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Sub chronic oral toxicity study of a herbo-metallic ayurvedic formulation Swarna Guggulu in Wistar rats

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Safety of herbomineral formulations is being debated worldwide. Swarna Guggulu (SG) is a propriety ayurvedic formulation intended for use in arthritis and other neuromuscular disorders. The ingredients of SG have traditionally been used since long and are reported to be safe. In the present study, the safety profile of SG via repeated dose 90-day oral toxicity study was investigated in Wistar rats. Animals were divided into six groups. Aqueous extract of SG was administered orally once daily for 90 consecutive days to three group of animals at three dose levels (50, 250 and 500 mg/kg BW). One group served as high dose satellite reversal. One group each served as control and satellite control receiving milli-Q water. All the animals were observed for mortality and clinical sign of toxicity. Satellite groups were further observed for 28 days without treatment to detect any delayed toxicity or recovery from toxic effects if any. All the treated and control and satellite group animals exhibited a progressive gain in body weight and feed consumption throughout the dosing period and post-dosing recovery period. Abnormal breathing and lethargy were observed in one animal each in 500 mg/kg BW and satellite reversal group. These mortalities were, however, observed to be incidental findings. Laboratory parameters estimated for the treated and control animals on day 91 and satellite groups on completion of recovery period showed some significant changes in TLC, platelets, PCV and biochemical parameters that were comparable to control. These changes in haematology and biochemistry were inconsistent and therefore, considered incidental findings not related to test item. Urine parameters were found unaffected by treatment with SG. Necropsy of the surviving and found dead animals did not show any pathologically significant lesions. Histopathological examination of animals treated at 50 and 500 mg/kg showed lesions in some organs which were comparable with the control and hence, considered incidental findings. Based on the results, the NOAEL of SG, when administered orally once daily for a period of 90 days in both the sexes of wistar rats was found to be 500 mg/kg BW.

Keywords: Ayurveda, Herbomineral, Herbometallic, OECD guidelines, Repeated dose 90-day oral toxicity, Sub chronic oral toxicity, *Swarna guggulu*.

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Introduction

Swarna Guggulu (SG) is an ayurvedic herbomineral formulation intended for use in arthritis and other conditions that affect the joints and surrounding tissues including neuromuscular disorders. It comprises ingredients like calcined gold (*Swarna bhasma*), *Kumkuma* (*Crocus sativus* L.), *Ashvagandha* (*Withania somnifera* (L.) Dun) and *Mahayogaraj Guggulu* (a pharmacopoeial ayurvedic preparation) processed in decoctions of herbs *Eranda* (*Ricinus communis* L.) and *Rasna* (*Pluchea lanceolata* Oliv. & Hiern), which have been used traditionally for their beneficial effects in arthritis and joint health. Scientific studies on these ingredients report beneficial effects in arthritis and other neuromuscular conditions owing to their analgesic[1,2], antinociceptive[3] andantiinflammatory[4-7] properties. Previously, SG has been studied in preclinical efficacy studies, using dendritic cells which are professional antigen-presenting cells playing pivotal roles in the induction of protective immunity. Treatment of dendritic cells with SG resulted in considerable inhibition of LPS induced pro-inflammatory markers indicating potential anti-inflammatory activity and, thereby, suggesting SG to be a good option in the management of arthritis[8]. In the current study, we investigated the safety profile of SG via repeated dose 90-day oral toxicity study in Wistar rats.

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Study (GLP) compliance and ethical approvals

The study was conducted in compliance with the OECD Principles of Good Laboratory Practice

(GLP)[9], and OECD Environmental Health and Safety Publications at Dabur Research Foundation, Ghaziabad, India a registered facility for breeding and experiment of animals with the CPCSEA [(64/PO/br/s/99/CPCSEA)], Ministry of Environment and Forest, Govt. of India. All study procedures were compliant with the CPCSEA guidelines of India. Study design and protocol were approved by the Institutional Animal Ethics Committee (Approval No. IAEC/35/358; Date: 01/OCT/2015). The Animal welfare complied with all requirements of the applicable regulatory agencies including the animal welfare acts of India.

Material and Methods

Chemicals

Formaldehyde, sodium chloride, glacial acetic acid, sodium phosphate (Monobasic) (Rankem), isoflurane (Piramal Enterprises Limited), sodium phosphate (Dibasic), liquor ammonia, (Fischer Scientific), isopropyl alcohol, xylene, hematoxylin, eosin, DPX mount, ethanol (Merck), paraffin wax (German Company), homide (Indoco remedies Ltd.).

Experimental animals

Male and female Wistar rats aged between 8-9 weeks in weight ranges of 156.32 to 220.12 g (male) and 133.12 to 174.80 g (Female) obtained from Cadila Pharma, Ahmedabad, India were used in this study. Animals were housed in polypropylene cages (size 421 x 290 x 190 mm) with not more than three animals per group and acclimatized (8 days for males, 9 days for females, temperature 19.9 - 24.1 °C, relative humidity: 32 - 67 %, 12 h L/D cycle). The room temperature and relative humidity were measured daily by digital thermo-hydrometer and recorded. Throughout the study, the rats were given RO water and fed conventional pellet diet *ad libitum* (Golden Feeds, New Delhi).

Test item

Swarna Guggulu ((DRDC/AY/8049) (Dabur India Limited) was obtained as brown coloured tablets. Batch No. SB0190 of Swarna Guggulu Mfd: 04/16 and expiry 03/21 was used in this study. It was stored refrigerator between 5±3 ºC protected from light and moisture.

Preparation of test item

Test item (TI) was pulverized by mortar and pestle and transferred into measuring cylinder. The final volume was made up as required volume with Milli-q water. The nature of the test item was water soluble. Fresh test item was formulated daily. The dose volume of 1 mL/100 g was maintained throughout the study period

Treatment groups and dose administration

Animals were randomly allotted basis body weights into 6 (G1-G4, G5S & G6S) group. Groups G1-G4 comprised 10 animals each per sex. Satellite groups G5S and G6S comprised 5 animals/sex/group. G1 served as control, G5Sas satellite controls and G6S served as high dose satellite reversal.

TI was administered orally to animals of the groups G2, G3, G4 and G6S once daily for 90 consecutive days, using disposable syringes (1 mL, 2 mL and 5 mL) tipped with an oral gavage needle (18 gauge). Mili-Q water was administered to the control animals G1 and G5S at a dose volume of 10 mL/kg BW.

Justification for dose selection

Three dose levels: namely, low, mid and high (50, 250, and 500 mg/kg BW) were selected based on the human therapeutic dose (4 g/day) and converted allometrically to rat dose applying standard conversion factor considering human body weight (60 kg).

Observations

Clinical sign and mortality

Animals were observed once daily for clinical signs of toxicity and twice daily for mortality and morbidity throughout the study period. Clinical signs like home cage observation (posture, convulsion), handling observation (ease of removing from cage, handling reactivity, skin examination, piloerection, palpebral closure, eye examination, lachrymation and salivation) and open field observation (arousal, respiration, clonic movement, gait, rears, urination, defecation, mobility, stereotype and bizarre behavior).

In last week before study termination, sensory reactivity to stimuli of different types (approach response, touch response, tail pinch response air righting reflex, grip strength and motor activity assessment) were assessed in treated and satellite groups

Body weight

Body weight of all the animals was recorded on the first day of dosing, at study termination and weekly basis in between. Changes in body weight were calculated using the formula:

% Change in animal body weight=

$$\frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

Feed consumption

Feed consumption for the individual animal was recorded on a weekly basis

Ophthalmic examination

Ophthalmic examination was performed for all the treated and control animals, before the first treatment and before study termination.

Laboratory parameters

Approximately 2.5 mL of blood was collected from each animal for haematological and biochemical estimations. These tests were performed for all the animals of treatment and control groups (G1 to G4) while animals of satellite groups (G5S and G6S) were bled after the completion of reversal period (28 days). The animals were fasted overnight prior to blood collection. Animals were anaesthetized and blood collected from the retro-orbital sinus using capillaries. Blood was collected in tubes containing EDTA for haematological investigations and in tubes without anticoagulant for biochemical evaluation.

Haematological investigations

The blood samples were analyzed for the following parameters: Hemoglobin, PackedCell Volume (PCV/Hematocrit/HCT), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Total Leukocyte Count (TLC), Platelet count, Mean Corpuscular Hemoglobin (MCH) Differential Leukocyte Counts (DLC), Mean Corpuscular Hemoglobin Concentration (MCHC) and blood clotting time.

Blood biochemistry

Blood samples were collected into tubes without anticoagulant. The following estimations were done in the serum: Glucose, SGOT (AST), Total cholesterol, SGPT (ALT), Creatinine, Alkaline phosphatase (ALP), Urea, Total bilirubin, Sodium, Triglycerides, Potassium, Total proteins, Albumin. Samples for glucose estimation were collected in fluoride anticoagulant tubes.

Urinalysis

Urinalysis estimations were performed for all the treatment group animals and control group animals.

Necropsy

All the animals were sacrificed on day 91 except the satellite animals, which was sacrificed on 28 days of the recovery period. Necropsy was performed included examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. Gross examination of the organs and tissues (*viz*, eye, brain, adrenal, heart, testes/ovaries, epididymis/uterus, kidneys, liver, lungs, spleen, spinal cord, trachea, thymus, stomach, duodenum, jejunum, colon, thigh muscle, lymph node, urinary bladder, prostate and seminal vesicles) was done. The weights of adrenal, brain, heart, testes/ovaries, epididymis/uterus, kidneys, liver, thymus, and spleen were recorded as absolute values and their relative organ weights {(organ weight/body weight) x100} were calculated. All the collected organs were preserved in 10 % buffered formalin. The testes and eyes were preserved in modified Davidson's fixative.

Histopathological examination

All the preserved tissues; brain, adrenal, heart, testes/ovaries, epididymis/uterus, kidneys, liver, lungs spleen, spinal cord, trachea, thyroid, thymus, stomach, duodenum, jejunum, colon, esophagus, thigh muscle, lymph node, eyes urinary bladder, prostate, seminal vesicles were processed for paraffin embedding. 4-6 µm thick sections were cut and stained using hematoxylin and eosin dye according to standard methods for microscopic examination. Histopathology was carried out in all animals from control and high dose group (excluding satellite animals)

Statistical analysis

Data of detailed clinical observation (rears, urination, defecation, grip strength and motor activity assessment), body weights, feed consumption, haematology, biochemistry and organ weights were analyzed for differences among treated/control groups by the using in-house validated software. Data were analyzed for normal distribution and then homogeneity of variance by Bartlett's test followed by ANOVA and Dunnett's test, as required. Where data homogeneity was not met, data was analyzed using Kruskal Wallis test. Statistical significance of data was reported at the 5 % significance level (p<0.05).

Results and Discussion

Swarna Guggulu is proprietary herbomineral formulation. Therapeutic efficacy of ingredients of Swarna Guggulu like *Swarna Bhasma, Mahayogaraj Guggulu, Kumkuma* and *Ashwagandha* in arthritis and musculoskeletal disorders has been established basis

documented beneficial effects in classical texts of *Ayurveda* as well as published modern literature[10-15]. In the current study, we investigated the safety profile of SG via repeated dose 90-day oral toxicity study in Wistar rats.

General clinical observations and mortality

Two mortalities (rat no. 88 female from G4 high dose and 97 from high dose recovery) were found on day 56 and 50 respectively. However, no clinical signs of toxicity were observed in any of the treated and control groups animals except abdominal breathing and lethargy observed in animals prior to death. These mortalities were considered as an incidental finding.

Detailed clinical examination

Detailed clinical observation like home cage observation (posture, convulsion), handling observation (ease of removing from cage, handling reactivity, skin examination, piloerection, palpebral closure, eye examination, lachrymation, salivation) and open field observation (arousal, respiration, clonic movement, gait, rears, urination, defecation, mobility, stereotype, bizarre behaviour) carried out in all animals once before the first treatment and thereafter weekly till 90 day and further 28 days, for satellite group, did not reveal any treatment-related changes.

Sensory reactivity to stimuli of different types (auditory, visual and proprioceptive stimuli) recorded before first treatment and during last week of exposure period did not exhibit any treatment-related changes in comparison to control. The rearing, urination, defecation, grip strength and motor activity assessment in male and female rats in any of the treated group did not reveal any statistically significant changes in comparison to the control group.

Body weight

Animals of both the sexes in all the treated and satellite groups showed a progressive increase in body weight during the study. Weekly mean values of body weight and body weight per cent changes of all the treated animals were found statistically non-significant in both the sexes when compared with control group (Fig.1a and b). The comparable gain in weight showed that the SG did not have major toxic effects.

Feed consumption

No significant change in feed consumption was observed in male rats in any of the treatment groups compared with control group animals except G3 which showed a significant increase during week 5 and in G2 and G3 during week 9. In female satellite group rats, a significant increase in feed consumption was recorded during weeks 8, 9 and 16 and a significant decrease was recorded in week 1. Feed consumption in all other treatment groups was unaffected and found to be comparable with the control group. These changes in feed consumption were considered as incidental findings (Fig. 2a and b).

Ophthalmic examination

No abnormality was detected during the ophthalmic examination in any of the treated animals or control animals prior to dosing and before termination.

Haematological investigations

Interim and terminal evaluations of laboratory parameters such as haematology, biochemistry and urinalysis were performed for all the animals from control (G1) and treatment groups (G2-G4) on day 60 and 91 of treatment and for satellite groups after completion of 28 day recovery period. Evaluation of hematological parameters on day 60 revealed significant decrease in neutrophils in G3 male (G1 – 27.00 %; G3 - 22.10 %) TLC in G2, G3 and G4 female (G1 -15.30, G2 – 11.95, G3 – 11.33 and

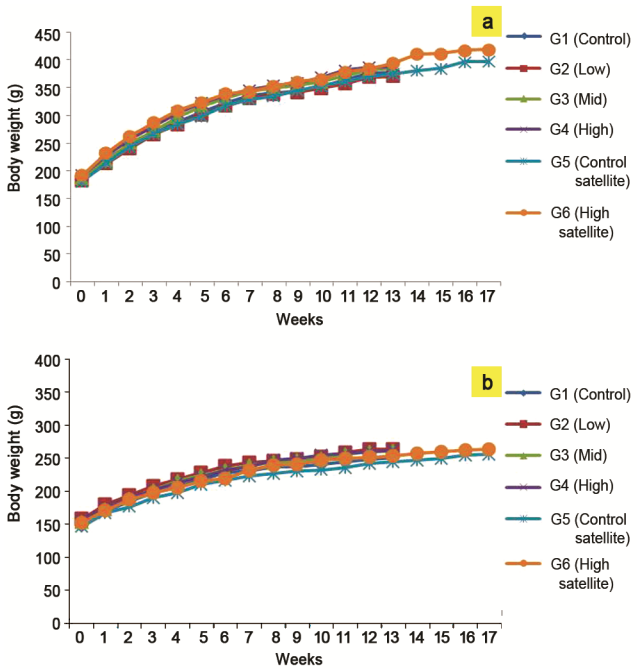

Fig. 1(a-b) — Effect of subchronic administration of Swarna Guggulu (DRDC/AY/8049) for 90 days on body weight of female and male rats. Data are expressed as Mean±Standard deviation

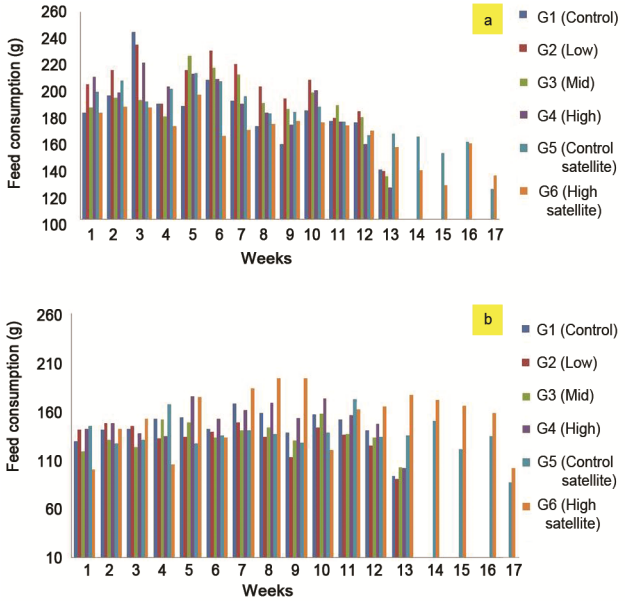

Fig. 2(a-b) — Effectofsub chronic administration of Swarna Guggulu (DRDC/AY/8049) for 90 days on feed consumption of female and male rats. Data are expressed as Mean±Standard deviation

G4 -12.42 k/µL), animals when compared with control group.

Evaluation of haematological parameters on day 91 revealed a significant decrease in monocytes G2 males (G1- 6.35 % and G2- 5.13 %) and significant increase in platelets in G2 and G3 male (G1 – 1098.90; G2 -1290.70 and G3 1280.40 k/µL) animals when compared with control group.

In the satellite recovery group, PCV values were significantly decreased in G6 female (G5 – 43.80; G6 – 41.35 %) animals when compared with the control satellite group. All other parameters were not significant and found to be comparable with control groups. Changes observed in the above parameters were inconsistent or not observed in satellite groups, hence considered as incidental findings (Table 1a-c).

Biochemical investigations

Evaluation of biochemical parameters of male rats on day 60 revealed significant increase in albumin in G3 and G4 (G1 - 4.15, G3 - 4.45 and G4 - 4.59 gm/dL) and decrease in sodium G3 and G4 (G1 – 147.30, G3 – 145.60 and G4 – 144.50 m Eq/L) whereas in female rats, a significant increase was observed in albumin (G1 – 4.33, G4 – 4.85 gm/dL), SGOT (G1 – 105.77, G4 – 163.93 IU/L) and bilirubin (G1 – 0.14, G4 – 0.24 mg/dL) and decrease in sodium (G1 – 147.20, G4 – 144.11 m Eq/L) was observed compare with control group.

Evaluation of biochemistry parameters on day 91 revealed significant decreased SGOT in the male of G4 (G1 – 249.21, G4 – 193.12 IU/L) and creatinine in the female of G3 and G4 (G1- 0.54, G3 – 0.48 and G4 – 0.47 mg/dL) was observed compared with control group.

A significant increase in albumin in the male of G3 (G1 – 4.21 and G3 – 4.48 gm/dL) and sodium in the female of G3 and G4 (G1 – 146.60, G3 – 148.50 and G4 – 150.22 m Eq/L) was observed compared with control group.

A significant increase in sodium was observed in male rats (G5 – 145.00, G6 148.40 m Eq/L) from satellite treatment group compared to control satellite group.

The above changes are incidental findings hence considered as not treatment-related. Changed observed in above biochemical parameters were considered as incidental findings as these changes were not supported by histopathological findings (Table 2a - c).

Urinalysis

Examination of urine parameter on day 60, day 91 and on completion of the recovery period, no abnormality was detected in all the urine parameter like appearance, volume, pH, specific gravity, glucose, protein, blood in the treatment groups compared with control group.

Organ weights and relative organ weights

Mean value of absolute organ weight did not reveal any significant alteration except a significant increase in absolute weight of heart (G3 Male and G4 Male) and kidney (G4 Male) and a significant decrease in adrenal weight (G4 Female) when compared with control.

No significant differences were observed in relative organ weights of both sexes in treatment groups when compared with control group except a significant decrease in the brain (G3 Female and G4 female).

The above changes were incidental findings and, hence, not considered as treatment-related (Table 3a-b).

Gross pathology

After completion of study, all surviving animals were terminally sacrificed by the carbon dioxide asphyxiation and thereafter animals were necropsies. There was no abnormality observed at the external gross pathological examination in any animals of either sex.

Table 1 — Summary of Hematology Parameters

Parameters	G1 (Control)				G2 (Low Dose)				G3 (Mid Dose)				G4 (High dose)				G5 (Satellite Control)				G6 (High Dose Recovery Satellite)			
	Male		Female		Male		Female		Male		Female		Male		Female		Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hemoglobin (g/dL)	15.85	1.92	15.28	0.80	16.00	0.81	15.64	0.42	16.41	0.40	15.56	1.04	16.40	0.97	15.24	0.94	15.30	0.45	16.28	1.01	14.70	0.22	15.30	0.45
TLC (K/uL)	11.12	2.02	8.94	1.57	12.07	1.70	7.83	2.14	11.23	2.19	10.13	1.64	11.89	1.64	11.68	3.93	7.88	1.39	10.86	3.22	6.36	1.34	7.88	1.39
Neutrophil (%)	24.23	3.30	21.03	4.85	21.68	4.62	20.52	3.51	22.63	5.02	22.34	5.15	27.03	13.18	21.40	3.21	26.60	3.14	23.80	4.03	24.38	3.34	26.60	3.14
Lymphocyte (%)	66.59	3.62	72.22	5.13	70.27	5.82	72.39	5.14	68.74	5.66	70.82	5.88	64.49	13.18	71.52	3.92	64.46	3.02	66.26	4.71	65.95	4.65	64.46	3.02
Eosinophil (%)	1.77	0.47	1.51	0.34	1.66	0.42	1.93	1.02	1.56	0.27	1.98	0.50	1.34	0.47	1.80	0.44	1.88	0.81	1.80	0.37	1.98	0.59	1.88	0.81
Monocyte (%)	6.35	0.98	4.20	0.79	5.13↓	1.00	4.15	1.14	5.89	0.65	3.89	1.12	5.95	0.98	4.36	0.89	5.84	1.92	7.02	1.21	6.50	0.99	5.84	1.92
Basophil (%)	1.06	0.44	1.04	0.32	1.26	0.32	1.01	0.24	1.18	0.24	0.97	0.32	1.19	0.34	0.92	0.33	1.22	0.45	1.12	0.36	1.20	0.27	1.22	0.45
RBC (M/uL)	8.72	1.16	7.88	0.40	8.85	0.35	8.02	0.33	9.08	0.36	7.90	0.49	8.98	0.58	7.83	0.57	8.07	0.16	9.37	0.45	7.64	0.22	8.07	0.16
PCV (%)	45.65	5.58	42.78	1.97	46.38	2.16	43.87	1.29	46.92	1.30	43.61	2.52	46.93	2.03	43.26	2.42	43.80	1.14	46.74	2.74	41.35↓	1.02	43.80	1.14
Platelet count (K/uL)	1098.90	145.24	1281.60	143.60	1290.70↑	96.28	1357.60	196.18	1280.40↑	202.93	1410.10	180.65	1257.8	190.74	1259.44	132.89	1254.00	62.45	1321.80	184.68	1236.25	136.02	1254.00	62.45
Clotting Time (Min/Sec)	1.51	0.34	1.24	0.13	1.37	0.22	15.64	0.42	1.44	0.30	15.56	1.04	1.51	0.34	15.24	0.94	1.44	0.31	1.44	0.31	1.48	0.35	1.44	0.31

↓= Significant Decrease; ↑= Significant Increase

Table 2 — Summary of Biochemistry Parameters

Parameters	G1 (Control)				G2 (Low Dose)				G3 (Mid Dose)				G4 (High dose)				G5 (Satellite Control)				G6 (High Dose Recovery Satellite)			
	Male		Female		Male		Female		Male		Female		Male		Female		Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood Glucose (mg/dL)	128.35	14.43	143.04	21.97	130.42	17.05	125.85	14.96	128.62	13.12	140.02	20.09	118.42	15.17	125.31	26.84	136.65	15.98	129.74	33.25	131.05	9.49	133.25	24.88
Albumin (gm/dL)	4.21	0.13	4.55	0.18	4.29	0.20	4.54	0.35	4.48 ↑	0.16	4.60	0.43	4.32	0.27	4.47	0.25	4.24	0.19	4.47	0.15	4.20	0.18	4.65	0.16
Total Bilirubin (mg/dL)	0.13	0.03	0.19	0.03	0.11	0.04	0.20	0.06	0.09	0.04	0.17	0.04	0.11	0.03	0.17	0.03	0.14	0.03	0.18	0.04	0.12	0.02	0.19	0.03
Total Cholesterol (mg/dL)	39.44	5.54	44.71	7.05	41.83	6.33	47.14	12.02	35.85	7.28	47.30	9.97	35.63	8.20	47.21	9.49	39.60	4.62	43.92	12.06	49.96	9.16	40.16	3.62
Creatinine (mg/dL)	0.41	0.04	0.54	0.06	0.41	0.04	0.50	0.05	0.39	0.02	0.48↓	0.05	0.39	0.05	0.47↓	0.05	0.36	0.05	0.41	0.10	0.36	0.07	0.51	0.32
SGOT (IU/L)	249.21	39.10	138.74	21.92	218.37	63.84	135.17	37.47	216.45	32.22	126.92	23.75	193.12↓	43.41	141.39	22.27	183.13	35.36	147.48	14.47	140.20	29.59	153.54	38.28
SGPT (IU/L)	63.23	7.06	39.20	6.85	70.79	51.25	45.82	10.36	62.32	18.02	42.49	7.53	54.62	7.29	41.92	10.46	49.14	6.60	30.79	3.90	45.65	8.87	32.61	5.14
Total Protein (gm/dL)	6.14	0.44	6.23	0.32	6.46	0.61	6.55	0.71	6.34	0.57	6.47	0.54	6.25	0.44	6.46	0.67	6.15	0.51	7.18	0.43	6.54	0.67	7.09	0.16
Urea (mg/dL)	48.79	8.07	59.02	6.08	46.76	3.81	57.13	11.42	51.40	5.09	60.67	9.38	49.50	9.24	67.68	10.00	49.20	4.57	54.79	6.07	52.24	9.74	55.18	7.14
BUN (mg/dL)	22.91	3.79	27.71	2.85	21.94	1.78	26.82	5.36	24.03	2.35	28.48	4.41	23.24	4.34	31.77	4.70	23.10	2.14	25.12	1.99	24.53	4.57	25.91	3.36
Sodium m (Eq/L)	148.20	0.92	146.60	1.35	147.90	1.52	146.90	0.88	149.20	1.93	148.50↑	1.58	149.50	1.27	150.22↑	2.17	145.00	0.71	145.60	1.34	148.40 ↑	1.67	147.50	1.29
Potassium (m Eq/L)	5.85	0.51	5.41	0.54	5.88	0.25	5.63	0.53	6.04	0.34	5.86	0.48	6.29	0.40	5.86	0.53	5.84	0.64	5.20	0.32	5.92	0.33	4.93	0.19

↓= Significant Decrease; ↑= Significant Increase

Table 3 — Summary of relative organ weight (%): Male animals

Organ	Group	G1		G2		G3		G4		G5S		G6S	
	Dose	Control		50		250		500		Control satellite		High dose recovery satellite	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Brain	Mean	0.567	0.853	0.593	0.811	0.543	0.743 ↓	0.566	0.749 ↓	0.493	0.735	0.482	0.732
	SD	0.049	0.096	0.061	0.114	0.051	0.061	0.030	0.074	0.053	0.043	0.047	0.064
Heart	Mean	0.320	0.373	0.354	0.380	0.356	0.384	0.351	0.351	0.339	0.347	0.312	0.374
	SD	0.021	0.039	0.030	0.035	0.038	0.034	0.050	0.036	0.016	0.043	0.042	0.013
Adrenal	Mean	0.017	0.036	0.018	0.032	0.017	0.029	0.018	0.028	0.017	0.039	0.017	0.043
	SD	0.003	0.007	0.004	0.007	0.002	0.002	0.002	0.007	0.003	0.008	0.004	0.004
Testis	Mean	0.841	0.069	0.851	0.067	0.896	0.066	0.899	0.056	0.845	0.071	0.665	0.073
	SD	0.166	0.011	0.118	0.012	0.125	0.013	0.060	0.022	0.062	0.008	0.315	0.006
Epididymis	Mean	0.339	0.297	0.346	0.283	0.340	0.259	0.350	0.260	0.358	0.309	0.387	0.378
	SD	0.055	0.082	0.027	0.052	0.046	0.055	0.040	0.053	0.056	0.067	0.098	0.082
Kidneys	Mean	0.739	0.776	0.739	0.770	0.788	0.768	0.803	0.762	0.721	0.689	0.718	0.701
	SD	0.062	0.047	0.081	0.044	0.042	0.066	0.068	0.046	0.030	0.031	0.062	0.041
Liver	Mean	2.977	3.342	2.971	3.060	2.908	3.114	3.133	3.118	2.858	2.981	2.923	2.988
	SD	0.424	0.356	0.309	0.272	0.130	0.305	0.321	0.374	0.275	0.316	0.302	0.150
Thymus	Mean	0.121	0.148	0.117	0.146	0.112	0.148	0.134	0.300	0.092	0.100	0.087	0.104
	SD	0.022	0.033	0.025	0.021	0.030	0.019	0.013	0.441	0.011	0.017	0.013	0.019
Spleen	Mean	0.175	0.200	0.195	0.210	0.187	0.206	0.182	0.207	0.188	0.189	0.168	0.230
	SD	0.026	0.033	0.024	0.042	0.012	0.017	0.015		0.016		0.024	0.008 ↑

↓= Significant decrease; ↑= Significant increase

Internal examination of found dead animal No. 97 showed multiple white foci in all the lobes of lungs and found dead animal No. 88 showed dark red coloured lungs with greyish green colour fluid in the pleural cavity and ruptured oesophagus.

Internal examination of the terminally sacrificed animals of either sex across various experimental groups (G1-G6) did not reveal any abnormality of pathological significance.

Histopathological evaluation

Microscopic examination of various organs/tissues from dead animal found in 500 mg/Kg BW did not reveal any abnormal pathology except diffuse myositis in the esophagus, multifocal pigmented histocytes in lungs and diffuse MNC infiltration in the trachea (Fig. 3a-f). Tissues of found dead animal in satellite control group were completely autolyzed. Hence, histopathology was not performed.

Microscopic examination of various organs/tissues from vehicle control G1 and G4 groups revealed certain pathological changes such as MNC infiltration in alveoli and bronchi, pigmented histiocytes, increased activity of bronchial lymph tissue and fibrosis (lungs), basophilic tubules and MNC infiltration (kidneys), testicular atrophy and tubular cell depletion (testes), oligospermia, cellular debris, sperm granuloma (epididymis), extramedullary hematopoiesis, MNC infiltration (liver), MNC infiltration (prostate and trachea), myositis (esophagus) of varying degree. These findings recorded in various organs of experimental animals were mostly non-specific, infrequent in nature. Moreover, the rates of occurrence of the findings recorded in high dose group were either very low or comparable to the concurrent vehicle control group. Hence, all these findings could be considered as spontaneous or incidental in nature representing the normal physiological/metabolic or congenital changes encountered in rats of this age kept under laboratory conditions (Table 4a-b).

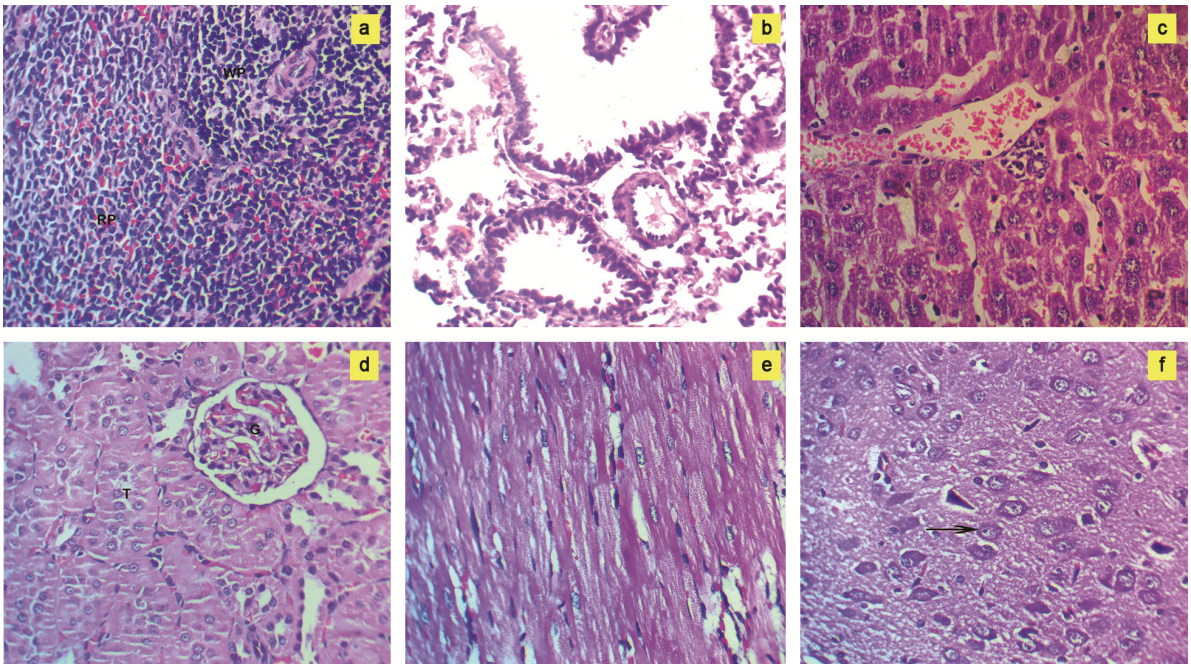

Fig. 3 — Histopathology of vital organs: a) Spleen, b) Lung, c) Liver, d) Kidney, e) Heart, f) Brain

Table 4 — Summary of Histopathological Findings

Organs	Histopathology Findings	Male Animals		Female Animals	
		G1 (N=10)	G4 (N=10)	G1 (N=10)	G4 (N=10)
Lungs	MNC infiltration in bronchi and alveoli	5	3	0	8
	MNC infiltration in bronchi	0	1	4	0
	MNC infiltration in alveoli	1	0	0	0
	Pigmented histocytes	1	3	1	2
	Increased activity of BALT	2	5	0	1
	Fibrosis	1	0	0	0
Kidneys	Basophilic tubules	0	1	0	0
	MNC infiltration in pelvis	0	0	2	0
Testes	Tubular atrophy	1	2	0	0
	Tubular cell depletion	1	0	0	0
Epididymis	Oligospermia	2	2	0	0
	Cellular debris	2	2	0	0
	Sperm granuloma	0	1	0	0
Liver	Extra medullary haematopoiesis	1	0	0	0
	MNC infiltration	1	1	0	0
Prostate	MNC infiltration	1	0	1	5
Trachea	MNC infiltration	0	1	0	0
Oesophagus	Myositis			0	1
Brain, spinal cord (at three levels: cervical, mid-thoracic and lumbar), pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, liver, pancreas, kidneys, adrenals, spleen, heart, trachea and lungs, aorta, testes, epididymis & prostate (male animals), urinary bladder, lymph nodes, peripheral nerve, section of bone marrow, skin and eyes, uterus & ovaries (female animals)	NAD	2	2	3	0

MNC- Mononuclear cells, NAD - No Abnormality Detected.

Conclusion

Safety of herbo-mineral formulations has often been debated. In the current study, Swarna Guggulu was studied in subchronic oral toxicity studies to assess its long-term safety. Basis results of this study, Swarna Guggulu was found safe on subchronic oral administration in NOAEL(No Observed Adverse Effect Level) dosage greater than 500 mg/kg BW when administered orally once daily for a period of 90 days in both the sexes of Wistar rats.

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