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Acute Oral Toxicity studies and dose fixation of *Hibiscus rosa sinensis* ethanolic flower extract in non-obese diabetes induced Wister rats.

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Abstract:

Diabetes mellitus includes various diverse disorders commonly exhibiting episodes of hyperglycaemia and glucose intolerance, as a result of insulin deficiency, defective insulin action, or both[1]. Such complications arise due to defects in the regulatory mechanisms which control the storage and mobilization of glucose and other metabolic fuels. The catabolism and anabolism of carbohydrates, lipids and proteins as a result of the defective insulin secretion is also one of the major causes of the disease [2, 3]. Hibiscus rosa-sinesis Linn. (Malvaceae) is a glabrous shrub widely cultivated in the tropics and has been known for its diverse medicinal properties, however, it is important to access the toxicity of the ethanol extract of wild variety of Hibiscus rosa sinensis flowers via oral toxicity test which has been carried out in the present study.

Keywords: Acute toxicity, Hibiscus rosa-sinensis L, , Malvaceae, lipid profile, Kidney profile, biochemical parameter.

Introduction:

Diabetes mellitus is a cartel of various diverse disorders commonly exhibiting episodes of hyperglycaemia and glucose intolerance, as a result of insulin deficiency, defective insulin action, or both[1]. Such complications arise due to defects in the regulatory mechanisms which control the storage and mobilization of glucose and other metabolic fuels. The catabolism and anabolism of carbohydrates, lipids and proteins as a result of the defective insulin secretion is also one of the major causes of the disease [2, 3]. Classification of diabetes mellitus is based on its aetiology and clinical symptoms. Four types or classes of diabetes mellitus viz type 1diabetes, type 2 diabetes, gestational diabetes, and other specific types have been classified[1]. Type 1diabetes is said to account for only a minority of the total percentage of diabetes in a population although it is the major type of the diabetes in younger age groups in majority of developed countries. The incidence of type 1 diabetes is increasing in both rich and poor countries. Furthermore, a shift towards type 1 diabetes occurring in children at earlier ages is inevitable [1].

This ailment is an important cause of mortality, morbidity, and health-system costs in the world. Therefore, there is an urgent need to implement population-based interventions that prevent diabetes, enhance its early detection, and use lifestyle and pharmacological interventions to prevent or delay its progression to complications. To motivate such actions, one of the global targets set after the 2011 UN High-Level Meeting on Non-Communicable Diseases (NCDs) is to halt, by 2025, the rise in the age standardised adult prevalence of diabetes at its 2010 levels[4].

***Hibiscus rosa sinensis* (L) as a potential traditional medicine and need of research:**

Hibiscus rosa-sinesis Linn. (Malvaceae) is a glabrous shrub widely cultivated in the tropics.

It is well accepted that the leaves and flowers of *Hibiscus rosa-sinesis,* have hair growth promoting and antigreying properties [5,6]. Moreover in India, the herbal products in the market intended for hair growth include the extract of various parts of Hibiscus rosa sinesis, Adhirajan et al. reported that the leaf extract of *Hibiscus rosa- sinesis* has a potential effect on maintaining the hair growth in in-vivo and in-vitro methods[7] *Hibiscus rosa- sinensis* (Fam. Malvaceae) is a perennial ornamental shrub available throughout India. Various parts of this plant, like leaves, flowers and roots, have been known to possess medicinal properties like aphrodisiac, menorrhagic, oral contraceptive, laxative, etc. [8] Several articles and ancient literature have shown that the flowers of this plant possess antifertility activity, like anti-implantation, abortifacient, in rodents[9].

Objective of study:

Although, many studies have been undertaken in the past to investigate the pharmacological potential of traditional herbal remedies, however, rather little work has been done to assess their potential toxicities. There is now growing evidence that many herbal medicines do cause serious toxicity to their users, so it becomes quite necessary to evaluate the potential effects of the Hibiscus flower extract under study. [10,11]

The current study is focused on Acute Oral Toxicity studies and dose fixation in non-obese diabetes induced Wister rats using Hibiscus rosa sinensis ethanolic flower extract in order to determine its effect on these ailing rats with diabetes.

Fig- Hibiscus rosa sinensis (Linn.) Flower wild variety.

Material and method:

Preparation of the flower extract:

100 grams of the whole flower powder stored in airtight bottle was extracted each time in a soxhlet apparatus installed at the Department of Zoology, Ranchi University, Ranchi with petroleum ether (60-80 ºC) till complete extraction. Successively, the defatted flower material was extracted with chloroform, ethyl acetate and then with 95% ethanol. The ethanolic extract obtained was dark brown in colour which was then concentrated under reduced pressure using a rotary evaporator concentrator to get a brown semisolid mass of the crude extract. The extract so obtained was kept under refrigeration below 10ºC.

3.2 Induction of Diabetes in High Fat diet Rat groups

The rats were injected with alloxane monohydrate (150 mg/kg) intraperitoneal injection) dissolved in sterile normal saline. As alloxane is capable of producing fatal hypoglycemia due to massive pancreatic insulin release, animals were treated with 30 percent glucose solution orally at different time intervals after six hours of alloxane induction, and 5 percent glucose solution was kept in bottles in their cages for the next 24 hr to prevent hypoglycemia. Fasting blood glucose level was recorded daily using a glucometer in the morning at 8 a.m. for one week. Animals developed a stable hyperglycemia after 4-5 days. Only those animals with blood glucose >250 mg/dl were selected for study purpose. The diabetic animals were divided into 3 groups of 5 animals each.

Acute Toxicity Study

This study was taken as per the up-and-down-procedure of Organization for Economic Cooperation and Development (OECD) guidelines 425.[12] In the acute toxicity study, a single oral dose of 5000 mg/kg of extract was given to five rats at 48 h intervals. The animals were observed individually for any clinical signs of toxicity or mortality for 14 days.

Animals were observed at least once during the first 30 min after dose, periodically during the first 24 h, and daily thereafter, for a total of 14 days for any clinical signs of toxicity or mortality.

The blood lipid profile and biochemical parameters of animals were studied at the end of the experiment.

Observation:

Lipid profile parameters in control group and group treated with (5000mg/kg bw) post 14 days of the experimental protocol-

LIPID PROFILE PARAMETERS	5000mg/kg bw(V) (C) I	(HRSFE)II
	14 days	14 days
TC(mg/dl)	130.48±0.96	80.2±1.2
TG(mg/dl)	129.22±1.03	80.42±2.27
HDL(mg/dl)	21.5±0.53	21.7±0.68
LDL(mg/dl)	82.13±0.99	30.47±2.5
VLDL(mg/dl)	24.84±0.28	16.08±0.45

The results were expressed as mean ± S.E.M.

Kidney profile parameters in control (group I) & HRSFE treated (group II) post 14 days of the experimental protocol-

Kidney profile	(C) I	(HFDD) II
	14 days	14 days
Urea(mg/dl)	22.26±1.72	52.09±1.65
Creatinine(mg/dl)	0.7±0.02	2.3±0.05

The results were expressed as mean ± S.E.M

Results and Discussion:

In general, the safety studies on herbal medicines have been performed in the form of acute and sub-acute toxicity tests in animals like mic and rats. [13]

The acute toxicity test revealed that oral administration of a single 5000 mg/kg bw dose of *H. rosa-sinensis* ethanol flower extract (HRSEFE) to five male albino Wiser rats during the 14 days of treatment.

The treated rats did not bring out any signs of toxicity or mortality during the 14 days observation period. No death was observed within first 24 h in any of the cases. Skin fur, mucous membranes, eyes, respiratory, circulatory, nervous systems and somatomotor activity and behaviour pattern were also found to be normal.

No tremors and convulsions were observed in the test animals. Overall results suggested the LD_{50}value as 5000 mg/kg. The therapeutic dose was calculated as $1/10^{th}$ (500 mg/kg) of the lethal dose for further studies.

In the present study, we investigated the acute oral toxicity of *H. rosa-sinensis* ethanol flower extract in wister rats. The acute effects are generally observed soon after a single exposure of test agent. Therefore, the LD50 of extract may be considered to be greater than 5000 mg/kg. According to the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals, the substances having an LD50 value greater than 2000 mg/kg are considered as relatively safe.[14] In some relative studies carried out likewise, the LD50 values of therapeutic herbal extracts have been found to be greater 2000mg/kg, similar to this case and as per the GHS criterion, these extracts have been considered to be reasonably safe on acute exposure.[15]

Conclusion:

The results of this study suggest that for traditional medicinal purpose, only a low dose of H. rosa-sinensis leaf extract (i.e., 500 mg/kg) should be considered as safe for use.

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