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Chronic Toxicity Study For Tamra Bhasma (A Generic Ayurvedic Mineral Formulation) in Laboratory Animals

M.K. Vahalia*, K.S. Thakur S. Nadkarni, V.D. Sangle

Shree Dhootapapeshwar Ayurvedic Research Foundation, Arogya Mandir, Tilak Road, Panvel-410206, India

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Abstract

Tamra Bhasma – a generic Ayurvedic mineral formulation was studied for its toxicity in laboratory animals. Chronic toxicity was conducted in albino rats (wistar strain). In this study Tamra Bhasma was administered orally, daily to different groups of albino rats in TD (Tamra Bhasma) and 2 TD (Tamra Bhasma 2 x Therapeutic Doses) doses for 3 months. Tamra Bhasma was found to be relatively safe at these dose levels. There was no mortality. No significant behavioural changes were noted in any of the group studied. No major alterations were observed in haematology, serum biochemical, necropsy and histopathology at the administered dose level. The overall chronic toxicity study data indicates that the test substance at its TD and 2TD level is very well tolerated since no toxicity symptoms of serious cause could be observed in any of the parameters analysed.

Key Words: Tamra, Chronic Toxicity, Ayurvedic formulation

Introduction

Bhasma has its unique place in Ayurvedic Therapeutics. Bhasmas are basically made from metals and minerals [Dhatus and Khanij Dravya]. The process of Bhasmikaran is used to transform Dhatus and Khanij into Bhasmas. Synthesis of Tamra bhasma involves treating metallic copper with Herbal juices and then repeated calcinations in presence of air in controlled environment to a form traditionally known as 'bhasma' [1]. Bhasma- an integral part of Ayurveda describes about using metals & minerals for chronic disorders in various combinations, dosages forms & at various levels of purities. Tamra Bhasma which is Ushna, Teekshna & Srotoshodhak acts on liver. It induces secretion, circulation of Yakrut Pitta (Bile) properly. It is effective in reducing any inflammation or edema with its 'Lekhan' property. Deficiency of copper in the body causes weight loss, bone disorders, microcytic hypochromic anaemia, hypopigmentation, graying of hair and demyelination of nerves etc [2]. Safety and efficacy depends upon the methodology adopted for the preparation and any deviation from the classical preparation method will not yield desired results. Tamra Bhasma has been in use since long for many types of liver and gastro intestinal tract disorders [3]. The present study was aimed at screening the drug for its safety/toxicity studies in animal model.

Materials and Methods

Tamra Bhasma used for study was manufactured by Shree Dhootapapeshwar Ltd., Panvel, Navi Mumbai, India. The drug was prepared as per classical literature [1]. The test substance was dispensed in plastic bags labelled properly and was dispatched to an approved Animal House facility no.136/1999/CPCSEA, Biomedical Services Unit, Shree Dhootapapeshwar Ayurvedic Research Foundation, for Toxicity studies without interruption of the custody chain. Test substances were coded to blind the identity of the drug.

A total of 18 Wistar strain albino rats of either sex with average body weight 150 to 250 g were obtained from in-house breeding facility of SDARF. They were maintained in ideal laboratory conditions in the prevailing ambient temperature (22±02ºC) and humidity (65±05%). The experiments were carried out in accordance with the guidelines of the Institute's Animal Ethics Committee after obtaining its permission. Dose for experimentation was calculated with reference to suggested human doses i.e.60 - 125 mg. For toxicity study, higher dose was selected to ascertain its safety potential. The animals were divided into three groups (Group-I, II and III) containing six rats in each. Group-I rats received Tamra Bhasma at a dose equivalent to human therapeutic dose (TD- 2.25 mg/200g), Group-II rats received 2 times the dose equivalent to human therapeutic dose (2TD- 4.5 mg/200 g), p.o. for 90 days. Group-III was kept as control. Test drug was given as suspension in distilled water by gavage with a control group receiving the vehicle (distilled water). Toxicity was evaluated by observing the test drug effect on body weight and gross behaviour changes, gross and histological appearance of vital organs (brain, heart, lungs, liver, spleen, kidney and adrenals). Further effect on different biochemical variables like total cholesterol, triglycerides, urea nitrogen, creatinine, alkaline phosphatase (ALP), total protein, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine phosphokinase were estimated with the help of an autoanalyzer (ERBA CHEM-5, Trans Asia Biomedicals Ltd).

Electrolytes Sodium (Na) and Potassium (K) were analysed using a flame photometer. The haematological parameters like total white blood cell count (WBC), Total red blood cell count (RBC), packed cell volume (PCV), Absolute Erythrocyte indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and haemoglobin were analysed using "Erba hemolab-8 Hematology Analyser"(US Tech Inc, Washington, MD, USA). 0.08ml blood was drawn from supra-orbital plexus of animals and mixed with 0.02ml of EDTA (33.33 mg/ml). The sample was fed to auto-analyzer and readings were recorded. Differential WBC counts were estimated manually.

The rats were sacrificed by exsanguinations under carbon dioxide anaesthesia. Complete necropsies were carried out on all animals. Tissues were preserved in 10% neutral buffered formalin. The tissues examined were: brain, heart, lungs, liver, spleen, kidney, adrenals. Tissues were subjected to microscopic examination in this study and were embedded in paraffin wax, sectioned at five micrometers and stained with haematoxylin and eosin.Organs like Kidneys, liver, adrenals, heart, brain, lungs and spleen from all animals were dissected free of fat and weighed as soon as possible to avoid drying. Values of these organs as percent of necropsy body weights were also estimated (relative organ weights).

Statistical method

The data were statistically analysed by one way analysis of variance. Data are expressed as Mean ±S.D. from 6 rats. P value of <0.05 was considered to be statistically significant [4]. Specific differences between treatments were examined by the Sidak-Bonferroni multiple comparison *post hoc* test. Calculations were made using SPSS (SPSS Inc.1989).

Results and Discussion
Hematology

Values of haemoglobin, hematocrit, total and differential leukocyte counts, total erythrocyte counts and erythrocyte indices of male and female rats from groups treated with Tamra Bhasma (TD) & Tamra Bhasma 2 x Therapeutic Doses (2TD) were found to be comparable with those of the control group rats (Table 1).

Clinical Chemistry

The values of total protein, aspartate aminotransferase, alkaline phosphatase, glucose, urea nitrogen, creatinine, creatinine phosphokinase, cholesterol, lactate dehydrogenase, triglycerides, sodium and potassium of rats treated with Tamra Bhasma (TD) and Tamra Bhasma (2TD) were found to be comparable with those of the control group rats. The Alanine aminotransferase (ALT) value of TD rats was significantly lower when compared to control (P< 0.05) but there was no significant difference in ALT value of 2TD group with control group rats (Table 2).

All the thirteen Biochemical parameters except ALT studied in treatment groups were observed to be in consonance with control group. Variation of ALT value occurred as remote incidence only in TD group. Taking these values in to account it may be inferred that the results do not indicate any serious pathological condition.

Organ Weights

The absolute & relative values of lungs from all treatment groups were found to be lowered as compared to control group. Absolute and relative weights of kidneys were found to be increased in all treatment groups as compared to control group but were not statistically significant at P value of 0.05. Significant (P< 0.05) increased relative weights of heart was noted in animals treated with Tamra Bhasma (2TD). The values of absolute and relative weights of adrenals, brain and spleen of male and female rats from treatment groups were found to be comparable with those of the control group rats (Tables 3 and 4).

Gross Pathology

Gross pathological findings noted in rats at necropsy in this study included reddening of lungs and small size adrenal in TD and Control group. However, these changes occurred as isolated incidences and did not appear to be treatment related (Table 5).Since no degenerative changes could be observed in these organs even in histological examinations, toxicity can be ruled out. This happened as isolated incidences in one of the TD group and Control group, thus did not appear to be treatment related.

Histopathology

The microscopic examination of tissues revealed some incidental findings, such as acute congestion in liver, kidneys and lungs, round cell infiltration in liver and kidneys in the treated and control group rats and was considered unrelated to exposure to the test article (Table 6). These observations were incidental and were considered unrelated to exposure to the test substance. Further the microscopic examination of Adrenals, Spleen, Brain and Heart showed normal cell structure without any sign of degeneration.

Body Weight

Body weight gain was observed in control as well as test drug administered groups. The body weight gain was slightly higher in 2TD group (Table 7). The observed difference was found to be statistically non-significant. No significant behavioural changes were observed in any of the group studied. Body weight change is an important index for assessment of toxicity During the study none of the 18 animals died. This clearly indicates that it has no serious organ degeneration or body function affecting potential. Besides this the test drug did not produce any significant changes in the cytoarchitecture of any organ studied at various dose levels.

The overall chronic toxicity study data indicates that the test substance at its TD and 2TD level is very well tolerated since no toxicity symptoms of serious cause could be observed in any of the parameters analysed. The cause for concern is the acute congestion and round cell infiltration reported in histopathological examination of tissues (liver, kidneys). The fact that these effects observed were not dose dependent in nature and occurred in control group also, the result rules out serious toxicity potential especially at the dose levels employed in therapeutic indications.

The safety of the drug Tamra bhasma reported may be attributed to *bhasmikarana* process which converts the metal into its specially desired chemical compound which eliminates the toxicity of the metal and has the necessary medicinal benefits [5-6].

Another investigation carried out to study physico-chemical changes occurring during bhasma manufacturing indicate that the complete process of bhasma preparation (shodhan and bhasmikaran) leads to the removal of free copper from the system. It begins the synthesis of copper sulphides along with some new metallic complexes, which are yet to be characterized [7].

Table 1: Summary of Heamatology Data

Group	Hb	PCV	Total RBC	RBC Indices			Total WBC	Differential WBC (%)			
				MCH	MCV	MCHC		N	L	E	M
	(g/dl)	(%)	(x10⁶/cmm)	(pg)	(fl)	(g/dl)	(x10³/cmm)				
Tamra Bhasma (TD)	12.15 ±0.49	37.18± 1.25	7.09 ±0.55	17.18 ±0.95	52.63 ±2.99	32.70 ±1.08	8.50 ±1.54	27.17 ±4.49	70.33 ±4.18	0.67 ±0.82	1.83 ±0.75
Tamra Bhasma (2TD)	10.60 ±0.49	37.08 ±1.10	6.86 ±0.43	15.48 ±1.11	54.15 ±2.71	28.62 ±1.93	9.15 ±1.12	26.50 ±5.09	69.67 ±4.32	1.33±1.03	2.50 ±1.05
Control	11.60 ±0.46	37.90 ±1.19	7.13 ±0.65	16.35 ±1.44	53.47 ±4.50	30.62 ±0.75	8.50 ±1.07	25.00 ±4.05	71.00 ±4.56	1.50 ±0.55	2.50 ±1.38

Values are expressed as mean ±standard deviation (n=6)

Table 2: Summary of Clinical Chemistry Data

Group	Total Protein (g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Glucose (mg/dl)	UN (mg/dl)	Cholest (mg/dl)	Creatinine (mg/dl)
Tamra Bhasma (TD)	6.16 ±0.19	53.04* ±5.87	120.42±4.90	118.10±12.9 5	84.38 ±2.72	16.37 ±0.83	68.40 ±4.39	1.01 ± 0.07
Tamra Bhasma (2TD)	6.29 ±0.27	61.42 ±7.61	127.02 ±19.67	127.02 ±17.86	82.23 ±4.28	17.52 ±1.49	73.03 ±9.31	0.94 ±0.05
Control	6.29 ±0.26	65.16 ±4.01	135.75 ±24.24	133.78 ±22.61	86.30 ±7.95	15.84 ±1.60	73.68 ±9.58	0.95 ±0.10

Values are expressed as mean ±standard deviation (n=6)
* Significantly different from control (P< 0.05)

Table 2: (Contd.)

Group	Lactate dehydrogenase (IU/L)	Creatinine Phosphokinase (IU/L)	Triglycerides (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)
Tamra Bhasma (TD)	541.90 ±125.78	175.85 ±29.87	65.98 ±11.80	147.33 ±9.14	5.25 ±0.96
Tamra Bhasma (2TD)	516.82 ±106.66	193.82 ±28.48	58.42 ±7.77	144.83 ±7.41	5.75 ±0.90
Control	642.65 ±62.36	191.97 ±18.13	61.78 ±12.07	144.33 ±8.02	5.75 ±1.34

Values are expressed as mean ±standard deviation (n=6)

Table 3: Summary of Absolute organ weights (g)

Group	Lungs	Adrenals	Spleen	Brain	Kidneys	Liver	Heart
Tamra Bhasma (TD)	1.62 ±0.38	0.04 ±0.01	0.84* ±0.21	1.85 ±0.06	1.96 ±0.50	9.49 ±2.31	1.02 ±0.29
Tamra Bhasma (2TD)	1.25 ±0.14	0.05 ±0.01	0.74 ±0.10	1.82 ±0.12	1.97 ±0.50	8.96 ±2.15	1.03 ±0.21
Control	1.82 ±0.61	0.05 ±0.01	0.63 ±0.06	1.87 ±0.06	1.70 ±0.44	8.14 ±1.77	0.94 ±0.24

Values are expressed in following sequence: mean, standard deviation (n=6)
* Significantly different from control (P< 0.05)

Table 4: Summary of Relative organ weights (% of Body Weights)

Group	Lungs	Adrenals	Spleen	Brain	Kidneys	Liver	Heart
Tamra Bhasma (TD)	0.54 ±0.06	0.01 ±0.01	0.28 ±0.06	0.63 ±0.12	0.65 ±0.05	3.15 ±0.36	62.74 ±11.44
Tamra Bhasma (2TD)	0.43 ±0.12	0.02 ±0.01	0.25 ±0.08	0.62 ±0.14	0.64 ±0.06	2.93 ±0.29	82.56* ±19.39
Control	0.62 ±0.21	0.02 ±0.01	0.22 ±0.05	0.65 ±0.16	0.57 ±0.09	2.73 ±0.42	54.55 ±14.75

Values are expressed in following sequence: mean, standard deviation (n=6)
* Significantly different from control (P< 0.05)

Table 5: Summary of Necropsy Findings: Incidence

Group	Tamra Bhasma (TD)	Tamra Bhasma (2TD)	Control
No. of Animals Examined / No. of animals in the Group	6 /6	6 /6	6 /6
No abnormality detected	4 / 6	6 /6	4 / 6
Adrenal (right) - small	1 / 6	-	-
Lungs – moderate reddening	1 / 6	-	2 / 6

Table 6: Histopathology
Summary: Fate: Terminal sacrifice: Incidence of microscopic Findings

Findings	No. of rats with finding / total no. of rats treated		
Group	Tamra Bhasma (TD)	Tamra Bhasma (2TD)	Control
No Abnormality Detected	1 / 6	1 / 6	2 / 6
Lungs – acute congestion	3/6	3 / 6	3 / 6
Kidneys – round cells infiltration	1 / 6	-	-
Kidneys – acute congestion	1 / 6	-	-
Liver – round cells infiltration	3 /6	1/6	2/6
- acute congestion	2 / 6	2 / 6	-

Table 7: Body Weights
Average body weights of Animals after Chronic Administration of Tamra Basma

Initial body wt			Final body wt		
Control	TD	2 TD	Control	TD	2 TD
178.33	178.33	178.33	275.83	275.0	280.83
+13.29	+ 11.69	+ 11.69	+ 57.82	+ 53.19	+ 61.43

TD - Therapeutic Dose
2 TD   - 2 x Therapeutic Doses

Conclusions

The test drug is well tolerated since no changes of serious nature could be observed in any of the parameters noted down. This clearly indicates that the formulation has no serious toxicological implications.

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