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Pharmacological Study

Repeated dose oral toxicity of *Trivanga Bhasma* in Swiss albino mice

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Abstract

Trivanga Bhasma, a metallic preparation containing *Bhasmas* of *Naga* (lead), *Vanga* (tin) and *Yashada* (zinc), was studied for repeated dose toxicity in Swiss albino mice to estimate No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL). A total of 80 Swiss albino mice of either sex with an average body weight of 28-30 g were equally divided into four groups (Group I, II, III, and IV). Group I served as control and was given vehicle (honey: water in 2:3 ratio) Group II, III, and IV received *Trivanga Bhasma* @ 7.8, 39.5, and 78 mg/kg body weight for 90 consecutive days. The effect of drug was assessed on body weight, feed and water consumption changes, hematological, and histopathological parameters. At the end of the study, all animals were sacrificed and examined for gross pathological changes. Histopathological evaluation was performed for control and high dose group. *Trivanga Bhasma* was found to be safe. No significant clinical signs were noted in all groups studied. No major alterations were observed during histopathological evaluation. Hence, dose rate of 78 mg/kg body weight was established as NOAEL. It is suggested to carry out a toxicity study at possible higher doses and in a different species so as to establish target organ of toxicity.

Key words: Mice, *Naga*, toxicity study, *Trivanga Bhasma*, *Vanga*, *Yashada*

Introduction

Bhasmas are Ayurvedic drugs prepared by using various minerals and metals along with specified herbs. The minerals and metals used in these preparations may be toxic in nature at higher dose or at cumulative dose. The purpose of toxicity study is two-fold, that is, to find out the effect caused and the level of exposure at which the effect is observed. Long-term toxicity studies are designed to detect effects on organs or body systems and the dose range over which the effect develops due to repeated administration for long period. The data collected from toxicity studies will help to understand dose-toxicity response and to establish a safe dose for use in clinical trials.[1]

Trivanga Bhasma is a classical Ayurvedic preparation containing *Bhasmas* of three metals, namely, lead, zinc, and tin indicated in diabetes mellitus and urinary disorders.[2] These metals undergo various processes described in classical literature to yield desired product. Finally, these *Bhasmas* are tested following Ayurvedic specifications before being used as a drug for treatment. As these preparations contain substantial amount of heavy metals, it is obvious to be concerned about safety of these drugs.[3] However, simple measurement of heavy metal content of the drug does not explain how it may be acting *in-vivo*.[4] Hence, it is essential to study its effects in biological system, which will give some idea about target organ of the toxicity. Meticulous perusal of available literature indicated that there is paucity of data on toxicity studies of *Trivanga Bhasma*, hence, present study was undertaken with an objective to evaluate the toxicological profile, identify the target organs of toxicity and to establish No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL) after administration of *Trivanga Bhasma* in mice for 90 consecutive days.

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Materials and Methods

Trivanga Bhasma was provided by the Central Council for Research in Ayurvedic Sciences, New Delhi along with chemical analysis as described in Table 1.

Table 1: Chemical analysis of *Trivanga Bhasma*

Name of the test	Result
Description	
Color	Yellowish
Odor	Nil
Identification	Yields positive for Pb, Sn, Zn
Particle size	39.41% passed through 80 mesh and 12.76% passes through 120 mesh
Loss on drying at 105°C	0.1801%
Total ash content	99.57%
Acid insoluble ash content	94.3654%
Water soluble ash content	1.2509%
Qualitative analysis	
Iron	Positive
Calcium	Positive
Free metal content	Negative
Assay of elements	
Sn	38.26%
Zn	17.067%
Pb	31.67%
Si	3.54%
Mn	356.98 ppm
Cl	0.34%
Ca	3.98%
Fe_2SO_3	3.15%
Al	1.68%
Ni	161.084 ppm
Cu	679.69 ppm
Cd	106.67 ppm
Ayurvedic specifications	
Lusterless	Non-luster
Rekhapurnatva	Positive

ppm: Parts per million

An approval for the study was obtained from Institutional Animal Ethics Committee (IAEC) (No 6-17/2003-CRI/Tech/777 dated February 2, 2009). A total of 80 Swiss albino mice of either sex with body weight ranging from 28 to 30 g were obtained from Institutional Animal Breeding Facility. Mice were acclimatized for 7 days. Temperature and relative humidity were maintained at 25 ± 1°C and 40-70%, respectively and illumination were controlled to give approximately a sequence of 12 hours light and 12 hours dark.[5] Mice were individually housed in polypropylene cages (27 × 19 × 14 cm) with lids and rice husk bedding. Pelleted rodent diet obtained from National Institute of Nutrition, Hyderabad, was provided along with deionized water using plastic nozzle bottles *ad libitum*.

The animals were weighed and randomly divided into four groups of 10 animals/sex each in such a way that mean body weights are equal and total weight variation should not exceed ±20% of the mean. Chronic toxicity study was conducted by single daily administration of test drug @ 7.8 mg/kg i.e. Therapeutically Equivalent Dose (TED), 39 mg/kg (TED × 5), and 78 mg/kg (TED × 10) body weight along with vehicle control for 90 consecutive days. The dose suggested for *Trivanga Bhasma* is 60 mg/day, which was converted to mice therapeutic dose using standard dose extrapolation method.[6] The test drug was given as suspension in vehicle [honey mixed with water (2:3)] by gavage, and control receiving vehicle.

All animals were observed for morbidity and mortality twice daily. General clinical observations were made twice a day at the same time throughout 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. The behavior of the animals was recorded. Body weights and feed consumption of each animal were recorded at the start of study and thereafter at weekly intervals. Differential Leukocyte Count (DLC) was done manually. Animals were

Table 2: Weekly body weight record of males (in gm)

Duration (week)	Control group		TED×10 (78 mg/kg)		TED×5 (39 mg/kg)		TED (7.8 mg/kg)	
	n	Mean±SD	*n*	Mean±SD	*n*	Mean±SD	*n*	Mean±SD
Initial	10	29.60±1.65	10	28.40±1.35	10	28.50±2.07	10	30.80±3.91
1	10	29.90±1.85	10	28.80±1.48	10	28.20±2.04	10	28.40±2.80
2	10	28.80±2.25	10	28.70±1.57	10	27.70±2.50	10	28.30±2.87
3	10	29.10±1.79	10	28.30±1.57	10	27.20±2.90	10	28.70±2.91
4	10	28.70±1.16	10	29.10±0.74	10	28.50±2.64	10	28.90±2.92
5	10	29.10±1.97	10	29.10±1.29	10	28.40±3.34	10	28.90±3.41
6	10	30.42±2.06	10	31.13±1.79	10	29.70±3.16	10	29.68±3.53
7	10	30.50±2.32	10	31.10±2.23	10	29.90±2.60	10	29.90±3.18
8	10	30.36±2.01	10	32.54±1.84	10	29.50±3.06	10	30.48±3.35
9	10	30.78±2.04	10	31.70±0.82	10	29.20±3.62	9	30.89±3.18
10	10	30.92±1.97	10	32.90±1.60	10	29.40±3.60	9	30.22±3.27
11	10	30.90±2.04	10	33.30±1.83	10	29.90±4.70	8	30.75±3.66
12	10	31.23±1.97	10	33.60±1.65	10	30.00±4.11	8	27.63±5.07
13	10	31.80±2.07	10	33.60±1.90	10	29.80±5.16	8	30.38±3.78

Data: Mean±standard deviation, TED: Therapeutically equivalent dose

Table 3: Weekly body weight record of females (in gm)

Duration (week)	Control group *n* =10	TED×10 (78 mg/kg) *n*=10	TED×5 (39 mg/kg) *n*=10	TED (7.8 mg/kg) *n*=10
Initial	24.80±2.30	25.30±1.64	24.70±1.42	23.80±1.32
1	25.30±1.89	24.80±1.23	23.90±1.79	23.50*±1.43
2	24.70±2.11	24.10±1.85	24.10±1.79	22.90±1.37
3	24.50±1.84	23.80±1.55	25.50±1.65	24.50±1.65
4	25.20±1.99	25.80±1.14	25.90±1.45	24.10±2.08
5	25.80±1.23	25.70±2.06	25.90±1.20	25.20±1.81
6	25.67±1.41	25.80±1.48	26.30±2.06	25.68±1.56
7	25.60±1.35	25.00±1.56	25.80±2.39	24.98±1.23
8	25.54±1.50	26.30±1.83	26.00±2.05	26.16±1.71
9	26.30±1.64	25.70±2.16	26.90±2.51	26.30±1.64
10	25.34±1.61	25.90±1.60	26.60±2.41	25.80±1.81
11	25.94±2.22	26.20±1.93	26.60±2.41	26.20±1.87
12	25.58±1.93	26.50±1.51	26.80±2.25	26.50±1.65
13	26.26±1.98	26.50±1.90	27.40±2.37	26.90±2.03

Data: Mean±standard deviation, TED: Therapeutically equivalent dose, *The mean difference is significant at the 0.05 level

Table 4: Organ weight record of males (in gm)

Organ	Control group *n*=10	TED×10 (78 mg/kg) *n*=10	TED×5 (39 mg/kg) *n*=10	TED (7.8 mg/kg) *n*=8
Liver	1.52±0.25	1.60±0.21	1.33±0.28	1.45±0.26
Brain	0.42±0.02	0.43±0.02	0.39±0.04	0.41±0.02
Thymus	0.04±0.02	0.03±0.02	0.03±0.01	0.04±0.03
Heart	0.20±0.04	0.21±0.03	0.18±0.03	0.19±0.02
Kidneys	0.45±0.04	0.50±0.04	0.46±0.07	0.44±0.06
Spleen	0.08±0.02	0.08±0.03	0.06±0.02	0.08±0.02
Testis	0.19±0.02	0.20±0.04	0.17±0.03	0.16±0.04
Epididymis	0.08±0.01	0.10±0.02	0.09±0.04	0.09±0.02
Adrenals	0.005±0.003	0.01±0.01	0.00±0.00	0.01±0.00

Data: Mean±standard deviation, TED: Therapeutically equivalent dose

Table 5: Organ weight record of females (in gm)

Organ	Control group *n*=10	TED×10 (78 mg/kg) *n*=10	TED×5 (39 mg/kg) *n*=10	TED (7.8 mg/kg) *n*=10
Liver	1.23±0.32	1.20±0.15	1.21±0.22	1.23±0.17
Brain	0.45±0.02	0.43±0.05	0.43±0.02	0.46±0.02
Thymus	0.04±0.02	0.04±0.02	0.05±0.02	0.04±0.02
Heart	0.15±0.02	0.15±0.02	0.16±0.02	0.15±0.01
Kidneys	0.31±0.04	0.30±0.02	0.33±0.03	0.33±0.03
Spleen	0.09±0.05	0.09±0.04	0.07±0.02	0.08±0.02
Ovaries	0.05±0.05	0.05±0.04	0.04±0.01	0.04±0.01
Uterus with cervix	0.12±0.08	0.15±0.07	0.17±0.05	0.16±0.05
Adrenals	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00

Data: Mean±standard deviation, TED: Therapeutically equivalent dose

Table 6: Hematological parameters of males

Parameter	Control group *n*=10	TED×10 (78 mg/kg) *n*=10	TED×5 (39 mg/kg) *n*=10	TED (7.8 mg/kg) *n*=8
Neutrophills	15.70±1.252	17.30±1.767	16.30±1.337	13.00±7.040
Eosinophills	01.50±0.527	01.40±0.516	01.20±0.632	00.90±0.738
Lymphocytes	75.30±2.669	74.40±2.541	74.40±2.757	60.20±31.752
Monocytes	07.30±1.494	07.00±1.764	07.30±1.636	05.30±3.268
Hemoglobin %	11.89±6.14	13.08±4.97	14.25±6.33	12.18±3.92

Data: Mean±standard deviation, TED: Therapeutically equivalent dose

Table 7: Hematological parameters of females

Parameter	Control group *n*=10	TED×10 (78 mg/kg) *n*=10	TED×5 (39 mg/kg) *n*=10	TED (7.8 mg/kg) *n*=10
Neutrophills	15.50±1.080	15.40±1.265	14.70±1.337	16.00±1.633
Eosinophills	01.20±0.422	01.10±0.568	01.30±0.483	00.90±0.316
Lymphocytes	74.80±2.700	74.50±2.677	76.40±3.204	74.40±2.716
Monocytes	06.30±2.214	07.80±2.394	07.10±2.234	07.40±1.955
Hemoglobin %	14.72±5.00	13.63±6.63	12.92±6.42	16.34±5.69

Data: Mean±standard deviation, TED: Therapeutically equivalent dose

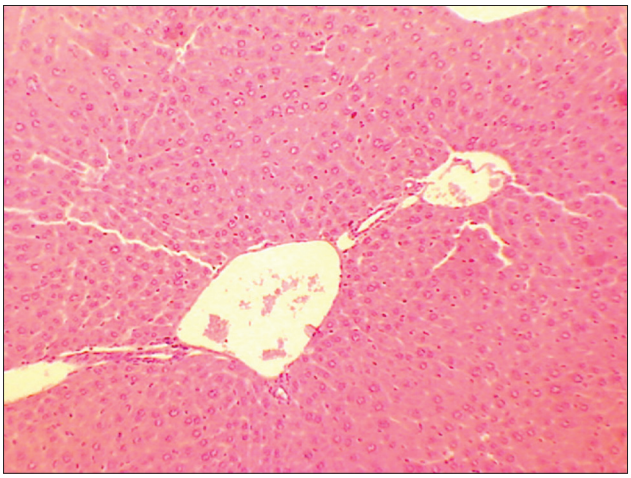

Figure 1: Control liver

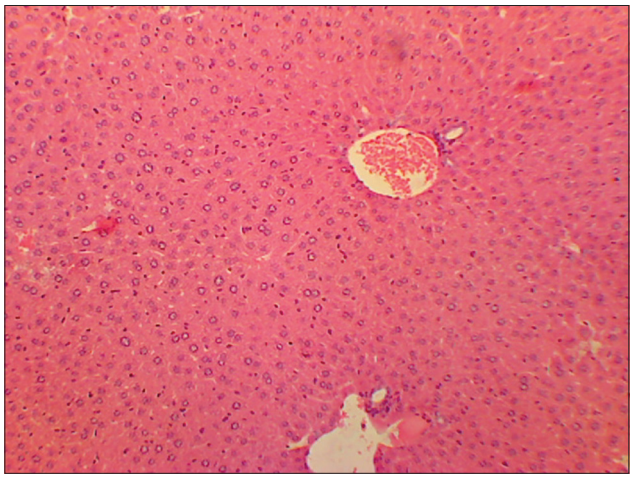

Figure 2: High dose (TED ×10) liver

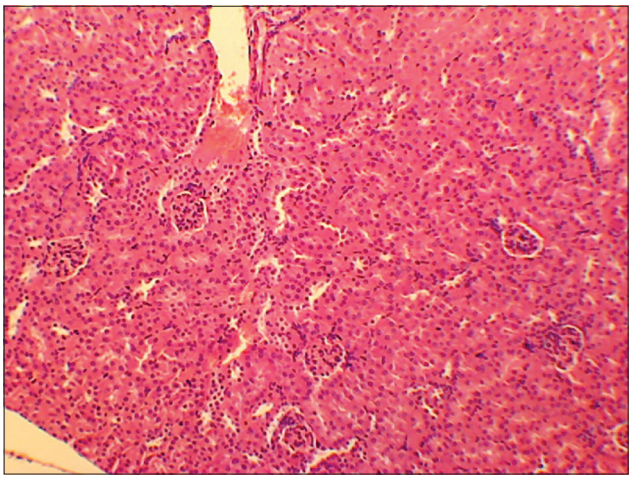

Figure 3: Control kidney

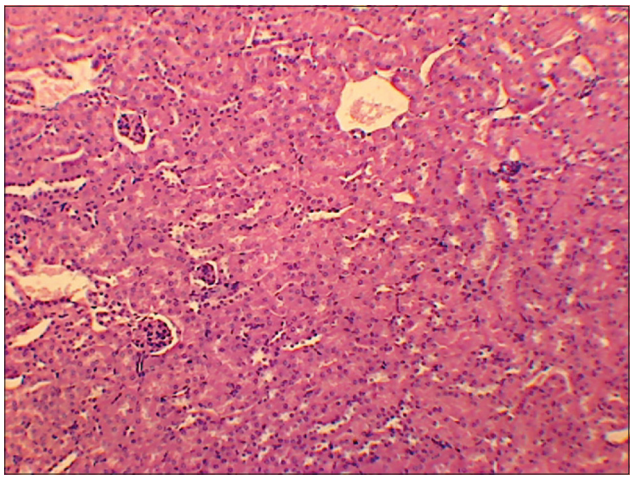

Figure 4: High dose (TED ×10) kidney

sacrificed on 91st day of the study, and were subjected to a detailed gross pathological examination. The tissues were weighed and collected in 10% neutral buffered formalin. Testes were collected in modified Davidson's fluid and subsequently transferred to 10% neutral buffered formalin after 24 hours. Organs from Control group and High Dose group were processed as per Registry of Industrial Toxicology Animal-data (RITA) guidelines[7] and subjected to histopathological evaluation. In-life phase observations were recorded and analyzed statistically by Student's *t* test followed by analysis of variance (ANOVA).[8]

Results

No abnormality in clinical signs was detected across all the groups throughout the study and during neurological examination except excitation and subsequent death on next day of one male in therapeutic dose group in 8th week. Another male from therapeutic dose group was found dead in 11th week of the study. However, gross pathological examination revealed no abnormal changes. Except these two, no mortality was found in any of the study groups. No treatment related effect on body weights [Tables 2 and 3], feed consumption, water

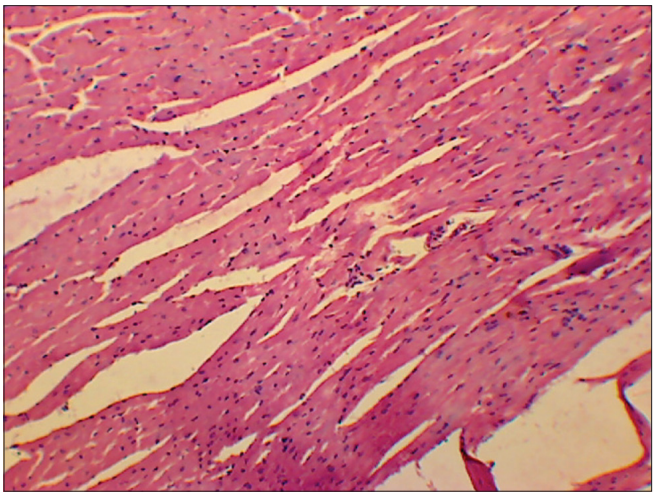

Figure 5: Control heart

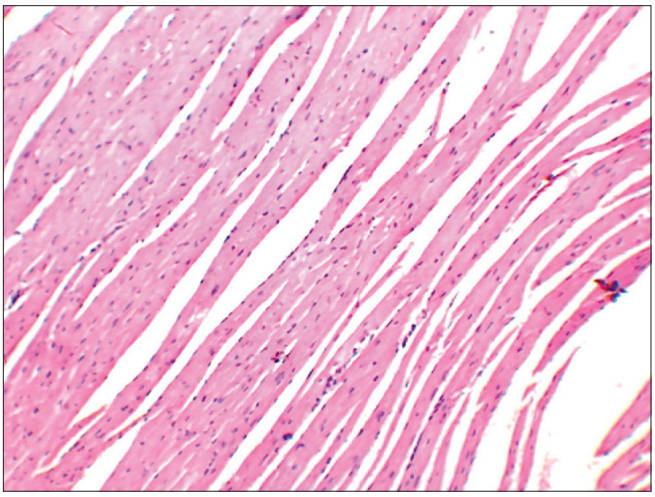

Figure 6: High dose (TED ×10) heart

consumption, fecal consistency, organ weights [Tables 4 and 5], and hematological parameters [Tables 6 and 7] were observed on comparison to control group. No treatment-related adverse effect was recorded upon detailed histopathological evaluation [Figures 1-6].

Discussion

Trivanga Bhasma contains heavy metals lead (31.67%), tin (38.36%), and zinc (17.1%) and is prepared in compliance with Ayurvedic classical literature.[2] However, no treatment-related effect was observed up to 10 times therapeutic dose levels. Present study indicates 78 mg/kg body weight as NOEL for *Trivanga Bhasma* in Swiss albino mice. Body weight change is an important index of assessment of toxicity.[4] In this study; the test drug even at the highest dose level studied showed normal weight gain. This indicates the drug has not caused any adverse effect on normal physiological functions of the body. Histopathological evaluation also indicated normal cyto-architecture and hence, it is concluded that *Trivanga Bhasma* is safe at dose rate of 78 mg/kg.

The dose selected in this study was extrapolation of the human therapeutic dose, which was found to be safe. The dose conversion factor was based on surface area. It is suggested to carry out a toxicity study at higher doses so as to establish target organ of toxicity. Mice were selected as biological system because of its easy availability, small size, and large background data. However, it is difficult to study all desired parameters in Mice owing to its small size and less blood volume. It is suggested to carry out study in different biological system for example, rats.

Conclusion

It is concluded that *Trivanga Bhasma* is generally well tolerated at dose rate of 78 mg/kg (TED × 10) in mice.

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हिन्दी सारांश

त्रिवंग भस्म को ९० दिनों तक देने से स्विस अल्बिनो चूहों में होने वाली विषाक्तता का अध्ययन

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त्रिवंग भस्म भारी धातुओं जैसे जस्ता, सीसा और टिन से बना होता है । इस भस्म का स्विस अल्बिनो चूहों में नोएल (NOEL-No Observed Effect Level) अनुमानित करने हेतु विषाक्तता अध्ययन किया गया । कुल ८० चूहों को (४० नर तथा ४० मादा) जिनका औसत वजन २८–३० ग्राम था, चार वर्गों (प्रथम, द्वितीय, तृतीय, चतुर्थ) में समान रूप से विभाजित किया गया । नियंत्रित (प्रथम) वर्ग के चूहों को शहद–पानी का मिश्रण (२:३ अनुपात) में दिया गया । द्वितीय, तृतीय तथा चतुर्थ वर्ग के चूहों को त्रिवंग भस्म क्रमशः ७.८, ३९.५ और ७८ मिलीग्राम/कि.ग्रा. भार के हिसाब से ९० दिनों के लिए निरन्तर दिया गया । भस्म के प्रभाव के आंकलन करने हेतु शरीर का भार, फ़ीड और पानी का सेवन, तथा हिमाटोलाजिकल और हिस्टोपैथोलोजिकल मानकों का निरीक्षण किया गया । अध्ययन के अंत में सभी प्राणियों को मानवीय पद्धति से मृत करके उनकी नेक्रोप्सी की गई तथा ग्रॉस पॅथॉलॉजी निरीक्षण किया गया । नियंत्रित वर्ग और द्वितीय वर्ग (उच्चतम मात्रा दर वर्ग) के प्राणियों का हिस्टोपैथोलोजिकल मूल्यांकन किया गया । किसी भी वर्ग के प्राणियों ने कोई भी महत्वपूर्ण नैदानिक लक्षण अथवा रोग लक्षण नहीं दिखाए । नियंत्रित वर्ग और द्वितीय वर्ग के प्राणियों के हिस्टोपैथोलोजिकल मूल्यांकन में कोई भी प्रमुख परिवर्तन नहीं पाया गया । इसलिए, ७८ मिलीग्राम/कि.ग्रा. शरीर भार की मात्रा दर को नोएल के रूप में स्थापित किया गया । त्रिवंग भस्म को उपरोक्त मात्राओं में स्विस अल्बिनों चूहों में सुरक्षित पाया गया । त्रिवंग भस्म का अधिक उच्च मात्रा दर में एक अलग प्रजाति में विषाक्तता अध्ययन किया जाये, ताकि भस्म की विषाक्तता और लक्ष्य अंग को स्थापित किया जा सके ।