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Research Protocol for Experimental Study of Acute and Sub chronic Dermal Toxicity of Mahabhallatak Tail in Wistar Albino Rats

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Abstract

Ayurvedic medicinal preparations that contain Vishadravyas (toxic substances) like Bhallataka (Semecarpus anacardium Linn.) are popular in the treatment of chronic skin diseases. Although their therapeutic value is important, they may have potential toxicological risks during long-term use and thus require scientific evaluation based on modern parameters. One of the classic formulations, as shown in Kushtaroga, is Mahabhallataka Taila, which includes Bhallataka as one of its key ingredients, which has strong pharmacological and irritant effects. The objective of the current research is to determine acute and subchronic dermal toxicity of Mahabhallataka Taila in Wistar albino rats with an aim of determining its safety profile. The acute dermal toxicity was measured based on OECD guidelines with 14 days observation period and subchronic toxicity was measured based on repeated dermal application, 90 days observation and 28 days recovery. Such parameters as clinical symptoms, body weight, food consumption, hematological and biochemical indicators, and histopathology were evaluated. It is likely that the study will offer scientific data regarding the dermal safety, and assist in establishing the potential local or systemic toxicity of long-term use, which will support safe therapeutic use and the global acceptance and standardization of Ayurvedic forms.

Keywords: Bhallataka, Semecarpus anacardium, Mahabhallataka Taila, Dermal toxicity, Ayurveda, Agadtantra, Wistar rats.

1. Introduction

The use of medicinal plant preparations has been rampant especially in the treatment of various diseases like skin disorders, arthritis and other chronic illnesses. Nevertheless, the widespread use or prolonged use of medicinal plants through the use of crude drugs, particularly at the beginning of the use, can occasionally cause severe toxicity in humans. Experimentation to guarantee the safety of drugs and food has its roots in ancient times. Ayurvedic writings explain preclinical testing procedures like Vishanna Pariksha, where the testing was carried out on animals (Shastri, 2017). As the use of natural products continues to increase globally, the safety, efficacy, and quality control of the ASU (Ayurveda, Siddha, and Unani) drugs have become a key concern in both the health authorities and the health care systems of the population (Central Council for Research in Ayurvedic Sciences [CCRAS], 2018). Non-clinical evaluation of drug safety usually consists of standard animal toxicological studies. In vivo data obtained from animal studies are more indicative of toxicity and can be considered important safety markers. The research and study of Ayurvedic medicines with no long history of documented use or studied beforehand should be done according to the guidelines on the evaluation of the safety of herbal medicines by WHO. As a result, adverse effects analysis must be regarded as a priority point when it comes to systematic safety assessment of such therapies (WHO,2002). Agadtantra, one of the eight branches of Ashtanga Ayurveda, deals with the toxicological aspects of medicinal plants as well as poisonous substances having therapeutic indications. Vishadravyas are employed in herbo-mineral preparations which are prescribed in the treatment of different dermal clinical manifestations of Dushivisha and Garavisha. Based on this, the current research paper by the title, Experimental Study of Acute and Subchronic Dermal Toxicity of Mahabhallataka Taila in Wistar Albino Rats has been conducted. Bhallataka (Semecarpus anacardium Linn.), an Upavisha, is a famous medicinal plant in Ayurveda and the Siddha medicine system. It has been used since ancient times both internally and externally in the form of Lepa, Ghrita and Taila in various medicinal formulations in the treatment of Kapha Vikara, Arsha, Kustha, Krimi, Gulma, Grahani, Vata Vyadhi and many more (Shastry, 2008). Maha-bhallataka Taila is a formula listed in Rasaratna Samuccaya under Kushta Roga Chikitsa Adhyaya, which is recommended to treat different kinds of Kushta Roga (Mishra, 2021). Charaka says that even a powerful poison can be a great medicine when administered rightly and even a medicine can be a poison when used in a wrong way ((Tripathi, Pandeya, 2000). Vishadravyas like Bhallataka are used in medicine due to their qualities like Ashukaritwa (immediate action), Ushna (hot potency) and Tikshna (penetrating nature), which assist the drug to travel fast within the body and promote the therapeutic effect. These formulations are occasionally administered over a period of time, either topically or orally, in chronic dermatological diseases like Dushivishajanya and Garavishajanya Twacha Vikara and Kushta. Such formulations might have potentially toxic substances such as Bhallataka and their prolonged use can have deleterious effects on other body systems or organs as well as the skin. The need of the time is to re-test the safety of ancient classical medicines on modern scientific parameters in order to determine their scientific validity. Thus, acute dermal toxicity (14-day study) and subchronic dermal toxicity (90-day study and 28-day recovery period) studies will be conducted to determine the safety profile of Mahabhallataka Taila. The study will be useful in determining the safety profile of this Ayurvedic formulation. As the world is becoming more accepting and using Ayurveda, Siddha, and Unani (ASU) medicines, the safety, efficacy, and quality

control of these products are becoming a matter of concern in regulatory bodies and the scientific community. The WHO guidelines are also keen on the importance of systematic safety appraisal of herbal medicines, especially that have not been adequately scientifically proven. Pre -clinical toxicity testing in animal models is significant as it gives valuable safety information and can be relied upon to give an idea on potential toxicity in humans. Agadatantra is a branch of Ayurveda that directly concerns the toxicology of drugs, including those that are toxic as well as therapeutic, known as Vishadravyas, that is, substances that are toxic and therapeutic, depending on how they are properly processed and administered. Bhallataka (Semecarpus anacardium Linn.) is an Ayurveda preparation that is classified as Upavisha, which is widely used in different Ayurvedic preparations because of its powerful therapeutic effects. One such formula is Mahabhallataka Taila, which was used in chronic dermatological disorders and is indicated in Kushtaroga. But since we have the Bhallataka that has irritant and possibly toxic properties, there is a risk of long-term harmful dermal application unless the safety of this compound is confirmed scientifically. As such formulations are frequently employed over long time periods in chronic skin diseases, it becomes important to assess their dermal toxicity. Thus, the current research will assess the acute and subchronic toxicity in Wistar albino rats of Mahabhallataka Taila with an aim of determining its safety profile based on the current toxicological parameters. The research will be useful in supporting the scientific evidence of safe therapeutic use of this classical Ayurvedic formulation.

1.1 Aim and Objectives

Aim: To study the acute and Sub chronic dermal toxicity of Mahabhallatak Tail in Wister albino Rats

1.2 Primary Objectives

1. To evaluate the acute dermal toxic effects of Mahabhallataka Taila in Wistar albino rats.
2. To evaluate the sub chronic dermal toxic effects of Mahabhallataka Taila in Wistar albino rats.

1.3 Secondary Objectives

1.To carry out the preparation and standardization of Mahabhallataka Taila.
2.To evaluate the effects of Mahabhallataka Taila on hematological, biochemical, and histopathological parameters in Wistar albino rats.

2. Materials and methods

2.1 Study Design and Setting

The current research is intended to be an experimental study done in an animal research laboratory that has been recognized by government. Wistar albino rats will be used as the study population and the study will last three years after the research protocol is approved.

2.2 Source of Data and Scientific Basis

The present study has conducted a comprehensive study of ancient Samhita Granthas and other related current scientific literature. Research studies in the recent past about the topic have also been taken into consideration to have a holistic understanding of the formulation and its effects. Moreover, the final product, Mahabhallataka Taila, is

going to be standardized in a government-approved laboratory to be able to guarantee its quality, safety, and reproducibility.

2.3 Collection and Authentication of Raw Materials

Bhallataka (Semecarpus anacardium Linn.) and Til Taila (Sesame oil) will be bought in the local market. The collected samples of Bhallataka will be identified and authenticated at the Department of Botany of the respective university as well as the Department of Dravyaguna of the home institute to ensure the authenticity of the raw materials.

2.4 Preparation and Standardization of the Drug

The department of Rasa Shastra and Bhaishajya Kalpana of the home institute will prepare the experimental drug, Mahabhallataka Taila. The said formulation will then be subjected to standardization by a government-approved laboratory to maintain consistency, quality, and safety.

2.5 Acute Dermal Toxicity Study Design

The acute dermal toxicity test will be carried out based on the OECD Guideline 402 (OECD, 2017). The study will choose a limit dose of 2000mg/kg body weight of rats. The drug will be applied as a single dermal application and applied covering about 10 percent of total body surface area using between 0.5 to 1 ml of the oil. The procedure will be carried out on a 2 x 1 cm patch where a thin and uniform layer will be ensured. The animals will be monitored over a period of 24 hours after administration of the drug to determine any toxicity.

2.6 Experimental Animals and Protocol (Acute Study)

The acute toxicity research will be conducted on Wistar albino rats of the albino strain that will be acquired at a recognised government animal research laboratory. The animals will weigh between 150- 200 grams and will be aged between 6–8 weeks. The study will consist of 10 animals (5 male and 5 female rats). It is planned to administer the drug as a single dose of 2000 mg/kg body weight, and observe the animals over a 24 hours period to see whether it has any toxic effects.

2.7 Subchronic Dermal Toxicity Study Design

Subchronic dermal toxicity test will be carried out according to OECD Guideline 411 (OECD, 1981). The drug will be applied dermally with three dose levels in a duration of 90 days. Oil will be applied to a 2 x1 cm patch on the body to cover 10 percent of the body area uniformly by using 0.5-1 ml of oil. A satellite group of 10 animals (5 males and 5 females) will receive the high dose level and will be followed up at least 28 days post-treatment to determine the reversibility, persistence or delayed expression of toxic effects.

2.8 Experimental Animals and Protocol (Subchronic Study)

The subchronic test is going to be done with a total of 50 Wistar albino rats of albino strain, which will be acquired in a recognized government animal research laboratory. The animals will be 150-200 grams and equal number of male and female rats will be used. The animals will be separated into five groups, each with 10 animals. The three dose levels of the drug will be given dermally within a period of 90 days. The study will observe the animals regularly over the period in which the study will be conducted to check whether they are showing any clinical signs of toxicity.

2.9 Assessment of Toxicological Parameters

In acute and subchronic studies, animals will be monitored to detect any toxicity, the onset, the degree and the duration of the toxic effects. The changes of the skin and fur, eyes and mucous membranes, respiratory and circulatory processes, and behavioral patterns will be observed. Morbidity and mortality of animals will also be monitored. To identify recovery, persistence or delayed toxicity subchronic animals in the satellite group will be monitored an extra 28 days untreated. The skin reactions will be graded as per the OECD Guideline 404. Any animals that perish during the study will undergo necropsy whereas the surviving animals will be sacrificed at the end of the study to further assess them.

2.10 Hematological and Biochemical Analysis

At the end of the period of study, the blood samples will be taken under light ether anaesthesia and analyzed. Hematological parameters which include haemoglobin percentage, total number of red blood cells, total number of white blood cells, platelets, and clotting parameters will be measured. The biochemical analysis will involve the clinical biochemistry tests, specifically liver and kidney tests, before and after the time of the experiment.

2.11 Histopathological Examination

At the end of the study, all animals will be sacrificed and subjected to necropsy. Several vital organs such as the heart, lungs, liver, spleen, kidneys, and skin will be meticulously removed, weighed separately and preserved in 10% formalin. These will be further subjected to histopathological examination to identify the presence of any structural or cellular abnormalities that may be related to the test drug.

2.12 Statistical Analysis

Statistical analysis of all experimental data such as body weight, organ weight, hematological parameters, and biochemical parameters will be done using Statistical Package for Social Sciences (SPSS) software version 23. Student t-test will be used to test the difference between means and analysis of variance (ANOVA) will be used to test the differences between and within groups. Experimental groups will be compared with the control group using Dunnett test.

2.13 Ethical Considerations

The research will be carried out in line with CPCSEA procedures and OECD 402 and 411 Guidelines. The study will start only after prior approval is received by the Institutional Animal Ethics Committee (IAEC). The required approval of the Institutional Ethics Committee (IEC) will also be obtained.

3. Discussion

The present study will be discussed with the classical and pharmacological properties of Mahabhallataka Taila along with its Ras, Guna, Virya, Vipaka, Prabhava and Rogaghnata properties as written in Ayurvedic literature. It will be necessary to critically analyze the results of the animal experiments, with special focus on the outcomes of the acute and subchronic tests of dermal toxicity. The correlation between the dose of the test material and the intensity of observed abnormalities, such as behavioral alterations, clinical effects, body weight changes, mortality effects, and other general or specific toxic effects will be determined. Moreover, the findings of the experimental studies will be expounded and discussed against the background of

the scientific knowledge available to present a complete picture of safety profile of Mahabhallataka Taila.

4. Conclusion

The findings of the acute dermal toxicity test and subchronic dermal toxicity test of Mahabhallataka Taila will be compared and contrasted. On the basis of this comparison, as well as the statistical analysis of the results of the experiment, a final conclusion will be made on the safety profile of the formulation.

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