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Research Article

PRE-CLINICAL TOXICITY STUDY OF TAMRA BHASMA ON ALBINO WISTAR RATS

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ABSTRACT

The use of metals in traditional medicine is very often seen as a matter of concern these days. Metals are processed through various steps like Shodhana, Marana, Amritikaran etc., to convert them into Bhasmas, which are then used as a medicine in Ayurveda for internal consumption. Bhasmas are unique Ayurvedic metallic preparations with herbal juices/fruits widely used for treatment of variety of chronic ailments. One of the extensively used routine Ayurvedic practice is Tamra (Copper) Bhasma. If it is not prepared properly or Shodhana procedure is not done properly, it acts as poison. To indicate its toxic potential, Ashtamahadoshas (eight major ill effects) have been quoted in classics and due emphasis have been given to its Shodhana procedure. In present study, Tamra Bhasma prepared by Rasamarit type under Kupistharasayana method was adopted. The observation was that Tamra Bhasma at dose TED i.e 5.5mg/kg showed non-toxic effect as indicated by its morphological, behavioral, biochemical, hematological and histopathological studies in wistar rats.

Keywords: Shodhana, Tamra Bhasma, Rasamarit, Kupistharasayana.

INTRODUCTION

Ayurveda is the science made up of Ayush (life) i.e. knowledge of life and Veda (knowledge). An Ayurvedic system adopts a holistic approach towards health care by balancing the physical, mental and spiritual functions of the human body[1].

Ayurvedic system of medicine the oldest system among all other life sciences originated in India thousands of years ago. Ayurveda enjoyed a big revolution in the form of origin of Rasashastra (Alchemy/Vedic Chemistry) since the 3rd century. It is one of the parts of Ayurveda, which deals with herbo-mineral / metal / non-metal preparations called Bhasma. Rasayana (Immunomodulation and anti-aging property) and Yogavahi (ability to target drugs to the respective site) are characteristics of a properly made herbo-mineral / metal / non-metal preparation. These preparations are also nontoxic, gently absorbable, adaptable and digestible in the body[2].

Use of mercury and several other minerals came in to practice. These metals and mineral have been used after certain difficult processing. The medicaments prepared through such techniques had several advantages over earlier ways of treatments. These have been fast but safe in action and much effective in very small doses. Even today these ayurvedic formulations have very special place in Ayurvedic medical practice and often show miraculous results in difficult situations.

According to this medicinal system, metal based drugs known as 'Bhasma' involve the conversion of a metal into its mixed oxides. During these transformations, the zerovalent metal state gets converted into a form with higher oxidation state and the most important aspect of this synthesis (known traditionally as 'bhasmikarana') is that the toxic nature (i.e. systemic toxicity causing nausea, vomiting, stomach pain, etc.) of the resulting metal oxide is completely destroyed while inducing the medicinal properties into it.

Tamra Bhasma is prepared by method called Rasamarit as mentioned in literature of Rasashastra of Ayurvedic granthas, i.e in presence of mercury, copper wire, gomutra, sulphur, and citrus lemon juice. Some of the metals used in medicine have the potential to produce the adverse effect. Hence during their transmutation to drug, it is essential to evaluate the margin of safety between the dose level that produces therapeutic effect and that produces adverse effect.

With this view in mind the aim of the study was to assess the safety toxicity of Tamra Bhasma prepared by Kupistharasayana method in albino rats.

MATERIALS AND METHODS

Animals

Wistar albino rats of either sex weighing between 200-250 gm. were procured from Bombay Veterinary College. They were maintained under standard laboratory conditions of 25 ±1°C, relative humidity of 45-55% and photo period (12 h dark/12 h light). Commercial pellet diet (Jay Trading co. Panchavati, Nashik, India) and purified distilled water were provided *ad libitum*. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi,

India, and approved by the Institutional Animal Ethical Committee (MGV/PC/CPCSEA/XXX11/02/2016/02)

Drugs and Chemicals

Tamra Bhasma (Sample gifted by Ayurvedic Sanshodhan Vibhag), Gum Acacia (Analytical grade), all chemicals and reagents for antioxidant studies were of analytical grades and purchased from Sigma Chemicals (St. Louis, MO, USA). Biochemical Kits for Creatinine Kinase and CK-MB Isoenzyme(CK-MB), Lactate Dehydrogenase (LDH), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Serum Triglyceride Kit, Lipid Profile Kit, Alkaline Phosphatase (ALP), Total Protein, Bilirubin, Serum Uric acid, Serum Creatinine, were purchased from Sweety Surgicals, Nashik.

Experimental Design

Animals were divided into 4 groups of 8 animals each and treated for 60 days.

Group I (Control) received 5% Gum Acacia (10ml/kg, p.o) daily once for 60 days.
Group II (Therapeutic dose- TED) received Tamra Bhasma (5.5 mg/kg, p.o) daily once for 60 days.
Group III (Intermediate dose- TED X5) received Tamra bhasma (27.5 mg/kg, p.o) daily once for 60 days.
Group IV (Fatal dose-TED X 10) received Tamra Bhasma (55 mg/kg, p.o) daily once for 60 days.

The change in body weight was recorded every 10 days. At the end of the treatment schedule, the animals were subjected for behavioral studies, sacrificed and subjected to relative organ weight, antioxidant, biochemical, haematological and histopathological studies.

Percentage Change in body weight, changes in behavioral phenotype and relative organ weight

Body weight of each animal was determined before treatment, and at every 10^{th} day (up to 60 days) and before sacrifice. Behavioral changes were also studied by means of open field and elevated plus maze test[3].Percent change in body weight was recorded at the end of treatment schedule. Brain, Heart, Liver, Lungs and Kidney tissue of each animal were dissected out and weighed to calculate relative organ weight.

Preparation of serum and tissue homogenate

The animals were sacrificed 60 days after the treatment of bhasma. Blood samples were withdrawn by cardiac puncture and collected in EDTA tubes (2ml) and Clot activator tube (4ml). They were subjected to haematological studies from Manas Pathology. Serum was separated by centrifugation at 3000 rpm for 10 min. The serum samples were maintained at (-20 °C) to be used for measurement of various biochemical markers for organ function. Known amount of tissue (Brain, Heart, Liver, Lung& Kidney) was weighed and homogenized in ice cold 0.1 M Tris-HCl buffer for estimation of lipid peroxidation activity (LPO) and Reduced glutathione (RGSH).

Antioxidant Parameters

Lipid Peroxidation (LPO)

0.1 ml of post mitochondrial supernatant portion was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCL reagent and placed in water bath for 15 min, cooled and centrifuged at R.T. for 10 min at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535nm [4].

Reduced glutathione (RGSH)

1.0 ml of post mitochondrial supernatant portion was added to 1ml of 10% TCA and centrifuged. 1.0 ml of supernatant was treated with 0.5ml of Elman's reagent and 3 ml of phosphate buffer (pH 8.0). The color developed was measured at 412nm[5].

Biochemical Assays: 4ml of blood were collected from cardiac puncture in clot activator tubes, to separate serum to be used for assessment of CK-MB[6], LDH[7], AST[8], ALT[8], Lipid Profile[9], ALP[10], Total Protein[10], Serum Bilirubin[11], uric acid[12] and Creatinine[13]using standard biochemical kits (Sweety Surgicals).

Haematological studies

1 ml of blood samples were collected in EDTA tube and studied for various hematological tests like Hb count, RBC count, TLC count, platelet count and blood glucose at Manas Pathology, Manas Hospital, Nashik.

Histopathological examination

Soon after sacrifice of the animal the brain, heart, lungs, liver and kidney tissues were removed immediately and fixed in 10% formalin solution and sent for histopathological examination. These tissues were embedded in paraffin wax, cut into fine thin sections of 3-5µm thickness and were stained with hematoxylin-eosin and observed for histological changes by taking photograph under 40 X magnification[14].

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was done using one-way analysis of variance, followed by Dunnett's multiple comparison tests. $p<0.05$ was considered significant.

RESULTS

Percent change in body weight

There was a significant ($P<0.05$) decrease in percent body weight of rats given Tamra Bhasma (TED X 5 and TED X10) as compared to Control group **[Figure 1]**.

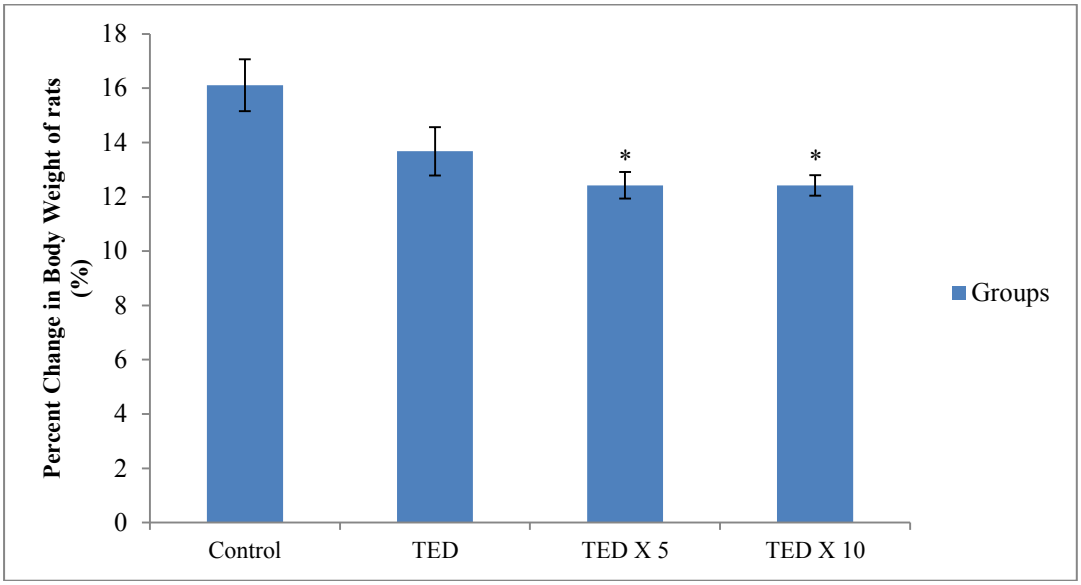

Figure 1: Effect of Tamra Bhasma (TED, TED X 5, TED X 10), on % Change in body weight in rats.
N=8, All data were subjected to ANOVA followed by Dunnett's test, the observations are mean ±SEM. *p<0.05 as compared to Control group. Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose(27.5mg/kg,p.o), TED X 10= Lethal dose(55mg/kg, p.o).

Behavioral Phenotypes

Open field test and Elevated plus maze test: The behavior of animal in open field test and elevated plus maze test is shown in [Table 1]. There were no significant changes (P<0.05) in any of the parameters as compared to control group.

Table 1: Effect of Tamra Bhasma (TED, TED X 5, TED X 10) in Open field and Elevated plus maze test

Treatment Group. (mg/kg)	Open field apparatus No of squares traversed	Elevated plus maze Transfer Latency (sec)
Control 5% Gum Acaica (10ml/kg)	34.75 ± 2.67	17 ± 1.5
TED (5.5 mg/kg)	30.62 ± 2.86	20.12 ± 1.76
TED X 5 (27.5 mg/kg)	28.38 ± 2.07	20.25 ± 2.21
TED X 10 (55 mg/kg)	28.88 ± 2.67	19.88 ± 1.92

N=8, all data were subjected to ANOVA followed by Dunnett's test. The observations are mean±SEM. * p<0.05 as compared to control treated group.
Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose(27.5mg/kg,p.o), TED X 10= Lethal dose(55mg/kg,p.o).

Relative organ weight (Brain, Heart, Liver, Lung and Kidney)

There was a significant (P<0.05) decrease in relative organ weight of Brain in rats given Tamra Bhasma (TED X 5 and TED X10) and significant (P<0.05) decrease in relative organ weight of heart in rats given Tamra Bhasma (TED, TED X 5 and TED X10) as compared to Control group [Table 2].

Table 2: Effect of Tamra Bhasma in relative organ weight of wistar rats

Organs	Control	TED	TED X 5	TED X 10
Brain	1.73 ± 0.07	1.58 ± 0.08	1.28 ± 0.03*	1.34 ± 0.02*
Heart	0.74± 0.02	0.61 ±0.04	0.55 ± 0.04*	0.44 ±0.01*
Liver	9.03 ± 0.42	7.74 ±0.64	7.37 ± 0.64	7.87 ± 0.39
Lung	1.28 ± 0.02	1.34 ± 0.10	1.34 ±0.08	1.43 ± 0.11
Kidney	1.87 ± 0.26	1.27 ± 0.07	1.27 ± 0.07	1.41 ± 0.07

N=4, All data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. * p<0.05 as compared to control group. Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose(27.5mg/kg,p.o), TED X 10= Lethal dose(55mg/kg, p.o).

Antioxidant Studies

In-vivo antioxidant studies like Lipid Peroxidation (LPO), Reduced Glutathione (RGSH) levels were estimated by performing various standard procedures and the results obtained are illustrated below.

Antioxidant study in Brain Tissue of Wistar rats

Tissue LPO activity showed a statistically significant ($p<0.05$) decrease in Tamra Bhasma TED X 5 and TED X 10 treated group as compared to the Control group [Table 3]. Tissue RGSH activity showed a statistically significant ($p<0.05$) decrease in Tamra Bhasma TED X 5 and TED X 10 treated group as compared to the Control group. [Table 4]

Table 3: Effect of Tamra Bhasma on anti-oxidant status (LPO) in various tissues of Wistar rats.

Treatment Group (mg/kg)	LPO (n Moles/mg of wet tissue)				
	Brain	Heart	Liver	Lungs	Kidney
Control 5% Gum Acacia (10ml/kg)	112.0 ± 3.83	77.64 ±0.19	184.0 ± 0.76	96.01 ± 3.22	141.1 ± 13.8
TED (5.5 mg/kg)	105.3 ± 3.68	79.75 ± 1.15	145.9 ± 5.66*	90.49 ± 1.53	125.4 ± 1.92
TED X 5 (27.5 mg/kg)	72.66 ± 4.41*	69.02 ± 3.06	110.8 ± 1.15*	31.06 ± 0.38*	80.13 ± 1.15*
TED X 10 (55 mg/kg)	49.46 ± 0.77*	51 ± 0.38*	74.57 ± 2.11*	16.49 ± 0.76*	71.12 ± 1.34*

N=4, All data were subjected to ANOVA followed by Dunnett's test, the observations are mean ±SEM. * $p<0.05$ as compared to Control group. Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose(27.5mg/kg,p.o), TED X 10= Lethal dose(55mg/kg, p.o), LPO= Lipid Peroxidation

Table 4: Effect of Tamra Bhasma on anti-oxidant status (RGSH) in various tissues of Wistar rats.

Treatment Group (mg/kg)	RGSH (n Moles/mg of wet tissue)				
	Brain	Heart	Liver	Lungs	Kidney
Control 5% Gum Acacia (10ml/kg)	757 ± 20	1235 ± 75	1467 ± 110	1212 ± 20	527 ± 10
TED (5.5 mg/kg)	757 ± 20	1177 ± 15	1492 ± 20	1110 ± 25*	399.5 ± 17.5*
TED X 5 (27.5 mg/kg)	329.5 ± 2.5*	362 ± 5*	697 ± 5*	347 ± 35*	219.5 ± 12.5*
TED X 10 (55 mg/kg)	284.5 ± 7.5*	209. ± 7.5*	189.5 ± 2.5*	162 ± 10*	54 ± 18*

N=4, All data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. * $p<0.05$ as compared to Control group. Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose(27.5mg/kg,p.o), TED X 10= Lethal dose(55mg/kg,p.o), RGSH= Reduced Glutathione

Biochemical Assays
Assessment of Biomarkers

There was a non-significant ($P<0.05$) decrease in CK-MB activity in rats given Tamra Bhasma at different dose as compared to Control group **[Table 5]**.

Table 5: Effect of Tamra Bhasmachanges in levels of bio markers (for brain, lungs and kidney) in Wistar rats.

Treatment Groups (mg/kg)	CK-MB (U/L)	S.Creatinine mg/dl	S.Uric Acid mg/dl
Control 5% Gum Acacia (10ml/kg)	62.75 ± 5.25	0.95 ± 0.15	5 ± 0.1
TED (5.5 mg/kg)	56.6 ± 3.1	1.1 ± 0.1	5.05 ± 0.25
TED X 5 (27.5 mg/kg)	53.15 ± 2.65	0.95 ± 0.15	4.35 ± 0.45
TED X 10 (55 mg/kg)	40.95 ± 0.75	1.35 ± 0.05	5 ± 0.2

N=5, all data were subjected to ANOVA followed by Dunnet's test. The observations are mean ±SEM. * $p<0.05$ as compared to Control group. Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose(27.5mg/kg,p.o), TED X 10= Lethal dose(55mg/kg, p.o), CK-MB= Creatinine Kinase and CK-MB Isoenzyme

There was a significant ($P<0.05$) decrease in ALP level of Tamra Bhasma TED X 5 and TED X 10 treated group as compared to the Control group **[Table 6]**.

Table 6: Effect of Tamra Bhasma changes in biomarkers (for liver) in Wistar rats

Treatment Groups (mg/kg)	ALP U/L	S. Bilirubin U/L			Total Protein U/L		
		Total	Direct	Indirect	Total	Albumin	Globulin
Control 5%Gum Acacia (10ml/kg)	136.6 ± 1.9	1.05 ± 0.05	0.45 ± 0.05	0.6 ± 0.1	6.85 ± 0.04	3.45 ± 0.05	3.4 ± 0.05
TED (5.5 mg/kg)	132.6 ± 2.6	1.1 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	6.95 ± 0.05	3.55 ± 0.05	2.9 ± 0.5
TED X 5 (27.5 mg/kg)	123.2 ± 1.6*	0.1 ± 0.05	0.45 ± 0.05	0.55 ± 0.05	6.95 ± 0.05	3.55 ± 0.05	3.35 ± 0.05
TED X 10 (55 mg/kg)	120 ± 2.45*	1.1 ± 0.1	0.45 ± 0.05	0.65 ± 0.05	6.9 ± 0.2	3.55 ± 0.15	3.3 ± 0.05

N=5, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean ±SEM. * p<0.05 as compared to Control group. Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose (27.5mg/kg, p.o), TED X 10= Lethal dose (55mg/kg, p.o), ALP = Alkaline Phosphatase.

There was a significant (P<0.05) decrease in LDH activity in rats given Tamra Bhasma at dose TED as compared control group [Table 7].

There was significant (p<0.05) increase in AST and ALT activity in rats given Tamra Bhasma at a dose TED, TED X 5 and TED X 10 treated group as compared to the Control group **[Table 7]**.

Table 7: Effect of Tamra Bhasma changes in biomarkers (for heart) in Wistar rats.

Treatment Groups (mg/kg)	LDH U/L	AST U/L	ALT U/L
Control 5% Gum Acacia (10 ml/kg)	3463 ± 25	24.05 ±1.85	25.3 ±1.1
TED (5.5 mg/kg)	3040 ±53*	46 ± 1.85*	47.25 ±1.65*
TED X 5 (27.5 mg/kg)	2998 ±130.5	43.85 ±1.45*	42.9 ±0.1*
TED X 10 (55mg/kg, p.o)	3327 ±37.3	40.25 ±0.2*	40.25 ±0.35*

N=5, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean ±SEM. * p<0.05 as compared to Control group. Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose(27.5mg/kg,p.o), TED X 10= Lethal dose(55mg/kg, p.o), LDH = Lactate Dehydrogenase, AST =Aspartate Amino-transferase, ALT = Alanine Amino-transferase.

Assessment of Hematology

There was a significant (P<0.05) increase in Total Leucocyte count (TLC) level in rats given Tamra Bhasma at dose TED X 5, and TED X 10 as compared control group **[Table 8]**.

Table 8: Effect of Tamra Bhasma changes in Hematology tests of Wistar rats

Treatment Groups (mg/kg)	Blood Glucose	Hb gm/dl	TLC /cmm	RBC gm/dl	PLT 103/cmm
Control 5% Gum acacia (10 ml/kg)	85.5 ± 0.95	12 ± 0.2	1.96 ± 0.65	6.47 ± 0.24	1018 ± 45.5
TED (5.5 mg/kg)	78.85 ± 3.05	10 ± 3.6	6.8 ± 2	6.15 ± 2.19	705.5 ± 306.5
TED X 5 (27.5 mg/kg)	71.8 ± 0.45	11.25 ± 1.35	9.45 ± 0.55*	6.19 ± 0.24	674 ± 217
TED X 10 (55 mg/kg)	70.5 ± 5.5	12.25 ± 0.65	8.2 ± 0.5*	6.92 ± 0.40	868 ± 26

N=5, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. * p<0.05 as compared to Control group. Control = 5% Gum Acacia, TED=Therapeutic effective dose (5.5mg/kg, p.o), TED X 5=Median effective dose(27.5mg/kg,p.o), TED X 10= Lethal dose(55mg/kg, p.o), HGB = Hemoglobin, TLC = Total Leucocyte count, RBC = Red Blood Cells, PLT = Platelets

Histopathological Examination

Histopathology studies of H&E stained brain, heart, liver, lung and kidney sections (40X) of the Tamra bhasma treated groups (TED, TED X 5, TED X 10) are shown in Figure (2-21)

Histopathological examination of Brain

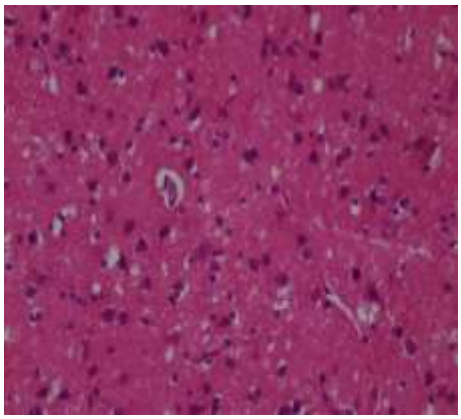

Figure 2: Group I (Control)

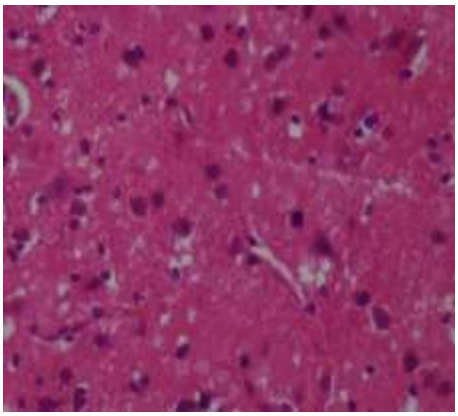

Figure 3: Group II (TED)

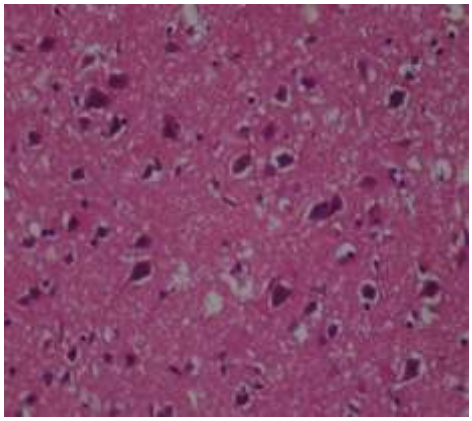

Figure 4: Group III (TED X 5)

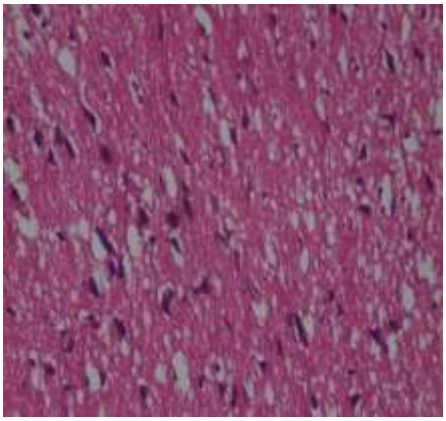

Figure 5: Group IV (TED X 10)

Histopathological studies of H&E stained cerebral tissue (40X) of the control group showing the normal neuronal density. On the contrary Tamra bhasma treated TED X 5, and TED X 10 groups presented signs of vaculation, demyelination, and loss of neurons. (Fig 2-5)

Histopathological examination of Heart

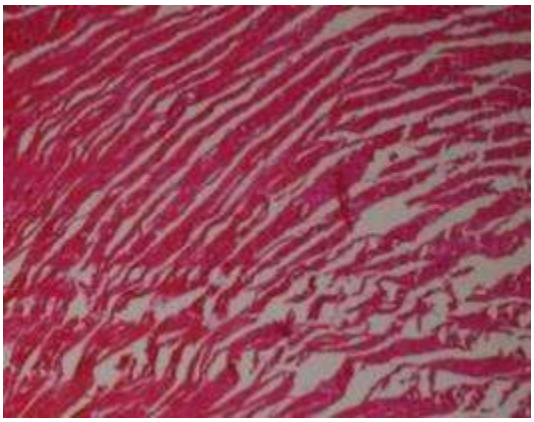

Figure 6: Group I (Control)

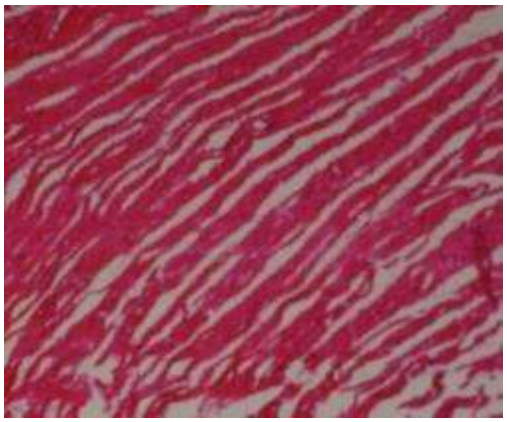

Figure 7: Group II (TED)

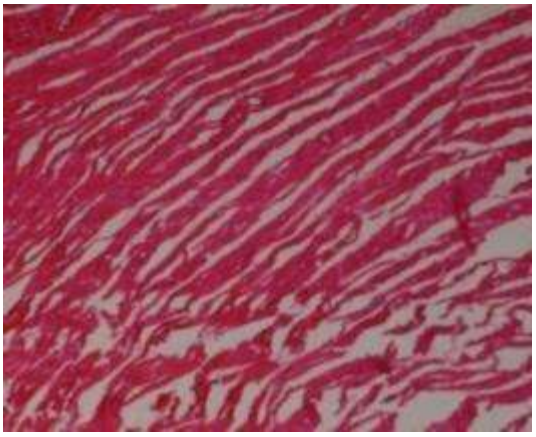

Figure 8: Group III (TED X 5)

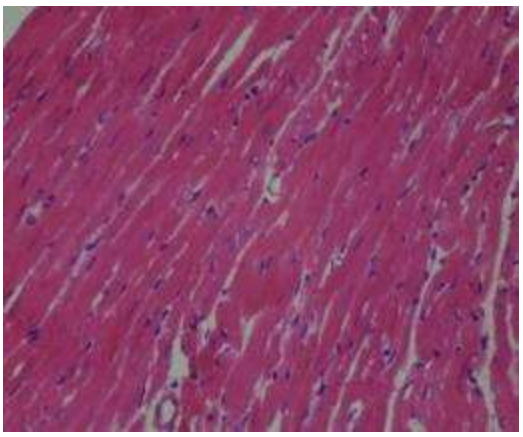

Figure 9: Group IV (TED X 10)

Histopathological studies of H&E stained cardiac tissue (40X) of the control group showing the normal architecture of heart and the normal arrangement of the layers of the myocardium and having normal cellularity. On the contrary Tamra bhasma treated TED X 10 group presented a sign of atrophy with smaller cellular nuclei. (Figure 6-9)

Histopathological examination of Liver

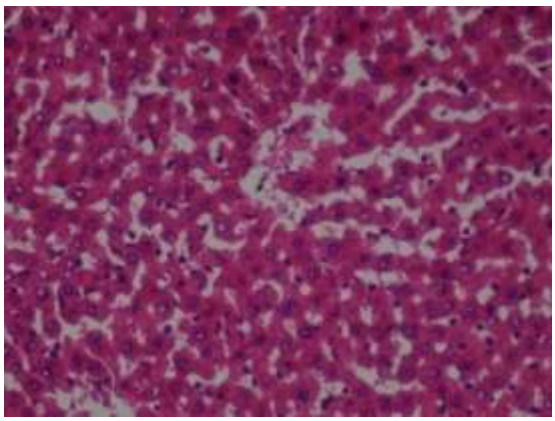

Figure 10: Group I (Control)

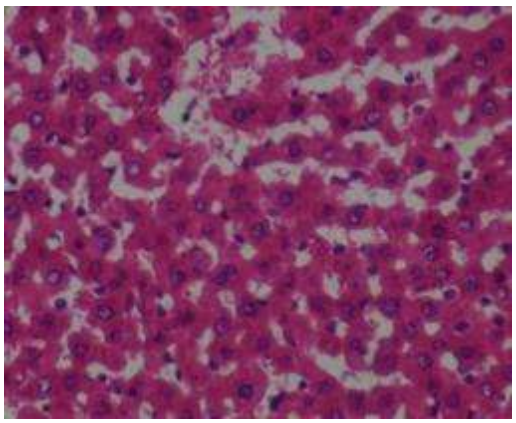

Figure 11: Group II (TED)

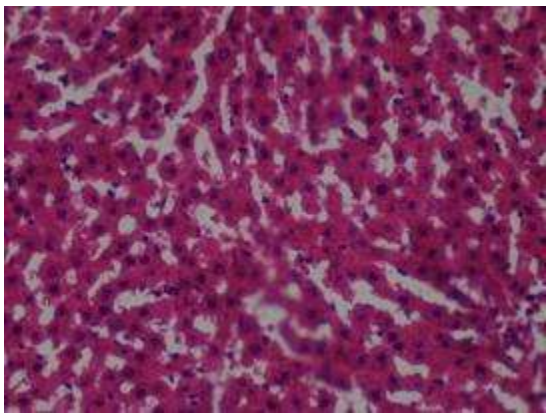

Figure 12: Group III (TED X 5)

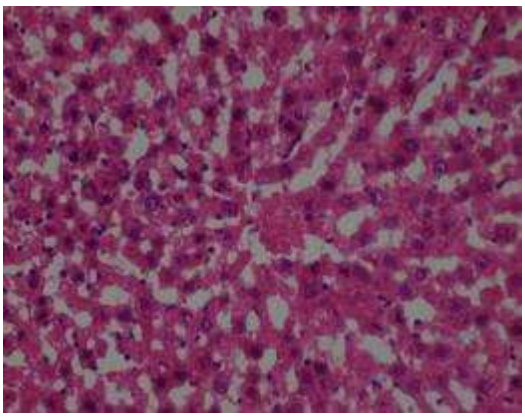

Figure 13: Group IV (TED X 10)

Histopathological studies of H&E stained liver tissue (40X) of the control group showing the normal architecture of liver and the normal arrangement of the layers of the hepatocytes. On the contrary Tamra bhasma treated TED groups presented signs of very mild fatty changes, while Tamra bhasma treated with TED X 5 group shows fatty changes and minimal inflammation, and TED X 10 group shows more necrosis and fatty changes. (Figure 10-13)

Histopathological examination of Lung

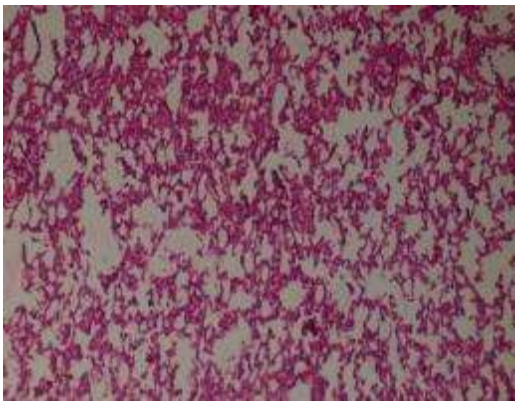

Figure 14: Group I (Control)

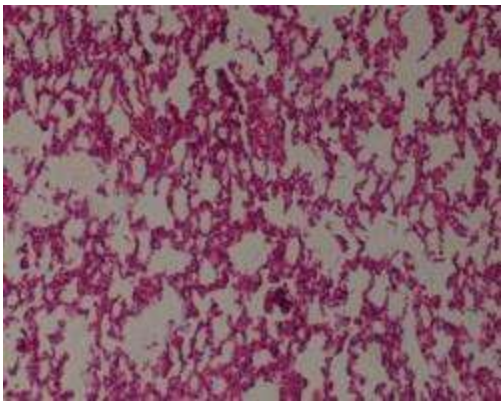

Figure 15: Group II (TED)

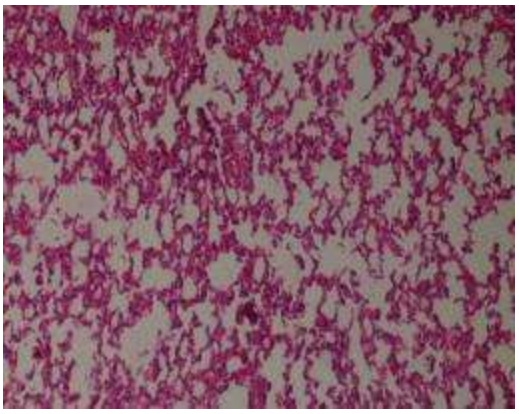

Figure 16: Group III (TED X 5)

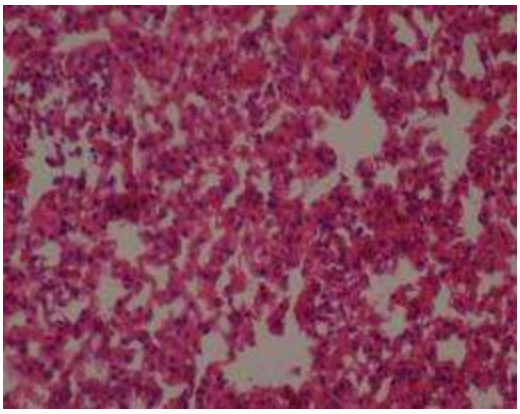

Figure 17: Group IV (TED X 10)

Histopathological studies of H&E stained lung tissue (40X) of the control group showing the normal architecture of lung and the normal arrangement of the cells of the lungs. On the contrary Tamra bhasma treated TED X 10 groups presented a signs of congestion and edema. (Figure14-17)

Histopathological examination of Kidney

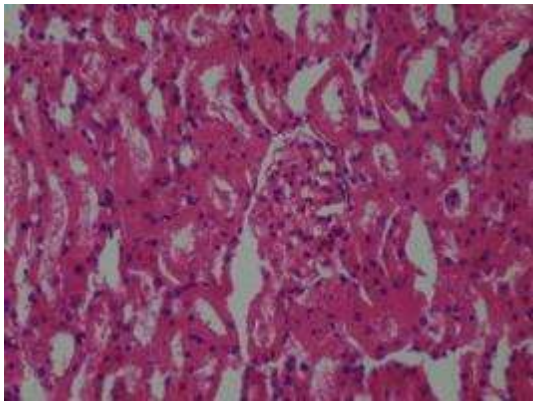

Figure 18: Group I (Control)

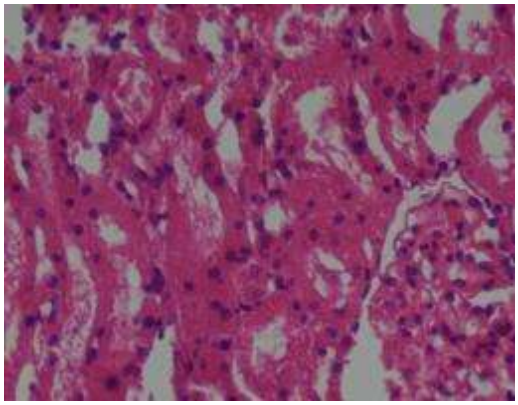

Figure 19: Group II (TED)

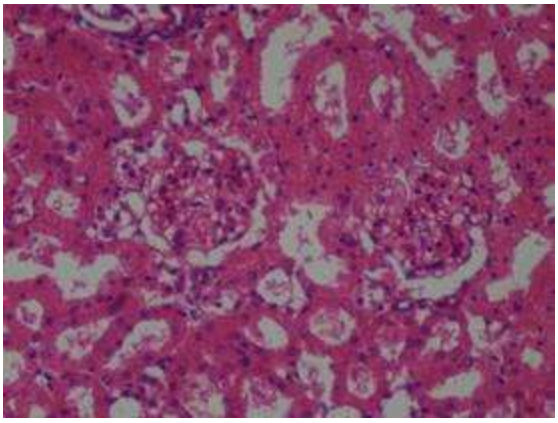

Figure 20: Group III (TED X 5)

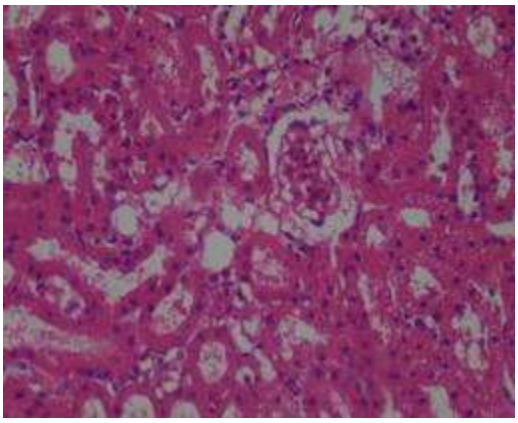

Figure 21: Group IV (TED X 10)

Histopathological studies of H&E stained kidney tissue (40X) of the control groups showing the normal architecture of kidney and the normal arrangement of the cells of the nephrons and tubules. On the contrary Tamra bhasma treated TED X 5and TED X 10 groups presented a sign of tubular degeneration, casts and glomerular congestion. (Figure 18-21)

DISCUSSION

Natural products and their active principles are a source for new drug discovery and treatment of diseases have attracted attention in recent years. Medicinal use of spices/ herbs has been increasing in developed countries from ancient past. Some of the natural products find their use not as pharmaceuticals (real medicine) but as a novel class dietary supplement or nutraceutical that fall well into the concept of function foods. Gradually metals were also identified and incorporated for medicinal purpose. But the use of metals was recognized as toxic, hence to reduce its toxicity, no. of Shodhana and Marana procedures were adopted which is mentioned in Rasashastra (Alchemy). The main concept of Rasashastra lies in the transformation of base lower metals into noble higher metals and to use them for strengthening the body tissues and nourish them[15].

According to this medicinal system, metal based drugs known as bhasma involves the conversion of metal into its mixed oxides. The product obtained is called bhasma and the process is called bhasmikarana[16].

Behavioral study on open field test and elevated plus maze test were done and there was no significant change in locomotion as compared to control group. This indicated that bhasma has not affected any of the neurological functions. Percent change in body weight was significantly ($p<0.05$) decreased in Tamra bhasma treated TED X 5 and TED X 10 groups as compared to control group**.** Relative organ weight of brain and heart in Tamra Bhasma treated (TED X 5, TED X 10) rat was significantly decreased which may be related to the possible ill effects of Tamra Bhasma, while treatment with Tamra Bhasma with TED has not caused any significant change in body weight and relative organ weight of brain and heart in Tamra Bhasma treated rat. Relative organ weight and percent change in body weight determination are commonly used tools in toxicity, while the purpose of relative organ weight analysis is to detect any direct treatment effect on the organ weight over and above any indirect effect caused by the effects of the treatment on body weight[17].

Several neuronal, cardiac, hepatic, pulmonary and renal marker enzymes are used to assess any toxicity associated with these organs.
The antioxidant status of brain, heart, liver, lung and kidney tissue were used to determine any toxic stress faced by these organs.

Decrease in lipid peroxidation in Brain, Heart, Liver, Lung and kidney tissue of Tamra Bhasma treated rats indicates decrease damage due to peroxides to the lipid membranes of cells. This results in decrease in membrane permeability, destruction of cell surface receptors and ligands for vital messengers causing toxic effects and decreased functions of the brain, heart, liver, lung and kidney cells[18]. Treatment with Tamra Bhasma showed significant protective effect by decreasing the lipid peroxidation in brain, heart, liver, lung and kidney. Reduced Glutathione (RGSH) is highly abundant in all cell compartments and is the major soluble antioxidant. Reduced GSH/Oxidized GSH ratio is a major determinant of oxidative stress. GSH shows its antioxidant effects in several ways. It detoxifies hydrogen peroxide and lipid peroxides via action of GSH-Peroxidase. Reduced glutathione was significantly decreased in brain, heart, liver, lung and kidney tissue of Tamra Bhasma treated rats, which reveals imbalance between oxidants and defense mechanism.

Significant decrease in LDH activity of Tamra Bhasma treated rats is an indicator of cardiac dysfunction as compared to control group reported by Chaudhari *et al* (2014)[19]. The LDH is a sensitive marker of cardiac function. The LDH activity was significantly decreased in Tamra Bhasma treated group TED as compared to control group. Significant increase in activity of AST, ALTin Tamra Bhasma treated rats indicates cardiac dysfunction as compared to control group animals. The AST and ALT enzyme is a sensitive marker of cardiac function[20]. Therefore, the increase in the serum AST and ALT activity might perhaps be an indication of heart damage. This increase could also be explained by free radical production which reacts with polyunsaturated fatty acids of cell membrane leading to impairment of mitochondrial and plasma membranes resulting in enzyme leakage[21].

The result seemingly agrees with the reports of Farombi and Onyema (2006), and Onyema *et al.* (2006)[22, 23] that the activity of serum AST and ALT increased in rats that were fed with Tamra Bhasma probably due to the finding that Tamra Bhasma treated oxidative stress in the heart. The serum AST and ALT shows functional activity of heart. An increase in the activities of these enzymes indicates an effect due to the doses.

Significant decrease in HDL level in Tamra Bhasma treated rats were reported previously by Chaudhari *et al* (2014)[19] and this study also projects decrease in HDL level in rats as compared to control group. In addition, the observed increase in the serum AST and ALT activity may be indicative of myocardial infarction as suggested by Rodwell and Kennelly (2003)[24].

A decrease in the serum ALP activity in all Tamra Bhasma treated groups as compared to control group animals was observed. In addition, the significant reduction in serum ALP activity by Tamra Bhasma perhaps indicate the absence of cholestasis (lack of bile flow) as previously reported by Kaneko (1989)[25]. Cholestasis may result from the blockage of the bile duct or from a disease that impairs bile formation in the liver itself. Thus, the possible absence of cholestasis with Tamra Bhasma intake could not be explained by the observation in the present study of possible liver damage in rats treated with Tamra Bhasma as indicated by increased serum ALT and AST activities. However, increase in the markers of liver damage without an increase in the marker of cholestasis have been reported in rats and interpreted as evidence of ongoing hepatocellular toxicity in the absence of significant cholestasis[26].

In hematological study, there were no significant changes in any of the TED groups in Hb, RBC, TLC, Platelet counts as compared to control.

The histopathological changes in Tamra bhasma treated TED group showed normal architecture of brain, heart, liver, lung and kidney. Histopathology of brain showed its normal structure and neurons. Heart showed normal myocardial muscle with normal nuclear appearance, Liver showed normal hepatocytes, nucleus, and normal central vein, Lung showed normal connective tissue, alveolar ducts, & pulmonary veins, and kidney showed normal architecture of nephron, glomeruli, & tubules. However, TED X10 has disturbed the normal architecture of the tissues.

Thus, in view of the above discussion Tamra bhasma at dose TED i.e 5.5mg/kg showed non-toxic effect as indicated by its morphological, behavioral, biochemical, hematological and histopathological studies in wistar rats, whereas the higher doses were found to have toxic effect.

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CONCLUSION

The present study provides an overview of the safety toxicity of Tamra Bhasma in laboratory rats by assessing its morphological, behavioral, biochemical, hematological and histopathological parameters.

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