Section: Anatomy

Original Research Article

HISTOPATHOLOGY, BEHAVIOURAL AND QUALITATIVE ANALYSIS BY SPECTROMETRY OF POORA PARPAM - AN INDIGENOUS MERCURY CONTAINING SIDDHA DRUG

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Abstract

Background: Poora Parpam is one of the notable medicines in treating arthritis, syphilis, and jaundice. Thus, we have evaluated the toxicity profile and acute and long-term toxicity studies of the drug "POORA PARPAM" on an animal model. **Materials and Methods:** We have collected, authenticated, and purified Poora Parpam. We have prepared an extract and tested the acute and long-term toxicity of "poora parpam" in an animal model. We have performed liver and renal function tests and histopathological analyses. **Result:** After HR SEM analysis, the particle size of the Poora Parpam was 1-10 µ (micron). In the Acute toxicity study, no abnormal signs developed in Swiss albino mice at ten times more than the therapeutic dose level (0.216 mg/animal) within 24 hrs. At the end of the study, no mortality and reduction in body weight of control and test group animals were observed. The Long-term toxicity study showed no significant changes in behavioural signs, haematological parameters, biochemical investigation, body weight, food, and water intake. The histopathological study on the organs such as the heart, lungs, kidney, brain, and liver was normal in X and 5 X groups compared with the control group. In the 10 X group, only the brain showed mild edema with separated glial cells, and the other organs showed no abnormal histological variation. **Conclusion:** Acute and long-term toxicity of Poora Parpam is less toxic. Hence further studies on Poora Parpam will be conducted for its scientific validation and global acceptance.

INTRODUCTION

The Siddha system of medicine is one of the oldest medical systems in India. "Siddha" comes from the word "Siddhi", which means an object to be attained perfection. Siddhi generally refers to Astamaa Siddhi. Those who attained or achieved the above-said powers are known as Siddhars. Siddha's system concludes body, as a whole, is made of five elements: the fundamental principles of creation, protection, and destruction. This unparallel knowledge and uncommon development which the Siddhars had attained in this branch of science from the very ancient times are reflected in their works as Vatham (Alchemy), Vaidhiyam (Treatment), Yogam, and Gnanam (Spirituality).[1-6]

Siddhars are specialized in the treatment of poisons. Their experience speaks from the way of diagnosis, describing the signs and symptoms of poison in humans. The literature survey reveals their vast knowledge in treating poison, including metal, mineral, herbal and food. They also specified general antidotes for all kinds of poison. According to Siddha literature, Poora Parpam is given to all types of Vathadisease, Gunmam (peptic ulcer), Soothaga nominal, and Neerilivu (diabetes mellitus).[4-9]

To this day, arthritis is one of the lifestyle diseases commonly affecting the elderly. In the incidence of arthritis among the age group 25 to 35, osteoarthritis is the second most prevalent disease after diabetes. And these growing populations with arthritis are to be treated with appropriate medicine. According to Siddha literature, Poora Parpam efficiently manages

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arthritis and its related complications.[3,5,7-9] In this aspect, Poora Parpam may be a better choice of drug. Since modern society is against using mercurial drugs as medicine, this study is more important. We have evaluated the toxicity profile and acute and long-term toxicity studies of the drug "POORA PARPAM" on an animal model.

MATERIALS AND METHODS

The raw drug was collected from a country drug merchant shop in Chennai. The raw drug Pooram was authenticated by Siddha Central Research Institute, Chennai. For purification, Piper betel leaves and Pipernigrum seeds were ground and made into a poultice. Then one litre of water was taken in a mud pot, and the poultice was mixed. The raw drug Pooram in cloth was suspended in the decoction. The vessel was constantly heated till decoction reduced by three fourth of its volume. Finally, the Pooram was removed from the cloth, washed with clean water, and dried in sunlight.

5g of sample was taken in 250 ml of the clean beaker, and 50 ml of distilled water was added. Then it was boiled well for about 10 min. Then it is allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation is used for the qualitative analysis of acidic and basic radicals.

Quantitative analysis was done at SAIF, IIT Madras, including HR SEM and experimental procedure.

Acute and long-term toxicity studies were carried out on Poora Parpam. The toxicity studies were evaluated after getting permission from The Institutional Animal Ethical Committee clearance. (1248/ac/09/CPCSEA/04/IAEC2011).

Acute toxicity was carried out in Swiss albino mice of either sex with a single exposure of 10 times more than the recommended therapeutic dose of the test drug. The study duration was 14 days. The study was carried out as per the WHO guidelines.

Test animals were obtained from The King Institute, Chennai, and kept at the animal house, National Institute of Siddha, Chennai. All the animals were kept under standard environmental conditions (22 ± 3˚c). The animals had free access to water, and a standard pellet diet (Sai Meera foods Pvt. ltd, Bangalore)—the principles of laboratory animal care were followed.

The animals were randomly divided into two groups. Each group consists of 10 animals (5 per sex in each group). The first group was kept as a control, and another group was treated with a test drug. The animals were allowed an acclimatization period of 7 days to laboratory conditions before the initiation of treatment. The females were nulliparous and non-pregnant. By cage number, animal number, and individual marking on fur with picric acid, animals were identified.

The animals were housed in polypropylene cages provided with bedding of husk. Dark and light cycle each of 12 hours was maintained. The oral route was selected because it is the normal route of clinical administration. The Poora Parpam is white-coloured, without taste and odour. The test substance is insoluble in water, and the drug is dissolved in 10% aqueous tween 80 solutions to obtain and ensure uniformity in drug distribution.

Poora Parpam was suspended in 10% aqueous tween 80 solutions with uniform mixing, and it was administered to the test group in a single oral dose. The control groups received an equal volume of the vehicle. The animals were weighed before giving the drug. The dose level was calculated according to body weight and surface area. Since the clinical dose was 12 mg/day, it was converted to an animal dose (0.216mg/animal) and then administered to the test group animals.

Observations were made and recorded systematically and continuously as per the WHO guideline after test drug administration. Animals were observed individually for the first 4 hours and then periodically during the study. Mortality and abnormal signs were observed during the study period. All the animals were sacrificed at the end of the study, and an autopsy was done. The individual weight of animals was determined before the test drug administration and daily for 14 days.

S. No	Groups	No of Rats
1	Vehicle control	6 (3 male, 3 female)
2	1X Therapeutic dose (0.1296 mg/animal)	6 (3 male, 3 female)
3	5X Therapeutic dose (0.648 mg/animal)	6 (3 male, 3 female)
4	10X Therapeutic dose (1.296 mg/animal)	6 (3 male, 3 female)

The long-term toxicity study was carried out for one month because the clinical duration of human consumption of the test drug was eight days. The long-term toxicity study was conducted at different dose levels 0.1296 mg/animal, 0.648 mg/animal, and 1.296 mg/animal. The selected doses were calculated according to the rat's body weight and surface area. The human therapeutic amount of Poora Parpam is 12mg/day.

Poora Parpam has been suspended in an aqueous tween 80 solutions (10%). It was administered to groups at dose levels of X therapeutic dose (0.1296 mg/animal), 5X therapeutic dose (0.648 mg/animal), and 10X therapeutic dose (1.296 mg/animal). The control animals were administered vehicle only. The administration was given orally using an oral gavage once daily for 30 days.

Experimental animals were observed throughout the study and recorded for the various parameters. All animals were observed twice daily for mortality during the entire study period. The weight of each rat was recorded on the first day, at weekly intervals throughout the study and the end of the study. From the data, mean body weights and percent, body gain

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was calculated. The quantity of food and water consumed by animals of different doses was recorded weekly. Food and water consumed per animal were calculated for the control and the treated dose groups.

At the end of the study, the blood samples were collected by cardiac puncture using a syringe. The collected blood samples were kept in vacutainer blood samples were centrifuged at 3000 rpm for 10 minutes, and collected serum was used for laboratory investigations. Renal and liver function tests were performed.

All the animals were sacrificed under ether anaesthesia at the end of the study. Necropsy of all animals was carried out, and the morphological changes of organs, including liver, kidneys, brain, heart, and lungs, were noted.

Tissue samples of organs from control and treated animals were preserved in 10% formalin to prepare sections using a microtome. The animals' organs, liver, kidneys, spleen, brain, heart, and lungs were preserved. They were subjected to histopathological examination.

The organ pieces (3 to 5 microns) were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in a tissue processor and then cleaned in benzene to remove absolute alcohol. Embedding was done by passing the cleared sample through three cups containing molten paraffin at 50˚c, then a cubical block of paraffin made with L moulds, followed by microtome slicing and mounting on the slides. The slides were stained with Haematoxylin –Eosin stain.

Findings such as biochemical parameters were subjected to one-way ANOVA followed by Dunnett's "t" test using a computer software programme-INSTATV3 version

RESULTS

Behavioural signs of acute toxicity in Swiss albino mice treated with Poora Parpam were tested. It indicated the presence of alertness, grooming, gripping strength, touch responses, pain response, pinna reflex, corneal reflex, pupillary size, normal skin colour, and absence of mortality in control. In contrast, alertness, grooming, gripping strength, touch responses, restlessness, pain response, defaecation, pinna reflex, corneal reflex, pupillary size, normal skin colour, and absence of mortality were reported in acute dose (216mg/ animal) group [Table 1].

Table 1: Behavioral signs of Acute toxicity study in Swiss albino mice treated with Poora Parpam

Treatment group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Control	+	–	–	+	+	+	–	–	–	+	–	+	+	+	–	–	–	–	N	A
Acute dose (216mg/ animal)	+	–	–	+	+	+	+	–	–	+	+	+	+	+	–	–	–	–	N	A

1. Alertness, 2. Aggressiveness, 3. Passivity, 4. Grooming, 5 Gripping strength, 6. Touch responses, 7. Restlessness, 8. Tremors, 9. Convulsion, 10. Pain response, 11. Defaecation, 12. Pinna reflex, 13. Corneal reflex, 14. Pupillary size, 15. Lacrimation, 16. Salivation, 17. Urination, 18. Writhing, 19. Skin colour, 20. Mortality;

2. + Presence of activity, N - Normal, - Absence of activity, A - Absent

After HR SEM analysis, the particle size of the Poora Parpam was analyzed as 1-10 µ (micron). In the Acute toxicity study, no abnormal signs developed in Swiss albino mice at ten times more than the therapeutic dose level (0.216 mg/animal) within 24 hrs. At the end of the study, no mortality and reduction in body weight of control and test group animals were observed. The Long-term toxicity study showed no significant changes in behavioural signs, haematological parameters, biochemical investigation, body weight, food, and water intake. The lymphocyte count was increased in test groups, but it was not statistically significant compared with the control group. The histopathological study on the organs such as the heart, lungs, kidney, brain, and liver was normal in X and 5 X groups compared with the control group. In the 10 X group, only the brain showed mild edema with separated glial cells, and the other organs showed no abnormal histological variation.

Then, we performed a renal function test of Wister rats in a long-term toxicity study treated with PooraParpam, which indicated a non-significant difference between the control and experimental groups [Table 2].

Table 2: Renal function test of Wister rats in long-term toxicity study treated with Poora Parpam

Parameters	Control	X - group	5x - group	10x - group	P-value (p)*
UREA (mg/dl)	28 ± 16	26 ± 3	28±7	30 ± 6	N.S
CREATININE(mg/dl)	0.76 ± 0.25	0.69 ± 0.10	0.66 ± 0.09	0.72 ± 0.12	N.S
URIC ACID(mg/ dl)	2.51 ± 0.20	2.57 ± 0.36	2.38 ± 0.50	2.43 ± 0.37	N.S
CALCIUM(mg/ dl)	8.8 ± 0.8	8.9 ± 0.8	8.7 ± 0.6	9.1 ± 0.6	N.S
POTASSIUM(mg/dl)	2.7 ± 0.4	2.7 ± 1	2.6 ± 0.5	3.1 ± 0.6	N.S

Further, the liver function test of Wister rats in a long-term toxicity study treated with Poora Parpam indicated a non-significant difference between the control and experimental group [Table 3].

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Table 3: Liver Function Test of Wister rats in long-term toxicity study treated with Poora Parpam

Parameters	Control	X - group	5x - group	10x - group	P-value (p)*
T.BILIRUBIN(mg/ dl)	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	N.S
D.BILIRUB IN(mg/dl)	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	N.S
I. BILIRUB IN(mg/ dl)	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	N.S
SGOT(U/ dl)	67 ± 25	70 ± 23	58 ± 16	57 ± 16	N.S
SGPT(U/ dl)	77 ± 31	86 ± 27	66 ± 23	62 ± 22	N.S
ALP(U/ dl)	132 ± 6	142 ± 25	138 ± 8	134 ± 20	N.S
T.PROTEIN(mg/ dl)	7.0 ± 0.9	6.7 ± 0.3	6.8 ± 0.5	7.1 ± 0.4	N.S
ALBUMIN(mg/ dl)	3.3 ± 0.6	3.4 ± 0.4	3.2 ± 0.3	3.3 ± 0.2	N.S
GLOBULIN(mg/ dl)	3.7 ± 0.3	3.3 ± 0.2	3.6 ± 0.5	3.7 ± 0.3	N.S

Histopathology

Histopathological analysis of HEART for Plate. a. CONTROL sections show normal myocardial fibers and blood vessels, Plate.b. X group section shows normal myocardial fibers and blood vessels, Plate.c. 5X group section shows normal myocardial fibers and blood vessels, and Plate.d. 1OX group section of the heart shows myocardial fibers with congested blood vessels and haemorrhage.

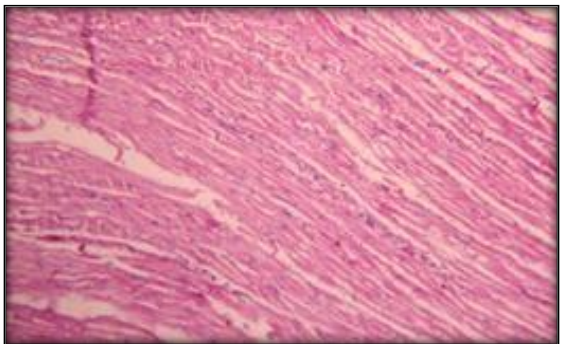

Plate. a. Heart -CONTROL - Normal

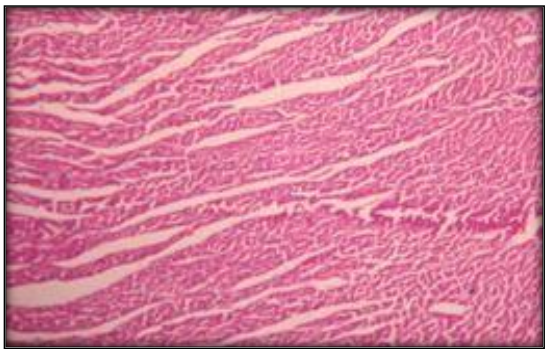

Plate. b. Heart -X group-Normal

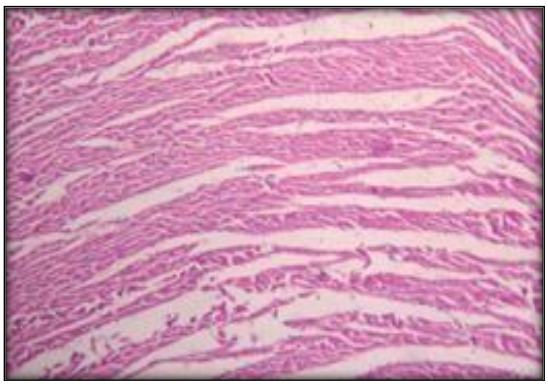

Plate. c. 5X group - Normal

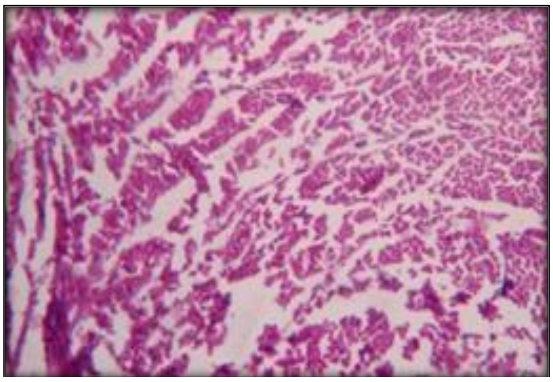

Plate.d.10X group showing normal myocardial fibers with Congested blood vessels and haemorrhage

Further, histopathological analysis of the KIDNEY for Plate. a. CONTROL specimen shows renal parenchyma with normal appearing cortex and medulla, Plate. b. X group specimen shows renal parenchyma with normal appearing cortex and medulla and Plate.c. 5X group specimen shows renal parenchyma with normal appearing cortex and medulla. However, Plate. d. 10X group section from the kidney shows congested glomeruli. Tubules appear normal. The interstitium shows congested vessels with an area of haemorrhage.

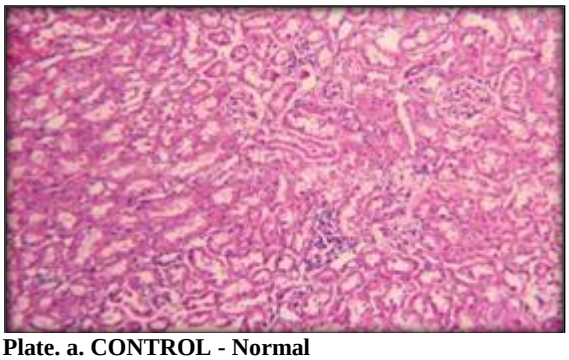

Plate. a. CONTROL - Normal

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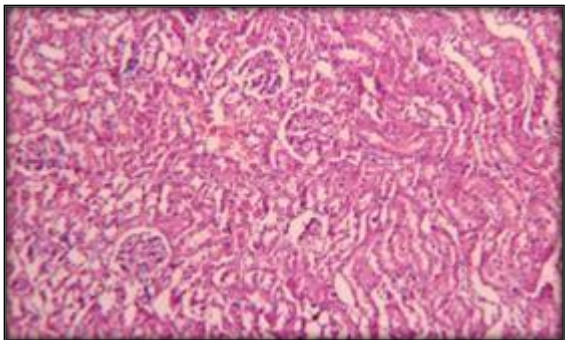

Plate. b. X group - Normal

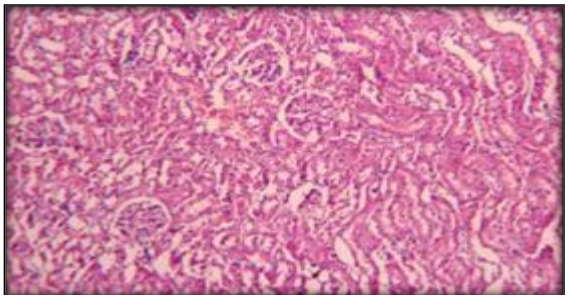

Plate. c. 5X group - Normal

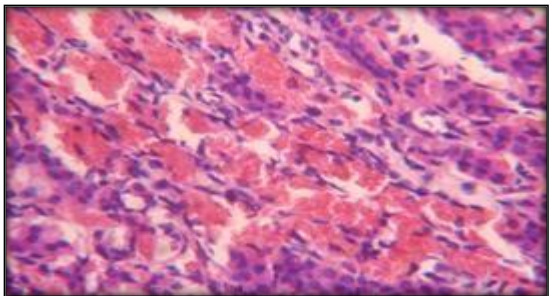

Plate.d. 10X group showing congested blood vessels and a haemorrhage

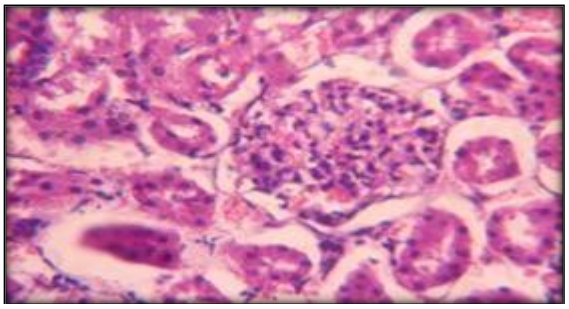

Plate.e.10X group - high power - showing congested glomeruli

Histopathological analysis of LIVER for Plate.a. CONTROL section from the liver shows normal-appearing hepatocytes, sinusoids, kupffer cells, and portal triad. Plate.b. X group section from the liver shows normal-appearing hepatocytes, sinusoids, kupffer cells, and portal triad, Plate.c. 5X group section from the liver shows normal-appearing hepatocytes, sinusoids, kupffer cells, and portal triad, and Plate.d. 10X group section shows normal-appearing hepatocytes, sinusoids, sinusoids, kupffer cells, and portal triad.

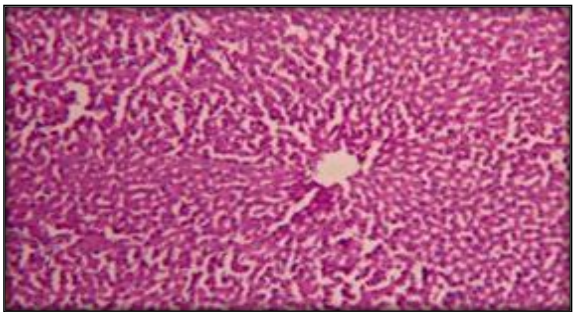

Plate. a. CONTROL - Normal

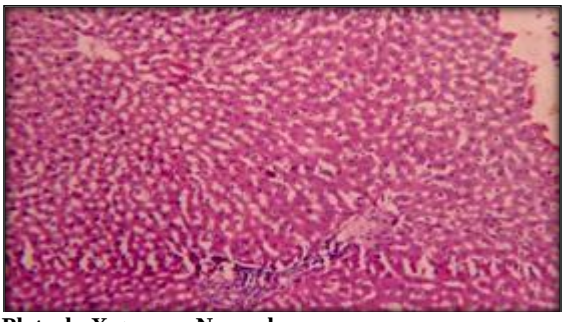

Plate. b. X group - Normal

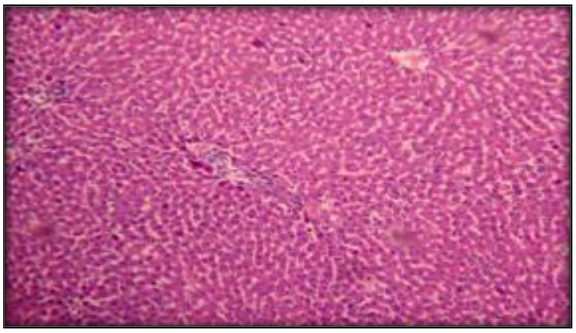

Plate.c.5X group - Normal

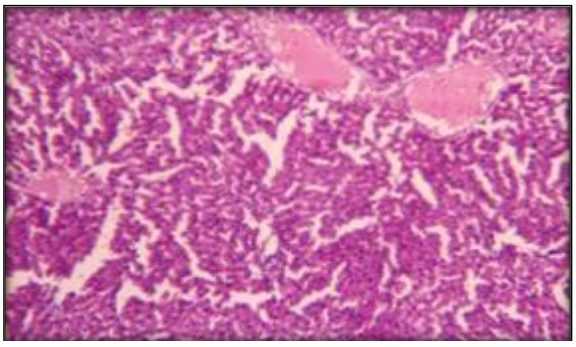

Plate.d.10X group - Normal

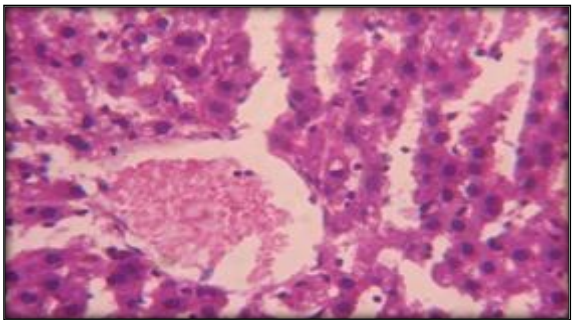

Plate.f.10X group high power showing normal portal a triad with radiating sinusoids

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Histopathological analysis of LUNG for Plate .a. CONTROL section from the lung shows normal-appearing bronchioles, alveoli, interstitium, and blood vessels. Plate .b. X group section from the lung shows normal-appearing bronchioles, alveoli, interstitium and blood vessels, Plate .c. 5X group section from the lung shows normal-appearing bronchioles, alveoli, interstitium, and blood vessels, Plate .d. 10X group section shows bronchioles, alveoli, interstitium with dilated, congested blood vessels. Focal lymphoid aggregations are present

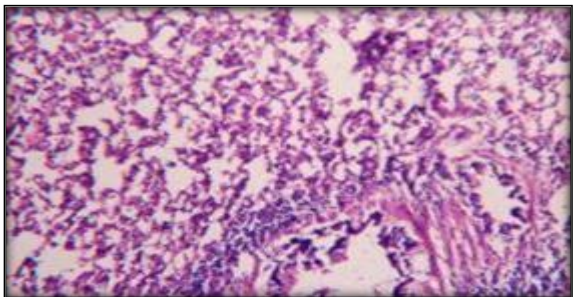

Plate. a. CONTROL - Normal

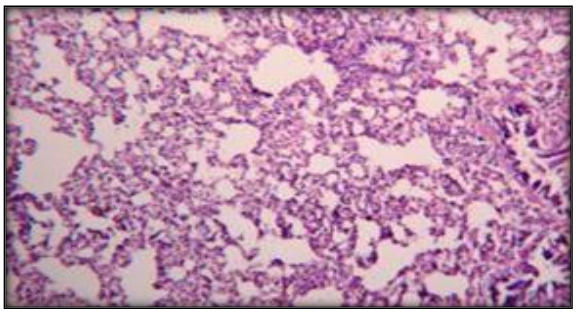

Plate. b. X group - Normal

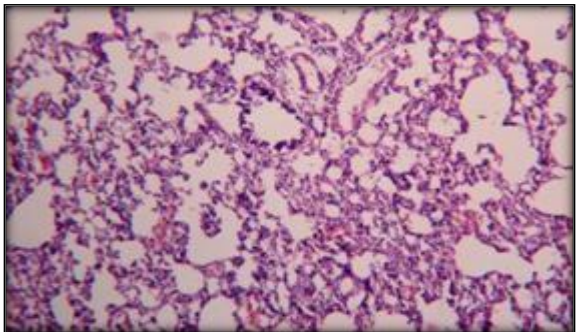

Plate. c .5X group - Normal

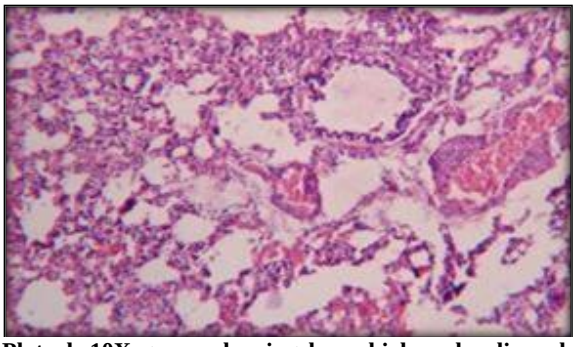

Plate.d. 10X group showing bronchioles, alveoli, and interstitium showing dilated, congested blood vessels. Focallymphoid aggregations were present

Histopathological report of the BRAIN for Plate .a. CONTROL sections show the normal cerebral parenchyma and cerebellum structure. Plate .b. X group section shows the normal structure of cerebral parenchyma and cerebellum—plate .c. 5X group section shows the normal structure of the cerebral parenchyma and cerebellum. Plate.d. 10X group section of the brain shows mild edema in the cerebral cortex with separated glial cells.

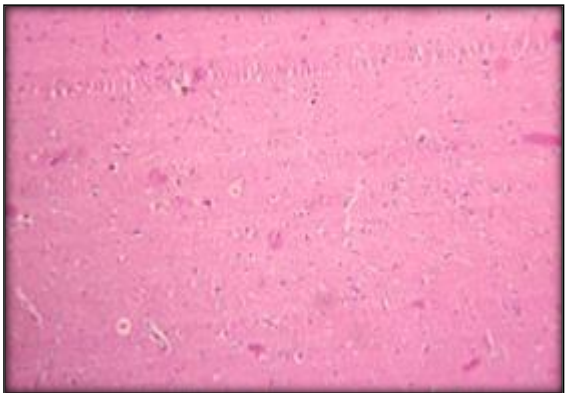

Plate. a. Control group - Normal

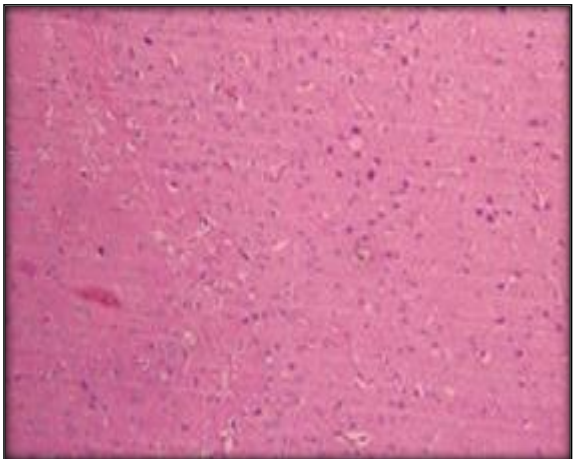

Plate. b. X group - Normal

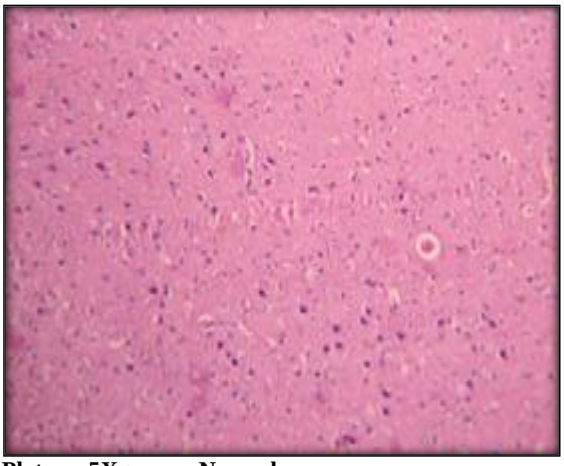

Plate. c. 5Xgroup - Normal

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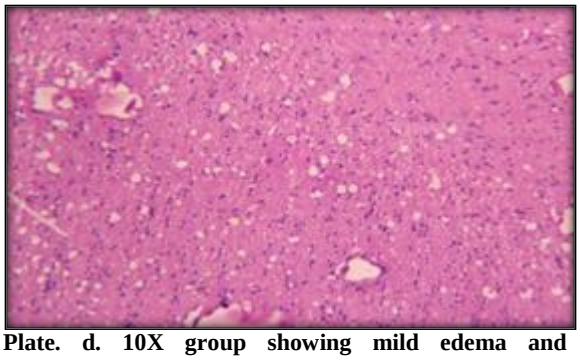


Plate. d. 10X group showing mild edema and separated glial cells

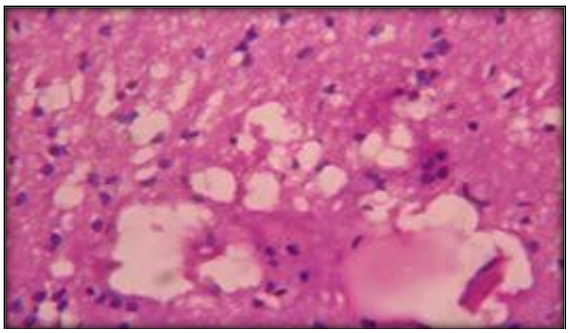
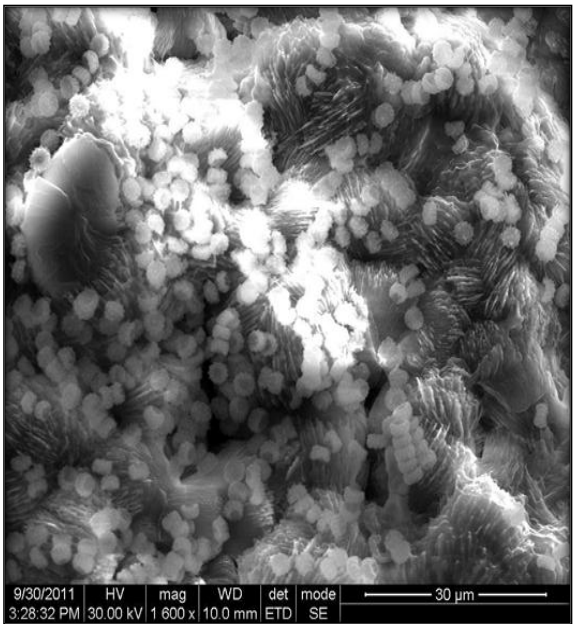


Plate. e. 10X group - high power – Showing edematous cerebral cortex with separated glial cells

Quantitative analysis: HR SEM Analysis - Determination of particle size of Pooram Parpam



As of Poora Parpam, the particle size ranges from 1 to 10. (micron). The Parpam has a uniform distribution of particles. The particles' flat surface makes it simple for the gastrointestinal tract to absorb them. As a result, the medicine will have a higher bioavailability.

Table 4: Inductively coupled plasma optical emission spectrometry

Elements	Wavelength in nm	Unpurified Pooram (ppm)	Purified Pooram (ppm)	PooraParpam (ppm)
Arsenic	As193.696	BDL*	BDL*	BDL*
Calcium	Ca 317.933	18.654	16.425	15.856
Cadmium	Cd 226.502	BDL*	BDL*	BDL*
Mercury	Hg 253.652	204.175	98.251	3.187
Iron	Fe 238.204	1.856	1.658	1.356
Potassium	K 766.490	47.853	46.522	45.426
Sodium	Na 589.592	20.689	19.853	17.952
Phosphate	P 213.617	11.751	10.856	9.784
Lead	Pb 230.204	BDL*	BDL*	BDL*

* BDL = below the detection limit, ppm – Parts per million

DISCUSSION

PooraParpam was subjected to qualitative, quantitative, and toxicity studies in this study. The qualitative analysis includes chemical analysis of unpurified, purified, and Pooram Parpam. Quantitative analysis includes acute and long-term toxicity studies carried out in rodents per WHO guidelines.

HR SEM analysis of PooraParpam reveals a 1-10 µ (micron) particle size. The particles were homogenously distributed in the Parpam. And smooth surface of the particles enables their easy absorption in the gastrointestinal tract. Hence the drug will have increased bioavailability.

In the Acute toxicity study period, no abnormal signs developed in Swiss albino mice at ten times more than the therapeutic dose level (0.216 mg/animal) within 24 hrs. No mortality or reduction in the body weight of animals was observed in the study period. At the end of the Long-term toxicity study, animals were sacrificed, and blood samples were collected and investigated. The organs were collected and sent for histopathology study.

There was no mortality observed at the given dose level. A long-term toxicity study indicates no abnormal behavioural signs were observed during the study period. The test drugPoora Parpam results in no mortality in both groups. The histopathological study of the organs heart, lungs, kidney, brain, and liver showed a normal study in both groups.

All the reports were statistically calculated. There were no significant changes in the liver and renal function tests. The histopathological study on the organs such as the heart, lungs, kidney, brain, and

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liver was normal in control, X, and 5 X groups. In the 10 X group, the brain shows mild edema in the cerebral cortex with separated glial cells. The lung showed focal lymphoid aggregations and congested blood vessels, and the interstitium was dilated. The kidney shows congested glomeruli, and the interstitium shows congested blood vessels with an area of haemorrhage. The heart shows congested blood vessels and haemorrhage.

The overall outcome of the toxicological profiling gives important evidence-based data that demonstrates the medicine is relatively non-toxic, does not appear to induce organ damage, and does not cause mortality when administered to study animals for short and extended periods. As a result of the findings, it was determined that this drug was safe and would not cause any substantial toxicity-related side effects when used to manage the chronic disease condition long-term.

CONCLUSION

As a result of this study, it has been concluded that the acute and long-term toxicity of Poora Parpam is less toxic, and the therapeutic dose level mentioned in the literature is safer for human consumption. Hence further studies on Poora Parpam will be conducted for its scientific validation and global acceptance.

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