SAFETY STUDY OF A SELECTED AYURVEDIC FORMULATION: MAHASUDARSHAN GHAN VATI

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ABSTRACT

Mahasudarshan Ghan Vati is a classical preparation of fourty five ingredients, it has been in use since long as an antipyretic in Ayurvedic practice. The drug was screened for its safety/toxicity studies in acute and chronic models in albino rats. The limits of all the four heavy metal content are found to be within the permissible limits. No mortality and behavioural changes were observed upto seven days at all the three dose level, in acute toxicity study. Chronic toxicity study revealed that, the drug has no serious toxicity potential to most of the important organs in therapeutic doses. However, at 10 fold higher than the normal therapeutic dose, depression of testicular function was observed.

Keywords: Ayurvedic preparation, Albino rats, Acute toxicity, Chronic toxicity, Mahasudarshan ghan vati.

INTRODUCTION

Initially, the CCRAS has conducted safety/toxicity studies of nine ayurvedic and siddha preparations. The present study is very much relevant in the background of the heavy metal content above the limits in some of the marketted drugs of Indian System of Medicine (ISM) published earlier[1].

The findings of present studies will be helpful in clearing misconception among physicians, scientists and consumers as well. The chemical analysis for heavy metal contents and evaluation of safety/ toxicity profiles of these drugs biologically through acute and chronic toxicity studies at CCRAS institutes and to cross check at other identified institutes were conducted.

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Mahasudarshan ghan vati, one of the ayurvedic classical preparations is widely used in the treatment of all types of fever, delusion, drowsiness, giddiness, thirst, dyspnea, cough, anaemia, cardiac diseases and jaundice[2]. Since, there is no literature available with regards to its safety/toxicity; the present studies were undertaken for its heavy metal contents and safety/toxicity studies.

MATERIALS AND METHODS

Animals

The animals were obtained from the animal house facility of Gujrat Ayurved University, Jamnagar. The animals were maintained in ideal laboratory conditions, light period of 12 h/day, temperature 22 ± 3^0 and humidity 30-70%. The experiments were carried out in accordance with the guidelines of the Institutes Animal Ethical Committee after obtaining its permission.

Drug

Mahasudarshan ghan vati (MSG) was procured from the open market of New Delhi. The name of the manufacturer, location and lot number were recorded and allotted in coded form for heavy metal analysis and safety studies.

Estimation of heavy metal

To determine the metal concentration in the drug, sample was analyzed by atomic absorption spectrophotometer[3].

The sample was weighed and taken in porcelain basin and ashed in muffle furnace at 525°. The ash was extracted with 1:1 HCl solution and then volume was made up. Heavy metals of the extracted solution were estimated with atomic absorption spectrophotometer. For estimation of arsenic hydride, generator was used.

Acute toxicity studies

Swiss albino mice (20-32g) of either sex in groups of 8 were used for the test. The animals were divided into four groups. The drug mahasudarshan ghan vati (MSG) was suspended in distilled water and was administered in the doses of 84.5 (MSG), 422.5 (MSGx5) and 845 (MSGx10) mg/kg, orally once daily. Dose for experimentation was calculated by dose conversion table[4] with reference to suggested human doses (i.e.650 mg/day). Thereafter, all the animals were observed carefully for seven days and mortality rate noted daily. They were closely observed for gross behavioural changes and other signs of toxicity in first 24 h[5,6].

Chronic toxicity studies (90 days)

Charles Foster strain albino rats of either sex weighing 160-200g in groups of 10 for each dose level of mahasudarshan ghan vati (MSG) were used for experiments. Chronic toxicity was evaluated after single daily administration of test drug at 58.5 (MSG), 292.5(MSGx5) and 585 (MSGx10) mg/kg,p.o. for 90 days. Dose for experimentation was calculated by dose conversion table[4] with reference to suggested human doses (i.e.650 mg/day). Test drug was given in distilled water by gavage with a control group receiving the vehicle (distilled water). All the rats were maintained on pranav agro industries "Amrut" brand rat feed given *ad libitum* with water. Toxicity was evaluated in terms of body weight and gross behaviour, gross and histological appearance of vital organs (brain, thymus, heart, lungs, liver, stomach, spleen, kidney, testis, and ovary)[7], biochemical changes (blood sugar, s. total cholesterol, s. triglyceride, s. urea, s. creatinine, s. alkaline phosphatase, SGOT, SGPT, total protein, s. albumin and s. globulin and haematological parameters (WBC, RBC, platelet count, lymphocyte percentage, MCV, monocyte percentage, hematocrit, PCT, granulocyte percentage, MCH, MPV, MCHC, lymphocyte count, MRBC, haemoglobin). The qualitative analysis of urine, estimation of sodium and potassium in urine, pH of urine and urine microscopy were studied[8].

RESULTS AND DISCUSSION

Heavy Metal Analysis

Table I lists the heavy metals contained in mahasudarshan ghan vati viz. lead, mercury, arsenic and cadmium concentrations analyzed by CCRAS. All the heavy metal contents in mahasudarshan ghan vati (MSG) are within the permissible limits.

Table I: Heavy metal estimation in mahasudarshan ghan vati (tablet)

	Pb (10ppm)*	Hg (1ppm)*	As (10ppm)*	Cd (0.3ppm)*
CCRAS[1]	9.96ppm	0.07ppm	6.96ppm	0.27ppm

** Limits of heavy metals* [9]

[1] Technique used for estimation automic absorption spectrophotometer

Acute toxicity

No mortality was observed upto seven days at all the three-dose levels. The approximate LD_{50} of test drug was thus found to be much higher than 845 mg/kg, body weight. There were no gross behaviour changes.

Chronic toxicity

No significant behavioural changes were observed in any of the groups studied. During the study period, 11 animals (2 control, 4 MSG, 1 MSGx5 and 4 MSGx10) died during experiment, but no apparent discernible changes could be observed

during necropsy. A moderate increase in food intake and body weight was observed in all the test drug administered groups in comparison to control group (Table II).

A decrease in weight of thymus was observed in all the groups as compared to the control. A statistically significant increase in the spleen weight was observed in all test drug administered groups in comparison to control. An apparent increase in the testis weight was observed in all the test drug administered groups in comparison to control (Table III).

A statistically significant decrease in serum blood glucose was observed in MSG × 05 and MSG × 10 treated groups. However MSG treated group, was found to be statistically non-significant. A decrease in the serum cholesterol level was observed in all the three groups. There was decrease in the serum creatinine level in MSG× 10 administered groups and was found to be statistically significant in comparison to control group. The test drug at the entire dose level studied did not affect the serum alkaline phosphatase activity to significant extent in comparison to control group.

No change in S.G.O.T level was observed in the MSG × 10 administered groups in comparison to control group. S.G.P.T. level in MSG and MSG × 10 administered groups was observed decrease in comparison to control group and was found to be statistically non-significant. The test drug administration did not affect serum total protein albumin content to significant extent in comparison to control group (Table IV).

Table II: Effect of different dose level of mahasudarshan ghan vati on body weight in albino rats

Groups	Dosage (mg/kg)	Body weight (g)		Body weight change in (g)	Body weight change in (%)
		Initial	At the end of Study		
Control	D.water	203.33±08.33	222.66±11.16	19.33 ± 05.81	-----
MSG	58.5	172.66±05.85	232.67±16.22	60.00 ± 13.84*	210.39 ↑
MSG× 05	292.5	180.00±11.21	236.67±13.62	56.67 ± 09.14**	193.17 ↑
MSG× 10	585.0	191.66±08.72	229.33±18.01	41.00 ± 12.64	112.10 ↑

Data: Mean ± SEM **P< 0.05, **P<0.01*

Table III: Effect of different dose level of mahasudarshan ghan vati on organs weight (mg) of albino rats

Name of organs	Control (D.water)	MSG (58.5mg/kg)	MSG × 05 (292.5mg/kg)	MSG× 10 (585mg/kg)
Thymus (mg)	620.83 ± 42.15	520.00 ± 27.80	544.00 ± 16.17	593.33 ± 38.61
Liver (g)	05.32 ± 00.23	05.57 ± 00.35	05.40 ± 00.31	05.57 ± 00.29
Spleen (mg)	406.83 ±09.20	517.00 ± 28.14**	496.66 ±43.48*	577.33 ± 40.74**
Kidney (mg)	1281.66 ± 75.38	1347.00 ± 100.75	1331.66 ± 75.91	1406.66 ± 122.38
Testis (mg)	1203.33 ± 112.89	2590.00 ± 60.00***	1667.00 ± 301.43	2193.33 ± 612.81

P<0.05; **P<0.01&*P<0.001*

Table IV: Effect of different dose level of mahasudarshan ghan vati on biochemical parameters in albino rats

Parameters studied	Control	MSG (58.5mg/kg)	MSG × 05 (292.5mg/kg)	MSG× 10 (585.0mg/kg)
Blood sugar (mg/dl)	103.67 ±11.26	81.50 ± 02.72	64.83 ± 05.46*	69.00 ± 02.79*
S. Total cholesterol (mg/dl)	66.67 ± 05.61	53.00 ± 05.17	57.00 ± 04.49	60.17 ± 05.75
S. Triglyceride (mg/dl)	72.33 ± 10.99	82.33 ± 14.74	96.00 ± 09.05	124.00 ± 30.31
S. Urea (mg/dl)	51.33 ± 07.65	48.00 ± 01.84	49.17 ± 01.96	54.66 ± 01.80
S. Creatinine (mg/dl)	01.00 ± 00.03	00.92 ± 00.03	01.02 ± 00.03	00.88 ± 00.03*
S. Alkaline Phosphatase (IU/L)	133.83 ± 34.53	104.66 ± 22.69	138.17 ± 35.33	160.00 ± 61.24
S.G.O.T. (IU/L)	467.83 ± 190.99	289.50 ± 22.12	277.17 ± 13.14	459.00 ± 77.50
S.G.P.T.(IU/L)	105.00 ± 17.08	81.33 ± 06.50	117.67 ±13.43	88.00 ± 09.90
Total Protein(g/dl)	07.98 ± 00.29	07.30 ± 00.27	07.92 ± 00.06	08.02 ± 00.31
S. Albumin(g/dl)	04.45 ± 00.26	04.01 ± 00.24	04.08 ± 00.28	04.67 ± 00.31

**P<0.05*

Table V: Effect of different dose level of mahasudarshan ghan vati on haematological parameters in albino rats

Parameter	Control	MSG (58.5mg/kg)	MSG × 05 (292.5mg/kg)	MSG× 10 (585mg/kg)
W.B.C($10e^3$/ mcL)	00.81 ± 00.13	00.73 ± 00.09	01.21 ± 00.17	01.63 ± 00.41
R.B.C($10e^6$/ mcL)	06.78 ± 00.44	05.93 ± 00.51	06.48 ± 00.33	07.98 ± 00.50
Lymphocyte percentage	98.15 ± 00.88	96.58 ± 01.43	95.52 ± 01.10	94.67 ± 02.58
M.C.V(fl)	69.95 ± 01.65	77.57 ± 02.17*	75.02 ± 01.65**	68.75 ± 00.85
Monocyte percentage	00.40 ± 00.19	00.83 ± 00.26	00.70 ± 00.18	00.93 ± 00.21
Haematocrit(%)	47.03 ± 01.99	45.48 ± 02.80	48.38 ± 01.46	51.53 ± 10.13
Granulocyte percentage	01.52 ± 00.75	02.60 ± 01.32	03.78 ± 01.85	04.38 ± 02.38
M.C.H(Pg)	13.53 ± 00.54	15.30 ± 00.80	15.32 ± 00.54*	13.73 ± 00.43
Lymphocyte count ($10e^3$/ mcL)	0.78 ± 00.13	00.68 ± 00.10	01.08 ± 00.14	01.55 ± 00.40
M.R.B.C%	01.37 ± 00.09	00.83 ± 00.21*	00.94 ± 00.11**	01.43 ± 00.53
R.D.W. (%)	06.72 ± 00.34	06.51 ± 00.30	07.62 ± 00.15*	09.52 ± 02.26
Haemoglobin (g/dl)	09.08 ± 00.27	08.88 ± 00.36	09.86 ± 00.23*	08.23 ± 00.58

**P<0.05;* ***P<0.01*

Table VI: Effect of different dose level of mahasudarshan ghan vati on urine pH, sodium and potassium excretion in albino rats

Groups	Dosage (mg/kg)	Urine pH	Sodium excretion (mEq/L)	Potassium excretion (mEq/L)
Control	Distilled water	08.50 ± 00.35	29.36 ± 12.35	62.04 ± 19.74
MSG	58.5	09.00 ± 00.00	37.70 ± 05.80	79.30 ± 14.09
MSG × 05	292.5	06.87 ± 00.37*	14.35 ± 04.32	69.71 ± 11.50
TED × 10	585.0	08.83 ± 00.17	24.52 ± 03.96	63.95 ± 11.72

Data: Mean ± SEM ** P<0.01*

Mahasudarshan ghan vati did not affect RBC counts significantly in comparison to control group. Statistically significant increase in MCV was observed in MSG and MSG× 05 administered groups in comparison to control group. An apparent increase in mean cell haemoglobin was observed in all tests drug groups in comparison to control group. However, only the increase observed in MSG × 05 administered groups was found to be statistically significant. Statistically significant increase in haemoglobin content in comparison to control group in MSG × 05 group and a marginal statistically non-significant decrease was observed in MSG and MSG × 10 administered groups (Table V).

At the dose of MSG and MSG × 10 of the drug, a marginal increase in urine pH was observed and was found to be statistically non-significant. A decrease in urinary excretion of sodium and potassium levels was observed and found to be statistically non-significant at MSG × 05 and MSG × 10 dose levels. However, in MSG treated group an apparent and statistically non-significant increase in sodium excretion and decrease in potassium excretion was observed (Table VI).

The photomicrographs of liver and kidney are shown in Fig. 1 and Fig. 2 respectively.

The test drug did not produce any significant changes in the cytoarchitecture of any organ except the sections of testis from MSG × 10 administered group with marked degenerative changes and decrease in spermatogenesis. The increase in the proportion of interstitial cells was also observed (Fig. 3).

Bone marrow smear did not reveal any significant changes in the microscopic profile in control group and compared to treated group. Incidence of cells with micronuclei was observed but very rare (Fig. 4).

CONCLUSION

Daily administration of mahasudarshan vati for 90 days did not reveal any serious toxic symptoms in rats at therapeutic dose level. No gross changes in their feeding habits or behaviour were noticed. However there was a dose related weight gain in all the drug treated groups compared to the control group. Other drug related effects included a significant decrease in macro RBC and increase in the MCV. However there was increase in the MCV, may be an adaptive response due to slight decrease observed in RBC count. In TED and average dose, five significant changes were observed. There were significant decrease in macro RBC and increase in the MCV, Hb, RDW and MCH. These changes considering that the magnitude of change is not remarkable and is not observed at higher dose level need not be considered as significant from pathological point of view.

During the experiment, mortality was observed in all the groups which were not dose dependent. No apparent discernible changes could be observed in

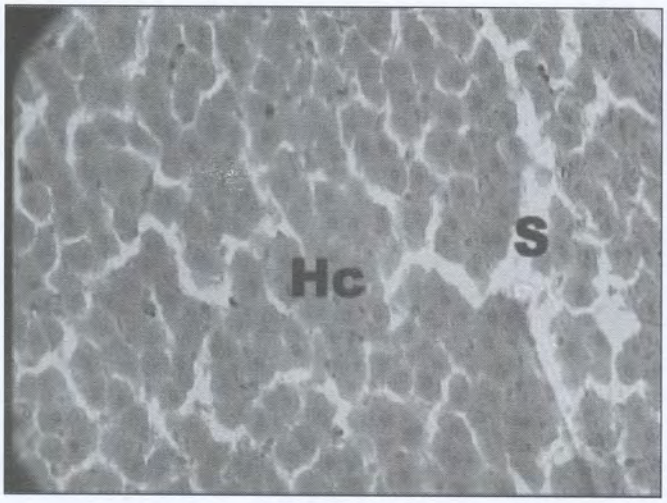


Fig. 1A

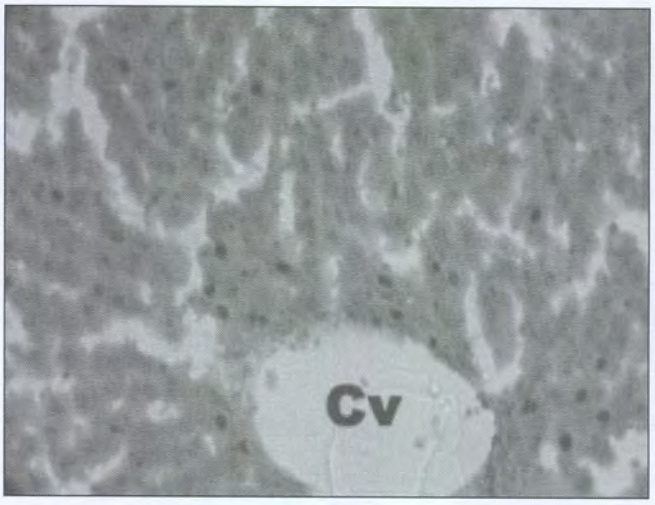


Fig. 1B

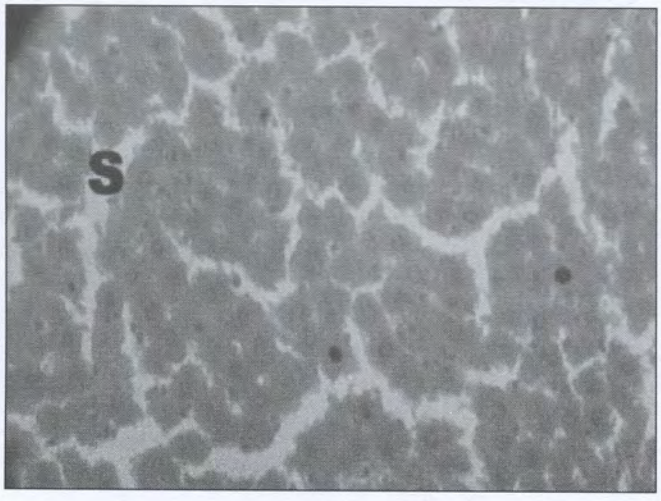


Fig. 1C

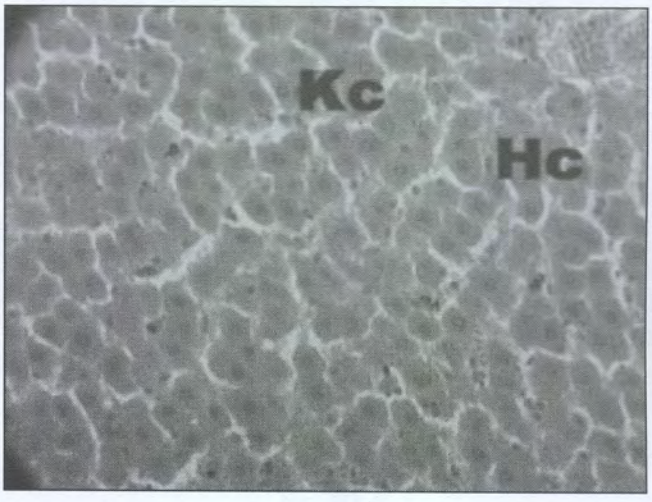


Fig. 1D

Fig. 1: Photomicrographs of liver

Fig. 1A: Photomicrograph of representative sections of liver of albino rats from control group. (1× 400 magnification) Hc-hepatic cell; Kc-kupffer cell; S-sinusoid Note: normal cyto-architecture

Fig. 1B: Photomicrograph of representative sections of liver of albino rats from msg group. (1× 400 magnification) Kc-kupffer cell; S-sinusoid Note: normal cyto-architecture

Fig. 1C: Photomicrograph of representative sections of liver of albino rats from MSG × 05 group. (1× 400 magnification) Kc-kupffer cell; S-sinusoid Note: normal cyto-architecture

Fig. 1D: Photomicrograph of representative sections of liver of albino rats from MSG × 10 group. (1× 400 magnification) Hc-hepatic cell; Hc-hepatic cells Note: almost normal cyto-architecture

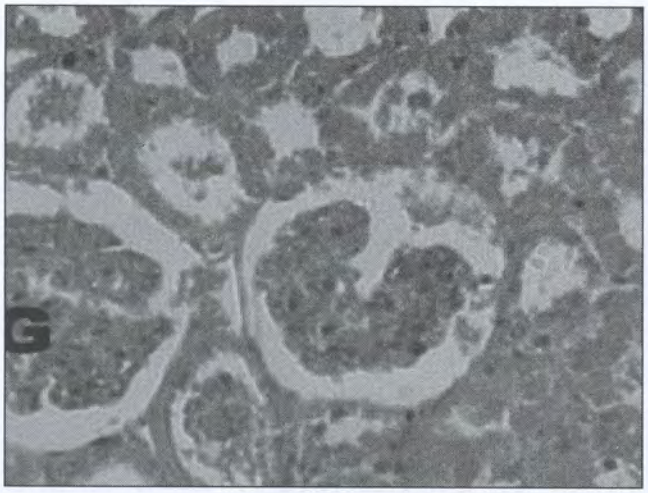
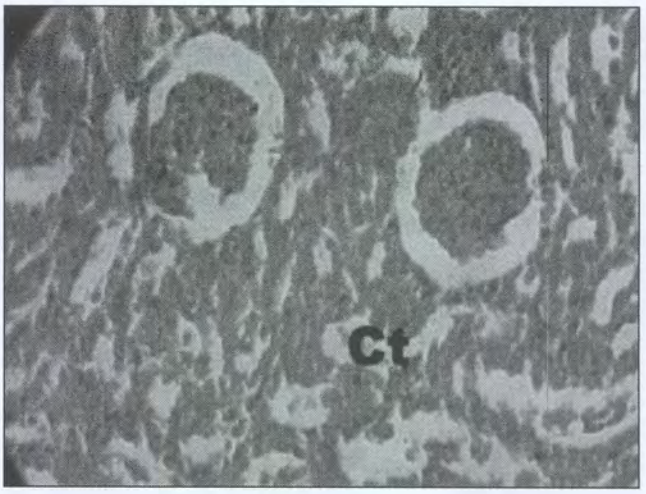


Fig. 2A



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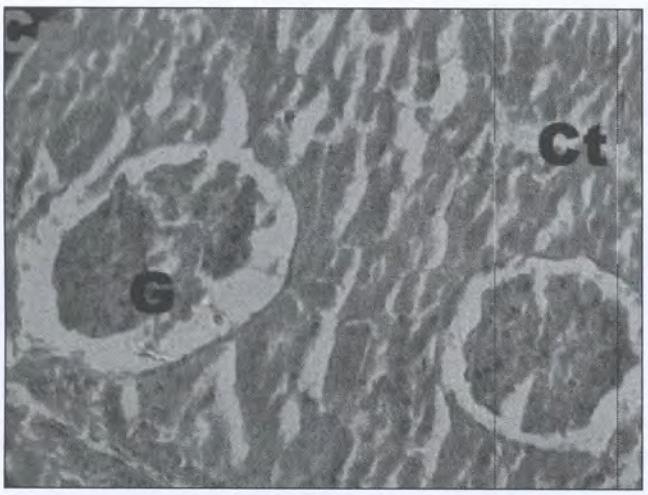

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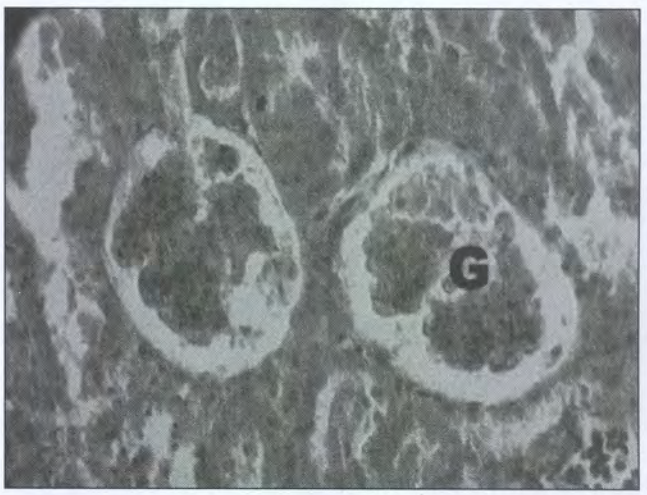

Fig. 2D

Fig. 2: Photomicrographs of kidney

Fig. 2A: Photomicrograph of representative sections of kidney of albino rats from control group. (1× 400 magnification) Cp-capsule; Ct-convoluted tubule; G-glomerulus Note: normal cyto-architecture

Fig. 2B: Photomicrograph of representative sections of kidney of albino rats from MSG group. (1× 400 magnification) G-glomerulus Note: normal cyto-architecture

Fig. 2C: Photomicrograph of representative sections of kidney of albino rats from MSG× 05 group. (1× 400 magnification) Note: normal cyto-architecture

Fig. 2D: Photomicrograph of representative sections of kidney of albino rats from MSG × 10 group. (1× 400 magnification) Ct-convoluted tubule; G-glomerulus Note: normal cyto-architecture

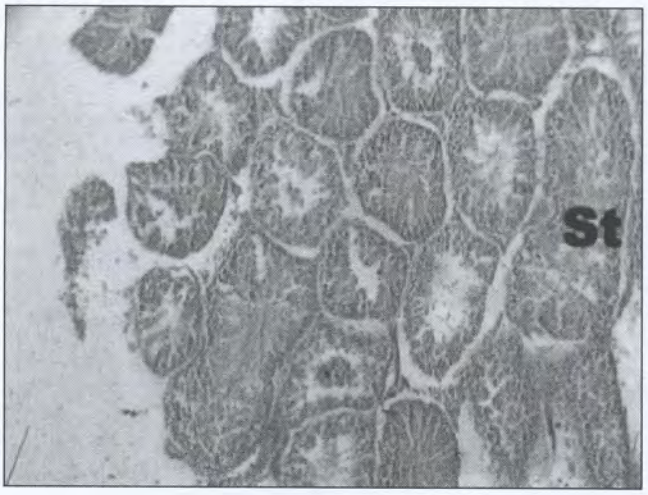

Fig. 3A

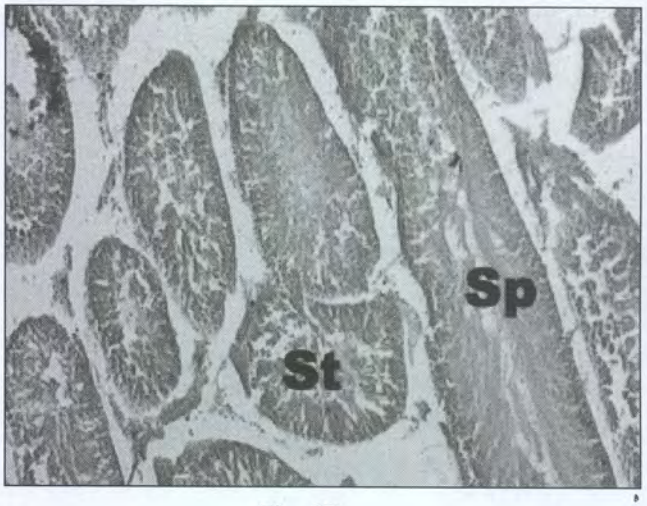

Fig. 3B

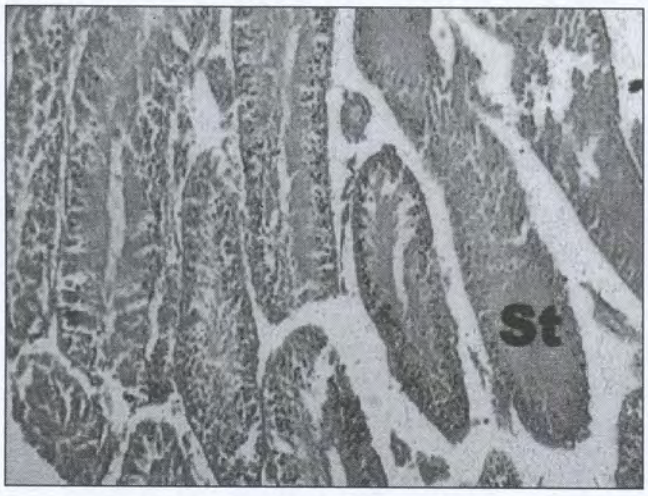

Fig. 3C

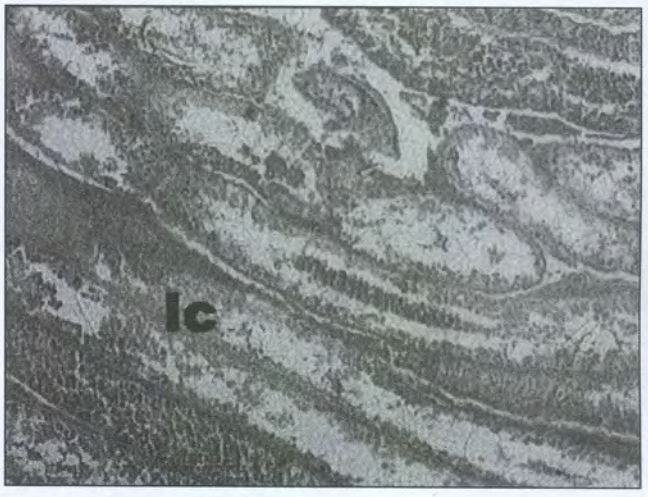

Fig. 3D

Fig. 3: Photomicrographs of testes

Fig. 3A: Photomicrograph of representative sections of testis of albino rats from control group. (1× 400 magnification) Sp-sperms

Fig. 3B: Photomicrograph of representative sections of testis of albino rats from MSG group. (1× 400 magnification) Ic-interstitial cells

Fig. 3C: Photomicrograph of representative sections of testis of albino rats from MSG × 05 group. (1× 100 magnification) St-seminiferous tubule, Ic-interstitial cell; Sp-sperms

Fig. 3D: Photomicrograph of representative sections of testis of albino rats from MSG × 10 group. (1× 100 magnification)

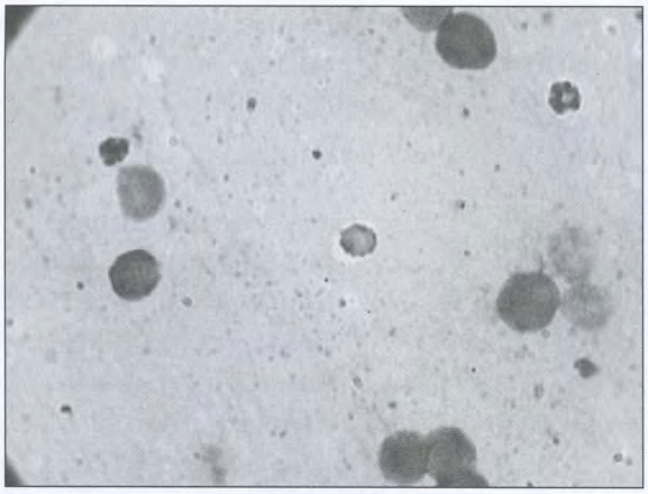

Fig. 4A

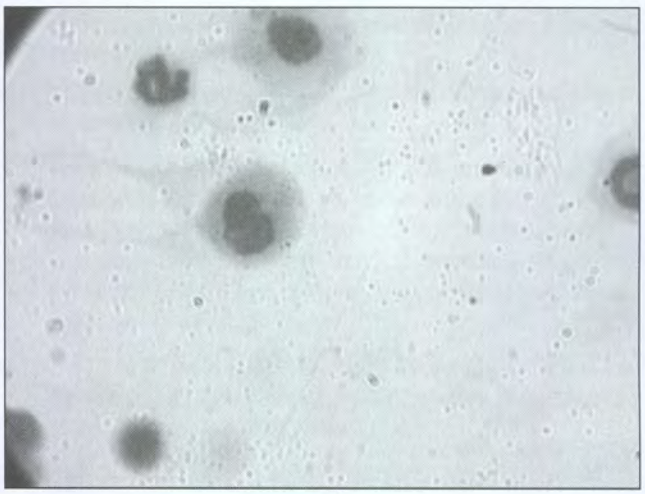

Fig. 4B

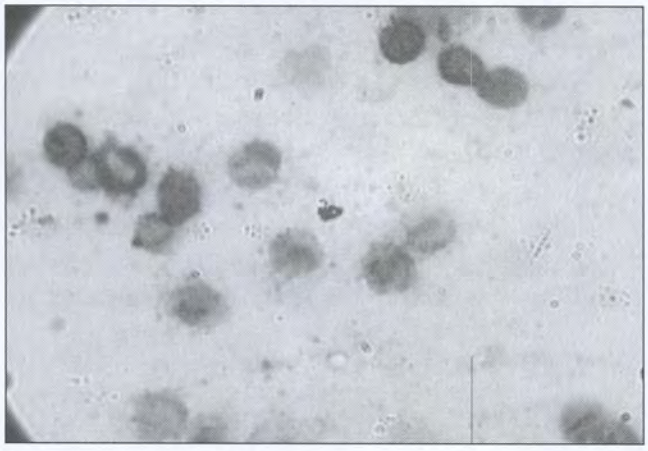

Fig. 4C

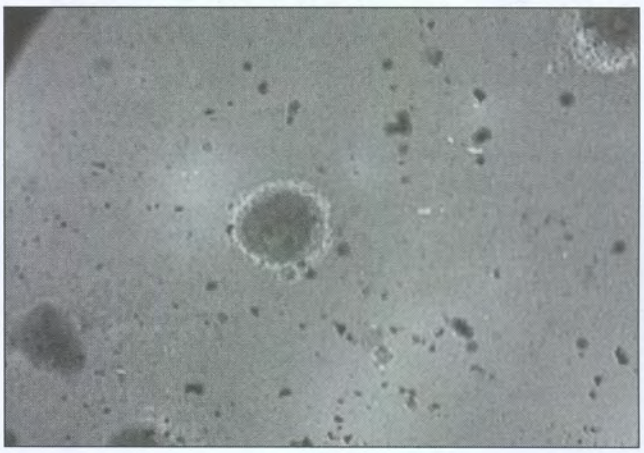

Fig. 4D

Fig. 4: Photomicrographs of bome marrow smear

Fig. 4A: Photomicrograph of bone marrow smear of albino rats from control group

Fig. 4B: Photomicrograph of bone marrow smear of albino rats from MSG group

Fig. 4C: Photomicrograph of bone marrow smear of albino rats from MSG × 5 group

Fig. 4D: Photomicrograph of bone marrow smear of albino rats from MSG × 10 group

all the groups during necropsy and the mortality may be due to non drug related reasons and seems to be due to multi-organ failure.

Surprisingly the changes observed were not prominent in the animals, which survived the course of treatment. On the basis of the available data it can be concluded that the test drug has no serious toxicity potential to most of the important organs at the therapeutic dose level. Besides these, there were no apparent gross lesions at necropsy and histological examination of liver, kidney, heart, spleen, brain, thymus and stomach did not reveal any pathological changes at therapeutic dose level. This might be due to the presence of metals in the formulation in oxide form which is non toxic. To confirm the exact nature or hydrolytic/non-hydrolytic form of the metals needs to be analyzed by using the sophisticated instrument.

However, at very high dose level depression of testicular function may be a major problem which was observed by histopathology.

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REFERENCES

1. Saper R.B., Kales S.N., Paquin J., Burns M.J., Eisenberg D.M., Davis R. B., Philips R.S., Heavy metal content of Ayurvedic herbal medicine products., **JAMA,** 2004:292:P: 2868-2873.
2. Mahasudarshan churna: **Sharngadhara samhita madhyam khand**-CH:6/26-36
3. Kenaway M.M.,Hafez M.A.H.,Akl M.A. and Lashein R.R. Determination by AAS of some trace heavy metal ions in some natural and biological samples after their preconcentration using newly chemically modified chlormethylated polystyrene-anion-exchanger. **Anal. Sci.,** 2000, 16 (5), P: 493.
4. Paget and Barnes, (1964), Evaluation of Drug Activities: Pharmacometrics eds,Laurance and Bacharach, Vol.1.Academic Press, New York.
5. Kale S. R. (1994), Practical pharmacology and toxicology. Nirali Prakashan, Pune.
6. Ghosh M. N. (1984), Fundamentals of experimental pharmacology. Scientific Book Agency, Calcutta. II.
7. Harshmohan (2000.), Textbook of pathology. IV Edition. Jaypee Brothers. New Delhi.
8. Raghuramulu N., Nair, M.K., Kalyanasundaram S. (Eds) (1983). A manual of laboratory techniques. National institute of nutrition, Hyderabad, India.
9. World Health Organization (WHO), (2004b), Guidelines for Quality standardized herbal formulations.