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Acute and Subacute Toxicity Study of Manasamitra Vataka without Musk in Experimental Animal Models

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ABSTRACT

Manasamitra Vataka (MMV), a compound Ayurvedic formulation is used in Ayurveda as intellect promoter and musk is an important ingredient of this preparation. In the present study, Manasamitra Vatka without musk (MMVWOM) was evaluated for safety in experimental animals. The test drug was evaluated for acute toxicity (single exposure) at dose of 2000 mg/kg body weight in Swiss mice and subacute toxicity (consecutive exposure for 28 days) at doses 130 -1300 mg/kg body weight in Wistar rats. Mortality, Signs of toxicity, mortality, body weight and feed & water consumptions were recorded at weekly intervals during subacute toxicity study. The test drug was found to be safe as evidenced by absence of morbidity and mortality at the tested dose levels during acute and subacute toxicity studies in Swiss mice and Wistar rats respectively. Hematological and biochemical investigations carried out at the termination of subacute study did not reveal significant changes between test groups and control group except for variations in polymorphs and lymphocytes ratio in intermediate dose group. The safety of the test drug was further evident with the normal histopathology findings of major internal organs. Deletion of the musk from the preparation MMV did not make any difference with respect to its safety in experimental animals.

INTRODUCTION

Manasamitra Vataka (MMV) is an Ayurvedic medicine used to improve memory & intellect and to combat speech problems in children. This medicine contains 73 ingredients (Table 1) and the dose is 1 tablet per day (Ayurvedic Formulary of India, 2003) Clinical studies have proved the efficacy of Manasamitra Vatka in the management of generalized anxiety disorder (Tubaki *et al*, 2003).

Musk, one of the ingredients of this preparation is the dried secretion of the preputial follicles of Musk deer (*Moschus moschiferus*, Family: Cervidae). Musk is very costly and there is a dearth in its availability due to its high demand in perfumery industry. Further, there is growing concern by animal welfare activists and naturalists over the killing of musk deers and their possible extinction in coming decades. The present trial has been carried out to establish the safety of the test drug Manasamitra Vatka without Musk (MMVWOM) in the absence of musk. The study also serves as a preliminary step towards screening of MMVWOM for its intellect promoting activity.

MATERIALS AND METHODS

Animals

Adult male and female Swiss albino Mice and Wistar albino rats were used for acute and subacute toxicity studies respectively. The animals were procured from Small Animal Breeding Station, Veterinary College, Mannuthy, Thrissur, India. Animals were quarantined for 10 days and were caged individually with access to standard pelleted feed and water *ad libitum.*

Test drug

Manasamitra Vatka without musk (MMVWOM) was prepared at 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) meeting held at National Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala.

Acute Toxicity study

Limit test at dose rate of 2000 mg/kg was carried out in mice as per OECD guideline 423 (OECD 2001). A total of 6 mice were used in the study. The test drug was administered to 3 female animals once orally and the animals were observed for a period of 72 hours for mortality and for signs of toxicity. The procedure was repeated at the same dose in 3 male mice.

Subacute toxicity study

Repeat dose oral toxicity study was carried out in Wistar albino rats as per OECD guidelines No.407 (OECD 2008). Animals were divided into four groups each consisting 12 rats (6 males and 6 females). The test drug was administered at doses of 130 (Therapeutic dose group), 650 (Average dose group) and 1300 (High dose group) mg/kg to the test groups once daily for 28 consecutive days. Distilled water was administered to animals in control group.

During the study period, animals were observed for mortality and signs of toxicity. Body weights and feed consumption of individual animals were recorded at weekly intervals.

Blood samples were collected from fasted animals through retro-orbital puncture under ether anesthesia on 29th day for hematological and biochemical studies. Animals were sacrificed. Detailed postmortem was carried out and internal organs were collected, weighed and preserved in 10% formalin for histopathology.

Statistical analysis

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

RESULTS AND DISCUSSION

Acute toxicity study

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

Subacute Toxicity study

Wistar Rats of either sex did not show any signs of toxicity throughout the period of 28 days and no mortalities were observed too. There was significant (P<0.05) increase in body weight gain of male rats of Average Dose (AD) group as compared to control group at the end of III week. But no such increase was observed in male rats of other test groups (Table 2). Similarly, Female rats of different test groups too did not show any significant variation in body weight (Table 3).

Male and female rats in AD group showed significant (P<0.05) increase in Polymorph percentage and corresponding significant (P<0.05) decrease in lymphocyte percentage as compared to Control group. Serum samples of male rats in HD group showed significant (P<0.05) increase in Hemoglobin and Total red cell count (Table 4 and Table 5). Rats in other test groups did not show such variations.

There were no significant changes in biochemical parameters between test groups and VC group except for significant (P<0.05) increase in SGOT and SGPT levels in male rats of AD group. The same could not be concluded as blood samples from male and female rats of other test groups did not show such increase. In HD group, female rats in HD groups showed significant (P<0.05) increase and the male rats showed relative increase in total protein levels (Tables 6 & 7).

Significant differences were not observed with respect to relative organ weights between control group and test groups (Tables 8 & 9).

Histopathology of vital internal organs did not reveal any pathological changes consequent to test drug administration (Fig. 1 – 5).

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
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. Effect of MMVWOM on Weekly % body weight gain in male Wistar rats

Weeks	0-1	0-2	0-3	0-4
VC	6.79 ± 0.678	16.76 ± 2.307	26.01 ± 2.167	37.33 ± 1.903
TD	6.27 ± 0.592	15.66 ± 0.872	27.29 ± 1.22	39.8 ± 1.312
AD	7.39 ± 0.696	19.5 ± 1.698	33.72* ± 2.409	46.38 ± 3.603
HD	8.63 ± 0.56	19.38 ± 1.372	31.25 ± 2.395	44.01 ± 3.214

*P<0.05 (Average of 6 Values)

Table 3. Effect of MMVWOM on Weekly % body weight gain in female Wistar rats

Weeks	0-1	0-2	0-3	0-4
VC	2.06 ± 0.658	7.03 ± 0.56	11.59 ± 0.616	18.22 ± 0.868
TD	2.69 ± 0.549	6.92 ± 0.938	13.34 ± 0.978	21.3 ± 1.216
AD	2.63 ± 0.68	7.84 ± 0.964	13.48 ± 0.781	19.97 ± 0.693
HD	2.61 ± 0.527	7.25 ± 0.71	11.92 ± 0.683	19.73 ± 1.44

(Average of 6 Values)

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

	TLC ($x10^3$)	POLY (%)	LYM (%)	PCV (%)	HB (g %)	TRC (10^6)	Platelets (10^5)
VC	4.24 ± 2.13	22.33 ± 2.96	77.67 ± 2.96	35.5 ± 1.82	11.67 ± 0.12	3.3 ± 0.1	1.8 ± 0.2
TD	4.35 ± 1.55	24 ± 1.59	76 ± 1.59	34.83 ± 1.19	11.58 ± 0.05	3.2 ± 0	1.7 ± 0.2
AD	4.40 ± 1.71	30.17* ± 0.70	69.83* ± 0.70	33.5 ± 1.0	11.58 ± 0.05	3.2 ± 0	1.8 ± 0.2
HD	4.62 ± 1.54	29.33 ± 2.20	70.67 ± 2.20	42.5 ± 3.53	12.33* ± 0.30	3.6* ± 0.1	1.6 ± 0.1

* P<0.05 (Average of 6 Value)

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

	TLC ($x10^3$)	POLY (%)	LYM (%)	PCV (%)	HB (g %)	TRC (10^6)	Platelets (10^5)
CT	3.91 ± 2.54	24.17 ± 1.85	75.83 ± 1.85	36 ± 2.22	11.82 ± 0.09	3.32 ± 0.08	1.75 ± 0.15
TD	4.20 ± 1.98	22.17 ± 1.38	77.83 ± 1.38	33 ± 1.06	11.62 ± 0.05	3.23 ± 0.02	1.92 ± 0.23
AD	4.47 ± 1.56	31.33* ± 2.45	68.67* ± 2.42	35 ± 1.86	11.6 ± 0.14	3.267 ± 0.09	2.12 ± 0.20
HD	4.42 ± 1.28	29.83 ± 1.94	70.166 ± 1.94	39 ± 1.79	11.98 ± 0.25	3.47 ± 0.12	2.02 ± 0.19

* P<0.05 (Average of 6 Values)

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

	Glucose (mg %)	SGOT (IU/L)	SGPT (IU/L)	Creatinine (mg %)	Total Protein (g %)	Prothrombine Time (Sec.)
CT	114.7 ± 8.14	125 ± 5.08	52.5 ± 2.96	0.98 ± 0.031	6.1 ± 0.1	16 ± 1.9
TD	109.8 ± 8.38	120.5 ± 1.15	49.5 ± 1.84	0.88 ± 0.03	6 ± 0.2	20 ± 2.2
AD	105.5 ± 8.20	142..84* ± 3.07	64.67* ± 4.45	0.98 ± 0.05	6 ± 0.1	15 ± 0.9
HD	107.8 ± 7.52	116.7 ± 5.45	57.83 ± 3.38	1.03 ± 0.02	6.3 ± 0.2	15 ± 1.9

*P<0.05 (Average of 6 Values)

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

	Glucose (mg %)	SGOT (IU/L)	SGPT (IU/L)	Creatinine (mg%)	Total Protein (g %)	Prothrombine Time (Sec.)
CT	111.2 ± 3.46	124 ± 1.23	58 ± 3.13	1.05 ± 0.02	6.02 ± 0.32	15.5 ± 1.52
TD	121.3 ± 3.01	120 ± 1.11	47.67 ± 1.84	0.98 ± 0.02	6.42 ± 0.20	15.33 ± 1.33
AD	109.5 ± 7.81	131.84 ± 5.75	53.33 ± 4.55	1 ± 0.04	6.55 ± 0.24	19 ± 1.91
HD	97.33 ± 2.78	129 ± 3.14	47.67 ± 3.02	1.03 ± 0.02	7* ± 0.23	18 ± 2.13

*P<0.05 (Average of 6 Values)

Table 8. Effect of MMVWOM on relative organ weights (gms) in male Wistar rats

	Heart	Lung	Liver	Spleen	Kidneys	Testes
VC	0.38 ± 0.02	0.91 ± 0.07	4.31 ± 0.27	0.41 ± 0.07	0.98 ± 0.05	1.23 ± 0.02
TD	0.35 ± 0.02	0.73* ± 0.03	4.0 ± 0.22	0.30 ± 0.02	0.92 ± 0.06	1.18 ± 0.05
AD	0.35 ± 0.02	0.75 ± 0.05	4.38 ± 0.31	0.4 ± 0.04	0.95 ± 0.06	1.23 ± 0.12
HD	0.37 ± 0.01	0.80 ± 0.06	4.39 ± 0.32	0.78 ± 0.42	0.99 ± 0.05	1.36 ± 0.04

(Average of 6 Values)

Table 9 .Effect of MMVWOM on relative organ weights (gms) female Wistar rats

	Heart	Lung	Liver	Spleen	Kidneys	Ovaries
VC	0.40 ± 0.03	0.94 ± 0.07	4.32 ± 0.30	0.36 ± 0.02	0.83 ± 0.04	0.11 ± 0.05
TD	0.35 ± 0.03	0.9 ± 0.08	4.17 ± 0.38	0.37 ± 0.03	0.84 ± 0.04	0.06 ± 0.01
AD	0.35 ± 0.01	0.80 ± 0.03	3.74 ± 0.22	0.28 ± 0.03	0.81 ± 0.02	0.06 ± 0.01
HD	0.38 ± 0.01	0.91 ± 0.03	4.25 ± 0.28	0.44 ± 0.02	0.87 ± 0.07	0.07 ± 0.01

(Average of 6 Values)

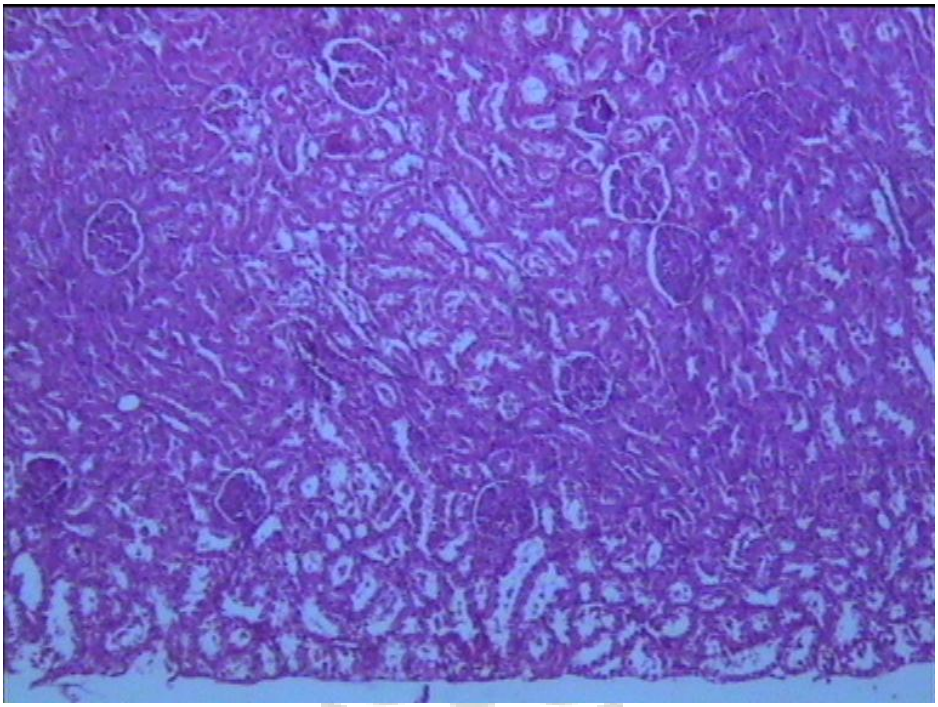

Fig 1. Histopathology of section of Kidney (1300 mg/kg b.w.) of a male rat showing normal glomeruli and renal tubules.

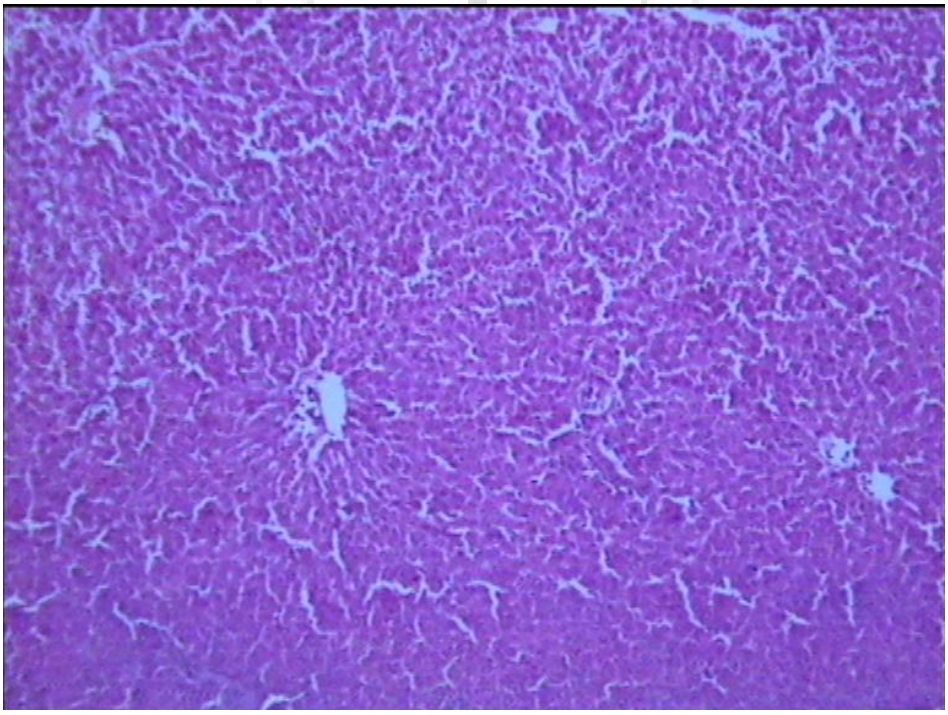

Fig 2. Histopathology of section liver (1300 mg/kg b.w.) of a male rat showing normal portal areas and central venous system

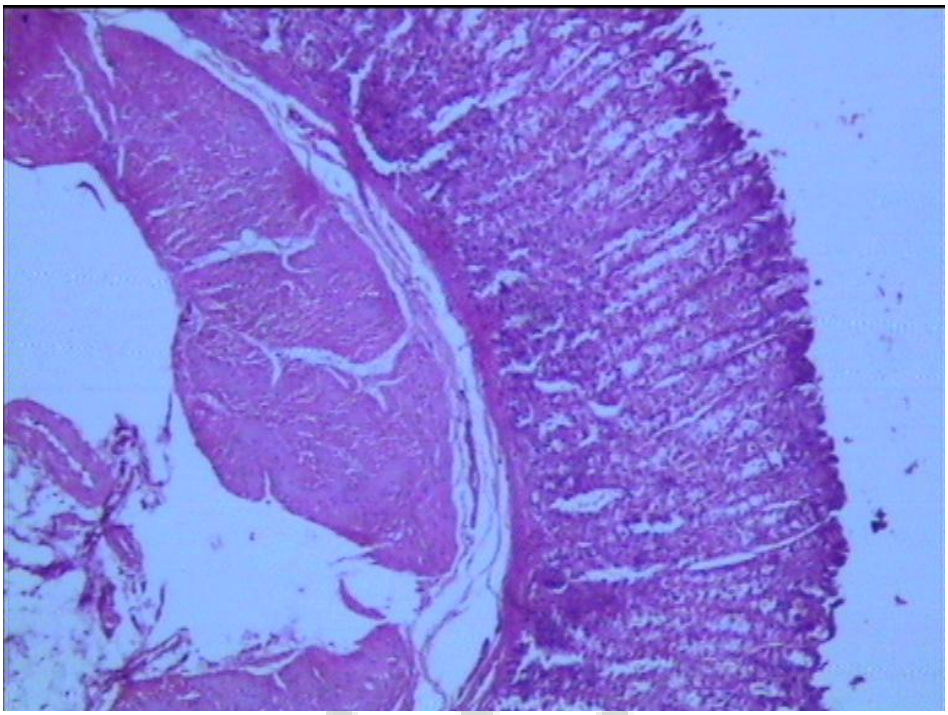

Fig 3. Histopathology of section stomach (1300 mg/kg b.w.) of a female rat showing normal mucosal and muscle layers.

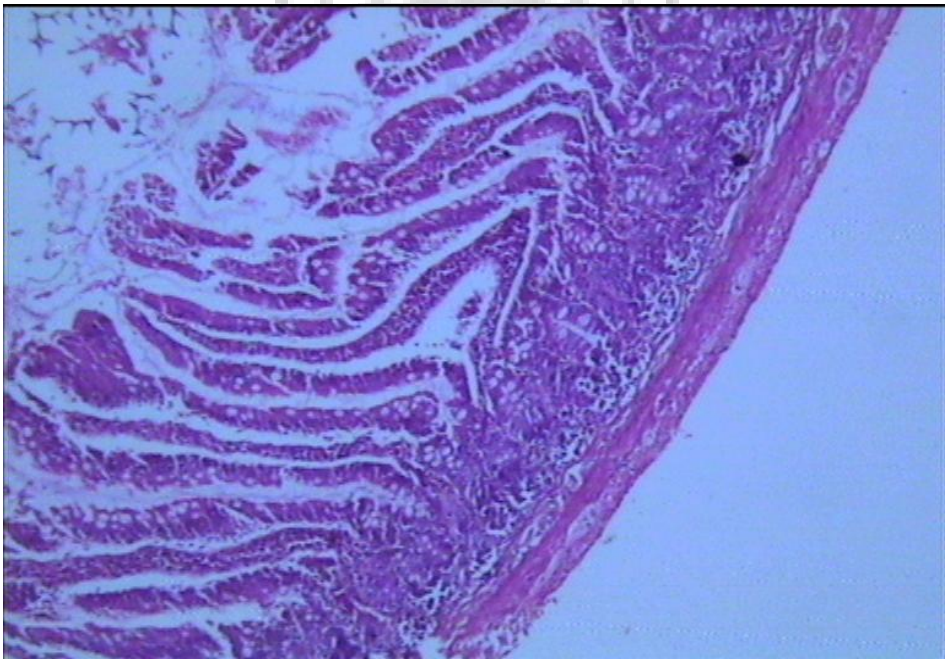

Fig 4. Histopathology of section intestine (1300 mg/kg b.w.) of a female rat showing normal mucosal glands and villi lined by columnar cells.

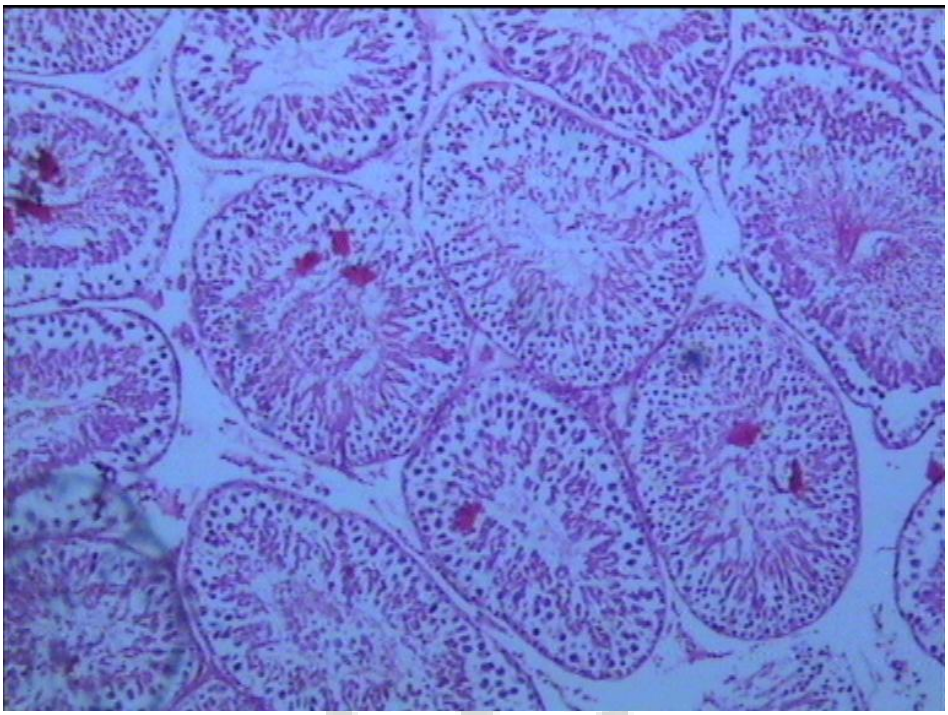

Fig. 5. Histopathology of section testis (1300 mg/kg b.w.) of a male rat showing seminiferous tubules containing normal sertoli cells.

CONCLUSION

In the present study, single exposure to MMVWOM up to dose of 2000 mg /kg body weight per oral was found to be safe in Swiss mice. During subacute toxicity study, 28 days oral administration of MMVWOM up to dose of 1300 mg/kg body weight did not cause any adverse effects or lethality to Wistar rats. The deletion of musk from the preparation Manasamitra Vataka did not affect safety of the drug in experimental animals during the study.

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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.