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EVALUATION OF SUB ACUTE TOXICITY STUDY ON SUYAMAKKINI CHENDHURAM

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ABSTRACT

Plant medicine is the oldest form of health care known to mankind. In many countries, herbal medicines and related products are introduced into the market without safety or toxicological evaluation. The objective of this 'Sub-Acute Toxicity Study of *Suyamakkini Chendhuram*on Wister Rats' was to assess the toxicological profile of the test item when treated as a single dose. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time. In conclusion, *Suyamakkini Chenduram* extract can be considered safe, as it did not cause either any lethality or adverse changes with general behavior of rats and also there were no observable detrimental effects (100, 150, to 200 mg/kg body weight) over a period of 28 days. Our results have demonstrated that the *Suyamakkini Chenduram*extractis relatively safe when administered orally in rats.

KEYWORDS: Sub-Acute Toxicity, *Suyamakkini Chendhuram*on, Hematology, Clinical biochemistry, Histopahology.

INTRODUCTION

Subacute toxicity tests are intended to evaluate the toxicity of the chemical after repeated administration and also to help in establishing doses for the longer-term subchronic studies. Most subacute studies utilize three to four different dosages of the chemicals, administered by mixing it in the feed. For rodent studies, 6 animals of each sex are usually used at each dose, whereas for dogs three dosages and three to four animals per sex are used. The chemical is

typically administered for 14 days, after which the animals are killed and complete clinical chemistry and histopathology analyses are performed (Lu, 1996; OECD, 2008; Colerangle, 2017). Plants used in traditional medicine, therefore, have a critical role in the maintenance of health all over the world. The drugs of herbal, herbo-mineral, and animal origin have been used by the traditional healers to maintain health and treat diseases since antiquity. Such medicines are widely used in Africa and Asia, including India and China (Pati and Gaikwad, 2010). Due to the adverse side-effects, and also the development of resistance against synthetic drugs, the uses of plant-derived drugs are becoming popular in developed countries also (Dias and Takahashi, 1994). However, the latest surveys have indicated many medicinal plants also showed adverse effects (Ankush et al., 2013; Nath and Yadav, 2015). This raises concerns about the potential toxic effect resulting from chronic use of such medicinal plants. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used clinically or preclinically, is a crucial part of its assessment of potential toxic effects.

MATERIALS AND METHODS

The study was conducted on 24 Wister rats. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *ad libitum* in the Animal at M/s. Venkateshwara Enterprises Pvt. Ltd, Bangalore. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

Table 1: Numbering and Identification.

Group No	Animal Marking
1 (CONTROL)	H,B,T(MALE) H,B,T (FEMALE)
2 (LOW DOSE (100 mg/kg) *Suyamakkini Chendhuram)*	H,B,T(MALE) H,B,T (FEMALE)
3 (MIDDLEDOSE (150mg/kg) *Suyamakkini Chendhuram)*	H,B,T(MALE) H,B,T (FEMALE)
4 (HIGH DOSE (200mg/kg) *Suyamakkini Chendhuram)*	H,B,T(MALE) H,B,T (FEMALE)

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals:

Cage No	Group No	Animal Marking	Sex
1	I (CONTROL) DISTILLED WATER	H,B,T H,B,T	Male Female
2	II (LOW DOSE(100 mg/kg) *Suyamakkini Chendhuram)*	H,B,T H,B,T	Male Female
3	III (MIDDLEDOSE(150mg/kg) *Suyamakkini Chendhuram)*	H,B,T H,B,T	Male Female
4	IV (HIGH DOSE MIDDLEDOSE(200mg/kg) *Suyamakkini Chendhuram)*	H,B,T H,B,T	Male Female

DOSES

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

Table 2: Dose level.

TEST GROUP	DOSE TO ANIMALS (mg/kg body-weight/day)	NUMBER OFANIMALS
Group-1	Control (Distilled Water).	6 (3MALE and 3 FEMALE)
Group-II	LOW DOSE(100 mg/kg) *Suyamakkini Chendhuram)*	6(3MALE and 3 FEMALE)
Group-III	MIDDLE DOSE(150mg/kg) *Suyamakkini Chendhuram)*	6(3MALE and 3 FEMALE)
Group-IV	HIGH DOSE(200mg/kg) *Suyamakkini Chendhuram)*	6(3MALE and 3 FEMALE)

The test item was administered as single dose. After single dose administration period, all animals were observed for 28 days.

Dose Preparation

Suyamakkini Chendhuram was added in distilled water and completely dissolved to for oral administration. The dose was prepared of a required concentration before dosing by dissolving ***Suyamakkini Chendhuram***, in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each rat as single dose using a needle fitted onto a disposable syringe of appropriate size at the following different doses. The concentration was

adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

OBSERVATIONS

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatic motor activity and behaviour.

Clinical Signs Of Toxicity

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes. Clinical signs of toxicity daily for 28 days.

Food Intake

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

Water Intake

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

Bodyweight

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection

Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

Laboratory Studies

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 6 rats from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Haematological parameters like RBC, WBC, and PLATELETS

etc…. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, UREA and CREATININE.

Clinical Biochemistry

The Hematological and clinical Bioparameters were analysed using Autoanalyser.

HISTO PATHOLIGICAL STUDY

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 24 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after visceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ Weights

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

RESULTS AND DISCUSSION

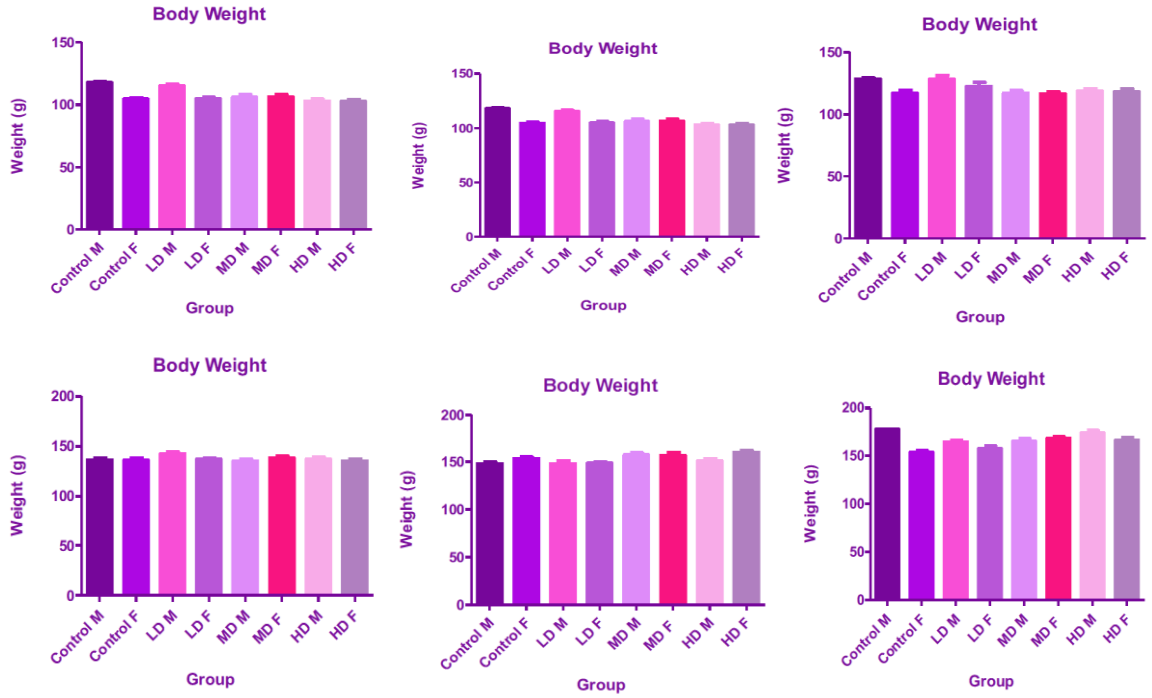


Fig: 1: Effect of Extract On Body Weight on "0, 7, 14, 21 and 28" days.

Body weight

Result of body weight determination of animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Organ Weight

Group Mean Relative Organ Weights (% of body weight) are recorded in Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

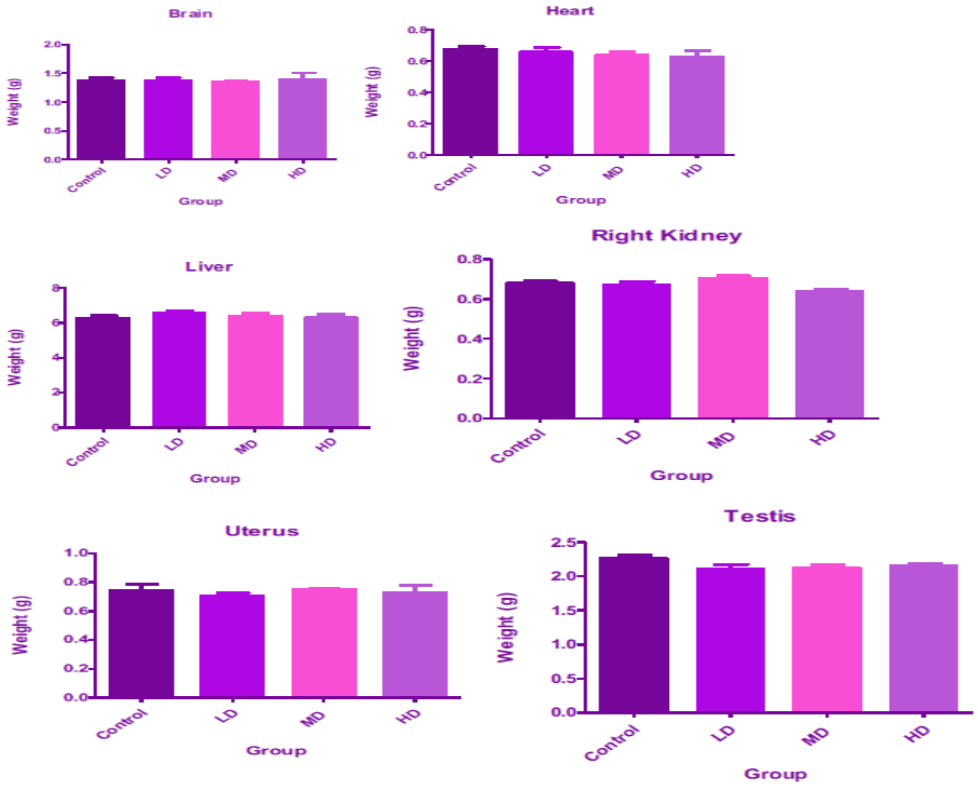


Fig. 6: Effect Of Sub-Acute Doses (28 Days) Extract of *Suyamakkini Chendhuram* On Organ Weight (Physical Parameter).

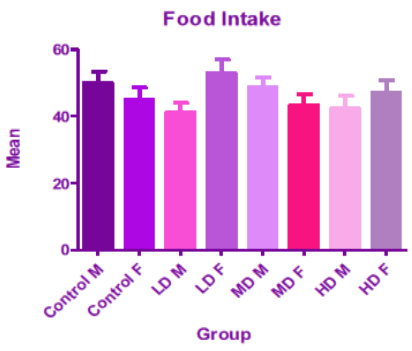


Fig : 7EFFECT OF SUB-ACUTE DOSES (28 DAYS) OFEXTRACT OF *Suyamakkini Chendhuram* ON FOOD INTAKE

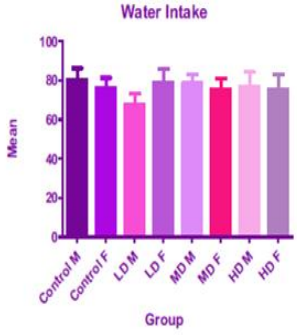


FIG: 8 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF EXTRACT OF *Suyamakkini Chendhuram* ON WATER INTAKE.

Food Consumption

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

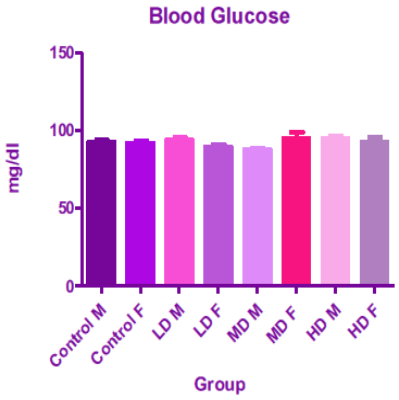


Fig : 9 Effect of extract on Blood Glucose Leval Of *Suyamakkini Chendhuram*

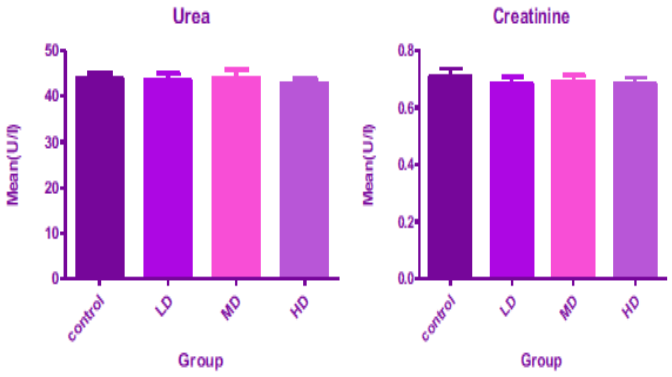


Fig: 10 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF EXTRACT OF *Suyamakkini Chendhuram*ON BIOCHEMICAL PARAMETERS

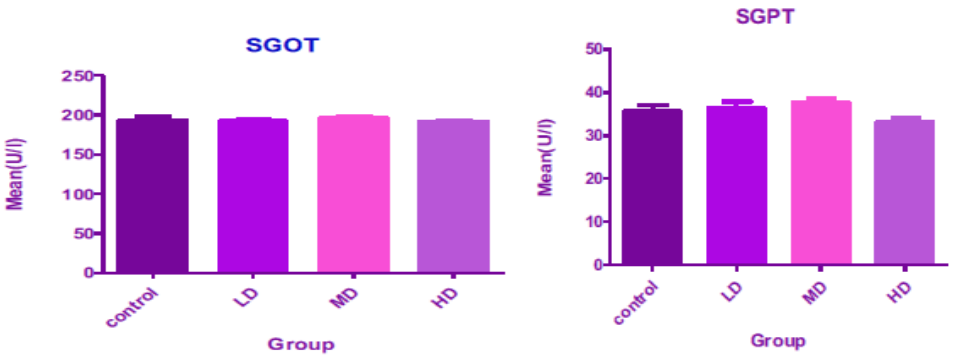
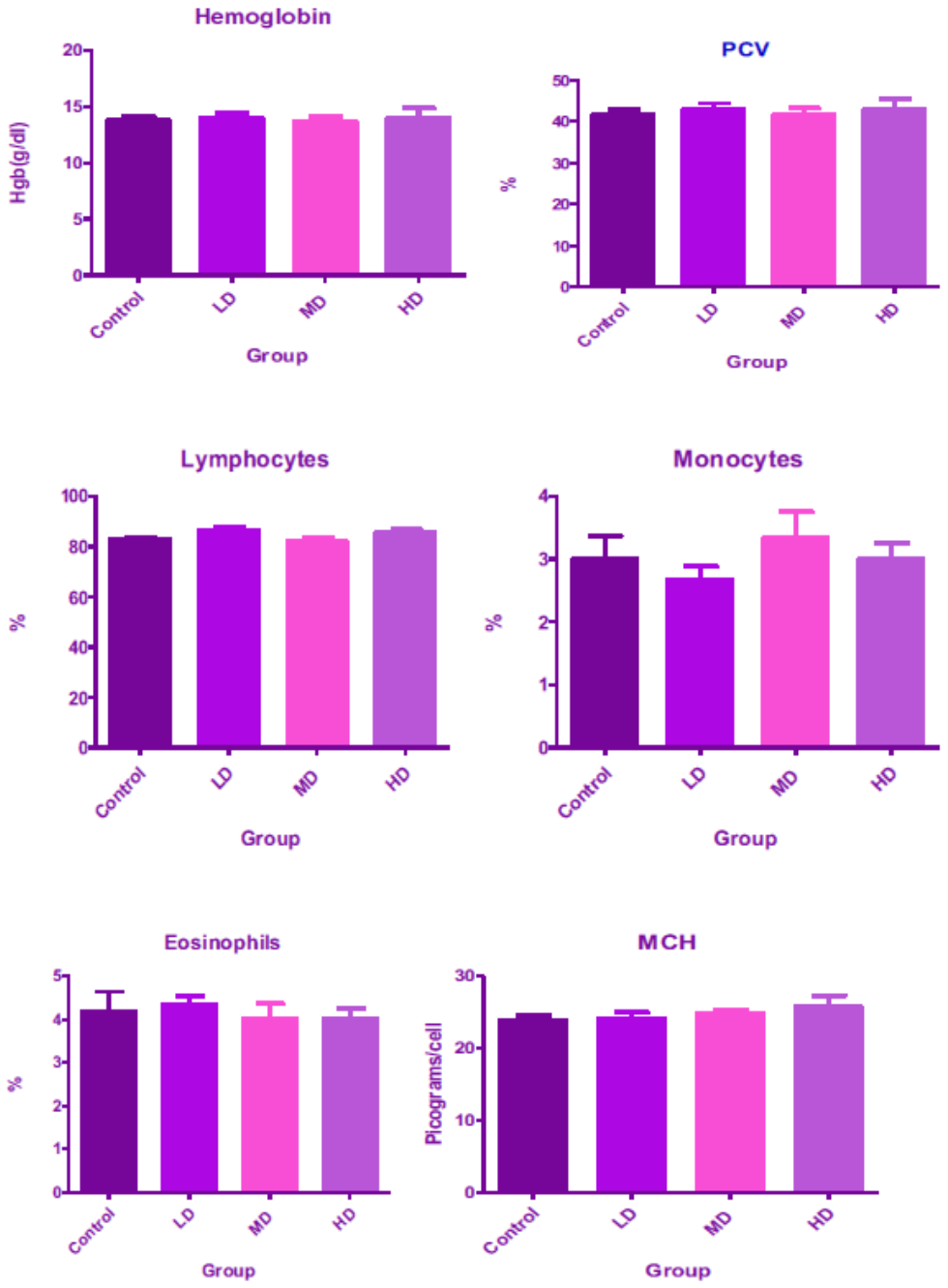


Fig: 10 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF EXTRACT OF *Suyamakkini Chendhuram*ON BIOCHEMICAL PARAMETERS

BIOCHEMCIAL INVESTIGATIONS

Results of Biochemical investigations conducted on 29th day and recorded in revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.



Fig, 11: EFFECT OF SUB-ACUTE DOSES (28 DAYS) OFEXTRACT OF *Suyamakkini Chendhuram* ON HAEMATOLOGICAL PARAMETERS.

Haematological Investigations

The results of Haematological investigations conducted on 29th day revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

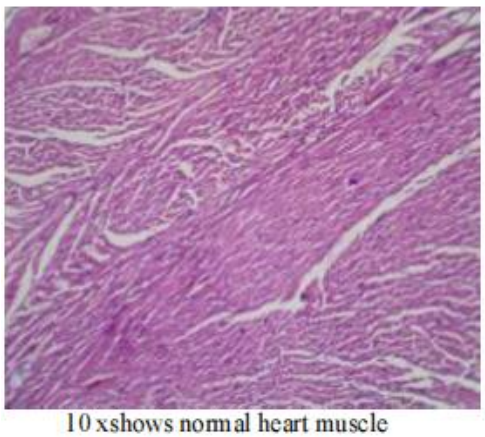
HISTOPATHOLOGY

The target organs liver were collected and preserved in 10% formalin for the Histopathological examination. The organs from control and drug treated animals were preserved in 10% neutral formalin for Histopathological examination.

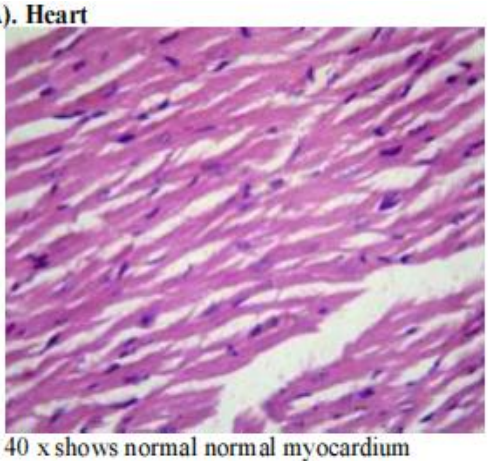
TOXICITY STUDY

Group –I- CONTROL

(A). Heart



10 xshows normal heart muscle

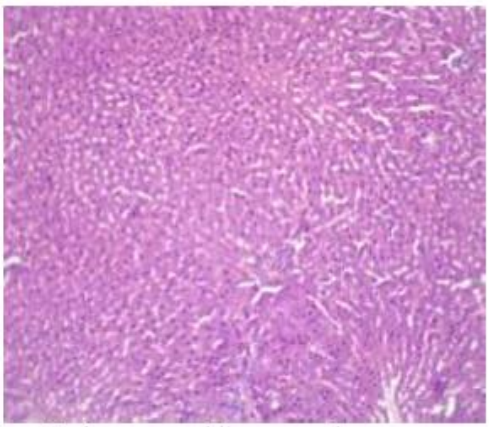


40 x shows normal normal myocardium

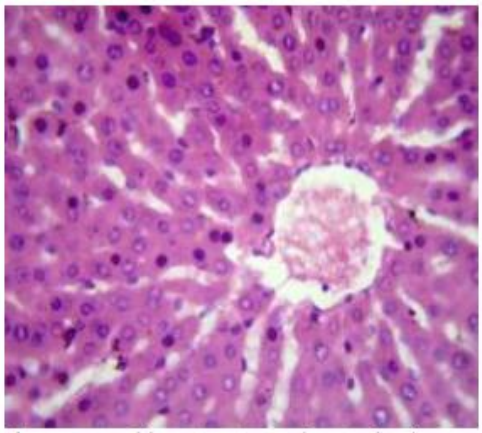
MICROSCOPIC APPEARANCE

Group –I – Control: Section studied from the heart shows normal myocardial tissue. There is no disarray of muscle fibres, no inflammation or evidence of ischemia/ necrosis. No myocarditis or pericarditis noted. No evidence of toxicity.

Group –I- CONTROL (B). Liver



10xshows normal liver parenchyma

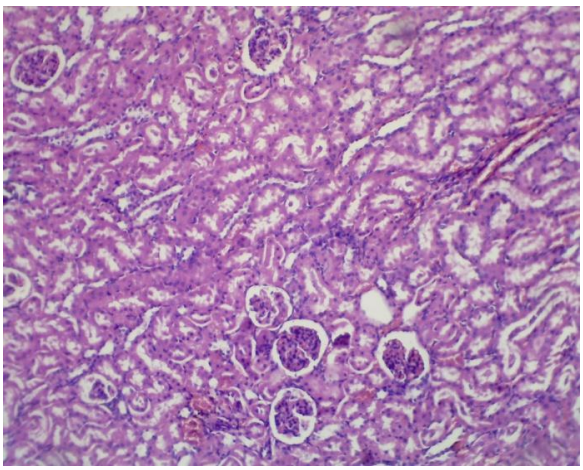


40xshows normal hepatocytes and central vein

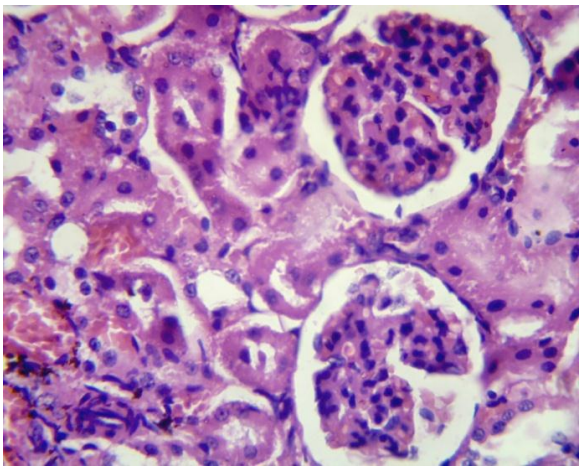
MICROSCOPIC APPEARANCE

Group –I – Control: Section studied from the liver shows normal lobular architecture. The sinusoids shows normal morphology. The portal tracts shows unremarkable. The central vein shows normal morphology. There is no fibrosis. There is no lobular inflammation, necrosis or granulomas in the liver parenchyma.

(C). Kidney



10x shows normal cortex and medulla



40x shows normal glomeruli and tubules

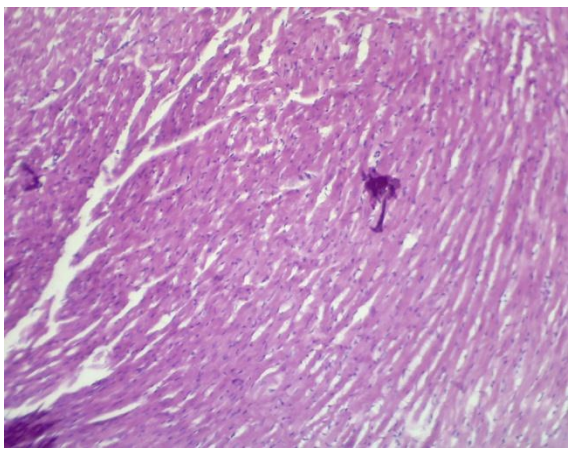
TOXICITY STUDY

MICROSCOPIC APPEARANCE

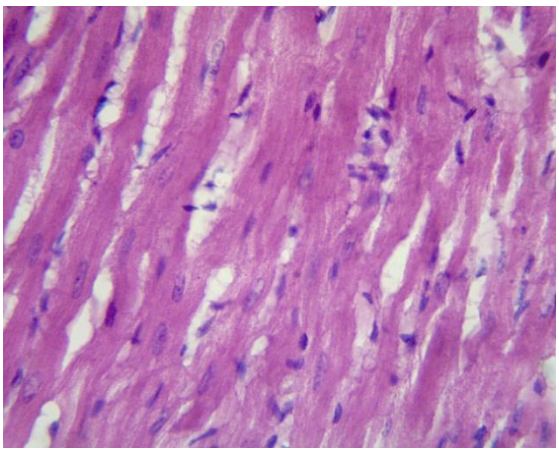
Group –I – Control: Section studied from the kidney shows normal cortex and medulla. The glomeruli shows normal morphology. The tubulo interstitial compartments are also normal morphology. There is no evidence of inflammation or tubular necrosis noted.

Group –II- LOW DOSE (100 mg/kg)

(A). Heart



10x shows focal loss of architecture



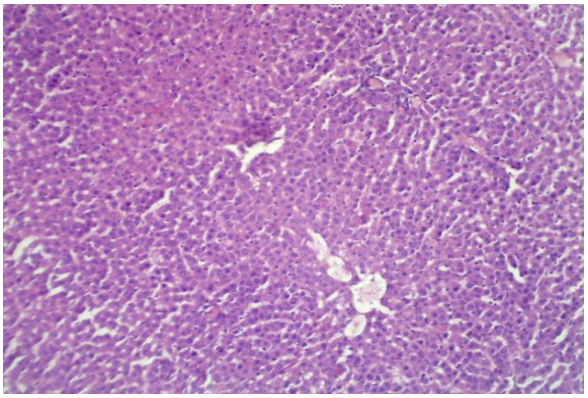
40x shows mild inflammation

MICROSCOPIC APPEARANCE

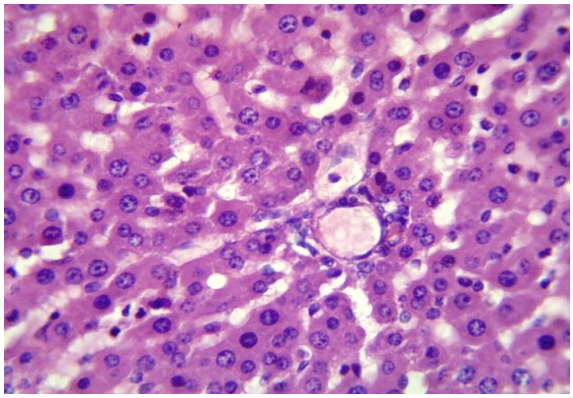
Group –II – LOW DOSE (100 mg/kg): Section studied from the heart shows normal myocardial tissue. The blood vessels show congestion. Focal areas shows mild loss of architecture with inflammation composed oflymphocytes.

Group –II- LOW DOSE (100 mg/kg)

(B). Liver



10xshows lobular architecture



40x shows binucleation and cytoplasmic vacuolation

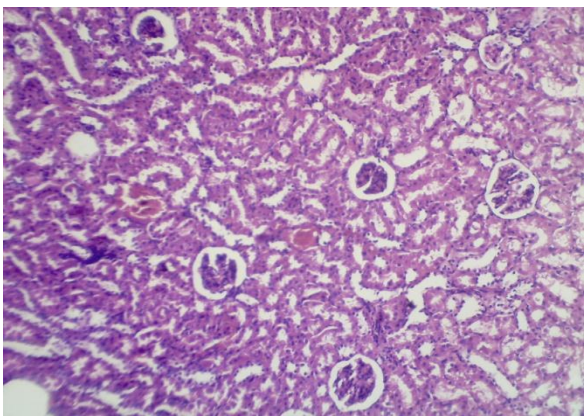
TOXICITY STUDY

MICROSCOPIC APPEARANCE

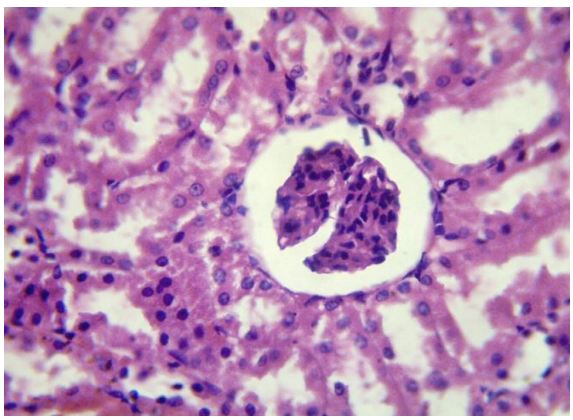
Group –II – LOW DOSE (100 mg/kg): Section studied from the liver shows normal lobular architecture. The sinusoids shows mild dilatation. The portal tract shows unremarkable. The central vein shows congestion. Indvidual hepatocytes shows cytoplasmic vacuolation and binucleation.

Group –II- LOW DOSE (100 mg/kg)

(C). Kidneys



10xshows normal cortex and medulla



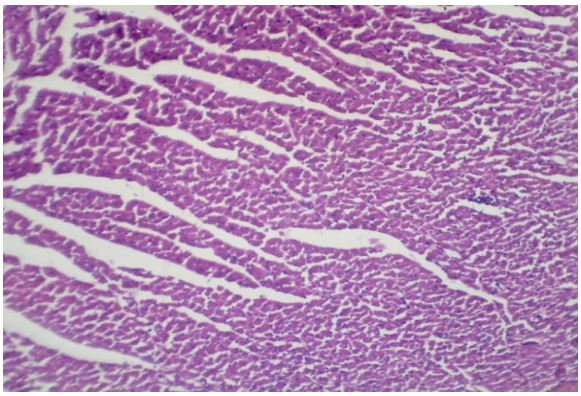
40x shows normal glomeruli

MICROSCOPIC APPEARANCE

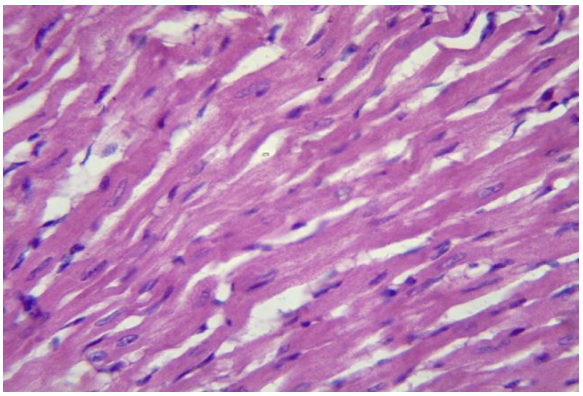
Group –II – LOW DOSE (100 mg/kg): Section studied from the kidney shows normal cortex and medulla. The glomeruli shows normal morphology. The tubulo interstitial compartments shows unremarkable. The blood vessel shows congestion. There is no evidence of inflammation or tubular necrosis noted.

Group –III – MIDDLE DOSE (150mg/kg)

(A). Heart



10x shows normal myocardium



40 x shows normal myocardium with myocytes

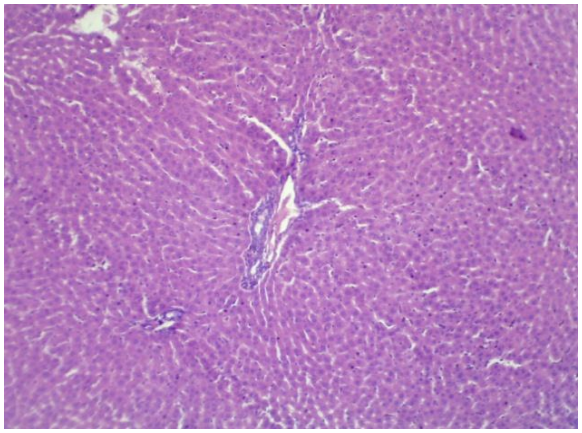
TOXICITY STUDY

MICROSCOPIC APPEARANCE

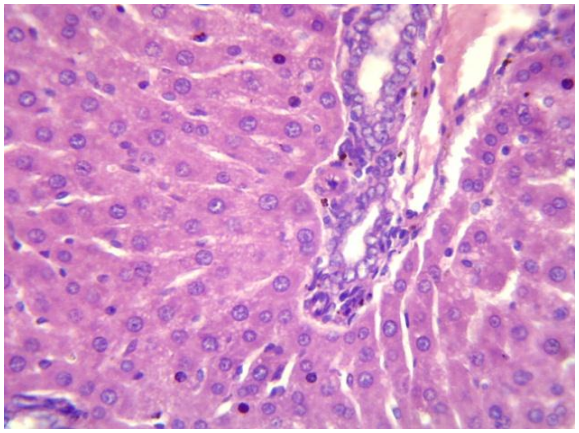
Group –III – MIDDLE DOSE (150mg/kg): Section studied from the heart shows myocardium with normal myocytes. The blood vessels are unremarkable. There is no evidence of ischemia/ necrosis and no loss of architecture.

Group –III- MIDDLE DOSE (150mg/kg)

(B). Liver



10xshows lobular architecture



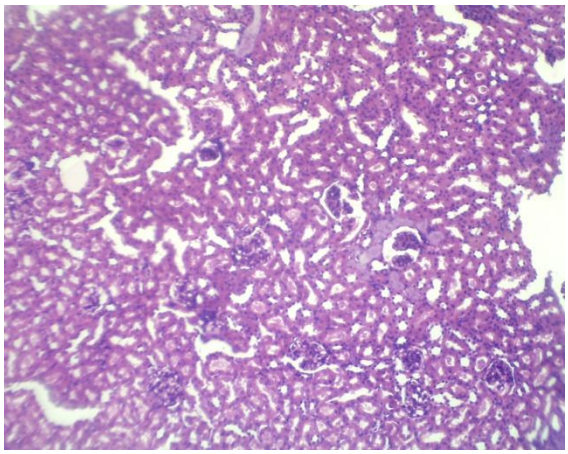
40x shows bile duct hyperplasia

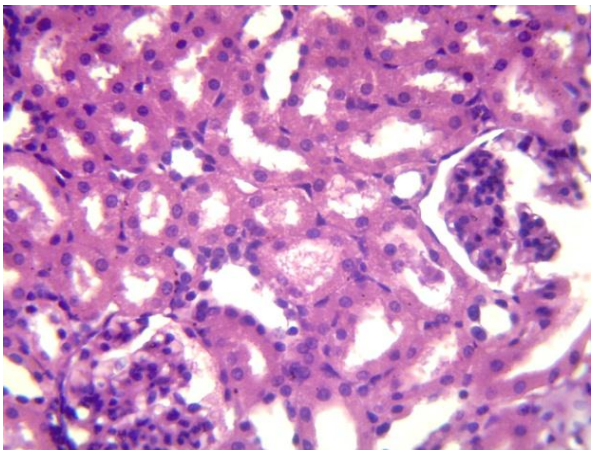
MICROSCOPIC APPEARANCE

Group –III – MIDDLE DOSE (150mg/kg): Section studied from the liver shows normal lobular architecture. The sinusoids shows dilatation. The portal tract shows bile duct hyperplasia. The central vein shows mild congestion. Individual hepatocytes are unremarkable.

Group –III- MIDDLE DOSE (150mg/kg)

(C). Kidney



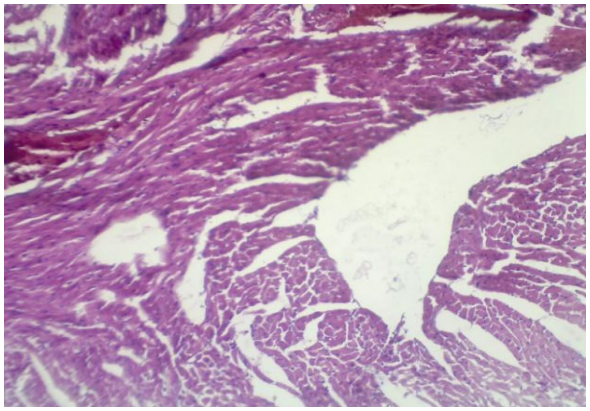


MICROSCOPIC APPEARANCE

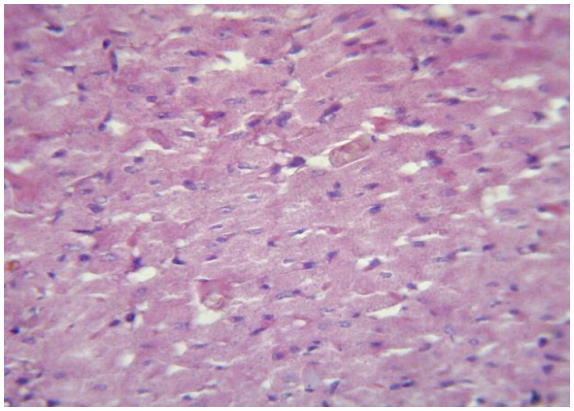
Group –III – MIDDLE DOSE (150mg/kg): Section studied from the kidney shows normal cortex and medulla. The glomeruli shows normal morphology. The tubules are normal morphology. The interstitial compartment shows unremarkable. Blood vessels are congested There is no evidence of inflammation or tubular necrosis noted.

Group –IV- HIGH DOSE (200mg/kg)

(A). Heart



10x shows normal myocardium



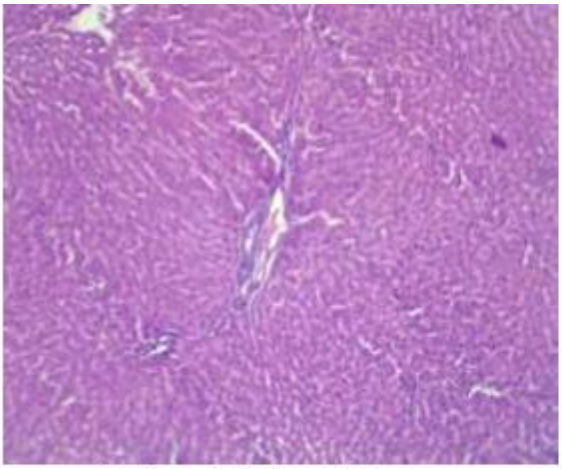
40 x shows normal myocardium with myocytes

ICROSCOPIC APPEARANCE

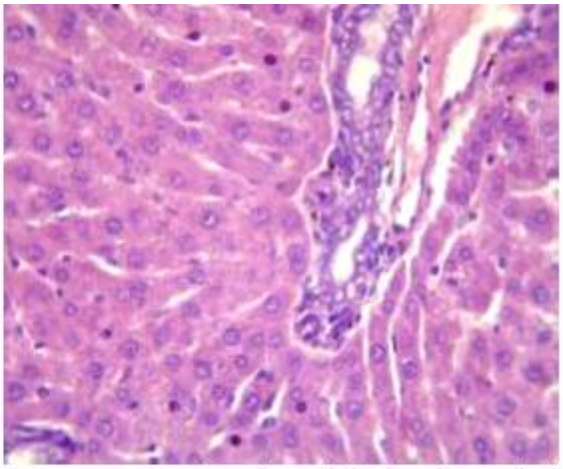
Group –IV – HIGH DOSE (200mg/kg): Section studied from the heart shows normal myocardial tissue with myocytes with mild loss of architecture. No inflammation or evidence of ischemia/ necrosis.

Group –IV- HIGH DOSE (200mg/kg)

(B). Liver



10xshows lobular architecture

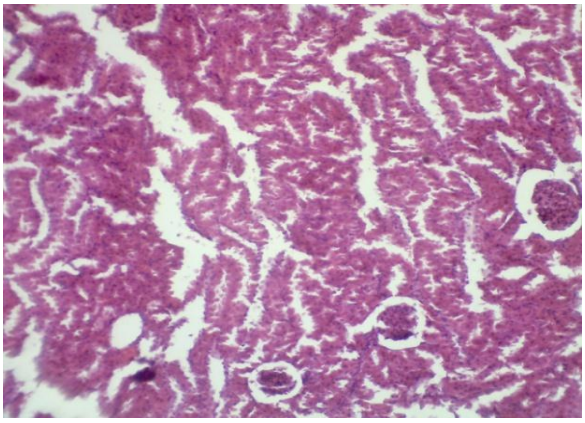


40x shows bile duct hyperplasia

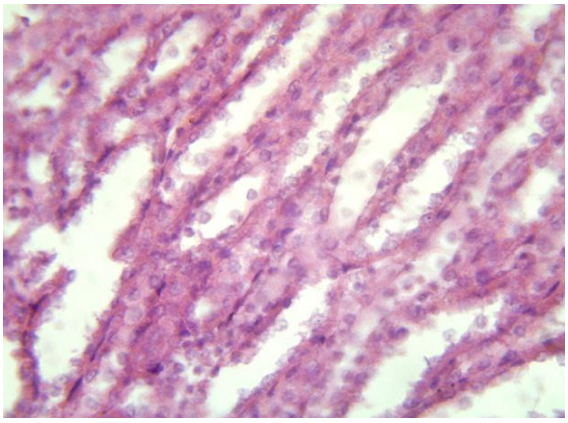
MICROSCOPIC APPEARANCE

Group –IV – HIGH DOSE (200mg/kg): Section studied from the liver shows normal lobular architecture. The sinusoids show dilatation. The portal tracts shows bile duct hyperplasia. The central vein shows congestion. Individual hepatocytes are unremarkable.

Group –IV- HIGH DOSE (200mg/kg)
(C). Kidneys



10xshows normal cortex and medulla



40x shows normal glomeruli

Fig. 12: Histopathological Observations Effect Of Suyamakkini Chendhuram On Histopathological Changes In Rat Organs.

MICROSCOPIC APPEARANCE

Group –IV – HIGH DOSE (200mg/kg): Section studied from the kidney shows normal cortex and medulla. The glomeruli shows normal morphology. Both the tubules are normal morphology. Blood vessels are unremarkable. There is no evidence of inflammation or tubular necrosis noted.

1. BIOCHEMICAL ANALYSIS

The presence of given sample ***SUYAMAKKINI CHENDURAM*** contains sulphate, Chloride, Iron Ferrous and un saturation compound.

MICROBIOLOGICAL RESULT

SUYAMAKKINI CHENDURAM sensitive to Escherchia coli, and moderate sensitive to Klebsiella pneumonia, resistant to staphylococcus aureus.

3. SUB ACUTE TOXICITY STUDY

From sub acute toxicity study, it was observed that the administration of **Suyamakkini Chenduram** at a dose 100mg/kg, 150mg/kg, 200mg/kg(L/M/H) to a rat. From the sub acute toxicity study, it was **200mg/kg**to the rats do not produce drug- related toxicity, it shows mild variations according to the dose in the organ weight, Haematological investigations and Biochemical investigations. No Histopathological changes in Brain / Lungs / Kidney and spleen. Except that the mild inflammatory changes are occurred in the liver and heart. These changes are unremarkable during the course of drug administration. Both acute and sub acute toxicity study were conducted in KMCH College of Pharmacy, Coimbatore.

Sub Acute Toxicity Study

1. All the animals from control and all the treated dose groups up to 500 mg/kg survived throughout the dosing period of 28 days.
2. No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.
3. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.
4. Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days
5. Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment.

6. Biochemical analysis conducted at the end of the dosing period on day 29 no abnormalities attributable to the treatment.
7. Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.
8. Histopathological examination revealed normal architecture in comparison with control and treated animal.

CONCLUSION

Sub Acute Toxicity Study

In conclusion, ***Suyamakkini Chenduram*** extract can be considered safe, as it did not cause either any lethality or adverse changes with general behavior of rats and also there were no observable detrimental effects (100, 150, to 200 mg/kg body weight) over a period of 28 days. Our results have demonstrated that the ***Suyamakkini Chenduram*** extractis relatively safe when administered orally in rats.

Further Scope

Effectiveness of Siddha medicine should be ruled out through pharmacological study. Active principle which is responsible for activity and clinical studies for the good clinical practice.

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