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Acute and subchronic toxicity study of *Tamra Bhasma* (incinerated copper) prepared with and without *Amritikarana*

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ABSTRACT

Background: *Tamra Bhasma* (TB) is one among herbo-metallic preparations extensively used in routine ayurvedic practice. In the present era, *Bhasma* preparations used in ayurvedic system of medicines are always under stern observations for containing heavy metals which may raise the question of safety aspect.
Objective: In the present study, TB prepared with and without *Amritikarana* was subjected to toxicity study to ascertain the role of *Amritikarana* on safety profile of TB in rats.
Materials and methods: Both the samples of TB were administered to rats for 28 consecutive days at the doses of 5.5, 27.5, and 55 mg/kg. The effects of both drugs were assessed on ponderal changes, hematological, serum biochemical, and histopathology of various organs.
Results: Results showed that both the samples of TB did not produce any sign and symptoms of toxicity at therapeutic dose level (5.5 mg/kg) and therapeutic equivalent dose (TED) × 5 (27.5 mg/kg) while at higher dose of TED × 10 (55 mg/kg) TB has mild toxicity in liver, kidney, heart, and thymus on repeated administration for 28 days in rats. The sample without *Amritikarana* has more magnitude of toxicity than the sample with *Amritikarana*.
Conclusion: From the present study, it is concluded that TB with *Amritikarana* was found to be relatively safer than TB without *Amritikarana* at different dose levels in rats and hence suggest for safely use in humans at therapeutic dose level. It proves the role of *Amritikarana* in the preparation of TB.

1. Introduction

Ayurveda advocates therapeutic uses of mineral and metallic preparations in many diseases since century in clinical practice. Knowing the possibilities of toxic effects, seers emphasized on following set of exclusive pharmaceutical procedures such as *Shodhana* (purification and/or detoxification), *Marana* (incineration and/or calcination), and *Amritikarana* that converts the metals and minerals into *Bhasma* (calcined powders) [1,2]. *Bhasmas* are unique preparations which are safely being practiced in Ayurveda without any noticeable side effects can be considered as a testimony to their safety, but no objective verifiable data exist to support many such claims. Preclinical studies of ayurvedic drug provide scientific basis for their traditional use and to prove that they are safe and efficacious [3].

Tamra Bhasma (TB) (incinerated copper) is one among such ayurvedic herbo-metallic preparation used in the treatment of *Udara* (ascites), *Pandu* (anemia), *Svasa* (asthma), and *Amlapitta* (hyperacidity) [4]. Though wide therapeutic utility of TB has been mentioned in classics, it is reported as poison as or more than that if not processed or purified properly as per classical methods [5]. To indicate its toxic potential, *Ashtamahadoshas* (eight major ill effects) have been quoted in classics [6]. Previous studies reported safety of TB in animal models [7,8]. Role of *Shodhana* in safety of TB was also reported in animals [9]. Though number of studies have been carried out in direction of safety of *Bhasmas*, concerns are always being raised on ayurvedic formulations for the presence of heavy metals [10,11]. *Amritikarana* is exclusively mentioned for TB and *Abhrak Bhasma* which is said to eliminate all the blemishes from the end

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product. There is a need to provide scientific basis to establish the impact of this procedure. Hence, the present study is aimed to evaluate the acute and subchronic toxicity studies of TB prepared with and without *Amritikarana* in rats.

2. Materials and methods

2.1. Drugs and chemicals

Two samples of TB with and without *Amritikarana* were prepared by following standard guidelines as prescribed in ayurvedic classics. Copper scraps with 99.89% pure copper was procured from local industrial area, Jamnagar, India. It was subjected to general and specific purification procedure followed by incineration after mixed with purified sulfur, *Kajjali* (black sulfide of mercury), and juice of *Citrus jambhiri* Lush. In *Amritikarana*, it was mixed with half part purified sulfur and juice of *C. jambhiri* Lush, kept in the corm of *Amorphophallus campanulatus* Linn. It was subjected to heat treatment and labeled as *Tamra Bhasma* with *Amritikarana* (TBA) [12]. Another sample was subjected up to *Marana* and labeled as TB without *Amritikarana* [13]. All chemicals used in the study were of analytical grade.

2.2. Animals

Wistar strain albino rats of either sex, weighing 200 ± 20 g, were used as per the guidelines of the Institutional Animal Ethics Committee (IAEC). The animals were obtained from the animal house attached to the pharmacology laboratory, IPGT and RA, Jamnagar. The animals were maintained under ideal husbandry conditions in terms of standard conditions of temperature (23 ± 2 °C), relative humidity (50–60%) and exposed to 12 h light and dark cycles. All animals were exposed to the same environmental conditions and were maintained on standard diet and drinking water *ad libitum*. The experimental protocol was approved by the IAEC/14/2013/16 as per guideline of committee for the purpose of control and supervision of experiments on animals in India.

2.3. Dose fixation

As per classical guideline, the therapeutic clinical dose of TB is 30 mg twice a day (60 mg/day) [14]. The suitable dose for rats was calculated by referring to table of Paget and Branes [15] and was found to be 5.5 mg/kg body weight of rat (considered as TED). The test drug was administered orally (licking) along with honey as adjuvant with the help of oral cannula.

2.4. Acute toxicity study

Young, healthy, nulliparous, and nonpregnant 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 guideline [16]. The rats were observed closely for behavioral 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. Subchronic toxicity

Animals were divided into seven groups, each comprising three male and three females. Rats were randomized into six groups, each consisting of six rats comprising three male and three female. Group I was kept as control group, received vehicle as honey (2 ml/kg, orally). Group II to IV were administered with test drug TB without *Amritikarana* along with adjuvant at TED (5.5 mg/kg, orally), TED × 5 (27.5 mg/kg, orally), and TED × 10 (55.0 mg/kg, orally), respectively. Group V to VII were administered with test drug, TBA along with adjuvant at TED (5.5 mg/kg, orally), TED × 5 (27.5 mg/kg, orally), and TED × 10 (55.0 mg/kg, orally), respectively. The suspensions of test drugs were administered orally once a day for 28 consecutive days [17].

Initial body weight of all animals was recorded. General behavioral pattern was observed once a week by exposing each animal to open arena. On the 29th day, animals were weighed again and anesthetized with diethyl ether. Supraorbital plexus was punctured under light anesthesia and blood was collected by capillary in two different types of tubes, one containing anticoagulant fluid for hematological parameters and another plain tube for serum biochemical investigations. 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.

Hematological analysis was performed by using an automatic hematological analyzer (Swelab, Sweden). Total red blood cell (TRBC), hemoglobin (Hb), hematocrit, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, white blood cell (WBC), neutrophils, lymphocyte percentage, eosinophils percentage, monocyte percentage, 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 analyzer (BS-200, Lilac Medicare Pvt. Ltd., Mumbai). The studied parameters were blood glucose [18], urea [19], creatinine [20], total cholesterol [21], high-density lipoprotein (HDL)-cholesterol [22], triglyceride [23], very-low-density lipoprotein (VLDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, total protein [24], albumin, globulin [25], alkaline phosphatase [26], serum glutamic oxaloacetic transaminase (SGOT) [27], serum glutamic pyruvic transaminase (SGPT) [28], uric acid [29], direct bilirubin [28], total bilirubin [30], and serum calcium [31].

All the important internal organs were carefully dissected namely, liver, kidney, heart, lungs, trachea, intestine spleen, thymus, lymph node, testis, seminal vesicle, prostate, uterus, and ovary. After noting signs of gross lesion and ponderal changes of major organs, all were transferred to 10% phosphate buffered formalin solution for fixation and later on subjected to dehydrating, wax embedding, sectioning, and staining with hematoxylin and eosin for histological evaluation. The slides were viewed under trinocular research Carl-Zeiss's microscope at various magnifications to note down the changes in the microscopic features of the tissues.

2.6. Statistical analysis

The data are expressed as mean ± standard error of mean for six rats per experimental group. One-way analysis of variance was used to compare the mean values of quantitative variables among the groups followed by Holm–Sidak multiple *t*-test for unpaired data by using Sigmastat software (version 3.5, Systat Software Inc.) to determine significant difference between groups at $P < 0.05$.

3. Results

3.1. Acute toxicity study

The results of acute toxicity showed that both the samples of TB along with adjuvant did not affect any behavioral changes and other parameters during entire experimental period of 14 days.