ORIGINAL ARTICLE

Evaluation of acute toxicity of *Tribhuvana-Mishrana* in albino rats

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Abstract

Background: *Tribhuvana- Mishrana* (TM) is a complex herbo-mineral preparation used as an antipyretic agent. It is an effective remedy for Sannipata (~vitiation of all doshas/severe condition of any disease), Sarva Jvara (~all types of fever), and Pratishyaya (~coryza). **Objective:** The present work was conducted to establish the safety aspect of the use of TM. **Study and Design:** TM is the combination of three drugs - Tribhuvan Kirti Rasa (TKR), Godanti Bhasma (GB), and Sudarshan Ghanvati (SGV). For the preparation of TM, TKR, GB and SGV were prepared separately by an assortment of Ayurvedic procedures such as Shodhana (~purification), Marana (~calcination), and Bhavana (~lavigation). Then, fine powder of above three mentioned drugs was mixed homogenously as per the reference of Ayurveda Formulary of India. The resultant product was subjected to acute toxicity study. **Materials and Methods:** Acute toxicity study was conducted in Wister strain of albino rats as per the OECD guidelines 423. Criteria of assessment included behavior changes, hematological changes, and histopathological changes. Histopathological studies of different organs include brain, liver, kidney, and spleen were also conducted to observe pathological changes if any. **Results:** In acute toxicity study TM was found to be safe. Rats did not showed any signs of toxicity. All hematological parameters, namely, hemoglobin%, total leukocyte count, differential leukocyte count, and total count were found to be within normal limits. Histopathological study of different organs revealed normal cytoarchitecture.

Key words: Herbo-metallic formulation, histopathology, toxicity, *Tribhuvan-Mishran*

INTRODUCTION

Indian systems of medicine have long use of herbs, metals, and minerals as the base of their medicinal formulas. However, with changing times, it requires even more scientific validation regarding their safety profile, especially Herbo-metallic preparations are most targeted. In contemporary system of medicine, for any ailment drug is prescribed in a small dose and for short duration but it has adverse effects more than the benefits. Hence, in current era, everyone is looking toward Ayurveda for efficacious treatment in small dose without any side effects. Herbo-metallic formulations fulfill this requirement due to their innate qualities such as quick action, lesser dose, tastelessness, prolonged shelf-life, and better palatability.[1]

There are many single drugs and compound formulations described in the classical text for Jvara (~fever) in different dosage schedules such as Churna (~powder), Vati (~tablets), and Rasaushadhi (~herbo-metallic). Among them, Tribhuvan Kirti Rasa (TKR), Godanti Bhasma (GB), and Sudarshan Ghana Vati (SGV) are widely used in practice for the treatment of Jvara (~fever). On the behalf of this Ministry of Ayush, Government of India prepared a combination of the above three formulations known as *Tribhuvan-Mishran* (TM),[2] which is found to be safe and efficacious in different diseases but these claims have not been validated. The present study was designed to establish the toxicity profile of antipyretic preparation named as TM.

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MATERIALS AND METHODS

Collection of Raw Materials

All the ingredients were procured from the Khari Baoli Market, Old Delhi, India. For Bhavana Dravya fresh leaves of Nimba (*Azadirachta indica* A. Juss.), Dronapushpi (*Leucas cephalotes* (Roth) Spreng), Tulasi (*Ocimum sanctum* L.), Datura (*Datura stramonium* L.), and Nirgundi (*Vitex negundo* L.) which were collected from Rajaji National Park Shyampur, Haridwar, Uttarakhand, India, and rhizome of Adraka (*Zingiber officinale* Roscoe) procured from local market of Haridwar. All the drugs were authenticated by the subject expert.

Preparation of TM

For the preparation of TM, TKR,[3] GB[4] and SGV[5] were prepared separately as per the classical references. Then, fine powder of above three mentioned drugs was mixed homogenously in the ratio of 1:1:2 as per the reference of Ayurveda Formulary of India[6] [Table 1].

Experimental Animals

Albino rats of Wister strain of both sex weighing between 150 and 200 g were used in this study. They were procured from the animal house of "Institute of Biomedical and Industrial Research," Jaipur, Rajasthan. The animals were kept under standard condition of 22°C + 2°C and relative humidity 50 + 60%. The rats had free access to food (Pranav agro mills "Amrut" brand rat pellets) and water *ad libitum* with 12 h light and dark cycle. All animals were acclimatized for at least 5 days before the start of the study. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) with Approval no. ibir/iaec/2015/II/4 and performed according to the CPCSEA guidelines for care and use of animals. Dose of TM was escalated as per conversion of human dose to experimental animal dose.[7]

Acute Toxicity of TM

Acute toxicity of TM was carried out as per the OECD guidelines 423 on Wister strain of albino rats for 14 days. Total, 6 albino rats of both sexes, weighing 150–200 g were divided randomly into two groups, containing three animals each. All animals in Group I were treated with 300 mg/kg TM. Animals of Group II were given 2000 mg/kg TM. Single dose of the drug was administered orally according to the stated dosage schedule. Gross behavior and exitus (death) were recorded for 14 consecutive days.

Table 1: Ingredients of TM

Ingredients	Quantity	
TKR	(1 part)	TM
GB	(1 part)	
SGV	(2 part)	

TM: *Tribhuvan-Mishran*, TKR: Tribhuvan kirti Rasa, GB: Godanti Bhasma, SGV: Sudarshan Ghanvati

Behavioral Observation

All observations were systematically recorded with individual records being maintained for each animal, namely, changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern and observations regarding any tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

Laboratory Investigation

All the behavioral, hematological and histopathological studies were conducted in "Institute of Biomedical and Industrial Research," Jaipur, Rajasthan.

Hematological Analysis

Hematological analysis was performed using an automatic hematological analyzer. Samples of blood were drawn at the beginning and termination of the experiment. Parameters studied included hemoglobin, white blood cells (WBC) count, red blood cells (RBC) count, neutrophils count, lymphocytes counts, eosinophils count, monocytes count, basophils counts, and platelets count were investigated by complete blood count.

Histopathological Study

All the vital organs were carefully dissected, cleaned, weighed, and transferred to Bouin's solution for preservation and make slides. The slides were scanned in Carl Zeiss's microscope (Germany) under different magnifications. Changes if any, in cytoarchitecture were noted down.

RESULTS AND DISCUSSION

For the treatment of Jvara (~fever), number of formulations of different dosage forms from simple Churna to Bhasma and Rasaushadhi are advocated in Ayurveda. Furthermore, in traditional practice, Vaidhyas (~physicians) prescribe combination of drugs for the treatment of different diseases. TM is one such combination in which three dosage forms, namely, Vati, Bhasma, and Rasaushadhi are incorporated in single combination. This combination is claimed to have tremendous antipyretic potential by different Vaidhyas

(~physicians), and hence, this combination by the name of "TM" has been included in Ayurveda Formulary of India Part III by Ministry of Ayush, Government of India.[6] As no scientific data are available for its antipyretic role, the author of the present research work has studied its antipyretic and antimicrobial activity.[8] Before commencing

Table 2a: Behavior observations of test sample TM at dose 300mg/kg

Observation (average value)	30 min	4 h	24 h	48 h	1 week	2 weeks
Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil
Morbidity	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil	Nil	Nil

Table 2b: Behavior observations of test sample TM at dose 2000 mg/kg

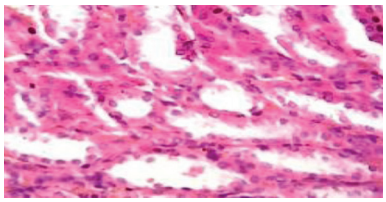
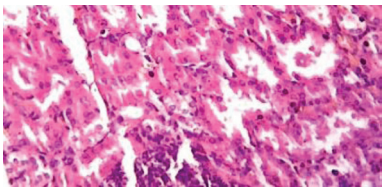
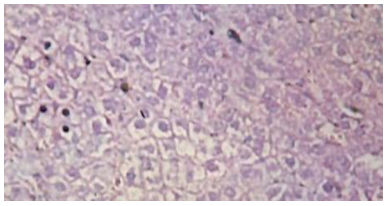
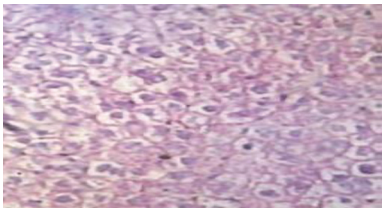
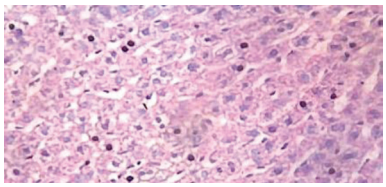
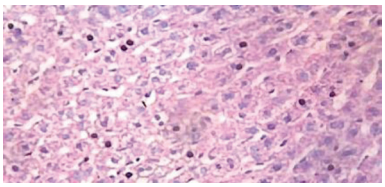
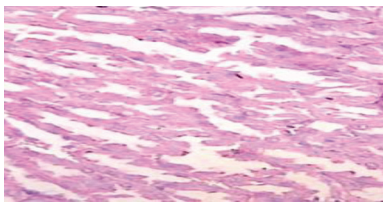
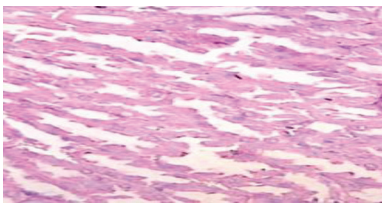
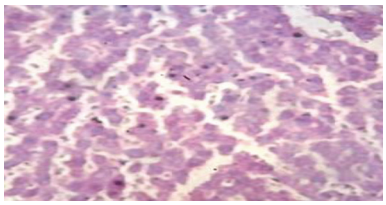
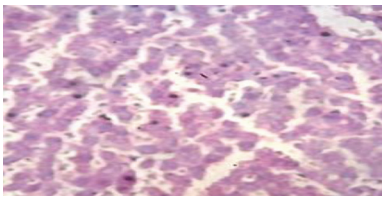
Observation (average value)	30 min	4 h	24 h	48 h	1 week	2 weeks
Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous Membrane	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil
Morbidity	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil	Nil	Nil

Table 3: Hematological observations on the 7th day and 14th day at dose 300 mg/kg and 2000 mg/kg of test sample TM

Hematologic parameters	300 mg/kg (Mean)	2000 mg/kg (Mean)	300 mg/kg (Mean)	2000 mg/kg (Mean)	Normal range
	7th day	7th day	14th day	14th day	
Hemoglobin	14.2	14.9	13.6	15.5	11.5–16.1 g/dl
WBC	9.8	7.9	8.9	8.5	6.6–12.6×10^3/mm^3
RBC	7.3	8.3	7.4	7.9	6.76–9.75×10^6/mm^3
Neutrophils	3.1	3.6	3.9	2.9	1.77–3.38×10^3/mm^3
Lymphocytes	5.6	7.1	8.4	8.4	4.78–9.12×10^3/mm^3
Eosinophil	0.04	0.03	0.03	0.07	0.03–0.08×10^3/mm^3
Monocytes	0.02	0.03	0.03	0.04	0.01–0.04×10^3/mm^3
Basophils	0.0	0.0	0.00	0.00	0.00–0.03×10^3/mm^3
Platelets	367	386	451	384	150–460×10^3/ml

WBC: White blood cells, RBC: Red blood cells, TM: *Tribhuvan-Mishran*

Table 4: Observations on histopathological analysis on the 14th day at dose 300 mg/kg and 2000 mg/kg of test sample *Tribhuvan-Mishran*

300 mg/kg	2000 mg/kg
	
Kidney: Cells have normal architecture of renal glomeruli with intact Bowmans capsule. Brush bordered cuboidal epithelium lining the proximal convoluted tubules	
	
Spleen: Cells showing normal morphology of lymphocyte, red blood cells, neutrophils, macrophages, and platelets	
	
Heart: The nuclei appeared normal, the connective tissue was normal, the nuclei and cardiac muscle fibers were well arranged	
	
Liver: It was observed that the sections conformed to normal histological features. The sinusoids in the sections of the treated rats are devoid of occlusions and are not distorted	
	
Brain: The section of the cerebellum showed the normal histology of the cerebellum and its layers - the outer cerebellar cortex and inner medulla	

the above-mentioned study, namely, antipyretic and antimicrobial the author also carried out acute toxicity study as the combination contain certain metals, minerals, and toxic herbs like Vatsanabha in it.

Oral acute toxicity study of TM was carried out as per the OECD guidelines 423 on Wister albino rats for 14 days. TM at dose 300 mg/kg and 2000mg/kg was found safe with no morbidity and no mortality and no any behavioral changes were found as per shown in Table 2a and b. All the hematological parameters with hemoglobin, WBC, RBC, neutrophils, lymphocytes, eosinophil, monocytes, basophils, and platelets were found to be within normal limit as manifest from Table 3. Histopathological findings of body organs, namely, kidney, spleen, heart, liver, and brain in all sections revealed normal cytoarchitecture.

Vatsanabha, one of the contents of TM, is reported to have cardiac depressant properties,[9] but in Ayurveda Vatsanabha is used after a special procedure of Shodhana (~purification

and potentiation) in which it is treated with Gomutra by the dipping (Table 4).[10]

One study has reported that Vatsanabha after Shodhana process with Gomutra converts Aconite into a compound with cardiac stimulant property.[11,12] In another study, Shodhana by both Gomutra and Godugdha makes Aconite devoid of cardiac and neuromuscular toxic effects without affecting its antipyretic activity.[13] Simultaneously, Gomutra potentiates the Vata-kapha hara properties of Vatsanabha, being a Vata-kapha hara Dravya itself. Vatsanabha specifically indicated in inflammatory fevers and due to its swedajanana property, it possesses good antipyretic activity.[10]

Another compound of TM, i.e., was also evaluated for its acute toxicity, antiulcer, and antipyretic activity in experimental animals which revealed that in the acute oral administration of GB showed no mortality in mice in the period of 14 days. GB also showed significant ($P < 0.001$) protection in cold restrain stress-induced ulcer. A significant ($P < 0.001$) reduction in Jvara (~fever) in the rat was also produced by GB.[14]

The key ingredient of SGV (third component of TM) is Swertia chirata which constitute 50% of this total composition is known mostly for its bitter taste caused by the presence of different bioactive compounds such as amarogentin (most bitter compound till date)[15] and swerchirin.[16] Both compounds were proved to possess antipyretic, antibacterial, and antiviral activities.[17]

Results of the present study showed that TM is the combination of herbal, metals, and mineral dosage forms even in the preparation of metals and mineral or toxic herbs was found to be safe.

The reason for non-toxic nature could be as metals in Ayurvedic formulation is not present in elemental form.[18,19] Physicochemical state of the heavy metals in the form of Ayurvedic medicine is totally different from the known physicochemical forms of that metal.[20-23]

Critical analysis of the observations mentioned above reveals that TM did not impart any untoward effect.

CONCLUSION

The results of present study are coherent with the Ayurvedic literature. There were no significant changes in behavioral, biochemical, and histopathological parameters of TM treated rats, thus proving its safety. Hence, TM can be used at recommended dose and duration. The author recommends undergoing subacute and chronic study of the combination before coming on to the final conclusion.

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