WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 2085-2095. Research Article ISSN 2277– 7105

LONG TERM ORAL TOXICITY STUDY OF *MIRUTHAR SINGI PARPAM* IN WISTER ALBINO RATS

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Article Received on
15 May 2015,

Revised on 07 June 2015,
Accepted on 29 June 2015

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ABSTRACT

Hemorrhoid (Piles) is one of the leading lifestyle disorders affecting most of the common people. In Siddha system of medicine, *MIRUTHAR SINGI PARPAM* (MSP), a herbo mineral (lead) preparation is mostly used for treating Hemorrhoids. Herbo-mineral formulations are gaining worldwide importance due to its nano form, increased bioavailability, minimal side effect and less dosage. We already evaluated the preparation, standardization and acute toxicity study (72hr) of MSP and so the present study was conducted to check the safety of this drug in the long term (90days) use. For that four different group of animals in which MSP was administered orally once a day at various doses (0.702 mg/kg, 3.51 mg/kg, and 7.02 mg/kg) and control animals received only saline water for 90 days. Throughout the experimental period no mortality was observed. Food and water intake of the experimental group animals were found in normal range. Hematological, biochemical parameters and histopathological evaluation of the organs was performed in all groups of animals. No impairment in Haemopoietic Lipid profile, Liver function and Kidney function was observed in study group animals. Histopathological study revealed that Heart, Lung, Stomach, Liver, Brain and Kidney organs did not show any signs of toxicity. MSP was found to be non-toxic in testing doses and hence a safe herbo-metal preparation for long term therapeutic use.

KEYWORDS: Siddha, Hemorrhoids, Long term, Toxicity.

INTRODUCTION

In Herbal Medicine, Siddha system has attained greater importance as an alternative to conventional therapy. Siddha system of medicine is an ancient medical system of Dravidian origin which is prevalent mainly in Southern parts of India, especially in Tamil Nadu. The drug sources are mainly obtained from plants, animal products, minerals and metals. They have been used extensively for many centuries in different forms for various conditions after thorough evaluation of the drug by traditional way. Siddha system emphasizes the dose regimen and pertinent vehicle for every medicine intake. Metals have been used as therapeutics since time immemorial. To optimize the safe use, 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.[1] To determine the safety and efficacy of these herbal metal medicines many screening methods were employed and established the active component in the products.[2] '*MIRUTHAR SINGI PARPAM*" (MSP) is one of the important formulations of metal herbal (Sulfide of Lead, Galena) drug preparation mentioned in *Anupoga Vaithiya Navaneetham* part –IV by Hakeem Abdullah Sayabu. Some lead compounds are experimental neoplastigens and tumorigens. Lead poisoning is one of the commonest of occupational diseases and it is a cumulative poison. Increasing amounts build up in the body and eventually reach a point where symptoms and disability occur. Sulfides have variable toxicity. Sulfides of the heavy metals are generally insoluble and hence have little toxic action except through the liberation of hydrogen sulfide. Galena based substances from plant sources has excellent clinical efficiency in cholelithic disease conditions.[3] MSP preparation, mode of processing, steps in standardization of the drug, phytochemicals screening, and acute toxicity study was already evaluated.[4] This medicine is indicated specially for Hemorrhoids. The prevalence of symptomatic hemorrhoids increased from 4.4% in the general population to 36.4%.[5] The pathophysiology is not completely understood other than the structural and vascular changes that is involved. Conservative treatment typically consists of increasing dietary fiber, oral fluids to maintain hydration, analgesics, sitz baths and rest. Increased fiber intake improves outcomes,[6] but conventional drugs sometimes cause serious side effects. To combat such critical anorectic problems, a comprehensive approach through alternative system has been extended with definite and a positive outcome. It is such a simple, safe and effective remedy for anal fistula and it is becoming universally acceptable day by day. The Indian Council of Medical Research (ICMR) has validated the unique and effective approach of alternative system.[7] However, the scientific validation of its safety in long term has not been established so far. So the

present study gives detailed information on the toxicological profile of *Miruthar singi parpam* by repeated oral toxicity studies in rats.

METHODOLOGY

EXPERIMENT ANIMAL

Wister albino rats of either sex weighing 100g – 200g were obtained from the animal house of King Institute of Preventive Medicine, Guindy, Chennai. Institutional ethical clearance for the present study was obtained (IAEC no-1243/ac/09/04-2010). All animals were kept in the standard environmental condition (23±2°C) with Relative humidity between 30% and 70% and standard light cycle (12hrs light, 12 hrs dark). They were freely allowed to have water and food (Pellet feed from Sai meera foods Pvt Ltd, Bangalore). Totally 40 rats were selected randomly and divided into four groups. They were identified by individual marking on fur with picric acid. Group I served as a control, contain 10 animals (5 per sex) other 3 test groups II, III and IV contain 30 animals that is each test group contain 10 animals (5 per sex) for treating up to 3 months. The females were nulliparous and non-pregnant. They were kept seven days prior to dosing to allow acclimatization to the experimental laboratory condition. Control group I animals received normal saline 10 ml/kg. Test dose for rats were calculated as per body surface area. Group II animals were administered with 0.702 mg/kg (X) of *Miruthar singi parpam* (MSP) while Group III and IV animals received 3.51 mg/kg (5X) and 7.02 mg/kg (10X) respectively.

Administration of Dose

Miruthar singi parpam was suspended in 10% tween 80 solutions in distilled water. The test drug was administered orally to animals at the dose levels X, 5X, and 10X. The test substance suspensions were freshly prepared every two days for 3 months. Animals were put to fast prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered .The control animals were administered vehicle only by oral (gavage). The toxicological effect was assessed on the basis of mortality. Administration was by oral gavage once daily for 90 consecutive days. Experimental animals were kept under observations throughout the course of study for the following: Clinical signs and mortality, body weight, food and water consumption.

Body weight changes

Initially, the body weights of the individual animals were recorded on the 1st day of the study before the administration of drug. Thereafter, at the end of the 2nd, 3rd, and so on up to 14th

week of the experimental period, animal weights were recorded and compared to that of control animals**.** Water consumption and amount of food intake per animal was calculated.

Haematological studies

At the end of treatment period, blood samples were collected by retro orbital puncture of all control and experimental rats in the heparinized test tubes and Hematological parameters were determined using Hematology analyzer.

Biochemical studies

In non heparinized tubes the blood was collected and centrifuged for 10 min at 3000 rpm. To analyze the liver enzymes and kidney function test the serum was separated. Bio chemical parameters were determined using auto analyzer.

Necropsy

All the animals were sacrificed at the end of the study under ether anesthesia. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, stomach, and lung were recorded.

Histopathology

Tissue samples of organs from control and treated animals at the dose level of X, 5X, 10X were preserved in 10% formalin. The organs including liver, kidneys, spleen, brain, heart, stomach, lung of the animals were preserved and they were subjected to histopathological examination. The organ pieces (3-5 µm thick) were fixed in 10% formalin for 24h and washed in running water for 24h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin**.**

Statistical analysis

Findings such as clinical signs of body weight changes, water and food consumption, and hematology and blood chemistry were subjected to One-way Anova followed by Post hoc test (Tukey HSD) using R statistical computer software.

RESULT AND DISCUSSION

Clinical signs

Animals showed no toxic clinical signs during the dosing period of 3 months.

Mortality

All animals of control group and all the treated dose groups survived throughout the dosing period of 3 months. There was no death of animal.

Body weight

Results of body weight determination of animals Table-1 of control and different dose groups was found to be comparable and significant changes were observed in the dose group of 10X ($P<0.05$) in 5^{th}, 6^{th} and 11^{th} week. However, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Table: 1 Body weight (g) changes of albino rats exposed to *Miruthar singi parpam*

	Weight (gms/rats)			
Weeks	**Control**	**X-(0.702mg/kg)**	**5X-(3.21mg/kg)**	**10X-(7.02mg/kg)**
1week	118.34±6.12	117.12±8.37	115.08±6.91	114.33±9.10
2week	115.88±9.18	119.32±8.24	118.52±9.83	117.37±12.91
3week	119.88±7.10	118.0±8.12	123.0±8.00	123.0±10.11
4week	120.33±5.16	121.66±9.83	120±14.14	123.33±13.66
5week	119.12±9.18	123.34±12.50	122.16±10.75	126.00±14.78*
6week	123.31±7.10	124.43±9.10	125.50±9.34	128.35±9.91*
7week	129.0±10.88	128.0±10.87	130±12.00	129.0±8.16
8week	125.0±9.18	126.0±10.41	128±11.13	128.0±10.38
9week	127.31±10.88	128.43±10.10	126.50±11.34	128.35±12.91
10week	129.02±11.13	128.43±17.0	126.50±18.4	127.5±12.91
11week	118.34±6.22	119.0±7.04	121.0±10.95	124.0±6.36*
12week	123.33±13.66	121.32±8.24	124.43±9.10	125.37±12.12
13week	129.33±61.6	131.66±83	130.0±4.14	132.33±3.66

*Values are expressed as mean □□SD (One way ANOVA followed by TukeyHSD test).*P<0.05, **P<0.01, ***P<0.001 vs. control; N=6*

Food consumption

During dosing period, the quantity of food (Table-) consumed by animals in different dose groups was found to be comparable and not significantly changed with that by control animals.

Table2. Food (g/day) intake of albino rats exposed to *Miruthar singi parpam*

	Food (gms/rats)			
Weeks	**Control**	**X-(0.702mg/kg)**	**5X-(3.21mg/kg)**	**10X(7.02mg/kg)**
1 week	35.51±2.87	39.79±3.68	38.46±2.40	39.30±2.17
2 week	39.98±3.18	38.00±2.11	41.66±3.46	41.2±2.09
3 week	36.64±2.00	36.34±2.57	39.79±3.68	39.80±2.88
4 week	38.12±2.80	38.41±2.47	41.98±3.00	39.44±2.50
5week	39.56±3.10	40.30±3.39	39.98±3.18	40.45±2.99
6week	38.12±2.80	39.00±2.68	40.00±3.48	40.5±6.90
7 week	37.98±2.18	38.00±2.11	39.66±3.46	40.2±2.4
8week	39.98±3.18	40.68±3.54	41.33±6.05	42.16±4.91
9week	36.12±2.80	38.41±2.77	40.98±3.00	39.44±3.60
10week	39.12±2.80	38.41±2.77	39.98±3.00	39.44±3.60
11week	37.12±2.80	38.00±2.68	38.68±3.48	40.5±6.90
12week	38.98±3.18	39.00±2.11	37.66±3.46	38.2±2.08
13week	37.12±2.18	39.41±2.57	40.98±3.08	39.44±3.63

*Values are expressed as mean □□SD (One way ANOVA followed by TukeyHSD test).*P<0.05, **P<0.01, ***P<0.001 vs. control; N=6*

Water consumption

Water consumption by the animals in different dose group was within normal levels throughout study period and found no changes significantly with control group.

Table3. Water (ml/day) intake of albino rats exposed to *Miruthar singi parpam*

	Water (ml/day)			
Weeks	**Control**	**X-(0.702mg/kg)**	**5X-(3.21mg/kg)**	**10X(7.02mg/kg)**
1week	48.20±3.80	54.23±3.21*	50.16±2.46	49.11±2.68
2week	47.10±2.54	43.14±2.40	46.30±3.08	47.38±2.30
3week	45.10±2.15	46.19±2.08	44.37±3.10	48.10±2.08
4week	41.0±3.02	46.68±2.58	47.67±2.00	46.11±2.19
5week	41.0±3.02	45.60±2.40	44.22±2.39	44.15±3.66
6week	46.42±2.18	43.1±2.33	45.33±2.16	46.0±2.31
7week	45.10±3.74	43.30±4.42	47.18±3.10	43.88±2.49
8week	45.2±3.27	42.18±4.10	47.31±5.15	45.10±4.00
9week	44.51±2.30	46.2±3.27	48.33±4.08	44.16±2.04
10week	45.07±3.20	44.16±4.34	47.00±3.44	44.67±4.18
11week	41.0±3.02	45.60±2.40	44.22±2.39	44.15±3.66
12week	39.00±2.11	39.00±2.11	37.66±3.46	38.2±2.08
13week	42.2±3.27	38.48±2.54	37.93±3.45	38.88±3.91

*Values are expressed as mean □□SD (One way ANOVA followed by TukeyHSD test).*P<0.05, **P<0.01, ***P<0.001 vs. control; N=6.*

Hematological investigations

Table shows the result of hematological parameters of various dose group animals which was conducted on day 91 and this revealed that there was a significant (P<0.05) increase in the RBC count in the dose group of 10X. WBC count also have been found a significant (P<0.05) increase in the same dose group of animals. The haemopoietic system serves as important target for toxic chemicals and is a sensitive index for pathological conditions both in humans and animals.[8] In the present study, treatment with MSP did not produce any alteration in haematological parameters (i.e. RBC, WBC, haemoglobin, haematocrit etc.), which indicate that MSP did not affect blood cells nor their production. Changes in clinical biochemistry and hematological parameters holds significant role in determining the toxicity induced by drugs.[9]

Table4. Hematological investigations of albino rats exposed to *Miruthar singi parpam*

Parameters	Control	X-0.702 mg/kg	5X-3. 51 mg/kg	10X-7.02 mg/kg
Red blood cell (x10^6/mm3)	4.00±0.19	3.95±0.16	4.22±0.62	4.53±0.66*
HB (%)	12.2±10.6	13.4±.5	12.4±.9	13.4±.6
Leukocytes (x10^6/mL)	8432±978	8486 ±1807	8647±2670	9036±1354*
Platelets/uL	2.5180 ±8273	2.4083±0.2886	2.6688±0.5380	2.5125±.0566
MCV (fl)	88±5	92±5	91±2	92±3
DLC (%)				
P (%)	57±4	57±5	58±5	59±3
L (%)	35±4	34±5	33±6	35±6
E (%)	5±3	7±1	7±6	8±2
PCV (%)	42±4	42±6	44±3	42±4
BLOOD SUGAR (R)	66.4±3.6	64.8±2.3	65.7±5.4	66 .8±3.2

*Values are expressed as mean □□SD (One way ANOVA followed by TukeyHSD test).*P<0.05, **P<0.01, ***P<0.001 vs. control; N=6*

Biochemical Investigations

Biochemical investigations in Table 5a, 5b, 5c show that there was no significant change in lipid profile, liver function test and Renal function test in all experimental groups of animals when compared to the control group. Transaminases (AST and ALT) are good indicators of liver function status and biomarkers to predict the possible toxicity of drugs.[10] Any elevation pertaining to these enzymes indicate their outflow into the blood stream due to damage in liver parenchymal cells. There were no changes in the AST and ALT levels which reveal that MSP did not affect liver function or metabolism. Serum electrolytes such as sodium, potassium and chloride were found to be normal between the experimental groups. Similarly,

the serum electrolyte levels were found to be well within the clinical range of rats which reflects that MSP has no adverse effect on ionic homeostasis.

Table 5a: LIPID Profile of albino rats exposed to *Miruthar singi parpam*

Dose (mg/kg)	Control	X-0.702 mg/kg	5X-3.51 mg/kg	10X-7.02mg/kg
Total cholesterol(mg/dL)	57.49±6.0	57.88±2.99	58.85±1.81	57.67±1.35
HDL(mg/dL)	20.26±1.78	20.75±0.41	20.54±0.72	20.00±0.68
LDL(mg/dL)	23±7	23±8	21±4	23±6
VLDL(mg/dl)	7.22±1.04	7.88±0.34	7.32±0.65	7.94±0.14
Triglycerides (mg/dl)	1276±18	126±16	125±26	128±16

*Values are expressed as mean □□SD (One way ANOVA followed by TukeyHSD test).*P<0.05, **P<0.01, ***P<0.001 vs. control; N=6*

Table5b: Liver Function Test of albino rats exposed to *Miruthar singi parpam*

Dose (mg/kg)	Control	X-0.702 mg/kg	5X-3.51 mg/kg	10X-7.02 mg/kg
Total Bilirubin (mg/dL)	0.8±0.2	0.7±0.1	0.8±0.3	0.8±0.4
Bilirubin direct (mg/dL)	0.15±0.2	0.18±0.01	0.15±0.04	0.14±0.02
Bilirubin indirect(mg/dL)	0.29±06	0.28±06	0.28±04	0.33±03
ALP (U/L)	7.4±0.4	7.5±0.7	7.6±0.6	7.4±0.4
SGOT (U/L)	68±24	68±23	69±16	67±14
SGPT(U/L)	76±30	74±26	76±24	75±20
Total Protein(g/dl)	7.0±0.8	7.5±0.3	7.4±0.5	7.1±0.4
Albumin(g/dl)	3.7±0.4	3.6±0.4	3.8±0.3	3.8±0.3
Globulin(g/dl)	2.3±0.5	2.4±0.3	2.2±0.1	2.6±0.3
GGT(U/L)	15±2	16±1	14±1	14±2

*Values are expressed as mean □□SD (One way ANOVA followed by TukeyHSD test).*P<0.05, **P<0.01, ***P<0.001 vs. control; N=6*

Table5c. Renal Function Test of albino rats exposed to *Miruthar singi parpam*

Dose mg/kg	Control	X-0.702 mg/kg	5X-3.51mg/kg	10X-7.02mg/kg
Urea(mg/dL)	29±14	28±4	29±7	30±6
Creatinine (mg/dL)	0.74±0.26	0.76±0.8	0.75±0.10	0.78±0.12
Na m.mol	143.12±7.30	141.2±4.38	144.10±4.10	141.15±4.18
K m.mol	139.12±7.30	141.2±4.38	143.10±4.10	141.15±4.18
Cl m.mol	21.60±2.84	19.45±1.50	20.7±1.20	19.25±2.18

*Values are expressed as mean □□SD (One way ANOVA followed by TukeyHSD test).*P<0.05, **P<0.01, ***P<0.001 vs. control; N=6*

Organ Weight

Group Mean Relative Organ Weights (% of body weight) are recorded in Table-6. Organ weight of the study group animals treated with the dose level of X, 5X, 10X did not change

significantly during the experiment period when compared with control group at the end of the study.

Table6. Organ Weight of albino rats exposed to *Miruthar singi parpam*

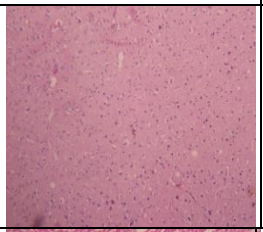
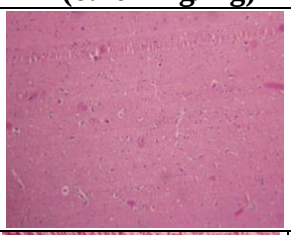
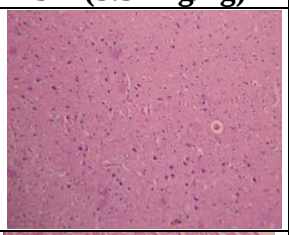
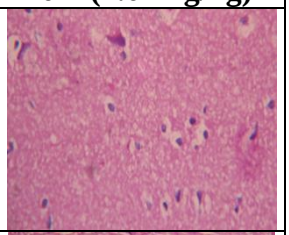
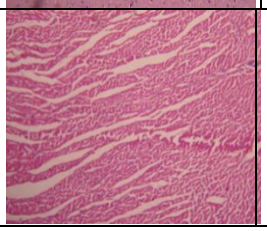
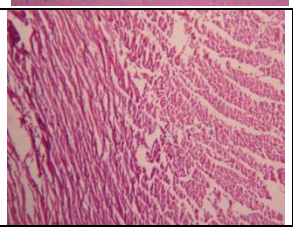
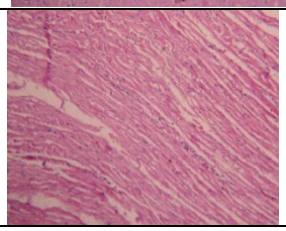
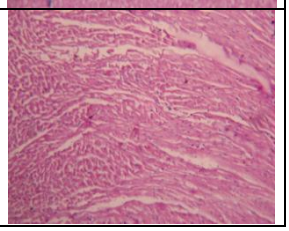
Dose mg/kg	Control	X-0.702 mg/kg	5X-(3.51mg/kg)	10X-(7.02mg/kg)
Brain (g)	1.60±0.18	1.62±0.15	1.59±0.14	1.60±0.15
Liver (g)	5.01±0.15	4.98±0.11	4.96±0.14	5.04±0.20
Heart (g)	0.63±0.05	0.62±0.04	0.64±0.05	0.62±0.54
Spleen (g)	0.63±0.07	0.64±0.05	0.62±0.06	0.63±0.06
Stomach (g)	1.41±0.10	1.38±0.07	1.42±0.08	1.39±0.10
Kidney (g)	0.71±0.05	0.72±0.04	0.70±0.04	0.69±0.05
Lung (g)	1.15±0.05	1.14±0.18	1.16±0.26	1.15±0.19

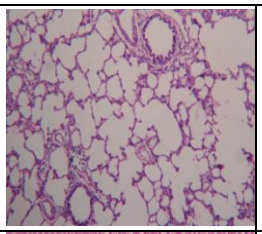
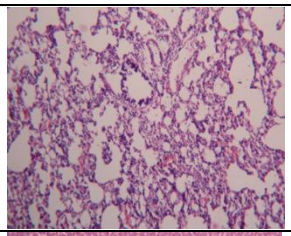
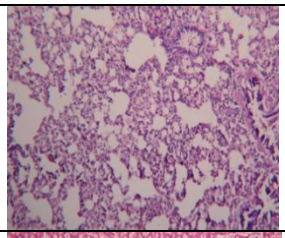
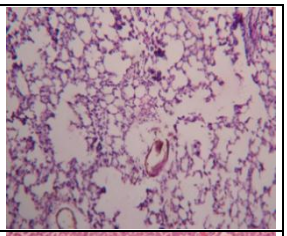
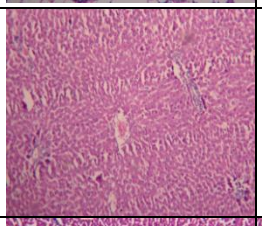
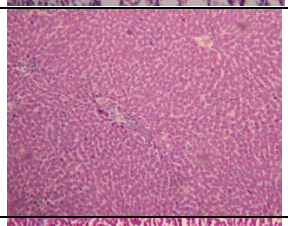
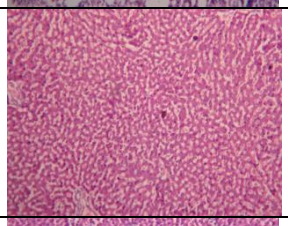
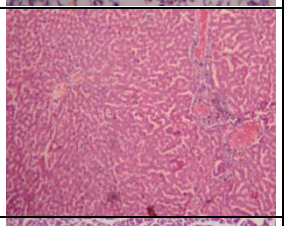
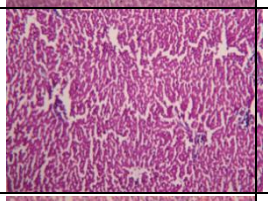
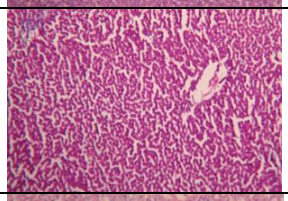
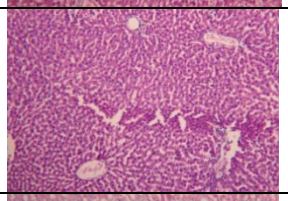
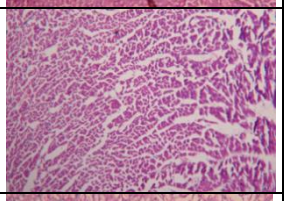
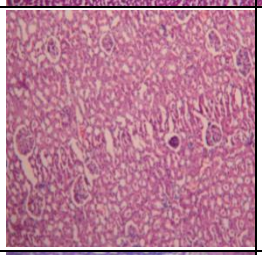
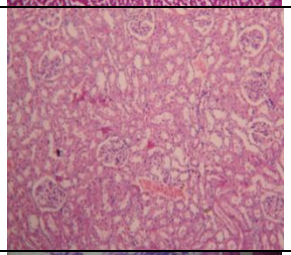
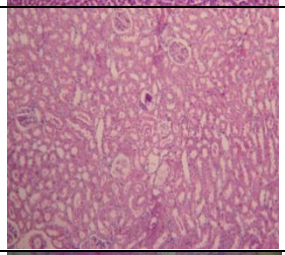
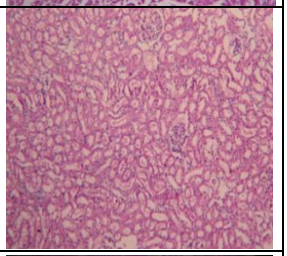
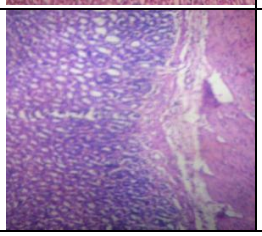
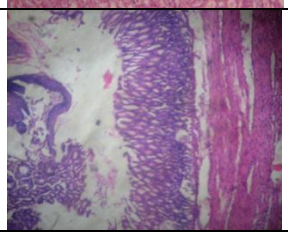
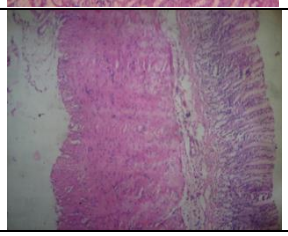
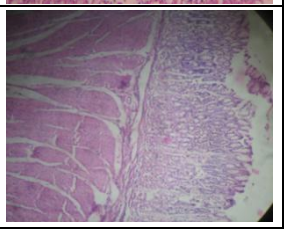
Values are expressed as mean □□SD (One way ANOVA followed by TukeyHSD test).*P<0.05, **P<0.01, ***P<0.001 *vs.* control; N=6

Histo-Pathological study

Gross pathological examinations of animals in dose level of X, 5X, 10X (Figure 1) do not reveal any structural abnormalities. Histo-pathological studies provide supportive evidence for biochemical and haematological observations.[11] To elucidate the safety of the medicine this analysis is very important. Chronic usage of Lead affect the brain and kidney in humans, but the histopathology report of current studies in animals reveal that medicine does not affect the organs like kidney and brain when compared with control. Analysis on spleen, lung, heart, liver, and stomach in experimental group of animals does not indicate any abnormality.

Figure1: Histo Pathological Analysis of *Miruthar singi parpam*

Tissues	Control	X-(0.702 mg/ kg)	5X-(3.51mg/kg)	10X-(7.02mg/kg)
Brain				
Heart				

Lung				
Liver				
Spleen				
Kidney				
Stomach				

CONCLUSION

The present study shows that the herbo metal siddha preparation of MSP does not induce any toxic manipulation on experimental animals. Dosage which used in animals was five times higher than prescribed level for human. From these we can infer and hypothesize that this drug is nontoxic in nature and can be used as a therapeutic agent in treating the reported diseases effectively.

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