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Impact of *Amritikarana* Procedure on Safety Aspect of *Somanathi Tamra Bhasma*

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Abstract

Somanathi Tamra Bhasma is a unique method of preparation of *Tamra Bhasma*. In the classics, it is mentioned that if *Tamra Bhasma* has been used without *Amritikarana* in therapeutics, it produces ill effects. Hence it should be recommended after *Amritikarana* process only. Actual impact of this procedure in the preparation of *Somanathi Tamra Bhasma* has not evaluated till date. This is the first attempt towards preparing *Somanathi Tamra Bhasma* with *Amritikarana* (STBA) and evaluate its safety. Three batches of STBA were prepared as per the classical guidelines. In acute toxicity, STBA was administered at a maximal dose of 2000 mg/kg to overnight fasted rats and observed closely for behavioural changes, signs of toxicity and mortality if any, continuously for the first six hours and thereafter periodically up to 14 days. In the repeated dose 90-days oral toxicity evaluation, the test drug was administered daily at the doses of 67.5, 337.5 and 675 mg/kg along with *Pippali Churna* and honey as an adjuvant to rats for 90 days in divided dose followed by a 30-day recovery period. Animals were sacrificed on the 91st day and haematological, serum biochemical parameters and histopathology of organs were studied. In acute toxicity, STBA at the dose of 2000 mg/kg did not produce any observable toxic effects or mortality. STBA did not produce any significant changes in haematological, biochemical and histopathological parameters at TED and TED x 5 dose levels but mild fatty and inflammatory changes were observed in liver at TED x 10 dose level. These changes were returned to normal at recovery groups. Based on these observations, it can be concluded that STBA is safe at therapeutic dose levels when used judiciously along with specified adjuvants.

Keywords: Ayurveda, *Amritikarana*, Safety, *Somanathi Tamra Bhasma*, *Tamra Bhasma,* Toxicity

1. Introduction

Tamra is one of such metal in Ayurveda which if properly processed and detoxified is useful in many diseases. But if not processed properly, leads to serious hazardous effects. The toxic nature of *Tamra Bhasma* in its impure state has been described as *Ashtamahadosha* (eight ill effects) in *Ayurvedic* classics[1]. To make it therapeutically fit, *Tamra* has to pass through a set of pharmaceutical processes like *Shodhana*, *Marana* etc., as stated in classics. Possible impurities that remain in *Bhasma* after the *Marana* process are advocated to be removed by another specialized pharmaceutical procedure known as *Amritikarana*[2]. *Somanathi Tamra Bhasma* is one such unique method for preparation of *Tamra Bhasma* comprising processed *Tamra* (copper), *Parada* (mercury), *Gandhaka* (sulphur),

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Haratala (arsenic trisulphides), *Manahshila* (arsenic disulphide) as an integral ingredient. It is recommended in the management of *Udara* (ascites), *Pandu* (anemia), *Grahani* and *Yakritavikara* (liver diseases)[3]. There is need to evaluate safety of such formulations containing ingredients mentioned in Schedule E[1][4].

Few research works were carried out on the safety of *Somanathi Tamra Bhasma* but they have not followed *Amritikarana* process in the pharmaceutical procedure. But, *Somanathi Tamra Bhasma* without *Amritikarana* was found toxic at histopathological level at higher doses when administered with water[5]. Role of *Anupana* (adjuvant) is also well reported and ancient Ayurvedic scholars have always emphasized on administration of herbo mineral formulations with suitable adjuvants[6]. Impact of *Amritikarana* has been also evaluated in the preparation of *Tamra Bhasma* upto genetic level[7,8]. Actual impact of *Amritikarana* in the preparation of *Somanathi Tamra Bhasma* (STBA) has not been evaluated till date. Considering this, a study has been planned to evaluate acute and repeated dose 90-days oral toxicity study of STBA along with *Pippali Churna* as *Sahapana* to generate safety evidences.

2. Materials and Methods

2.1 Drug Preparation

STBA was prepared by following standard classical guidelines in Ayurveda texts. Formulation composition of STBA is depicted at Table 1. All the raw materials were procured from the Pharmacy attached to the Institute and authenticated by the subject experts as per criteria given in classics[9-13]. *Tamra*, *Parada*, *Gandhaka*, *Haratala* and *Manahshila* were processed as per classical guidelines[14-19]. Processed mercury and sulphur were triturated together till formation of *Kajjali* (black sulphide of mercury). Then, the powder of processed *Haratala* and *Manahshila* was mixed in *Kajjali* and triturated well till the formation of homogenous mixture. Processed *Tamra* and prepared mixture were kept in earthen pot (*Garbha Yantra*) and subjected to heat for 12 hours in *Valuka Yantra* (traditional heating instrument)[20]. Prepared *Somanthi Tamra Bhasma* has been further processed for *Amritikarana*. It was mixed with half part purified sulfur and juice of *C. jambhiri* Lush, kept in the form of *Amorphophallus campanulatus* Linn. It was subjected to heat treatment and labeled as *Somanthi Tamra Bhasma* with *Amritikarana* (STBA)[21].

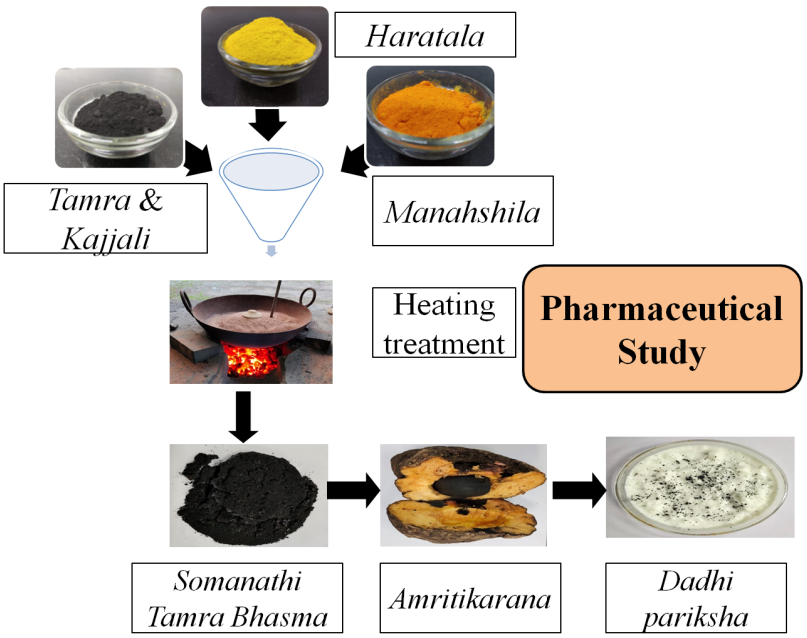


Figure 1. Schematic presentation of *Somanathi Tamra Bhasma* preparation.

2.2 Experimental Animals

Wistar albino rats weighing between 200 ± 20 g were used for experiment as per the guidelines of the Institutional Animal Ethics Committee (IAEC). The animals were obtained from the animal house attached to the Pharmacology Laboratory of the institute. The animals were maintained under ideal husbandry conditions in terms of standard conditions of temperature (23 ± 3 ºC) and relative humidity (50-60 %) and 12 h light and dark cycles. Animals were fed *ad libitum* with VRK brand rat pellet feed supplied by Keval Sales Corporation, Vadodara and drinking water *ad libitum*. The experimental protocol was approved as IAEC/28/2021/17as per guidelines of the committee for the purpose of control and supervision of experiments on animals in India.

2.3 Dose Fixation

As per classical guidelines, the human therapeutic dose of STBA is 750 mg with *Sahapana* (375 mg STBA + 375 mg *Pippali Churna*) per day[22]. The suitable dose for rats was calculated by referring to table of Paget and Branes[23] and found to be 67.5 mg/kg body weight of rat (considered as TED). The test drug was mixed with honey[24] (4 ml/kg) and licked orally with the help of 1 ml syringe in divided dose daily for 90 days. Groupwise dosage levels are depicted at Table 2.

2.4 Acute Toxicity

Young, healthy, nulliparous, and non pregnant wistar strain albino female rats were selected and acclimatized for 7 days before the experiment. Both test drugs along with adjuvant were orally administered at limit dose of 2000 mg/kg to overnight fasted female rats by following Organization for Economic Cooperation and Development (OECD) 425 guidelines[25]. The rats were observed closely for behavioural changes, signs and symptoms of toxicity, and mortality, if any continuously for the first 6 h and thereafter periodically up to 14 days.

2.5 Repeated Dose 90-Days Oral Toxicity Study

The study was carried out by following OECD 408 guidelines[26]. Wistar strain albino rats of either sex weighing between 200 ± 20 g were selected and randomly categorised into 5 groups, each comprising 3 males and 3 females. Group I was served as vehicle control and received *Pippali Churna* and honey (4 ml/kg), Group II to V were administered with test drug along with *Anupana*

Table 1. Ingredients and composition of *Somanathi Tamra Bhasma*

Sr. No	Ingredients	Latin/English Name	Quantity
1	*Shuddha Tamra*	Processed copper	1Part
2	*Shuddha Parada*	Processed mercury	1Part
3	*Shuddha Gandhaka*	Processed sulphur	1Part
4	*Shuddha Hartala*	Processed arsenic trisulphide	½ Part
5	*Shuddha Manahshila*	Processed arsenic disulphide	1/4th Part

Table 2. Detail of dosage for different groups

Groups	Dose (orally)	Description
Group I (VC)	4 ml/Kg	Honey + *Pippali Churna*
Group II (TED)	67.5 mg/kg	STBA + *Pippali Churna* and honey
Group III (TED X5)	337.5 mg/kg	
Group IV (TEDX10)	675 mg/kg	
Group V (RTEDX10)	675 mg/kg	

at TED (67.5 mg/kg), TEDx5 (337.5 mg/kg), TED x10 (675 mg/kg) and recovery group (675 mg/kg) respectively in divided dose. Test drug were administered orally twice a day for 90 consecutive days. Body weight of all animals was recorded prior initiation of the experimentation. Additional 6 animals were kept in the TED x 10 treated group (Group V) for observation after the treatment period of reversibility or persistence of any toxic effects. The duration of this post-treatment period was fixed as 30 days.

General behavioural pattern was observed once a week by exposing each animal to open arena. Body weight, food intake, water intake and any other symptoms were observed throughout the study period for assessment of toxicity or adverse effects of drugs, if any. On 90th day, all animals of Groups I to IV were kept for overnight fasting. Next day, animals were weighed again and anesthetized with diethyl ether. Supraorbital plexus was punctured under light anaesthesia and blood was collected by capillary in two different types of tubes, one containing anticoagulant fluid for haematological parameters and another plain tube for serum biochemical investigations. The final body weight of each animal was taken. Then, the rats were sacrificed and the abdomen was opened through midline incision to record the autopsy changes followed by dissecting out the important organs.

Haematological analysis was performed by using an automatic haematological analyser (Swelab, Sweden). Total Red Blood Cell (RBC), haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell, neutrophils, percent lymphocytes, eosinophils, monocytes, Packed Cell Volume (PCV) and platelet count were measured from the blood samples. Serum biochemical parameters were carried out by using fully automated biochemical random-access analyser (BS-200, Lilac Medicare Pvt. Ltd., Mumbai). The studied parameters were blood glucose[27], total cholesterol[28], triglyceride[29], High Density Lipoproteins (HDL) cholesterol andvery low-density lipoprotein cholesterol[30], serum glutamic oxaloacetic transaminase[31], Serum Glutamic Pyruvic Transaminase (SGPT)[32], alkaline phosphatase[33], total bilirubin[34], direct bilirubin[35], indirect bilirubin[36], blood urea[37], serum creatinine[38], total protein[39], albumin[40], globulin[41], uric acid[42] and serum calcium[43].

Liver, kidney, heart, lungs, spleen, thymus, testis, seminal vesicle, prostate, uterus and ovary were dissected carefully. After noting signs of gross lesions and ponderal changes of these organs; all were transferred to 10% phosphate buffered formalin solution for fixation and later on subjected to dehydrating, wax embedding, sectioning and staining with haematoxylin and eosin for histological evaluation. The slides were viewed under trinocular research Carl Zeiss microscope at various magnifications to note down the changes in the microscopic features of the tissues.

2.6 Statistical Analysis

The results are expressed as Mean ± standard error of mean. Statistical comparisons were carried out by paired and unpaired student 't' test as well as one way analysis of variance followed by Dunnett's multiple 't' test as post-hoc test in Sigma stat software (version 3.5, Systat Software Inc.) to determine significant difference between groups at $p < 0.05$.

3. Results and Discussion

Normal progressive body weight gain was observed in STBA treated groups with adjuvant *Pippali Churna* and honey, which suggest that the drug is devoid of any serious toxic effect on rats in acute toxicity study. According to UN classification substances having oral LD50 of more than 2000 mg/kg is designated as low hazard potential and categorized as UN 6.1 PG III[44]. So that STBA along with adjuvant can be categorized as a low health hazard potential substance.

Behavioural changes were not observed in any of the treated groups during the course of repeated dose 90-days oral toxicity in comparison to VC group. No mortality was observed in any of the experimental groups. Progressive weight gain was observed in all treated groups. Changes in body weight are an important factor to monitor the health of an animal. Loss of body weight is usually considered as the first sign indicating the onset of an adverse effect. The percentage change in body weight pattern in test drug treated groups did not differ significantly from the changes observed in the control groups, which suggest the absence of serious toxic effect of STBA during repeated administration in rats.

Nutritional status of an individual is dependent on dietary intake and effectiveness of metabolic processes. Bodyweight indicates the health status of any living being and increase in body weight indicates a normal

progressive health status of the animals[45]. Administration of STBA at different dose levels has shown insignificant changes in relative weight of seven organs except significant increase in weight of spleen at TED X 10R and kidney at TED and TEDX10 dose levels. STBA at all dose levels produced insignificant decrease in relative weight of uterus in TED, TED X 10 and TED X 10R dose levels while other histopathological changes were not distinct in comparison to control group. The drug at all dose level shows a progressive and significant increase in body weight compared to the normal control group. The drug did not affect the relative weight of heart, liver, prostate, seminal vesicles and uterus at the significant level except on spleen and kidney at all dose level in comparison to the control group. The non-significant differences recorded in the organ weight indicating that the repeated oral administration of test drug for 90 days, did not cause any deleterious effect on organs. (Table 3).

Effect of STBA on haematological parameters (Table 4) revealed that out of the eight parameters studied, significant decrease in neutrophil counts was observed at TED X 5 dose levels. Significant increase in WBC, lymphocyte and MCH at TED X 5, TED X 10 dose levels however, all the values were returned to normal level in recovery groups. All other parameters studied were not altered to significant level and found within normal range. Honwad S *et al.,* carried out the chronic toxicity study of *Somanathi Tamra Bhasma*, in that study MCHC and platelet count significantly decreased at TEDx5 and TEDx10 dose level suggesting toxicity of the drug over haematological parameters, but in present study no any such changes was observed at all the dose levels, it may the impact of specialized procedure *Amritikarana*[46,47]. This indicates that the test drug is devoid of any serious haematological toxicity, even at higher dose on repeated administration.

Table 3. Effect of STBA on organ weight (g) of albino rats in repeated dose 90-days oral toxicity study

Organ Name	Control	STBA TED	STBA TEDx5	STBA TEDx10	STBA TEDx10 R
Heart	285.01 ± 13.20	336.29 ± 13.42	298.33 ± 9.27	308.26 ± 7.89	336.16 ± 22.40
Liver	2.96 ± 0.13	3.14 ± 0.13	2.81 ± 0.18	3.00 ± 0.10	2.72 ± 0.15
Spleen	176.04 ± 10.93	198.58 ± 13.47	183.21 ± 14.01	208.16 ± 22.17	270.78 ± 25.18
Kidney	622.41 ± 17.79	724.23 ± 28.24	630.44 ± 49.07	741.31 ± 49.30	706.93 ± 42.10
Thymus	68.10 ± 2.49	82.68 ± 17.50	77.61 ± 9.42	74.45 ± 8.02	87.68 ± 26.46
Testis	853.82 ± 59.96	851.70 ± 59.76	1050.77 ± 61.99	902.88 ± 45.59	745.27±68.96
Prostate	205.03 ± 42.35	189.47 ± 17.49	229.89 ± 9.83	178.83 ± 11.95	310.81 ± 33.13
Seminal vesicle	291.22 ± 31.2	259.74 ± 42.59	397.44 ±131.20	336.05 ± 48.07	499.54 ± 142.56
Uterus	305.54 ± 21.40	296.02 ± 41.54	264.80 ± 16.35	305.19 ± 36.21	377.82 ± 19.98

Table 4. Effect of STBA on haematological parameters of albino rats in repeated dose 90-days oral toxicity study

Parameter	Control	STBA TED	STBA TEDx5	STBA TEDx10	STBA TEDx10 R
WBC Count(/mm^3)	11816.66 ± 474.28	12933.33 ± 1259.27	15383.33 ± 1071.88	18166.66 ± 1143.87	14183.33 ± 1460.68
RBC (mil/cum)	7.93 ± 0.22	8.22 ± 0.16	8.67 ± 0.19*	8.16 ± 0.25	8.23 ± 0.49
Haemoglobin (g %)	13.82 ± 0.69	14.88 ± 0.29	15.25 ± 0.26	14.20 ± 0.39	14.10 ± 0.63
PCV (%)	43.93 ± 0.58	44.26 ± 0.68	45.93 ± 0.84	42.38 ± 1.08	41.20 ± 1.89
MCV (fl)	52.80 ± 0.84	53.88 ± 0.31	53.01 ± 0.57	51.98 ± 0.83	53.38 ± 0.80
MCH (pico gm)	16.68 ± 0.30	18.10 ± 0.08	17.60 ± 0.22	17.41 ± 0.32	18.28 ± 0.24
MCHC (%)	33.91 ± 0.72	30.58 ± 3.02	33.21 ± 0.20	33.48 ± 0.16	34.23 ± 0.31
Platelet count (10^6/mm^3)	1113.66 ± 109.62	1171.00 ± 73.63	1171.16 ± 37.61	1213.50 ± 38.78	1172.50 ± 104.55

Administration of STBA on biochemical parameters (Table 5) has revealed significant decrease in blood sugar level at TED X5 dose level. Accumulation of some toxic metals was found in various biological samples of Type 2 Diabetes patients other than essential metals. The reasons like pollution and industrialization might be the source of exposure of these toxic metals like Lead (Pb), Nickel (Ni), Cadmium (Cd) and Arsenic (As). These metals can disturb glucose uptake and alter glucose regulatory mechanisms[48]. Required or increased amount of copper is helpful in improving renal functions and reduces the risk of poor glucemic control[49]. After 90 days of test drug administration, a significant (P>0.05) decrease in blood sugar level at TEDx5 dose level suggest role of copper in glucose metabolism. Copper play's important role in carbohydrate metabolism by stimulating insulin binding, hexose transport and lipogenesis *in vivo* and *in vitro*[50]. This may be the reason behind decreased blood sugar level[51] but since this was reversed in the recovery group, the effects cannot be categorized under deleterious/toxic effects of drugs on the glucose level.

Exposure to cadmium (Cd), lead (Pb), manganese (Mn) etc. significantly increases the concentration of liver transaminase activity and bilirubin in male wistar rats along with oxidative stress in rat liver. Histological injury, lipid peroxidation, glutathione depletion and mitochondrial dysfunction are the main hepatotoxicity's observed in various rat models due to various heavy metals. Some studies evaluated combined cytotoxicity of heavy metal mixtures on human liver HL7702 cells and observed synergistic, antagonistic or additive effects of toxicity[52]. The liver contains the highest amount of SGPT in comparison to other tissues. SGPT is a cytosolic enzyme, also present in the liver; the proportion in the liver is greater than in the heart and skeletal muscle. Elevation of Serum SGPT is indicative of liver injury due to leakage of this enzyme from the tissue into the serum. Elevations in SGPT are usually associated with cell necrosis in many tissues[53]. But in the present study, STBA did not produce any significant changes in SGPT level, which suggest that the drug is not having any toxic effect. Raised levels of serum SGPT and SGOT are plausible markers of necrotic lesions in the hepatocyte's cells[54]. Honwad S *et al.,* carried out the chronic toxicity study of *Somanathi Tamra Bhasma*, in that study SGOT and Alkaline phosphatase level significantly increased at

Table 5. Effect of STBA on biochemical parameters of albino rats in repeated dose 90-days oral toxicity study

Parameter	Control	STBA TED	STBA TEDx5	STBA TEDx10	STBA TEDx10 R
Blood sugar (mg/dL)	90.00 ± 3.77	89.16 ± 5.64	69.16 ± 4.34	81.33 ± 5.61	83.83 ± 6.38
Cholesterol (mg/dL)	57.00 ± 4.97	64.83±8.87	62.83 ± 7.87	65.50 ± 6.86	60.66 ± 9.24
Triglycerides (mg/dL)	65.00 ± 6.57	114.16 ± 12.82	117.0 ± 18.73	132.83 ± 15.01	108.40 ± 24.25
HDL-Cholesterol (mg/dL)	30.98 ± 2.49	18.68 ± 2.03	12.65 ± 1.87	18.75 ± 2.42	16.56 ± 3.08
VLDL-Cholesterol (mg/dL)	13.00 ± 1.31	22.83 ± 2.56	23.40 ± 3.74	26.56 ± 3.00	20.80 ± 4.56
Blood Urea (mg/dl)	33.66 ± 1.94	31.83 ± 2.60	37.83 ± 2.28	29.83 ± 1.57	30.16 ± 2.10
Uric acid (mg/dL)	1.23 ± 0.07	1.10 ± 0.07	1.34 ± 0.05	1.22 ± 0.06	1.24 ± 0.08
Creatinine (mg/dL)	0.77 ± 0.02	0.68 ± 0.02	0.69 ± 0.02	0.71 ± 0.02	0.77 ± 0.02
SGPT (IU/L)	48.50 ± 2.40	58.33 ± 4.59	45.83 ± 7.12	42.00 ± 3.73	44.33 ± 3.66
SGOT (IU/L)	109.00 ± 4.47	126.33 ± 4.21	134.00 ± 4.03	125.66 ± 9.96	132.83 ± 23.05
Total Protein (g/dL)	8.05 ± 0.19	8.13 ± 0.29	8.52 ± 0.38	8.46 ± 0.36	8.45 ± 0.47
Albumin (g/dL)	3.52 ± 0.14	3.61 ± 0.10	3.64 ± 0.10	3.61 ± 0.17	3.55 ± 0.23
Globulin (g/dl)	4.53 ± 0.23	4.51 ± 0.19	4.87 ± 0.34	4.84 ± 0.22	4.90 ± 0.29
Alkaline phosphatase (IU/L)	106.83 ± 5.39	146.00 ± 8.66	99.50 ± 15.35	110.00 ± 13.90	134.83 ± 18.90
Total Bilirubin (mg/dl)	0.18 ± 0.01	0.19 ± 0.01	0.28 ± 0.03	0.16 ± 0.01	0.17 ± 0.01
Calcium(mg/dl)	10.30 ± 0.18	10.21 ± 0.11	10.51 ± 0.11	10.51 ± 0.12	9.99 ± 0.22

TEDx5 and TEDx10 dose level suggesting toxicity of the drug over liver functions, but in present study significant increase in SGOT and alkaline phosphatase level was seen in STBA treated group at TED and TEDx5 dose level and both are non-significantly altered in the TEDx10 and recovery group[55]. Suggesting the impact of *Amritikarana* procedure at higher dose levels. From this we can interpret that STBA is safe at therapeutic dose and have mild to moderate untoward effect at higher dose levels. However, the injury occurred to liver cells in higher dose is reversible.

Test drug exhibited a significant increase in total bilurbin level at TEDx5 and TEDx10 dose levels but these changes were returned to normal level at TEDx10R dose level similarly indirect bilirubin level was significantly increased at TEDx5 dose level but these changes were significantly decreased at TEDx10R dose level. similarly, creatinine level was significantly decreased at TED and TEDx5 dose level which was reversed at TEDx10 and TEDx10R dose level. Honwad S *et al.,* carried out the chronic toxicity study of *Somanathi Tamra Bhasma*, in that study creatinine level was increased at all dose levels and significantly increased at TEDx5 and TEDx10 dose level suggesting toxicity of the drug over renal functions, but in present study creatinine value was significantly decreased at TEDx5 and TEDx10 dose level, it may the impact of specialized procedure *Amritikarana*[56,57]. Hence it can be interpreted that the drug is devoid of any hepatic and renal toxicity. Test drug did not show any significant changes in the levels of blood urea, uric acid and calcium.

Serum biochemical analyses are useful to check the possible alterations in hepatic and renal functions if any. They are also helpful in toxicity screening of the test drugs to generate safety evidences. From HDL, cholesterol is transferred to VLDL, IDL, LDL and chylomicrons remnants with the aid of Cholesteryl Ester Transfer Protein (CETP). Thus, cholesterol ends up finally in the liver[58]. A significant (P>0.05) decrease in the HDL cholesterol was noted at all dose level in comparison to control. A significant (P>0.01) increase was noted in the VLDL cholesterol level at TED, TEDx5 and TEDx10 and non-significant increase in TEDx10R dose levels in comparison to control group. The decrease in HDL-cholesterol level and increase in LDL by test drug at a higher dose level suggests that it is better to use the test drug at Therapeutic dose level. This indicates that the test drug's effects on lipid turnover may be due to its effect on the liver as observed by an increase in serum transaminase activity[59,60]. Test drug produced more effect on HDL-cholesterol which may have good therapeutic application and will be quite useful in patients with hypercholesterolemia conditions. However, the intensity of adverse effects is mild to moderate grade because there were no significant effects observed on bilirubin and alkaline phosphatase level and values of the above biochemical parameters are still within the normal range.

The results of histopathological studies revealed that fatty and inflammatory changes were observed in liver at TEDx10 dose level. However, this change was not observed in at lower dose levels and recovery group in rats, which suggest that the drug is devoid of drastic toxicity at therapeutic dose level. Sections of other organs were exhibited normal cytoarchitecture at TEDx10 and TED X 10R dose levels. *Somanathi Tamra Bhasma* which was found toxic even at lower doses when administered for shorter duration in previous studies, same drug when administered even in higher dosage found safe at therapeutic dose and intensity of pathological changes were very minimal and this may be considered as impact of *Amritikarana*. Considering this, it can be said that STBA is safe at TED level, and mild changes can manifest at higher dose levels. Ayurveda classics have also emphasized cautious administration of such formulations in right doses with suitable adjuvants.

4. Conclusion

The current study has demonstrated the impact of *Amritikarana* procedure in the safety of STBA when administered at therapeutic dose level along with *Pippali Churna* (*Sahapana*) and honey as an adjuvant. STBA may produce mild pathological changes when administered for longer duration at higher (TED×10) doses. Though the observed pathological changes were reverted after withdrawal of the drug; it is advisable to administer STBA in specified therapeutic dose along with a suitable vehicle (honey) for shorter periods. Care needs to be observed while administering the drug in patients with hepatic, cardiac and renal impairments. It is also inferred that such formulations are not to be administered continuously for a longer period and observing a gap of few days is advisable.

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