Research Article

TOXICOLOGICAL STUDIES OF TRADITIONAL AYURVEDIC PREPARATION TAMRA BHASMA; COMPARATIVE EFFECTS OF COMPLETELY PROCESSED AND INCOMPLETELY PROCESSED BHASMA

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ABSTRACT

The bhasmas are one among Ayurvedic preparations used for many disorders without producing serious adverse effects in ancient days. The present work aims to investigate the differential effects of completely processed Tamra Bhasma to incompletely processed Tamra Bhasma on animals. The animals were divided into 15 groups of 6 in each as 3 schedules of treatment (30, 60 and 90 days). After the specified period of treatment, the animal was euthanized and blood was collected through retro orbital route. The one portion of collected blood was used for WBC count and other part is used for separation of serum. The collected serum was used for the estimation of Copper, SGOT and SGPT determinations. The liver and kidneys are dissected and kept at -20^{o}. A part of liver and kidney was fixed to investigate histopathological studies and remaining portion was homogenized for the estimation of Copper in tissue. There was significant increase in the SGOT and SGPT levels in animals treated with incompletely processed Tamra Bhasma. The Processed Tamra bhasma is one it is claimed to have Hepatoprotective activity so it should decrease the enzyme levels. But if bhasma is not processed, it increases the enzyme levels and cause hepatotoxic effect. The concentration of copper in liver and kidney were also increased more significantly in animals treated with incompletely processed Tamra bhasma when compared to control animals and animals treated with processed bhasma. The above mentioned changes were also supported by histological changes in kidney and liver. Histological studies suggested that, more prominent changes were seen in animals treated with incompletely processed bhasma when compared to completely processed bhasma. Findings of the study suggest that, incompletely processed bhasma is toxic if used clinically. To get beneficial effects of bhasmas, it is must to process the bhasma completely.

Keywords: Tamra bhasma, bhasma, copper, toxicology, traditional preparation

INTRODUCTION

It has taken nearly 5000 years for the world to wake up to the possibilities of Ayurveda. People across the globe are becoming increasingly disgruntled with the detrimental effects of the drug therapies of modern system of medicine. The necessity of an alternative medicinal system is being felt throughout the world. Ayurveda is one among the alternative system to modern medicine. However, the globalization of Ayurveda is not merely because of the failures of the modern medicine. Ayurveda preaches the art of living for humans so as to have a good health and better life. As we all know that Ayurveda an ancient science is the gift of India to the world.[1]

The Rasashastra is one among the Ayurvedic branch, which describes in detail the significance of minerals to human body, their origin and the methods to convert them into the form of Bhasmas. The use of minerals as drugs developed with this branch of Ayurveda. According to the historians, Stone Age was followed by Iron Age which means after the Stone Age people started using metals for various purposes. Iron Age not only introduced the use of metals for preparing weapons, receptacles and ornaments but also for medicinal purposes. It is important to mention that, Hindus were the pioneers to use the metals for the medicinal purposes. [2]

Rasashastra explains about preparation of Ayurvedic medicine and it includes the extraction of metals from their minerals, their purification and conversion into digestible metallic bhasma. The process of manufacturing bhasma consists of *satvapatana* (metal exrtraction) and *bhasmikarana* (conversion to non toxic form). The processing of metals can be classified into *sodhana* (purification), *marana* (conversion to non toxic fine powder), *mardana* (preparation of intermediate mixture), *putapak* (reactions at high temperature) and in few cases amrithikarana. At the end of processing, this microfine medicinal product has easy digestive power and quick reaction with the bile juices.[3]

Nagarjuna, father of Rasashastra, introduced clinical use of metallic preparations (Bhasmas) who was actively involved in the metallic transformations along with his team, also achieved tremendous success in developing the metals, minerals and their preparations as the best therapeutic agents of the age. In his time mineral therapy (Rasa Chikitsa) had been recognized as Daivi Chikitsa (divine therapy or therapy of choice) by all concerned, considering its superiority over the other types of treatments. Hence, the treatment with all the drugs of mineral group has been considered superior than treatment with other types of drugs.[2]

Bhasma is a powder of substance obtained by calcination. In this section, it is applied to metals, minerals and animal products which are by special processes, calcined in closed crucible in pits and with cow dung cakes.

Calcination is the drying process by roasting which produces a powder. Although Bhasmas have been used as effective drugs for centuries without any significant adverse effects, certain factors related to their preparation have remained in disguise.

Though the Bhasmas have been well established forms of drugs, but current research is limited to the study based on Ayurvedic point of view. Very few reports are available where attempts have been made to understand the chemical properties of the material. These reports fail to throw light on the physical and chemical characteristics of these materials which could help in determining the mechanism involved in their preparation and the unsolved mysteries related to the use of Bhasmas. So, the studies on this concept are essential to flourish Ayurveda and due to the fact that these drugs could cure certain chronic diseases for which the modern medicine has no full proof remedy.[1] Previously, the Bhasmas were being prepared on small scale by the Ayurvedic physicians (vaidya) themselves, but now they are manufactured on large scale in pharmaceutical industries. This new approach has created several problems, because the use of new appliances has not been standardized regarding the quality of these Bhasma preparations.

For standard bhasma preparations, there is need for a scientific approach which may be defined as

- ❖ Physical standardization and elemental analysis of raw material and bhasmas.
- ❖ Determination of oxidation state of metals
- ❖ Pharmacokinetics of the metallic component of the bhasma
- ❖ Accumulation studies of metals in different tissues and organs
- ❖ Effect of bhasmas on normal physiological and antioxidant parameters
- ❖ Therapeutic uses of the bhasmas on the recommended disease model at cellular and molecular level
- ❖ Role of bhasmas as drug carriers and in immunomodulation

These studies will provide evidence for the safety behind the use of bhasmas and also provide knowledge regarding their mechanism of action. An effort should be made to educate the regulatory authorities and the consumers about the fact, so that they can judiciously differentiate between the myth and reality associated with use of bhasmas.[4]

The preparation of Bhasmas involves incineration of metals for several times until the required quality is achieved. The commercial formulators may stop the processing of bhasma abruptly for making it more economical, but in other way it produces the adverse effects due to the incomplete conversion of metal into non toxic bhasma which may cause accumulation of metals leading to the several problems. The present work aims to investigate the differential effects of completely processed Tamra Bhasma with incompletely processed Tamra Bhasma on animals. The objectives of the work are as follows.

- To analyze the comparative effects of the completely processed and incompletely processed bhasmas.
- As a step towards establishing these materials as potential medicines to the world.
- To understand the mechanism involved in their quantization.
- To resolve the unsolved mysteries related to use of bhasma.
- To evaluate toxic effects of incompletely processed bhasmas.

MATERIALS AND METHODS

Animals

Wistar rats of either sex were used for the study. The animals were procured from Drug Testing Laboratory, Bangalore. They were housed in the animal house of the Government College of Pharmacy at least 2 weeks prior to the study, so that animals could adapt to the new environment. Animals were maintained and handled as per CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) guidelines.

Animal house was well maintained under standard hygienic conditions, at temperature (22+ 1^{o}C) room humidity (60%±10%) with 12 hour day and night cycle. The rats were provided with commercial food pellets and purified water *ad libitum.* Cleaning and sanitation work was done on alternate days. Paddy husk was provided as bedding material, which was changed everyday. The cages and water bottles were maintained clean. The specifications of the animals are given in table 1.

Table 1: Specifications of the Animals

Species	Rats
Strain	Wistar
Age	5 to 6 months
Body weight	150 to 250g
Number of animals in each group	Six
Number of groups	Fifteen
Vehicle for bhasmas	2% Acacia in distilled water
Water and food	*ad libitum*

Drugs:

- Completely processed Tamra bhasma
- Incompletely processed Tamra bhasma

Chemicals:

- Zinc estimation kits (chema diagnostica)
- Copper estimation kits (chema diagnostica)

- SGOT estimation kits (span diagnostics)
- SGPT estimation kits (span diagnostics)
- Di potassium EDTA
- WBC diluting fluid
- Concentrated nitric acid
- 2- mercapto ethanol (1% v/v)
- Alcohol (90%)
- Formalin solution (10%)

The Bhasmas were procured commercially and are used in this study. The completely processed Bhasma is manufactured by Sri Dhoothpapeshwar Private Limited, Bombay. The incompletely processed Bhasma is procured from Janatayu Pharma, Uttar Pradesh.

Completely Processed Bhasma (Figure 1)

The Tamra Bhasma manufactured and marketed by Sri Dhoothpapeshwar Private Limited, Bombay was used for the study.

Incompletely Processed Bhasma (Figure 2)

These are not completely processed; their processing are stopped at particular level intentionally and used for the study and this is not a marketed product.

Incompletely Processed Tamra Bhasma used in the study was undergone Sodhana and Marana process but not amrithikarana.

Figure 1: Completely processed Tamra Bhasma.

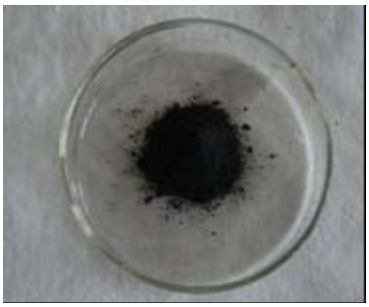


Figure 2: Incompletely processed Tamra Bhasma

METHODS

Quality control Tests for Bhasmas

The science of Ayurveda itself specifies certain tests to ascertain the quality of Bhasmas. All these tests mentioned in the texts are aimed to make sure that, the Bhasma converted to light from heavy, fine from bulky, digestible and absorbable from undigestible and un absorbable. The tests may be physical or chemical. Applicable tests for Tamra bhasma are mentioned below and were performed with both completely processed and incompletely processed bhasmas.

Physical tests:

- Varitaratwa
- Rekhapurnathwa
- Gatarasatwa

Chemical tests:

- Reaction with Curd

Varitaratwa

Jalaptava is the synonym used for this test. It should be present in all the prepared Bhasmas. The meaning of this term is to float over the surface of water. If a Bhasma floats over the surface of water, it can be regarded as standard one. Take a beaker full of water and allow it to become quiet. Now pour the Bhasma powder slowly over the water surface and see whether all the particles of the powder are floating over water surface or some of them sink into the water. If the entire particles float then the Bhasma is considered to be of the best quality, otherwise some more putas are to be given to make the Bhasma up to the standard. Here the surface tension of the water plays an important role. i.e, the particles of the Bhasma have become so fine that they cannot break the surface tension of the water in the ordinary way. After attaining this stage the Bhasma should be recommended for internal use. [2, 5,6]

Rekhapurnathwa

This indicates the fineness of the Bhasma. Here the Bhasma powder is rubbed in between the thumb and the fingers. If the particles of the Bhasma enter the furrows of the fingers it is presumed that they may also be absorbed into the system and then the process of marana may be considered complete. [2, 5,6]

Gatarasatwa

After the completion of the marana process, generally the Bhasmas are without any taste. To test this, a portion of the Bhasma should be put on the tip of the tongue to detect its taste if any. In case of Tamra Bhasma, this test is most important as the Tamra Bhasma having an astringent taste cannot be considered suitable for clinical use. If such Bhasma is used internally may produce nausea, vomiting, vertigo and burning sensation in the human subjects. [2, 5,6]

Reaction of Tamra Bhasma with curd

A pinch of prepared Tamra Bhasma was put on the surface of the curd which is kept in beaker for 24 hrs. The colour of the curd around Tamra Bhasma needs to be observed for any change in the color of the curd. [2,5]

Toxicity evaluation in mice

Therapeutic dose for Tamra Bhasma was well known through earlier studies. The same dose was used in the study based on the available sources as mentioned below.[7,8]

Dose 1: 5 mg/kg body weight of rats

Dose 2: 10mg/kg body weight of rats

Experimental design

Tamra Bhasma (completely and incompletely processed) was given orally to rats according to their body weights in the form of suspensions with 2% acacia in water for following groups of animals. Control animals received acacia solution alone.

Groupings

I. 30 days treatment schedule:

Group 1: Normal rats receive acacia solution.

Group 2: Normal rats receives Tamra Bhasma (P) dose 1.

Group 3: Normal rats receives Tamra Bhasma (P) dose 2.

Group 4: Normal rats receives Tamra Bhasma (IP) dose 1.

Group 5: Normal rats receives Tamra Bhasma (IP) dose 2.

II. 60 days treatment schedule:

Group 1: Normal rats receive acacia solution.

Group 2: Normal rats receives Tamra Bhasma (P) dose 1.

Group 3: Normal rats receives Tamra Bhasma (P) dose 2.

Group 4: Normal rats receives Tamra Bhasma (IP) dose 1.

Group 5: Normal rats receives Tamra Bhasma (IP) dose 2.

III. 90 days treatment schedule:

Group 1: Normal rats receive acacia solution.

Group 2: Normal rats receives Tamra Bhasma (P) dose 1.

Group 3: Normal rats receives Tamra Bhasma (P) dose 2.

Group 4: Normal rats receives Tamra Bhasma (IP) dose 1.

Group 5: Normal rats receives Tamra Bhasma (IP) dose 2.

(P)→Processed bhasma

(IP)→ Incompletely processed bhasma

After the specified period of treatment, the animal was euthanized and blood was collected. The one portion of collected blood was used for WBC count and other part for separation of serum. The collected serum was used for the estimation of Copper, SGOT and SGPT determinations. The liver and kidneys are dissected and kept at -20^{o}. A part of liver and kidney was fixed to investigate histopathological studies and other portion was homogenized for the estimation of Copper.

Parameters:

- WBC counting.
- Copper concentration in serum.
- SGOT & SGPT.
- Copper concentration in liver and kidney homogenates.
- Histopathological studies.

The procedures to carrying out above parameters are given below.

WBC counting

This test is performed on a blood sample drawn from retro orbital route. The blood (approximately 0.5 ml) was collected in the tube containing 10 µl of 1% solution of dipotassium EDTA as anticoagulant. The blood and anticoagulant was mixed properly and added 20µl of this mixture to the tube containing 380µl of WBC diluting fluid and mixed well and incubated for 10 minutes. The 10 µl of incubated mixture was put on the Neuber's chamber and number of WBC cells counted in 4 squares. The total WBC count per mm^3 was calculated by using dilution factor and volume correction factor.

Copper concentration in serum

Copper concentration in serum was determined using commercially available kits by colorimetric method using Elico SL 159 UV-Visible spectrophotometer.

SGOT & SGPT

SGOT and SGPT concentration in serum was determined using commercially available kits by colorimetric method using Elico SL 159 UV-Visible spectrophotometer.

Copper concentration in liver and kidney homogenates

Approximately 500 mg of wet liver and kidney were homogenized separately with 5 ml of water containing 1% (w/v) 2-mercaptoethanol in a tissue homogenizer and subsequently frozen and thawed three times. After centrifugation, the insoluble material was re-suspended in an additional 5 ml of the same extraction fluid, stirred properly in cyclo mixer and again centrifuged. The extraction procedure was then repeated. The total extracted fluid was approximately 15 ml; the concentration of copper in this tissue homogenate was done by commercially available kits.[9]

Histopathological studies

The part of liver and kidney used for the histopathological studies, the tissues were processed to prepare slides. The slides were used for the interpretation of the results.

RESULTS AND DISCUSSION

RESULTS

1. Physical and Chemical Quality control Tests

Results of the Physical and chemical tests conducted to make sure the quality of the bhasmas are given in table 2.

- **Varitaratwa (floatability)**

Completely processed Tamra Bhasma (Figure: 3a to 3b)

Figure 3a **Figure 3b**

Incompletely processed Tamra Bhasma (Figure: 3c to 3d)

Figure 3c **Figure 3d**

- **Reaction of Tamra Bhasma with curd**

Method

A pinch of prepared Tamra Bhasma was put on the curd which is kept in beaker for 24 hrs. The colour of the curd near Tamra Bhasma was observed. The results are as follows with processed and incompletely processed Tamra Bahamas. Observations are presented in Figures 4a to 4h.

Completely processed Tamra Bhasma:

On day one:

Figure 4a **Figure 4b**

After 24 hours:

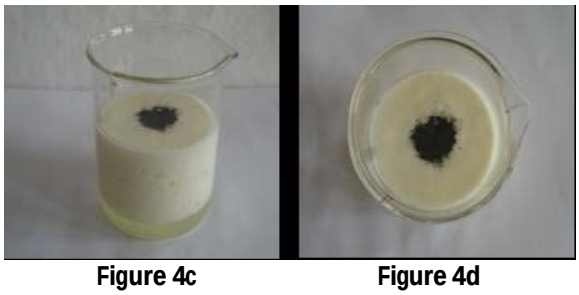


Figure 4c **Figure 4d**

Incompletely processed Tamra Bhasma:

On day one:

Figure 4e **Figure 4f**

After 24 hours:

Figure 4g **Figure 4h**

Results of *In-vivo* Studies

WBC Counting

WBC count was significantly increased in animals treated with incompletely processed Tamra bhasma compared to control animals and animals treated with completely processed Tamra bhasma. Increase in the WBC count was more significant with dose 1 than at dose 2 in animals treated with incompletely processed Tamra bhasma. Although, decrease in the WBC count observed in animals treated with completely processed bhasma, the decrease in WBC count was least significant. (Table 3 and Figure 5)

SGOT Concentration

The decrease in SGOT levels were significant in animals received processed Tamra bhasma at dose 1 and 2. The SGOT levels were increased in all groups receiving incompletely processed Tamra bhasma. The increase in the enzyme level in animals treated with incompletely process bhasma was not significant except in 60 days treatment schedule. (Table 3 and Figure 6).

SGPT Concentration

In all groups the SGPT level was reduced, the reduction in SGPT level was significant only in animals treated with processed Tamra bhasma at dose 2. There was a significant increase in the SGPT levels observed in animals treated with incompletely processed Tamra bhasma at dose 1 in 30 days treatment schedule. (Table 3 and Figure 7).

Copper in Serum

Increased concentration of copper in serum was highly significant in groups treated with incompletely processed Tamra bhasma at both dose 1 and 2. But in groups

treated with processed Tamra bhasma at both doses were not shown any significant changes in serum copper concentration for 30 and 60 days treatment schedule. In animals treated for 90 days, the increase in the copper concentration in serum was significant. (Table 4 and Figure 8).

Table 2: Comparative results of Physical and Chemical tests of Completely processed and Incompletely processed Tamra bhasmas.

Parameters	Tamra Bhasma (processed)	Tamra Bhasma (Incompletely processed)
Colour	Black	Dark ash colored
Fineness	Moderately fine	Moderately fine
Rekhapurnathwa	Some particles could enter furrows of the finger	Some particles could enter furrows of the finger
Gatarasatwa	Tasteless	Tasteless
Varitaratwa	Floatable in water (Figure 3a-3b)	Sink in the water (Figure 3c-3d)
Reaction with Curd	There was no change in the color of the curd was observed after 24 hours of contact (Figure 4a -4d)	There was bluish green color developed around the bhasma in curd after 24 hours (Figure 4e-4h)

Table 3: Results of WBC count and SGOT and SGPT determinations at all the treatment schedules.

30 days treatment schedule				
S.NO	**Groups**	**WBC count**	**SGOT (IU/L)**	**SGPT (IU/L)**
1	NORMAL	12770 ± 1864	64.83 ± 3.928	48.00 ± 0.3651
2	TBP D I	7492 ± 754.3*	65.33 ± 3.293*	47.50 ± 0.5627**
3	TBP D II	8425 ± 962.4	55.00 ± 0.7303	46.17 ± 0.3073
4	TBIP D I	26640 ± 3582**	71.17 ± 1.195	52.83 ± 1.515*
5	TBIP D II	17850 ± 2058	68.83 ± 0.4773	48.33 ± 1.085
60 days treatment schedule				
S.NO	**Groups**	**WBC count**	**SGOT (IU/L)**	**SGPT (IU/L)**
1	NORMAL	7917 ± 611.8	56.67 ± 0.3333	48.17 ± 0.9804
2	TBP D I	8067 ± 655.4	58.50 ± 0.6191*	47.33 ± 0.8028
3	TBP D II	9008 ± 488.4	57.00 ± 0.8165	47.83 ± 0.7032
4	TBIP D I	19280 ± 1449***	73.17 ± 1.195***	48.00 ± 1.000
5	TBIP D II	12040 ± 1220*	75.67 ± 1.256***	49.00 ± 0.8165
90 days treatment schedule				
S.NO	**GROUPS**	**WBC count**	**SGOT (IU/L)**	**SGPT (IU/L)**
1	NORMAL	12480 ± 988.8	74.17 ± 3.859	53.50 ± 1.607
2	TBP D I	8817 ± 664.8*	60.17 ± 2.548*	49.50 ± 0.9220
3	TBP D II	10010 ± 1129	62.67 ± 1.116*	48.67 ± 0.6146*
4	TBIP D I	14280 ± 626.7	81.67 ± 1.585	50.50 ± 1.500
5	TBIP D II	9092 ± 823.1*	80.50 ± 1.544	50.67 ± 0.8028

Table 4: Concentration of Copper in Serum, Liver and Kidney after all treatment Schedules

COPPER CONCENTRATION (30 DAYS TREATMENT SCHEDULE)						
S.No	**PARAMETERS**	**NORMAL**	**TBP D I**	**TBP D II**	**TBIP D I**	**TBIP D II**
1	Copper in serum µg/dl	149.7 ± 9.117	154.3 ± 2.996	148.7 ± 8.800	206.8 ± 6.253**	189.5 ± 6.244***
2	Copper in liver µg/500mg	23.09 ± 0.04333	23.09 ± 0.6589	21.91 ± 0.2200**	22.21 ± 1.074	30.99 ± 1.890*
3	Copper in kidney µg/500mg	19.95 ± 1.283	20.03 ± 0.5763	19.00 ± 0.6058	20.72 ± 1.239	20.83 ± 1.371
COPPER CONCENTRATION (60 DAYS TREATMENT SCHEDULE)						
S.No	**PARAMETERS**	**NORMAL**	**TBP D I**	**TBP D II**	**TBIP D I**	**TBIP D II**
1	Copper in serum µg/dl	192.0 ± 3.055	176.3 ± 7.940	181.8 ± 5.793	229.3 ± 3.887***	234.0 ± 3.416***
2	Copper in liver µg/500mg	23.26 ± 0.3157	23.44 ± 1.216	21.30 ± 0.8300	25.27 ± 0.8713	33.43 ± 0.8289***
3	Copper in kidney µg/500mg	20.94 ± 0.6850	16.36 ± 1.354*	18.78 ± 0.9590	18.89 ± 0.1100*	23.03 ± 0.4819
COPPER CONCENTRATION (90 DAYS TREATMENT SCHEDULE)						
S.No	**PARAMETERS**	**NORMAL**	**TBP D I**	**TBP D II**	**TBIP D I**	**TBIP D II**
1	Copper in serum µg/dl	165.5 ± 12.33	205.5 ± 12.18*	216.2 ± 10.35*	271.0 ± 10.15***	272.3 ± 9.715***
2	Copper in liver µg/500mg	22.65 ± 1.530	22.57 ± 0.3414	22.70 ± 0.3060	28.85 ± 1.454*	35.48 ± 1.549**
3	Copper in kidney µg/500mg	19.15 ± 0.7024	18.05 ± 0.5094	21.13 ± 0.6967	22.48 ± 1.926	25.67 ± 1.529*

Note: Students t test, * p<0.05, **p<0.01, ***p<0.001Vs control.
TBP D I: Tamra Bhasma (processed) dose I, TBP D II: Tamra Bhasma (processed) dose II,
TBIP DI: Tamra bhasma (incompletely processed) dose I, TBIP D II: Tamra bhasma (incompletely processed) dose II

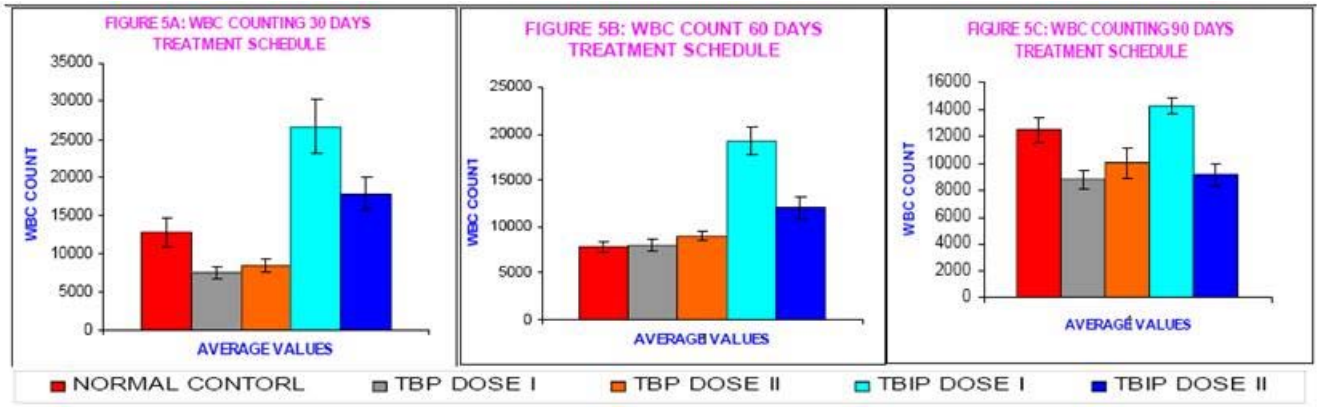


Figure 5: Results of WBC count at all treatment schedules

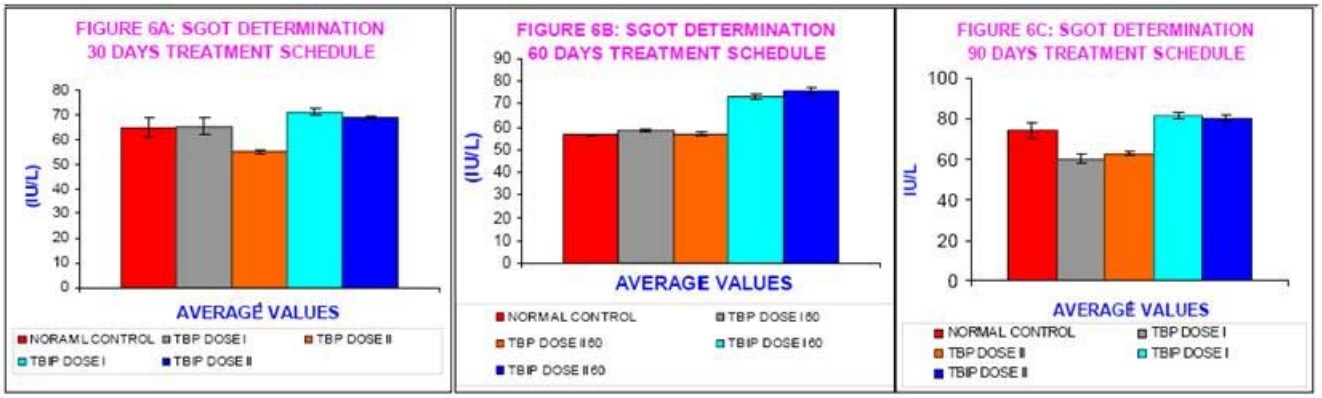


Figure 6: Results of SGOT determination at all treatment schedules

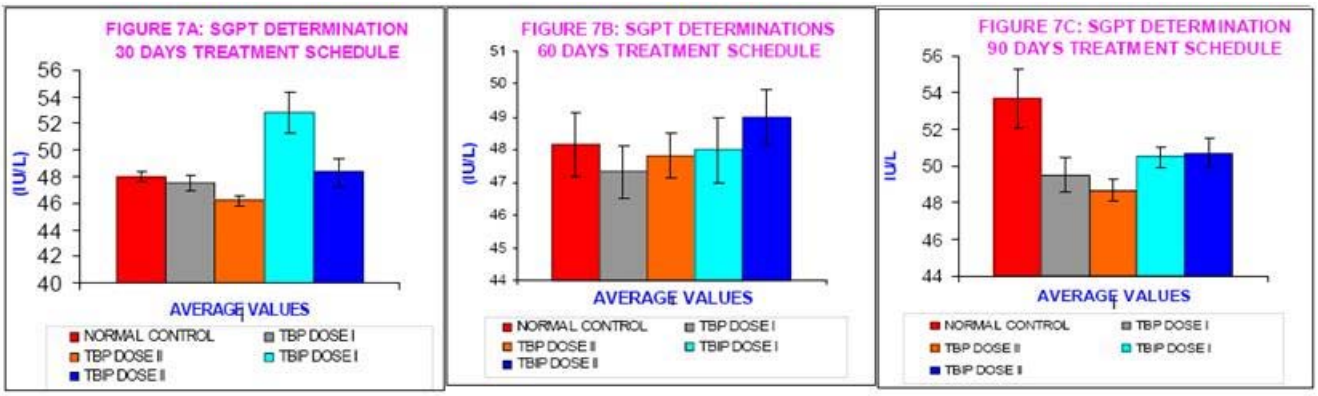


Figure 7: Results of SGPT determination at all treatment schedules

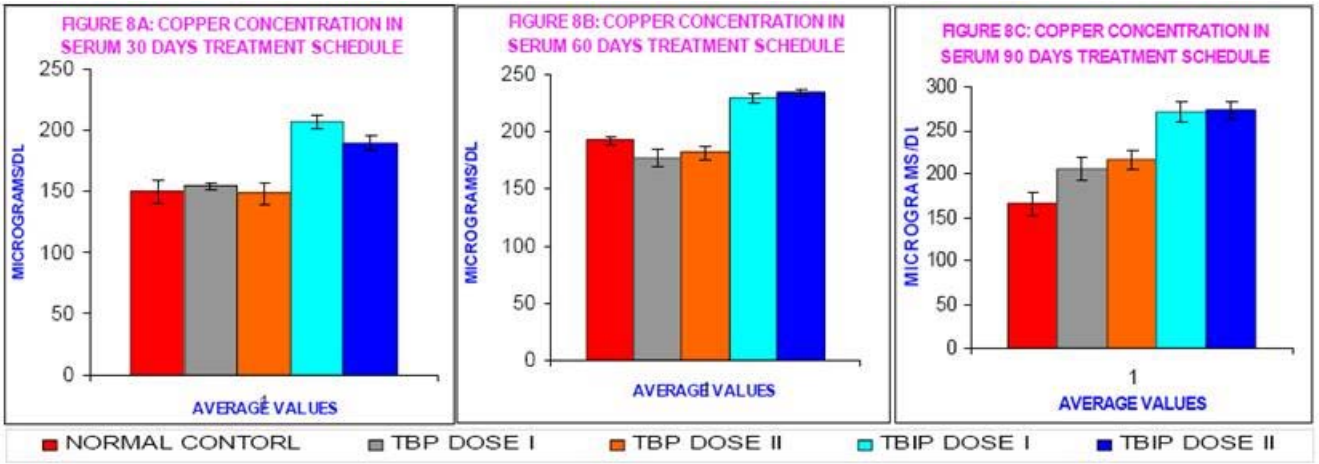


Figure 8: Determination of copper concentration in serum at all treatment schedules

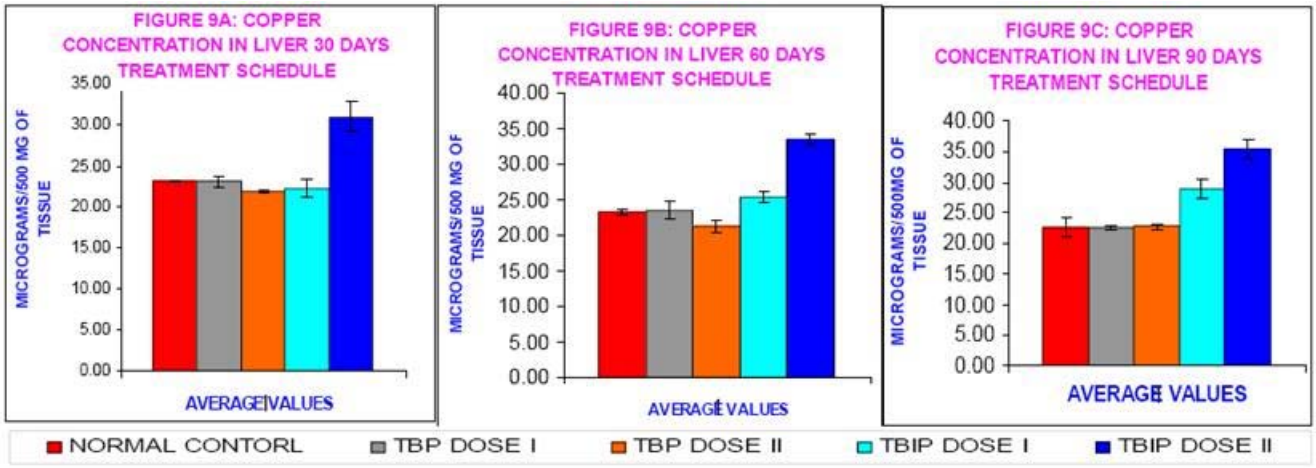


Figure 9: Determination of copper concentration in liver tissue at all treatment schedules

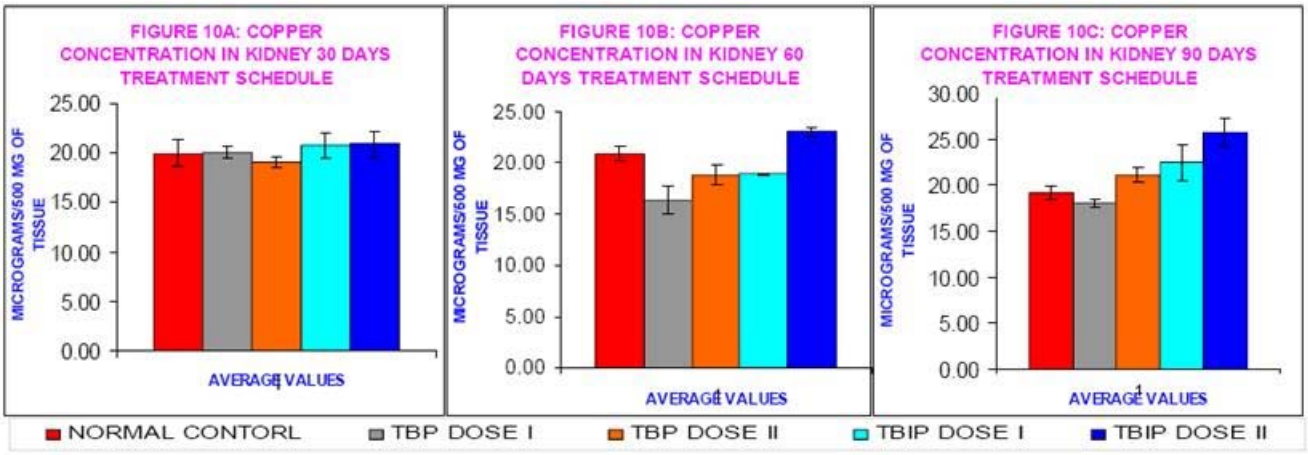


Figure 10: Determination of copper concentration in kidney tissue at all treatment schedules

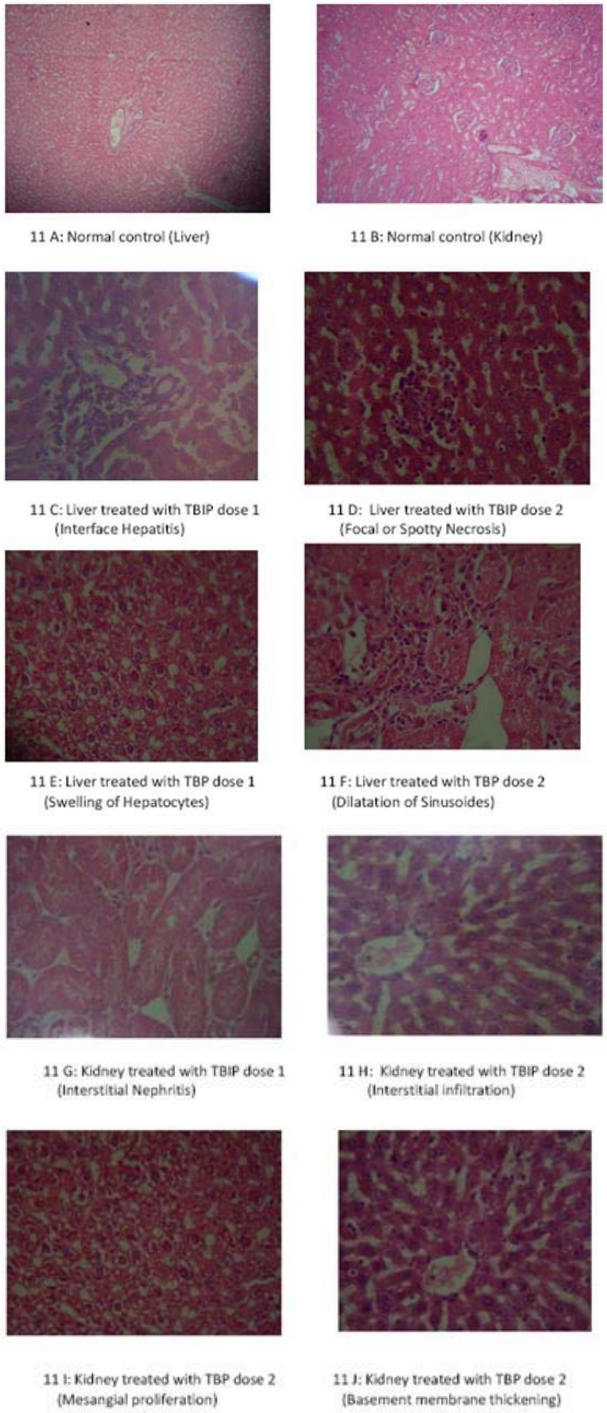
Table 5: Results of Histopathological studies in Liver tissue

S. No	Groups	Histological changes in liver
1	Normal	Normal
2	TBP DI	Interface hepatitis, Intracytoplasmic eosinophilic infusions, Swelling of Hepatocytes, Wire loop appearance and Mild steatosis.
3	TBP D II	Portal tract inflammation, Interface hepatitis, Swelling of Hepatocytes, Dilatation of sinusoides and Mild steatosis.
4	TBIP D I	**Interface hepatitis, **Swelling of Hepatocytes, Focal or spotty necrosis and Interface hepatitis.
5	TBIP D II	Enlarged Hepatocytes, **Cellular swelling, Balloon degeneration, **Prominent sinusoids, Interface hepatitis, edema and **Focal or spotty necrosis.

Table 6: Results of Histopathological studies in Kidney tissue

S. No	Groups	Histological changes in kidney
1.	Normal	Normal
2.	TBP D I	Basement membrane thickening, Tubular atropy with necrosis, Atropy of glomeruli, Wire loop appearance, Tubular epithelial disruption.
3.	TBP D II	**Tubular atropy, Increase in eosinophilia, Tubular vacculation, Basement membrane thickening, **Wire loop appearance, Focal atropy and Mesangial cell proliferation.
4.	TBIP D I	Tubular necrosis, Prominent capillary tubes, Increase eosinophilia, Atropy of glomeruli, Increased vacculation, Disruption of tubules, **Mesangial cell proliferation, **Wire loop appearance, ** Increase eosinophilia, and **Interstitial Nephritis
5.	TBIP D II	Atropy of Glomeruli, Basement membrane thickening, Cellular swelling, Interstitial edema with inflammation, **Wire loop appearance, **Increase eosinophilia, Edema, **Mesangial cell proliferation and **Globalization.
**** prominent changes** TBP D I: Tamra Bhasma (processed) dose I, TBP. D II: Tamra Bhasma (processed) dose II, TBIP D I: Tamra bhasma (incompletely processed) dose I, TBIP D II: Tamra bhasma (incompletely processed) dose II		

Figure 11: Results of Histopathological studies



Copper in Liver

There was significant increase in liver copper concentration in animals treated with incompletely processed Tamra bhasma at dose 1 and 2. But no significant changes in liver copper concentration of animals treated with processed Tamra bhasma at dose 1 and 2. (Table 4 and Figure 9)

Copper in Kidney

Increase in kidney copper concentration was significant only in animals treated with incompletely processed Tamra bhasma at dose 2. But changes in other groups were not significant. (Table 4 and Figure 10)

Results of Histopathological studies

The liver and kidney tissues were used for the histopathological studies; the tissues were processed to prepare slides. The slides were used for the interpretation of the results. The results of the histopathological studies are given in table 5, 6 and figure 11.

DISCUSSION

There is burning desire for the drugs in all times for the treatment of different diseases. The drugs used for the therapeutic purpose should not cause serious adverse effects or serious damages. The switching of people from modern medicine to Ayurvedic medicine is increasing because of the serious adverse effects and cost of modern medicine system. The bhasmas are one among Ayurvedic preparations used for many disorders without producing serious adverse effects in ancient days. The preparation of bhasmas involves different methods and steps, these methods and steps are very laborious and time consuming. It is bit difficult to manufacture bhasmas and to follow certain conditions of the preparations as given in the ancient texts. It is also noted that the cost of the preparation increases with the more stringent manufacturing process to produce good quality bhasmas.

To make Bhasma cost-effective, nowadays Ayurvedic manufacturers may stop processing of bhasmas abruptly. Although it reduces the duration of time to prepare Bhasma, have a great impact on the therapeutic effect of the bhasma prepared by this way. In spite of having impact on therapeutic activity of bhasma, it certainly leads to serious adverse effects. This study aims to differentiate the effects of fully processed and incompletely processed Tamra bhasma on animals. The findings included in this study are determination of SGOT, SGPT and Copper concentrations in serum followed by in liver and kidney homogenates. Histopathological studies were also carried out.

There was significant increase in the SGOT and SGPT levels in animals treated with incompletely processed Tamra bhasma. The Processed Tamra bhasma is one it is claimed to have Hepatoprotective activity and our study supported the same. But if Bhasma is not processed, it increases the enzyme levels and cause hepatotoxicity. This clearly indicates that, the incompletely processed bhasmas are very harmful and may cause liver or kidney damage.

There was more significant increase in copper in serum of animals treated with incompletely processed Tamra bhasma. The increase in copper level was dose dependant and also depends on the duration of treatment (30, 60 and 90 days) when compared to animals treated with completely processed Tamra bhasma. This clearly indicates the differential effects of the processed and incompletely processed bhasmas.

The concentration of copper in liver and kidney homogenates were also increased more significantly in animals treated with incompletely processed Tamra

bhasma when compared to control animals and animals treated with processed bhasma. The increase in copper concentration in liver homogenate was highly significant in 60 and 90 days treatment schedules. The increase in copper concentration in kidney homogenate was significant in 90 days treatment schedule. This clearly indicates that, the long term treatment is very harmful with incompletely processed bhasmas. Increase in copper levels is also found in animals treated with processed bhasmas at higher doses. But they are less significant when compared to incompletely processed bhasmas.

The above mentioned changes are also supported by histological studies in kidney and liver. Histological changes suggest that, more prominent changes were seen in animals treated with incompletely processed bhasma when compared to completely processed bhasma.

CONCLUSION

Tamra bhasma is used to treat/manage many disorders/diseases. It is one of the abundantly used bhasma for its therapeutic value. Processing of bhasma is very crucial in deciding whether, the bhasma is useful or not. The incompletely processed Tamra bhasma showed many toxic effects when compared to processed bhasma. In this study, the toxicity of the incompletely processed bhasma is evaluated, with respect to the serum levels of SGPT, SGOT and copper. In addition, copper concentration in liver and kidney homogenates in treated animals were also evaluated.

Incompletely processed bhasma shown to have significant increase in SGPT, SGOT and copper concentration in serum of animals and increased copper concentration in liver and kidney homogenates. This increase in copper accumulation in kidney and liver indirectly increases the concentration of SGPT, SGOT in serum. The accumulated copper caused damages to the kidney and liver which was supported by histopathological studies. The histological changes in animals treated with incompletely processed bhasma were more prominent when compared to normal and processed bhasma treated animals.

These findings suggest that, the incompletely processed bhasmas are very toxic if used clinically. To get beneficial effects of bhasmas, it is must to process the bhasma completely. The further experimental studies are must to evaluate effects of the incompletely processed bhasmas on different organs and at different doses. As this study utilizes the calorimetric analysis, there is need for better analytical method suitable for precise analysis of metals and their preparations.

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