Original Article

# Effect of *Tamra Bhasma* (Calcined Copper) on Ponderal and Biochemical Parameters

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## ABSTRACT

**Introduction:** *Tamra Bhasma* (TB) and its forms like *Somnathi Tamra Bhasma* (STB), etc., are in vogue since centuries in *Ayurveda*. The present study is carried out to evaluate the effect of TB and STB in different dose levels on ponderal and biochemical parameters in wistar strain albino rats to provide scientific basis for its safety profile. **Materials and Methods:** TB and STB were prepared as per the classical guidelines and administered to wistar strain albino rats for 45 consecutive days. Blood was collected and rats were sacrificed on the 46$^{th}$ day. Ponderal and biochemical parameters were studied. **Results:** Results showed significant decrease in serum cholesterol, High Density Lipoprotein (HDL) cholesterol, triglycerides, total protein, and serum alkaline phosphatase levels. Comparatively, all the differences in between the groups are insignificant and no pathological changes at ponderal and biochemical levels were observed. **Conclusion:** Based on these observations, it can be said that these formulations can be safely used in cases of hyperlipidemia.

**Key words:** *Bhasma*, biochemical parameters, copper, ponderal parameters, *Somnathi Tamra Bhasma*, *Tamra Bhasma*

## INTRODUCTION

Metals (like mercury, iron, copper, lead, zinc, etc.) and minerals (like mica, arsenic, chalcopyrite, etc.) in the form of *Bhasmas* are an integral part of Ayurvedic therapeutics. As these *Bhasmas* are prepared by following the classical procedures of repeated calcinations, they are chemically mixed with oxides of one or more metals[1] and are associated with a number of trace elements. Therapeutic utility of properly processed *Bhasmas* and their hazardous effects under inappropriate use when used in impure form is well documented in Ayurveda.[2] Despite of this, concerns are being expressed frequently regarding the metal toxicity and safety of traditional preparations containing *Bhasmas*.[3-6] *Tamra Bhasma*, one of such metallic preparations of *Ayurveda* is useful in the treatment of *Udara* (ascitis), *Pandu* (anemia), *Svasa* (bronchial asthma), and *Amlapitta* (hyperacidity), etc.[7] It is an integral component in Ayurvedic formulations like *Kalyansundara Rasa*, *Hridayarnava Rasa,* etc., used for cardiac and lipid disorders.[8,9]

*Tamra* is attributed with *Ashtamahadoshas* (eight blemishes).[10] Hence, one has to be careful while handling this metal. Though, the role of incinerated copper in hepatoprotection and lipid peroxidation is reported, effect on biochemical parameters is not reported.[11] Considering this, the present study is aimed at screening the ponderal and biochemical changes in Swiss albino rats after administration of *Tamra Bhasma* (TB) and *Somnathi Tamra Bhasma* (STB) at different dose levels.

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## MATERIALS AND METHODS

### Test drugs

Both the trial drugs were prepared in the laboratory of *Rasashastra* and *Bhaishajyakalpana*, Institute for Post Graduate Teaching and Research in Ayurveda (I.P.G.T. and R.A), Gujarat Ayurved University, Jamnagar by following standard guidelines as prescribed in classical Ayurvedic literature.

Copper wire with 99.89% pure copper was procured from Amber Electricals, Jamnagar. It was processed through classical procedures of *Shodhana* (purification procedure), *Marana* (incineration process), and *Amritikarana* (necterization process) to prepare *Tamra Bhasma* and labeled as *Shodhita Tamra* (SHTB).[12-14] Another sample was processed for *Marana* avoiding the initial steps of *Shodhana* and labeled as *Ashuddha Tamra* (ATB). STB, another familiar copper formulation was prepared by *Kupipakva* method.[15]

### Animals

Wistar strain albino rats of either sex weighing 200 ± 20 g were obtained from the animal house attached to the pharmacology laboratory, I.P.G.T. and R.A, Gujarat Ayurved University, Jamnagar and were exposed to natural day and night cycles with ideal laboratory conditions in terms of ambient temperature and humidity. Animals were fed *ad libitum* with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water. The experiment was carried out after obtaining permission from Institutional Animal Ethics Committee (IAEC 07/2010/05/MD) and care of animals was taken as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

### Dose fixation and schedule

The animal dose for rats was calculated by considering therapeutic doses of trial drugs (TB, STB) and referring to table of Paget and Barnes.[16] On this basis, dose of both the test drugs for rats was found to be 5.5 mg/kg and 13.5 mg/kg. The test drugs were administered in the form of suspension in distilled water orally with the help of rubber catheter attached to a disposable syringe. For the preparation of stock solution, both the test drug samples were taken in requisite quantity in small porcelain mortar and 0.5 ml of 5% gum acacia suspension was added, grounded for 5 minutes and the volume was made up with distilled water, so as to contain 5.5 mg/ml and 13.5 mg/ml test drugs.

### Experimental design

Rats were randomly assigned into eight groups. Group I served as positive control (water control, WC) receiving tap water and normal food. Group II, III, IV received TB prepared from ATB in different doses and Group V, VI, VII received TB prepared from SHTB in different doses. Group VIII received STB at five Therapeutically Equivalent Dose (TED) levels [Table 1]. Body weight of all the animals was recorded initially and at the end of the study. General behavioral pattern was observed on every week by exposing each animal to an open arena. At the end of experimental period, all the animals were euthanized and gross pathological observations were performed.

### Serum biochemical analysis

At the end of experimental period, animals were anesthetized with diethyl ether and blood was collected from supraorbital plexus in plain tube for serum biochemical investigations, including blood sugar, urea, creatinine, cholesterol, triglycerides, HDL, bilurubin, serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), S. alkaline phosphatase (SAP), total protein, and uric acid were analyzed by auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai)

### Statistical analysis

The results were presented as Mean ± SEM in each group. Statistical comparisons were performed by both paired, unpaired Student's *t*-test, and one-way analysis of variance (ANOVA) with Dunnets' multiple *t*-test as post-hoc test by using Sigma stat software (version 3.1) for all the treated groups with the level of significance set at $P < 0.05$.

## RESULTS AND OBSERVATIONS

### Body weight

Insignificant weight gain was observed in control group while the weight was insignificantly reduced in other groups except Group II. Animals treated with ATB loose body weight significantly [Table 2].

**Table 1: Test drug posology**

| Group | No of animals | Drug | Dose (mg/kg) | Duration |
|---|---|---|---|---|
| I | 6 | WC | - | 45 days |
| II | 6 | ATB | 5.5 | |
| III | 6 | | 27.5 | |
| IV | 6 | | 55 | |
| V | 6 | SHTB | 5.5 | |
| VI | 6 | | 27.5 | |
| VII | 6 | | 55 | |
| VIII | 6 | STB | 67.5 | |

Abbreviation: WC = Water control, ATB = *Ashuddha Tamra Bhasma*, SHTB = *Shodhita Tamra Bhasma*, STB = *Somnathi Tamra Bhasma*

### Biochemical parameters

HDL was found to be reduced with all dose levels of ATB and with 10 TED of SHTB. Physiological levels of HDL were maintained with the treatment of other trial drugs. Insignificant changes were observed in blood glucose, serum cholesterol, and triglycerides in all groups. Animals treated with SHTB TED and SHTB 5 TED showed significant increase in serum creatinine and decrease in serum alkaline phosphatase (ALP). STB-treated group was also found be significant in decreasing ALP level. All other biochemical parameters were not affected to a significant extent in all the treated group in comparison to the control group [Table 3].

## DISCUSSION

Metals may be toxic in their native or free form but not their *Bhasmas* because they have different compound forms. Thus, they are incorporated in herbomineral formulations for their specific therapeutic role and used successfully in the treatment of many diseases since a long period. Though metallic preparations are therapeutically used since long, there is a need to document their safety profiles.

TB is one among such herbometallic formulations used for treatment of anemia, cardiac, liver, and lipid-related disorders as an important ingredient in compound formulations or singly. As seers of Ayurveda claimed its therapeutic effectiveness in above pathological manifestations, the present study was designed to assess comparison of ponderal and biochemical parameters of TB and STB. Non-significant decrease in the body weight was observed in all samples. These results justify the role of TB in *Lekhana* (scraps excessive fat) property.

Significant decrease in serum HDL cholesterol level was found in ATB TED, ATB TED × 5 and SHTB TED × 10 groups. This showed that they may impair the transfer of cholesterol from both very-low-density lipoprotein (VLDL) and tissue to HDL fraction or it may be promoting the metabolism of this fraction by enhancing the activity of the key enzymes involved in HDL cholesterol metabolism. In contrast to this, SHTB TED and TED × 5 did not show any significant changes in HDL level [Figure 1].

Administration of TB in ATB TED, SHTB TED, and SHTB, STB-treated groups at TED × 5 dose levels showed significant decrease in serum ALP level but they did not affect this enzyme activity to significant extent at higher dose levels, hence, the extra hepatic cause for decreased activity of this enzyme may be involved. Changes in SGOT, SGPT, bilurubin level (total and direct) were found to be statistically insignificant [Figure 2]. Both ATB and SHTB group showed significant decrease in serum total protein level only at higher doses (TED × 10) but it is to be noted that they did not produce any significant changes at TED and even at 5 TED in all other test drugs, showing importance of dosage forms in drug toxicity [Figure 3].

**Table 2: Effect of test drugs on the body weight of albino rats recorded during toxicological study**

| Group | Treatment | Body weight (g) | | 't' value | 'P' value |
|---|---|---|---|---|---|
| | | Initial | Final | | |
| I | Water control | 208.33±6.5 | 226.3±17.5 | −1.046 | 0.344 |
| II | TED ATB | 215.0±14.3 | 190.7±16.8 | 5.255 | 0.003*@ |
| III | TED×5 ATB | 196.7±3.3 | 181.8±11.3 | 1.361 | 0.232 |
| IV | TED×10 ATB | 203.3±8.8 | 184.7±8.5 | 2.189 | 0.08 |
| V | TED SHTB | 215.0±5.0 | 212.4±9.1 | 0.581 | 0.593 |
| VI | TED×5 SHTB | 211.7±9.8 | 201.0±10.09 | 1.661 | 0.158 |
| VII | TED×10 SHTB | 208.3±10.1 | 209.3±7.5 | −0.123 | 0.907 |
| VIII | TED×5 STB | 202.00±3.9 | 199.7±3.630 | 1.053 | 0.341 |

Data: Mean±SEM, *$P$<0.05 (Paired $t$-test), @$P$<0.05 (ANOVA test). ATB = *Ashuddha Tamra Bhasma*, SHTB = *Shodhita Tamra Bhasma*; STB = *Somnathi Tamra Bhasma*; TED = Therapeutically equivalent dose

**Table 3: Effect of test drugs on biochemical parameters of albino rats recorded during toxicological study**

| Parameters | NC | TED ATB | TED×05 ATB | TED×10 ATB | TED SHTB | TED×05 SHTB | TED×10 SHTB | TED×5 STB |
|---|---|---|---|---|---|---|---|---|
| Blood glucose (mg/dL) | 117.5±8.9 | 108.7±2.1 | 116.8±5.5 | 110.9±4.2 | 108.2±9.7 | 116.0±12.4 | 98.5±15.5 | 99.167±2.651 |
| S. cholesterol (mg/dL) | 77.5±9.9 | 63.3±6.02 | 51.5±4.4* | 56.0±3.7 | 91.2±8.2 | 78.5±16.5 | 53.8±5.6 | 63.500±5.920 |
| S. triglyceride (mg/dL) | 97.5±11.9 | 61.7±6.9* | 93.0±11.07 | 94.2±10.4 | 121.2±22.6 | 87.0±14.6 | 124.3±18.9 | 93.0±8.77 |
| S. HDL (mg/dL) | 39.2±5.4 | 26.3±2.2* | 24.8±2.2*@ | 27.0±3.3 | 37.3±3.7 | 32.7±3.7 | 24.0±3.3*@ | 33.67±3.242 |
| S. Urea (mg/dL) | 100.3±11.4 | 112.0±10.5 | 114.8±11.8 | 115.8±12.6 | 96.0±5.7 | 97.3±6.9 | 83.0±3.4 | 83.33±7.149 |
| S. creatnine (mg/dL) | 0.6±0.2 | 0.6±0.07 | 0.6±0.03 | 0.6±0.06 | 0.7±0.03* | 0.7±0.03* | 0.6±0.03 | 0.67±0.03 |
| S.G.P.T. (IU) | 77.3±6.9 | 89.0±9.8 | 93.5±4.08 | 83.7±6.2 | 62.7±3.4 | 70.8±8.3 | 86.2±15.3 | 73.83±4.722 |
| S.G.O.T. (IU) | 332.0±42.2 | 279.2±9.9 | 321.7±25.6 | 335.3±54.9 | 246.5±9.9 | 302.0±19.9 | 309.0±36.6 | 283.33±27.85 |
| Total protein (g/dL) | 7.6±0.3 | 7.9±0.26 | 7.15±0.1 | 6.9±0.1* | 7.7±0.2 | 7.5±0.1 | 6.7±0.2*@ | 7.45±0.118 |
| S. Alkaline phosphatase (IU/L) | 236.2±20.7 | 176.7±16.4* | 300.3±60.3 | 250.0±29.8 | 146.7±11.4**@ | 146.8±30.3*@ | 170.3±34.72 | 146.5±5.277*@ |
| S. bilirubin (T) (mg/dL) | 0.7±0.2 | 0.9±0.2 | 0.6±0.1 | 0.9±1.1 | 0.5±0.04 | 0.5±0.04 | 0.5±0.04 | 0.467±0.0422 |
| S. bilirubin (D) (mg/dL) | 0.2±0.04 | 0.3±0.6 | 0.2±0.03 | 0.7±0.5 | 0.15±0.02 | 0.15±0.02 | 0.2±0.2 | 0.150±0.0224 |
| S. Uric acid (mg/dL) | 2.1±0.4 | 2.6±0.5 | 1.7±0.3 | 2.7±0.4 | 1.6±0.1 | 1.6±0.2 | 1.9±0.4 | 1.233±0.196 |

Data: Mean±SEM, *$P$<0.05 (unpaired $t$ test), @$P$<0.05 (ANOVA test). ATB = *Ashuddha Tamra Bhasma*, SHTB = *Shodhita Tamra Bhasma*, STB = *Somnathi Tamra Bhasma*, TED = Therapeutically Equivalent Dose, SGPT = Serum glutamic pyruvic transaminases, SGOT = Serum glutamic oxaloacetic transaminase

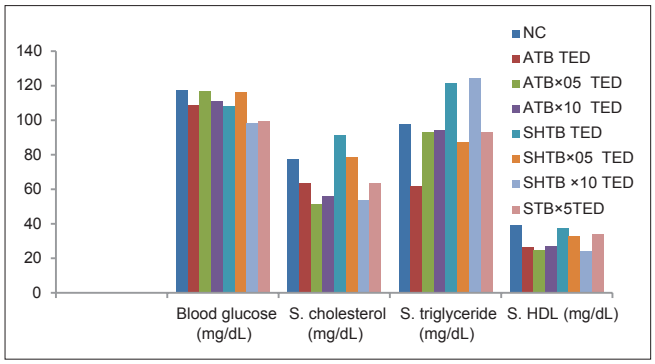


**Figure 1:** Effect of *Tamra Bhasma* on biochemical parameters related to lipid metabolism

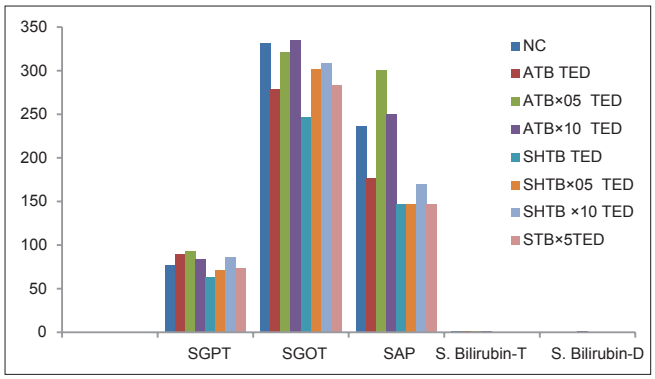


**Figure 2:** Effect of *Tamra Bhasma* on biochemical parameters related to liver functions

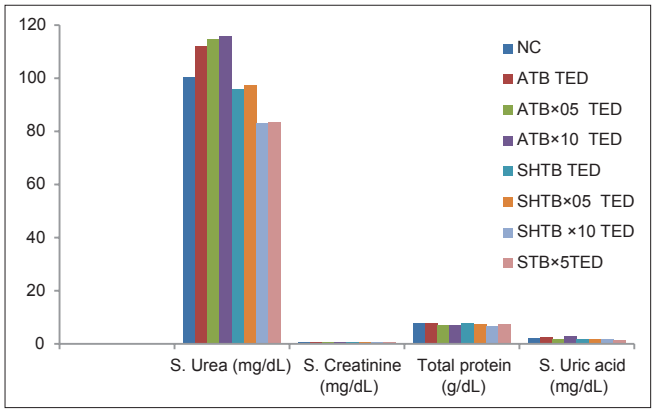


**Figure 3:** Effect of *Tamra Bhasma* on biochemical parameters related to renal functions

It indicates that the drug has no significant effect on parameters related to liver function when administered for 45 days. All these observations reveal safety of the formulations at therapeutic dose levels.

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