Research Article

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TUTTHA BHASMA AS A LIPID-LOWERING AGENT: A PRECLINICAL STUDY ON WISTAR ALBINO RATS

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ABSTRACT

Background: Hyperlipidemia affects 25-30% of urban and 15-20% of rural Indians, primarily those aged 30-60 years. Conventional treatments often have adverse effects, whereas Ayurveda offers a cost-effective, complication-free alternative. Tuttha bhasma with its kashaya and madhura rasa, laghu guna, and kapha-pitta-hara properties, acts as rasayana, lekhana, medohara, and nadibalyakara. Traditionally indicated in medo roga, this study is aimed to evaluate Tuttha bhasma's efficacy in lowering lipid levels, preventing atherosclerosis, and reducing the risk of myocardial infarction. Materials and Methods: Shodhana (Purification & processing) of ingredients is an essential step before usage, transforming raw drugs into safe, bioactive, therapeutic forms. The test drug Tuttha bhasma (incinerated copper sulphate), was prepared by homogeneously mixing processed tuttha, gandhaka, and tankana, followed by triturating it with Lakucha swarasa and subjected to kukkuta puta (incineration by following specific temperature pattern). The antihyperlipidemic activity of Tuttha bhasma was investigated in Wistar albino rats with hyperlipidemia induced by a high-cholesterol diet, using atorvastatin as the standard drug. The study parameters included body weight, heart weight, serum lipid profile, and histopathology of the heart was studied. Results and Conclusion: This study yielded significant results in serum VLDL, LDL, cholesterol, triglycerides, SGPT, creatinine, and ultra-sensitivity CRP levels. Significant improvements were observed in serum HDL cholesterol. Tuttha bhasma has lekhana (scraping) and rasayana action, contributing to its nadibalyakara (cardiovascular strengthening) effects, particularly in the coronary arteries.

Keywords: Tuttha, Medoroga, Hypolipidemic, Anti-atherosclerotic, Antihyperlipidemic, Experimental study, Copper Sulphate, Atorvastatin.

INTRODUCTION

In India, approximately 25-30% of urban and 15-20% of rural subjects are suffering from hyperlipidemia. It is prevalent in the people age group 30 to 40 years and markedly high among the people aged above 60 years[1]. Hyperlipidemia involves an imbalance of cholesterol levels, including low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in the blood. Elevated LDL-C can lead to buildup of plaques within the arteries and is associated with an increased risk of atherosclerotic cardiovascular disease, including coronary artery disease, myocardial infarction or stroke[2]. Though conventional medicines have proven to reduce the complications of hyperlipidemia, they are associated with numerous adverse effects, including gastrointestinal events, musculoskeletal pain, respiratory infections, and headache[3].

Ayurveda treats the root cause of the disease with fewer complications through cost-effective treatment accessible to everyone. In Rasashastra, formulations are designed in such a way that they meet the demands of a changing society. Having already proven, rasaoushadis are effective and safe after proper processing. Due to prompt action, small doses, and tastelessness, it's effective in incurable diseases and chirakala sthita vyadhi (chronic diseases). Hence, mineral preparations are preferred over herbal formulations[4]. Clinically, the therapeutic efficacy of copper, mica, and chalcopyrites in managing hyperlipidemia is well-established[5]. To enhance bioavailability, these minerals are often administered in the form of bhasma. The reduced particle size of bhasma facilitates easier absorption into the systemic circulation, thereby optimizing its therapeutic potential.

Tuttha (copper sulphate) is one of the maharasa in Rasashastra. Tuttha bhasma has been described as medohara, lekhana, nadibalyakara and rasayana[6]. It is used to treat hridroga (cardiac disorders), especially those related to medoroga (lipid disorders). Previous studies reported safety[7] and the effectiveness of Tuttha bhasma as wound healing[8], antifungal, and antibacterial properties[9]. Yet, its antihyperlipidemic and cardio-protective activity remains unexplored. Hence, the study was undertaken to evaluate the efficacy of Tuttha bhasma in lowering lipid levels to prevent coronary heart blocks due to atherosclerosis and preventing myocardial infarction.

MATERIALS AND METHODS

Test Drug- Tuttha bhasma preparation

Tuttha bhasma was prepared by following standard guidelines as prescribed in Ayurvedic classics. Raw Tuttha (copper sulphate) was subjected to processing- nirmalikarana[10] (to remove external impurities) and shodhana (purification) was done by triturating with decoction of Triphala (three myrobalans) for 7 times[11] followed by incineration by mixing equal quantity of shuddha gandhaka (processed sulphur) and shuddha tankana (processed borax) triturated with juice of lakucha (*Artocarpus lakoocha* Roxb) pellets were made and later placed in sharava samputa (encased earthen crucible) and sandhi bandana (sealing of sharava samputa) was done subjected to kukkuta puta[12] (incineration by

following specific temperature pattern). After 10 kukkuta puta, Tuttha bhasma was obtained and was analyzed as per classical parameters of bhasma pariksha.

Experimental Animal

Wistar albino rats were acclimatized and maintained with 24°C ± 2°C,70% RH and 12/12h light and dark cycle throughout the study at an animal house at the Department of Pharmacology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, India, as per the protocol outlined in publication of the committee for the purpose of control and supervision of experiment on animal standard guidelines (CPCSEA) and approval was obtained from Institutional Animal Ethical Clearance Committee (IAEC) Approval No: SDMCRA/IAEC/TB-R-10.

Dose fixation for rats

The therapeutic clinical dose of Tuttha bhasma is ¼ ratti[13] (30 mg). According to Paget and Barnes Table 1964[14], the rat dose was calculated by extrapolating the human therapeutic dose in rat dose (2.7 mg/kgBw); Therapeutic double dose - 5.4 mg/kgBw.

Experimental protocol (Antihyperlipidemic activity)

Thirty healthy Wistar albino rats were randomly allocated into five groups, each consisting of six rats in each group.

1. **The first or normal control group** (NC) rats were administered a normal diet, rat and mouse pellets and water ad libitum.
2. **The second or positive control group** (PC) rats were administered a high-cholesterol diet that contained 40 % cholesterol suspension in vanaspati (hydrogenated vegetable oil). The suspension was administered at a dose of 1 ml/100g rat daily for 28 consecutive drugs, orally, in the morning and evening sessions.
3. **The third group, or standard group** rats, were administered Atorvastatin 5 mg/kg (0.005g × body weight of rats in grams) along with a hyperlipidemic diet.
4. **The fourth group** or **test group- Tuttha bhasma therapeutic dose** (TBTED) rats were given Tuttha bhasma at a dose of 0.0027 gm × body weight of rats in grams in the morning session and the cholesterol suspension in the morning and evening sessions.
5. **The fifth group of Tuttha bhasma therapeutic Double dose** (TBTED×2) rats were given Tuttha bhasma at a dose of 0.0054 gm × body weight of rats in grams and the cholesterol suspension in the morning and evening sessions, following the animal dosage formula for 28 consecutive days along with the normal diet.

On the 29th day, after overnight fasting, the animals were sacrificed with ether overdose after collecting the blood from the retro-orbital plexus. The heart was excised (Figure 1b), cleaned, weighed, and transferred to a 10% formalin solution before being sent for histopathological investigations.

Statistical analysis

The data obtained were expressed in mean ± SEM and statistically analyzed using one-way ANOVA followed by Dunnet's multiple post hoc 't' test using Graph pad prism version 6.01 and 'P' < 0.05 were considered statistically significant.

RESULTS

The antihyperlipidemic study revealed no mortality in the treatment groups compared to the NC group. Furthermore, all groups, including the positive control, Standard, and Test drug groups, exhibited zero mortality. A significant reduction in body weight was noted in the Standard and TBTED groups compared to the positive control group. Conversely, the TBTED×2 group exhibited a non-significant increase in body weight (Table 1). No behavioural changes were observed in any treated groups during the Antihyperlipidemic study. Statistically, a highly significant decrease in heart weight was observed in the PC group compared to the NC group.

In contrast, a significant increase in heart weight was noted in the TBTED and standard groups relative to the NC group (Table 2). This suggests that the test drug possesses cardio-protective properties, improving cardiac function. Tuttha Bhasma demonstrated significant benefits in treating hyperlipidemia. It offered liver protection by reducing SGOT levels and ensured kidney safety by showing no adverse effects on kidney function. Additionally, it improves the lipid profile by lowering LDL cholesterol and increasing HDL cholesterol. Tuttha bhasma also decreased serum triglyceride levels and exhibited anti-inflammatory effects by reducing CRP levels. A histopathological study revealed that the test drug reduces inflammatory infiltration, degeneration, cellular alteration, and congestion in the heart tissue compared to PC and the standard control group (Figures 3-6).

Table 1: Effect of Tuttha bhasma on Body Weight

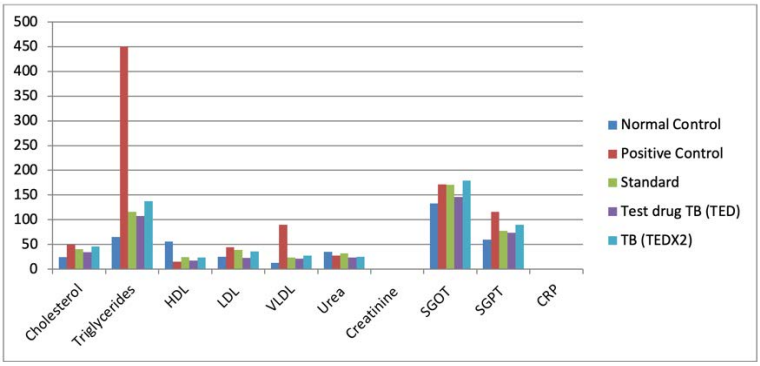
Groups	Body weight (Mean ± SEM)	% Changes
Normal control	26.48 ± 4.63	-
Positive control	20.29 ± 4.15	23.37↓@
Standard	5.09 ± 1.75*	74.9↓#
Test drug TBTED	12.04 ± 3.12	40.66↓#
TB (TED×2)	22.85 ± 5.37	12.61 ↑#
**P<0.01; @-compared with Normal Control (NC); #-compared with Positive Control (PC); SEM: Standard error of mean		

Table 2: Effect of Tuttha bhasma on Heart Weight

Groups	Heart Weight (Mean ± SEM)	% Changes
Normal control	0.94 ± 0.02	-
Positive Control	0.54 ± 0.01**	42.02↓@
Atorvastatin	0.77 ± 0.02**	41.65↑#
TBTED	0.93 ± 0.04**	70.64↑#
TBTED×2	0.58 ± 0.02	7.33↑#
**P<0.01; @-compared with NC; #-compared with PC; SEM: Standard error of mean		

Table 3: Effect of Tuttha bhasma on Biochemical parameters

Parameters	Groups (Mean ± SEM)				
	NC	PC	Standard	TBTED	TBTED×2
Cholesterol mg/dL	24.33 ± 4.52	50 ± 3.55**	40.5 ± 3.45	34.66 ± 6.05	45.5 ± 3.73
Triglycerides mg/dL	65.33 ± 7.78	450.5 ± 86.61**	116.3±11.27**	107.5±16.14**	137.5±12.41**
HDL	55.83 ± 0.47	14.83 ± 1.32**	24 ± 3.18*	17.4 ± 2.43	23.5 ±2.71*
LDL	24.88 ± 4.19	44.16 ± 3.62	39.16 ± 4.02	23.06 ± 10.56*	35.83 1.24
VLDL	13.06 ± 1.55	90 ± 17.26**	23.3 ± 2.30**	21.5 ± 3.23**	27.66 2.41**
Urea mg/dL	34.83 ± 0.47	27.16 ± 1.81**	31.66 ± 0.88	23.83 ± 2.18	24.83 1.90
Creatinine mg/dL	0.55 ± 0.06	0.605 ± 0.02	0.66 ± 0.01	0.59 ± 0.03	0.34 0.03**
SGOT IU/L	133.16 ± 6.90	171.33 ± 16.99	170.33 ± 5.59	146 ± 18.42	179.5 ± 11.10
SGPT IU/L	59.83 ± 5.39	116.16 ±22.24**	77.16 ± 3.94	73.83 ± 5.91*	90.16 ± 5.95
Ultra sensitivity CRP	0.60 ± 0.05	0.73 ± 0.05	0.47 ± 0.03**	0.22 ± 0.02**	0.35 ± 0.018**
**P<0.01; @-compared with NC; #-compared with PC; SEM: Standard error of mean					



Graph 1: The effect of Tuttha Bhasma on Biochemical Parameters.

Histopathological changes of all groups have been shown in Figures 2-5.

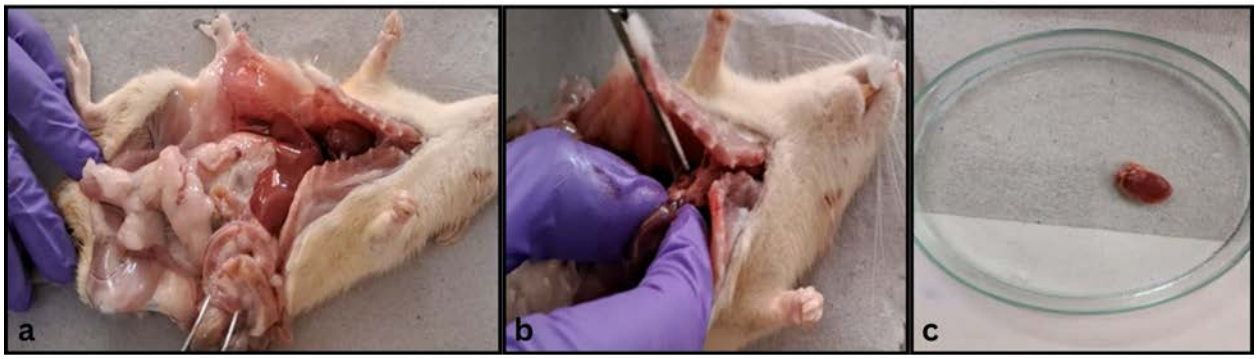


Figure 1: Dissection of heart for histopathological study; a. Fat deposition in a positive control group, b. Excising the Heart for Histopathological Investigations, c. Excised Heart in Petri dish

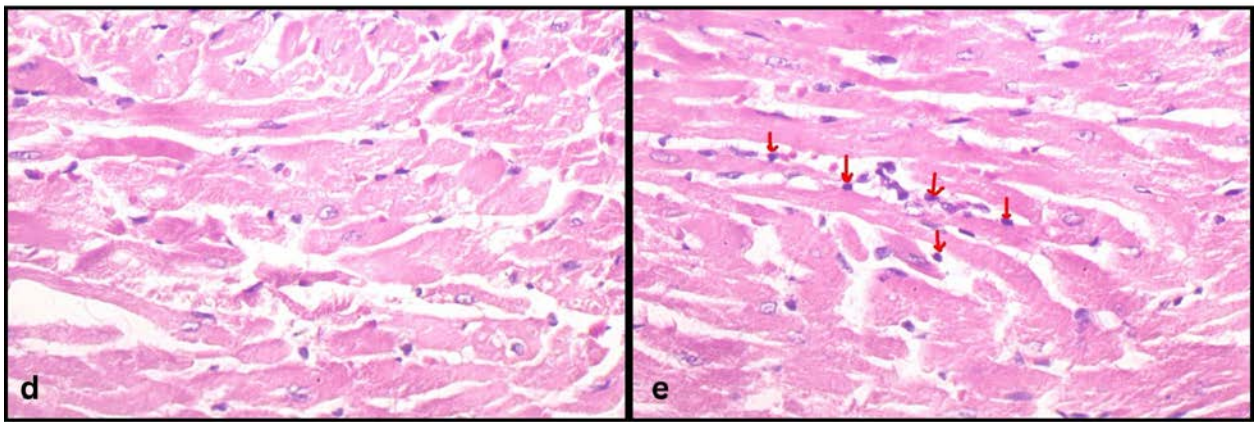


Figure 2: Histopathological image of Positive Control Group with 400X magnification; d. Area with degenerative changes, e. Mild inflammatory infiltrate

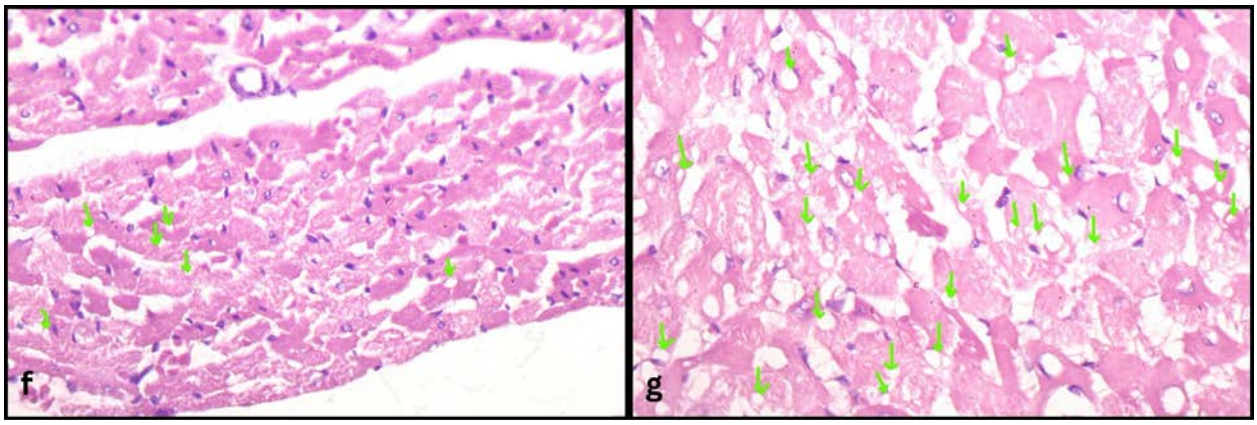


Figure 3: Histopathological image of Standard Group with 400X magnification; f. Area with degenerative changes, g. Severe vacuolation

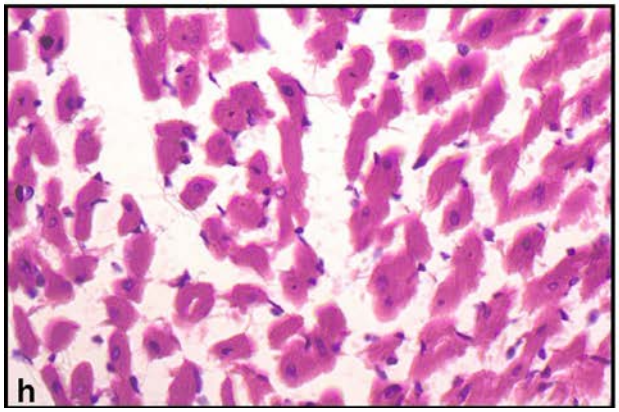


Figure 4: Histopathological image of Tuttha Bhasma's Group with 400X magnification; h. reduced histological changes

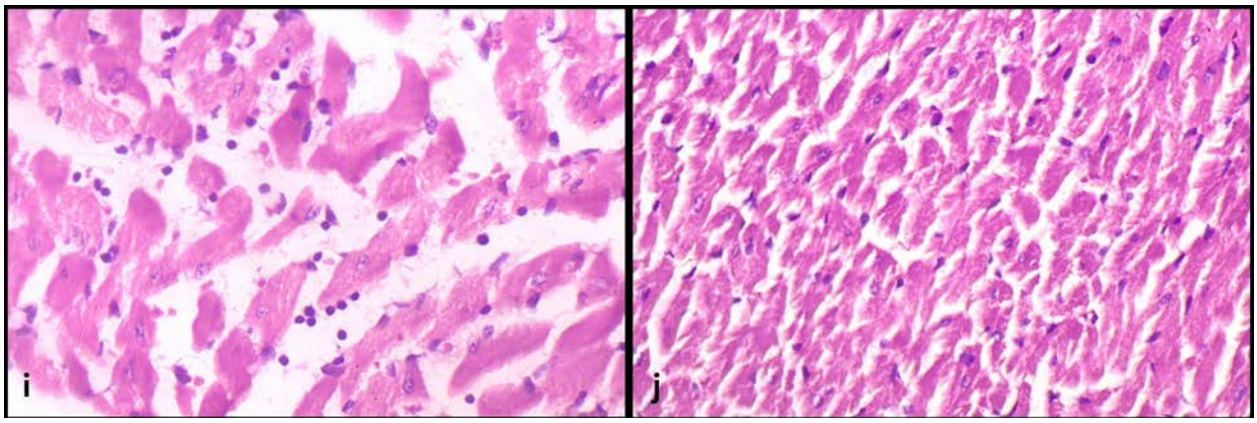


Figure 5: Histopathological image of TB TED×2 group with 400X magnification; i. Area reduced degenerative changes, j. Area with reduced inflammatory changes

DISCUSSION

The process of tuttha shodhana involved bhavana with Triphala kwatha (decoction of three myrobalans), which served as a liquid medium for wet grinding, facilitating smooth grinding and eliminating dust hazards. This process also enabled achieving a finer particle size than dry grinding. Furthermore, the liquid media's active constituents form an organo-metallic complex with the primary drug, enhancing its bioassimilation and therapeutic effect. Triphala's constituents, including alkaloids, phytosterols, tannins, saponins, ellagic acid, and citric acid, contributed to its potential hypolipidemic effect. In copper sulphide nanoparticle synthesis, sulphur compounds and copper sulphate are used[15]. Sulphur facilitates the reduction of copper (II) ions to elemental copper through electron donation[16]. The addition of dehydrated borax stabilizes the copper, preventing oxidation. Lakucha, rich in citric acid, acts as a reducing agent. It enhances the solubility and bioavailability of CuS nanoparticles[17], potentially augmenting lipid-lowering efficacy.

High triglyceride levels elevate cardiovascular risk by promoting atherosclerosis, primarily through the action of triglycerides and their metabolic byproducts. These smaller particles penetrate the arterial intima more effectively, exacerbating atherosclerotic burden and contributing to atherosclerotic disease pathology[18]. When comparing the TBTED group with the PC group, TBTED shows significant improvements. TBTED reduces high triglyceride and LDL levels and improves HDL levels. It also

lowers inflammatory markers like CRP, which is much higher in the PC group. These results suggest that Tuttha bhasma is beneficial in managing cholesterol levels, reducing inflammation, and lowering cardiovascular risks, especially atherosclerosis. There was a non-significant elevation of SGOT in the PC group, indicating liver damage. However, treatment with Tuttha bhasma reduced SGOT levels, suggesting hepatoprotective effects.

The Test drug group demonstrated a slight advantage over the Standard group, achieving further reductions in cholesterol and triglycerides, providing an anti-inflammatory effect, and offering liver protection as evidenced by lower SGOT and SGPT levels. Overall, TBTED appears to have a slight edge over the Standard group in improving lipid levels and reducing inflammation.

The TBTED×2 (therapeutic double dose) group shows significant changes in all the parameters except SGOT compared to the PC Group. Regarding lipids, TBTED×2 slightly lowers triglycerides and raises HDL (good cholesterol) more than TBTED. The higher dose of TBTED×2 seems to have a dual effect; it slightly increases liver enzyme levels (SGOT), possibly due to higher metabolic or liver strain, but at the same time, it shows a more favourable outcome for kidney function, as evidenced by the lower creatinine levels. It also reduces CRP (inflammation marker) more than TBTED, indicating a more potent anti-inflammatory effect. This could suggest that the higher dose works differently on the liver and kidneys, with a slightly more taxing effect on the liver but a beneficial impact on the kidneys.

Differences are seen in body weight changes between the TBTED and TBTED ×2 groups. The TBTED group showed a 40.66% decrease in body weight, indicating a moderate weight loss effect from the test drug at the therapeutic dose. On the other hand, the TBTED×2 group, which received double the therapeutic dose, showed a 12.61% increase in body weight. This suggests that the higher dose of the test drug had a positive effect, leading to weight gain rather than weight loss. The contrasting outcomes imply that the higher dose may have a rejuvenating or weight-preserving influence, possibly linked to the rasayana effect, which is believed to support health and vitality. Overall, these results suggest that while the standard dose of the test drug might lead to some weight loss, the higher dose appears to help preserve or increase body weight, possibly by improving metabolic functions or reducing the extent of weight loss.

Histopathology: Tuttha bhasma reduced inflammatory infiltration, degeneration, and cellular alteration in heart tissue, confirming its cardioprotective effects (Figures 2-5).

Probable mode of action: In treating medoroga (obesity), the line of treatment is based on samprapti vighatana (understanding the disease pathogenesis and breaking it). To manage medoroga, a drug should address vikrita meda (altered fat metabolism), prakupita Vayu (aggravated Vata), and tikshna jathragni (imbalanced digestive fire). Tuttha bhasma, with its rasayana property (rejuvenating), fulfils the guru cha apatarpana criteria for samprapti vighatana. Therefore, Tuttha bhasma is an ideal drug of choice for managing medoroga.

Tuttha bhasma's action on dosha and dhatu is multifaceted. It balances Kapha and Pitta due to its tikshna (sharp), laghu (light), and ruksha(dry) properties. It's ushna virya (warming potency), which also counteracts Vata. It corrects imbalances in rasa dhatu and facilitates nourishment of subsequent dhatus through its katu vipaka (pungent) and madhura rasa (sweet), which acts as a rasayana. Its tikshna property, ushna virya, and kashaya rasa (astringent) stimulate Agni (digestive fire), particularly medodhatvagni (fat metabolism), promoting lekhana (scraping) of excess fat and removing srotorodha (channel blockages). This action strengthens nadis (blood vessels), especially coronary arteries and supports overall well-being.

CONCLUSION

TB at therapeutic dose has shown lipid-lowering, anti-inflammatory and cardioprotective effects and has a significant role in reducing cardiovascular risk. To assess treatment safety, serum liver transaminases were examined; they did not adversely affect SGOT and SGPT enzymes, indicating a lack of hepatotoxicity. On the other hand, Tuttha bhasma at double dose demonstrates more substantial effects on kidney function, particularly in lowering creatinine, but increases SGOT. Given the safety concerns raised for many of the drugs aimed at the cardiovascular system, the satisfactory safety profile of Tuttha bhasma at the therapeutic dose is a promising finding for lack of toxicity, given future clinical studies in humans.

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