Comparative anti-hyperlipidemic activity of Tamra Bhasma (incinerated copper) prepared from Shodhita (purified) and Ashodhita Tamra (raw copper)

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In Ayurveda, metals are converted into Bhasmas for internal consumption by processing them through various processes like Shodhana (purification and/or detoxification), Marana (incineration), etc. and then used in the treatment of various diseases. These procedures not only decrease the possible harmful effects of metals but also said to increase their bio-availability and thus efficacy. In Rasashastra classics, due emphasis has been given to the Shodhana procedure. One of the most popularly used metallic preparations is Tamra Bhasma (incinerated copper) and it is said to be very harmful if its Shodhana is not done or if it is improperly prepared. Tamra Bhasma has been advocated in the treatment of Medoroga (lipid disorders), Hridroga (cardiac disorders), etc. and role of copper in lipid disorders is well documented fact. In the present study, a comparative anti-hyperlipidemic activity of unpurified (Ashodhita) and purified (Shodhita) Tamra was carried out to know the effect of Shodhana on efficacy. The hyperlipidemia was induced by feeding high fat diet in Wistar strain albino rats. The parameters including body weight, weight of various organs, serum lipid profile and histopathology of liver, kidney, heart and aorta were studied. The results of this study suggests that Tamra Bhasma prepared from Shodhita Tamra is having significant anti-hyperlipidemic activity, while Ashodhita Tamra Bhasma is lack of such effects. Also Ashuddha sample proved to possess cardio-toxic effect. This shows that the Rasashastriya Shodhana procedure have definite role in not only increasing the efficacy of the drug but also in removing the toxicity.

Keywords: Bhasma, Copper, Hyperlipidemia, Rasashastra, Shodhana, Tamra.

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Introduction

Hyperlipidemia is the presence of high levels of cholesterol in the blood. It is a metabolic derangement, not a disease. It can be secondary to many diseases and can contribute many forms of diseases, most notably cardiovascular diseases¹. One percent drop in serum cholesterol reduces the risk for cardiac heart disease (CHD) by two percent. Low HDL cholesterol (<35 mg/dl); obesity (>30% overweight); HDL levels (>60 mg/dl) and high LDL (>160 mg/dl) are positive risk factors for CHD while negative risk factors include high HDL levels (>60 mg/dl)². Large proportion of the deaths from cardiovascular (CV) diseases is attributed mainly to coronary heart disease and direct manifestation of atherosclerosis³. The treatment of hyperlipidemia depends on the patient’s cholesterol profile. Statin, fibrates, niacin, bile acids, ezitimibe etc. are the antihyperlipidemic agents which reduce cholesterol level with different condition¹.

In Ayurveda, different formulations are in use for the treatment of Medoroga (lipid disorders) since centuries. Advent of Rasashastra (Ayurvedic pharmaceutics) from 7 A.D. onwards successfully made the usage of many metals, minerals, gems, poisonous substances, etc in the treatment of various diseases. To make them suitable for human use, these substances should undergo Shodhana (purification and/or detoxification) procedure as described in classics of Rasashastra. These processes have dynamic effect on the pharmacological activities of the drug.

Tamra Bhasma (TB) (incinerated copper) is one of such drug which is widely used in treatment of Kushtha (Skin disorders), Kshaya (General debility), Pandu (Anaemia), Sthauylya (Obesity), Netrarogas (eye diseases), etc⁴. Various formulations having

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TB as an ingredient are indicated in the treatment of Hridrogas (cardiac disorders), viz. Hridayarnava Rasa, Prabhakara Vati, Kalyana Sundara Rasa. These formulations are frequently being practiced by the Ayurvedic physicians in cardiac disorders and the efficacy is anticipated because of the presence of TB. Lipids and cholesterol are directly related to obesity (Sthaulya) Tamra (copper) has been described as Medopaha (destroyer of fat/lipids), Lekhana (scraps excessive fat), Sthaulyapaha (destroyer of obesity) in different texts of Rasashastra. In modern research also it is found that there is a special role of copper in lipid metabolism, its deficiency raises blood cholesterol and a diet high in copper has a beneficial effect on blood cholesterol. These references directly indicate it as a lipid lowering agent.

Screening of literature revealed that, till date no pharmacological work has been reported on the role of Shodhana procedure of metals on their efficacy especially on TB. Hence to know the effect of Shodhana on efficacy, in the present study a comparative anti-hyperlipidemic activity of unpurified (Ashodhita) and purified (Shodhita) Tamra was carried out.

Materials and Methods

Test drugs
Copper wire of 99.89% purity was procured from local electrician. TB was prepared from this copper wire by subjecting it to Samanya (general), Vishesha (specific) Shodhana, Marana and Amritikarana procedures (coded as STB). Another sample of TB was prepared from the same copper wire by following only Marana and Amritikarana procedures without subjecting to the Shodhana procedure (coded as ATB).

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. & R.A, Jammagar. They were exposed to natural day and night cycles, with ideal laboratory conditions in terms of ambient temperature (22 ± 02°C) and humidity (50-60%). 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 the permission from Institutional Animal Ethics Committee (Approval number: IAEC 07/2010/05/MD) and care of animals was taken as per the CPCSEA guidelines.

Dose fixation and schedule
The clinical dose of TB as mentioned in classics is 60 mg per day. The suitable dose for rats was calculated by referring to table of Paget and Barnes (1964) which becomes 5.5 mg/kg. The test drugs (ATB and STB) were made fine suspension in deionized water by adding few drops of gum acacia as suspending agent to suitable concentration depending up on body weight. The test drugs were administered orally with the help of gastric catheter sleeved to syringe.

Anti-hyperlipidemic activity evaluation
The selected animals were divided into four groups of six animals each. First group was kept as normal control (NC) which received only deionized water. To second group hyperlipidemic diet was administered and served as cholesterol control (CC) group. Third group received hyperlipidemic diet and ATB (5.5 mg/kg), while fourth group received hyperlipidemic diet and STB (5.5 mg/kg). Test drugs were administered at morning hour and hyperlipidemic diet was administered at evening hours for 20 consecutive days. The hyperlipidemic diet included hydrogenated vegetable oil (Vanaspati Ghee - ‘Raag’ brand, Batch No. BA 70, Adani Wilmar Ltd., Gujarat) and cholesterol extra pure powder (Batch No. 14022, Suvridinath Laboratories, Baroda) made in to 20% suspension in coconut oil (Parachute coconut oil, Batch No. PSO73, Goa). The suspension was administered at the dose of 0.5 mL/100 g rat. On the 21st day, after overnight fasting, the animals were weighed and blood was collected from retro-orbital plexus under ether anesthesia. Serum was collected from blood for biochemical investigations like serum total cholesterol, serum triglyceride and serum HDL cholesterol by an auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai). References given in the kit literature mentioning the basis of the methods on which test procedures were as: serum total cholesterol, serum triglyceride and serum HDL cholesterol by an auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai). References given in the kit literature mentioning the basis of the methods on which test procedures were as: serum total cholesterol, serum triglyceride and serum HDL cholesterol by an auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai). References given in the kit literature mentioning the basis of the methods on which test procedures were as: serum total cholesterol, serum triglyceride and serum HDL cholesterol by an auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai). References given in the kit literature mentioning the basis of the methods on which test procedures were as: serum total cholesterol, serum triglyceride and serum HDL cholesterol by an auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai). References given in the kit literature mentioning the basis of the methods on which test procedures were as: serum total cholesterol, serum triglyceride and serum HDL cholesterol by an auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac Medicare Pvt. Ltd., Mumbai).
were cut and each section was stained with hematoxylin and eosin. The slides were viewed under trinocular research microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues.

**Statistical analysis**

The data were expressed as mean ± standard error mean (SEM). The significance of differences among the groups was assessed using unpaired student’s t as well as one-way ANOVA followed by Dunnett’s test. P values less than 0.05 were considered as significant.

**Results**

In normal control group, progressive gain in body weight was observed in comparison to initial body weight (Table 1). In contrast significant increase in body weight was observed in cholesterol control rats in comparison to both initial values as well as water control group. Treatment with ATB failed to attenuate cholesterol rich diet induced weight gain, while treatment with STB apparently attenuated it. To know the effect of test drugs on various organs, the relative weight of liver, heart and kidney were measured and the outcome is provided in Table 2. Marked and statistically non-significant increase in relative weight of liver was observed in cholesterol control group in comparison to normal control group. Treatment with both ATB and STB failed to attenuate cholesterol rich diet induced weight gain, while treatment with STB apparently attenuated it. As an outcome, administration of exogenous cholesterol rich diet resulted in significant increase of various serum lipid profiles in cholesterol control group in comparison to control group (Table 3). Treatment with ATB did not attenuate any of parameters to significant extent, while treatment with STB significantly attenuated almost all serum lipid profiles in comparison to cholesterol control group. Histopathological section from control group shows normal cytoarchitecture of liver, kidney, heart and aorta (Plate 1a,e; Plate 2a,e). In contrast, hyperlipidemic diet produced macro and micro fatty changes in liver, cell infiltration and fatty changes in kidney and cell infiltration and fatty changes in majority of sections of heart (Plate 1b, f; Plate 2b).

Some sections of aorta of cholesterol control group shows larger tunica adventitia (Plate 2f). ATB treated group does not show any significant attenuation of pathological changes in liver, heart and kidney caused due to hyperlipidemic diet (Plate 1c, g; Plate 2 c, g), whereas STB treated group showed almost normal cytoarchitecture of liver, kidney, heart and aorta (Plate 1d, h; Plate 2c, h).

**Discussion**

Elevated levels of different types of lipids have been implicated in the production of atherosclerosis. In this condition the blood vessel wall thickens due to accumulation of lipid in its wall ensuing inflammatory reaction. This leads to loss of elasticity of blood vessel wall and becomes the cause of many cardiovascular system (CVS) complications such as myocardial infarction, stroke, peripheral vascular disease which account for significant mortality in developed and developing countries. Through extensive studies it has been proved beyond doubt that lowering the elevated plasma lipid levels is highly effective in reducing coronary artery diseases (CAD) mortality and other CVS events mentioned.

| Table 1—Effect on body weight |
|---|---|---|
| **Groups** | Initial body weight | Final body weight | Actual change in body weight (g) |
| NC | 203.0 ± 6.4 | 210.00 ± 4.10 | 07.06 ± 2.26 |
| CC | 199.0 ± 6.9 | 213.30 ± 7.30 | 14.3 ± 2.40 |
| ATB | 201.3 ± 8.5 | 229.30 ± 14.20 | 28.0 ± 7.60 |
| STB | 194.3 ± 5.5 | 203.30 ± 8.20 | 09.0 ± 7.40 |

Data: Mean ± SEM, *P<0.05 (Compared with normal control group)

| Table 2—Effect on weight of liver, heart and kidney |
|---|---|---|
| **Groups** | Weight of liver (mg/100 g) | Weight of heart (mg/100 g) | Weight of kidney (mg/100 g) |
| NC | 3061.8 ± 102.9 | 335.1 ± 6.3 | 755.0 ± 20.3 |
| CC | 3346.2 ± 209.8 | 300.19 ± 11.8 | 684.1 ± 18.6 |
| ATB | 3429.5 ± 103.5 | 370.5 ± 46.2 | 694.3 ± 23.6 |
| STB | 3550.2 ± 250.7 | 350.9 ± 28.4 | 756.2 ± 33.7 |

Data: Mean ±SEM

| Table 3—Effect of test drugs on serum lipid profile |
|---|---|---|---|---|---|---|
| Groups | Cholesterol (mg/dL) | Triglyceride (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | VLDL (mg/dL) |
| NC | 66.3 ± 3.8 | 767.6 ± 6.9 | 40.3 ± 5.3 | 41.3 ± 10.9 | 15.3 ± 3.4 |
| CC | 86.2 ± 2.7 | 1762.2 ± 8.6 | 44.0 ± 2.5 | 77.4 ± 10.0 | 35.2 ± 4.2 |
| ATB | 84.0 ± 2.7 | 1398.2 ± 14.2 | 42.3 ± 1.6 | 69.6 ± 9.0 | 27.9 ± 6.9 |
| STB | 78.7 ± 2.8 | 1392.5 ± 5.9 | 43.7 ± 2.6 | 62.8 ± 6.6 | 27.8 ± 2.9 |

Data: Mean ±SEM, *P<0.05 One Way ANOVA with Dunnet’s t test (Compared with normal control group);
*P<0.05, **P<0.01 Unpaired t test (Compared with cholesterol control group)
Plate 1—a. Photomicrographs of sections of liver from normal control group (1×400 magnification) (Hc-Hepatocytes; Kc-Kupffer cell; S-Sinusoid, Normal cyto architecture); b. Photomicrograph of sections of liver from cholesterol control group (1×400 magnification) (CI-Cell infiltration; Severe cell infiltration and micro fatty changes); c. Photomicrograph of sections of liver from ATB treated group (1×400 magnification, Comparatively less fatty changes); d. Photomicrograph of sections of liver from STB treated group (1×400 magnification, Normal cyto architecture); e. Photomicrographs of sections of kidney from normal control group (1×400 magnification) (G-Glomerulus; Ct-Convoluted tubule); f. Photomicrographs of sections of kidney from cholesterol control group (1×400 magnification) (Fc-Fatty changes; CI-Cell infiltration, Cell infiltration and micro-fatty changes); g. Photomicrograph of sections of kidney from ATB treated group (1×400 magnification); h. Photomicrograph of sections of kidney from STB treated group (1×400 magnification)
Plate 2–a. Photomicrographs of sections of heart from normal control group (1×400 magnification) (Mc-Myocardium; b. Photomicrograph of sections of heart from cholesterol control group (1×400 magnification) (Cl-Cell infiltration); c. Photomicrograph of sections of heart from ATB treated group (1×400 magnification); d. Photomicrograph of sections of heart from STB treated group (1×400 magnification); e. Photomicrographs of sections of aorta from normal control group (1×400 magnification); Smf- Smooth muscle fibre; TA- Tunica adventia; f. Photomicrographs of sections of aorta from cholesterol control group (1×400 magnification); g. Photomicrograph of sections of aorta from ATB treated group (1×400 magnification) ; h. Photomicrograph of sections of aorta from STB treated group (1×400 magnification).
Diet induced hyperlipidemia is considered as better animal model for investigating antihyperlipidemic activity because the hyperlipidemia induced by diet is more similar to human situation. Serum cholesterol levels increase by accelerating the biosynthesis of saturated fats in the diet when taken in excess, whereas diet containing polyunsaturated fatty acids lowers the cholesterol level. This may explain the significant elevation of serum cholesterol, serum triglycerides, serum LDL and serum VLDL levels in comparison to control rats on normal diet in the present study. Similarly, several other workers have also reported increased blood and tissue levels of cholesterol after feeding high fat diet for varying period.

Administration of hyperlipidemic diet led to significant increase in body weight of cholesterol control group albino rats when compared to normal control rats. Treatment with ATB failed to attenuate cholesterol rich diet induced weight gain, while treatment with STB apparently attenuated it. This indicates that the test drug STB has antagonizing effect against hyperlipidemic diet induced changes in the body weight. Administration of ATB failed to attenuate the increased serum lipid profile, while serum triglycerides, LDL and VLDL cholesterol levels were significantly attenuated by treatment with STB. The observed hypolipidemic activity of STB is further evidenced by histopathological examination of liver, heart, kidney and aorta. Organs from STB group showed significant attenuation effect on hyperlipidemic diet induced pathological changes whereas ATB not only failed to prevent hyperlipidemic diet induced pathological changes in these organs but it is also cardio toxic (evidenced by myocarditis) which is not present in cholesterol control group. The above profile indicates that STB can not only reverse hyperlipidemic diet induced changes in liver, heart and kidney, but also is devoid of cardio toxic effect.

Hypercholesterolemia, a high cholesterol diet and oxidative stress increase serum LDL levels resulting in increased risk for development of atherosclerosis. The first line defensive enzymes against the free radical produced during the oxidative stress are the antioxidant enzymes, mainly superoxide dismutase (SOD) and catalase. Like other metals copper is also considered as an essential element of body for normal physiological functions. Deficiency of copper leads to anaemia, nervous weakness, weakness in connective tissue and the hypo activity of lysyl oxidase, cytochrome C oxidase, SOD, etc. Researches have established the linkage between cholesterol metabolism and copper utilization. Hypercholesterolemia from copper deficiency in several species has been found in at least 22 independent laboratories world wide. TB is the rich source of copper. Previous studies have reported that, TB inhibits lipid peroxidation and induces the activity of SOD; thus proving it as a strong antioxidant agent.

Observed anti-hyperlipidemic activity of STB may be attributed to involvement of one or more mechanisms, viz. by interfering with the absorption of the cholesterol from dietary sources, by interfering with the re-esterification or incorporation of fatty acids to form chylomicrons in the intestinal epithelial cells, by interfering with the formation of endogenous triglycerides in the tissues by inhibiting the enzyme diacylglycerol transferase, by interfering with the transport of triglycerides from endoplasmic reticulum to microsomal site which is by microsomal triglyceride transport protein, by inhibiting the activity of the lipoprotein lipase at different sites, by inhibiting the activity of the rate limiting enzyme in cholesterol bio-synthesis - HMG-CoA (3-hydroxy 3-methyl 3-methylglutaryl CoA).

Conclusion

Tamra Bhasma prepared from Shodhita Tamra possesses significant anti-hyperlipidemic activity, while Ashodhita Tamra Bhasma is lack of it besides it is having cardio toxic property. This shows that the Rasashastra Shodhana procedure have definite role in not only increasing the efficacy of the drug, but also in removing the toxicity. Further comparative antihyperlipidemic activity with established antihyperlipidemic drug is required to provide some insight into the probable mechanism involved in activity profile.
References