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Research Article

TOXICITY STUDY OF COMBINED HERBAL EXTRACTS

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Abstract:

The present study was under taken to ascertain the safety aspect of the combined drug through acute and repeated toxicity studies. We have combined aqueous extracts of *Eugenia jambolana* (EJ), *Momordica charantia* (MC) and *Gmelina Arborea* (GA). The group A contains EJ, MC, GA into 1:1:1 proportion, group B contains EJ, MC group C contains EJ and MC group D is normal control. Acute toxicity study was carried out in Swiss albino mice and repeated toxicity study was carried out in albino rats at different dose levels. The parameters studied were haematological, biochemical and histopathological in case of repeated toxicity study, and mortality and behaviour change in acute toxicity. In acute toxicity study the test drug did not produce mortality up to the dose of 5000 mg kg-1 (orally) in mice. The result or data obtained during the study indicate no significant changes observed in hematological, biochemical and histopathological parameters.

Key Words: *Eugenia jambolana* (EJ), *Momordica charantia* (MC), *Gmelina Arborea*, (GA), toxicity

INTRODUCTION:

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorders causing hyperglycemia, which is due to impaired carbohydrate "glucose" utilization resulting from a defective or deficient insulin secretary response. Along with hyperglycemia and there are also abnormalities in serum lipids. The disease causes morbidity and long-term complications and an important risk factor for cardiovascular diseases[1]. To-date there are different groups of oral hypoglycemic drugs and insulin for clinical use, having characteristic profiles of side effects[2,3].

Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing the demand for complementary and alternative medicine with antidiabetic activity and less side effects. Numerous herbal preparations have been shown to affect blood glucose levels through various mechanisms, although they are usually limited by toxicity or relative lack of efficacy compared with standard medications

Numbers of plants have been mentioned in Ayurveda for the treatment of diabetes. *Momordica charantia* (MC) a well know herb and used in number of antidiabetic formulations.[4, 5] Also it has been documented in the literature that it shows insulinmimetic activity.[6] *Eugenia jambolana(EJ)* is also traditionally used for the treatment of diabetes mellitus.5,[7] There are recent publications and also few studies going on to access antidiabetic activity of *Gmelina arborea* bark.[8] Individuals toxicities of all three plant extracts has been published in the literature and confirmed that the aqueous extracts of these plant does not produce any toxicity up to 2000 mg/kg does.[9] Considering this the present study was conducted to assess the acute and repeat dose toxicity of combined aqueous extracts of *Momordica charantia* (fruit), *Eugenia jambolana* (seeds) and *Gmelina arborea* (stem bark). These toxicity studies were conducted to confirm safety of selected aqueous extract in mice and rats before testing the same in hyperglycemic rats for antidiabetic activity.

MATERIALS AND METHODS

Selected Plants and extracts*: Aqueous extracts of Plant:*

- ***Momordica charantia fruit (MC)-Fruit***
- ***Eugenia jambolana seed (EJ)-Seeds***
- ***Gmelina arborea bark (GA)-Stem bark***

1. Combined in a ratio of 1:1:1 (EJ+MC+GA)
2. Combined in a ratio of 1:1 (EJ+MC)

Preparation of plant Materials and Extracts: All the plant materials were collected from Pune and Thane district of Maharashtra. The plant material was then air dried at room temperature. The dried plant material was grounded into a fine powder. The Seeds of *Eugenia jambolana,* fruits of *Momordica charantia* and stembark of the *Gmelina arborea were* authenticated by Maharastra Association for the Cultivation of Science, Agharkar Research Institute, Pune (Maharastra). For future reference a voucher specimens were prepared and deposited in the Department of Pharmacology, SPTM, NMIMS University, Mumbai. The powdered materials were used to prepare the aqueous extract. The extracts were prepared under Good Manufacturing Practice condition at Phytoconcentrates Pvt. Ltd, Ahmadabad (India).

Experimental Animals: Albino mice of either sex were used for the acute toxicity study. Male albino rats of either sex were used for repeat dose toxicity study Animals were maintained in the Animal House, under standard conditions (temperature 25◦C±2◦C, relative humidity 75%±5%, and 12-h light-dark cycle). During the experiments animals were provided with standard rodent pellet diet (Amrut feed) water *ad libitum*. The study was conducted after obtaining prior approval from the institutional Ethical Committee in accordance (IAEC) with the National Institute of Health “Guide for the Care and Use of Laboratory Animals” (NIH publication no. 86-23, 1985).

Acute toxicity: Swiss albino mice of both sexes were randomly divided into ten groups as mentioned in the table-1, each containing five animals (Weight: 25±5 g,

age: 6–8 weeks). The aqueous extract's were administered orally at doses of 300, 2000, and 5000 mg/kg of body weight (OECD, 423).[10] Distilled water was administered to control group. The general behaviour of the mice was continuously monitored for 1 h after dosing, periodically during the first 24 h with special attention given during the first 4 h (Hilaly et al., 2004), and daily thereafter, for a total of 14 days. Cage side functional observation batteries (FOBs) such as convulsion, vomiting, diarrhea, paralysis, breathing difficulties, bleeding, irritations, and abnormal posture, were also observed. Changes in the normal activity of mice and their body weights were monitored and the time at which signs of toxicity or death appeared recorded. Signs of toxicity and mortality were observed daily for 14 days, with food and water intake ad libitum. During the study, food consumption was evaluated at on daily basis. Body weights of the animals were also recorded regularly. All surviving animals were euthanized with diethyl ether at day 14 and various organs like the liver, lungs, heart, spleen and kidneys were removed, weighed and carefully examined macroscopically for any abnormal, pathological signs of toxicity.

Table- 1 Doses and groups selected for Acute Toxicity Study

Group No	Test Extract	Doses studies mg/kg
A	EJ (aqueous extract of seeds) + MC+ GA (1:1:1)	300, 2000, 5000
B	EJ (aqueous extract of steam bark) + MC + GA (1:1:1)	300, 2000, 5000
C	EJ+MC 1:1	300, 2000, 5000
D	Normal Control	Dist. Water

Repeat Dose Toxicity study: Fifty albino rats were used for study and randomly assigned into four groups mentioned in table:2 below containing five animals in each (body weight (BW): 150-200 g; age: 6-8 weeks old). Treatment groups were administered aqueous extract orally by gavage once a day for 28 days. A group of animals, serving as control, received normal saline (0 mg/kg BW); the second (Low dose), third (Medium dose) and fourth (High dose) group received the

combined aqueous extract at doses of 500, 1000 and 2000 mg/kg BW respectively. All animals were observed for morbidity and mortality, twice daily. Different Signs noted include, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. selfmutilation, walking backwards) were also recorded. At the end of study surviving animals were fasted overnight, and anesthetized for isolation of vital organs for histopathological observations. Blood were collected from the right ventricle for biochemical analysis.

Table : 2 Doses and groups selected for Repeat Dose Toxicity study

Group No	Test Extract	Doses studies mg/kg
A	EJ (aqueous extract of seeds) + MC+ GA (1:1:1)	500, 1000, 2000
B	EJ (aqueous extract of stem bark) + MC + GA (1:1:1)	500, 1000, 2000
C	EJ+MC 1:1	500, 1000, 2000
D	Normal Control	Dist. Water

Body Weight, Food and Water Intake were measured once a week. Haematological analysis was performed using automatic hematological analyzed (Sysmex, Japan) at baseline and end of the study. All the major biochemical parameters (like Blood glucose, creatinine (CRE), blood urea nitrogen (BUN), alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) total billirrubin (Bil), total protein (PRO), albumin (ALB)) were analyzed at the baseline and end of the study using autoanalyzer (Erba Chem 7, Germany)

At the end of study all the animals in the study were subjected to a full, detailed gross necropsy which included careful examination of the external surface of the

body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, lungs, kidneys, adrenals, gonads, spleen, heart and brain of all animals were removed and their wet weights were taken immediately after dissection to avoid drying. Liver, kidney, stomach, intestine, spleen, pancreas, adrenal, lungs, heart, brain and gonads were fixed immediately in 10% formalin for routine Histopathological examination.

RESULTS AND DISCUSSION :

Acute toxicity: The present investigation showed that all the combinations i.e A, B and C were non-toxic when administered to mice as a single oral dose at 300, 2000 and 5000 mg/kg .Oral administration of these extracts at given dose did not produce mortality or symptoms of toxicity According to Hilaly et al. (2004), this study of oral administration the no-observed adverse effect (NOAE) dose was found as 5000 mg/kg of body weight and same dose can be considered as maximum tolerated dose (MTD).

There were no statistically significant difference in the body weights and food consumption trend was observed for all the groups. There were no significant biologically significant changes observed when compared to control group and other groups in acute toxicity study. There was no mortality in any of the tested doses at the end of the 14 days of observation.

Repeat Dose Toxicity: No deaths or significant changes in general behaviour or other physiological activities were observed at any point in the present study. Neither clinical signs of any toxic or adverse effect were noticed throughout the study.

Body weight and food intake: On administration of combined extract at doses 500, 1000, and 2000 mg/kg of BW for 28 days did not produce any changes in body weight from initial body weight and food intake in the control and treated groups of rats.

Hematological and biochemical parameters of rats: The hematological analysis showed no significant changes of RBC, Hb, HT, WBC, and platelets in the treatment group compared to the control group. The leukocyte differential count showed no difference between groups. The biochemical analysis showed no significant differences in any of the parameters examined in either the control or treated group.

Organ weights and Pathological examinations: There were no significant differences between the control and treated groups in the organ weights in test and control groups. Pathological examinations of the tissues on a gross basis indicated that there were no detectable abnormalities. No alterations were seen in the microscopic examination of the internal organs, the cellular architecture was not changed in both groups. All animals survived until the scheduled euthanasia and no gross pathological alteration was found in the internal organs. Organ weight revealed that aqueous extract at the test doses used did not produce organ swelling, atrophy or hypertrophy.

Histopathological examination of liver, kidney, and pancreas in the control and the animals treated with the extracts showed no lesion that could attribute to the effect of oral administration of the aqueous test extracts for 28 days. So the lowest

observed adverse effect level of combined extracts is ranges between 500 to 2000 mg/kg following oral administration.
The results if repeat dose toxicity study indicates that the test extracts has no effect on organ weight and body weight. The results of biochemical studies indicate that the extracts have no significant adverse effect on studied parameters. The histopathological examination liver, kidney, pancreas in the control and treated groups shows no differences suggesting that the extract at those dose tested did not result in any adverse toxicological effect on these organs.

Therefore the combined extract A, B and C at dose level 500, 1000 to 5000 mg/kg of body is considered as safe or with negligible toxicity level. Drugs with LD50 1000 and 2000 mg/kg of body weight are considered as safe according to the report of Clark and Clark (1977) and OECD guidelines respectively.

The result of acute toxicity study indicates that the LD50 of aqueous test extract's is greater than 5000 mg/kg oral dose. The results of repeated dose toxicity study suggest that the all three test extracts are safe and nontoxic at tested dose i.e between 500 to 2000 mg/kg body weight for oral administration The results of both the studies therefore suggest that the test extracts in selected combination is nontoxic and safe in oral formulation. So the finding of both the acute oral and 28 days sub-chronic toxicity study could be an indication that the tested aqueous extracts has some high level of safety margin in oral formulation this justifying is wide use in herbal formulation's.

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