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SUB-ACUTE TOXICITY STUDY OF PARANGIPATTAI RASAYANAM

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ABSTRACT

Parangipattai Rasayanam is widely used as anti-vaathaa drug (Anti-inflammatory and analgesic). Both individually have not shown any toxicity on long term administration. A study was planned to assess the safety of drug by doing sub-acute toxicity study in mice with 28 days oral administration three dose. Food consumption, body weight, histopathological examinations were studied. There was significant increase in body weight, food consumption was observed. Liver, Heart, Lungs, Pancreas, Spleen, Stomach, Intestine, Kidneys, Urinary bladder, Uterus were normal on histopathological examination. Sub-acute toxicity studies in Wistar albino rats did not reveal any toxicity.

KEY WORDS

Parangipattai Rasayanam, Sub-acute toxicity, Histopathological examination.

I. INTRODUCTION

Indian medicinal plants have been subjected to detailed chemical, pharmacological and therapeutic investigations. Parangipattai [smilax china, Linn] it have anti-vaatha property as well as analgesic in wide use. In the past, a number of studies have been carried out on these plants for testing their pharmacological activities [1-4]. Though some isolated toxicity studies are reported of individual agents [5]. Therefore, in order to substantiate the claim of safety of this drug, this particular study was planned as Sub- acute toxicity studies.

II. MATERIALS AND METHODS

Animals	: Male and Female Wistar albino rats
Age	: 6-8 weeks
Weight	: 150-200 gms
Gender	: Both male and female
Number of animals	: Rat: 40
Acclimatization period	: 7 Days
Clinical duration	: 28 days

Sl. No	Group	No of Rats
1	Vehicle control	10 (5male, 5 female)
2	1xTherapeutic dose (180mg)	10 (5male, 5 female)
3	5xTherapeutic dose (900mg)	10 (5male, 5 female)
4	10xTherapeutic dose(1800mg)	10 (5male, 5 female)

a. Animal source

Test animals were obtained from the animal laboratory of the King Institute, Chennai, [6] and stocked at Animal house, National Institute of Siddha, Chennai. All the animals were kept under standard environmental condition (27± 2^0C). The animals had free access to water and standard pellet diet (Sai Meera foods Pvt. Ltd., Bangalore) [7]. The principles of laboratory animal care were followed and the Institutional ethical committee approved the use of

animals and the study design. (1248/ac/09/CPCSEA/4-08/2011 - 20/12/2011)

b. Identification of animal

The animals were identified by cage number, animal number and individual marking.

c. Housing and Environment

The animals were housed in polypropylene cages provided with bedding of husk .Dark and light cycle each of 12 hours.

d. Administration period

The period of administration of the test substance to animals depends on the expected period of clinical use. Since the clinical dose of the test drug is 28 days and as per WHO guidelines.

e. Dose selection

The results of acute toxicity studies in Swiss albino mice indicated that **Parangipattai Rasayanam** was nontoxic and no behavioral changes, mortality was observed. On the basis of these results, the doses were selected for the study as per WHO guidelines.

III. Preparation and administration of dose

Parangipattai Rasayanam was mixed with milk. It was administered to animals at dose levels of 1xTherapeutic dose (180mg), 5xTherapeutic dose (900mg) and 10xTherapeutic dose (1800mg).The control animals were administered milk only. Administration was by oral (gavage) once a day for 28 days

a. Methodology by Randomization, numbering and grouping of animal

The animals were randomly divided into four groups for dosing up to 28 days. Each group consist of 10 animals (5 per sex in each group) were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

b. Observation

Experimental animals were kept under observation throughout the course of study for the following:

***i.* Body weight**

Weight of each rat was recorded on day one and at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data mean body weights and percent body gain were calculated.

***ii.* Food and water consumption**

The quantities of food consumed by groups consisting of an animal for different doses were recorded at weekly intervals. Food consumed per animal was calculated for control and the treated dose groups.

***iii.* Clinical sings**

All animals were observed daily for clinical signs. The time of onset, intensity and duration of the symptom if any were recorded

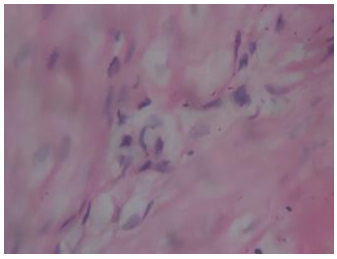
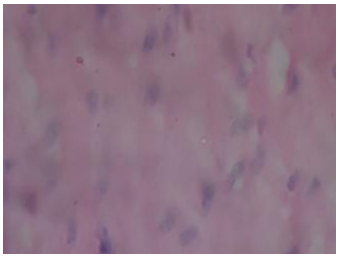
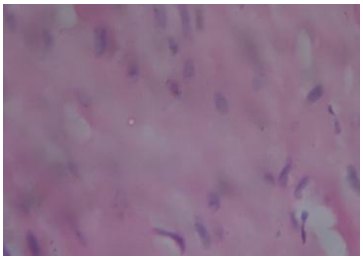
***iv.* Mortality**

All animals were observed twice daily for any mortality during entire course of study.

c. Histopathology

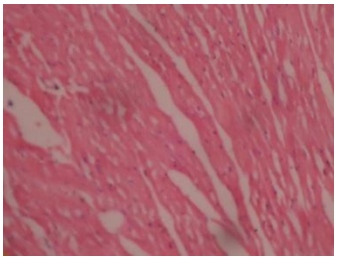
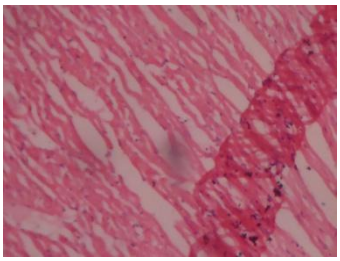
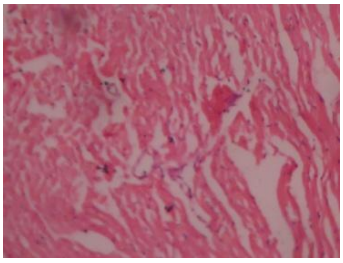
Tissue samples of organs from control and treated animals were preserved in 10% formalin for preparation of sections using microtome. The organs included liver, kidneys, heart, lungs, spleen, intestine, pancrease, and stomach of the animals were preserved and they were subjected to histopathological examination [figure 1 to 8].

The organ pieces (3-5 micron) were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in 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 50^0C and then a cubical block of paraffin made by the L moulds it was followed by microtome and the slides were stained with haematoxylin–eosin stain. Stained sections of each organ were examined under light microscope at high (40X) power magnification. All the histopathological slides were prepared at Department of Pathology, Vels University, Chennai [8].

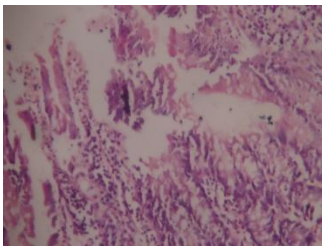
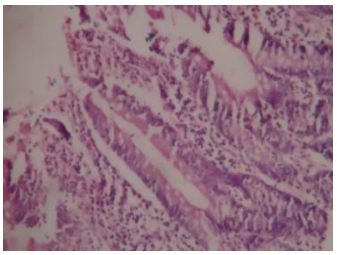
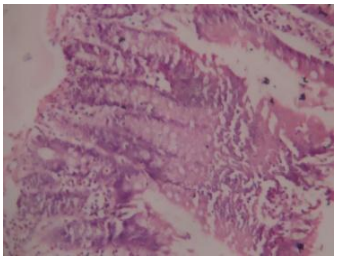
(i) Plate A (ii) Plate B (iii) Plate C

Figure 1: Histopathology of Stomach for (i) Plate A – Treated on 1x; (ii) Plate B – Treated on 5 X; (iii) Plate C – Treated on 10 X

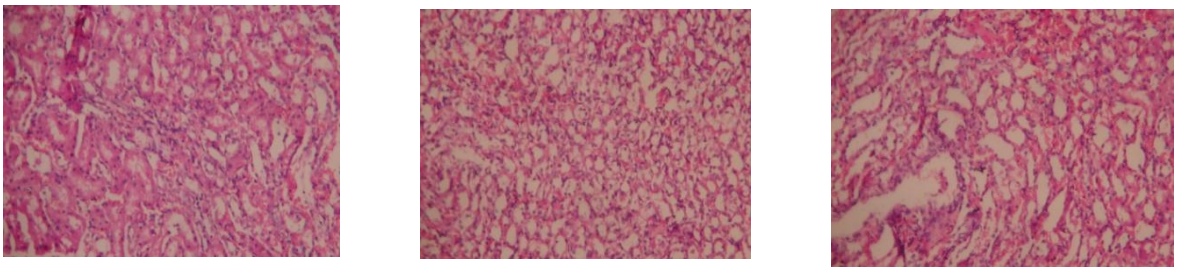
(i) Plate A (ii) Plate B (iii) Plate C

Figure 2: Histopathology of Heart for (i) Plate A – Treated on 1x; (ii) Plate B – Treated on 5 X; (iii) Plate C – Treated on 10 X

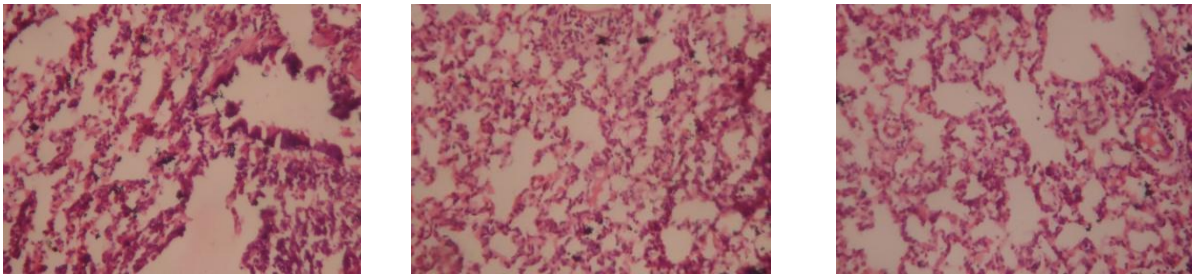
(i) Plate A (ii) Plate B (iii) Plate C

Figure 3: Histopathology of Intestine for (i) Plate A – Treated on 1x; (ii) Plate B – Treated on 5 X; (iii) Plate C – Treated on 10 X



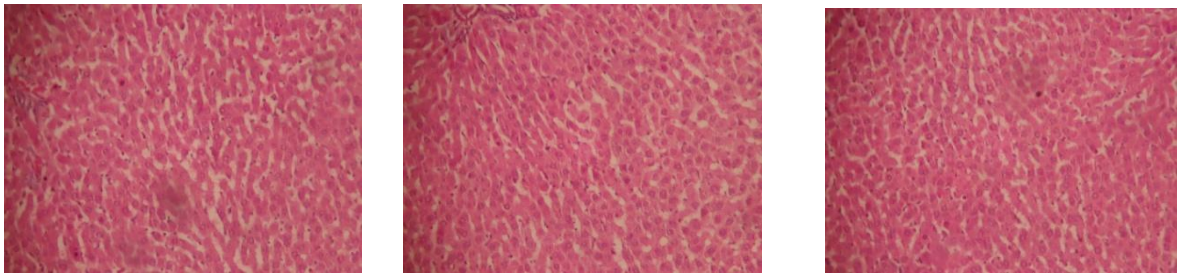
(i) Plate A (ii) Plate B (iii) Plate C

Figure 4: Histopathology of Kidney for (i) Plate A – Treated on 1x; (ii) Plate B – Treated on 5 X; (iii) Plate C – Treated on 10 X



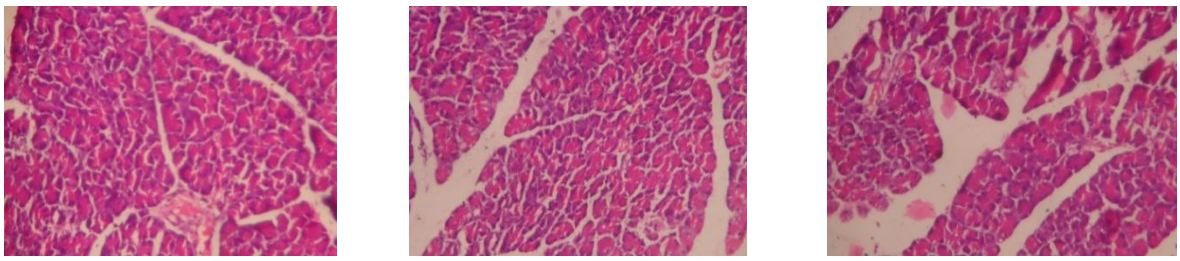
(i) Plate A (ii) Plate B (iii) Plate C

Figure 5: Histopathology of Lung for (i) Plate A – Treated on 1x; (ii) Plate B – Treated on 5 X; (iii) Plate C – Treated on 10 X



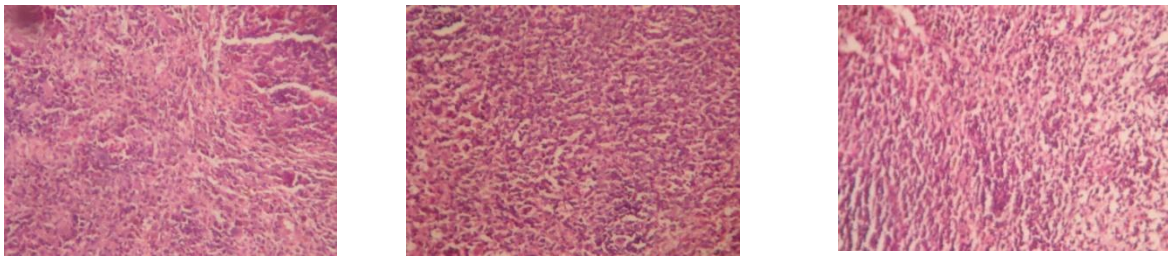
(i) Plate A (ii) Plate B (iii) Plate C

Figure 6: Histopathology of Liver for (i) Plate A – Treated on 1x; (ii) Plate B – Treated on 5 X; (iii) Plate C – Treated on 10 X



(i) Plate A (ii) Plate B (iii) Plate C

Figure 7: Histopathology of Pancreas for (i) Plate A – Treated on 1x; (ii) Plate B – Treated on 5 X; (iii) Plate C – Treated on 10 X



(i) Plate A (ii) Plate B (iii) Plate C

Figure 8: Histopathology of Spleen for (i) Plate A – Treated on 1x; (ii) Plate B – Treated on 5 X; (iii) Plate C – Treated on 10 X

IV. RESULTS AND CONCLUSION

Parangipattai Rasayanam at the three dose 180mg, 900mg, 1800mg /animal did not exhibit any mortality in rats. No behavior changes were noted for the first 4 hours and for the next 24 hours and throughout the study period of 28 days. No weight reduction was noted before and after the sub - acute study duration. Reflexes were found to be normal before and after the study. All other observations were found to be normal before and after the study. In, Histopathological examination the organs of the animal such as Liver, Heart, Lungs, Pancreas, Spleen, Stomach, Intestine, Kidney following conditions see below.

a. 180MG TREATED (Low dose)

Kidneys: shows normal renal tissue with glomeruli and tubules.

Spleen: shows normal spleen with lymphoid aggregation.

Liver: shows almost normal hepatocytes and occasional binucleate cells.

Stomach: shows normal mucosal glands.

Lungs: shows normal alveoli.

Heart: shows normal cardiac muscle bundles.

Intestine: Shows normal Intestinal mucosal lining with mild exudates.

Pancreas: shows normal acini with islets of β-cells

Impression: normal study

b. 900MG TREATED (Mid dose)

Kidneys: shows renal tissue with focal tubular damage, interstitial inflammatory collection. Glomeruli show epithelial proliferation.

Liver: shows hepatocytes with focal mild fatty change.

Spleen: shows congestion with lymphoid hyperplasia.

Stomach: shows near normal mucosal gland with mild exudates.

Lungs: shows congested alveolar wall with mild thickening and mild emphysematous changes.

Pancreas: shows pancreas with acini and normal islets.

Heart: shows congestion and mild inflammatory infiltration in between cardiac muscle bundles.

Intestine: Shows normal Intestinal mucosal lining with mild exudates.

Impression: normal study

c. 1800MG TREATED (High dose)

Stomach: shows stomach with superficial erosion and congestion.

Heart: shows hypertrophic cardiac muscle bundles.

Spleen: shows lymphoid hyperplasia.

Liver: shows marked dilatation of sinusoids, degeneration of hepatocytes, necrosis.

Kidneys: shows renal tissue with tubular epithelial damage.

Pancreas: shows atrophic islet cells.

Lungs: shows congestion, narrowed alveolar space and thickened alveolar wall.

Intestine: Shows normal Intestinal mucosal lining with mild exudates.

Impression: normal study

In conclusion, it can be said, that sub-acute toxicity studies in Wistar albino rats did not show any significant toxic effect and thus substantiate the claim of safety of the drug.

V. ACKNOWLEDGEMENT

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