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Evaluation of anti-diabetic activty of ethanolic and methanolic extracts of leaves of curcuma inodora against streptozotocin induced diabetic rats

Sabbani.Vidya[1], A.Ramesh[2], S.Shobharani[3]
[1]*Department of Pharmacology, Sitha Institute of Pharmaceutical Sciences, Bachupally, Hyderabad. Telangana ,India*
[2]*Department of Pharmacology, Vishnu Institute of Pharmaceutical Education & Research, Medak. Telangana ,India*
[3]*Department of Pharmaceutical Chemistry, IST, CPS, JNTUH, Hyderabad. Telangana ,India*

Abstract: *The present research was focused to evaluate the antidiabetic activity of ethanolic and methanolic leaf extractsof Curcuma inodora in streptozotocin -induced diabetic rats. Ethanolic and methanolic leaves extract of Curcuma inodora (100,200,400 mg/kg) were administered to streptozotocin -induced diabetic rats for 14 day and blood glucose levels were estimated daily at regular intervals up to 14 days through out the treatment using Glucose peroxidation method. Treatment of streptozotocin induced diabetic rats with ethanolic leaf extract of* Curcuma inodora *caused a significant decrease in blood glucose levels at a dose of 200mg/kg and 400mg/kg when compared with standard glibenclamide drug . Treatment of streptozotocin induced diabetic rats with Methanolic leaf extracts of* Curcuma inodora *caused less significant decrease in blood glucose levels at a dose of 200 & 400mg/kg when compared with standard glibenclamide drug .The maximum reduction in the blood glucose was observed 6th hour to 10th hour after oral administration of 200 and 400mg/kg b.w of ethanolic extracts of leaves of curcum a inodora . These results suggest that ethanolic leaf extract of cururma inodora reduced elevated blood glucose levels more significantly compared to methanolic leaf extract in streptozotocin induced diabetic rats in a dose dependent manner .*

I. Introduction:

Carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin[1]. Diabetes mellitus is the sixth leading cause of death globally [2]. Several drugs have been used in the management of the disease. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems [3]. Traditionally, a number of plants have been used in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically [4]. More than 800 plants have been studied for their ant diabetic potentials [5] . Curcuma inodora is an attractive plant with large, showy flowers; scentless turmeric is also cultivated as an ornamental and used in traditional medicine for the treatment of muscular pain. Tubers are mixed with water to form a paste, which is applied locally. It is also used in the treatment of psychosomatic disorders and constipation[6-12].

II. Materials and Methods

Collection of Plant Material

The plant, Curcuma inodora , was collected from narsapur forest in narsapur ,Medak district in the month of September.The plant was authenticated by Dr.Madhana Shetty , Department of Botany, Sri Venkateshwara University ,Tirupathi .

Preparation of extraction [13]

The plant material first washed with water thoroughly to remove dirt and soil deposits and dried under shade until complete removal of moisture content,such dried plants were powdered by mechanically and passed through sieve no 80. 500gm of powdered extract with 300 ml of 90% ethanol solvent heated to 60-70ºC by using soxhlet apparatus for 48 hours. The extract thus obtained was concentrated with the help of rotator vacuum evaporator and stored in –2ºC and different dose levels of plant extracts were prepared in 1% gum acacia solution and were used for studies.

Animals

Wistar albino rats of either sex (150-200 g) were maintained under controlled conditions for all sets of experiments.The rats were allowed to take standard laboratoryfeed and water ad libitum.

The experimental protocol was approved by the institutional animal ethics committee of Vishnu institute of pharmaceutical sciencves,Narsapur,Medak , which wasregistered with Committee for the purpose of control and supervision of experiments on animal(CPCSEA),Govt. of India

Acute Toxicity Studies [14,15]

Determination of acute toxicity (LD50) The oral acute toxicity of alcoholic and Methanolic extracts were determined in albino Wistar albino rats (150-250 g), maintained under standard husbandry conditions. The animals were fasted 3 hrs prior to the experiment and "Up and Down" procedure of OECD Guidelines No. 425 method of CPCSEA was adopted for toxicity studies. Animals were administered with single doses of alcoholic and aqueous extracts in different groups and observed for the mortality during 48 hrs study period (short term) toxicity. Based on the short term profile the doses for the next group of animals were determined as per OECD Guidelines No. 425. All the animals were observed for long term toxicity (14 days) and from the observed LD50 doses of, alcoholic and methanolic extracts 1/10th &1/5th doses were selected for the present study. The animals were also closely examined for signs of intoxication, lethargy, behavioral modification and morbidity.

Induction of Diabetes[16-18]

Diabetes was induced by a single intra-peritoneal dose of 60 mg/kg of b. w of streptozotocin (STZ)dissolved in 0.1M fresh cold citrate buffer (pH4.5) into 12 hr fasted rats. The blood sampleswere taken on third day from retro orbital plexusof the rats for the estimation of blood glucose levels by using the auto analyzer. Rats withdiabetes having hyperglycemia (i.e. with bloodglucose of 185 to 360 mg/dl) were taken for the experiment **.**

Evaluation of anti-diabetic activity of the ethanolic and methanolic leaf extracts of curcuma inodora[19].

The Diabetic animals were randomly divided into eight groups with 5 rats in each group and treated as follows for 14 days continuously orally.

Group I : Control group: 5 ml of Gum acacia suspension.
Group II : Ethanolic leaf extract of Curcuma inodora (ECI) (100 mg/kg/day).
Group III : Ethanolic leaf extract of Curcuma inodora (ECI) (200 mg/kg/day)
Group-IV : Ethanolic leaf extract of Curcuma inodora (ECI) (400 mg/kg/day)
Group V : Methanolic leaf extract of Curcuma inodora (MCI) (100 mg/kg/day).
Group VI : Methanolic leaf extract of Curcuma inodora (MCI) (200 mg/kg/day).
Group VII : Methanolic leaf extract of Curcuma inodora (MCI) (400 mg/kg/day).
Group VIII : Glibenclamide (10mg/kg)

The fasting Blood Glucose Levels of all the rats were recorded at regular intervals during the experimental period. For acute study, the Blood Glucose Levels were monitored after 2, 4, 6 and 8 ,12 h of administration of a single dose and at the end of 1, 3, 5, 7 and 14 days for prolonged treatments.

Collection of Blood Samples and Serum Glucose Estimation[20-22]

The blood samples (0.5ml) were collected for every time intervals of 0, 2, 4, 8, 12, hr in 1ml Eppendorf's tubes. Serum was separated by centrifuging at 3000 rpm for 10 minutes. 30 µl of serum sample and 3 ml of working glucose reagent were taken in to a dry and clean test tube and incubated for 10 minutes at 37o C. The pink color developed was measured by using auto analyzer.

III. Statistical Analysis

The values were expressed as mean±SEM. The data was subjected to the analysis of variance(one way ANOVA) to determine the significanceof changes followed by students "t"-test[23-25**]**.The statistical significance of difference between two independent groups were calculated for the determination of blood glucose levels.

IV. Results

The extracts did not produce any toxic signs during the observation period for 24 hours in any of the rats they were tested. Hence it was concluded that the extracts are safe upto 2000mg/kg. The mean blood glucose levels of control and drug treated animals after oral administration of different extracts at different doses (100, 200 and 400 mg/kg b.w) of Curcuma inodora leaves at various time intervals (0, 2, 4, 8, 12, hrs) are shown in Table 1 and Figure 1.

The statistical significance of decrease in blood glucose levels was calculated with respect to initial blood glucose levels. Oral administrationof 1% gum acacia suspension did not change the blood glucose levels

of rats. The blood glucose levels of diabetic rats treated with Glibenclamide (10 mg/kg b.w) showed significant ($P<0.001$) decrease in blood glucose levels at 6 -12th hrs, more significantly. Nevertheless, the reduction in mean blood glucose levels was less significant at 12 hrs.

The ethanolic leaf extract of Curcuma inodora at a dose of 100 mg/kg b. w ,showed less significant ($P<0.05$) decrease in blood glucose levels at 6th hr and more significant ($P<0.01$) decrease in blood glucose level at 8th hr. However,oral administration of 200 mg/kg & 400 mg/kg b. w of leaves of Curcuma inodora showed less significant ($P<0.05$) decrease in blood glucose levels at 4th hour more significant ($P<0.01$) decrease in blood glucose levels at 6 th hr and highly significant ($P<0.001$) decrease in blood glucose levels at 8th hr.

The Methanolic extract of leaves of Curcuma inodora a dose of 100,200 & 400 mg/kg b. w, showed less significant ($P<0.05$) decrease in blood glucose levels.

The decrease in blood glucose levels produced by the oral administration of different doses (100, 200 and 400 mg/kg b.w) at various time intervals for 14 days shown in Table 2 and Figure 1.

V. Discussion

The data obtained from the test carried out on STZ diabetic rats clearly showed that the ethanolic extracts of Curcuma inodora leaves at dose levels of 100, 200 and 400 mg/kg b.w produced a significant reduction of blood glucose level than methanolic extracts. Comparing the results of 100, 200 and 400 mg/kg b. w ethanolic extracts of Curcuma inodora leaves in diabetic rats, it was found that the ethanolic extract at 400 mg/kg b.w showed highly significant ($P<0.001$) decrease in blood glucose levels when compared to control STZ induced diabetic animals which was comparable with the standard glibenclamide (10 mg/kg b.w). The lowest blood glucose levels were observed at 6th and 8th hrs after oral administration of ethanolic leaf extracts of curcuma inodora the reduction of blood glucose levels in diabetic rats was in a dose dependent manner compared to glibenclamide which is used in treatment of Type II diabetes mellitus. The standard drug Glibenclamide stimulates insulin secretion form beta cells of islets of langerhans. From the study, it is suggested that the possible mechanism by which the plant extracts decrease the blood glucose level may be by potentiation of insulin effect either by increase in pancreatic secretion of insulin form beta cells of Islets of langerhans. Histopathological studies of pancrease and kidney are under study. The bioactive principle will be identified and characterization will be done based on their chemical nature.

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Table: 1 Antidiabetic effect of leaf extracts of Curcuma inodora on blood glucose level of streptozotocin -induced diabetic rats during acute study (mean ± SEM) (n=5).

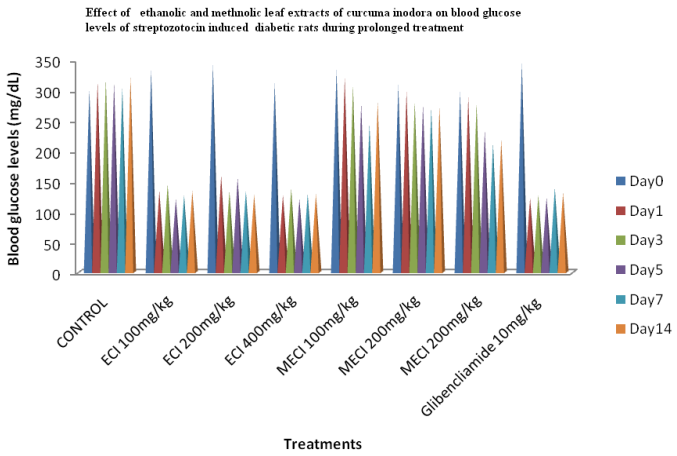
Treatments	Dose mg/kg	Blood glucose levels (mg/dL) in hours					
		0hr	2hr	4hr	6hr	8hr	12hr
Control(1% gum acacia)	5 ml	311 ± 0.123	324 ± 3.87	318 ± 2.89	312 ± 6.67	305 ± 2.14	321 ± 3.54
ECI	100	298 ± 3.78	231 ± 3.56	222 ± 4.34	219 ± 1.98	197 ± 0.45	174 ± 4.56
ECI	200	342 ± 1.45	289 ± 4.56	234 ± 1.34	156 ± 6.34	134 ± 1.45	128 ± 1.35
ECI	400	312 ± 1.78	301 ± 2.56	278 ± 1.45**	127 ± 3.65**	129 ± 2.54**	123 ± 1.89
MCI	100	334 ± 5.78	321 ± 8.09	308 ± 2.98	278 ± 3.89	245 ± 4.67	281 ± 4.523
MCI	200	309 ± 3.78	298 ± 1.09	281 ± 2.09	276 ± 2.90	271 ± 2.90	272 ± 3.98
MCI	400	298 ± 6.78	289 ± 5.56	278 ± 1.23*	234± 1 .65*	213 ± 2.54	219 ± 1.23
Glibenclamide	10	345 ± 2.56	291 ± 3.45	147 ± 2.67 **	149 ± 3.67**	142 ± 3.78 **	159 ± 1.56

Values are expressed as mean ± SEM, n = 5; n= Number animals per treatment;
** Significantly different from Control, p<0.01

Table: 2 Effect of leaf extracts of Curcuma inodora on blood glucose level of streptozotocin -induced diabetic rats during prolonged treatment

Treatments	Dose mg/kg	Blood glucose levels (mg/dL) in days					
		Day0	Day1	Day3	Day5	Day7	Day14
Control(1% gum acacia)	5 ml	301 ± 0.123	314 ± 1.87	318 ± 4.98	312 ± 7.17	305 ± 1.11	321 ± 1.19
ECI	100	334 ± 3.78	134 ± 3.56	145 ± 4.34	121 ± 1.98	127 ± 0.45	134 ± 4.56
ECI	200	342 ± 1.45	159 ± 4.56	134 ± 1.34	156 ± 6.34	134 ± 1.45	128 ± 1.35
ECI	400	312 ± 1.78	127 ± 2.56**	138 ± 1.45**	121 ± 3.65**	129 ± 2.54**	129 ± 1.89 **
MCI	100	334 ± 5.78	321 ± 8.09	308 ± 2.98	278 ± 3.89	245 ± 4.67	281 ± 4.523
MCI	200	309 ± 3.78	298 ± 1.09	281 ± 2.09	276 ± 2.90	271 ± 2.90	272 ± 3.98
MCI	400	298 ± 6.78	289 ± 5.56	278 ± 1.23*	234± 1 .65*	213 ± 2.54*	219 ± 1.23 *
Glibenclamide	10	345 ± 2.56	122 ± 3.45 **	127 ± 2.67 **	123 ± 3.67**	139 ± 3.78 **	131 ± 1.56**

Values are expressed as mean ± SEM, n = 5; n= Number animals per treatment;
** Significantly different from Control, p<0.01


Effect of ethanolic and methnolic leaf extracts of curcuma inodora on blood glucose levels of streptozotocin induced diabetic rats during prolonged treatment
Blood glucose levels (mg/dL)
350
300
250
200
150
100
50
0
CONTROL
ECI 100mg/kg
ECI 200mg/kg
ECI 400mg/kg
MECI 100mg/kg
MECI 200mg/kg
MECI 200mg/kg
Glibenclamide 10mg/kg
Treatments
Day0
Day1
Day3
Day5
Day7
Day14