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Sub Acute (28 Days) and Chronic (90 Days) Toxicity Studies of Ayurvedic Bhasma Rasamanikya In Wistar Rats

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ABSTRACT

Rasamanikya is a herbo mineral preparation used extensively in Ayurvedic treatment. This is prepared using haratala (arsenic tri oxide) by the process of bhavana (wet trituration) and exposure to high temperature. This drug in minute quantities is said to be effective against respiratory ailments, skin diseases and fever. Rasamanikya was evaluated for safety in Wistar rats by sub acute (28 days) and chronic toxicity (90 days) studies. The test drug was given up to three times of its therapeutic dose orally. The test drug did not cause mortality or signs of toxicity in Wistar rats in both the studies. No major changes were observed with respect to hematology, and biochemical parameters. Post mortem of the animals did not reveal any gross pathology of internal organs. Histopathology changes observed in few groups were minimal and were of reversible type. Rasamanikya was found to be safe up to 54 mg/kg body weight in Wistar rats.

Keywords: Rasamanikya, Wistar rats, Sub acute toxicity, chronic Toxicity.

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INTRODUCTION

Herbo mineral preparations occupy a prominent place in the Ayurvedic systems of medicine. Bhasmas are the metallic preparations treated with herbal juice and exposed to heat. They are the nano particles of Ayurveda and are recommended for treatment of chronic ailments. Rasamanikya is one among the frequently used rasakalpas. It is found to be beneficial in different types of jwaras (fevers) .It is used as effective medicine in ailments such as dyspnoea, cough, skin ailments including chronic wounds and fissures[1].

The very mention of metal and minerals in Ayurvedic preparations makes many to conclude the product as toxic and unfit for human use. But if consumed in therapeutic doses under able Ayurvedic supervision, these preparations were found to be absolutely safe. The present study is an attempt to prove the safety of Rasamanikya in Wistar rats.

MATERIALS AND METHOD

Animals

Wistar Rats of either sex procured from 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.

Test drug

Rasamanikya prepared as per Abhrakapatra method was used in the trial (Rasatarangini. 11: 90-93)[2].

Purified Haratala (Arsenic tri oxide) was triturated with the juice of Ash gourd (*Biniscasa hispida* (Thunb.). Cogn.) and the triturated mixture were washed twice with warm water. The mixture was cooled and ground into coarse crystals.

Two coarse crystals (around 130 mg) were kept between the sheets of mica and the sheets were sealed on all four sides and heated on coal fire. When the grains turn reddish, the grains were taken out and the process was repeated for all the grains. Cooled grains were made into fine powder.

Experimental groups and dose calculation

The human dose of the Rasamanikya is 60 mg per day and the dose equivalent to rats was calculated as per the method of Ghosh[3]. The dose of the test drug was 5.4 mg/kg BW. 27 mg/kg BW and 54 mg/kg BW in test dose, average dose and high dose groups respectively. The test drug was dissolved in diluted honey and applied on the tongue of each animal.

The rats were divided into four groups namely Vehicle Control (VC) group, test dose (TD) group, Average dose (AD) group and high dose (HD) group each comprising of 12 animals (6 Male and 6 Female).

Sub acute toxicity study

The study was carried out as per Schedule Y[4].Test drug and vehicles were administered to the experimental groups for a consecutive period of 14 days. 50% of the animals were euthanized on day 15 to assess immediate toxicity. Remaining 50% of the animals were euthanized on the 29th day of the study to evaluate delayed onset of toxicity.

Chronic toxicity study

The study was carried out as per OECD guideline 408[5].Test drug and vehicle were administered to the experimental groups for a period of 91 days consecutively. All the animals were euthanized at the termination of the trial.

Clinical Observation

Animals were observed for signs of mortality and clinical signs of toxicity during the study period. Behavioral and physiological responses were monitored daily and weekly feed consumption and body weight gain were recorded as per OECD guideline 407 [5].

Blood collection, haematology and Biochemical Investigations

Blood samples were collected from fasted animals at the termination of respective studies under ether anaesthesia. Total Leucocyte Count (TLC), Polymorph Percentage, Lymphocyte percentage, Packed Cell Volume , Haemoglobin (HB)levels, Total red Cells Count (TRC) and platelets Count, Serum Glucose, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Creatinine, Total Protein (TP) and Prothrombin Time (PT) were analyzed at Biochemistry division of the Institute.

After blood collection, animals were euthanized by cervical dislocation, and detail post morten examination was carried out. Vital organs viz., Heart, Lung, liver, Spleen. Kidneys, Testes, ovaries, brain etc. were weighed and tissue samples were collected in 10% formalin for histopathology studies.

RESULTS AND DISCUSSION

Mortality and clinical signs of toxicity

Pre terminal deaths and signs of toxicity were not observed in any of the animals during sub acute and chronic toxicity studies. Animals were found active and did not show any signs of toxicity or abnormalities with respect to physical appearance and physiological parameters like, temperature and respiration. Lacrimation and salivation were not observed. Animalsshowed normal locomotor and rearing activities.

Body weight and feed consumption

No significant difference was observed between test groups and control group in weekly bodyweight gain with respect to day 1 during sub acute and chronic toxicity studies (Tables 1-2)

The animals in the sub acute study did not show any change with respect to % feed intake, Female animals in the chronic study showed increase in the feed intake in the average dose group during VIII and IX week of the study compared to their male counterparts. (Tables 3-4)

Table 1: Weekly body weight gain as compared to day 1 during Sub acute toxicity study.

	1 Week	2 Week	3 Week	4Week
VC	0.83±0.83	7.50±1.12	11.67±1.67	16.67±2.11
TD	1.66±1.05	8.33±1.05	13.33±1.05	17.50±1.71
AD	1.67±1.05	9.17±1.54	14.17±2.01	19.17±2.39
HD	1.67±1.05	7.50±1.12	12.50±1.12	16.67±1.05

(Average of 6 values)

Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Table 2: Weekly body weight gain as compared to day 1 during chronic toxicity study

Weeks	VC		TD		AD		HD	
	Male	Female	Male	Female	Male	Female	Male	Female
0-1	22.17±1.05	17.74±1.51	19.407±0.76	18.56±1.18	25.99±1.56	21.63±1.15	24.95±1.01	20.57±1.22
0-2	48.72±2.56	37.23±3.19	48.69±1.84	37.05±1.38	54.43±1.63	38.28±2.17	52.45±1.957	36.83±1.62
0-3	75.485±4.206	50.76±3.006	76.43±3.452	50.78±2.72	80.75±3.52	51.89±2.22	73.64±4.21	50.77±2.68
0-4	106.72±5.45	59.40±3.66	101.46±5.29	62.32±3.49	113.86±4.68	63.95±2.95	107.94±6.05	60.06±3.45
0-5	128.22±5.84	66.54±4.04	115.87±5.46	69.80±3.28	133.80±4.28	71.20±2.91	122.42±5.48	67.87±3.83
0-6	140.05±4.99	71.17±4.20	133.05±6.09	73.76±3.77	149.01±3.66	76.05±2.82	139.91±6.70	74.23±3.96
0-7	155.59±5.47	76.91±4.25	142.75±5.50	82.71±3.76	160.69±3.13	83.58±3.52	150.28±7.99	80.26±4.01
0-8	174.11±6.63	81.64±4.71	158.02±5.75	88.00±4.24	171.84±3.26	90.65±3.48	163.62±8.11	86.68±3.47
0-9	182.80±8.47	85.16±4.48	171.15±4.92	92.10±3.92	185.65±3.60	93.58±4.01	173.86±8.06	91.95±4.05
0-10	194.64±8.10	89.89±4.85	187.33±6.08	95.07±3.71	199.65±4.97	99.71±4.44	189.05±9.53	95.99±3.94
0-11	203.21±8.17	94.07±4.96	191.83±6.51	99.15±3.91	210.55±6.21	101.37±4.19	194.69±9.42	98.89±3.67
0-12	213.69±9.84	98.62±4.49	201.99±6.30	106.67±3.96	218.55±6.69	107.15±3.62	205.41±8.51	108.03±4.49
0-13	219.73±9.82	101.54±5.05	206.48±6.09	107.88±4.81	220.39±6.81	107.15±3.62	216.37±8.86	108.65±4.50

(Average of 10 values) Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Table 3: Weekly % feed consumption in grams during Sub acute toxicity study

	1 Week	2 Week	3 Week	4 Week
VC	67.50±1.22	67.52±0.89	66.42±1.53	68.76±1.65
TD	67.88±1.59	67.87±2.14	68.03±0.78	70.30±1.61
AD	64.40±0.95	67.67±1.45	68.82±0.89	67.19±1.46
HD	69.59±1.68	65.56±1.57	67.62±0.99	68.79±1.33

(Average of 6 values) Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Table 4: Weekly % feed consumption in grams during chronic toxicity study.

Weeks	VC		TD		AD		HD	
	Male	Female	Male	Female	Male	Female	Male	Female
1	55.56±6.48	45.12±4.43	41.99±4.84	54.15±3.79	58.57±4.10	58.51±4.69	53.28±2.55	53.62±3.21
2	49.583±5.09	51.31±2.30	40.47±3.15	39.38 *±4.23	47.01±4.58	40.00*±2.96	50.63±4.04	43.30±2.77

3	30.97±3.61	34.76±3.12	34.58±3.01	34.95±2.46	29.17±3.04	33.96±2.61	27.96±2.06	31.97±1.65
4	41.50±3.20	25.19±2.31	40.05±2.83	27.20±2.93	36.37±3.62	25.29±2.04	31.98±3.06	26.18±2.30
5	46.96±2.04	38.31±2.073	45.019±3.24	30.07±2.16	46.88±2.01	29.87±2.01	46.23±2.48	34.88±3.41
6	39.27±2.04	37.57±2.14	37.97±1.98	29.74±2.74	36.37±2.84	30.74±1.83	36.96±2.04	32.09±2.79
7	35.92±1.581	33.02±2.01	37.44±2.44	29.63±3.39	38.70±1.91	33.91±2.26	40.87±2.47	28.69±1.48
8	37.88±0.73	29.45±2.12	38.41±1.03	33.01±2.15	37.78±1.69	38.16*±2.71	38.12±2.50	35.63±1.67
9	28.93±1.06	26.28±3.23	27.96±1.12	37.83±2.88	28.39±4.05	45.95**±6.20	27.67±0.93	40.76±3.56
10	50.41±2.09	50.08±1.84	53.74±2.61	50.26±1.40	53.43±1.86	49.36±2.32	52.31±1.93	49.23±1.88
11	51.40±1.47	53.55±1.68	54.13±1.34	54.45±1.50	51.35±1.52	53.72±1.42	52.43±1.33	55.38±1.99
12	50.64±1.49	53.70±1.60	54.47±4.56	54.45±1.32	50.93±1.61	53.70±130	52.55±1.28	55.55±2.15
13	49.86±1.77	52.69±1.22	54.44±1.86	53.35±1.20	51.71±1.61	52.18±1.29	53.24±0.90	53.13±2.08

* P<0.05, ** P<0.01

(Average of 10 values) Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Hematology

During Sub acute Toxicity study, significant differences were not observed with respect to haematological parameters. During Chronic Toxicity Study, Significant difference was not observed between the test groups with respect to haematological parameters except for Significant (P<0.01)decrease in Total Leukocyte Count in AD and HD groups as compared to Control, while TD showed no difference. Other haematological parameters did not vary significantly (Tables 5-6).

Table 5: Hematology values during Sub acute Toxicity study.

	TLC ($x10^3$)	POLY(%)	LYM(%)	PCV(%)	HB(g %)	TRC (10^3)	Platelets(10^6)
VC	5.66±0.30	20.5 ±1.06	79.5 ±1.06	43 ±0.45	14.9±0.24	5.13 ±0.07	1.57±0.87
TD	5.77±0.18	21.2 ±1.19	78.8 ± 1.19	43 ±0.9	14.8 ± 0.26	5.13 ±0.07	1.61±0.73
AD	5.05±0.21	19.7 ±2.01	80.3 ±2.01	43 ±1.7	14 ±0.92	5.19 ±0.17	1.59±0.58
HD	6.50±0.27	20±1.06	80±1.06	45±1	14.1±0.26	5.24±0.09	1.57±0.50

(Average of 6 Values)

Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Table 6: Hematology values during Chronic Toxicity study

	TLC ($x10^3$)	POLY(%)	LYM(%)	PCV(%)	HB(g %)	TRC(10^5)	Platelets(10^5)
VC	5.36±0.23	24±1.04	76±1.04	39.65±1.07	14.02±0.38	4.51±0.11	1.65 ±0.08
TD	4.86±0.17	22±1.01	78±1.03	40.6±1.32	13.91±0.33	4.50±0.08	1.87±0.17
AD	4.57**±0.10	23±0.82	77±0.87	42.25±1.16	14 .48±0.29	4.66±0.08	1.97±0.13
HD	4.58**±0.14	23±0.77	77±0.92	42.2±1.27	14.53±0.29	4.67±0.08	1.76±0.11

** P<0.01 (Average of 20 Values)

Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Clinical Chemistry

Blood samples of Wistar rats under sub acute toxicity study did not show significant variations in biochemical parameters except for Significant (P<0.05) decrease in SGOT in TD group as compared to control group. Serum biochemistry values did not vary significantly between the rats in control and test groups during chronic toxicity study (Tables 7-8).

Table 7:Clinical chemistry during Sub acute Toxicity study

	Glucose (mg %)	SGOT(IU/L)	SGPT(IU/L)	Creatinine (mg %)	Total Protein (g %)	Prothrombine Time (Sec.)
VC	121±14.5	166±11.4	57.17±5.343	0.88±0.03	6.6±0.3	11.17±0.667
TD	133±15.2	128*±4.16	50.33±3.57	0.83±0.02	6.7±0.3	11.62±1.04
AD	146±10.1	149±5.5	85.83±3.60	0.78±0.03	6.4±0.2	10.83±0.36
HD	134±11.5	145±11.7	61.67±2.37	0.8±0.03	6.4±0.2	11.67±1.14

(Average of 6 Values)

Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Table 8: Clinical chemistry during Chronic Toxicity study

	Glucose (mg %)	SGOT (IU/L)	SGPT (IU/L)	Creatinine (mg %)	Total Protein (g %)	Prothrombine Time (Sec.)
VC	77.15±5.47	181±6.74	50.9±2.91	0.83±0.03	6.84±0.20	16.97±0.09
TD	84.25±7.52	155.5±5.41	54.5±4.18	0.82±0.02	6.87±0.14	16.2±1.36
AD	71.35±6.86	174.2±9.05	52.05±3.45	0.86±0.03	6.93±0.15	15.15±0.26
HD	76.7±7.04	175.3±8.88	57.8±3.55	0.835±0.03	7.31±0.22	16.2±1.17

(Average of 20 Values)

Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Relative organ Weights

In Sub acute toxicity study , Significant ($P<0.01$) increase was observed in AD and HD groups with respect to Liver weight relative to body weight as compared to control group, but no such difference was observed in TD group. The relative weights of other organs did not vary significantly. In Chronic Toxicity study, significant differences were not observed between control and test groups with respect to organ weights relative to body weight in both male and female rats (Tables 9-11).

Table 9: Organ wt in % Body weight during Sub acute Toxicity study

	Heart	Lung	Liver	Spleen	Stomach	Kidneys	Ovaries	Testes	Brain
VC	0.35±0.02	0.71±0.06	3.28±0.08	0.26± 0.02	1.02±0.02	0.85± 0.02	0.07± 0.01	1.02± 0.04	1.20±0.02
TD	0.34±0.01	0.70±0.02	3.34± 0.13	0.27± 0.01	1.05±0.01	0.87± 0.01	0.06± 0.01	1.19± 0.04	1.21±0.04
AD	0.32±0.01	0.79±0.13	3.96**± 0.09	0.25± 0.01	0.95±0.05	0.80±0.04	0.05±0.01	0.85± 0.18	1.17±0.24
HD	0.31±0.02	0.68± 0.05	3.98**± 0.15	0.23± 0.01	0.92±0.03	0.77±0.03	0.05±0.01	1.05± 0.03	1.06±0.03

** P<0.01 (Average of 6 Values)

Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Table 10:Organ wt in % Body weight during Chronic Toxicity study in male rats.

	Heart	Lung	Liver	Spleen	Stomach	Kidneys	Testes	Brain
VC	0.31±0.01	0.77±0.06	2.99±0.09	0.23±0.01	0.66±0.02	0.73±0.02	1.00±0.04	0.83±0.03
TD	0.30±0.01	0.87±0.08	2.90±0.09	0.24±0.01	0.67±0.02	0.76±0.03	0.95±0.07	0.86±0.02
AD	0.30±0.01	0.75±0.05	2.84±0.09	0.23±0.01	0.69±0.02	0.76±0.03	0.97±0.04	0.86±0.02
HD	0.31±0.02	0.75±0.04	2.87±0.07	0.23±0.01	0.67±0.02	0.74±0.03	0.93±0.03	0.83±0.02

(Average of 10 values)

Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Table 11: Organ wt in % Body weight during Chronic Toxicity study in female rats.

	Heart	Lung	Liver	Spleen	Stomach	Kidneys	Ovaries	Brain
VC	0.35±0.02	0.76±0.05	2.89±0.09	0.26±0.01	0.80±0.03	0.77±0.03	0.06±0.01	1.00±0.04
TD	0.33±0.01	0.93±0.09	2.79±0.05	0.26±0.01	0.76±0.03	0.76±0.03	0.06±0.01	0.96±0.04
AD	0.33±0.01	0.89±0.04	2.91±0.11	0.25±0.02	0.80±0.02	0.78±0.02	0.06±0.01	1.00±0.03
HD	0.325±0.009	0.80±0.03	2.89±0.07	0.24±0.01	0.76±0.03	0.73±0.02	0.06±0.01	0.96±0.04

(Average of 10 values)

Sub acute (28 days) and chronic (90 days) toxicity studies of Ayurvedic bhasma Rasamanikya in Wistar rats

Post mortem findings

No grass lesions were observed in different groups.

Histopathology:

Major histopathological lesions were not observed in Wistar rats. The minor changes that were observed include Focal collection of lymphocytes in interstitial tissue of lungs and slight sinusoidal congestion in spleen (Figure 1-12).

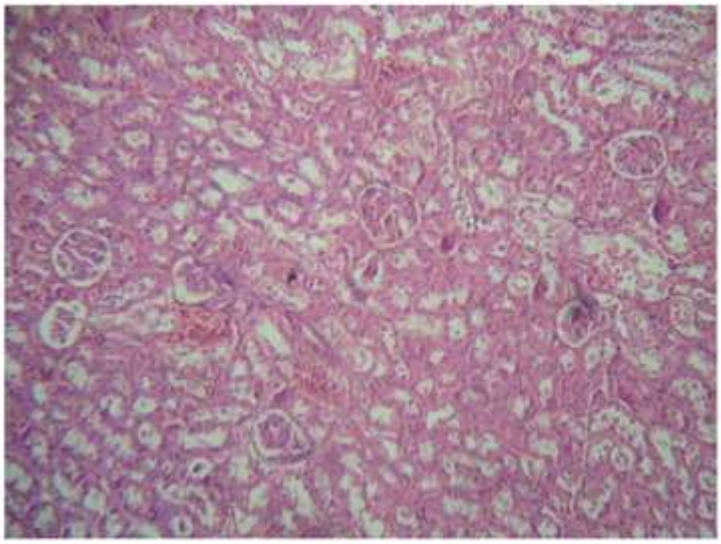

Figure 1: Section of Kidney of male rat in Control group showing normal glomeruli and Bowman's capsule (sub acute toxicity study).

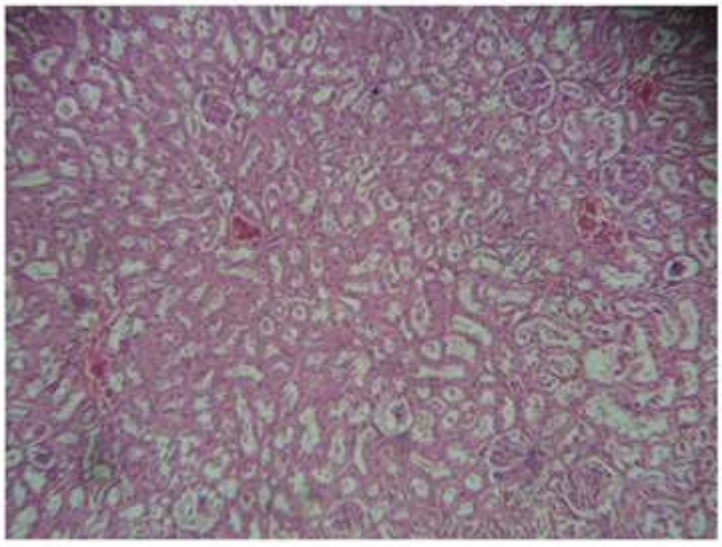

Figure 2: Section of Kidney of male rat in High dose group showing normal glomeruli and Bowman's capsule (sub acute toxicity study).

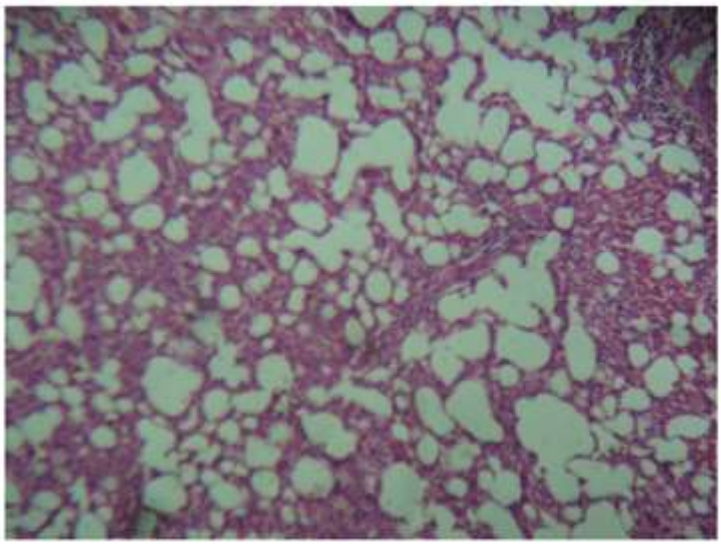

Figure 3: Section of Lung of male rat in Control group showing normal bronchioles and alveoli. Interstitial tissue shows focal collection of lymphocytes (sub acute toxicity study).

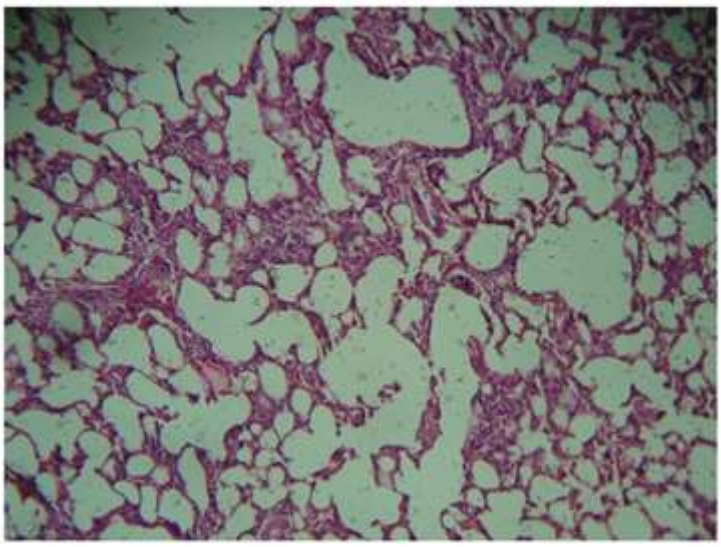

Figure 4: Section of lung of male rat in High dose group showing normal bronchioalveolar system. Interstitial tissue shows focal collection of lymphocytes (sub-acute toxicity study).

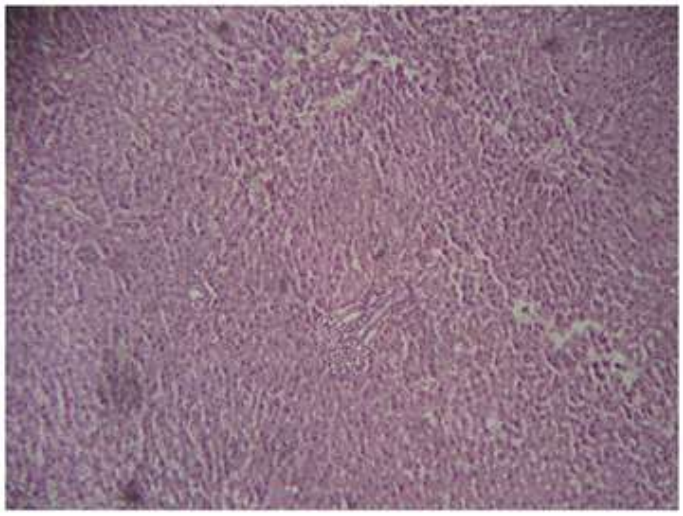

Figure 5: Section of Liver of a female rat in control group showing normal portal triads and central venous system (Sub-acute toxicity study).

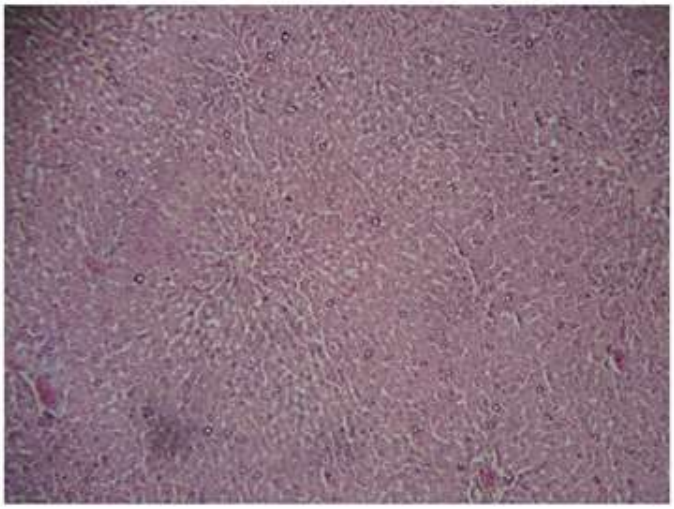

Figure 6: Section of Liver of a female rat in High dose group showing normal hepatocytes, sinusoidal spaces and kuffer cells (Sub-acute toxicity study)

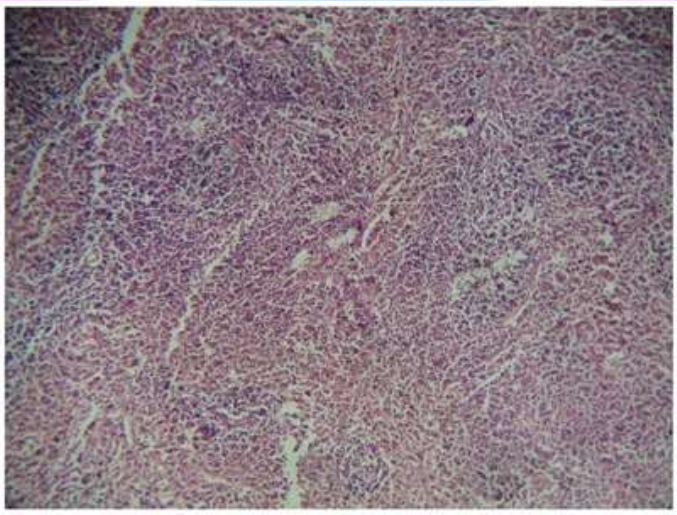

Figure 7: Section of Spleen of a female rat in control group showing lymphoid follicles with germinal centres and slight sinusoidal congestion (Chronic toxicity study).

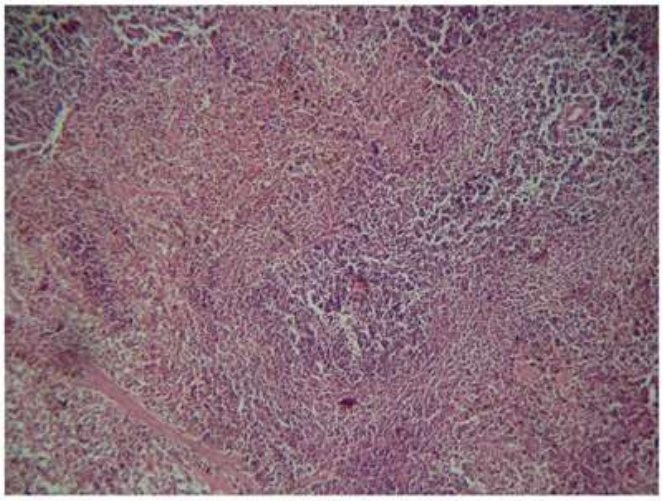

Figure 8: Section of Spleen of a female rat High dose group showing lymphoid follicles with germinal centres and slight sinusoidal congestion (Chronic toxicity study).

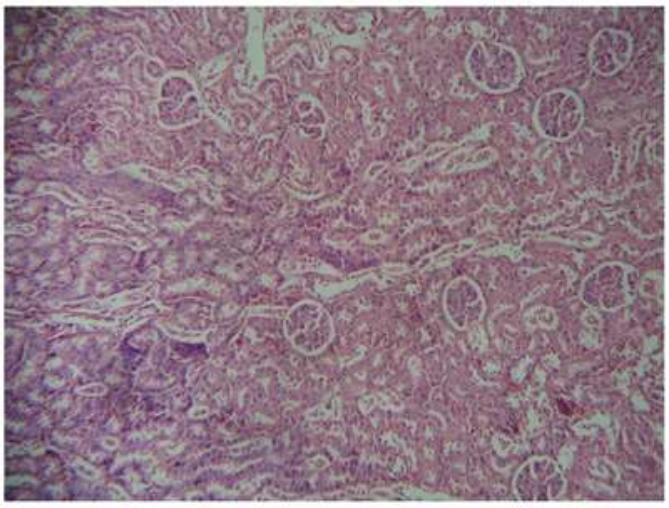

Figure 9: Section of Kidney of a male rat in control group showing normal glomeruli and Bowman's capsule (Chronic toxicity study).

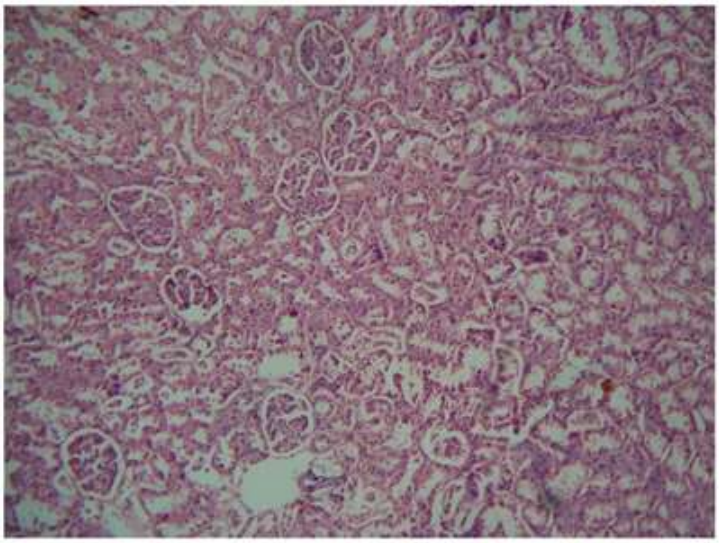

Figure 10: Section of Kidney of a male rat in High dose group showing normal glomeruli and Bowman's capsule and renal tubules (Chronic toxicity study).

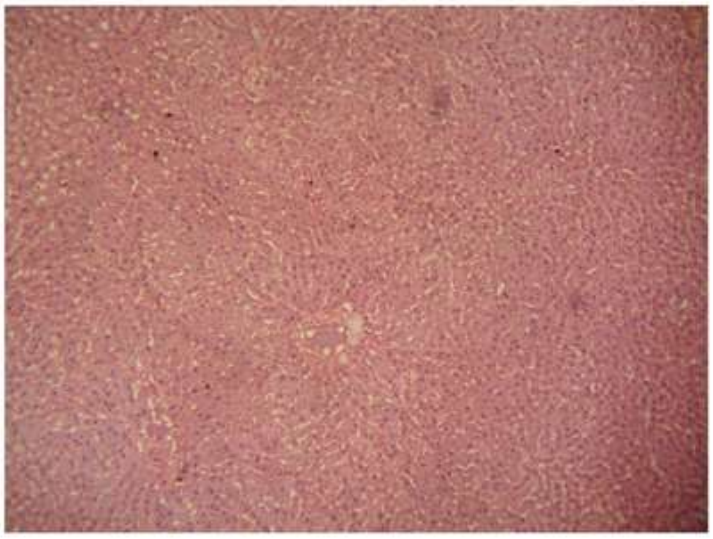

Figure 11: Section of Liver of a female rat in control group showing normal portal triads and central venous system (Chronic toxicity study).

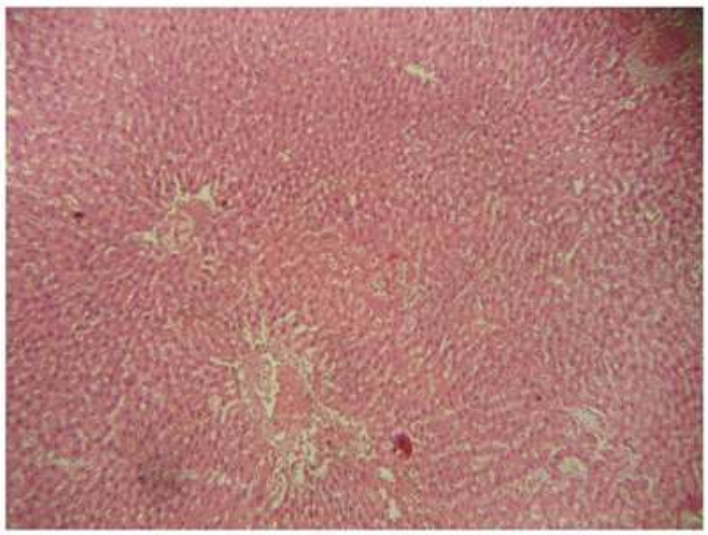

Figure 12: Section of Liver of a female rat in High dose group showing normal hepatocytes, sinusoidal spaces and Kuffer cells. (Chronic toxicity study).

CONCLUSION

No abnormal behavioural activity and pre terminal deaths were recorded in the rats exposed to the test compound up to 10 times of the intended therapeutic dose in sub acute and chronic toxicity. No significant difference was observed with respect to body weight gain and feed consumption between the test groups and control group in sub acute and chronic toxicity studies. The increase observed in feed intake of female rats in chronic toxicity study was only for a period of 2 weeks. Significant increase in weight of Liver relative to both body weight was found during sub acute toxicity study, but the same could not be concluded as the same were not observed in chronic toxicity study. Significant differences observed at few weeks of the study with respect to percent feed intake in female rats were in TD and AD groups and the same was not observed in HD groups. No major haematological changes were observed

between control and test groups except for decrease in the leukocyte count in Average dose and high dose groups, but the values were within normal physiological range. Serum biochemistry values did not vary significantly between the rats in control and test groups in both studies. Histopathological changes found in high dose group with respect to Lungs and spleen were minimal and the same findings were found in control animals too. The minor histopathological lesions found were of regenerative / attempt to repair type and these findings were in accordance with that Sud et al (2013) [6]. Histopathological findings of other organs were normal. The Ayurvedic bhasma was found to be safe in Wistar rats up to dose of 54 mg/Kg. body weight orally during sub acute and chronic toxicity studies.

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