Toxicity Study on Siddha Herbal Formulation of Madakku Karisalai Thailam in Wistar Rats

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Abstract

Madakku karisalai thylam (MKT) is a herbal siddha medicine which is used especially for lung diseases. This study was performed to analyze the acute (72hrs) and subacute toxicity (28 days) in wistar rats. The studies were carried out as per the OECD guidelines. In acute oral toxicity, wistar rats were administered a single dose of MKT (2000 mg/kg) orally. The clinical signs, behaviour, body weight and mortality were observed for 14 days. In subacute toxicity study, the wistar rats were administered with MKT for once daily up to 28 days. Clinical signs, body weight, feed, water consumption, haematology, serology, histo-pathology, organ weight were observed. In administration of MKT there was no death or signs of toxicity developed in wistar rats at the dose of 2000 mg/kg b.wt throughout the study period. No gross pathological changes have been seen in the internal organs of both control and treatment group. This study validate MKT is safe for therapeutic dosage.

Keywords: Siddha, Madakku Karisalai Thylam, Toxicity Studies.

1. Introduction

Siddha system of medicine is one of the traditional method of treating diseases in India. Siddha medicine not only treat only the physical body of the people, it also refresh's the mind and soul. The journey of Siddha medicine was started by Siddhar's in ancient times. This system is based on spiritual disciplines and ancient treatment practices. Siddhar's used plants, animal and mineral for treating the diseases. They also described the toxic nature of the substance their used and antidotes for them.

Main focus of Siddha system is on "prevention, rejuvenation and promotion" of the body for maintaining the healthy life. According to this system, the human body is formed by five elements [8] i.e Earth, water, air, fire and space which is known as "*Panjabhootham"*. The *Pancheekaranam* theory of Siddha science explains the origination and formation of these 5 elements [7]. This theory proposes 96 basic factors (*96 thathuvagal*) [9]. Based on this only the person will be treated. The Siddha system of medicine consists of 32 types of internal medicines and 32 types of external medicines. In internal medicines, *Thailam* is one of the type among them. The shelf life of Thailam is 1 year. *MADAKKU KARISALAI THAILAM* is a one among the Siddha medicine. It is used for the treatment of lung disease especially for

Bronchitis, in present scenario toxicological evaluation is need to prove the safety of drugs. The major ingredient of Eclipta prostrata (karisalai) has a potent immune modulator [6], anti-hyperlipidemic [6], anti-inflammatory, antioxidant [6] and broncho dilatory activity due to triterpenoids. It is also effectively used as a rejuvenating plant in Siddha medicine.

2. Materials and Methods

Raw Materials

The Plant of Eclipta prostrata *(*Karisalai) was collected from Kanchipuram District and it was authenticated by Botanist, NIS, Chennai. Sesame oil (Ell Ennai*)* was bought from a well reputed cold press oil shop at Chennai.

Preparation of MKT

Karisalai was properly purified and the medicine was prepared as per Siddha literature said in "*Marundhu seiiyyalum kalaium"*. The final product was named as Madakku karisalai thylam (MKT) which was subjected to Acute toxicity and Sub-acute toxicity study [3].

Experimental Animals

Healthy adult rats of Albino, weighing between 140-160 g were used in this toxicity study. The rats were procured from the animal house attached in the Department of Laboratory Animal Science, The Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk colony, Chennai 600051. The study was performed in the animal house, National Institute of Siddha, Tambaram Sanitorium, Chennai. A 12 hrs of light and dark cycle were maintained. Room temperature was maintained between 22°C ± 2°C. The animals were provided with proper food and water. The animals were kept in 100% ventilated polypropylene cages. All the animals were acclimatized for 7 days before the commencement of the study. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC). Administration Dose of Madakku karisalai thylam was calculated as per the conversion of human dose to experimental animal dose.

Acute Toxicity Study of MKT

Total of 15 Wistar rats of female were selected for the study. These rats are divided into five groups of 3 rat in each group. Group 1 rats are considered as "Control group", Group II rats were administrated with MKT orally at the dose of 5mg/kg b. wt, Group III rats were given 50 mg/kg b.wt of dose, Group IV of rats were given 300 mg/wt b.wt and Group V rats were given 2000 mg/wt b.wt of dose [Table no 1]. Single dose of MKT was given orally. Clinical signs, changes in behaviour and mortality were observed and recorded for 14 days.[1][2]

Table No. 1

Groups	No. of Rat
Group I : Control	3 Female
Group II: MKT* – 5mg/Kg b.wt	3 Female
Group III: MKT*– 50mg/kg b.wt	3 Female
Group IV: MKT* – 300mg/kg b.wt	3 Female
Group V: MKT* – 2000mg/kg b.wt	3 Female

MKT*- Madakku karisalai thailam

28 Repeated Oral Toxicity Study of MKT

40 Wistar rats (weighing from 140-160 g) of male and female were selected as shown in Table 2. Group I were considered as control group not treated with therapeutic dosage. Group II, Group III, Group IV were administered MKT orally i.e., 270mg/kg of the body weight, 540mg/kg of body weight, 1080mg/kg of body weight respectively for 28 days [Table no 2]. All the animals are monitored for first four hours after drug administration and observed twice a day for 28 days. Weekly once, body weight of the animals were monitored. Food and water intake of the animals were calculated daily. At the end of the study, all the rats were sacrificed and observed results were noted [1][2].

Table No. 2

Groups	No. of Rats
GroupI: Control	**10(5 Male + 5 Female)**
Group II: MKT* –270mg /Kg b.wt	**10(5 Male + 5 Female)**
Group III: MKT* –540mg /Kg b.wt	**10(5 Male + 5 Female)**
Group IV: MKT* –1080mg /Kg b.wt	**10(5 Male + 5 Female)**
MKT*– Madakku karisalai thailam	

Statistical Analysis:

The mean value changes in the body weight, organ weight, intake of food and water, biochemical and haematological were analysed and calculated by using one way ANOVA test followed by Dunnett's test to compare the mean value between the control and test group of the animals which shows P value <_0.005. Hence it was considered as statistically significant.

3. Results

In acute toxicity study of MKT, No signs of abnormality or behavioural changes were noted in all animals at the dose level of 2000 mg / Kg body weight of *Madakku Karisalai Thailam*. No changes were detected in the skin, fur, eyes and mucous membranes of all animals. No mortality was observed after administration of *Madakku Karisalai Thailam* up to 2000 mg/kg body weight, indicating that the LD50 of *Madakku Karisalai Thailam* is more than 2000 mg/kg body weight.

Habits, such as eating, drinking, sleep pattern, locomotion, were normal in all animals and no changes in body weight as compared to control group. At the end of the 14th day, necropsy was performed in which no abnormality was observed in test groups as compared to control group during the examination. There was no significant difference in the body weight of female rats between control and test group, but mild increase in weight of all male rats in both the group.

In repeated oral toxicity, all the rats were active and does not show any mortality during the trial period until 28 days. Water intake of all the male and female test animals were gradually increased when compared with control groups ($p = 0.0758$), ($p = 0.3293$). Food intake of the all the male rats of both the control group and test group were gradually increased during the study period and there were no significant changes observed between control and test drug treated group (i.e, Low Dose, Mid Dose, High Dose). Food intake was increased in low dose test group female group at **$p < 0.05$ and High dose test group females at *$p < 0.01$ ($p =$

0.0029) when compared with the control female. [Table 3] indicates organ weight of the all-test male animals was normal in level. P = 0.9953, considered not significant. Organ weight of all the female animals was normal. P = 0.9878, considered not significant. [Table 4] shows the haematological parameters of all the test animals were normal level when compared with control group. (p = 0.9992). In biochemical parameter analysis, [Table 5] shows the renal function test i.e., BUN, Serum creatinine for all the test animals were normal when compared with control group of animals (p = 0.9975). [Table 6] indicates Liver function test i.e., SGOT, SGPT, Total Bilirubin, the parameters of all the test animals were normal level. (p = 0.9941). But in lipid profile [Table 7], the parameters of all the test animals were in normal level. P value is 0.9833 Hence it was considered as not significant. [FIG1,2,3,4,5,6,7,8,9,10] shows that the organs like liver, lung, spleen, stomach, brain, testis, uterus, heart, ovary were normal without any abnormalities.

Table No: 3 Organ Weight

ORGANS	CONTROL		MID DOSE		HIGH DOSE	
	Male	Female	Male	Female	Male	Female
Brain	1.68±0.148	1.02±0.083	2.1±0.316	0.94±0.116	1.5±0.223	1.29±0.162
Heart	0.62±0.148	0.48±0.083	0.84±0.114	0.57±0.043	0.7±0.148	0.51±0.073
Lungs	2.42±0.286	1.32±0.192	2.36±0.288	1.23±0.153	2.24±0.207	1.36±0.133
Liver	5.84±0.378	5.26±0.24	5.56±0.194	5.37±0.408	5.9±0.316	5.67±0.482
Spleen	0.44±0.114	0.41±0.135	0.52±0.13	0.47±0.033	0.66±0.207	0.42±0.065
Kidney	1.68±0.192	1.26±0.207	1.22±0.204	1.27±0.119	1.24±0.084	1.41±0.055
Testis	3.06±0.169	0.1±0.02	2.5±0.282	0.13±0.03	3.3±0.264	0.17±0.02

Table No:4 Hematological Parameters

BLOOD PARAMETERS	CONTROL GROUP	LOW DOSE	MID DOSE	HIGH DOSE
RBC ($\times 10^6$ µl)	6.1±1.1	6.18±1.21	6.25±1.14	6.32±0.62
WBC ($\times 10^6$ µl)	7.91±2.45	9.05±2.11	8.89±1.99	9.92±2.59
PLT ($\times 10^6$ µl)	606.9±133.1	563.2±110.8	672.2±176.4	612.9±262.9
HB (g/dl)	12.2±0.9	11.33±1.4	12.23±1.24	14.21±0.87
MCH (pg)	18.12±2.39	26.21±17.1	35±50.99	18.62±2.41
MCV (fl)	57.7±6.24	56.55±4.25	53.45±12.03	61.85±8.36
N (fl)	2.27±0.49	2.16±0.71	2.1±0.57	2.08±0.76
E (%)	1.14±0.29	1.32±0.31	2.45±3.71	1.5±0.37
B (%)	0.2±0.42	0.2±0.42	0.3±0.48	0.2±0.42
L (%)	82.75±11.99	68.32±11.06	76.25±12.86	78.5±10.53
M (%)	2.99±1.18	3.15±1.19	3.06±1.19	3.51±1.25

Table No: 5 Renal Fuction Test

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BUN	13.41±2.26	12.06±1.09	15.44±2.11	14.77±2.81
S. Cr	0.54±0.18	0.41±0.2	0.55±0.22	0.55±0.21

Table No: 6 Liver Function Test

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T. BIL	0.38±0.2	0.39±0.16	0.37±0.11	0.32±0.16
SGOT	87.6±25.4	76±16.9	76.2±15.6	65.5±15.6
SGPT	23.5±8	33.2±6.2	29.3±9.21	22.2±6.07

Table No: 7 Lipid Profile Test

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T. Chol	128.8±13.75	138.66±9.09	124.8±16.9	110.9±9.51
HDL	62.7±9.01	58.7±7.4	58.2±7.81	56.1±9.56
LDL	53.3±15.65	62.7±16.09	54.3±16.87	48±5.99
VLDL	16.24±1.97	15.5±2.3	15.53±3.63	15.54±1.39
TG	35.5±8.02	31.3±3.05	25.2±6.23	26.4±2.91

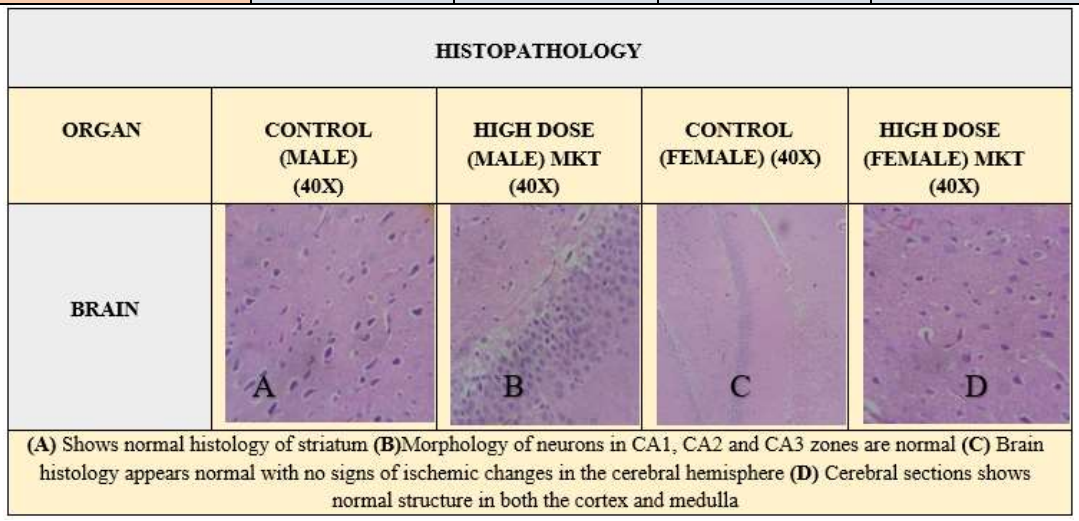


Figure : 1

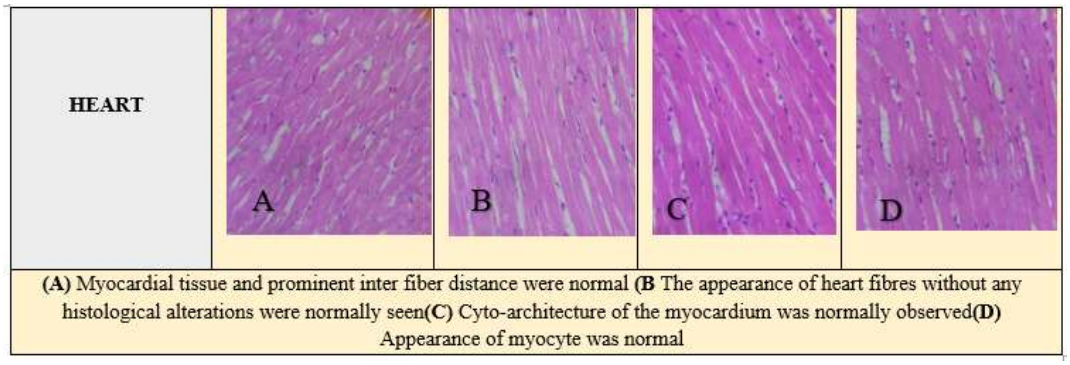
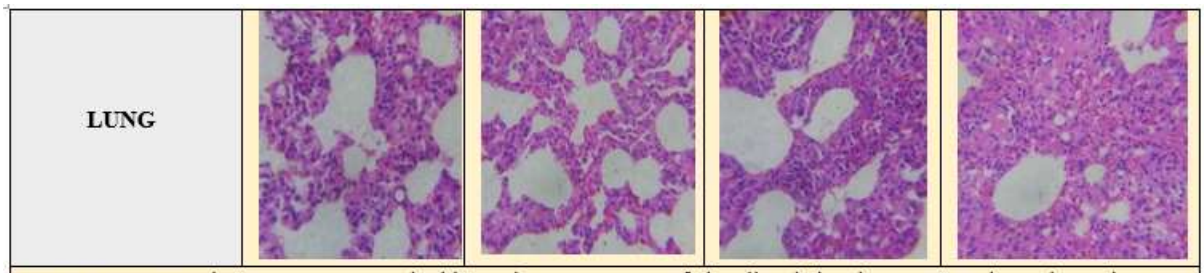
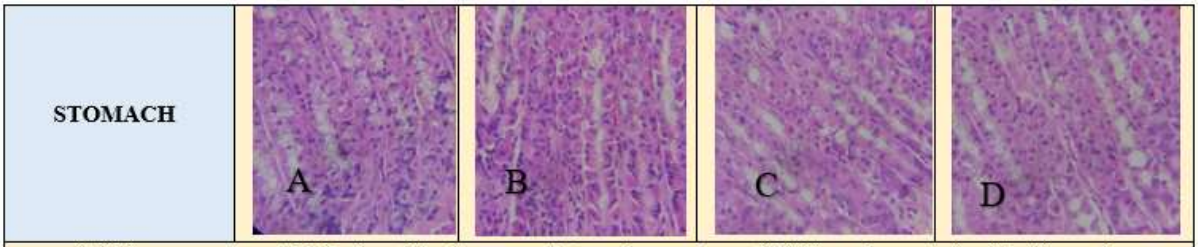


Figure : 2



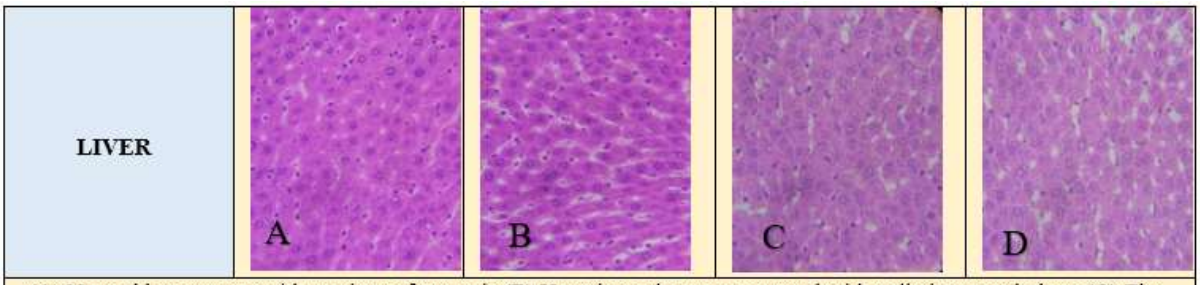
(A) Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac**(B)** Perivascular region appears normal, Alveolar septa and wall appeared widen and normal**(C)** Alveolar epithelium and capillaries appears normal **(D)** Alveoli with equidistant arrangement and prominent histology were normal

Figure : 3



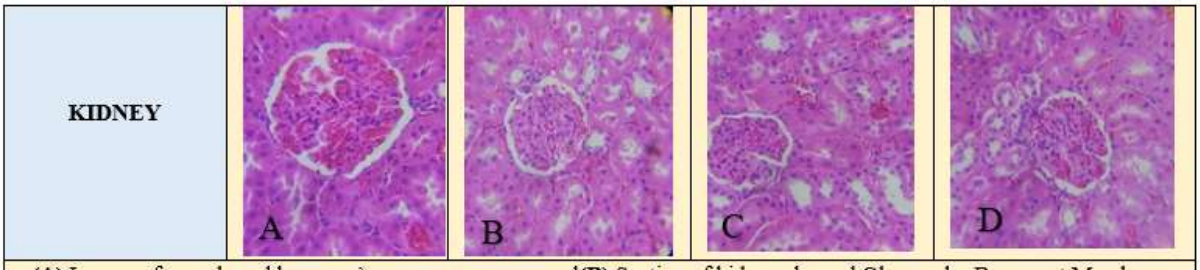
(A) The appearance of Pyloric and fundus zone of stomach were normal**(B)** Normal mucosal wall with regular arrangement of connective tissue were seen **(C)** Gastric epithelium and mucosa appear normal **(D)** Normal surface epithelium, mucosa and sub-mucosa

Figure : 4



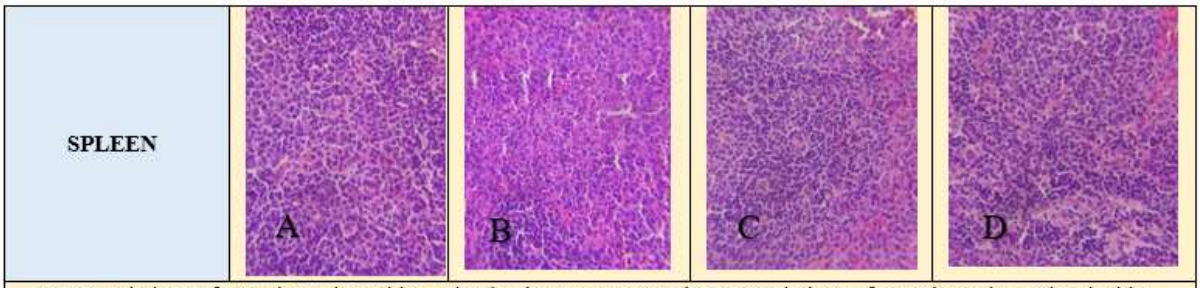
(A) Normal hepatocytes with no signs of necrosis **(B)** Hepatic cords appears normal with radiating morphology **(C)** The centrilobular hepatocytes appears normal with stained cytoplasm **(D)** Cytoplasm appears normal with widen portal tract

Figure :5



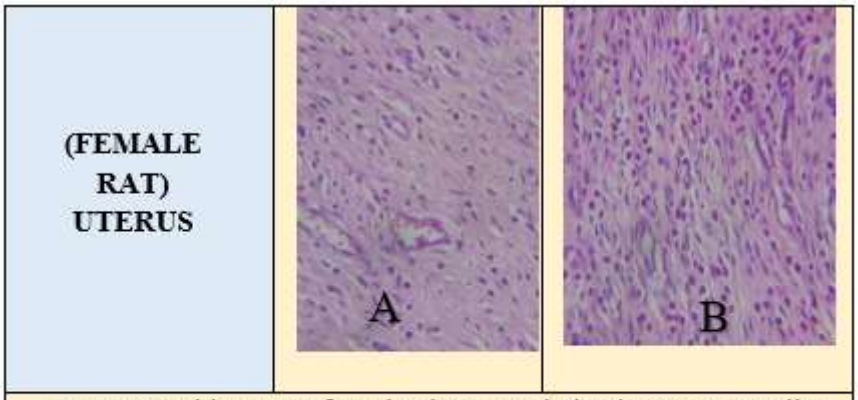
(A) Lumen of vessels and bowman's space appears normal**(B)** Section of kidney showed Glomerular Basement Membrane **(C)** Intact renal tubules as well as renal glomeruli were normal **(D)** Normal renal structure with rounded renal corpuscles formed of the Glomerulus and the Bowman's capsule

Figure : 6



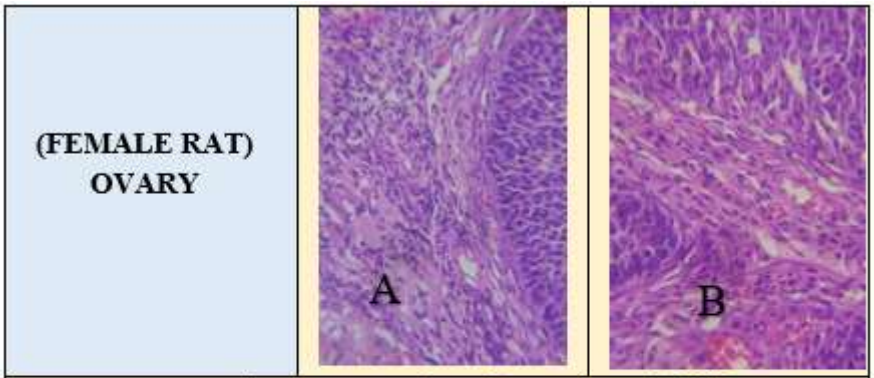
(A) Morphology of capsule, nodes, white and red pulp appears normal **(B)** Morphology of capsule, nodes, red and white pulp appears normal **(C)** Marginal zone at the interface of the red pulp with the periarterial lymphoid sheath (PALS) and follicles were observed clearly **(D)** Normal appearance of red pulp was seen which is composed of three dimensional meshwork of splenic cords and venous sinuses.

Figure :7



(A) Cyto architecture of uterine layers and glands were normally observed. **(B)** Endometrial stroma, glands, myometrium, perimetrium, lumen exhibits normal histological aspect of endometrium and myometrium

Figure :8



(A) Normal appearance of graafian and antral follicle waere observed **(B)** Section of ovary showing well follicular development, pre-ovulatory follicle surrounded by granulosa cells with normal appearance of zona pellucida and theca interna and externa.

Figure : 9

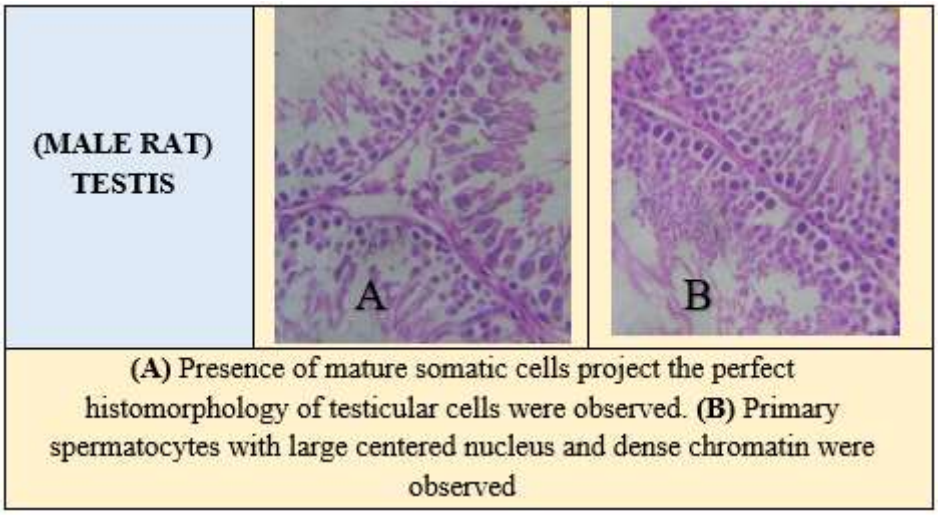


Figure : 10

4. Discussion

MKT is a siddha formulation having high potent therapeutic activity. The ingredients of the medicine are purified properly and prepared as per Siddha literature. The safety profile of MKT was evaluated, toxicity studies were performed for standardization. In acute oral toxicity the animals were treated with minimal dosage of MKT. The entire animals showed a normal condition up to the dose of 2000 mg/kg of the body weight without any abnormal signs and mortality throughout the study period. The animals were sacrificed at the end of the study, which shows no abnormality in gross pathology examination. Thus, the LD_{50} of Madakku Karisalai Thailam was more than 2000 mg/kg body weight. So according to GHS 5- Category hazard, the study medicine MKT can be classified under category V in which it includes chemicals with LD_{50}> 2000mg/kg.

Following completion of the acute oral toxicity study of MKT, 28-day oral toxicity study was carried out because multiple dose studies are useful in evaluating the safety of the study medicine. The determination of body weight during the period of toxicity study is of utmost importance, changes in the body weight is the indicator of adverse reaction of the trial medicine. In both acute and repeated oral toxicity study, there was no significant difference in abnormal body weight between the control and test group.

Water determination was important to maintain the rats' hydration throughout the study period. Improper intake of water may lead to dehydration which affects the health of the rat. There were no significant differences of water intake of the control and test group in this study.

Consumption of proper food indicates complete absorption of nutrients which is essential for physiological status of the animal, no significant changes of food intake occurred in all the male rats of control and treatment group. (Low dose, Mid dose and High dose). However, in female rats, the food intake gradually increased in low dose group and high dose group when compare to control group of females.

Haematological and biochemistry analysis are significant in determining the toxicity level induced by the drugs. Haematological parameters, includes RBC, WBC, Hb, MCH, MCV, Platelet etc., are widely used as a clinical indicator of physiological and pathological changes

that occurs in the body to rule out the diseases. Changes in the parameters helps in determining the side effects of the drug. In comparison with control group, haematological parameters of all animals in the test group were normal level. This shows that MKT does not affect any blood cells.

All animals in the test group were normal when compared with control group on renal function test, which indicates that the study drug does not affect the function of kidney.

Liver function test are used to diagnose and monitor liver diseases or damage. In determining the liver function test like Total bilirubin, SGOT, SGPT, the values were normal for all the animals. This proved that the medicine MKT did not affect the liver cells.

Lipid profile test is a blood test used to detect abnormalities of the lipids such as cholesterol, HDL, LDL, VLDL, Triglycerides. The lipid profile among the test group animals were normal when compared with the control group.

Organ weight analysis is important in toxicity studies for determining the harmful effects of test drug. It is the indicator of test drug effects in size, shape, function, deposition, necrosis of an organ. Organ weight of the treated group as compared to control group was not significantly different.

In histopathological examination of all the organ tissues, no abnormality was recorded. All the examined organs were normal in their structure without any changes. No toxicity signs were noted with respect to haematology, biochemistry, organ weight, gross and histopathology in MKT treated group.

In the previous study conducted on 2018, the alcoholic extraction of Eclipta prostrata - Karisalai (main herb in MKT) the minimum lethal dose when given orally was found to be greater than 2.0g/kg which shows no toxicity in rats and mice. [5]. According to the study conducted on 2016, Sesame oil (oil in MKT) the oral LD_{50} was greater than 5g/kg. Sesame seeds extract were not mutagenic with metabolic activation. Hence, the safety of sesame oil is concluded [4].

5. Conclusion

Acute toxicity study reveals that the siddha formulation MKT did not cause any change in the animals throughout the period of 14 days. The lethal dose (LD_{50}) of MKT is more than 2000mg/kg of body weight, hence it can be classified under category V of GHS hazard. No mortality was noted during the study period. Absence of significant abnormality in haematology, biochemistry, organ weight and histopathology of organ tissues. From the present study, it is concluded that MKT (Madakku Karisalai Thailam) is not toxic and hence it is safe to consume at therapeutic dose level.

Acknowledgments

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References:

[1] OECD Guidelines for the testing of chemicals section 4 Health effect Test no 423. Acute oral toxicity- Acute Toxic class method. Organization of economic corporative and development. Adopted 17-12-2001.

[2] OECD repeated oral toxicity test method in OECD guideline for the testing of chemicals No 407 Adopted 3-08-2008.

[3] Dr Deva asirvatham samuvel MD(s), Marundhu sei iyalum kalaium, Pg:No:1

[4] Mary Ann Liebert, Final report on the safety assessment of sesame oil, Journal of the American college of toxicology, volume 12, number 3 ,1993, pg no 269

[5] G.Bharathkumar, Acute toxicity of karisalai karpam in animal model, European journal of pharmaceutical and medical research, ejpmr, 2022, 9(10), 347-35

[6] Deepak Timalsina and Hari Prasad Devkota, Eclipta prostrata (L) L. (Asteraceae): Ethnomedicinalk uses, chemical constituents, and biological activities, Biomolecules. 2021 Nov 22; 11(11): 1738

[7] J Joseph Thas, Siddha medicine- background and principles and application for skin diseases, Clin Dermatol, 2008, pg no : 62-78

[8] Dr K S Uththamarayan, Thottrakirama aaraitchium siththa maruthuvamum, Thathuva araishi, pg no :125

[9] K.S.Murugesa Mudhaliyar, Siddha Materia Medica (Medicinal Plants Division),pg no:162,229