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EVALUATION OF ANTI-DIABETIC ACTIVITY ON *n*-BUTANOL FRACTION OF LEAF METHANOLIC EXTRACT OF *CURCULIGO ORCHIOIDES* (AMARYLLIDACEAE) IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Keywords:

Anti-diabetic activity, *Curculigo orchioides* methanolic extract, *n*-butanol, Streptozotocin, GOD-POD

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ABSTRACT: The aim of the present study to evaluate the anti-diabetic activity of methanolic extract *n*-butanol fraction of *Curculigo orchioides* (Amaryllidaceae) against in streptozotocin-induced diabetic rats. Fractions were administrated orally at 200 mg/kg and 100 mg/kg for 3weeks. The effects were compared with an oral dose of 10 mg/kg of glibenclamide. Blood glucose levels are a determination by GOD-POD kit method. In the results, our study indicates that *Curculigo orchioides n*-butanol fraction of leaf methanolic extract exhibited significant antihyperglycemic activities in streptozotocin-induced hyperglycemic rats without significant change in body weight. And shows the values are given in average body weight (g) ± SEM for groups of six animals each. A) Normal saline. B) Normal saline + streptozotocin. Significance *vs.* Control group *P < 0.01. **P < 0.005, ***P < 0.0001.

INTRODUCTION: Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. In the 19th century, chemical analyst and scientists started to extract and modify the active constituents from plants. Later, chemists began making their version of plant compounds. Nowadays almost one-fourth of pharmaceutical drugs are derived from botanicals. Recently, the World Health Organization estimated that 80% of people going towards the use of herbal medicines in different parts of the world, due to side effects of synthetic medicine [1].

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The number of diabetic patients is rapidly increasing, and in consequence, the control of their complications is a challenge. Diabetes mellitus is the world's largest growing metabolic disorder. After cardiovascular diseases and global cancer prevalence of diabetes has dramatically continued to increase [2]. Both fasting and postprandial glucose level control are critical to achieving proper long-term control in diabetic patients. In this regard, medicinal plant extracts have an ancient history of curing diseases and its complications [3].

The World Health Organization has been particularly attentive to the potential offered by herbal medicine, the main subfield of traditional medicine practiced in different countries. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [4-6]. *Curculigo* chiefly a paleotropical and subtropical genus of over twenty species, was established by Gaertner in 1788. *Curculigo* has emerged as a good source of

traditional medicines. Some uses of these plants in the traditional medicines have been validated by the pharmacological investigation. Phytochemical investigation of all species of the genus *Curculigo* has resulted in the identification of more than 110 compounds. The medicinal plants of this genus have shown wide spectrum pharmacological activities, including adaptive, immunostimulatory, taste-modifying and sweet-tasting, antioxidant, mast cell stabilization, antihistaminic and antiasthmatic, hepatoprotective and neuroprotective activity [7].

FIG. 1: ***CURCULIGO ORCHIOIDES***

Taxonomical Classification: [8]

Kingdom : Plantae
Sub-kingdom : Tracheobionta
Super division : Spermatophyta
Division : Magnoliophyta
Class : Monocotyledon
Subclass : Liliidae
Order : Liliales
Family : Amaryllidaceae
Genus : *Curculigo*
Species : *orchiodes*

The present investigation was undertaken to evaluate the anti-diabetic potential of *Curculigo orchioides*. Leaf methanolic extract of the *n*-butanol fraction on fasting blood sugar and serum biochemical analysis.

MATERIALS AND METHODS:

Plant Material: Fresh plants were collected from Yercaud hills Salem district Tamil Nadu and authenticated by Dr. V. Raju department of botany, Kakatiya University Warangal. Authentification number KUC/2015/4251.

Extraction: The stem and leaves, are shade-dried, and made course powder with the help of a dry mechanical grinder, and passed sieve number 60. The powdered stem and leaves were extracted using soxhlation method. The power defatted with petroleum ether (40-60 °C) and extract with ethanol. Extracts were evaporated to dryness and screenings were performed.

Animals: Female Swiss albino mice weighing 20-30 gm were used for oral acute toxicity study because shows greater sensitivity to treatment. Male Albino wistar rats weighing 200-250 gm were used for the anti-diabetic study. The animals were housed in standard aseptic clean environment condition are maintain and fed with standard rodent diet with water and *ad libitum*.

Toxicity Study: An acute oral toxicity study was performed as per the Organization for Economic Co-operation and Development (OECD) guidelines 423. By Acute toxic class method female Swiss albino mice of weighing 20-30 gm were used for the study. An acute toxic class method is a stepwise procedure with the use of three animals of each step. Average 2-4 steps may be necessary to allow determine the acute toxicity of the substance. Three animals were used for each step. The animal was placed individually and observed the first 24 h for any sign of toxicity, morbidity or mortality [9].

Assessment of Extracts on Streptozotocin-Induced Diabetic Animals: [10] Rats were made diabetic by a single intraperitoneal injection of 150 mg/kg). Streptozotocin was first weighed individually for each animal according to the weight and solubilized with 0.2 ml saline (154 mM NaCl) just before injection. Two days after streptozotocin injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 hours after streptozotocin injection. The blood sample was drawn at weekly intervals till the end of study (*i.e.,* 3 weeks).

Fasting blood glucose estimation and body weight measurement was done on the day of 1, 7 and 21 of the study. On day 21, blood was collected by cardiac puncture under mild ether anaesthesia from overnight fasted rats and fasting blood sugar was estimated. Serum was separated and analyzed for

serum cholesterol, serum triglycerides by enzymatic DHBS colorimetric method, serum HDL, serum LDL, serum creatinine, serum urea and serum alkaline phosphatase by hydrolyzed phenol amino antipyrine method.

Experimental Design: In the experiment, rats were divided into the five groups with six animals each

Group I: Positive control of Wistar rats received 1% w/v gum acacia 1 ml/kg for 15 days orally.

Group II: Diabetic control of Wistar rats received 1% w/v gum acacia 1 ml/kg for 15 days orally.

Group III: Diabetic rats received methanolic extract *n*-butanol fraction of *Curculigo orchioides* 100 mg/kg body weight once a day orally for 15 days.

Group IV: Diabetic rats received with the standard drug of Glibenclamide 0.5 mg/kg orally once a day for 15 days.

Rats fasted overnight, and the blood was withdrawn from the orbital sinus of the eye on the 5th day, 15th day and 20th day post-induction to determine by blood glucose GOD-POD kit method. The change in body weight was observed throughout the treatment period in experimental animals.

Statistical Analysis: All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as the mean ± standard error of the mean (SEM) and analyzed using Student 't' test.

RESULTS: Administration of streptozotocin (150 mg/kg, i.p) led to 1.5 - fold elevation of fasting blood glucose levels, which was maintained for a period of 3 weeks. Three weeks of daily treatment of extracts led to a dose-dependent fall in blood sugar levels by 25 - 62%. The effect seems to reach a maximum after 15 days of treatment and remained constant in the third week. Vehicle control animals were found to be stable in their body weight while diabetic rats showeda significant reduction in body weight during 21 days **Table 1**. streptozotocin caused weight reduction, which was reversed by n-butanol fraction os methanolic extract of *Curculigo orchioides* after 7 days of treatment. Serum cholesterol, serum triglycerides, serum LDL, serum creatinine, serum urea, and serum alkaline phosphatase levels were decreased significantly by glibenclamide ($p<0.001$), aqueous extract ($p<0.001$) and cold extract ($p<0.01$) of *Curculigo orchioides*, after 21 days of treatment compared with diabetic control. HDL levels were increased by glibenclamide ($p<0.001$), aqueous extract ($p<0.001$) and cold extract ($p<0.01$) compared with diabetic control **Table 2**.

TABLE 1: THE EFFECT OF 3-WEEK TREATMENT WITH *n*-BUTANOL FRACTION OF METHANOLIC EXTRACTS OF *C. ORCHIOIDES* ON BODY WEIGHT (g) AFTER *STREPTOZOTOCIN* INDUCED DIABETES IN RATS

Group no.	Treatment	Dose (mg/kg P.O)	Average body weight (g) ± SEM			
			Day 1	Day 7	Day 14	Day 21
I	Vehicle control	0.2 ml a	199.1±0.9	201.83±1.02	203.00±1.05	210.10±0.14
II	Diabetic control	0.2 ml b	202.2±0.24	178.00±0.58	164.33±0.24	136.03±0.25
III	Glibenclamide	10	204.8±0.42	196.00±0.31**	194.21±0.33***	181.00±0.22***
IV	N-butanol fraction	100	207.3±2.07	196.16±1.70*	190.21±1.72**	172.22±0.43**

Values are given in average body weight (g) ±SEM for groups of six animals each. a Normal saline, b Normal saline + streptozotocin. Significance *vs.* control group, * $p<0.05$. **$p < 0.01$, *** $p<0.001$.

TABLE 2: EFFECT OF *n*-BUTANOL FRACTION OF METHANOLIC EXTRACT OF *C. ORCHIOIDES* ON SERUM PROFILE IN STREPTOZOTOCIN INDUCED DIABETIC ALBINO RATS AFTER 21 DAYS OF TREATMENT

Group no.	Treatment	Dose (mg/kg P.O)	Serum cholesterol	Serum triglycerides	Serum HDL cholesterol	Serum LDL cholesterol	Serum creatinine	Serum urea	Serum alkaline phosphatase
I	Vehicle control	0.2 ml a	190.00±0.42	92.83±0.11	40.00±0.14	90.20±033	0.42±0.4	26.16±0.41	110.06±0.71
II	Diabetic control	0.2 ml b	290.13±1.15	210.13±1.21	30.00±1.44	180.16±10.01	1.95±1.71	72.00±1.41	294.50±0.24
III	Glibenclamide	10	136.83±0.61***	118.00±0.21***	46.20±0.8***	66.73±0.7***	0.51±0.1***	31.00±2.42***	140.26±0.1***
IV	*n*-butanol fraction	100	152.03±1.8***	105±1.51***	40.83±0.15***	96.16±0.47**	0.60±0.1***	29.99±1.2***	129.66±0.41***

Values are given in average body weight (g) ± SEM for groups of six animals each. a Normal saline. b Normal saline + streptozotocin. Significance *vs.* control group, * $p<0.05$, **$p<0.01$, *** $p<0.001$.

DISCUSSION AND CONCLUSION: In that *n*-butanol fraction os methanolic extract of *Curculigo orchioides* exhibited significant anti-hyperglycemic activities in streptozotocin-induced hyperglycemic rats without significant change in body weight. They also improved conditions of DM as indicated by parameters like body weight, and lipid profiles along with serum]. In streptozotocin-induced diabetes, (-)- epicatechin [creatinine, serum urea, and serum alkaline phosphatase. The number of

functionally intact β-cells in the islet organ is of decisive importance the development course and outcome of DM. It was also suggested that the regeneration of islet β-cells following destruction by streptozotocin might be the primary cause of the recovery of streptozotocin-injected guinea pigs from the effects of the drug. In diabetic rats which is comparable to that of standard drug of Glibenclamide. The standard drug stimulates insulin secretion from beta cells of islets langerhans. From the study plant extract decreases the blood glucose level may be stimulation of insulin action either by an increase in the pancreatic secretion of insulin.

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