: HISTOPATHOLOGY IN 10X THERAPEUTIC DOSE MALE GROUP.

DISSCUSSION

The preclinical toxicity studies are essential for determining safe dose for human use. To evaluate the safety profile of Adathodai Chooranam, acute and long term toxicity studies were performed. Adathodai Chooranam did not produce any toxic symptoms or mortality at the dose level of 2.6 gm/kg body weight for 14 days. In Long Term toxicity study no behavioural abnormalities or death was observed during the whole treatment period. Long term toxicity study assess the undesirable effect of continuous or repeated exposure of plant extracts or compounds over a portion of the average life span of experimental animals, such as rodents. Specifically they provide information on target organ toxicity and are designed to identify no observable adverse effect level.[6] Since the liver and Kidneys are the usual Organs of Metabolism and Excretion, they are easily affected by potentially toxic agents, their functions should be monitored in Long Term Studies.

Only moderate significant difference was observed in water intake among all groups of animals. But there is no difference between therapeutic dose and control group. Determination of food consumption was important in the study of safety of a product with therapeutic purpose as proper intake of nutrients essential to the physiological status of the animals and to the accomplishment of the proper response to the drug tested instead of a false response due to improper nutritional condition.[7] A uniform increase in food intake was observed in all the test drug administered groups when compared to control group during the treatment period of 28 days. On the basis of literature review of trial drug, most of the ingredients are having potent stomachic, stimulant and antioxidant action.[8] Therefore the observed increase in food intake may be due to the increased appetite. The body weight changes serve as a sensitive indication of the general health status of animals.[9] There was no weight changes on 7th day, 14th day, 21st day and 28th day in all test drug administered groups when compared to control group.

Evaluation of hematological parameters can be used to determine the extent of the deleterious effect of Adathodai Chooranam on the experimental animal. There is a moderate significant difference among all groups of experimental animals in RBC Count. But there is no difference between control and therapeutic dose group. No difference was observed in other hematological parameters like Total WBC Count, Differential Count, Hemoglobin and Platelets. There are no significant alterations in Hematological Parameters which indicate that Adathodai Chooranam did not affect blood cells production. The clinical biochemistry analysis was done to evaluate the possible alterations in hepatic and renal functions influenced by the test drug. Liver and Kidney function analysis is very important in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism.[10]

In the present study there was significant differences observed in blood sugar, total cholesterol, and triglycerides in all groups of animals. But there is no difference between control and therapeutic dose group. Moderate significant difference was observed in urea level between therapeutic dose and control groups,but the difference was within the normal expected range for the rat species used in this study which might be due to incidental . No difference in Creatinine level and total Protein was observed among all groups. Moderate significant difference among all group of experimental animals on Albumin level. But there is no difference between therapeutic and control group. There is difference among all groups of experimental animals on globulin and no difference between therapeutic dose and control groups.. Total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney and liver disease and many other conditions. There were no significant changes in total protein in rats treated with Adathodai Chooranam, which suggested that there was no sign of impaired renal function.Transaminases (SGOT and SGPT) are good indicators of liver function and bio markers to predict the possible toxicity of drugs. In serum bilirubin and SGOT, there is no difference in all groups of animals. There is difference among all groups of experimental animals on SGPT. But there is no difference between control and therapeutic dose group which reveal that Adathodai Chooranam did not affect Liver function. The normal levels of serum bilirubin concentrations at all does of the Adathodai chooranam used in this study are indicative of non adverse effect of the test drug on hemoglobin metabolism pathways. Absolute terminal organ weight and percent relative organ weight indicative of test compound caused changes in functioning of target organs, changes in phospholid metabolism, over or under secretion of enzymes and hormones hypo/hyperplasia, and possible tissue necrosis.[11] There is no difference in weight of Heart, Lungs, Kidney and Brain in all groups of animals. There is significant difference among different group of experimental animals on weight of Liver, Spleen, Stomach, Male Sex Organ and Female Sex organs. However there is no difference between therapeutic dose and control groups. .

Histopathological studies shown normal architecture in therapeutic dose and control groups. Liver has shown congestion and spleen has shown slightly hyperplastic. Histopathological result of stomach showed focal hyperplasia in non glandular area of stomach in 5x therapeutic dose male group. The focal hyperplasia may be due to stress induced because of repeated drug administration.[12,13] 5x therapeutic female group and 10x therapeutic female group exhibits hyperplasia and Squamous metaplasia in uterus. As per the literature evidence diffuse or focal (polypoid) hyperplasia of the endometrium is found as a spontaneous change in laboratory animals particularly in age advances. Hyperplasia is associated with age related decline in sex hormone levels in which there is often relative estrogen excess. Administration of exogenous estrogens or other xenobiotics with estrogenic effects also induces endometrial hyperplasia in both laboratory animals and in woman.[14] Hispathological result of kidney showed mild tubular epithelial cell degeneration in 10x therapeutic dose male group and the histopathological result of liver showed mild vacuolar degeneration. In the trial drug Adathodai Chooranam, one of the herbal ingredients is the seeds of embelia ribes. As per the literature survey administration of embelin at lower dose for short duration did not produce deleterious effect but its long term administration at higher dose was toxic with the following changes. The liver showed prominent hepatic congestion characterized by enlarged hepatic veins and accumulation of more blood within the hepatic sinusoids. The kidney also showed degenerative changes at the highest dose with the loss of cellular boundaries and hemosiderin deposition near the glomerular tuft. However the spleen revealed only mild to moderate pathological changes after the oral administration of embelin. The controls didn't show such changes.[15]

CONCLUSION

In acute and long term toxicity studies, mortality or signs of toxicity were not observed because of administration of Adathodai Chooranam. The single oral dose of 2.6 gm/kg body weight was considered as non toxic drug in acute toxicity study. Increase in the food intake was observed in all the groups in comparison to control group. This shows that the drug is well tolerated not have any side effects. Statistically there is no major difference in hematological and bio chemical parameters in test drug treated groups and control groups. There is no difference in organs weight between therapeutic dose and control group which suggest the safety profile of Adathodai Chooranam in therapeutic dose. Histopathological studies showed normal architecture in Target Organs like heart, lungs, stomach, kidney, brain, uterus and ovary in therapeutic dose group. Only the spleen showed slightly hyper plastic and the liver showed congestion. Analysis of the all data related to this long term toxicity study shows that the test drug adathodai chooranam is well tolerated at therapeutic dose level (270 mg/kg body weight). The data shows that there are no major toxicological indications at therapeutic dose level. However based on the histopathological results, the 5x therapeutic dose and 10x therapeutic dose may produce slight pathological changes in kidney, liver, stomach and uterus also. Hence it is proved the trial drug Adathodai Chooranam is safe in the dose of 1.5 g for human adult mentioned in the siddha literature. Clinically among 125 new Asthmatic cases 110 cases (88%) showed good response. 5(4%) cases showed moderate response and 10 cases (8%) showed poor response. The duration of treatment with the trial drug Adathodai chooranam can be extended from 30 days to 3 months for more efficacy. Therefore the drug can be subjected to chronic toxicity study in Animal models as per WHO guidelines. In vitro and In vivo pharmacological activities can be included for scientific validation of trial drug Adathodai chooranam.

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ABBREVIATIONS

ALT	Alanine transaminase
ANOVA	Analysis of Variance
AST	Aspartate transaminase
B.wt	Body Weight
CPCSEA	Committee for the purpose of control and supervisions of experiments on animals
EDTA	Ethylene Diamine Tetraacetic acid
G	Gram
HD	High Dose
HA	Haemagglutination titre
Hgb	Hemoglobin
HCT	Hematocrit
IAEC	Institutional Animal Ethical Committee
IMPCOPS	Indian Medical Practitioner's Co-operative Pharmacy and Stores
Kg	Kilogram
LD	Low Dose
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
Mg	Milligram
Ml	Milli liter
PCV	Packed Cell Volume
RBC	Red blood cells
SD	Standard Deviation
SGOT	Serum Glutamate Pyruvate Transaminase
SGPT	Serum Glutamate Oxaloacetate Transaminase
WHO	World health organization
WBC	White blood cells

Conflict of interest:
Nil

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