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SAFETY PROFILE OF SEENTHIL CHOORANAM ON REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY IN WISTAR ALBINO RATS

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ABSTRACT

The drug *Seenthil Chooranam* has been mentioned in the classical siddha text Agasthiyar Paripuranam – 400 (for *Megam* (Diabetic mellitus), *Eelai* (Tuberculosis), *Kasam* (Cough), *Elaipu* (Bronchial asthma), Eranda vayu (Scrotal swelling). The drug *Seenthil Chooranam* is prepared by mixture of herb and the animal product. In repeated dose of 90 days oral toxicity study results, all animals from the treated dose survived throughout the dosing period of 90 days. Animals from treated groups and control group shows overall weight gain throughout the study period of 90 days. The haematological and biochemical results shows no significant changes. But sugar levels were reduced significantly when compare to the control group, At the end of the study gross pathological examination of animals in control and treated groups did not reveal any abnormalities. The vital organs as liver, heart, kidneys, lungs and brain from the test group did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organs revealed normal histological appearance when compared with the control group.

KEYWORDS: Siddha Medicine, Toxicity and *Seenthil Chooranam.*

INTRODUCTION

The drug *Seenthil Chooranam* has been mentioned in the classical siddha text Agasthiyar Paripuranam – 400 (for *Megam* (Diabetic mellitus), *Eelai* (Tuberculosis), *Kasam* (Cough), *Elaipu* (Bronchial asthma), Eranda vayu (Scrotal swelling). The drug *Seenthil Chooranam* is prepared by mixture of herb and the animal product.

Safety is always a fundamental principle in the provision of any health-care treatment and procedures. Given the reality of wide use of traditional medicine worldwide, monitoring

safety of traditional medicines becomes important (WHO, 2017). As per Siddhars statement, continuous (at least one mandalam) use of medicine will cure the chronic disease. As per, the repeated dose of 90 days oral toxicity study will ensure the safety of the drug for the chronic use, based on that the drug *Seenthil chooranam* was evaluated for repeated dose 90 days oral toxicity study. More over in this toxicity study there is possibility to find the cumulative toxicity, it cannot be seen in acute and 28 days oral toxicity study.

In repeated dose of 90 days oral toxicity study result shows, all animals from the treated dose survived throughout the dosing period of 90 days. Animals from treated groups and control group shows overall weight gain throughout the study period of 90 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

The haematological results shows no significant changes in the values when compared to control group. The biochemical results revealed there were no significant changes in the values of different parameters with that of the control. But sugar levels were reduced significantly when compare to the control group because that drug traditionally used for diabetic condition, other values were within the normal biological and laboratory limits.

At the end of the study gross pathological examination of animals in control and treated groups did not reveal any abnormalities. The vital organs as liver, heart, kidneys, lungs and brain from the test group did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organs revealed normal histological appearance when compared with the control group.

In acute and repeated dose 28 days oral toxicity study the results revealed that the drug *Seenthil Chooranam* did not produce in any mortality at the dose of 2000mg/kg body weight. It conclusively indicated the LD50 value is greater than 2000mg/kg body weight. In repeated dose 28 days oral toxicity study was observed without any abnormalities and No-Observed Adverse Effect Level (NOAEL) were noted (Ushakanthan, 2017).

METHODOLOGY

Preparation and Administration of *Seenthil chooranam*

Ingredients

Seenthil (Tinospora cordifolia) - 10 *palam (*350gm) *Karisalai* (Eclipta Alba) - 10 *palam* (350gm) Earthworm (Eudrilus eugeniae) - 3 *palam* (105mg)

Preparation

Seenthil chooranam was suspended in melted Ghee with distilled water to obtain concentrations of 200mg/ml and administered to animals at the dose levels of the suspensions were freshly prepared every two days once for 90 days. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

Randomization, Numbering and Grouping of animals

Twenty four Wistar Albino Rats of both sex were selected. Six rats (Male -3, and Female-3) were in each group divided into 4 groups. The animals are randomly selected and kept in their cages for 7 days prior to the treatment to allow for acclimatization to the laboratory conditions. Each animal was marked with picric acid. The females were nulliparous and non-pregnant. Institutional Animal Ethical Committee approved Number**:** NIS/ IAEC-I/ 2016/ 08.

Group I was given vehicle only and served as a control and while other three groups were administered with *Seenthil chooranam* for 90 days at a dose of X, 5X, 10X respectively. (low, mid, high)

In the literature, the therapeutic dose of *Seenthil chooranam* is 2gm per day for human beings. The study carried out for the oral route because oral route is a proposed therapeutic route. As per the OECD guideline dose level that no observable toxic effects in the dose of 1000mg/kg, based on that double dose selected for the study. They were, mid dose (5X) and high dose (10X). X was calculated by multiplying the therapeutic dose (2000 mg) and the body surface area of the rat (0.018). i.e., 5X dose was 180mg/animal, 10X dose was 360 mg/animal. The acute toxicity study results revealed that no behavioral changes were observed up to the dose level of 2000mg/kg body weight in acute treatment. As per the results the Seenthil choornam was nontoxic.

OBSERVATIONS

The principles of laboratory animal care were followed. The weight of each rat was recorded on day 0 and weekly intervals throughout the course of the study. Observation were made and recorded systematically and continuously observed as per the guidelines. All animals were observed twice daily for mortality during entire course of study.

Laboratory Investigations

The laboratory investigations were carried out the end of the 90 day, they were fasted overnight. Blood samples were collected from orbital sinus for Heamatology and blood chemical analysis. Sodium heparin (200IU/ml) for blood chemical analysis and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samlpes were centrifuged at 3000 r.p.m. for 10 minutes. The Haematoogical parameters were determined by using Haematological analyzer. The Bio chemical parameters were determined by using auto-analyzer.

Histopathology

Both Control group and High dose group were accommodated for the histopathology changes. All the vital organs were immersed in 10% formalin for 24 h-48h for histopathological examination. After standard processing, the cut tissue was embedded in paraffin (Leica TP1020 tissue processor) and cut into 5 µm thick sections in a rotary microtome (Leica RM2255 - Fully Automated Rotary Microtome). The sections were stained with haematoxylin-eosin (Merck). Histological measurement and photographs were taken with Olympus CX31, Trinocular Biological Microscope (magnification 10x & 40 x).

Statistical analysis

Findings as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet't' test using a computer software programme (Graph Pad Prism5)

Repeated dose 90 days oral toxicity of *Seenthil chooranam* on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 Days. Various parameters were studied and the interpretation of the study result is discussed below.

RESULTS

Table 1: Changes of Body weight (g) of wistar albino rats exposed to *Seenthil Chooranam* for 90 Days.

Days	Groups			
	Control	Low dose	Mid dose	High dose
1	160.6±33.68	147.1	± 21.11	155.6± 13.57
15	172.8 ± 28.87	161.5	± 21.71	165.7 ± 29.01
30	180.8 ± 28.31	175.7	± 14.88	180.8 ± 32.11
45	204.5± 27.73	194.5± 29.76	203.7± 19.75	207.3± 22.75
60	217.6±33.68	213.6±23.68	225.6±33.68	214.6±23.78
75	235.8 ± 26.85	225.8	± 28.870	240.8 ± 28.87
90	248± 27.320	238.5± 27.320	250.4± 27.32	236.3± 36.32

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett's test.

Table 2: Haematological parameters of Wistar albino rats group exposed to *Seenthil Chooranam*.

Category	Control	Low dose	Mid dose	High dose
T.RBC	7.20±0.30	7.33±0.33	7.20±0.74	7.30±0.30
T.WBC(cells/cu.mm)	11.05±0.76	11.50±0.33	11.25±0.46	11.93±0.22
Platelets	3.61±0.30	3.70±0.27	3.65±0.16	3.41±0.43
PCV	37.74±.88	38.84±2.43	37.92±1.71	39.22±2.25
Hb (%)	12.58±0.29	12.95±0.82	12.65±0.58	13.08±0.75
MCV(%)	88.50±3.10	91.50±2.73	91.50±1.87	92.33±3.33
MCH(%)	31.83±1.72	30.83±2.79	31.67±3.72	31.50±1.05
MCHC	34.33±1.86	36.33±4.88	34.50±2.59	33.17±2.13

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett's test.

Table 3: Biochemical Parameters of of Wistar albino rats group exposed *Seenthil Chooranam*.

Biochemical Parameters	Control	Low Dose	Mid Dose	High Dose
Glucose (R) (mg/dl)	130.83±3.54	91.0±7.37*	81.83±8.18*	74.33±11.11*
T.Cholosterol(mg/dl)	108.67±11.43	116.00±11.01	107.33±11.31	117.17±5.11
TGL(mg/dl)	134.17±6.18	137.50±6.09	133.83±11.30	131.83±6.82
HDL	39.00±6.13	43.67±2.80	43.50±3.78	44.67±8.09
LDL	42.83±13.86	44.83±11.39	32.33±11.83	50.83±5.53
VLDL	26.83±1.23	27.50±1.22	26.76±2.26	26.87.±1.64
ALP	83.50±11.45	80.67±16.56	74.67±17.67	81.00±16.48

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett's test.

Histopathology of Control group animals

Heart

Low Power Magnification 10X **High Power Magnification 40X**

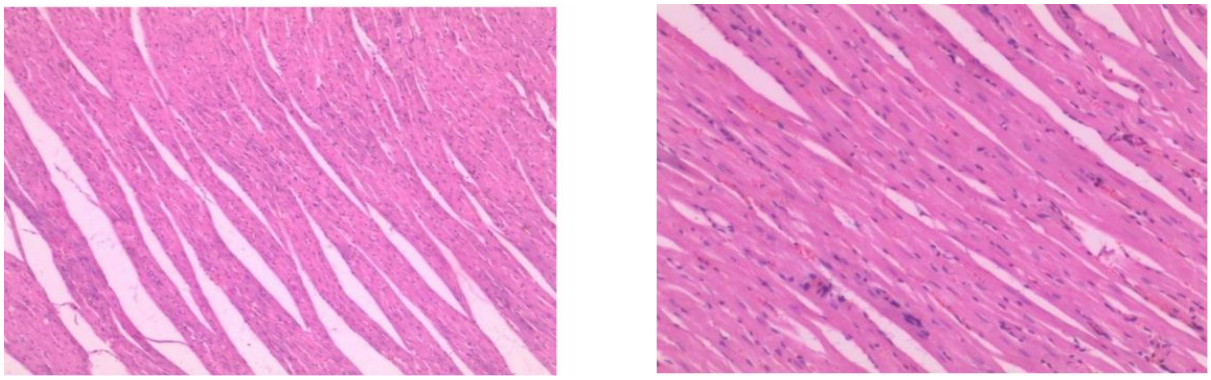

Figure 1.1

Kidney

Low Power Magnification 10X **High Power Magnification 40X**

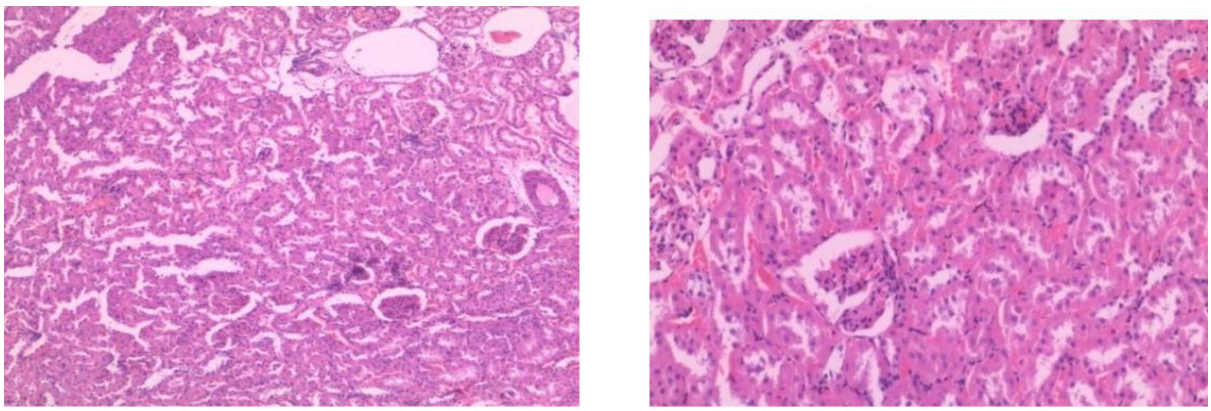

Figure 1.2

Liver

Low Power Magnification 10X **High Power Magnification 40X**

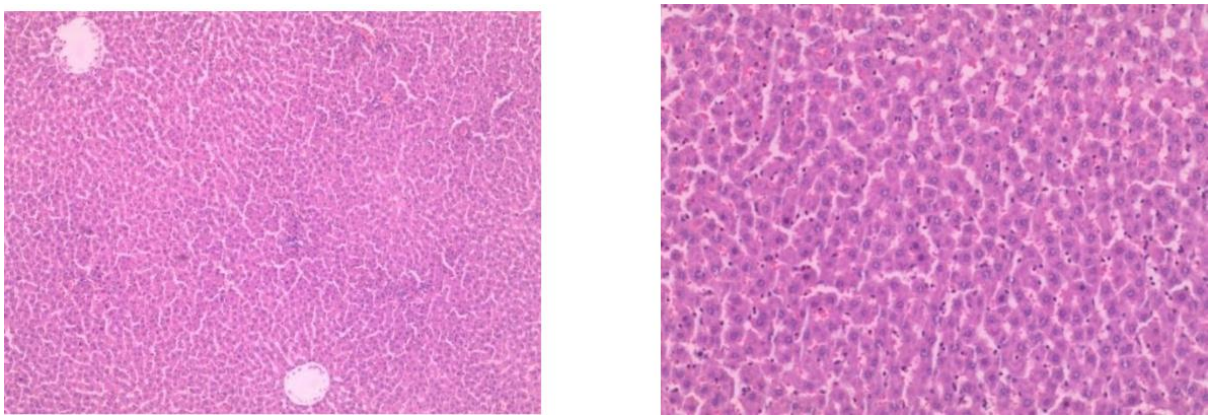

Figure 1.3

Lung

Low Power Magnification 10X **High Power Magnification 40X**

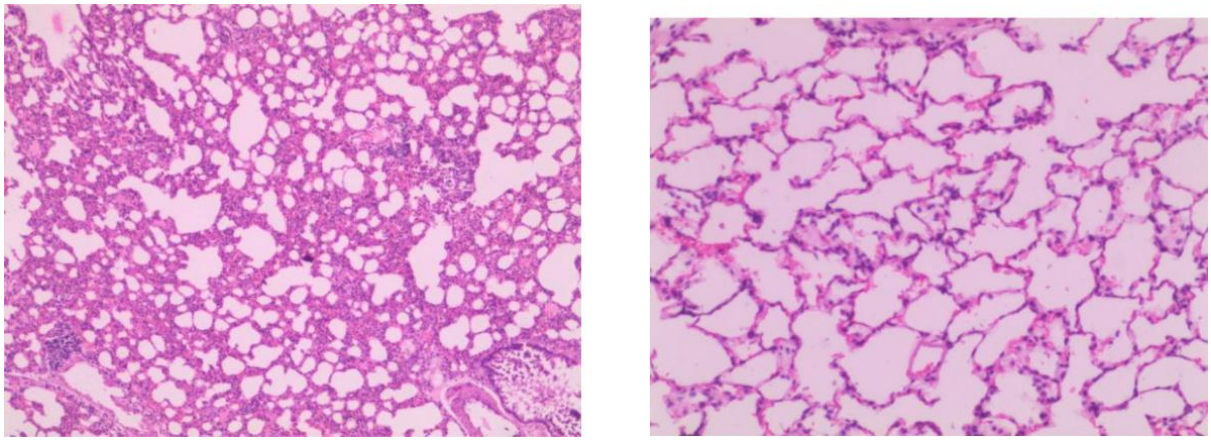

Figure 1.4

Brain

Low Power Magnification 10X **High Power Magnification 40X**

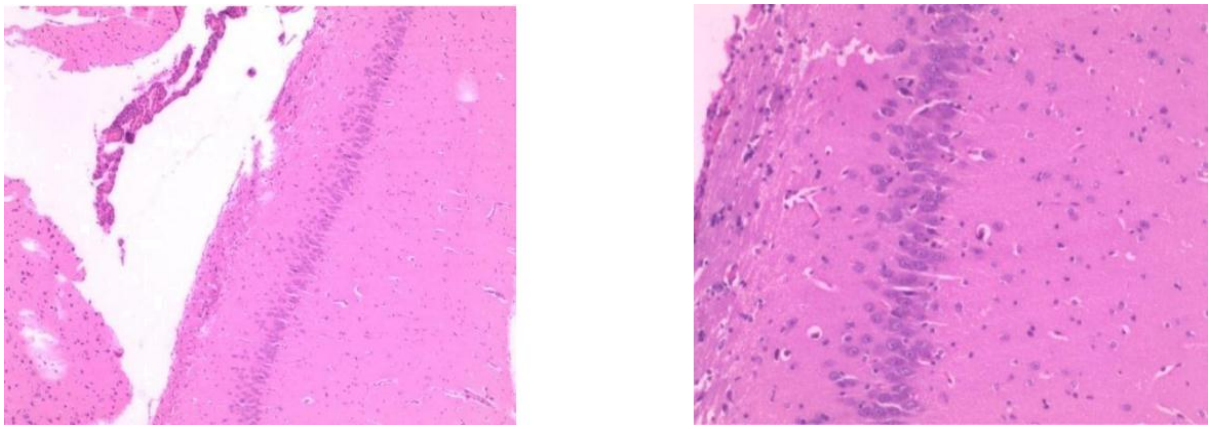

Figure 1.5

Histopathology of High dosed group animals

Heart

Low Power Magnification 10X **High Power Magnification 40X**

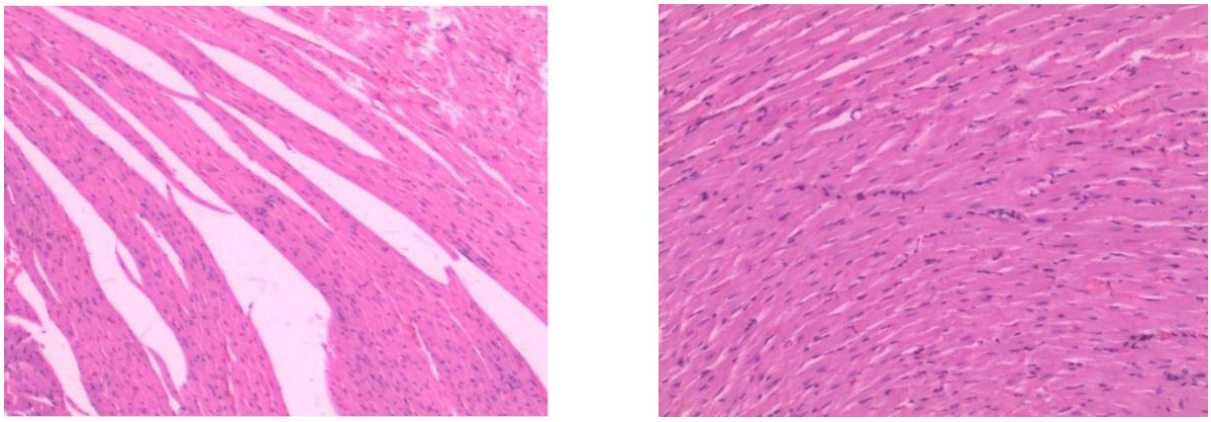

Figure 1.6

Kidney

Low Power Magnification 10X **High Power Magnification 40X**

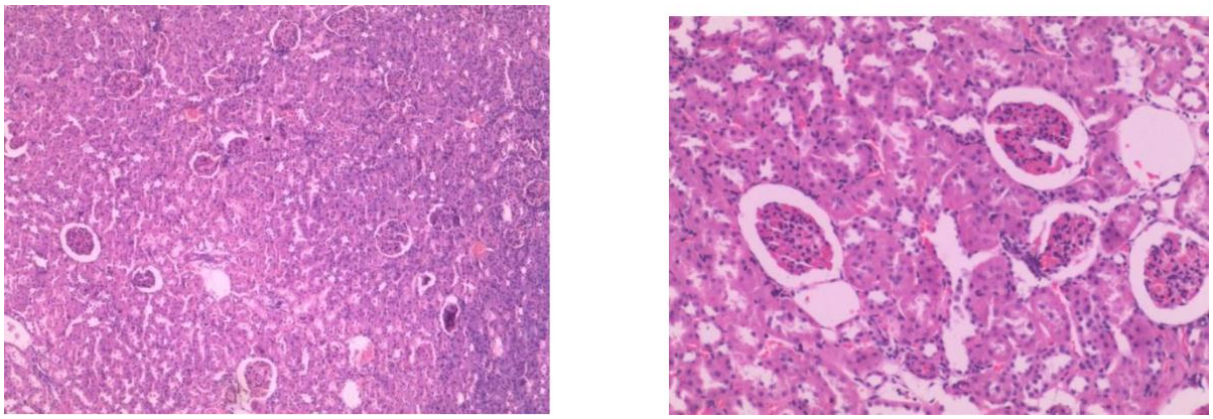

Figure 1.7

Liver

Low Power Magnification 10X **High Power Magnification 40X**

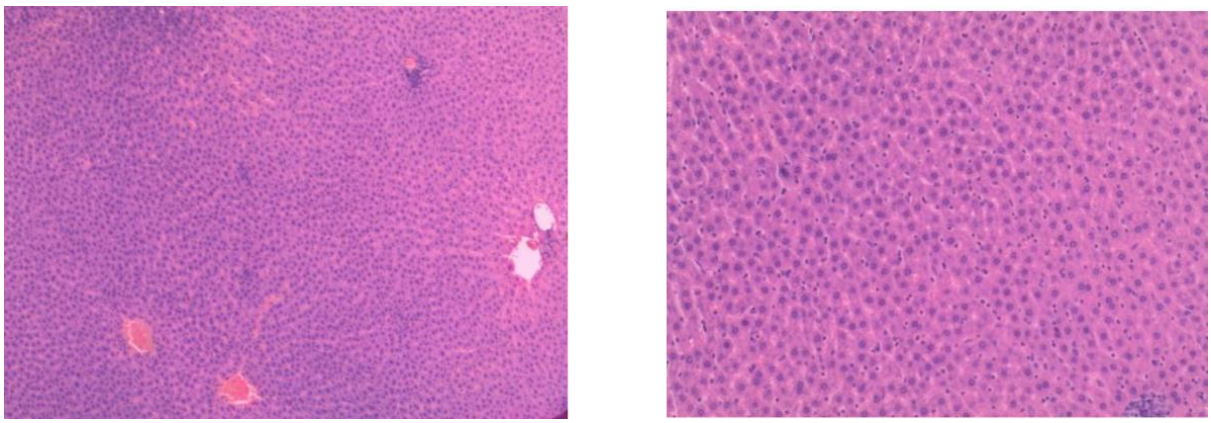

Figure 1.8

Lung

Low Power Magnification 10X **High Power Magnification 40X**

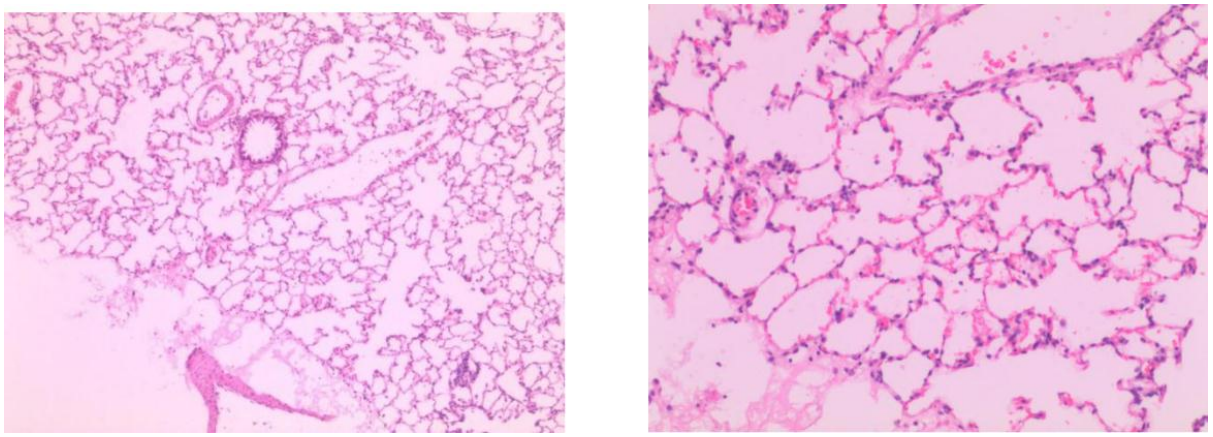

Figure 1.9

Brain

Low Power Magnification 10X **High Power Magnification 40X**

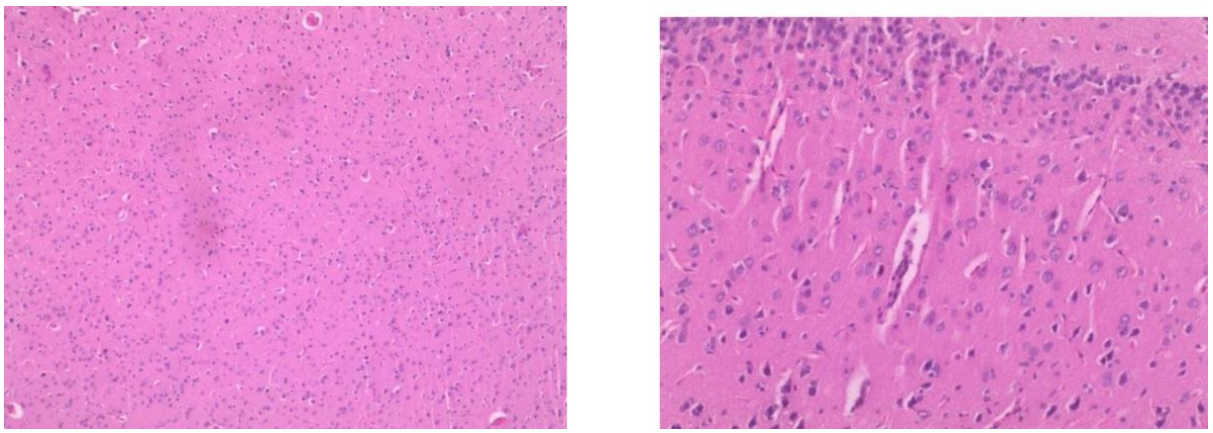

Figure 1.10

Interpretation of histopathology

Kidney: Appearance of glomeruli, tubules, interstitium and lumen was normal in both the samples with no signs of degeneration, interstitial connective tissue of both the sample appear normal.

Heart: Perfectly arranged myocardial fibers, clear transverse striation and normal structure were observed. Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear oval arrangement.

Liver: Hepatocyte appears with dark pigment chromatin in centri lobular and periportal region. Hepatic sinusoid and hepatic cord was normal.

Lung: Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis, perivascular region appears normal, Alveolar septa and wall appeared widen and normal No signs of lymphocyte cuffing.

No signs of airway secretion and bronchial secretion, Bronchial blood vessels and connective tissue appears normal with no signs of pulmonary edema.

Brain: Arrangement of the neurons appears intact with no signs of degeneration or apoptotic changes in both the samples, Cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm. No signs of ischemia or lesion were observed.

Body weight

The study shows overall weight gain throughout the study (Table 01) in both control and different dose group of *Seenthil chooranam*. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Haematological investigation interpretation

At the end of the study blood samples were collected for determining the blood count (Table 02) by Haematological analyzer the increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Biochemical investigation interpretation

The study results revealed there are no significant changes in the values (Table 03) of different parameters with that of the control. But sugar levels were reduced significantly. Other values were within the normal biological and laboratory limits.

Gross & Histopathology

Gross pathological examination for vital organs as liver, heart, kidneys, lungs and brain were removed at the end of the study in both control and treated groups. The organs. Carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.

DISCUSSION

The repeated 90 days oral toxicity study on rats for *Seenthil chooranam* were conducted. All animals from the treated dose survived throughout the dosing period of 90 days. Animals from treated groups and control group shows overall weight gain throughout the study period of 90 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

The haematological results shows no significant changes in the values when compared to control group. The biochemical results revealed there were no significant changes in the values of different parameters with that of the control. But sugar levels were reduced significantly when compare to the control group, other values were within the normal biological and laboratory limits

At the end of the study gross pathological examination of animals in control and treated groups did not reveal any abnormalities. The vital organs as liver, heart, kidneys, lungs and brain from the test group did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organs revealed normal histological appearance when compared with the control group.

CONCLUSION

In conclusion all animals from the treated dose survived throughout the dosing period of 90 days. There were no toxicologically significant changes in the body weight, haematological, biochemical and histopathological analysis this study. The study was concluded that the *Seenthil Chooranam* can be prescribed for the human with the dose of 2gm/ day and highly safety in chronic usage.

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