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Original Research Article

EVALUATION OF HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES OF TAMRA BHASMA (A COPPER BASED MINERAL FORMULATION) REPEATED DOSE TOXICITY STUDY IN WISTAR ALBINO RATS.

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ABSTRACT

Objective: The objective of this present study was to evaluate safety profile of Tamra Bhasma with main emphasis on biochemical and histopathological changes in Wistar albino rats.

Study design: The toxicity study was done by repeated dose on Wistar albino rats. Animals were made into three different categories or groups which contains ten rats in each group. Group I administered with 0.5% CMC & considered as vehicle control. Group II & III administered with Tamra Bhasma 1.98mg/kg & 9mg/kg respectively. These groups were administered the specific drug Tamra Bhasma for 28 consecutive days. On 28th day after one hour of last dosing, animals were sacrificed.

Data sources and extraction: The blood and important organs were subjected for biochemical and histopathological examinations.

Results: The repeated administration of Tamra Bhasma didn't produce remarkable toxicity at therapeutic dose level. However Tamra Bhasma at five times of therapeutic dose produced changes in biochemical parameters such as there is an increase in the serum creatinine level. This was without concomitant increase in serum urea level- which was found to be decreased. Total bilirubin level was found to be elevated. Kidney sections from Tamra Bhasma administered at 5 times of TED has shown necrotic changes in the tubular epithelium, vacuolization oedema in the interstial tissue and focal cell infiltration however, the cyto-architecture of the other important organs were not affected in a drastic manner.

Conclusion: The repeated administration of test drugs at therapeutic dose level confirms their relative safety; however at high dose level they may have some concern related to renal functioning and hence the benefits of repeated administration of Tamra Bhasma outweighed the toxic potential.

KEY WORDS: Tamra Bhasma, Histopathology, Serum urea, Total bilirubin, Creatinine, Tubular epithelium.

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INTRODUCTION

Innumerable references are available for the therapeutic efficacy of metal and mineral based drugs in modern and traditional system of medicines [1,2].

Rasashastra is one such branch of Ayurveda which deals with metal and mineral based drugs, their collection, preparations, testing and different clinical indications [3-7].

The steps involved in the preparation and use of metal based drugs are well documented in the classical texts like Rasatharangini. Many of the procedures are very elaborative and tedious, time consuming and hence in the present scenario scholars, pharmacists adopt short cut methods and employ modern techniques without verifying what impact the changes employed will have on both therapeutic and toxic profile of the final products obtained.

Market analysis shows that number of pharmacies is being manufacturing these products by different methods but these products are not standardised either on their chemical and structural point of view [8]. If not manufactured in proper manner the product may not be safe in the expected dose range. Therefore it is necessary to understand their current status by using modern analytical techniques for comparative study is important and also to analyse their safety aspects for human use [9] Tamra Bhasma is one among the important metallic preparations used in Indian System of Medicine and has widespread use in the treatment of diseases like anemia, skin disorders, dyspnea, Peptic ulcer, Fever, digestive impairment ,tuberculosis, liver and spleen disorders, eye diseases, lipid metabolic disorders, dyspepsia and cardiac disorders etc. [10].

The above information clearly shows that evaluation of safety and efficacy of mineral and mineral based preparations is considered as one of the most important area that deserves attention of the researchers in the field of bio-medical sciences. Thus in the present study mainly focused on the safety aspects of classically prepared *Tamra Bhasma* on repeated dose toxicity by mainly focused on biochemical and histopathological changes on important organs in Wistar albino rats.

MATERIALS AND METHODS

Test drug: Tamra Bhasma (a copper based mineral preparation) was manufactured by Sri Siddeshwara drugs, Cherpulssery, Palakkad, Kerala, by Dr. Sasi Kumar a renowed expert of Rasashastra. This drug was prepared according to classical reference mentioned in Bhasma Vignana by Yadavji. The batch number of Tamra Bhasma drug -25/November-2012 was used.

Dose fixation: The dose of Tamra Bhasma for the therapeutic purpose in human was 22 mg per day. By referring to the standard conversion table of Paget and Barnes (1964)[11] body surface area ratios the dose was calculated [12]. On the basis of this the rat dose was 1.98 mg/kg (therapeutic dose) and 9.9mg/kg (five times of therapeutic dose) was calculated. The test drug Tamra Bhasma was suspended in 0.5% CMC and based upon the body weight of animals (1ml/100g body weight) was administered orally by using oral catheter.

Experimental animal: For the experiment Wistar albino rats of both sexes weighing from 150-225 g body weight were procured from the animal house attached to pharmacology laboratory at SDM centre for research in Ayurveda and allied sciences, Udupi. All the animals were acclimatised to the standard laboratory conditions such as temperature of 25 ± 2°C and 55-60% of humidity and 12h day and night cycle throughout study. The animals were fed with rat diet and water ad libitum. The institutional animal ethical committee was approved experimental protocol with the reference number SDMCA/IAEC/CPCEA/GI 04/2011.

Study design: Wistar albino rats were divided into three different groups, each group contains 10 rats of which 5 male and 5 female. Group-I rats were considered as control, and received only vehicle (0.5% CMC). Group-II rats received *Tamra Bhasma* at therapeutic dose (TED) 1.98mg/kg, Group-III rats received 5 times of therapeutic dose (9.9mg/kg). The test drug *Tamra Bhasma* was administered for 28 consecutive days. On 28th day one hour after last dose, blood was withdrawn from retro-orbital plexus for biochemical investigation. At the end all animals from each group were sacrificed and important organs were collected for histopathol

ogical investigation [13-16].

Statistical analysis: The data was expressed in Mean ± SEM and analysed by one way ANOVA followed by Dunnet's Multiple't' test using Graph Pad Instant version 3.5 for determining the level of significance of the observed effects. A 'P' value of less than 0.05 was considered statically significant.

RESULTS

The repeated administration of Tamra Bhasma for 28 consecutive days didn't cause any significant change in the organs weight and observed changes were comparable with that of normal control. (Table-1a & 1b)

Table 1a: Showing the Effect of Tamra Bhasma on organs weight.

GROUP	Organs weight (g)			
	Brain	Lung	Heart	Liver
Control	1.82 ± 0.09	1.63 ± 0.01	0.86 ± 0.03	8.53 ± 0.46
Tamra Bhasma TED	1.75 ± 0.14	1.49 ± 0.09	0.84 ± 0.05	8.88 ± 0.48
Tamra Bhasma TED X 5	1.91 ± 0.07	1.49 ± 0.08	0.68 ± 0.05	8.2 ± 0.77

Data expressed in Mean ± SEM, one way ANOVA followed by Dunnet's multiple comparison't' test was employed for statistical analysis. TED-Therapeutic Dose, TED x 5- Five times of therapeutic Dose

Table 1b: Showing the Effect of Tamra Bhasma on organs weight.

GROUP	Organs weight (g)			
	Spleen	Jejunum	Testis	Uterus
Control	0.82 ± 0.14	0.75 ± 0.22	2.57 ± 0.15	0.8 ± 0.06
Tamra Bhasma TED	1.11 ± 0.21	0.49 ± 0.05	2.93 ± 0.14	0.67 ± 0.17
Tamra Bhasma TED X 5	0.98 ± 0.07	0.46 ± 0.03	2.72 ± 0.09	0.81 ± 0.12

Data expressed in Mean ± SEM, one way ANOVA followed by Dunnet's multiple comparison't' test was employed for statistical analysis. TED-Therapeutic Dose, TED x 5- Five times of therapeutic Dose

The following Total haematological parameters were examined at the end of experimentation such as haemoglobin, RBC, WBC, PCV, MCV, MCH, RADWCV, RDWSD and Platelet. Among the above parameters *Tamra Bhasma* administered at therapeutic dose shown significant increase in the total WBC count, MCH, RDWCV and at five times of therapeutic dose level significantly increased in the MCHC as compared to normal control. (Table-2a & 2b)

Table 2a: Effect of Tamra Bhasma on Haematological parameters.

Parameters	Hb (g/dl)	WBC (cells/cumm)	RBC (millions/cu.mm)	PCV (%)
Control	15.2±0.44	5516.67±826.81	7.55±0.27	43.45±1.30
Tamra Bhasma TED	16.01±0.45	12083.33±1671.2 *	7.80±0.22	43.97±0.59
Tamra Bhasma TED X 5	15.16±0.51	9400±2104.9	7.54±0.26	41.02±1.39

Data expressed in Mean ± SEM, *P<0.05, **P<0.01 in comparison to normal control.

Table 2b: Effect of Tamra Bhasma on Haematological parameters.

Parameters	MCV (Fl)	MCH (pg)	MCHC (%)	RDWCV (%)	RDWSD (%)	PLT (lakhs/cumm)
Control	57.65±0.62	20.12±0.28	34.95±0.19	12.55±0.2	25.87±0.59	7.01±0.13
Tamra Bhasma TED	56.58±1.12	20.53±0.43	36.33±0.6 *	14.78±0.9 **	29.13±2.09	6.96±0.69
Tamra Bhasma TED X 5	54.75±0.67	20.37±0.34	37.32±0.3 **	13.47±0.2	25.66±0.56	7.63±0.47

Data expressed in Mean ± SEM, *P<0.05, **P<0.01 in comparison to normal control.

Biochemical investigation in the serum revealed the repeated administration of Tamra Bhasma at both the dose levels increased the serum glucose, creatinine, total protein, total bilirubin and direct bilirubin level, however Tamra Bhasma administered at five times of therapeutic dose has significantly increased serum glucose, total and direct bilirubin level as compared to normal control. The serum urea level was significantly decreased in both dose levels and the serum creatinine was increased significantly as compared to normal control group. (Table-3a & 3b)

Table 3a: Effect of Tamra Bhasma on biochemical parameters.

Parameters	Glucose (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Control	100.50±10.86	115.5±9.52	134±15.99	283.33±48.55
Tamra Bhasma TED	122.55±11.51	128.33±7.87	110.83±2.95	457.33±81.61
Tamra Bhasma TED X 5	129.57±5.20 *	141.33±8.74	131.83±11.46	496.33±98.18

Data expressed in Mean ± SEM, *P<0.05, **P<0.01 in comparison to normal control.

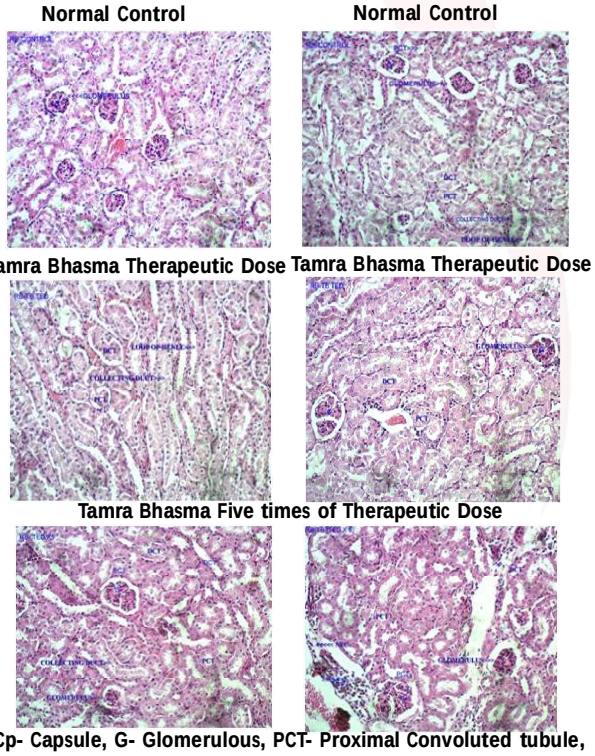
Table 3b: Effect of Tamra Bhasma on biochemical parameters.

Parameters	Urea (mg/dl)	Creatinine (mg/dl)	Sodium (mEq/L)	Potassium (mEq/L)	Total protein (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Control	57.16±3.25	0.6±0.07	139.33±0.50	4.28±0.11	5.33±0.59	0.11±0.01	0.14±0.01
Tamra Bhasma TED	39.18±2.90 **	0.67±0.06	141.83±0.60	4.38±0.21	7.83±0.18 **	0.11±0.01	0.21±0.02 **
Tamra Bhasma TED X 5	45.13±3.51 **	0.83±0.03 **	139.83±1.17	4.85±0.19	6.62±0.26 *	0.11±0.00	0.2±0.02 *

Data expressed in Mean ± SEM, *P<0.05, **P<0.01 in comparison to normal control.

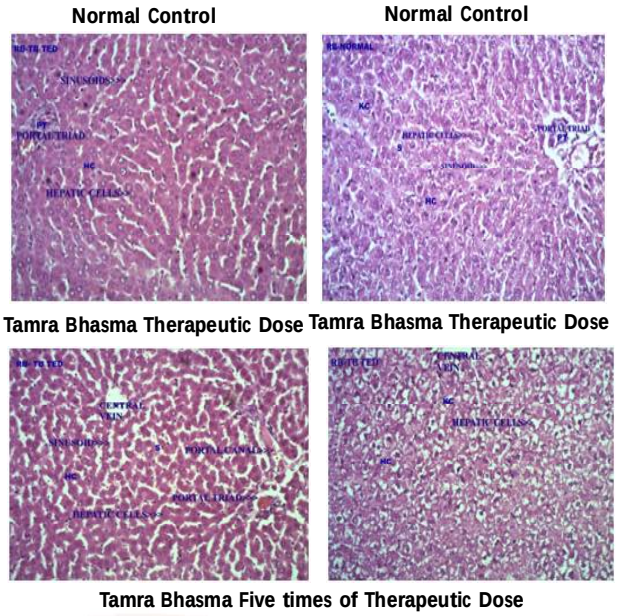
Effect of test drug Tamra Bhasma on histopathological changes in the important organs such as brain, heart, lungs, kidney, liver, spleen, jejunum, stomach, testis and uterus were carried out. The histopathological examination of spleen, lungs, liver, testis, uterus and jejunum didn't revealed any toxic changes and the cyto-architecture was almost normal as compared to normal control group. Tamra Bhasma administered at both dose levels has shown normal cyto-architecture with mild sinusoidal dilation in liver sections. The kidney sections from Tamra Bhasma administered group has shown nearly normal cyto-architecture, whereas at 5 times of TED has shown Necrotic changes in the tubular epithelium, vacuolisation oedema in the interstitial tissue and focal cell infiltration as comparable to normal control. The bone marrow sections of Tamra Bhasma administered at therapeutic dose group has shown moderate cellularity in comparison to control. Eosinophilic cells were rarely seen and megakaryocyte cells moderate in number. The myelomonocytic lineage found more in comparison to erythroid lineage. Tamra Bhasma administered at five times of therapeutic dose group has shown cellularity is moderate. Megakaryocytic lineage found more in comparison to erythroid cells lineage. Few reticulocyte cells found. Eosinophilic cells were rare. (Figure 1-3)

Fig. 1: Photomicrographs of Kidney.



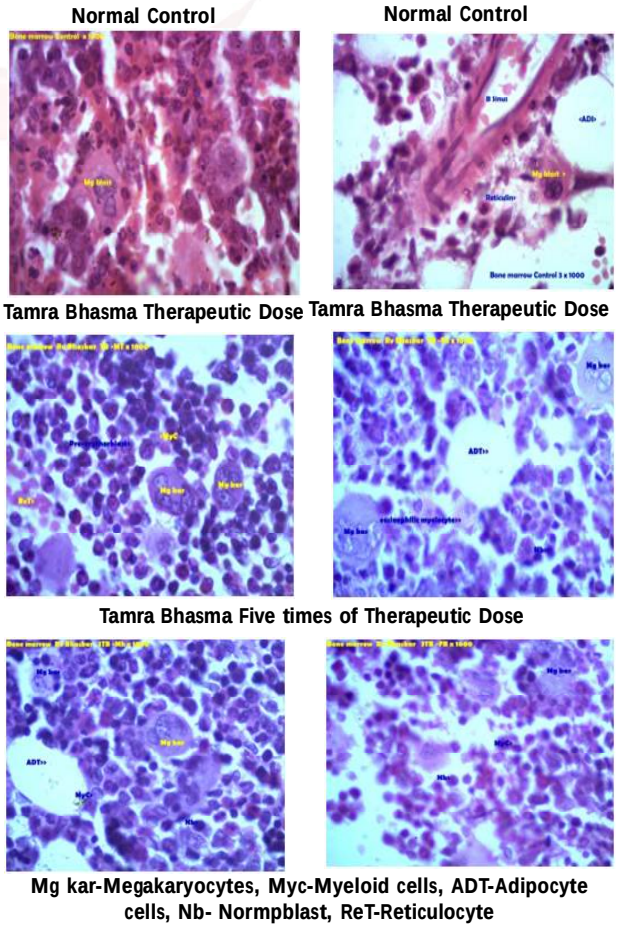
Cp- Capsule, G- Glomerulous, PCT- Proximal Convoluted tubule, DCT- Distal convoluted tubule

Fig. 2: Photomicrographs of Liver.



Hc-Hepatic cells, Kc- Kupfer cells, S- Sinusoid, Cl- Cell infiltration, Fc- Fatty Changes, PT- Portal traid

Fig. 3: Photomicrograph of Bone marrow.



Mg kar-Megakaryocytes, Myc-Myeloid cells, ADT-Adipocyte cells, Nb- Normpblast, ReT-Reticulocyte

DISCUSSION

Safety profile was evaluated based on the effect of Tamra Bhasma on following parameters such as haematological, biochemical and histopathological changes. The following haematological parameters such as haemoglobin concentration, erythrocyte count, total leukocyte count and differential leukocyte count, platelet count, MCHC, MCV and RDWCV were carried out. Among these parameters the Tamra Bhasma administered at therapeutic dose has showed significant increase in the WBC, MCHC and RDWCV concentration as comparison to normal control group. The normal range of MCHC is 28-36%. This elevation might be due to spherocytosis (it is a type of RBC that contains an abnormal amount of haemoglobin). If the haemoglobin is not stable this can cause marked raise in the MCHC. The elevation may also rise in case of deficiency of folic acid or Vit-B12. The changes though significant were only modest in magnitude and also not observed in other related parameters. Changes in RDW-CV were also not dose dependent indicating self-limiting nature of the observed [17,18].

The high level of WBC usually indicated inflammation and infectious state or a disease of bone marrow. In the present study Tamra Bhasma administered at therapeutic dose has significantly increased WBC; it might be due to inflammatory changes seen in some tissues. Another point to be noted here is that this elevation is not dose dependent and not observed at higher dose level. Based on this it can be suggested it as a limited nature change not important from pathological point of view. Thus the overall effect of the test drug Tamra Bhasma on the important haematological parameters do not have significant potential to cause blood related toxic effects [19].

Tamra Bhasma treated groups showed dose dependent significant changes in three of the recorded biochemical parameters- serum urea, serum protein and serum total bilirubin levels. Significant changes at higher dose level were observed in serum glucose and serum creatinine level

In the present study the test drugs administered in therapeutic and five times therapeutic did not elevate liver enzymes and are in comparable with that of normal control group. Thus this clearly indicates that the test drug do not have marked effect on liver functioning.

Tamra Bhasma administered at both the dose has significantly elevated total bilirubin (un-conjugated bilirubin). The total bilirubin represents > 90% is un-conjugated bilirubin. This indicates it might be due to increased haemolysis. This should have reflected in the form of decreased RBC and RBC related indices. This was not observed hence significant haemolysis may be ruled out. It may be due to decreased availability of endogenous conjugating factors especially glucuronic acid [20,21].

Tamra Bhasma administered at therapeutic dose and five times of therapeutic dose in comparison to normal control group there was a significant elevation of serum total protein. This was indicated that test drug might have a role in inducing inflammatory changes in repeated dose. However, the exact reason is not known [21,22].

The test drug Tamra Bhasma administered at therapeutic dose (TED) was showed nearly normal glucose level and in comparison to normal control group while the test drug Tamra Bhasma administered at five times of therapeutic dose (TED x 5) significantly elevated random glucose level in comparison to normal control group rats. The observed elevation might be due to inhibitory action on insulin secretion or antagonizing insulin action. It may not be a significant problem at therapeutic dose level; even at higher dose level it is only modest in magnitude. Analysis for the data pertaining to organ weight changes in 9 organs indicated that the test drug produce only non-significant increase in organs like brain, liver, spleen, testis and non-significant decrease in organs like lungs, kidney, jejunum and both the type of changes in uterus and heart. Organ weight is a sensitive index. Increased weight may be indicative of tissue or organ oedema, hypertrophy or hyperplasia. Decreased weight may be indicative of tissue loss due to necrosis or other causes. Since no significant effect could be observed in the Tamra Bhasma administered group in comparison to the normal control group it can be suggested that they do not have the potential to cause hypertrophy, hyperplasia, and oedema or tissue

loss. Microscopic sections of kidney from normal control groups of rats were showed normal cyto-architecture in both cortical and medullary regions. No remarkable changes could be seen in glomeruli or convoluted tubules. Kidney sections from Tamra Bhasma administered at 5 times of TED at both dose levels has shown necrotic changes in the tubular epithelium, vacuolization oedema in the interstial tissue and focal cell infiltration. However, the medullary region was not affected in remarkable manner. The above lesions were not extensive. This may be the reason for the observed elevation in serum creatinine level. The exact reason is not known. This kind of results is not frequently seen in clinical conditions even though they are used on extensive scale.

Liver microscopic sections showed normal structures except for the observation of mild cell infiltration in one section. The sections from Tamra Bhasma administered at TED & TED X5 dose exhibited almost normal cyto-architecture with mild sinusoidal dilatation. This corroborates with the data of biochemical parameters indicating relative good toleration of the drug by liver [23].

Tamra Bhasma administered at therapeutic dose group has shown overall cellularity is moderate to less in comparison to control and other groups. Eosinophilic cells were rarely seen. Few reticulocyte cells were found in two male groups. And megakaryocyte cells found more in number in two female groups. Myelomonocytic lineage found more in comparison to erythroid lineage in one group. Tamra Bhasma administered at five times of therapeutic dose group has shown cellularity is moderate. Megakaryocytic lineage found more in comparison to erythroid cells lineage. Few reticulocyte cells found. Eosinophilic cells were rare. But megakaryocyte cells were more in number in two male and two female groups [24].

CONCLUSION

From the repeated dose toxicity study we can conclude that Tamra Bhasma was relatively safer and did not produce any marked toxic effect, however at higher doses it produced some functional changes in kidney. Renal injury of moderate intensity was observed during histopathological examination. However, the higher dose used in the present study was much higher than routinely practiced clinical doses.

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Conflicts of Interests: None

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