WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 4, 919-927. Research Article ISSN 2277– 7105

SUB ACUTE ORAL TOXICITY STUDY OF SWARNA BHASMA IN WISTAR RATS

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Article Received on
23 Jan. 2017,
Revised on 12 Feb. 2017,
Accepted on 04 March 2017
DOI: 10.20959/wjpr20174-8088

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ABSTRACT

Swarna bhasma is an elixir / rasayana used in Ayurveda as rejuvenator and for therapeutic purposes since ancient times. In the present study, Swarna bhasma was evaluated for safety in Wistar rats by sub acute toxicity study. The test drug was administered to three groups of rats at therapeutic dose, 5 times of therapeutic dose and 10 times of therapeutic dose levels. No mortality or signs of toxicity was observed in Wistar rats during the study. Hematology and biochemical values did not alter significantly between test and control group. Internal organs did not reveal structural changes suggestive of toxicity upon gross examination and histopathology investigation. Swarna bhasma was found to be safe up to 13.5 mg/kg body weight in Wistar rats.

KEYWORDS: Swarna Bhasma, rats, Sub acute toxicity.

INTRODUCTION Gold, the precious metal is becoming increasingly important in many modern medical and beauty treatments. The mention of use of gold as medicine is found in all ancient classic treatise of Ayurveda,

including Charak Samhita (1500 BC), Sushruta Samhita (1000 BC) and Ashtang Hridaya (400 AD). Swarna bhasma, a calinated preparation of gold is used in the treatment of cardiac diseases, mental disorders, Conjuntivitis and skin diseases. In Swarna prashan a popular practice in India, Swarna Bhasma along with herbs like *Acorus calamus*, *Bacopa Monnieri*) mixed with honey and *ghee* is administered to the new born babies for enhancing immunity and intellectual development (Mahapatra et al, 2013). Analgesic activity (Bajaj and Vohora, 1998), antistress activity (Shah et al, 2005) and free radical scavenging activity (Mitra et al, 2002) of Swarna bhasma in experimental animals have been ascertained by various Studies. The present study is primarily aimed to assess the safety of Swarna bhasma upon oral administration in rats through sub acute toxicity study.

MATERIALS AND METHODS

Test drug

Swarna Bhasma was prepared by the process of shodana (purification) and calcination or incineration. In the process of Purification, the gold leaves are heated over fire till they turn red and dipped in tila taila (sesame oil) repeatedly for seven times. The process of heating and dipping was repeated seven times each with takra (butter milk), Gomutra (cow's urine), Kanji (sour gruel prepared from rice) and kulatha kwatha (decoction of Dolichos biflorus seeds). The purified gold leaves were triturated with purified mercury and lemon juice and the amalgam thus obtained was triturated further with purified sulphur. It was further grinded with lemon juice and finally calcinated by subjecting to repeated heating under high temperature to obtain bhasma. (Vagbhata's Rasaratna Samucchaya, 1962 and Sharma's Rasatarangini,1979).

Dose calculation

The human dose of the Swarna bhasma is 15 mg per day and the dose equivalent to rats was calculated (Ghosh, 1984). The test drug was administered at test dose level (test dose), 5 times of the test dose (Average dose) and 10 times of the test dose (High Dose). The test drug was administered orally at dose of 1.35 mg, 6.75 mg and 13.5 mg per kg body weight along with diluted honey in TD, AD and HD groups respectively.

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.

Ethical Clearance

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

Experimental design

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). 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. (Schedule Y, 2005).

At the termination of the experiment, animals were fasted and blood samples were collected by retro orbital plexus puncture under ether anesthesia for hematology and serum biochemistry.

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.

Hematology and serum Biochemistry

Blood samples collected under light ether anesthesia were analysed for Total Leucocyte Count (TLC), Polymorph Percentage, Lymphocyte percentage, Packed Cell Volume, Hemoglobin (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) at Biochemistry division of the Institute.

Post mortem and Histopathology

Animals were sacrificed by cervical dislocation and detailed post mortem examination was carried out. Vital organs such as heart, Lungs, Liver, spleen, stomach, Kidneys, Brain etc,

were individually weighed and tissue samples of the same were stored in 10% formalin for histopathology studies.

Statistical analysis

The data obtained during the trial was compared between groups through Analysis of variance (ANOVA) followed by Dunnet's test at P< 0.05 significance.

RESULTS AND DISCUSSION

No abnormal behavioral activity and pre terminal deaths were recorded in the rats exposed to the test compound up to 10 times of the intended therapeutic dose. Significant differences were not observed with respect to % body weight gain between test groups and control group. Significant differences were not observed with respect to % feed intake except for Significant decrease (P<0.05) in TD group during 4^{th} week of the trial as compared to control, but AD and HD groups did not show any difference.

Significant differences were not observed with respect to hematological parameters. Serum biochemical parameters too did not show significant variations except for Significant (P<0.05) decrease in serum Creatinine in AD and HD groups as compared to control group. The invitro studies carried out to investigate Red blood cell hemolysis, aggregation studies with blood cells, protein adsorption, complement C3 adsorption and platelet activation have indicated the safety of Swarna bhasma. (Paul and Sharma, 2011).

Significant differences were not observed with respect to organ weights relative to body weight in Wistar rats in control and test groups during sub acute study.

Significant difference was not observed with respect to organ weights relative to brain weight in test groups as compared to control. No significant compound related abnormalities in the samples of urine tested qualitatively for the presence of leukocytes, nitrite, urobilinogen, protein, blood, ketone, bilirubin, glucose, etc. before and after exposure to the test compound at all dose levels as compared to control group. No Major histopathological changes were observed in any of the organs from the rats that received high dose of the test drug as compared to that of control.

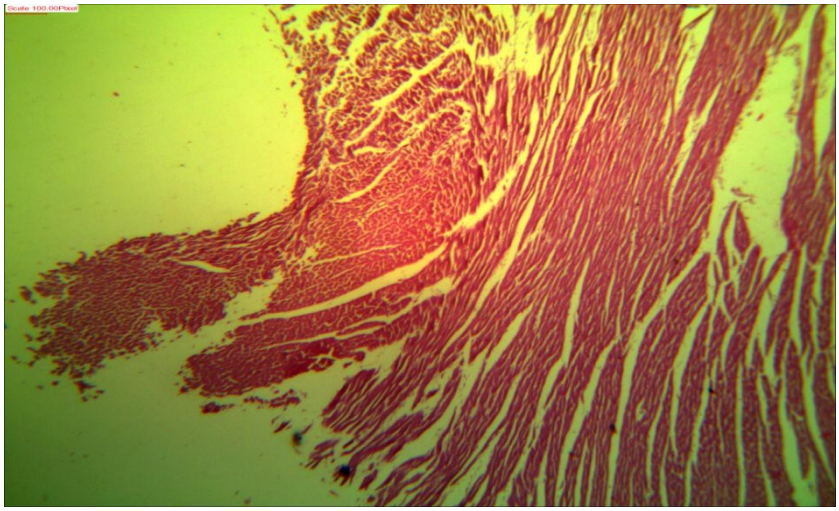


Fig: 1. Histopathology of section of Heart of male rat showing normal Endocardium, myocardium and pericardium.

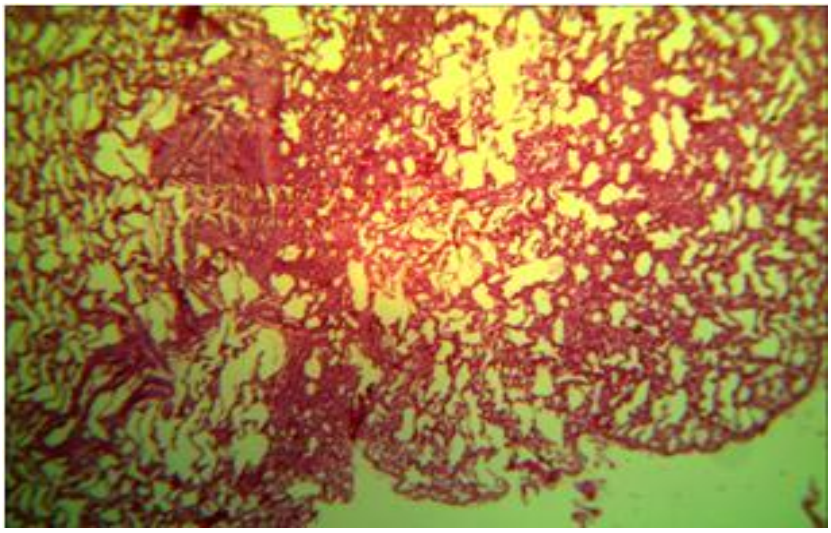


Fig 2. Histopathology of section of Lung of a female rat showing normal Bronchio-alveolar system (HD, Sub acute Toxicity).

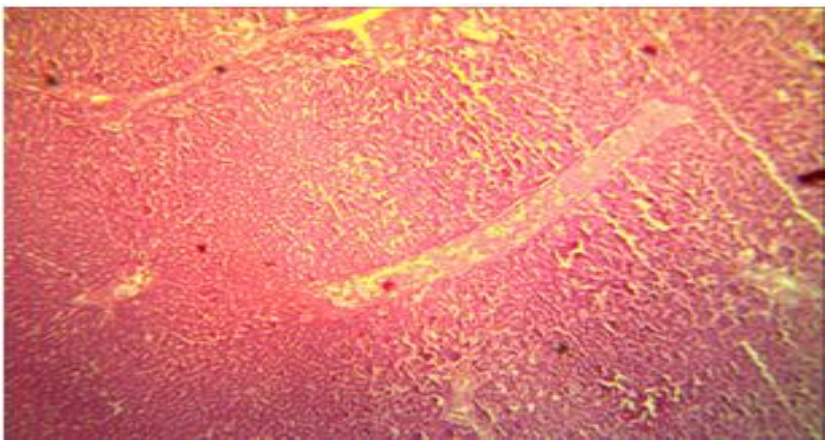


Fig: 3. Histopathology of section of Liver of a male rat showing normal Portal triads and sinusoidal

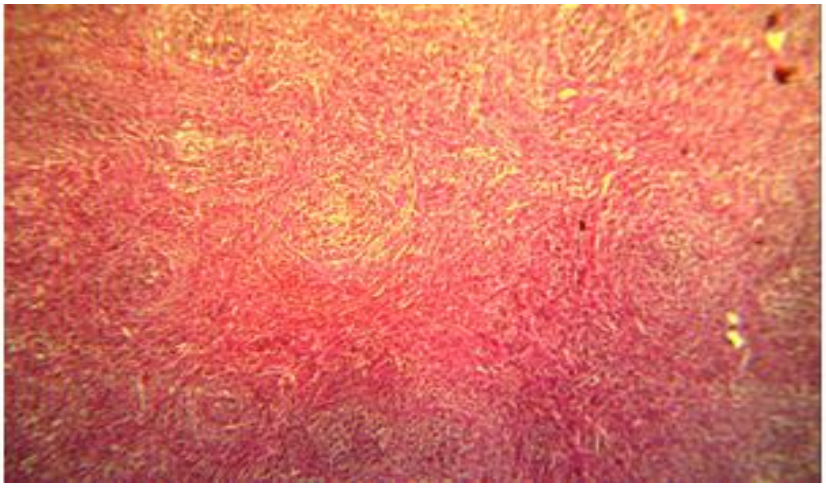


Fig 4. Histopathology of section of spleen of a female rat showing normal lymphoid follicles and germinal centres.

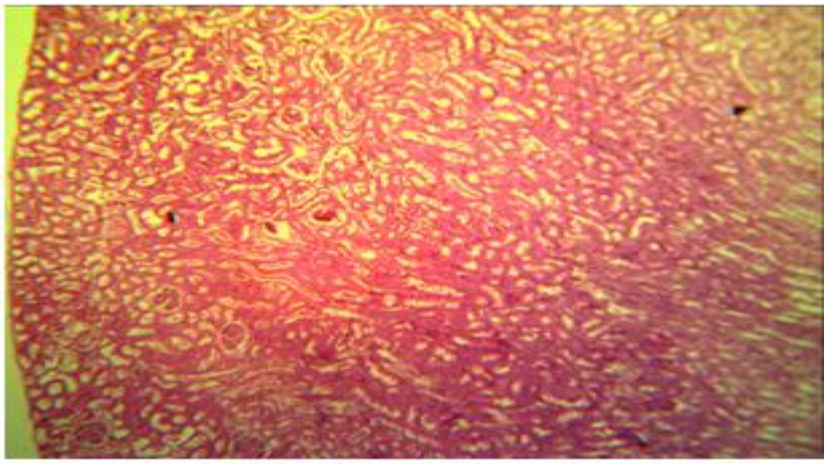
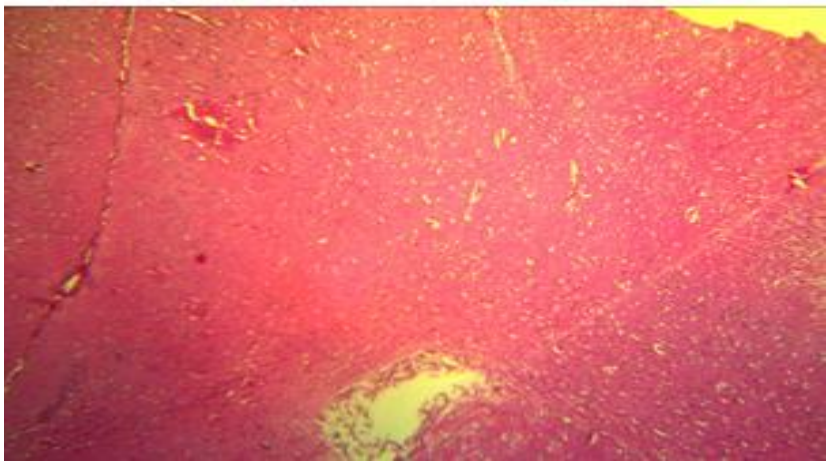


Fig: 5. Histopathology of section of Kidney of a male rat showing normal glomeruli and renal tubules



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

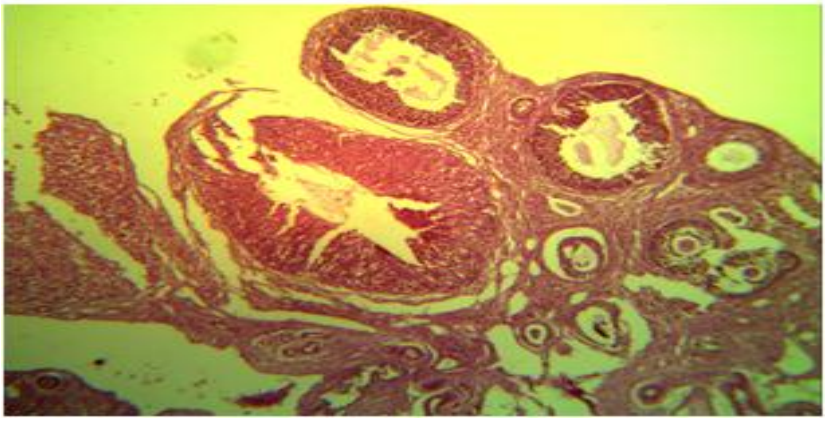


Fig 7. Histopathology of section of ovary of a female rat showing normal stroma and graffian follicles.

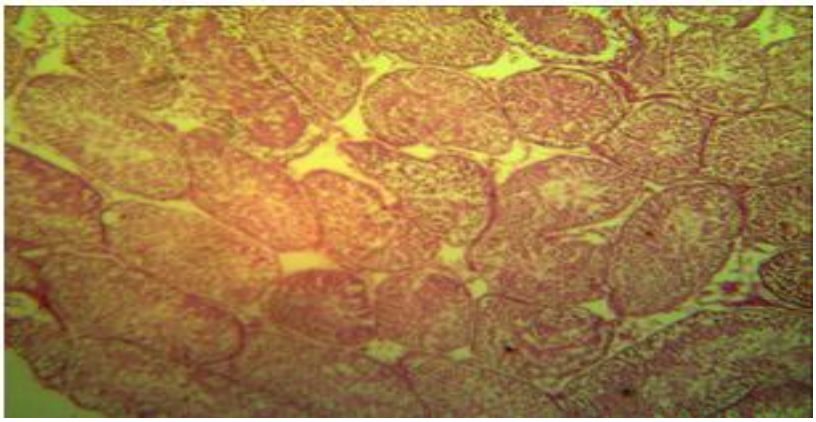


Fig: 8. Section of testis of a male rat showing seminiferous tubules of varying sizes with normal spermatogenesis.

Table: 1 Weekly % body weight gain as compared to day 1 during Sub acute toxicity Study in Wistar Rats.

Weeks	VC	TD	AD	HD
0-1	26.93±5.43	26.79±1.94	25.79±2.20	29.14±3.71
0-2	40.90±7.51	36.35±4.45	37.38±4.28	37.77±7.18
0-3	57.73±12.69	54.56±7.90	58.60±8.07	55.77±11.53
0-4	69.82±14.66	57.25 *±10.56	68.50±11.47	68.65±15.05

(Average of 6 Values)

Table: 2 Weekly % feed consumption in grams up to 28 days during Sub acute toxicity study in Wistar Rats

	VC	TD	AD	HD
I Week	87.11±10.26	76.22±3.23	82.21±4.99	79.72±7.87
II Week	76.69±5.96	63.05±3.98	71.67±5.50	70.55±5.29
III Week	72.15±8.38	61.93±3.79	73.55±5.19	69.93±5.30
IV Week	63.82±6.05	46.40*±3.02	55.30±3.75	56.41±3.53

*P<0.05 (Average of 6 Values)

Table: 3. Hematology parameters during Sub acute Toxicity study in Wistar rats

	TLC ($x10^3$)	POLY (%)	LYM (%)	PCV (%)	HB (g %)	TRC (10^3)	Platelets (10^6)
VC	6.37±0.28	29.67±1.61	70.33±1.61	34.17±1.01	14.75±0.25	4.38±0.14	2.15±0.04
TD	6.49±0.26	29.50±1.23	70.50±1.23	35.17±0.95	13.98±0.42	4.33±0.14	2.18±0.13
AD	6.33±0.41	31.83±1.49	68.17±1.49	37.00±1.71	14.13±0.58	4.02±0.07	2.05±0.03
HD	6.42±0.35	30.17±0.75	69.83±0.75	34.33±1.54	13.20±0.68	4.03±0.11	2.01±0.05

(Average of 6 Values)

Table 4. Clinical chemistry Parameters during Sub acute Toxicity study in Wistar rats

	Glucose (mg %)	SGOT (IU/L)	SGPT (IU/L)	Creatinine (mg%)	Total Protein (g %)	Prothrombine Time (Sec.)
VC	40.50±3.86	119.33±7.94	66.00±4.29	1.03±0.03	5.70±0.14	18.50±0.96
TD	40.50±2.20	121.00±10.31	53.33±2.29	0.85±0.05	5.85±0.10	20.17±1.01
AD	44.67±1.80	128.67±7.58	61.67±6.14	0.78**±0.02	5.70±0.16	18.83±1.05
HD	49.17±6.91	138.33±8.78	69.00±4.75	0.77**±0.02	5.65±0.18	20.00±0.52

**P<0.05

(Average of 6 Values)

Table: 5 Organ weight in % Body weight – Sub acute Toxicity study.

	Heart	lung	Liver	Spleen	Stomach	kidneys	Testes	Ovary	Brain
VC	0.41±0.03	0.70±0.03	3.33±0.16	0.26±0.02	0.90±0.05	0.80±0.04	0.80±0.35	0.07±0.01	1.29±0.08
TD	0.35±0.02	0.68±0.04	3.05±0.07	0.26±0.02	0.82±0.03	0.72±0.04	1.14±0.34	0.06±0.00	1.16±0.08
AD	0.40±0.02	0.77±0.04	3.21±0.16	0.26±0.01	0.88±0.06	0.76±0.06	1.20±0.12	0.07±0.00	1.11±0.08
HD	0.37±0.02	0.69±0.04	3.33±0.14	0.27±0.01	0.97±0.01	0.74±0.03	1.34±0.12	0.06±0.00	1.16±0.08

(Average of 6 Values)

CONCLUSION

Oral administration of Swarna bhasma up to dose of 13.5 mg per kg body weight for 14 consecutive days was found to be innoxious in Wistar rats. Physiological, hematological, biochemical and histopathological studies carried out as part of the study have ascertained the safety of the test drug up to the dose level tested.

ACKNOWLEDGEMENT

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

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