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Extraction, Isolation and Pharmacological Antidiabetic activity of Momordica Charantia L. Plant

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Abstract

Diabetes mellitus (DM) is the chronic conditions and one of cause for the precipitation of cardiovascular complications The reason the food's sugar is used is because insulin's regular activity is inadequate or malfunctioning. Due to aging populations, rising obesity rates, and changes in lifestyle, the number of cases with non-insulin dependent diabetes mellitus has dramatically grown. Momordica Charantia L,. (cucurbitaceae) employed in Indian traditional medicine to cure a number of ailments. Its fruit extract is used to treat diabetes. This study set out to evaluate the pharmacognocal, physic-chemical, phyto-chemical, and hypoglycemic qualities of a extract powder of Momordica Charantia L,. (fruit). The plant was procured from a nearby store. The plant medication was standardized in accordance with W.H.O. criteria. Male species of rats (Albino Wister) weighing approx. 150 g were used. Injections of alloxan (120 mg/kg, intraperitoneally) were used to induce diabetes. Tolbutamide (100 mg/kg p.o.) was given to three control groups: I was the N Control group, II was the DM control group, and III was the Std. control group. Group IV diabetic rats administered a dose of (300 mg/kg) of Momordica charantia (MCE) extract (fruit) hydro-alcoholically. Each therapy lasted for 21 days.When diabetic rats were given 300 mg/kg of Momordica charantia (MCE) fruit extract, their lipid, renal, and hepatic profiles reverted to normal, and their fasting blood glucose levels significantly (P̂0.01) dropped. This led to the normalization of other serum markers and a 45.25% decrease in blood glucose after 21 days of therapy. MCE has been shown to have a therapeutic effect against alloxan-induced diabetes by improving the histological changes of diabetic rats' pancreas and liver.

Keywords: Antidiabetic activity, Momordica Charantia L., Alloxan, extract of Fruit,etc.

Introduction

Among the world's top five causes of death is thought to be diabetes mellitus [1]. Diabetes Mellitus (DM) is a significant global health issue, with projections indicating that its prevalence will rise from 170 million in the year 2000 to 365 million by 2030 [2]The condition of disturbed metabolism that results in hyperglycemia, or abnormally elevated blood sugar levels, is typically due to a synthesis of hereditary and environmental variables [3]. Diabetes is a significant degenerative condition that impacts individuals across the globe is rapidly becoming the third most deadly disease [4]. With as many as 200 million cases reported worldwide and 16 million cases in the United States, it is the most prevalent endocrine condition. There is a clinical framework established for general medicine. Unlike traditional Western medical treatments, complementary and alternative medicine uses nutritional supplements and herbs. As per study up to 40% of DB patients use matching and substitute drug. [6].

Preparation of extract

Petroleum ether is used to defatten and coarsely grind dried fruits using a soxhlet method. In a Soxhlet system, the material was first defatted and then completely extracted using a 70% hydro alcoholic solution. At reduced pressure, the extract was concentrated.

Plant drug and extract Standardization

Further examinations, including sensory evaluations, microscopic analyses, ash content determinationTo verify the drug's purity, tests for heavy metals in the extract, extractive values in various solvents, and evaluations of microbiological contamination were carried out. Various compounds such as triterpenoids were identified in the extract through phytochemical methods [15–19].

Thin Layer Chromatography (TLC) Mobile Phase

The ratio of methanol to chloroform was 8.5:1.5 in order to determine the drug hydro-

alcoholic extracts' TLC patterns. To obtain the best TLC fingerprinting, a variety of viewing techniques were employed, including UV 255, UV 367 nm, and RF value (s) determination.

Extraction and Isolation

The 75 kg of fruit powder that had been freeze-dried was extracted using an 80% EtOH maceration process. The alcohol extract was progressively partitioned using n-BuOH and CH2Cl2 following filtering and reduced pressure evaporation.

Enzymatic Hydrolysis of Compound 5

Cellulose was employed to treat Compound of 60mg with Momordicoside for a duration of seven days at 37 °C acetate buffer with 0.1M conc solution. Subsequently, CHCl3 was utilized to extract the resulting reaction mixtures. Aglycone was separated by preparative TLC from each CHCl3 layer.

Assessment of Blood Sugar Levels and Glucose Tolerance Evaluation

Prior to the trial, the mice were deprived of food for a duration of five to seven hours. Blood samples for serum glucose were taken from the body of the mice and checked with Accuchek^{R} glucometer (Roche). After establishing the baseline blood glucose level at the 0-minute mark, the mice were administered injections into the peritoneal cavity of either Momordicodise S (5) at a dosage of given quantity of 100 mg/kg including Momordi-codise T (6) at a lower dosage of 10 mg/kg, AICAR at 500 mg/kg, or metformin at 200 mg/kg. The vehicle solution for each injection comprised 100 ml of quantity of 16% glycerol, 6% ethanol, and 83% saline. To observe effect on blood glucose levels over the next hour, an ipGTT was performed immediately following the sample collection. Mice that were insulin-resistant and fed a high-fat diet received a glucose dose of 2.0 g/kg, while those on a regular chow diet were given a glucose dose of 3.0 g/kg.

Animals

The IAEC authorized all experimental procedures under approved. The selected species of Male albino Wistar rats, which is weighing between 140 and 220 grams, were supplied by the vendor supplier. These animals were reserved in std sol with a temp maintained at 25 ± 2°C, RH ranging from 35% to 75%, and a light-dark cycle of 12 hours each. They were allowed to acclimate in the lab of the dept under normal conditions, receiving a standard diet and having access to water at all times.

Statistical analysis - Post-test TURKEY and One-Way ANOVA analyses were conducted on all data related to blood glucose and pathological estimates, presented as mean ± Std Err of Mean (S.E.M.). A significance level of $P < 0.01$ was established to evaluate differences between groups.

Histopath results of isolated parts

To preserve the liver and pancreatic tissue for detailed microscopic examination, small samples were collected and immersed in a 10% formalin solution. This initial step ensured proper fixation of the tissue. Subsequently, the tissues were further preserved in Bouin's fixative, a specialized solution lacking acetic acid, to prepare them for histological analysis. Hematoxylin and eosin (H&E) was used to stain tissue slices six microns thick for histological examination.

Result and Discussion

Table: Hydroalcoholic extracts Microbial analysis

Sr. no.	Micro-organism	Limit
1.	Total aerobic organisms	$1x10^5$
2.	Yeast/mould	$1x10^4$
3.	E. coli	Ab/g
4.	Sal. typhi	Ab/g
5.	P. aeruginosa	Ab/g
6.	S. aureus	Ab/g

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Structural Identification

Compound 1 were identified by HRESIMS as C36H60O10 (m/z 675.4063 [M+Na] +). The planar compound 1 structure was identified as previously released karaviloside XI based on their similar NMR results. Compound 1 was broken down using acid, resulting in the sugars allose and aglycone 4. Aglycone 4 was definitively identified through XRD analysis, confirming its structure and its relationship to the original compound. This analysis revealed that compound 1 is composed of 3-O-b-D-allopyranosyl-5 b, 19-epoxy cucurbita-6-ene-23(R), 24-(S), and 25-triol.

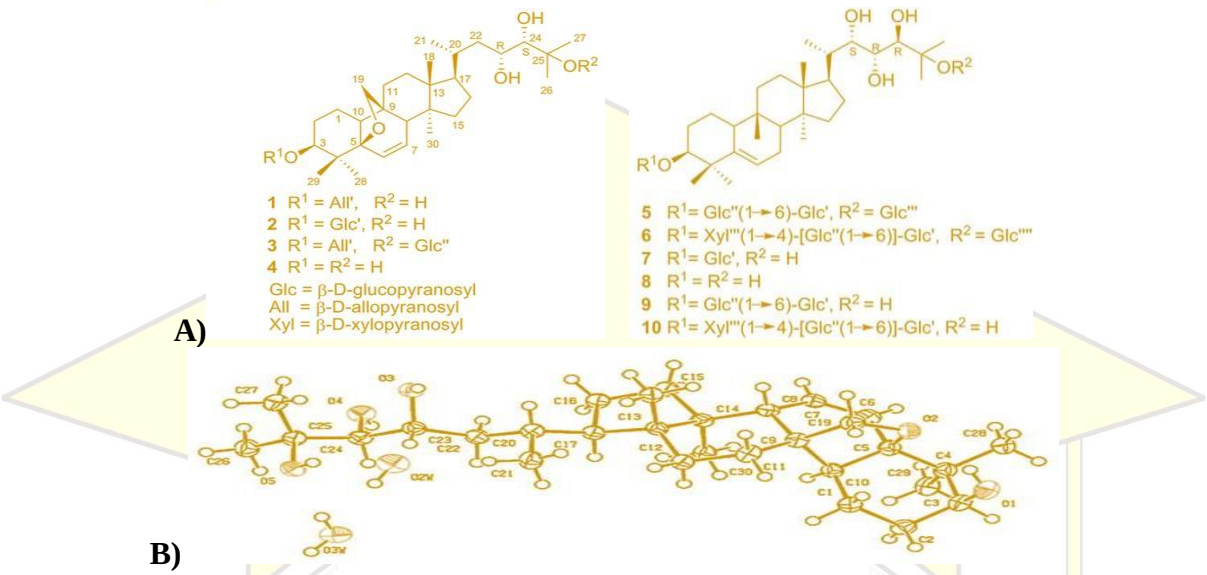

Fig: Structures of Cucurbitane-Triterpenoids of Bitter Melon Evaluating the Antidiabetic Properties in Animals

Based on these in vitro results, we investigated whether this medication could positively impact glucose and fuel metabolism in a living organism. In these studies, we concentrated exclusively on compounds 5 and/or 6, as we were unable to obtain the other components in adequate quantities for in vivo injection

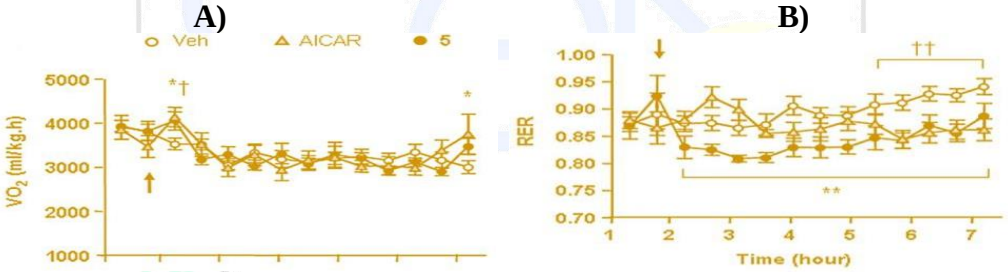

Figure. Comp. 5 effects on Body (Whole) VO2 and RER - Mice (A, B)

Animals were kept in metabolic chambers at 10:00 am. After a two-hour period, they received an injection of either comp 5 (100 mg/kg), AICAR (250 mg/kg), or saline (Veh) under the skin. The results displayed that compound 5 significantly reduced [mention the specific metric being measured] paralleled to the control group (Veh). This reduction was statistically significant, with p-values less than 0.05, 0.01, and 0.01 for different experimental groups (n = 6).

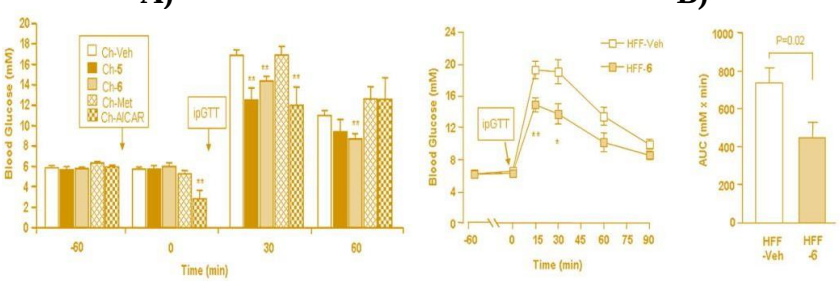

Figure 5. Comp. 5 and 6 Acute Effects on Serum Glucose in Mice

(A) For our experiments, mice fed a std. diet were fasted for 5-7 hours. After taking an initial blood sample, they received an injection of either AICAR (500 mg/kg), metformin (200 mg/kg), compound 5 (100 mg/kg), compound 6 (10 mg/kg), or a control solution (glycerol, ethanol, and saline). Sixty minutes after the injection, a glucose tolerance test was performed on the insulin- sensitive mice by administering a glucose solution. Another blood sample was taken immediately after the glucose injection.

(B) The study concentrated on the effects of a high-fat diet (HFF) on blood glucose levels in insulin-dependent mice. 2.0 g/kg of glucose was used in an intraperitoneal GTT. Statistical significance was defined as *p < 0.05 and **p < 0.01 when associated to the agreeing vehicle control groups. The sample size was 7 to 9 mice per group.

Acute Toxicity studies

When administered orally, MCE-treated rats did not exhibit any appreciable behavioral alterations. During the monitoring period, no deaths were reported.

Antidiabetic Study

Table: Treatment with standard medication and MCE for three weeks after alloxan-induced diabetic rats: impact on blood glucose levels

Sr.no.	Groups	00 Day	07 Days	14 Days	21 Days
1.	Normal C	78.34 ± 3.592	78.18 ± 2.640	79.34 ± 1.334	76.78 ± 0.998
2.	Diabetic C	317.3 ± 5.165	354.8 ± 6.098	368.7 ± 8.373	371.1 ± 8.938
3.	STD	359.4± 5.781*#	243.1 ± 22.59*#	198.1 ± 13.71*#	139.1 ± 12.45*#
4.	D+MCE	357.6± 8.056*#	272.9 ± 11.44*#	242.4 ± 11.57*#	204.9 ± 13.42*#

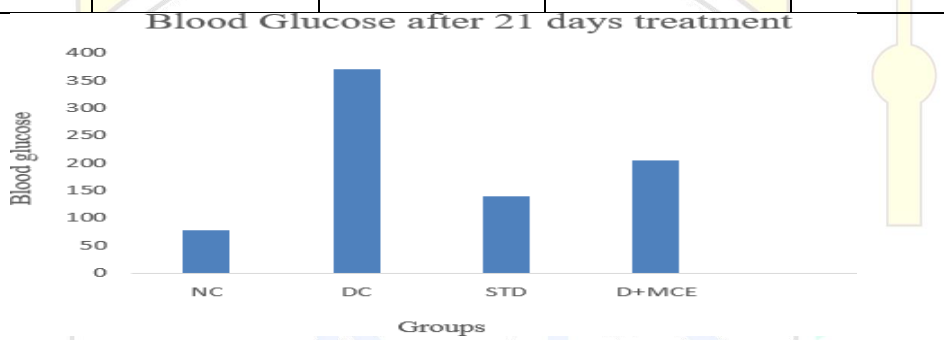

Fig. 6: An illustration of how MCE affects blood sugar levels.

Conclusion

This research shows that a hydroalcoholic extract made from the fruit of the bitter melon plant (70% v/v concentration) can effectively help manage diabetes and related health issues. Our results suggest that taking 300mg of this extract per kilogram of body weight daily can lead to long-term improvements in blood sugar control and potentially reverse some diabetic complications.

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