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

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ABSTRACT
The aim of present study to evaluate anti-diabetic activity of methanolic extract n-butanol fraction of *Curculigo orchioides*(*Amaryllidacae*) against in streptozotocin induced diabetic rats. Fraction were administrated orally at 200 mg/kg and 100mg/kg for 3weeks. The effects was compared with oral dose of 10mg/kg of Glibenclamide. Blood glucose levels are determination by GOD-POD kit method. In the results, our study indicates that *Curculigo orchioides n-butanol fraction of leaf methanolic extract* exhibited significant anti- hyperglycemic 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.

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

INTRODUCTION
Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological and molecular techniques. In 19th century, chemical analyst and scientists start to extract and modify the active constituents from plants. Later, chemists began making their own version of plant compounds. Nowdays almost one fourth of pharmaceutical drugs are derived from botanicals. Recently, 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] The number of diabetic patients is rapidly increasing, and in consequence the control of their complications is a challenge. Diabetes mellitus is world's largest growing metabolic disorder. After cardio vascular diseases and cancer global prevalence of diabetes has dramatically continued to increase.[2] Both fasting and postprandial glucose level control are critical to achieve a long-term proper control in diabetic patients. In this regard, medicinal plant extracts have an ancient history to cure 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. Ethno botanical 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* have emerged as a good source of the traditional medicines. Some uses of these plants in the traditional medicines have been validated by pharmacological investigation. Phytochemical investigation of all species of the genus *Curculigo* has resulted in identification of more than 110 compounds. The medicinal plants of this genus have showed wide spectrum pharmacological activities, including adaptive, immunostimulatory, taste-modifying and sweet-tasting, antioxidant, mast cell stabilization, antihistaminic and antiasthmatic, hepatoprotective and neuroprotective activity.[7]

Curculigo orchioides

Taxonomical Classification[8]
Kingdom - Plantae
Subkingdom - Tracheobionta
Superdivision - Spermatophyta
Division - Magnoliophyta
Class - Monocotyledon
Subclass - Liliidae
Order - Liliales
Family - Amaryllidacae
Genus - *Curculigo*
Specie-*orchiodes*

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

MATERIALS AND METHODS

I. Plant material

Fresh plant were collected from yercaud hills Salem dist tamil nadu and authentified by Dr.V.Raju department of botany, Kakatiya university Warangal. Authentification number KUC/2015/4251

II. Extraction

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

III. Animals

Female Swiss albino mice weighing 20-30gms were used for oral acute toxicity study because shows greater sensitivity to treatment. Male Albino Wistar rats weighing 200-250 gms were used for antidiabetic study. They animals were housed in standard aseptic clean environment condition are maintain and fed with standard rodent diet with water and ad libitum.

IV. Toxicity Study

An acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423. By Acute toxic class method female Swiss albino mice of weighing 20-30gms were used for the study. Acute toxic class method is a stepwise procedure with use of three animals of each step. Average 2-4 steps may be necessary to allow determine on the acute toxicity of the substance. Three animals were used for each step. The animal were placed individually and observed first 24 hours 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 animals according to the weight and solubilized with 0.2ml saline (154 mM NaCl) just prior to 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. Blood sample were drawn at weekly intervals till end of study (i.e. 3 weeks). Fasting blood glucose estimation and body weight measurement were done on day of 1, 7 and 21 of the study. On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated. Serum was separated and analyzed for serum cholesterol, se-rum 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 1ml/kg for 15 days orally. **Group II:** Diabetic control of Wistar rats received 1% w/v gum acacia 1ml/kg for 15 days orally. **Group III:** Diabetic rats received methanolic extract N-butanol fraction of *Curculigo orchioides* 100mg/kg body weight once a day orally for 15 days. **Group IV:** Diabetic rats received with standard drug of Glibenclamide 0.5mg/kg orally once a day for 15 days. Rats were 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 body weight was observed throughout treatment period in experimental animals.

Statistical Analysis

All the values of body weight, fasting blood sugar and biochemical estimations were expressed as mean ± standard error of 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 period of 3 weeks. Three weeks of daily treatment of extracts led to a dose-dependent fall in blood sugar levels by 25-62%. Effect seems to reach maximum after 15 days of treatment and remained constant in third week. Vehicle control animals were found to be stable in their body weight while diabetic rats showed 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 glibencalmide ($p < 0.001$), 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 *Curculigo 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 *Curculigo 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 HDLcholesterol	Serum LDLcholesterol	Serum creatinine	Serum urea	Serum alkalinephosphatase
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 bodyweight 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 regeneration of islet β-cells following destruction by *streptozotocin* may 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. 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 increase in pancreatic secretion of insulin.

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