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Acute Oral Toxicity Study of *Terminalia bellerica* Gum Polysaccharide In Swiss Albino Female Mice As Per OECD Guideline 423

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ABSTRACT

Toxicology is the important aspect of pharmacology that deals with the adverse effect of bio active substance on living organisms prior to the use as drug or chemical in clinical use. As per the OECD guidelines, in order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like mice, rat, guinea pig, dog, rabbit, monkey etc under various conditions of drug. Toxicological studies help to make decision clinically without its clinical trial as well as toxicity studies. The present study has been under taken to study the adverse or hazardous effects of polysaccharide extract from *Terminalia bellerica* gum (TBG) and establish the safety over, Swiss albino mice of female sex as per OECD guidelines 423. All the animals were acclimatized to laboratory conditions for one week prior to the experiment and mice were sequentially administered orally the extract of *Terminalia bellerica* gum (TBG) in dosage of 50, 300, 2000 mg/kg body weight. All the animals were observed for mortality and different signs of toxicity for 14 days. No mortality or any significant changes were observed at 2000 mg/kg body weight.

Keywords-OECD guideline 423, *Terminalia bellerica* gum, polysaccharide, Swiss albino mice

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INTRODUCTION

Medicines derived from different part of the plant have been used by the human being since thousand years ago. Initially plant medicines were taken in the form of crude drugs such as tinctures, elixirs, poultices, powders, and other herbal formulations. However, the use of herbal products should be based on scientific origin; otherwise they would be useless and unsafe. Furthermore, the irrational use of these herbal products may cause serious toxicity for humans. Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs. A typical example for a toxic herbal product are the leaves of *Atropa belladonna* and *Digitalis purpurea*, which show severe systemic toxicity if taken orally [1, 2].

Traditional use of herbal drugs may broadly be divided into three categories as follows:-

- Those are well known and have been widely used for many years.
- Those are not well known in the country but for which international experience is available.
- That represents a new compound hitherto not evaluated as to its safety and efficacy.

The first category mainly consists of foodstuff product (s), which has been in use for a long time as traditional herbal remedies, and the requirements are limited. In general, it these foodstuff products are generally safe for human and animal consumption and establishment of safety of these products is not so important. For the second category, views concerning the type of documents required to be presented may differ from country to country. So it is necessary that varieties of requirements will be elaborated for these products covering anything from reference in scientific literature confirming that the product is safe. To satisfy the demands for limited or shortened toxicological testing of these products, an investigation must be carried out on toxicity profile. The third group, where the authority is faced with a product not previously screened for its toxicological properties, toxicity studies of those product must have to be undertaken. Depending on the duration of drug exposure to animals, toxicological studies may be three types such acute, sub-acute and chronic toxicological studies. In acute toxicity studies, single dose of drug given in large quantity to determine immediate toxic effect. Acute toxicity studies are commonly used to determine LD50 of drug or chemicals and natural products. In sub-acute toxicity studies, repeated doses of drug are given in sub-lethal quantity for a period of 15 to 20 days. Sub acute toxicity studies are used to determine effect of drug on biochemical parameters of tissues. In chronic toxicity studies; drug is given in different doses for a period of 90 days to

over a year to determine carcinogenic and mutagenic Potential of drug [3-5].

Toxicity tests are mainly focused at discerning the compilations arising from the therapeutic use of the drug. Hence, a special attention should be paid to the solvent, dispersing agents, additives and excipients as the toxic effect may arise from these sources. During the period of the toxicity tests taking care of the animals is also of very much important task [6]

Terminalia bellerica in India has been used for centuries in the Ayurveda (a holistic system of medicine originating from India). The dried fruit and other parts used for medicinal purposes but the purified polysaccharide obtained from gums of *Terminalia bellerica* has no data for safety in animal till date. Hence, the following study of acute toxicity was performed to confirm the safety of TBG polysaccharide in animal [7].

MATERIALS AND METHODS

Extraction of gum polysaccharide

Dried gums were collected from the trunk bark of *Terminalia bellerica* plant from Similipal forest of Odisha and separated from fungal affected and other foreign object present during collection. They were converted into small pieces using a hammer. The pieces were then dried in oven dryer maintained between 40°C to 50°C for 72 h to obtain dry mass which were milled with a grinder to get the dry powder. The dried powder was passed through sieve number 40# and stored in an airtight container. The powder (1 kg) was extracted with boiling water three times. The aqueous extract was filtered through Whattman filter paper. The filtrate was concentrated in a rotary evaporator under reduced pressure, and then centrifuged at 3000 rpm for 15 min. The supernatant was precipitated with three volumes of acetone, and stored overnight at 4°C. The precipitate was collected and washed again three times with acetone to get crude polysaccharide (60.4 g). The crude polysaccharide was subjected to DEAE- Sephadex A-50 column chromatography, washed with H_2O and eluted with 1.0 M NaCl solution. Most of the pigments were absorbed in the column. The Elutes collected were concentrated under reduced pressure to an appropriate volume, and then dialyzed against distilled water. The remaining portion was lyophilized to afford total purified polysaccharide [8].

Purified polysaccharide isolated from dried plant gum of *Terminalia bellerica* was used as test drug in these experiments and propylene glycol was used as control vehicle.

Experimental Animals

Swiss albino mice of female sex weighing 20-25gms were used for toxicity study as Figure-1. This study was approved by the Animal Ethics Committee of Girijananda Choudhury Institute of

Pharmaceutical Sciences, Guwahati, Assam with CPCSEA Regn. No.1372/C/10/CPCSEA. Study approval No-GIPS/IAEC/10. Acute oral toxicity study was performed as per OECD guideline 423. The mice were housed in standard polypropylene cages having solid bottom in well-ventilated animal room. Sawdust was spread on the bottom of the cage to absorb urine and moisture from feces.

The animals were acclimatized to laboratory conditions for one week prior to the experiment. The temperature in the animal house was maintained at 25 ± 2˚C with a relative humidity of 30–70% and illumination cycle set to 12 h light and 12 h dark and other conditions are maintained as per Table 1.

Figure.1:- Swiss Albino Female Mice

Table1:- Laboratory Conditions as Per OECD 423

S.no.	Condition	Requirement
01	Room temperature	25 ± 2˚C
02	Humidity	30–70%
03	Light and dark period	12/12 Hrs
04	Bedding	Clean Sterilized Husk
05	Oral feed	Standard Pellet Chow
06	Distilled drinking water	Unlimited Supply

Noisy atmosphere was avoided as much as possible for healthy living condition of mice, as mice are very sensitive to noise. The mice were fed with standard pellet diet of composition mentioned as per Table .2.

Table.2:- Composition of Standard pellet diet

S.no.	Name of the ingredient	Percentage (%)
01	Wheat flour	63
02	Casein	15
03	Sucrose	10
04	Groundnut oil	05
05	Salt mixture	04
06	Shark liver oil	02
07	Vitamin mixture	01

Method of evaluation

Twelve female Swiss albino mice were acclimatized for a week in cleaned polypropylene cages and randomly divided into four groups of 3 animals each. The animals were fasted 4 hrs prior to dosing. Following the period of fasting, the animals were weighed to determine the appropriate weight of the test drugs to be administrated to animals. In all cases the maximum volume of the aliquot portion of test drug administrated did not exceed1ml/100mg of the animal body wt. Four doses were selected (5mg/kg, 50mg/kg, 300mg/kg and 2000mg/ kg) as per guideline. Three animals were used for each step.

Since there is no information of the toxicity of *Terminalia bellerica* gum polysaccharide for animals, it was decided to use the starting dose of 5mg/kg of *Terminalia bellerica* gum polysaccharide suspended in 1% w/v acacia. The *Terminalia bellerica* gum polysaccharide was administrated orally to first animal and the dosing for second animal was delayed to observe the toxicity of the previously dosed animal. Once no toxicity sign were observed in previously dosed animals, third animal was dosed.

Observations of the animals were done individually after dosing at least once during the first 30minutes, periodically during the first 24 hrs, with special attention given during the first 4 hours and daily thereafter for a total of 14 days. Since no toxic effects were observed for 24hrs on 5mg/kg group, the second group of animals received 50mg/kg orally and the animals were observed individually after dosing for toxic effects. Third group of animals received 300mg/kg orally and were observed for toxic effects. After observation of 24 hrs, fourth group animals were given with 2000mg/kg orally and were individually observed for toxic effects. As per guidelines, only when justified by special regulatory needs, the use of additional upper dose level of 5000mg/kg body weight may be considered. For the Animal welfare concern testing of animals, in ranges of 2000-5000mg/kg was discouraged [9, 10].

RESULTS AND DISCUSSION

The purpose of this study was to evaluate toxicity profile of the *Terminalia bellerica* gum polysaccharide. 14 day acute oral toxicity study was performed in Swiss albino mice. The LD50 of *Terminalia bellerica* gum polysaccharide were not further studied as it was found to be safe up to 2000 mg/kg on 24 h study basis [11].

It was observed that the animals fed with the *Terminalia bellerica* gum polysaccharide were found to be healthy. The animals were observed for any mortality and morbidity (convulsions, tremors, and grip strength and pupil dilatation etc given in Table no.3) at an interval of 12 h for

14 days. No unusual changes in behavior or in locomotors activity, ataxia, and signs of toxicity were observed during the 14 days period. No differences were found in growth behavior between the control and treatment group in 14 days of study. The body weight of male and female Swiss albino mice was found to be normal after treatment. There was no change observed in body weight of control and modified starch treated mice.

Table.3:- Acute toxicity study of *Terminalia bellerica* gum polysaccharide with respect to various sign of toxicity

Signs of Toxicity	Control group Normal saline	Test-I 50mg/kg	Test-II 300mg/kg	Test-III 2000mg/kg
Respiratory Blockage in Nostril	Nil	Nil	Nil	Nil
Dysponea	Nil	Nil	Nil	Nil
Aponea	Nil	Nil	Nil	Nil
Nostril Discharge	Nil	Nil	Nil	Nil
Motor Activities	Normal	Normal	Normal	Normal
Locomotion	Normal	Normal	Normal	Normal
Loss of rithing reflex	Normal	Normal	Normal	Normal
Anesthesia	Normal	Normal	Normal	Normal
Tremor	Normal	Normal	Normal	Normal
Toe Walking	Normal	Normal	Normal	Normal
Convulsion(Involuntary contraction)Clonic/tonic/tonic-clonic convulsion	Nil	Nil	Nil	Nil
Reflexes	Normal	Normal	Normal	Normal
Corneal	Normal	Normal	Normal	Normal
Eye lid closure	Normal	Normal	Normal	Normal
Rithing	Normal	Normal	Normal	Normal
Light	Normal	Normal	Normal	Normal
Auditory and sensory	Normal	Normal	Normal	Normal
Ocular Signs	Normal	Normal	Normal	Normal
Mitosis	Normal	Normal	Normal	Normal
Mydriasis	Normal	Normal	Normal	Normal
Lacrimation	Normal	Normal	Normal	Normal
Heart rate	Normal	Normal	Normal	Normal
Saliva Secretion	Normal	Normal	Normal	Normal
Analgesia	Nil	Nil	Nil	Nil
Hypo or Hyper-tonia	Nil	Nil	Nil	Nil
GIT- Solid	Nil	Nil	Nil	Nil
dried/Watery Stool	Normal	Normal	Normal	Normal
Emesis Red urine	Nil	Nil	Nil	Nil
Skin-Oedem/Erythrema	Nil	Nil	Nil	Nil

CONCLUSION

On the basis of the acute toxicity study we may conclude that *Terminalia bellerica* gum polysaccharide was nontoxic in female Swiss albino mice (which is most sensitive to the toxic effect of drug) and could be used in further study in Swiss albino mice within the dose of 2000mg/kg body weight. It can also be predicted that *Terminalia bellerica* gum polysaccharide could be similarly safe in Newzeland rabbit in terms of toxicity as we found in female Swiss albino mice.

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