INTERNATIONAL JOURNAL OF PHYTOTHEARPY RESEARCH
ISSN 2278 – 5701

Original Research Article

ACUTE AND SUB ACUTE TOXICITY STUDY OF MANASAMITRA VATAKA IN EXPERIMENTAL ANIMAL MODELS

Sanjaya Kumar Y.R*, Madhavikutty P, Thamizh Selvam N, Anand M, Venugopalan T N and Nair PKS

National Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala

ABSTRACT:
Manasamitra Vataka (MMV), a compound Ayurvedic formulation was evaluated for safety in experimental animals. In acute toxicity study, the test drug was administered at dose of 2000 mg /kg body weight in Swiss mice and the animals were observed for mortality and signs of toxicity. In sub acute toxicity study, the test drug was administered at doses 130 -1300 mg /kg body weight to Wistar rats for a period of 28 days. Signs of toxicity, mortality, body weight changes, and feed & water consumptions were recorded during this period. Hematological and biochemical investigations carried out at the termination of sub acute study did not reveal significant changes between test groups and control group. Significant increase in serum creatinine levels in rats receiving test drug at dose of 1300 mg/kg body weight could not be concluded due to absence of clinical signs and histopathology findings suggestive of kidney damage. In conclusion, the test drug was found to be safe as evidenced by absence of morbidity and mortality at the tested dose levels during acute and sub acute toxicity studies in Swiss mice and Wistar rats respectively.
Key words: Manasamitra Vataka, toxicity, hematology, serology, histopathology

INTRODUCTION:

Manasamitra vataka (MMV) is an Ayurvedic medicine used in treatment of psychosis, epilepsy, speech problems, and to improve memory and intellect in children. This medicine contains 73 ingredients (Table 1) and the dose is 1 tablet per day [1]. Clinical studies have proved the efficacy of Manasamitra vataka in the management of generalized anxiety disorder [2].

MATERIALS AND METHODS

Animals

Swiss albino rats and Wistar albino rats of both sexes were used in the trial. The animals were procured from Small Animal Breeding Station, Veterinary College, Mannuthy, Thrissur, India. After quarantine period, animals were caged individually

ISSN 2278 – 5701

with free access to pelleted feed and water.

Test drug

Manasamitra vataka was obtained from Pharmacy of National Research Institute for Panchakarma, Cheruthuruthy, Thrissur.

Ethical clearance

Necessary permission for conducting the present trial was obtained during Institutional animal Ethics Committee (IAEC) held at National Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala.

Acute Toxicity study

The test was carried out as per OECD guideline 423 [3]. A total of 12 mice were used in the study. To begin with 3 female animals were animals were administered with 2000 mg of test drug once orally and the animals were observed for a period of 72 hours for mortality and for signs of toxicity for a period of 10 days. The procedure was repeated at the same dose with 3 male mice.

Sub acute toxicity study

The test was carried out in Wistar albino rats according to OECD guidelines No.407 [4]. A total of forty eight rats were divided into four groups, each containing six male and 6 female rats. The test drug was administered at doses 130 (Therapeutic dose group), 650 (Average dose group) and 1300 (High dose group) mg/kg to the test groups once daily for a period of 28 days, whereas the control group received distilled water.

The animals were observed for mortality and signs of toxicity for a period of 28 days. Individual body weights of rat were taken initially and at weekly intervals till day 28. Feed consumption and body weight gain of individual animals were recorded at weekly intervals. Blood samples were collected under ether anesthesia from all the animals through retro orbital puncture on 29th day and animals were sacrificed.

Detailed postmortem was carried out. Internal organs were collected, weighed and preserved in formalin for histopathology.

Statistical analysis

The results were presented as Mean ± SEM. The statistical difference between the control and test groups were calculated by means of analysis of variance followed by Dunnet's test with minimal level of significance set at $P \leq 0.05$.

RESULTS AND DISCUSSION

No mortality and signs of toxicity was recorded in mice of either sex upon single exposure to the drug at dose of 2000 mg / kg body weight.

During sub acute toxicity study, rats did not show any signs of toxicity throughout the study period of 28 days and no mortalities were observed too. There was a significant ($P<0.05$) increase in body weight gain of male rats of High Dose (HD) group at the end of II, III and IV weeks as compared to those in Vehicle control group (VC) (Table 2). But, female rats did not show any significant increase in body weight gain (Table 3).

Male rats in HD group showed significant ($P<0.05$) decrease in Polymorph percentage and corresponding significant ($P<0.05$) increase in lymphocyte percentage as compared to those in other groups (Table 4). These differences were within normal range for rats and were not observed during hematology examination of female rats (Table 5).

There were no significant changes in biochemical parameters between test groups and VC group except for significant ($P<0.05$) increase in serum creatinine levels in HD group. This increase was observed in both male and female rats (Tables 6 & 7).

There was a significant ($P<0.05$) increase in spleen weight of male rats in HD group as compared to control. Similarly significant

ISSN 2278 – 5701

increase was observed in Kidneys and Ovaries weight in female rats of TD groups, but female rats in AD and HD groups did not show any such differences (Tables 8 & 9).

Histopathology examinations of vital organs did not reveal major changes indicative of tissue damage owing to test drug administration (Fig. 1 – 5). Histopathology of kidneys did not show any abnormalities and increase in serum creatinine in these groups could not be concluded. Further Rats (male and female) in the HD groups remained healthy and showed no signs of anorexia and weight loss.

Table 1. Ingredients of Manasamitra Vataka .(Ref: Sahasrayoga, Gutikaprakarana, 68)

Sida cordifolia L	Pluchea Lanceolata (D.C) Oliv & Hiern
Abutilon indicum L	Bhasma of silver
Aegle marmelos Corr.	Asphalatum
Desmodium gangeticum DC	Elephantopus scaber L.
Pravala Pishti (Coral ash)	Nelumbium nucifera Gaertn.
Clitoria ternatea L.	Microstylis wallichi Lindl
Adiantum lunulatu Burm	Fritillaria roylei Hook.
Swarna Bhasma (Gold ash)	*Lilium polyphyllum* D. Don
lnula racemosa Hook .f	Solanum indicum L.
Ash of deer horn	Solanu surratense Burm
Acorus calamus Linn.	Spheranthus indicus L.
Bhasma of copper iron pyrite	Andrographis paniculata Nees
Santalum album L	Swertia chirata (Roxb. ex Fleming) H. Karst
Pterocarpus santalinus L.f	Grewia asiatica L.
Paste of Pearl	Terminalia chebula Retz.
Bhasma of Iron	Terminalia bellirica Roxb.
Madhuca longifolia (Linn.) Machride	Emblica officinalis Gaertn.
Cinnamomum zeylanicum Blume	Tinospora Cordifolia (Thunb.) Miers
Piper longum linn	Decalepis hamiltonni Wight &Arn.
Cinnamomum camphora (L.) J.Presl.	Cryptolepis buchanani Roem. & Schult

ISSN 2278 – 5701

Prunus avium L.	Leptadenia reticulate Weight & Arn.
Citrullus colocynthis (L.) Schrad	Ceropegia juncea Roxb
Lodoicea maldivica (J.F. Gmeli.) Persoon	Withania somnifera Dunal
Vitex negundo L.	Curcuma longa Linn
Cyperus rotundus L.	Vetiveria zizanioides (Linn.) Nash
Vitis vinifera Linn	Habenaria intervmedia D. Don
Glycyrrhiza glabra L.	Cynodon dactylon (Linn.) Pers.
Adiantum lunulatum Linn.	Musk/Kasturi
Aervalanata (Linn.) Juss.ex Schultes	Ipomoea maxima (L.F.) G. Don
Syzygium aromaticum (L. Merrill & Perry	Crocus salivus Linn.
Ocimum tenuiflorum L.	Gentiana kurroo Royle.
Cuminum cyminum Linn	Cow milk
Goat Milk	

Table 2 .Weekly % body weight gain as compared to day 1 during Sub acute toxicity Study in male Wistar rats

Weeks	VC	TD	AD	HD	F Value
0-1	7.98±0.76	7.37±0.27	7.38±0.53	11.3±2.19	2.483
0-2	17.1±1.37	18.42±0.67	18.77±0.81	22.25±0.94**	5.004
0-3	25.08±1.4	29.46±1.08	31.31±1.83	38.9±2.38**	10.97
0-4	41.36±0.61	45.38±1.84	47.86±2.29	58.58±2.77**	12.98

**P<0.01 (Average of 6 Values)

ISSN 2278 – 5701

Table 3. Weekly % body weight gain as compared to day 1 during Sub acute Toxicity study in female Wistar rats

Weeks	VC	TD	AD	HD	F Value
0-1	2.91±0.92	2.91±1.49	4.36±0.07	7.22±2.87	1.466
0-2	9.82±1.05	10.16±0.94	9.43±0.73	15.69±2.84	3.305
0-3	17.29±1.05	17.43±0.34	16.69±0.78	23.2±2.49	4.619
0-4	25.58±1.90	26.14±0.51	24.67±1.01	30.83±3.00	2.179

(Average of 6 Values)

Table 4. Effect of MMV on hematological parameters in male Wistar rats

	VC	TD	AD	HD	F Value
TLC ($x10^3$)	4558±23	4067±25	3958±23	4258±17	1.419
POLY (%)	30±2.46	25.17±1.51	23.5±1.94	22±1.5*	3.316
LYM (%)	70±2.46	74.83±1.51	76.5±1.94	78±1.55*	3.316
PCV (%)	34±1.84	32±0.77	34.33±2.33	34.5±1.33	0.478
HB (g %)	11.88±0.08	11.62±0.08	11.67±0.12	11.6±0.05	2.210
TRC (10^6)	3.32±0.05	3.18±0.03	3.28±0.09	3.2±0.04	1.362
Platelets (10^5)	1.37±0.06	1.54±0.06	1.52±0.06	1.58±0.07	2.125

**P<0.01 (Average of 6 Values) (Average of 6 Values)

ISSN 2278 – 5701

Table 5. Effect of MMV on hematological parameters in female Wistar rats

	VC	TD	AD	HD	F Value
TLC (x10^3)	4867±178	4783±457	4642±311	4375±212	0.485
POLY (%)	22.83±2.07	24±2.72	24.17±2.75	20.5±1.84	0.505
LYM (%)	77.17±2.07	76±2.72	75.83±2.75	79.5±1.84	0.505
PCV (%)	35.67±2.04	32.17±0.48	32.17±0.83	34±2.11	1.185
HB (g %)	12±0.19	11.62±0.07	11.58±0.06	11.6±0.14	2.415
TRC (10^6)	3.35±0.10	3.22±0.03	3.22±0.03	3.3±0.08	0.977
Platelets (10^5)	2.05±0.14	2.18±0.35	2.43±0.28	2.11±0.31	0.355

(Average of 6 Values)

Table 6. Effect of MMV on Biochemical parameters in male Wistar rats

	VC	TD	AD	HD	F Value
Glucose (mg %)	112.8±11.18	96±6.01	103.3±7.214	100.5±5.11	0.847
SGOT (IU/L)	128.3±7.08	122.8±9.67	128.3±2.89	119±1.03	0.543
SGPT (IU/L)	69±2.62	65.5±5.86	62.33±3.11	56.16±2.60	2.064
Creatinine (mg%)	0.85±0.03	0.88±0.03	0.92±0.06	1.03±0.03*	3.726
Total Protein (g %)	5.9±0.07	6.72±0.31	6.57±0.36	6.25±0.13	2.089
Prothrombine Time (Sec.)	16.67±1.98	17.5±1.69	17.17±2.50	18.83±2.15	0.195

P<0.05 (Average of 6 Values)

ISSN 2278 – 5701

Table 7.Effect of MMV on Biochemical parameters in female Wistar rats

	VC	TD	AD	HD	F Value
Glucose (mg %)	111.7±3.60	102.2±6.73	90.6±7.67	119.7±9.55	2.999
SGOT (IU/L)	127.8±9.45	141.5±8.51	145±3.91	122.8±5.52	2.184
SGPT (IU/L)	60±3.50	62.67±5.58	64.5±1.65	51.83±2.93	2.286
Creatinine (mg%)	0.85±0.02	0.93±0.02	0.93±0.03	0.95±0.02*	4.314
Total Protein (g %)	7.13±0.47	6.98±0.41	6.75±0.17	7.42±0.34	0.579
Prothrombine Time (Sec.)	19.5±1.86	18.33±1.33	22.67±2.23	18.17±1.99	1.229

P<0.05 (Average of 6 Values)

Table 8 Effect of MMV on relative organ weights in male Wistar rats

	VC	TD	AD	HD	F Value
HEART	0.40±0.01	0.37±0.01	0.38±0.01	0.41±0.02	1.261
LUNG	0.64±0.03	0.65±0.04	0.60±0.03	0.68±0.03	1.408
LIVER	3.53±0.08	3.53±0.16	3.48±0.08	3.42±0.06	0.25
SPLEEN	0.38±0.02	0.43±0.02	0.41±0.02	0.48±0.02*	2.992
KIDNEY	0.75±0.03	0.75±0.01	0.79±0.01	0.83±0.03	2.748
TESTES	1.23±0.02	1.25±0.01	1.21±0.03	1.14±0.05	2.186

P<0.05 (Average of 6 Values)

ISSN 2278 – 5701

Table 9 .Effect of MMV on relative organ weights in female Wistar rats

	VC	TD	AD	HD	F Value
HEART	0.43±0.01	0.41±0.01	0.45±0.02	0.41±0.02	1.242
LUNG	0.79±0.02	0.78±0.03	0.86±0.06	0.82±0.08	0.424
LIVER	3.45±0.10	3.92±0.11	3.92±0.05	3.55±0.28	2.345
SPLEEN	0.40±0.01	0.43±0.03	0.45±0.02	0.42±0.01	0.798
KIDNEY	0.76±0.01	0.85±0.02*	0.80±0.03	0.83±0.02	3.695
OVARIES	0.07±0.01	0.09±0.01*	0.08±0.01	0.08±0.00	2.310

P<0.05 (Average of 6 Values)

ISSN 2278 – 5701

Fig 1. Histopathology section of Liver (1300 mg/kg b.w.) of a male rat showing normal portal areas and central venous veins.

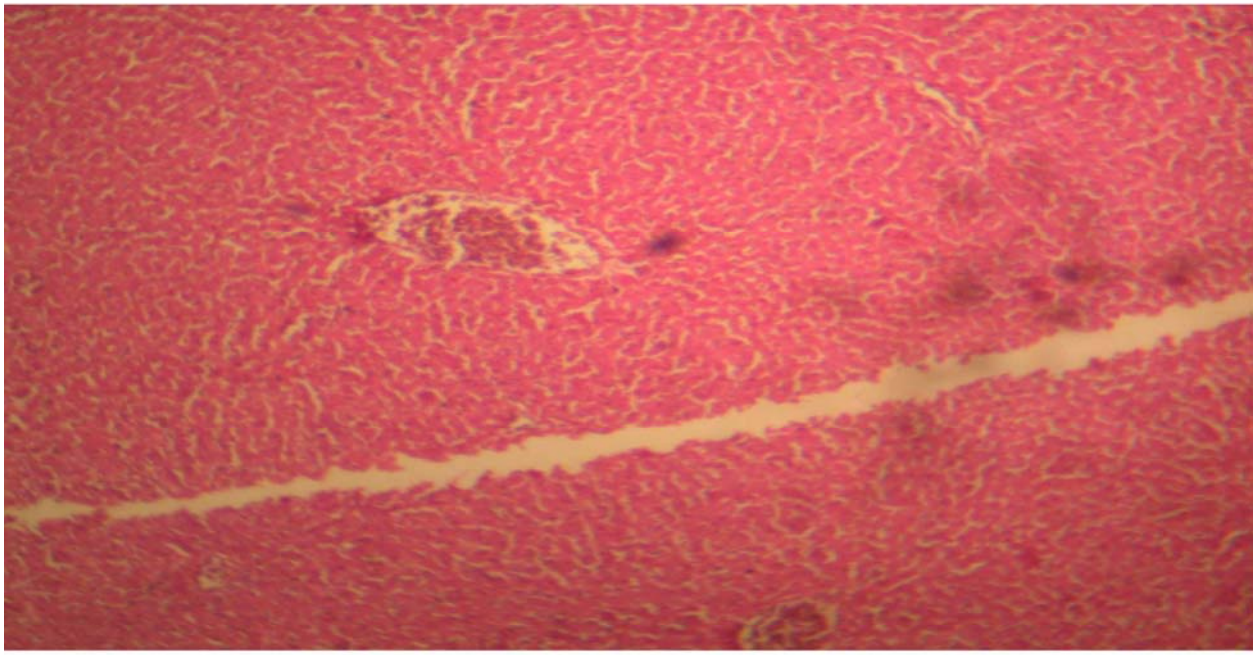
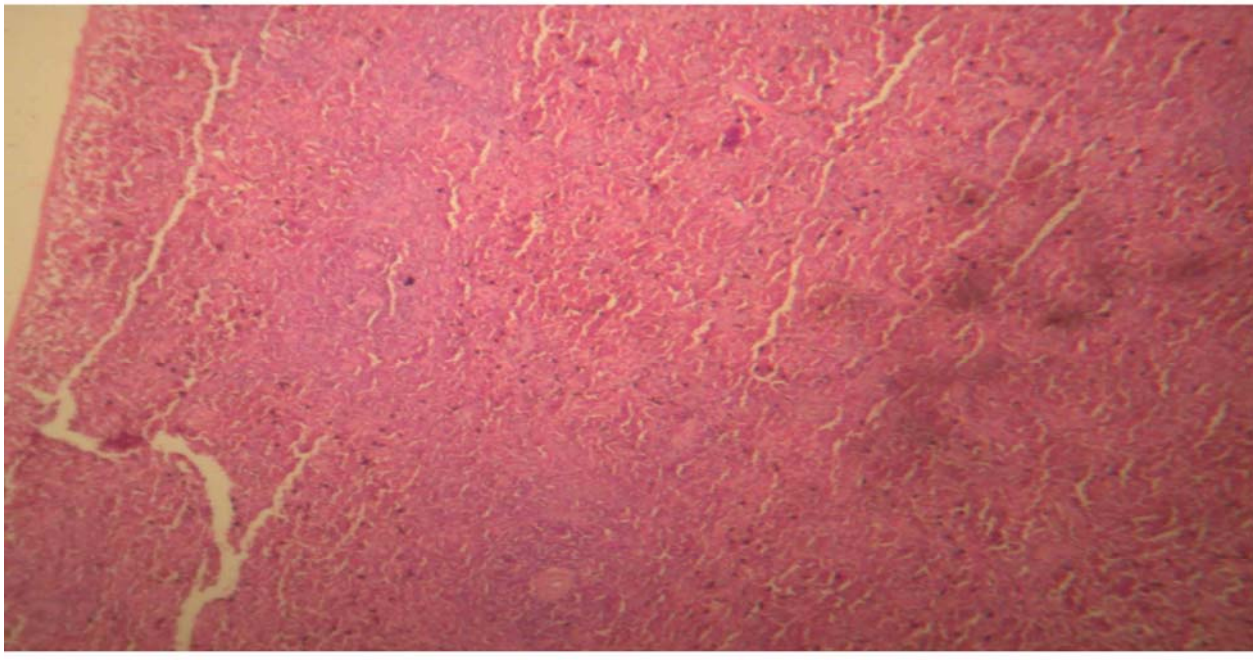


Fig 2. Histopathology of section of Spleen (1300 mg/kg b.w.) of a male rat showing normal lymphoid follicles and germinal centres.



ISSN 2278 – 5701

Fig 3. Histopathology of section of stomach (1300mg/kg b.w.) of a male rat showing normal mucosal, sub mucosal and muscle layers.

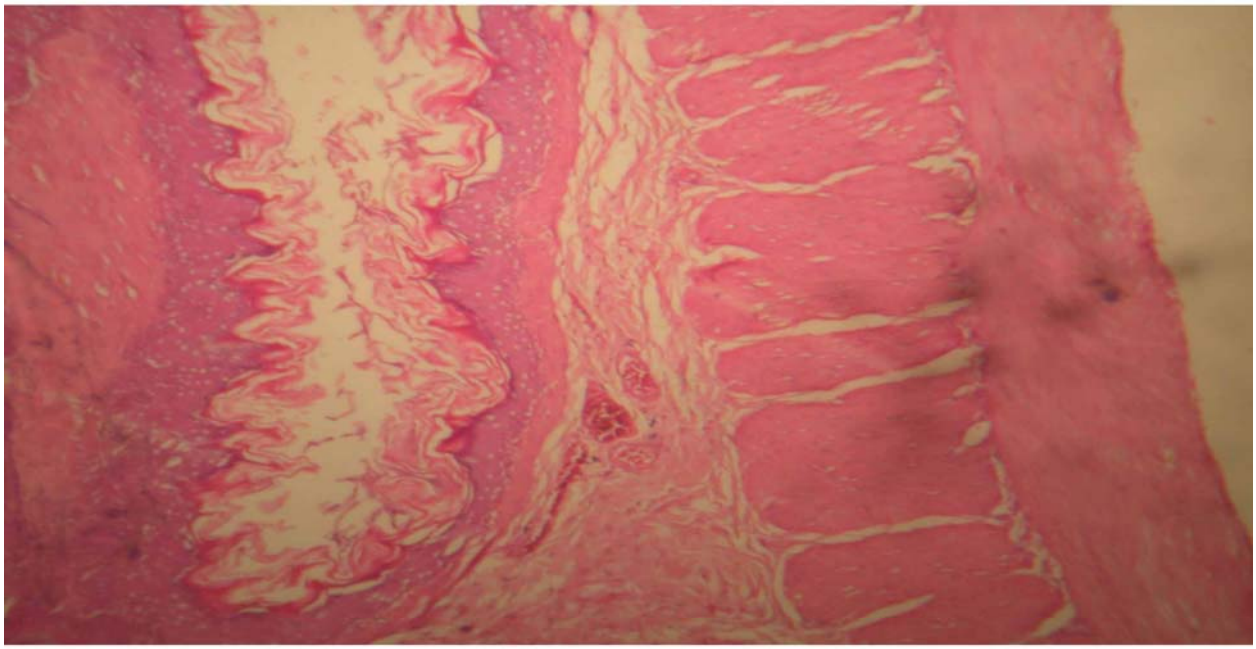
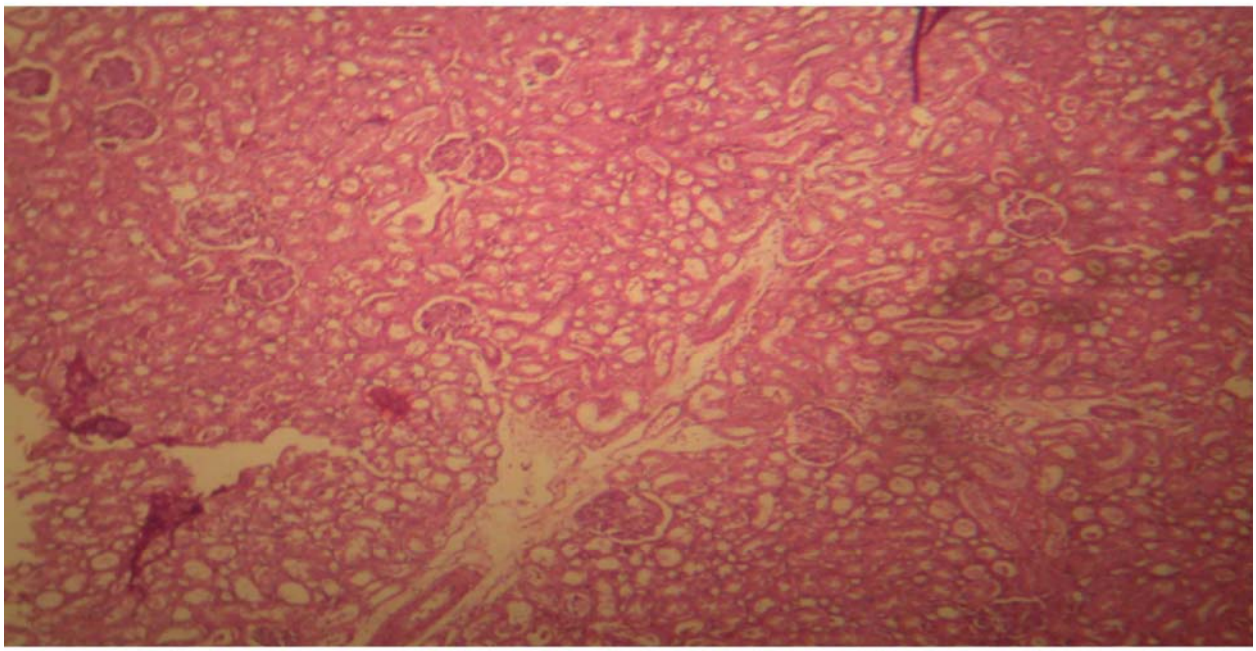
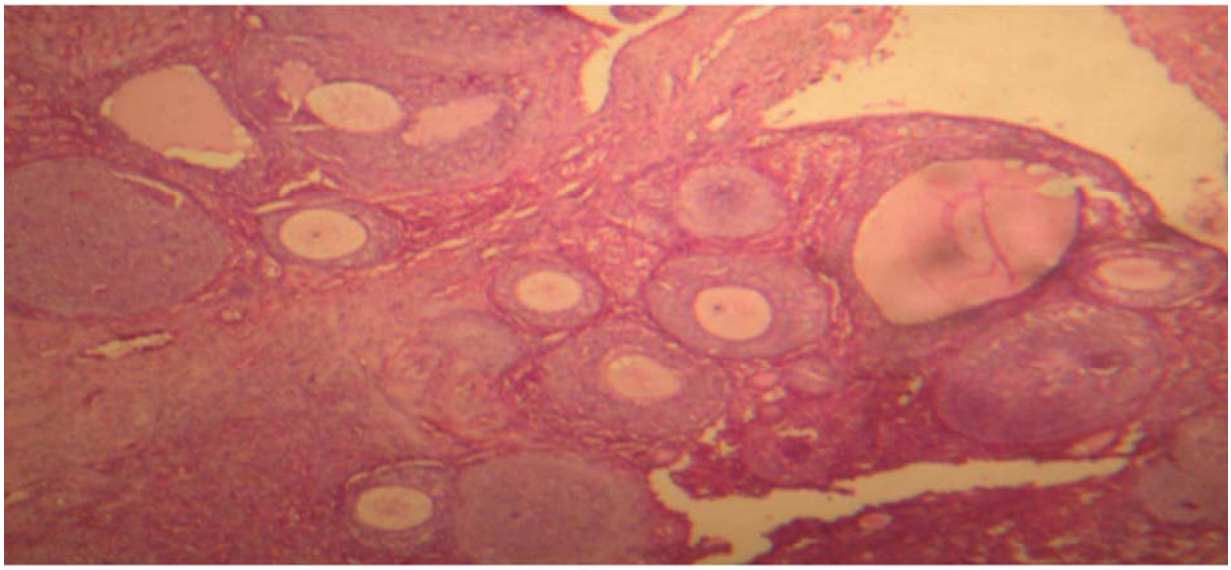


Fig 4. Histopathology of section of kidney of a female rat (130mg/kg b.w.) of a female rat showing normal glomeruli and renal tubules.



ISSN 2278 – 5701

Fig 5. Histopathology of section of ovary (650mg/kg b.w.) of a female rat showing normal graffian follicles and stroma.



ISSN 2278 – 5701

CONCLUSION

In this study, single exposure to Manasamitra vataka up to dose of 2000 mg /kg body weight per oral was found to be safe in mice. During sub acute toxicity study, 28 days oral administration of Manasamitra vataka up to dose of 1300 mg /kg body weight did not cause any adverse effects or lethality to Wistar rat.

ACKNOWLEDGEMENT

Authors are thankful to Director General, CCRAS and staff members of National Research institute for Panchakarma, Cheruthuruthy, Thrissur for their cooperation.

REFERENCES

1. The Ayurvedic Formulary of India, , 2nd Edition, Part 1, Dept. ISM&H, Ministry of H&FW, Government of India. New Delhi. 2003: 189.
2. Tubaki BR, Chandrashekar CR, Sudhakar D, Prabha TN, Lavekar GS and Kutty BM. Clinical efficacy of Manasamitra Vataka (an Ayurvedic medication)on generalized anxiety disorder with comorbid generalized social phobia : a randomized controlled study . J.Alter.Complement Med. 2003; 18(6):612-21.
3. OECD guidelines for the testing of chemicals Section 4 Health effects 2001 OECD, France.
4. OECD guidelines for the testing of chemicals Section 4 Health effects 2008 OECD, France.