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Toxicity Study of Swarna Bhasma, an Ayurvedic Medicine Containing Gold, in Wistar Rats

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Abstract

Objectives: This study was conducted to estimate No Observed Effect Level (NOEL) or no observed adverse effect level of Swarna Bhasma an Ayurvedic classical preparation of gold in Wistar rats. **Materials and Methods:** Swarna Bhasma was prepared as per classical Ayurvedic text and analyzed for physicochemical properties. A total of 40 Wistar rats of either sex with an average body weight of 150-200 g were divided into four groups with 10 animals of both sexes in each group. Group I served as control and was given vehicle (Honey:Water in 2:3 ratio) Group II, III, IV were orally administered Swarna Bhasma @ 13.5, 6.75, 1.35 mg/kg body weight for 90 consecutive days. The effect of the drug was observed on ponderal changes, hematological, and biochemical parameters. At the end of the study, all animals were sacrificed and examined for gross pathological changes. Histopathological evaluation was performed only for control and high-dose group and of animals that were moribund sacrificed. **Results:** Swarna Bhasma was found to be safe at all levels tested. No significant treatment-related clinical signs were noted in all groups studied. The food and water consumption was not affected at highest given dose, i.e., 13.5 mg/kg body weight. No major alterations were observed during histopathological evaluation. **Conclusion:** This study indicates 13.5 mg/kg body weight as NOEL for Swarna Bhasma in Wistar rats.

Keywords: Ayurvedic Classical Drugs, Swarna Bhasma, Toxicity Study

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1. Introduction

Swarna Bhasma is an Ayurvedic formulation prepared from elemental gold, its first reference dates back to the 8th century in a treatise called *Rasendramangalam* written by the great scholar Nagarjuna, which is considered as the authoritative text on Ayurvedic alchemy. *Rasendramangalam* also describes detailed methods of preparation and therapeutic uses of metals and minerals for various diseases (Sharma, 2008). In modern medicine, Chrysotherapy, that is, use of gold salts preparations containing gold viz., aurothioglucose, gold sodium thiomalate, and auranofin is used as an anti-rheumatic disease modifying agent but later came across repeated reports of adverse drug reactions (Forestier, 1935). The drawbacks of chrysotherapy are limited efficacy, very slow onset of action, and meticulous monitoring requirements. Physicians have suggested the use of such preparations with caution and exact supervision (Juan, 1984).

Saper *et al.*, (2004) reported that users of Ayurvedic drugs may be at risk of heavy metal toxicity, and hence quantification of heavy metals in Ayurvedic drugs should be performed. Lavekar *et al.*, (2010) reported that merely measurement of the heavy metal content of Ayurvedic preparations may not explain their behavior *in-vivo*. Hence, the need of comparative toxicity with known heavy metal compounds was emphasized. Accordingly, toxicity studies of Swarna Bhasma were conducted with an objective to find out the target organ of toxicity and to establish No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL) after oral administration in rats for 90 consecutive days.

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2. Materials and Methods

2.1 Test Drug Preparation and Analysis

Swarna Bhasma was provided by the Central Council for Research in Ayurvedic Sciences, New Delhi in a coded manner that was decoded along with chemical analysis report after completion of the study of Swarna Bhasma was prepared as per the method described in Ayurvedic Formulary of India (2004) classical Ayurvedic text that is given below.

2.2 Purification

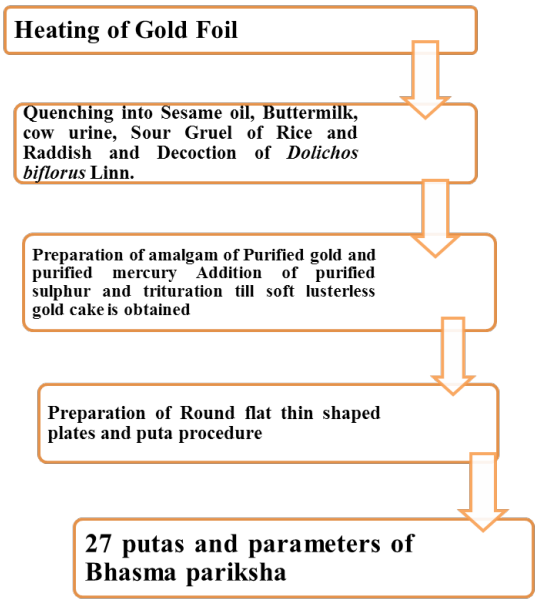
This process is called as "*Shodhana*" in classical Ayurvedic literature. In this process, thin foils of elemental gold were heated over the fire until red hot and were quenched by immediately immersing in sesame oil (oil obtained from the seeds of *Sesamum indicum* Linn). This process of heating and quenching was repeated 7 times. Later, a similar process was conducted using butter milk (takra), gomutra (cow urine), kanji [(sour gruel of rice *Oryzha sativa*) and radish (*Raphanus sativus*)], and kulattha (decoction of *Dolichos biflorus* L.) one after another subsequently for 7 times in each. The foils were washed with water after the completion of quenching.

2.3 Calcination

This process is called as "*Marana*" in classical Ayurvedic literature. Purified gold foils were cut into small pieces, and an equal quantity of purified mercury was added to it. Then, Nimbu Swarasa (fresh fruit juice of *Citrus limon* L.) of sufficient quantity was added to it and was triturated thoroughly. After proper amalgam formation, the residue of Nimbu Swarasa was washed out with water. Purified sulfur was added to the amalgam and subjected to trituration until soft, lusterless powder (Swarn Kajjali) was obtained.

This powder was later levigated with *Nimbu Swarasa* into a homogeneous, soft, semisolid mass. Round, flat, and thin shaped *Chakrikas* were prepared from this mass. These *Chakrikas* were placed inside an earthen pot, i.e., *Sharava* and were sun dried. The *Sharava* was covered with another inverted *sharav,* and their joints were sealed with clay-smeared cloth and again sun dried. Further, this assembly was subjected to the "*Puta Paka*" procedure. Puta procedure is a specific procedure in Ayurveda in which a pit of specific size is dug in an open place; half of the pit is filled with cow dung cakes and sealed. *Sharava* assembly containing small *Chakrikas* is placed in it. The remaining pit was filled with cow dung cakes. The cow dung cakes were ignited and allowed to cool by itself after complete combustion. After the self-cooling of Puta, the earthen pot was taken out, and *Chakrikas* were ground to prepare the powder.

Purified mercury (1/16th of that of used during 1st puta) and an equal quantity of purified sulfur was again added to that powder. *Nimbu Swarasa* was added to this mixture and levigated to prepare *Chakrikas* and subjected to Putapaka. The process was repeated 26 times. Champaka Varna (red color) Swarna Bhasma complying with all parameters of Bhasma Pariksha was obtained after 27 *Putas*. Details of the procedure are given in Flow Chart 1.



Flow Chart 1: Flow chart of Swarna Bhasma preparations algorithm.

3. Physico-Chemical Analysis of Swarna Bhasma

3.1 Qualitative Analysis

Physico-chemical analysis viz. description, estimation of moisture content, qualitative elemental testing, and Ayurvedic parameters was carried out by following standard methods as per Ayurvedic Pharmacopoeia of India WHO guidelines.

3.2 Quantitative Analysis

The quantitative elemental composition was performed on JY 2000 sequential inductively coupled plasma-atomic emission spectrometry (ICP-AES) Spectrometer (Horiba Jobin Yvon, France). The operating parameters of ICP-AES are as follows-RF power: 1000 w at 40.68 MHz, Plasma gas flow rate: 12 L/min, Nebulizer gas flow rate: 0.1 L/min, Sample uptake rate: 1.2 L/min, Slit width: 20 micron/20 micron (Entrance/Exit) and Monochromator: 0.64 m focal length, 2400 groves/mm, Czerny turner mounting.

3.3 Sample Preparation

Swarna Bhasma (0.5 g accurately weighed) was dissolved in 5 mL aquaregia and kept on a hot plate for 10 min. It was centrifuged, and the supernatant was separated. The residue was taken into polypropylene wide mouth bottle and treated with 2 mL concentrated hydrofluoric acid. It was heated on water bath for 2 h and evaporated to dryness. The residue was dissolved with few drops of nitric acid and transferred to a 50 mL plastic vial. The supernatant was added to this vial, and final volume was made up to 50 mL with ultra-pure de-ionized water. The sample solution was analyzed after appropriate dilution by ICP-AES for Au, Ca, Mg, and Al.

3.4 Toxicity Study

Ninety-day repeated dose oral toxicity study was performed in Wistar rats of both sexes. The approval for the study protocol was obtained from Institutional Animal Ethics Committee (IAEC) of NRIADD, Kolkata with ethical approval no IAEC/2010/04 dated 25/05/2011.

3.5 Housing and Environment

Wistar rats of either sex with body weight ranging from 150 g to 200 g were obtained from animal house facility of NRIADD, Kolkata. Rats were acclimatized for 7 days. Temperature and relative humidity were maintained at 25 ± 1°C, and 40-70 % respectively, and illumination was controlled to give approximately a sequence of 12 h light and 12 h dark. Rats were individually housed in polypropylene cages (27 cm × 19 cm × 14 cm) with lids and rice husk bedding. Pelleted rodent diet obtained from National Institute of Nutrition, Hyderabad, was provided along with de-ionized water using plastic nozzle bottles *ad libitum*.

3.6 In-Life Phase and Pathology

The animals were divided into four groups of 10 animals of both sexes each. Ninety days repeated dose oral toxicity study of Swarna Bhasma was conducted by daily single administration of the drug at 13.5 (High-Dose), 6.75 (Mid-Dose), and 1.35 (Low-Dose) mg/kg body weight along with vehicle control. The test drug was administered as suspension in vehicle, i.e., honey mixed with water (2:3) by gavage, and control receiving the vehicle. The dose calculation was as follows:

Therapeutic Dose for Human (70 kg body weight approximately): 15 mg/day.

Dose conversion factor from human to rats is 0.018 (Paget and Barnes, 1964).

Hence, the dose for rats (200 g body weight approximately) will be 15 × 0.018 = 0.27 mg per kg Dose for rats will be 1.35 mg.

The dose that was 5 times more than therapeutic, i.e., 1.35 × 5 = 6.75 was considered as Mid Dose and 10 times therapeutic dose, i.e., 1.35 × 10 = 13.5 was considered as Highest Dose for the study.

All animals were observed for morbidity and mortality twice daily. General clinical observations were made twice a day at the same time throughout the study. The animals were observed for changes in skin, fur, eyes, mucus membrane, occurrence of secretions, and excretions. For neurological examination, the animals were taken outside the cage in a standard arena and their behavior was recorded. Body weights and feed consumption of each animal were recorded at the start of the study and thereafter at weekly intervals. Hematological analysis and biochemical analysis were done at 30 days, 60 days, and 90 days, i.e., at the termination of the study. Animals were sacrificed on the 91st day of the study and were subjected to a detailed postmortem examination and histopathological analysis. In-life phase observations were recorded and analyzed statistically by Student's *t*-test followed by ANOVA.

4. Results

4.1 Test Drug Physico-Chemical Analysis

The prepared Swarna Bhasma is found in the form of a reddish brown powder having no characteristic odor. The qualitative analysis shows the positive test for the presence of gold. The assay of element revealed the presence of 62.6% gold, 0.7% calcium, 0.4% aluminum, and 0.2% of

magnesium. A little moisture content 0.11% was found when determined loss on drying at 105°C. The finished product has also qualified the Ayurvedic characteristics viz. *Rakhapurnatva* (the bhasma should be uniformly spread between finger lines of the index finger and the thumb) and lusterless.

4.2 Toxicity Study

No abnormality in clinical signs was detected across all the groups throughout the study. No mortality was found in any of the study groups. No abnormality was detected across the groups during neurological examination. No treatment-related effect on body weights, feed consumption, water consumption, fecal consistency, organ weights, biochemical, and hematological parameters was observed as compared to the control group. The results are described in Tables 1-4. No treatment-related and dosed-dependent adverse changes were observed upon detailed histopathological evaluation (Figure 1-4). Photomicrographs of the selected organs/tissues from vehicle control group and high-dose group are provided with this article.

Table 1. Weekly body weight of male rats (g) Mean±SD $^{n=10}$

Week	Vehicle control	High dose (13.5 mg/kg)	Mid dose (6.75 mg/kg)	Low dose (1.35 mg/kg)
Initial	155.80±41.13	155.40±33.06	156.30±29.24	154.80±34.30
1	184.50±39.53	187.82±36.06	184.94±28.99	183.28±37.36
2	213.70±36.99	215.84±34.25	213.52±27.40	215.66±31.49
3	237.18±35.22	237.94±33.21	241.54±24.73	242.94±32.20
4	256.70±35.14	257.32±32.70	262.06±22.61	259.20±25.56
5	273.52±31.79	271.94±30.68	279.20±20.29	274.82±27.46
6	290.54±32.76	289.02±33.38	295.58±17.80	289.76±27.00
7	303.38±32.94	302.60±30.20	306.98±17.28	301.42±25.79
8	313.66±31.52	313.90±34.26	317.52±18.34	317.96±30.42
9	321.34±33.41	322.16±32.25	322.32±20.23	315.14±26.69
10	333.52±32.96	333.12±30.99	336.53±20.98	327.36±25.14
11	342.64±34.65	342.48±32.72	344.96±18.78	331.78±30.39
12	351.24±35.81	349.06±31.99	354.35±17.72	346.60±27.21
13	358.26±36.94	353.97±34.40	360.60±18.26	354.26±28.37

Table 2. Weekly body weights of female rats (g) Mean±SD $^{n=10}$ except where stated

Week	Vehicle control	High dose (13.5 mg/kg)	Mid dose (6.75 mg/kg)	Low dose (1.35 mg/kg)
Initial	122.20±24.83	119.10±18.74	119.60±13.85	119.20±16.19
1	131.24±16.54	127.14±20.87	129.94±15.43	128.42±12.99
2	144.06±11.68	142.56±14.41	139.12±18.24	139.38±10.57
3	152.98±11.20	151.01±12.96	149.10±15.29	151.36±10.89
4	160.72±10.72	158.26±12.14	157.00±15.11	155.42±8.66
5	163.92±12.44	163.50±13.78	160.34±15.94	159.44±8.91
6	169.15±12.00	170.38±13.23	165.70±18.79	166.44±10.95
7	173.60±10.77	174.92±14.18	170.94±19.76	171.30±11.22
8	176.48±12.02	176.49±14.16	170.54±19.55	172.50±9.84
9	176.94±11.62	176.94±13.85	171.32±20.58	173.90±12.07
10	179.22±11.60	182.06±13.02	175.54±21.03	179.82 ±13.10
11	178.56±18.32	186.08±15.76	176.12±21.58	180.10±14.19
12	183.24±12.24*	187.50±19.67	178.50±19.93	182.96±12.61
13	184.29±13.53*	187.60±16.40	180.40±19.07	184.36±11.75

*n=9

Table 3. Organ weights of male rats (g) Mean±SD [n=10]

Organ	Vehicle control	High dose (13.5 mg/kg)	Mid dose (6.75 mg/kg)	Low dose (1.35 mg/kg)
Terminal body weight	333.96±37.64	335.22±34.61	344.52±19.72	335.39±29.03
Adrenals	0.057±0.013	0.063±0.017	0.061±0.015	0.062±0.012
Liver	10.555±1.171	10.817±1.429	10.962±1.484	10.975±1.009
Brain	1.891±0.218	1.893±0.162	1.847±0.189	1.949±0.075
Thymus	0.297±0.135	0.300±0.142	0.253±0.039	0.285±0.060
Heart	1.272±0.172	1.338±0.174	1.345±0.110	1.277±0.142
Kidneys	2.700±0.305	2.852±0.177	2.933±0.247	2.834±0.213
Spleen	0.974±0.224	1.030±0.253	0.963±0.288	1.092±0.311
Testes	3.04±0.83	3.28±0.18	3.25±0.16	3.24±0.18
Epididymis	1.277±0.189	1.369±0.109	1.435±0.125	1.382±0.115

Table 4. Organ weight of female rats (g)

Organ	Vehicle control	High dose (13.5 mg/kg)	Mid dose (6.75 mg/kg)	Low dose (1.35 mg/kg)
	Mean±SD [n=9]	Mean±SD [n=10]	Mean±SD [n=10]	Mean±SD [n=10]
Terminal body weight	176.44±12.00	179.26±15.75	170.51±17.67	174.32±10.66
Adrenals	0.059±0.011	0.062±0.012	0.064±0.011	0.062±0.014
Liver	5.548±0.326	5.791±0.993	5.604±0.921	6.028±0.648
Brain	1.721±0.199	1.788±0.109	1.774±0.123	1.738±0.137
Thymus	0.219±0.034	0.233±0.071	0.253±0.058	0.216±0.049
Heart	0.788±0.053	0.831±0.087	0.852±0.095	0.832±0.114
Kidneys	1.517±0.088	1.548±0.241	1.577±0.106	1.642±0.124
Spleen	0.585±0.128	0.665±0.108	0.689±0.129	0.600±0.150
Ovaries	0.146±0.037	0.154±0.019	0.150±0.046	0.153±0.026
Uterus (with cervix)	0.533±0.189	0.584±0.165	0.544±0.127	0.653±0.198

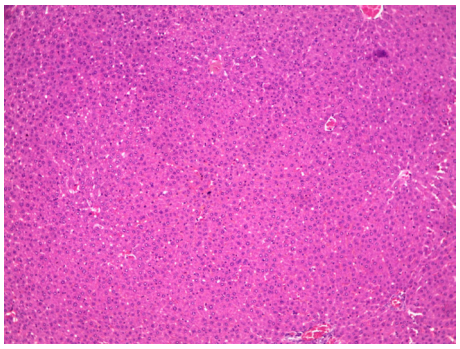


Figure 1. Liver from control group.

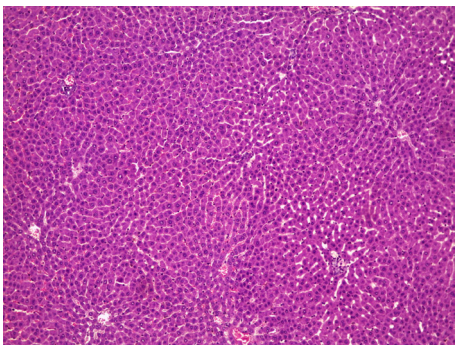


Figure 2. Liver from high-dose group.

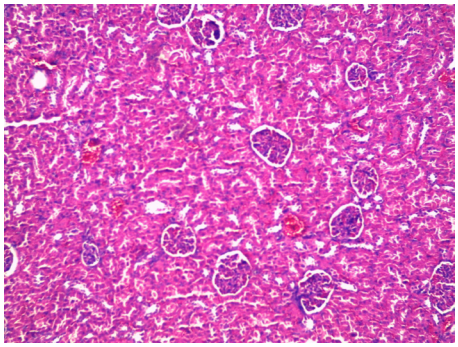


Figure 3. Kidney from control group.

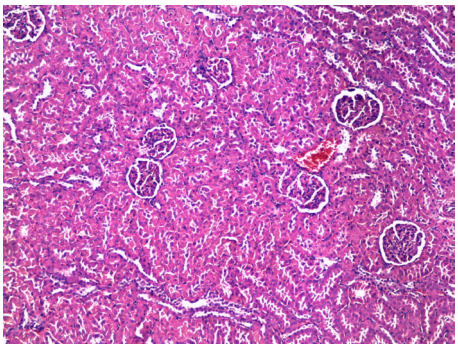


Figure 4. Kidney from high-dose group.

5. Discussion

Gold compounds used in modern medicine showed mucocutaneous reactions viz., exfoliative dermatitis and aphthous ulceration (Penneys *et al.,* 1974). Thirteen heavy metals including gold were reported as nephrotoxic (Schreiner, 1968). A time to tumor type of study was performed in rats at various doses of which 12 mg/kg body weight intramuscularly showed no gain in body weight after 3 months and 25% decrease in body weight at the time of necropsy in the same study at 24 mg/kg body weight intramuscular dose, body weights of females decreased by 10% at the end of the first week (Payne BJ and Sekella, 1978). Self-limiting gastrointestinal reactions such as frank diarrhea was the sign reported solely in oral gold therapy (Van Riel *et al.,* 1983). Various hematologic reactions such as eosinophilia, (Edelman *et al.,* 1983) thrombocytopenia, (Harth *et al.,* 1978) and aplastic anemia (Kay, 1976) were described when chrysotherapy was used. Parenteral administration of gold salts accumulates in the reticuloendothelial system, liver, spleen, and kidney (Juan, 1984). Nephrotoxicity due to deposition of immune complexes was reported with all gold compounds (Davis, 1988). Macrophages play an important role in beneficial or adverse effects of gold compounds (Tonn *et al.,* 1994). Nghiem *et al.,* (2012) reported accumulation of gold particles in Kupffer cells without a change in cellular morphology when gold nanoparticles were administered intravenously up to the dose of 5.84 mg/kg body weight for eight consecutive days. In the present study, chemical analysis showed Swarna Bhasma contained 62.6% gold, i.e., 13.5 mg/kg dose level contained 8.451 mg of gold that was administered per kg for 90 consecutive days in rats. Mitra *et al.,* (2002) studied the effect of daily oral dose of 0.5 mg/kg body weight of Swarna Bhasma up to 60 days in mice and reported no alteration in biochemical and histopathological parameters. No histopathological changes in any organ or tissues viz. kidney, liver, skin, ovary, uterus, testis, epididymis, spleen, intestine, stomach, adrenal gland, thymus, brain, lungs, and heart were noted in this study. However, in the present study body weight gain was normal among groups throughout the study period.

Thus, from results of the present study dose level of 13.5 mg/kg body, weight/day was determined as NOEL for Swarna Bhasma in Wistar rats. Hence, it can be concluded that Swarna Bhasma was safe up to dose rate of 13.5 mg/kg/day when administered orally for 90 consecutive days in Wistar rats. The results of the study further support the safety of Swarna Bhasma, which is being used traditionally since centuries without any adverse drug reactions that are common to other gold-containing therapeutic compounds. As per the Ayurvedic classical literature, detoxification procedures called "*Shodhana*" and "*Marana*" during drug preparation are responsible for decreasing the inherent toxic potential of gold and minerals used in Swarna Bhasma. It is suggested to carry out further toxicity studies of Swarna Bhasma at higher doses so as to establish target organ of toxicity and tissue distribution of gold.

6. Acknowledgment

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