Research Article

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PUNARNAVA MANDUR: TOXICITY STUDY OF CLASSICAL AYURVEDIC FORMULATION IN WISTAR RATS

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ABSTRACT
Punarnava Mandur is an iron containing classical Ayurvedic formulation which was studied for repeated dose oral toxicity study in Wistar rats for 90 days. Total 48 Wistar rats (24 male and 24 female) were selected based on the body weight and were randomly distributed into four groups followed by administration of Punarnava Mandur at the dose of 0, 90, 450, 900 mg/kg body weight for 90 consecutive days. Body weight, Weekly Feed and Water consumption, Clinical Chemistry, Hematology, Differential leukocyte Count, Reticulocyte count and Organ weights were recorded and analyzed statistically. At termination, rats were sacrificed, examined for gross pathological changes, organs were collected, weighed and processed for histopathological evaluation. There was no effect on body weights and feed consumption, no abnormal findings in the histopathological evaluation of high dose group animals but there was significant increase in weight of liver in females of high dose group as compared to control. Hence, the dose level 450 mg/kg of Punarnava Mandur was found as NOAEL (No Observable Adverse Effect Level). However, the NOEL (No Observed Effect Level) could not be established. It was suggested to carry out a toxicity study at possible higher doses so as to establish target organ of toxicity.
Keywords: Punarnava Mandur, Toxicity Study, NOEL, NOAEL

INTRODUCTION

Being ancient and time tested healthcare system, 'Ayurveda' is now in need of factual evidence base for being accepted globally. Global survey made under WHO, on national policy and regulation of Traditional Medicine emphasized lack of information sharing and method to evaluate safety and efficacy as common difficulties and challenges in form of traditional Knowledge of medicine [1]. In this situation, a properly planned toxicity study of Classical Ayurvedic drug considering global guidelines, using highest possible dose levels may explore target organ toxicity. Further, toxicity studies are essential part of regulatory requirements for marketing of the drug. The purpose of toxicity study is twofold i.e. to find out the effect caused and the level of exposure at which the effect is observed. Long term toxicity studies are designed to detect effects on organs or body systems and the dose range over which the effect develops due to repeated administration for long period. The data collected from toxicity studies will help to understand dose-toxicity response and to establish a safe dose for use in clinical trials.

Punarnava Mandur is a classical Ayurvedic formulation of various plant parts mixed with Mandur (Iron) Bhasma and cow urine (Gomutra). Mandur is ferric oxide which is boiled with cow's urine and then powder form of plant parts are mixed. Punarnava Mandur is indicated for various conditions like Anemia (Pandu roga), Mal-absorption Syndrome (Grahani), Inflammation (Sotha), Splenic Diseases (Pliha roga), Intermittent Fever (Vishamjwara), Hemorrhoids (Arsa), Skin diseases (Kushta) and worm infestation (Krumi)[2]. However, upon literature search for toxicity studies of Punarnava Mandur no reference was traceable. Hence, 90 days repeated dose oral toxicity study of Punarnava Mandur in Wistar Rats was conducted with objective to evaluate the toxicological profile to identify the target organs of toxicity and to establish No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL) after oral administration of Punarnava Mandur in Wistar rats for 90 consecutive days.

MATERIALS AND METHODS

48 (24 male and 24 female) Wistar rats were weighed and randomly divided into four groups in such a way that mean body weights are equal and total weight variation should not exceed ± 20% of the mean. All rats were acclimatized for seven days before initiation of the study. Optimum animal husbandry conditions, viz. maintenance of 12 hour light and dark cycle, maintenance of temperature 23 ± 2^0C and relative humidity maximum upto 70% were provided as per CPCSEA guidelines[3]. All rats were housed in polycarbonate cages and were provided feed (Mfg. NIN, Hyderabad) and water (Mfg. Bislery) ad-libitum. The study was performed after approval from Institutional Animal Ethical Committee (IAEC/2010/06 dated 10/03/2010). The dose for study was calculated with reference to the human therapeutic dose i.e. 1 g/day.

Punarnava Mandur which was provided by NIA, Jaipur was reddish black fine powder having cow's urine smell. Various ingredients of Punarnava Mandur are listed in table 1.

Table 1: Physico-Chemical analysis of Mandur Bhasma

Percentage of Total Iron (w/w)	31.79
Percentage of Ferric Iron (w/w)	31.44
Percentage of Ferrous Iron (w/w)	0.35

Table 2: Analysis of Gomutra

P^H	8.61
Specific Gravity	1.0180
Urea Nitrogen	0.0762 % w/w
Ammonia Nitrogen	0.340 % w/w
Total Nitrogen	0.4162% w/w
Total Microbial count	18 CFU ml
Fungal count	Nil
Staphylococcus aureus	Absent
E. coli	Absent
Salmonellae	Absent

The weighed quantity of Punarnava Mandur was suspended in vehicle i.e. distilled water and stirred for 40 minutes continuously in mortar and pestle. The suspension was stirred simultaneously during drug administration so as to maintain it uniformity and syrigebility. This suspension was administered to high dose, mid dose and therapeutic dose group rats with 900, 450, 90 mg/kg body weight for 90 consecutive days. The dose selected was derived from conversion of human therapeutic dose 1 g/day as per the method given [4].

All rats were daily observed twice for any clinical signs and mortality. Weekly body weights, feed and water consumption were recorded. Blood collection was performed at 30, 60 and 90 days for biochemical and hematological analysis. For biochemical examinations glucose, total protein, SGOT, SGPT, serum creatinine were evaluated by using Advia – 60 hematology analyzer (Siemens's, Germany). Prothrombine time was evaluated manually on 30th, 60th and 90th day. For hematological examination WBC count, RBC count, Hemoglobin, Haematocrit Platelet count, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration were measured on 30th, 60th and 90th day using the Advia – 60 Hematology Analyzer, Siemens's, Germany. Differential Leukocyte Count was performed manually which included neutrophil, eosinophil, lymphocyte, monocyte and basophil count on 90th day. Bone Marrow smear examination was performed manually and Myeloid: Erythroid ratio was calculated. At the end of 90 days dosing and in-life phase observation period, animals were sacrificed using CO_2 euthanasia followed by exsanguinations on 91st day of the study, and were subjected to a detailed post-mortem examination. The tissues were weighed and collected in 10% Neutral Buffered Formalin. Testes were collected in Modified Davidson's Fluid and subsequently transferred to 10% Neutral Buffered Formalin after 24 hours. Organs from control group and high dose group were processed as per RITA guidelines [5] and subjected to histopathological evaluation.

Body weight, Feed and Water consumption, Clinical Chemistry, Hematology, DLC (both percent and absolute) and Reticulocyte count (both percent and absolute) and Organ weights the data was analyzed using one way ANOVA followed by Dunnett's multiple comparison tests by Graph Pad Prism Software. Data of Bone Marrow was analyzed by Student's t test [6].

RESULTS

Classical Ayurvedic formulation Punarnava Mandur was tested for its toxicity by 90 days repeated oral administration in rats after reviewing the data of weekly body weights of male and female rats (Table 1) it was found that there was no significant increase in body weights, no significant effect on feed and water consumption (Table 2 and 3). Fecal consistency was normal throughout the study period. One rat from therapeutic Dose group died during 8th week of the study. But this death was not due to drug as one small abscess was found at the base of the tongue upon gross pathological observation.

No treatment related effect on organ weights (Table 4) of rats was observed in comparison to the control group except, there was significant increase in weight of liver in females of high dose group. Hematological and biochemical parameters were within biological limits (Table 5-9). Myeloid to Erythroid ratio was within biological limits in the animals of high dose as compared to control group (Table 10).

Gross and histopathology evaluations of various organs/tissues from male and female rats of control and high dose group did not show any lesion related to the treatment of Punarnava Mandur.

DISCUSSION

Punarnava Mandur was studied for 90 days repeated dose administration with 90, 450, 900 mg/kg body weight in both sexes of Wistar rats. This was first study aimed to evaluate target organ of toxicity and establish safety margins of this classical Ayurvedic formulation.

Main ingredient in Punarnava Mandur is Mandur Bhasma. Mandur Bhasma is prepared from an inorganic compound of iron i.e. ferric oxide. Its mineral form is called hematite. Physico-chemical analysis indicated that Punarnava Mandur contained 31.79 % of total iron of which 31.44% was ferric iron and 0.35% was ferrous iron. This reveals that total of approximately 286, 143 and 28.6 mg/kg of iron was being administered to the rats for 90 days continuously. In mice, LD_{50} of ferrous sulphate was recorded as 160 mg/kg b.wt.[7]. However, there was no reference traceable indicating toxic dose of ferric oxide in rats.

There was no effect on body weights, feed and water consumption. There were no abnormal findings in the histopathological evaluation of high dose group animals but there was significant increase in absolute and relative weight of liver in females of high dose group. Hepatocyte hypertrophy due to microsomal induction is common reason for increase in weight of liver in toxicity studies in rodents [5]. However, upon histopathological evaluation no effect was recorded on liver architecture. Punarnava Mandur was processed as per the Ayurvedic principles hence; there is possibility of interaction of metal and plant materials leading to decreased toxic effect [8].

Owing to these findings, the dose level 450 mg/kg of Punarnava Mandur was decided as No Observable Adverse Effect Level (NOAEL) for consecutive 90 days repeated administration in rats. The No Observed Effect Level (NOEL) was not established for Punarnava Mandur.

Table 2: Weekly Feed Weights (in Grams) of rats in 90 days repeated oral dose toxicity study of Punarnava Mandur

Group and Dose	Sex		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
Control	Male	Mean± SD	88.67± 8.82 (6)	89.00± 11.73 (6)	96.67± 9.46 (6)	96.83 ± 5.15 (6)	100.00± 0.00 (6)	105.30± 14.12(6)	103.80± 8.28 (6)	104.30± 7.89 (6)	105.20± 4.79 (6)	98.00 ± 9.06 (6)	105.00± 5.29 (6)	104.70± 6.02 (6)	95.33 ± 3.77 (6)
Control	Female	Mean± SD	63.33± 6.62 (6)	59.50± 5.68 (6)	63.33± 4.89 (6)	64.17 ± 5.67 (6)	60.17 ± 3.31 (6)	62.67 ± 8.91(6)	62.67 ± 7.17 (6)	63.17 ± 6.80 (6)	64.00 ± 7.07 (6)	62.67 ± 5.61 (6)	64.17 ± 4.58 (6)	61.67 ± 6.19 (6)	58.00 ± 5.02 (6)
HD (900 mg/kg)	Male	Mean± SD	92.17± 8.97 (6)	89.50± 8.50 (6)	93.33± 7.89 (6)	99.17 ± 1.33 (6)	98.17 ± 4.49 (6)	107.00± 11.37(6)	108.00± 9.08 (6)	108.80± 8.18 (6)	108.00± 6.90 (6)	103.80± 6.24 (6)	111.00± 6.87 (6)	107.80± 6.77 (6)	102.80± 8.54 (6)
HD (900 mg/kg)	Female	Mean± SD	64.17± 6.56 (6)	56.33± 3.45 (6)	62.67± 4.08 (6)	63.17 ± 6.11 (6)	63.33 ± 5.54 (6)	66.17 ± 6.34 (6)	65.67 ± 5.05 (6)	66.83 ± 6.85 (6)	67.50 ± 2.88 (6)	64.67 ± 4.32 (6)	68.17 ± 3.13 (6)	66.50 ± 3.02 (6)	64.83 ± 6.59 (6)
5TD (450 mg/kg)	Male	Mean± SD	62.67± 4.41 (6)	57.00± 3.58 (6)	64.00± 1.79 (6)	61.67 ± 3.83 (6)	61.33 ± 4.13 (6)	66.33 ± 6.71 (6)	64.00 ± 3.95 (6)	65.50± 4.09 (6)	67.00± 5.44 (6)	66.83± 8.75 (6)	65.67 ± 6.35 (6)	62.50± 4.09 (6)	61.83 ± 7.65 (6)
5TD (450 mg/kg)	Female	Mean± SD	90.50± 8.09 (6)	87.83± 6.97 (6)	94.33± 7.12 (6)	95.33± 4.23 (6)	96.33± 6.83 (6)	94.17± 9.70 (6)	96.50± 6.66 (6)	98.83± 7.73 (6)	100.30± 5.43 (6)	92.17± 8.28 (6)	97.83± 8.11 (6)	97.50± 5.05 (6)	92.17± 8.98 (6)
TD (90 mg/kg)	Male	Mean± SD	89.33± 8.89 (6)	87.17± 10.07 (6)	95.67± 5.09 (6)	97.83± 5.31 (6)	95.83*± 6.59 (6)	98.00± 10.88 (6)	98.33± 12.86 (6)	99.50± 14.63 (6)	101.00± 12.71 (6)	93.50± 14.46 (6)	94.00± 13.25 (6)	100.00± 10.53 (6)	94.00± 8.17 (6)
TD (90 mg/kg)	Female	Mean± SD	63.50± 6.41 (6)	58.33± 7.76 (6)	65.83± 4.83 (6)	66.00± 5.33 (6)	68.33± 5.72 (6)	67.67± 5.09 (6)	65.67± 2.73 (5)	67.83± 4.45 (5)	69.17± 4.75 (5)	62.67± 4.41 (5)	65.33± 3.45 (5)	66.83± 4.62 (5)	66.83± 4.54 (5)

(*) Significant at 5% level

Table 3: Weekly Water consumption ((in ml)) of rats in 90 days repeated oral dose toxicity study of Punarnava Mandur

Group and Dose			Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
Control 1	Male	Mean± SD	256.70± 29.44 (6)	270.00± 25.30 (6)	258.3± 42.15 (6)	298.3± 28.58 (6)	310.0± 20.00 (6)	323.3± 38.82 (6)	331.7± 24.83 (6)	293.30± 19.66 (6)	285.8± 33.23 (6)	270.80± 43.41 (6)	270.80± 48.62 (6)	275.0± 40.00 (6)	276.7± 29.44 (6)
	Female	Mean± SD	156.70± 12.11 (6)	146.70± 13.66 (6)	130.00± 18.97 (6)	206.70± 25.03 (6)	195.00± 25.10 (6)	210.00± 31.62 (6)	241.70± 42.15 (6)	193.30± 23.38 (6)	166.70± 14.72 (6)	150.00± 18.97 (6)	142.50± 12.55 (6)	130.00± 14.14 (6)	160.80± 31.37 (6)
HD (900 mg/kg)	Male	Mean± SD	235.00± 38.34 (6)	213.30*± 39.83 (6)	223.30± 41.79 (6)	278.30± 13.29 (6)	293.30± 40.33 (6)	304.20± 41.52 (6)	296.70± 58.88 (6)	245.00*± 33.91 (6)	268.30± 34.30 (6)	259.20± 48.42 (6)	265.80± 32.00 (6)	256.70± 38.82 (6)	290.00± 27.57 (6)
	Female	Mean± SD	165.00± 12.25 (6)	135.80± 12.81 (6)	132.50± 14.05 (6)	205.00± 28.11 (6)	201.70± 37.10 (6)	225.00± 49.30 (6)	241.70± 43.55 (6)	194.20± 14.97 (6)	189.20± 31.69 (6)	171.70± 26.39 (6)	163.30± 22.51 (6)	172.50± 47.51 (6)	175.00± 20.74 (6)
5TD (450 mg/kg)	Male	Mean± SD	258.30± 11.69 (6)	243.30± 22.51 (6)	253.30± 41.31 (6)	286.70± 10.33 (6)	296.70± 10.33 (6)	345.00± 35.64 (6)	325.00± 50.10 (6)	288.30± 19.66 (6)	302.50± 24.85 (6)	304.20± 22.00 (6)	296.70± 18.62 (6)	285.80± 8.01 (6)	263.30± 10.33 (6)
	Female	Mean± SD	150.80± 34.99 (6)	143.30± 35.59 (6)	145.80± 31.05 (6)	171.70± 27.87 (6)	215.00± 36.19 (6)	236.70± 31.57 (6)	190.00± 61.32 (6)	184.20± 19.08 (6)	168.30± 19.66 (6)	172.50± 28.94 (6)	160.80± 24.38 (6)	151.70± 22.06 (6)	180.80± 40.79 (6)
TD (90 mg/kg)	Male	Mean± SD	229.20± 30.73 (6)	192.50*± 31.90 (6)	220.80± 49.03 (6)	278.30± 43.55 (6)	290.00± 25.30 (6)	310.00± 49.40 (6)	301.70± 61.78 (6)	293.30± 24.83 (6)	288.30± 36.01 (6)	261.70± 37.10 (6)	266.70± 42.74 (6)	278.30± 48.34 (6)	270.00± 16.73 (6)
	Female	Mean± SD	166.70± 16.33 (6)	140.80± 22.00 (6)	143.30± 23.38 (6)	171.70± 23.17 (6)	231.70± 21.37 (6)	230.00± 49.40 (6)	195.80± 41.04 (5)	190.80± 32.62 (5)	194.20± 35.56 (5)	193.30*± 20.66 (5)	177.50*± 28.59 (5)	177.50± 37.65 (5)	167.50± 27.16 (5)

(*) Significant at 5% level

Table 4: Organ weight in 90 days repeated dose oral toxicity study of Punarnava Mandur

Group and Dose	Sex		Adrenals	Liver	Brain	Thymus	Heart	Kidneys	Spleen	Testis / Ovaries	Epi./Uterus (with cervix)
Control	Male	Mean± SD (N)	0.06±0.02 (6)	11.25±1.46 (6)	1.76±0.28 (6)	0.23±0.06 (6)	1.31±0.12 (6)	2.63±0.27 (6)	0.93±0.15 (6)	3.10±0.17 (6)	1.15±0.06 (6)
	Female	Mean± SD (N)	0.08±0.03 (6)	5.76±1.39 (6)	1.70±0.13 (6)	0.21±0.04 (6)	0.89±0.03 (6)	1.56±0.17 (6)	0.72±0.18 (6)	0.13±0.04 (6)	0.46±0.08 (6)
HD (900 mg/kg)	Male	Mean± SD (N)	0.06±0.01 (6)	12.14±2.15 (6)	1.88±0.10 (6)	0.22±0.04 (6)	1.25±0.06 (6)	2.72±0.21 (6)	1.02±0.16 (6)	3.22±0.19 (6)	1.20±0.07 (6)
	Female	Mean± SD (N)	0.06±0.01 (6)	7.46*±0.75 (6)	1.60±0.14 (6)	0.19±0.05 (6)	0.85±0.06 (6)	1.57±0.10 (6)	0.69±0.12 (6)	0.13±0.02 (6)	0.54±0.17 (6)
5TD (450 mg/kg)	Male	Mean± SD (N)	0.09±0.05 (6)	10.83±1.08 (6)	1.79±0.24 (6)	0.32±0.10 (6)	1.33±0.23 (6)	2.52±0.10 (6)	1.11±0.28 (6)	3.15±0.18 (6)	1.27±0.14 (6)
	Female	Mean± SD (N)	0.09±0.05 (6)	6.58±0.53 (6)	1.73±0.06 (6)	0.21±0.09 (6)	0.90±0.10 (6)	1.55±0.06 (6)	0.71±0.02 (6)	0.15±0.06 (6)	0.56±0.16 (6)
TD (90 mg/kg)	Male	Mean± SD (N)	0.07±0.04 (6)	9.51±1.52 (6)	1.64±0.22 (6)	0.22±0.09 (6)	1.24±0.11 (6)	2.35±0.22 (6)	1.07±0.37 (6)	2.94±0.14 (6)	1.15±0.06 (6)
	Female	Mean± SD (N)	0.06±0.03 (5)	6.04±1.11 (5)	1.70±0.04 (5)	0.21±0.05 (5)	0.83±0.09 (5)	1.44±0.16 (5)	0.57±0.16 (5)	0.11±0.04 (5)	0.51±0.19 (5)

(*) Significant at 5% level

Table 5: 30, 60, 90 Days Hematology Parameters of Male rats in 90 days repeated dose oral toxicity study of Punarnava Mandur

Group and Dose	Day		WBC (10^3 / mm^3)	RBC (10^6 / mm^3)	Hb (g/dl)	HCT (%)	PLT count (10^3 / mm^3)	MCV (µ mm^3)	MCH (pg)	MCHC (g/dl)
Control	30 days	Mean± SD	5.65 ±2.86 (6)	8.69 ±2.67 (6)	15.28 ±3.86 (6)	48.07 ±15.24 (6)	575.20 ±306.80 (6)	55.00 ±2.19 (6)	17.80 ±1.54 (6)	32.27 ±2.38 (6)
	60 days	Mean± SD	7.42 ±4.01(6)	8.41 ±0.50 (6)	14.38 ±1.20 (6)	42.43 ±3.48 (6)	678.30 ±146.60 (6)	50.33 ±1.97 (6)	17.12 ±0.90 (6)	34.05 ±1.42 (6)
	90 days	Mean± SD	7.00 ±1.78 (6)	11.77 ±2.16 (6)	19.10 ±3.43 (6)	60.27 ±11.96 (6)	637.00 ±237.80 (6)	51.17 ±1.72 (6)	16.25 ±1.11 (6)	31.82 ±1.66 (6)
High Dose	30 days	Mean± SD	8.27 ±1.76 (6)	7.75 ±0.52 (6)	14.80 ±0.99 (6)	42.08 ±2.63 (6)	821.20 ±94.38 (6)	54.33 ±1.37 (6)	19.08 ±0.60 (6)	35.12* ±0.25 (6)
	60 days	Mean± SD	5.65±1.52 (6)	10.31±0.99 (6)	17.38±1.42 (6)	53.58±5.91 (6)	506.20±106.10 (6)	51.83±0.98 (6)	16.88±0.63 (6)	32.58±1.53 (6)
	90 days	Mean± SD	5.40±2.45 (6)	10.56±1.47 (6)	17.08±2.19 (6)	53.10±8.32 (6)	645.20±118.60 (6)	50.67±1.03 (6)	16.37±0.67 (6)	31.72±1.47 (6)
5TD (450 mg/kg)	30 days	Mean± SD	6.62±1.74 (6)	7.12±1.19 (6)	13.43±1.91 (6)	38.30±6.39 (6)	828.50±339.10 (6)	53.67±1.51 (6)	18.98±0.98 (6)	35.23*±1.26 (6)
	60 days	Mean± SD	5.05±1.43 (6)	10.34±2.40 (6)	17.57±3.97 (6)	53.78±15.10 (6)	586.30±185.70 (6)	51.83±2.14 (6)	17.10±0.30 (6)	33.00±1.48 (6)
	90 days	Mean± SD	5.50±1.94 (6)	11.72±1.05 (6)	18.17±2.20 (6)	55.87±6.79 (6)	620.50±97.31 (6)	50.33±0.52 (6)	16.20±0.89 (6)	32.32±1.02 (6)
TD (90 mg/kg)	30 days	Mean± SD	6.15±1.87 (6)	8.46±0.34 (6)	15.17±0.65 (6)	44.18±2.12 (6)	676.20±121.60 (6)	52.17*±1.17 (6)	17.92±0.32 (6)	34.27±0.44 (6)
	60 days	Mean± SD	5.62±1.43 (6)	11.18*±2.04 (6)	18.55*±3.50 (6)	57.63*±10.52 (6)	595.20±137.00 (6)	51.50±1.05 (6)	16.33±0.87 (6)	32.17±0.95 (6)
	90 days	Mean± SD	5.72±1.30 (6)	11.51±1.85 (6)	18.43±3.55 (6)	60.40±4.84 (6)	597.20±57.24 (6)	49.83±2.40 (6)	16.05±0.86 (6)	32.12±0.56 (6)

(*) Significant at 5% level

Table 6: 30, 60, 90 Days Hematology Parameters of Female rats in 90 days repeated dose oral toxicity study of Punarnava Mandur

Group and Dose	Day		WBC (10^3 / mm^3)	RBC (10^6 / mm^3)	Hb (g/dl)	HCT (%)	PLT count (10^3 / mm^3)	MCV (µ mm^3)	MCH (pg)	MCHC (g/dl)
Control	30 days	Mean	4.73±1.45 (6)	7.50±0.41 (6)	14.38±0.54 (6)	39.42±1.36 (6)	693.50±110.40 (6)	52.50±2.26 (6)	19.23±1.24 (6)	36.48±0.99 (6)
		(±)SD								
	60 days	Mean	4.85±2.16 (6)	8.71±0.34 (6)	16.05±0.80 (6)	45.35±2.82 (6)	611.50±91.31 (6)	52.00±2.00 (6)	18.42±0.52 (6)	35.42±0.56 (6)
		(±)SD								
	90 days	Mean	3.23±0.67 (6)	8.71±1.42 (6)	18.05±4.58 (6)	46.90±9.00 (6)	546.30±166.00 (6)	55.00±4.65 (6)	17.77±2.14 (6)	35.22±1.66 (6)
		(±)SD								
High Dose	30 days	Mean	4.93±2.57 (6)	7.38±0.80 (6)	14.87±0.91 (6)	38.85±3.51 (6)	710.30±243.70 (6)	52.67±2.42 (6)	20.27±1.39 (6)	38.42±2.04 (6)
		(±)SD								
	60 days	Mean	3.15±0.81 (6)	9.09±1.03 (6)	16.57±1.71 (6)	47.83±6.34 (6)	577.30±122.30 (6)	52.50±1.52 (6)	18.23±0.69 (6)	34.73±1.27 (6)
		(±)SD								
	90 days	Mean	3.47±0.36 (6)	9.15±1.18 (6)	16.47±1.69 (6)	48.25±5.42 (6)	597.20±165.00 (6)	52.33±1.21 (6)	17.83±0.71 (6)	33.88±1.12 (6)
		(±)SD								
5TD (450 mg/kg)	30 days	Mean	6.08±1.75 (6)	8.02±0.73 (6)	15.08±1.25 (6)	41.03±4.29 (6)	723.20±140.60 (6)	51.33±2.07 (6)	18.83±0.68 (6)	36.80±0.88 (6)
		SD(±)								
	60 days	Mean	4.98±1.17 (6)	9.68±1.15 (6)	17.07±1.63 (6)	49.57±6.14 (6)	663.20±181.60 (6)	51.33±0.52 (6)	17.68±0.82 (6)	34.62±1.81 (6)
		SD(±)								
	90 days	Mean	3.67±0.61 (6)	9.30±1.13 (6)	16.55±2.15 (6)	47.68±6.36 (6)	639.80±136.80 (6)	50.67*±0.82 (6)	17.90±0.36 (6)	34.82±0.87 (6)
		SD(±)								
TD (90 mg/kg)	30 days	Mean	3.75±2.03 (6)	9.14*±1.76 (6)	16.22±2.68 (6)	48.17*±10.48 (6)	555.70±149.20 (6)	52.50±2.07 (6)	17.88±1.24 (6)	34.03±2.29 (6)
		SD(±)								
	60 days	Mean	2.47*±0.77 (6)	8.43±2.51 (6)	14.72±4.01 (6)	43.38±13.22 (6)	541.30±167.80 (6)	51.17±0.75 (6)	17.68±0.96 (6)	34.50±2.29 (6)
		SD(±)								
	90 days	Mean	3.07±0.43 (5)	9.82±1.47 (5)	16.42±2.59 (5)	49.22±8.74 (5)	617.50±163.40 (5)	51.67±0.82 (5)	17.18±0.39 (5)	33.22*±1.08 (5)
		SD(±)								

(*) Significant at 5% level

Table 7: 30, 60, 90 Days Biochemical Data Male rats in 90 days repeated dose oral toxicity study of Punarnava Mandur

Group and Dose	Day		Blood Glucose (mg/dl)	Total Protein (gm/dl)	SGOT (U/L)	SGPT (U/L)	Serum Creatinine (mg/dl)	Prothrombine Time (Sec.)
Control	30 days	Mean	137.80±15.04 (6)	7.25±0.56 (6)	170.00±18.58 (6)	52.50±7.31 (6)	3.17±0.54 (6)	9.67±1.86 (6)
		SD(±)						
	60 days	Mean	116.30±26.02 (6)	7.77±3.26 (6)	93.67±15.46 (6)	57.00±7.80 (6)	0.70±0.11 (6)	10.83±1.94 (6)
		SD(±)						
	90 days	Mean	97.17±24.57 (6)	8.37±1.50 (6)	87.50±19.18 (6)	39.50±5.01 (6)	0.62±0.19 (6)	10.50±1.52 (6)
		SD(±)						
High Dose	30 days	Mean	98.50*±22.36 (6)	6.12±0.28 (6)	188.80±41.49 (6)	56.50±18.39 (6)	2.35*±0.31 (6)	9.83±2.79 (6)
		SD(±)						
	60 days	Mean	109.30±16.69 (6)	6.92±2.01 (6)	85.33±15.13 (6)	39.50*±4.42 (6)	0.73±0.15 (6)	9.33±1.21 (6)
		SD(±)						
	90 days	Mean	91.83±24.58 (6)	7.93±1.12 (6)	80.17±10.63 (6)	37.00±6.16 (6)	0.60±0.06 (6)	10.50±1.52 (6)
		SD(±)						
5TD (450 mg/kg)	30 days	Mean	140.30±21.42 (6)	5.02*±0.88 (6)	144.70±14.00 (6)	64.83±6.56 (6)	3.00±0.43 (6)	9.83±1.17 (6)
		SD(±)						
	60 days	Mean	113.00±30.48 (6)	6.93±0.77 (6)	82.50±12.57 (6)	42.50*±7.42 (6)	0.63±0.12 (6)	9.00±1.67 (6)
		SD(±)						
	90 days	Mean	74.67±12.93 (6)	7.92±1.12 (6)	108.50±27.19 (6)	33.83±5.98 (6)	0.62±0.04 (6)	10.67±0.52 (6)
		SD(±)						
TD (90 mg/kg)	30 days	Mean	161.00±14.38 (6)	5.33*±1.16 (6)	190.50±19.32 (6)	55.00±7.77 (6)	3.88*±0.19 (6)	9.17±2.14 (6)
		SD(±)						
	60 days	Mean	114.50±16.74 (6)	7.12±0.95 (6)	101.50±30.09 (6)	47.67±10.56 (6)	0.68±0.12 (6)	9.00±1.41 (6)
		SD(±)						
	90 days	Mean	96.00±14.75 (6)	7.25±0.77 (6)	117.30±35.61 (6)	39.17±14.05 (6)	0.60±0.00 (6)	9.50±0.55 (6)
		SD(±)						

Table 8: 30, 60, 90 Days Biochemical Data Female rats in 90 days repeated dose oral toxicity study of Punarnava Mandur

Group and Dose	Day		Blood Glucose (mg/dl)	Total Protein (gm/dl)	SGOT (U/L)	SGPT (U/L)	Serum Creatinine (mg/dl)	Prothrombine Time (Sec.)
Control	30 days	Mean	149.00±14.60 (6)	7.02±0.36 (6)	141.20±15.90 (6)	36.50±3.15 (6)	3.07±0.16 (6)	11.17±0.98 (6)
		SD(±)						
	60 days	Mean	99.83±12.83 (6)	7.25±1.91 (6)	113.50±36.17 (6)	34.00±5.25 (6)	0.75±0.19 (6)	7.83±1.47 (6)
		SD(±)						
	90 days	Mean	94.50±19.62 (6)	8.25±0.90 (6)	86.33±13.19 (6)	29.17±8.33 (6)	0.65±0.14 (6)	9.83±0.98 (6)
		SD(±)						
High Dose (750 mg/kg)	30 days	Mean	114.80*±11.02 (6)	5.97*±0.37 (6)	144.20±16.92 (6)	46.67*±3.33 (6)	2.63±0.47 (6)	9.50±1.52 (6)
		SD(±)						
	60 days	Mean	107.00±16.44 (6)	7.23±1.48 (6)	80.83*±10.59 (6)	36.00±9.19 (6)	0.60±0.09 (6)	9.33±1.37 (6)
		SD(±)						
	90 days	Mean	94.50±21.64 (6)	8.33±1.34 (6)	91.67±15.45 (6)	33.67±4.13 (6)	0.58±0.10 (6)	9.83±1.60 (6)
		SD(±)						
5TD (450 mg/kg)	30 days	Mean	142.00±12.98 (6)	5.00*±0.20 (6)	142.20±8.16 (6)	43.17±3.97 (6)	3.17±0.31 (6)	12.67±2.50 (6)
		SD(±)						
	60 days	Mean	104.20±12.37 (6)	7.45±1.22 (6)	82.83±12.58 (6)	38.33±4.50 (6)	0.68±0.13 (6)	8.33±1.97 (6)
		SD(±)						
	90 days	Mean	76.50±30.47 (6)	7.92±1.07 (6)	97.00±22.94 (6)	31.17±5.81 (6)	0.58±0.16 (6)	10.50±1.64 (6)
		SD(±)						
TD (90 mg/kg)	30 days	Mean	148.00±23.41 (6)	5.88*±0.50 (6)	144.00±33.54 (6)	52.00*±10.00 (6)	3.67*±0.42 (6)	10.00±1.41 (6)
		SD(±)						
	60 days	Mean	106.70±25.48 (6)	6.93±1.07 (6)	93.67±14.77 (6)	40.17±6.82 (6)	0.62±0.12 (6)	9.50±1.64 (6)
		SD(±)						
	90 days	Mean	87.67±16.23 (5)	7.40±1.53 (5))	94.67±15.63 (5)	38.67±8.21(5)	0.60±0.09 (5)	8.83±1.33 (5)
		SD(±)						

(*) Significant at 5% level

Table 9: Differential Leukocyte Count Data (%) in 90 days repeated dose oral toxicity study of Punarnava Mandur

Group and Dose	Sex		Neutrophil	Eosinophil	Lymphocyte	Monocyte	Basophil
Control	Male	Mean± SD (N)	14.17±2.229 (6)	4.167±1.169 (6)	78.50±1.517 (6)	3.167±0.7528 (6)	0.0±0.0 (6)
	Female	Mean± SD (N)	14.00±0.6325 (6)	4.167±1.169 (6)	78.83±1.941 (6)	3.000±1.265 (6)	0.0±0.0 (6)
HD (900 mg/kg)	Male	Mean± SD (N)	15.17±0.9832 (6)	3.167±0.9832 (6)	78.33±2.338 (6)	3.667±1.033 (6)	0.0±0.0 (6)
	Female	Mean± SD (N)	12.83±2.137 (6)	3.667±1.033 (6)	80.50±2.881 (6)	3.000±1.414 (6)	0.0±0.0 (6)
5TD 450 mg/kg)	Male	Mean± SD (N)	13.67±1.751 (6)	4.000±0.6325 (6)	79.00±1.414 (6)	3.333±1.211 (6)	0.0±0.0 (6)
	Female	Mean± SD (N)	14.17±1.941 (6)	4.833±1.169 (6)	77.67±1.633 (6)	3.333±1.366 (6)	0.0±0.0 (6)
TD (90 mg/kg)	Male	Mean± SD (N)	14.33±0.8165 (6)	3.333±1.211 (6)	79.17±1.472 (6)	3.167±0.7528 (6)	0.0±0.0 (6)
	Female	Mean± SD (N)	13.67±1.211 (5)	4.500±0.8367 (5)	78.33±1.366 (5)	3.500±1.049 (5)	0.0±0.0 (6)

(*) Significant at 5% level

Table 10: Bone marrow evaluation of rats 90 days repeated dose oral toxicity study of Punarnava Mandur

	Group and Dose		Myeloid	Erythroid	M:E
Male	Control	Mean±SD(N)	285.50±19.99 (6)	199.50±41.61 (6)	1.51±0.46 (6)
	HD (900 mg/kg)	Mean±SD(N)	275.50±7.48 (6)	224.50±7.48 (6)	1.23±0.08 (6)
Female	Control	Mean±SD(N)	305.50±5.86 (6)	194.50±5.86 (6)	1.57±0.08 (6)
	HD (900 mg/kg)	Mean±SD(N)	286.30±9.11 (6)	213.80±9.09 (6)	1.34*±0.10 (6)

(*) Significant at 5% level

Table 1: Weekly Body Weights (in Grams) of rats in 90 days repeated oral dose toxicity study of Punarnava Mandur

Group and Dose	Sex		Initial Body Weight	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
Control	Male	Mean± SD (N)	127.70± 28.42 (6)	187.20± 31.16 (6)	214.30± 32.38 (6)	234.40± 28.62 (6)	245.50± 20.15 (6)	257.20± 19.55 (6)	282.60± 24.22 (6)	295.50± 18.64 (6)	305.00± 16.47 (6)	313.80± 23.45 (6)	326.10± 4.47 (6)	335.60± 25.00 (6)	344.70± 26.50 (6)	346.40± 25.00 (6)
	Female	Mean ± SD (N)	114.50± 25.65 (6)	140.70± 17.73 (6)	149.30± 16.15 (6)	152.80± 14.29 (6)	158.00± 13.91 (6)	164.30± 14.05 (6)	166.30± 14.51 (6)	171.00± 15.84 (6)	172.20± 15.51 (6)	172.40± 16.14 (6)	176.20± 17.32 (6)	180.50± 16.61 (6)	181.50± 1.63 (6)	182.50± 16.72 (6)
HD (900 mg/kg)	Male	Mean± SD (N)	127.40± 20.40 (6)	189.7± 17.03 (6)	207.40± 32.34 (6)	230.4± 27.10 (6)	244.9± 21.39 (6)	255.30± 18.52 (6)	281.8± 13.47 (6)	299.9± 13.59 (6)	312.8± 14.98 (6)	317.2± 13.15 (6)	329.3± 20.98 (6)	341.9± 19.72 (6)	349.1± 19.85 (6)	352.0± 22.61 (6)
	Female	Mean± SD (N)	114.20± 26.45 (6)	139.1± 16.00 (6)	147.90± 13.40 (6)	152.90± 11.48 (6)	156.3± 11.02 (6)	167.4± 11.37 (6)	170.3± 13.08 (6)	174.8± 12.01 (6)	179.5± 11.63 (6)	177.8± 9.63 (6)	184.0 ± 10.35 (6)	189.0± 10.85 (6)	191.3± 10.47 (6)	192.3± 10.58 (6)
5TD (450 mg/kg)	Male	Mean± SD (N)	127.80± 19.65 (6)	190.00± 18.25 (6)	213.20± 15.40 (6)	233.00± 13.27 (6)	250.10± 13.81 (6)	261.00± 12.29 (6)	280.2± 19.01 (6)	290.70± 18.04 (6)	299.90± 18.14 (6)	300.70± 19.74 (6)	308.40± 17.68 (6)	319.30± 15.41 (6)	329.80± 13.29 (6)	332.90± 13.28 (6)
	female	Mean± SD (N)	114.40± 28.01 (6)	139.20± 17.47 (6)	147.60± 14.48 (6)	154.20± 10.59 (6)	159.00± 10.54 (6)	163.70± 7.69 (6)	171.30± 8.83 (6)	174.80± 6.92 (6)	176.20± 9.57 (6)	177.80± 7.42 (6)	184.20± 8.00 (6)	187.10± 7.74 (6)	188.90± 9.28 (6)	188.30± 6.25 (6)
TD (90 mg/kg)	Male	Mean± SD (N)	127.40± 17.75 (6)	184.90± 15.99 (6)	212.60± 18.44 (6)	228.80± 14.39 (6)	253.60± 13.18 (6)	256.60± 13.74 (6)	278.70± 22.34 (6)	288.90± 25.98 (6)	296.50± 29.13 (6)	297.60± 30.72 (6)	310.90± 30.00 (6)	314.60± 36.34 (6)	320.40± 39.69 (6)	321.00± 36.77 (6)
	Female	Mean± SD (N)	114.10± 24.65 (6)	140.80± 20.06 (6)	148.30± 20.29 (6)	154.90± 18.40 (6)	160.80± 19.46 (6)	166.30± 18.12 (6)	171.90± 18.66 (6)	176.00± 17.21 (5)	177.50± 17.48 (5)	178.30± 18.27 (5)	181.80± 18.96 (5)	184.60± 16.95 (5)	187.90± 17.82 (5)	188.90± 16.47 (5)

(*) Significant at 5% level

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Abbreviations
HD: High Dose
TD: Therapeutic Dose

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