IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

TOXICOLOGICAL EVALUATION AND ORAL GLUCOSE TOLERANCE TEST OF HYDROALCOHOLIC EXTRACT AND FLAVONOID OF LEUCOMERIS SPECTABILIS LEAVES IN ALBINO WISTAR RATS

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Abstract: Aim: The present study aimed to investigate the toxicity and oral glucose tolerance test (OGTT) of *Leucomeris spectabilis* leaves hydroalcoholic extract and flavonoid on albino Wistar rats. Methods: Both extract and flavonoid were prepared and administered orally to experimental animals. The extract was tested for toxicity in rats at a dose of 1,000 & 2000 mg/kg and flavonoid at a dose of 5, 50, 300, and 1500 mg/kg body weight p.o. The hypoglycemic effects of both normal rats and orally glucose-induced hyperglycemic rats were compared with distilled water and glibenclamide. The blood glucose level was obtained by pricking the tail vein using a glucometer at times -30, 0, 30, 60, 120 and 240 minutes. Results: The dose of hydroalcoholic extract and flavonoid did not exert any sign or symptom of toxicity and the dead rat was not found. The body weight and food consumption were normal. The blood glucose levels of hydroalcoholic extract and flavonoid-treated groups were not different from control and glybenclamide treated. Conclusion: The findings of the present study can be concluded that the hydroalcoholic extract and flavonoid are practically non-toxic at a lower dose.

Index Terms **- Toxicity, Leucomeris spectabilis, Diabetes, Blood glucose.**

1 INTRODUCTION

1.1 Diabetes

Diabetes is a chronic condition brought on by either insufficient insulin production by the pancreas or inefficient insulin use by the body. Uncontrolled diabetes frequently causes hyperglycemia, also known as high blood glucose or raised blood sugar, which over time may severely damage multiple systems in the body, including the neurons and blood vessels. A total of 1.5 million deaths were directly related to diabetes in 2019, and 48% of these deaths occurred in those under the age of 70. Diabetes caused an estimated 460,000 renal disease deaths, and high blood glucose is responsible for 20% of cardiovascular fatalities (WHO). According to Ayurveda, numerous herbs have anti-diabetic potentials such as babul (Acacia arabica), bael (Aegle marmelos), church steeples (Agrimonia eupatoria), neem (Azadirachta indica), ash gourd (Benincasa hispida), beetroot (Beta vulgaris), fever nut (Caesalpinia bonducella), bitter apple (Citrullus colocynthis), and many others (Rizvi and Mishra 2013).

1.2 Acute toxicity studies

Acute toxicity studies are conducted to evaluate the effects of a single substance. Studies are carried out on two mammalian species (one nonrodent) to determine the short-term adverse effects of the drug when administered as a single dose or in multiple doses over 24 hours. Studies on acute toxicity provide data on the possibility of acute intoxication in people; an estimated safe acute dosage for people; the toxicity's most likely target organs; the progression of clinical findings induced by drugs; the right dosage for investigations on the toxicity of multiple doses; and variations in toxicity across species (Colerangle, J. B. (2017).

1.3 Oral Glucose Tolerance Test

Oral Glucose Tolerance Test (OGTT) is generally used to evaluate glucose tolerance, and how the body can store glucose by removing it from the blood. For both sexes, the test measures the plasma response to an intake of glucose, usually at 1 and 2 hours (Moini, J. 2019)but the test has also been used to obtain a contemporaneous estimate of insulin resistance. Without using an OGTT, nearly all diabetic patients are diagnosed based on their symptoms, physical examination, and random or fasting plasma glucose values. In contrast, patients with impaired fasting glycemia (intermediate fasting glucose values) are advised by the WHO and the UK to undergo formal glucose tolerance testing. As fasting plasma glucose concentrations lack diagnostic sensitivity, the test also has a special significance in the diagnosis of gestational diabetes mellitus (Wile& Wilding 2014).

1.4 Plant

Leucomeris spectabilis D. Don is a shrub with white flowers, commonly known as Showy White weed, Showy Agrimony in English; and Phusara & Phusiari in Hindi. Flowers are borne in rounded stalkess corymbs, 10-20 cm in diameter. Flower-cluster-stalks are densely woolly and have bracteoles. Flower-heads are 2-2.5 cm, involucral bracts about 10, nearly flat, linear-oblong blunt or pointed hairless. Florets are 1.2 cm. Branches are stout and grooved. Leaves are large, 10-35 by 3.5-10 cm, narrowed at both ends, leathery, hairless above; leaf-stalk very short. Leaves are elliptic or elliptic-lance-shaped densely velvety-woolly beneath. Seedpods are 4-6 mm long, slender, densely silky. Pappus is 1.2 cm, pale, hairs often contracted at the base. *Leucomeris spectabilis* is found in the Himalayas, from Kashmir to Nepal, at altitudes of 600-1700 meters (Flora of India, 1995).

2 MATERIALS AND METHODS

2.1 Preparation of *L. spectabilis* extracts

Fresh mature leaves of *Leucomeris spectabilis* were collected from Sambhal district (UP) India and authenticated by Dr. Sunita Garg, Former Chief Scientist, Head, RHMD, CSIR-NIScPR & Mr. R.S. Jayasomu, Chief Scientist, Head, RHMD, CSIR-NIScPR and a voucher specimen (NIScPR/RHMD/Consult/2022/4261-62)

The leaves were collected and dried in the shade. The dried leaves were powdered and extracted with 70% ethanol. The extract obtained was filtered through Whatman No.1 filter paper and evaporated in a rotary evaporator. The extract was stored at -20˚C until use. The extraction of *Leucomeris spectabalis* leaves flavonoids was done with slight modification according to Mistry S; 2021. The dried crude plant hydroalcoholic extract of 10 gm was diluted with distilled water and treated with 10% lead acetate solution with gentle stirring. The precipitated tannins were removed through filtration and the obtained filtrate was diluted again with distilled water and acidified by HCl. The acidic solution was boiled for a few hours. Sugar free flavones and flavonones were precipitated. After precipitation, butanol was added to the above and extracted using a separatory funnel, resulting in crude flavonoids. The obtained crude flavonoid was dried using a rotary evaporator.

2.2 Animals

Albino Wistar rats weighing 150-200 g, which are received from the Animal Research Facility Center (ARFC), School of Pharmacy, Monad University, Hapur (UP) India. The rats were housed under standard environmental conditions (at 25 ± 2°C, 40-60 % humidity with 12-h light/12-h dark cycle). All animals were given a standard laboratory diet with access to water ad libitum. The experimental protocol and the experiments performed on the rats were approved by the Institutional Animal Ethics Committee (IAEC), School of Pharmacy, Monad University, Hapur (UP) India (Reg. 1933/PO/Re/S/17/CPCSEA).

2.3 Acute Toxicity Test

Toxicity studies shall be carried out following OECD guideline 420. Rats will be divided into 6 groups each group consisting of 3 animals, and treated as follows-Animals will be treated with *Leucomeris spectabalis* hydroalcoholic extract (HAE) doses of 300 and 2000 mg/kg and *Leucomeris spectabalis* flavonoid (LSF) 5, 50, 300, and 1500 mg/kg p.o. once only. Immediately after dosing, the animals were closely observed for the initial 4 h after the administration and then once daily during the 14 days. The general behavioral changes closely observed were mainly hyperactivity, ataxia, convulsions, salivation, tremors, diarrhea, lethargy, sleep, and coma. They were then kept under observation for up to 14 days after drug administration to observe any mortality, body weight, and food consumption.

2.4 Oral Glucose Tolerance Test

The oral glucose tolerance test was performed on overnight fasting normal rats. Distilled water, HAE (200 mg/kg), LSF (150 mg/kg), and glibenclamide (2.5 mg/kg) were administered to three groups of rats, respectively (Sokolovska et al., 2012). Glucose (2 g/kg) was fed 30 min after pretreatment with distilled water, HAE, LSF, and glibenclamide (Narmadha and Devaki 2013). Blood glucose levels were measured at -30, 0, 30, 60, 120, and 240 min after glucose load to assess the effect of HAE and LSF on the blood glucose levels of the glucose-loaded animals. The blood glucose was measured using blood glucose test strips and a glucometer (Dr. Morepen glucoOne blood glucose monitor model BG 03).

2.5 Statistical Analysis

All data were expressed as mean + standard error of mean (SEM). Statistical analysis was carried out using an F-test (One-Way ANOVA) followed by a Scheffe's test. The criterion for statistical significance was at a p-value less than 0.05.

3 RESULTS AND DISCUSSION

3.1 Assessment of Acute Toxicity

The limit dose of 2000 mg/kg (HAE) and 1500 mg/kg (LSF) did not cause mortality or any sign or symptom of acute toxicity in the three rats dosed for a short period of 48 hrs. and a long period of 14 days (Table 1). The percentage increase in body weight and average food intake of control was not different with HAE and LSF (Table 2).

Table 1 14 days observation of animals receiving *Leucomeris spectabalis* hydroalcoholic extract and flavonoid

Toxicity Parameters	Treatment		
	Control (n=3)	HEA (2000 mg/kg) (n=3)	LSF (1500 mg/kg) (n=3)
Alertness	N	N	N
Irritability	-	-	-
Fearfulness	-	-	-
Touch Response	N	N	N
Restlessness	-	-	-
Abdominal Tone	N	N	N
Tremors	-	-	-

Writhing	-	-	-
Corneal reflexes	N	N	N
Defecation	N	N	N
Diarrhea	-	-	-
Urination	N	N	N
Food and water intake	N	N	N
Respiration rate	N	N	N
Pupil size and color	N	N	N
Pupil reaction to light	N	N	N
Skin color & texture	N	N	N
Fur color	N	N	N
Spontaneous activity	N	N	N
Heartbeat rate	N	N	N
Convulsions	-	-	-
Aggressiveness	-	-	-

Key: - N: - Normal; (-):- Not detected

Table 2 Change in body weight and food consumption in acute toxicity study of *Leucomeris spectabalis* HAE and LSF

Treatments	Control	1,000 mg/kg (HAE)	2,000 mg/kg (HAE)	05 mg/kg (LSF)	50 mg/kg (LSF)	300 mg/kg (LSF)	1,500 mg/kg (LSF)
% body weight change	14.30+2.34	13.91+5.03	14.20+3.00	14.59+0.52	15.40+0.58	14.04+1.03	14.78+4.32
Average food intake (g/day)	15.06+7.34	13.18+9.68	15.47+7.93	14.56+5.01	15.00+0.45	14.57+7.00	14.96+0.12

The values represent the mean + SEM.

3.2 Oral glucose tolerance test (OGTT)

Oral Glucose Tolerance Test, the blood samples were analyzed for glucose content at -30, 0, 30, 60, 120, and 240 minutes, respectively. The blood glucose levels of HAE (200 mg/kg) and LSF (150mg/kg) treated groups were not significant with control and Glibenclamide treated at -30, 0, 30, 120, and 240 minutes. The blood glucose levels at 60 minutes in HAE, LSF, and Glibenclamide treated were lower than the normal group. (Table 3)

Table 3 Blood glucose level oral glucose tolerance test study of *Leucomeris spectabalis* HAE and LSF

Treatment	Blood glucose levels (mg/dl)					
	-30	0	30	60	120	240
Control	82.17+3.12	81.17+4.07	148.83+7.33	127.83+7.24[a]	112.17+7.63[a]	83.17+3.16
HAE (200 mg/kg)	80.17+3.95	79.33+3.57	122.67+8.43	97.50+7.85[b]	89.50+4.96[b]	78.50+3.99
LSF (150 mg/kg)	81.16+2.15	80.34+4.05	120.54+2.09	95.00+1.34[b]	87.69+1.32[b]	77.05+2.10
Glybenclamide (2.5 mg/kg)	79.17+0.60	81.00+2.05	120.00+10.47	94.67+5.38[b]	87.43+2.30[b]	80.83+3.22

The values represent the mean + SEM. within the same column followed by the different superscript letters (a-b) are significantly different at the $p<0.05$.

3.3 DISCUSSION

Medicinal plants have become famous in healthcare and some have been falsely considered as safe as they are obtained from natural sources. Therefore, a toxicity study is required not only to identify the further range of doses in animal studies but also to explain the probable clinical signs evoked by the test compounds under investigation (Kumar et al., 2014). The results obtained from the acute toxicity study showed that the HAE and LSF demonstrated a high safety margin since the animals tolerated up to 2000 and 1500 mg/kg body weight of the extract and flavonoid orally. No significant differences were found in the body weight among normal rats. In addition, the food intake in the treated groups was decreased between normal rats. The assumption might be that HAE and LSF increased feed conversion efficiency in rats. In different clinical settings, the oral glucose tolerance test is used to assess insulin resistance and apparent insulin release (Stuvoll et al., 2000). The blood samples for the oral glucose tolerance test were analyzed for glucose content at -30, 0, 30, 60, 120, and 240 minutes, respectively. In the investigation using a single dose of HAE (200 mg/kg) and LSF (150 mg/kg), no noticeable hypoglycemia impact was seen in normal rats. It was compared with Glibenclamide, a drug that promotes the release of insulin and has been used for many years to treat diabetes.

4 CONCLUSIONS

As a result of the current study's findings can be concluded that the hydroalcoholic leaves extract and flavonoid of *L. spectabalish* are practically non-toxic at a lower dose which one 2,000 and 1500mg/kg respectively. A study in OGTT showed that the leaves of *L. spectabalish* reduced blood glucose levels not different from Glibenclamide treated. The single-dosed study of HAE dose at 200mg/kg and LSF, doses at 150 mg/kg produced no significant hypoglycemic effect in normal rats.

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