Acute and Sub Chronic (91 days) dermal toxicity study of Mahanarayana taila in Wistar rats

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Abstract - Mahanarayana taila, a compound Ayurvedic formulation was evaluated for safety in Wistar rats by acute and sub chronic (91 days) dermal toxicity studies. The test drug was applied externally and the animals were observed for the physical and clinical synptoms of toxicity in comparision to animals in control group. Neither mortality nor signs of toxicity were observed in Wistar rats during both the studies. Hematological, serum chemistry and qualitative urine examination showed no evidences of systemic toxicity. Skin and internal organs did not reveal structural changes suggestive of toxicity upon gross examination and histopathology investigation. Mahanarayana taila was found to be safe upon single and repeated dermal exposure in wistar rats during the study.

Key words: Mahanarayana taila, wistar rats, dermal toxicity

Introduction

Mahanarayana taila, a compound Ayurvedic formulation is used in treatment of Paraplegia, tremors, neck rigidity, facial palsy, lock jaw, wasting of hands and legs, insanity, teeth & tongue ailments, bloating, oligospermia, headache, glossal palsy and hump-back. It is also indicated in osteoarthritis, osteoporosis, fractures and tendon tear (1). In a clinical study, kati vasti (medicated enema) with mahanarayana taila has showed significant relief in patients with sciatica with vata predominance (2). In another study, administration of mahanarayana taila through nasya (nasal administration) and uttara vasti (enema through vaginal tract) for a fortnight has resulted in 66.66% and 28.57% ovulation in cases of female infertility with anovulatory cycle (3). Present study has been carried out to evaluate the safety of Mahanarayana taila in wistar rats upon external application.

Materials and Methods

Test drug

Mahanarayana taila, prepared as per the formulation in Ayurvedic formulary of India supplied by Central Council for Research in Ayurvedic Sciences, New Delhi was used during the trial (4)

Dose calculation

Surface area of the rat was calculated as per Meeh's formula. $1/10^{th}$ of the surface area was shaved from dorsal area of trunk and 1 ml of the test drug was applied as thin layer over the skin

Animals

Wistar Rats (male and female) were procured from small animal breeding station, Veterinary College, Mannuthy, Thrissur, Kerala were used in the trial. After quarantine period, animals were caged individually as per Committee for the purpose of Control and supervision of experiments on animals (CPCSEA) guidelines.

Ethical Clearance

The present trial was conducted with the approval of Institutional animal Ethics Committee (IAEC) meeting held at National Ayurveda Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala.

Experimental design

Acute Toxicity:

A total of 20 Wistar albino rats (10 Males and 10 Females) were randomized and equally divided into 2 groups. Gingelly oil, the base in the test drug was applied externally to animals (5 male and 5 female) in vehicle control group where as test drug was applied to those in test group once on first day of the experiment. The animals were observed for mortality and clinical signs of toxicity during next 14 days as per OECD guideline 402 (5).

Sub chronic Toxicity

The rats were divided into 2 groups namely Vehicle Control (VC) group and Test Dose (TD) group each comprising of 20 animals (10 Male and 10 Female). The study was carried out as per OECD guideline 411 (6).Test drug (mahanarayana taila) and vehicle (gingelly oil) were applied externally to the animals in experimental groups for a period of 91 days consecutively.

Animals were observed for signs of mortality and clinical signs of toxicity during the study

period. Weekly feed consumption and body weight gain were recorded.

Qualitative urine examination was carried out before exposure to the test compound and around 45^{th} day of the experiment using URISTIK A 10 reagent strips

At the termination of the trial, blood samples collected under light ether anesthesia were analysed Packed cell Volume(PCV), Hemoglobin (Hb) Red blood cell (RBC) count, Total white blood cell (WBC) count, Differential Leucocyte Count (Polymorph and Lymphocyte ratio) and Prothrombin Time (PT) Plasma Glucose (mg/dl), Creatinine (mg/dl), SGOT (U/L) and SGPT (U/L) , total protein (g/dl)were estimated using RA-50 auto analyzer (Bayer) were analysed at Biochemistry division of the Institute.

Animals were sacrificed by cervical dislocation and detailed post mortem examination was carried out. Vital organs such as heart, Lungs, Liver, spleen, Kidneys, testes and ovaries etc, were individually weighed and tissue samples of the same were stored in 10% formalin for histopathology studies.

Statistical analysis

The data obtained during the trial was compared between groups through student t test.

Results and Discussion

No pre terminal deaths were not recorded in animals upon dermal exposure to the test compound during acute and sub chronic toxicity studies .Physical and neurological examinations revealed no signs suggestive of toxicity.

No significant compound related abnormalities in the samples of urine tested qualitatively for the presence of leukocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubin, glucose, etc. before and after exposure to the test compound as compared to control group (Table 1).

There were no significant changes pertaining to body weight gain and feed intake between animals between control group and test group during acute toxicity study. Though body weight gain and feed intake were found to be on higher side in male rats of test group during first 3 weeks of the study it was normalised later and were on par with control group. No significant changes were observed in the hematology and serum chemistry profile of the test group as compared to control group (Tables 2 & 3).

The test drug did not affect the normal development of internal organs and significant differences were not observed with respect to the relative organ weights (Table 4).

There were no major evidences of histopathological changes in all organs studied in the rats exposed to the test compound as compared to unexposed test group. Histopathology of the skin did not reveal pathological changes and the normal regrowth of hair was observed in the rats post experimentation. (Fig.1-6).

Table 1. Urine examination (qualitative)

Groups		VC		TD	
Days		B E	A E	B E	A E
Leukocyte	Negative	100(20)	100(20)	100(20)	100(20)
	Trace	Nil	Nil	Nil	Nil
Nitrite	Negative	100(20)	100(20)	100(20)	100(20)
	Positive	Nil	Nil	Nil	Nil
Urobilinogen	Negative	100(20)	100(20)	100(20)	100(20)
	Trace	Nil	Nil	Nil	Nil
Protein	Negative	85(17)	90(18)	85(17)	85(17)
	Trace	15(03)	10(02)	15(03)	15(03)
Blood	Negative	100(20)	100(20)	100(20)	100(20)
	Trace	Nil	Nil	Nil	Nil
Ketone	Negative	90(18)	60(12)	85(17)	80(16)
	Trace	10(02)	40(08)	15(03)	20(04)
Bilirubin	Negative	100(20)	100(20)	100(20)	100(20)
	Small(+)	Nil	Nil	Nil	Nil
Glucose	Negative	100(20)	100(20)	100(10)	100(20)
	Trace	Nil	Nil	Nil	Nil

Values in Percentage ( ) No. of animals

Table 2. Hematology parameters during Sub chronic Toxicity study in Wistar rats (MEAN ± SEM)

GROUPS	VC	TD
Hb (g %)	12.8 ± 0.35	13.1 ± 0.35
PCV (%)	40 ± 0.7	40 ± 0.7
TRC (10^6 / cu.mm)	4.83 ± 0.05	4.77 ± 0.08
POLY (%)	29 ± 1	25.95 ± 1.178
LYM (%)	71 ± 1.2	74.55 ± 1.215
PT (Sec.)	14.9 ± 0.62	14.7 ± 0.52
Platele t (10^6 / cu.mm)	1.41 ± 0.28	1.40 ± 0. 27

(Average of 20 Values)

Table 3. Serum Biochemical Parameters during Sub chronic Toxicity study in Wistar Rats (MEAN ± SEM)

GROUPS	VC	TD
Blood Glucose (mg %)	121.1 ± 5.8	133.7 ± 4.7
Serum Creatinine (mg %)	0.9 ± 0	0.9 ± 0
SGOT (U/L)	121.3 ± 4.9	112.8 ± 5.7
SGPT (U/L)	40 ± 2	39 ± 2
Total Protein (g%)	6.3 ± 0.2	6.5 ± 0.1

(Average of 20Values)

Table 4. Organ wt in % Body weight – sub chronic Toxicity study in wistar rats (MEAN ± SEM)

Weeks	Male		Female	
	VC	TD	VC	TD
Heart	0.312 ± 0.009	0.294 ± 0.013	0.351 ± 0.008	0.332 ± 0.014
Lungs	0.758 ± 0.040	0.754 ± 0.035	0.947 ± 0.045	0.948 ± 0.060
Liver	3.432 ± 0.166	3.467 ± 0.220	3.475 ± 0.167	3.592 ± 0.235
Spleen	0.339 ± 0.029	0.295 ± 0.011	0.874 ± 0.283	0.411 ± 0.018
Kidneys	0.763 ± 0.024	0.789 ± 0.015	0.813 ± 0.021	0.858 ± 0.056
Testes	0.839 ± 0.034	0.829 ± 0.029	-	-
Ovaries	-	-	0.067 ± 0.005	0.052 ± 0.004

(Average of 10 values)

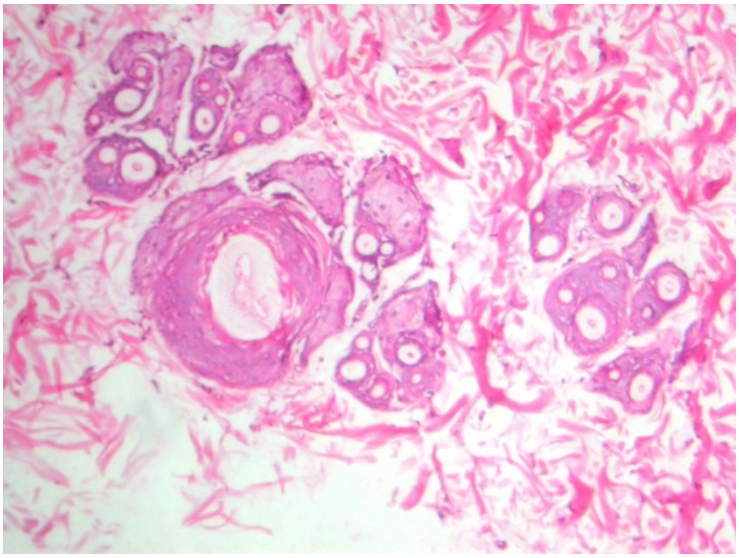

Fig 1. Section of skin of a female rat showing normal epidermis and dermis.

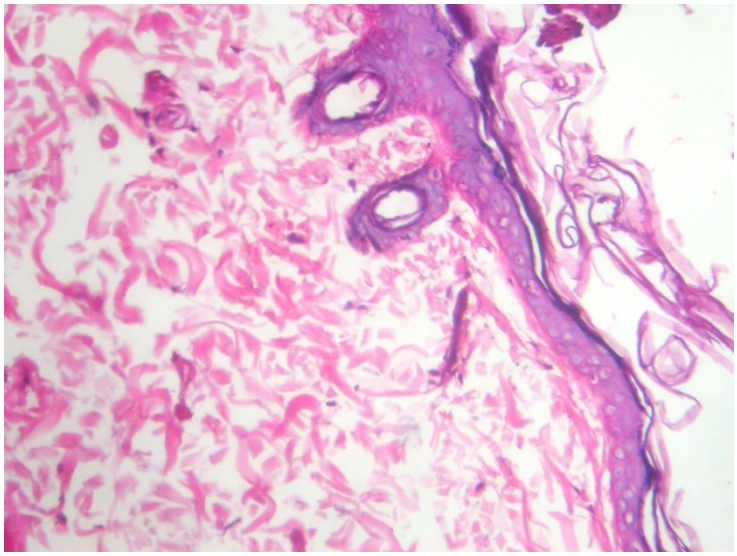

Fig 2. Section of skin of a female rat showing normal epidermis, dermis and dermal appendages.

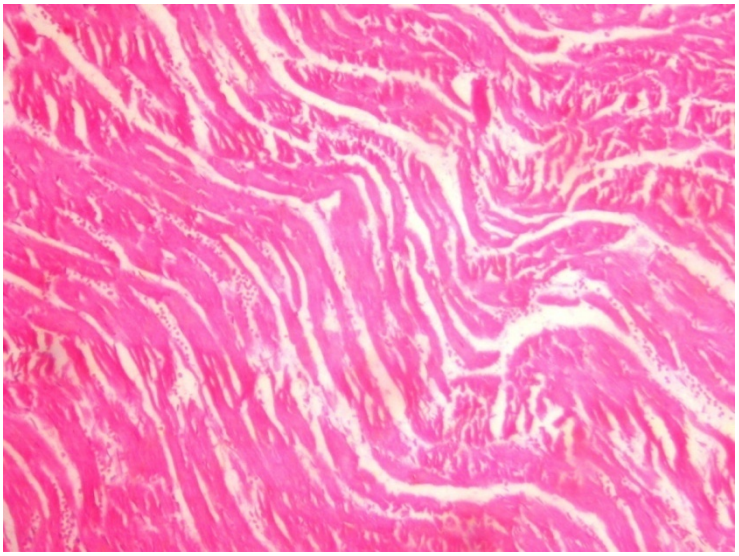

Fig 3. Histopathology of section of Heart of male rat showing normal Endocardium, myocardium and pericardium.

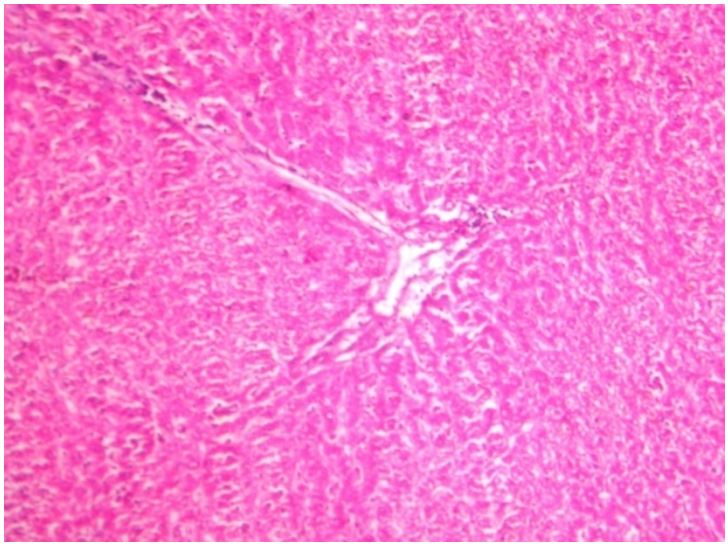

Fig 4. Histopathology of section of Liver of a male rat showing normal Portal triads and sinusoidal spaces.

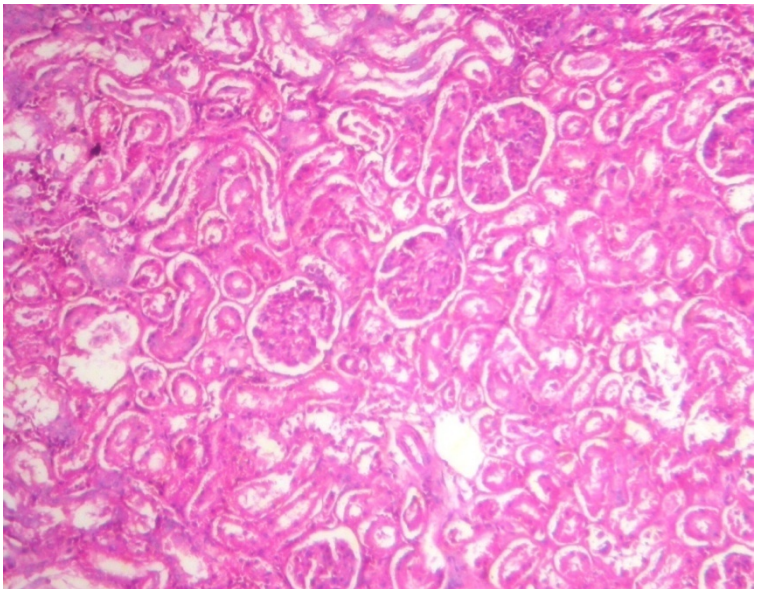

Fig 5. Histopathology of section of Kidney of a female rat showing normal glomeruli, Bowman's capsule and interstitial tissue.

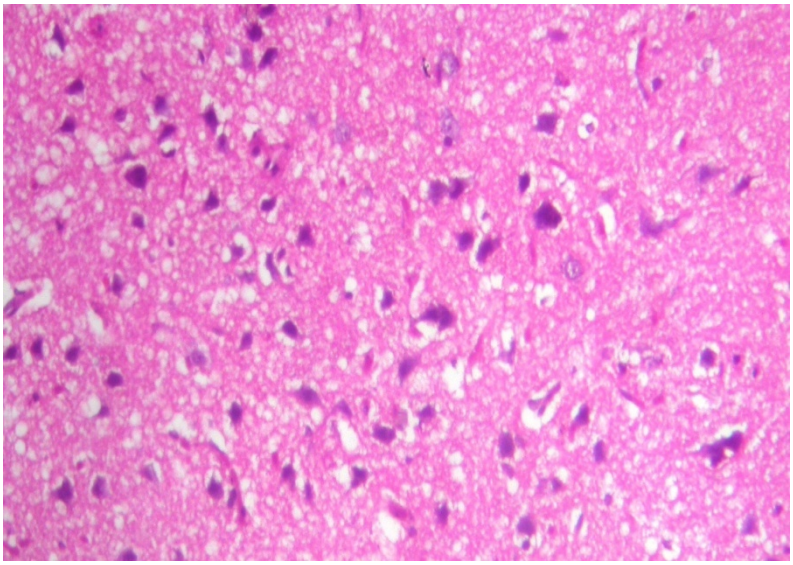

Fig 6. Histopathology of section of Brain of a female rat showing normal glial cells and astrocytes.

Conclusion

No abnormal behavioural activity and no pre-terminal deaths were recorded in wistar rats upon dermal exposure to test drug Mahanarayana taila during the course of acute and sub chronic toxicity study. Safety of the test drug at the tested dose level was confirmed by physiological, haematological, biochemical and histopathology findings.

Acknowledgement

The authors are grateful to Director General CCRAS, New Delhi for providing necessary facilities and encouragement.. Authors wish to express their thanks to Sri A.K. Ismail, DEO for statistical and typographical assistance.

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