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Antidiabetic activity of alcoholic root extract of *Caesalpinia digyna* in streptozotocin-nicotinamide induced diabetic rats

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ABSTRACT

Objective: The present investigation deals with evaluation of antidiabetic (Type 2) activity of standardized alcoholic root extracts of Caesalpinia digyna in STZ-nicotinamide induced diabetic rats. **Methods:** Alcoholic root extract of Caesalpinia digyna (ACD), obtained from Soxhlet extractor was standardized by HPLC. Type 2 diabetes was induced by single intraperitoneal injection of nicotinamide (110 mg/kg) followed by streptozotocin (65 mg/kg). Diabetic rats ware administered ACD at doses of 250, 500, and 750 mg/kg (p.o.) and different parameters such as normoglycemic and oral glucose tolerance test were evaluated. The study also included estimations of blood plasma glucose, lipid profile, liver glycogen, body weight and anti-oxidant status in normal and diabetic rats. **Results:** Normoglycemic rats did not reduce the blood glucose level, whereas oral glucose tolerance test showed better tolerance of glucose in treated rats. The alcoholic extract showed a dose dependent reduction in fasting blood glucose level i.e. more pronounced at 750 mg/kg ($P<0.05$). ACD showed significant reduction in plasma lipid like triglycerides, total cholesterol and improvement in high density lipo-protein cholesterol (HDL-C) in treated groups. The decrease in lipid peroxides and increase in superoxide dismutase (SOD) and catalase (CAT) in liver clearly showed the antioxidant potential while rat hemi-diaphragm glucose uptake study revealed increases in peripheral glucose uptake of treated rats. **Conclusions:** Results showed that standardized alcoholic extract of C. digyna possessed significant antidiabetic activity which may be attributed to increase in glycogen storage, hypolipidemic and antioxidant activity thus, rationalizing its traditional use.

1. Introduction

Caesalpinia digyna Rottler (Leguminosae) is a large, scandent, prickly shrub or climber, up to 10 m in height, growing wild in the scrub forests of the eastern Himalayas, Assam, West Bengal, Madhya Pradesh, and the Eastern Ghats of Andhra Pradesh. The root has marked astringent properties. It is given internally in phthisis and scrofulous affections; when sores exist, it is applied externally as well. It is also used in diabetes[1]. In some parts of the Burma the root, pounded and mixed with water, is drunk as a febrifuge and also said to have an intoxicating effect[1]. The drug also exhibits antifatigue effect in rats. The ethanol-water extract of roots inhibits the growth of Mycobacterium tuberculosis. Chemical investigations of the plant have shown the presence of caesalpinine A, cellallocinnine, ellagic acid, gallic acid, bergenin, bonducellin, intricatinol, and tannins. The root extract and bergenin isolated from it have shown significant antioxidant activity[2]. Bergenin isolated from the plant has been shown to posses antiulcerogenic, hepatoprotective, antiviral, antidiabetic/antiobesity (by in vitro inhibition of PTP1B (protein tyrosine phosphatase 1B), anti-arrhythmic, antioxidant, antiarthritic, burn wound healing and trypanocidal activities[3].

It is currently estimated that about 150 million people worldwide suffer from diabetes. This number is expected to be increase to 300 million by the year 2025. However, among the two major types of diabetes i.e. Type 1 and Type 2. Type 2 diabetes mellitus is the commonest form of diabetes constituting 90-95% of the diabetic population. It was also documented that the number of people diagnosed with Type 2 diabetes mellitus globally is estimated to be at 2-3% of the

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world population and is rising at a rate of 4-5% per year[4,5]. Hence, the present study was conducted to assess Type 2 antidiabetic activity of standardized alcoholic roots extract of C. digyna.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) and bergenin were obtained from Sigma-Aldrich Co., St. Louis, USA. Solvents were purchased from SD Fine Chemicals Ltd., Mumbai, India. All the chemicals used were of analytical grade, whereas other biochemical kits were obtained from Span Diagnostic Ltd, India.

2.2. Plant material

The dried roots of C. digyna were purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India, and identified by Prof. V. Chelladurai, Research Botanist, Palayamkottai, Tamilnadu, India. The voucher specimen was deposited in the herbarium of Department of Pharmaceutics, Banaras Hindu University for future reference (Specimen number-COG/CD-08).

2.3. Preparation of extract

The roots were chopped to small pieces and shade-dried. The dried roots were powdered and passed through sieve no. 20 and extracted (2.5 kg) in a Soxhlet extractor for three days using alcohol. The extract was than concentrated under reduced pressure to dryness. The alcoholic extract yielded a dark brown solid residue weighing 375.09 g (15% w/w). Extract was preserved in a desiccator till further use. Further preliminary phytochemical screening was conducted for the presence of various phytoconstituents[6]. Based on the results obtained from phytochemical screening, total phenolic (TP) content of extract was determined by Folin-Ciocalteu method[7].

2.4. Standardization of extract

Crude alcoholic extract of C. digyna was standardized by established method using bergenin as a standard[8]. HPLC analysis was performed with a Waters HPLC system, USA with PDA detector. A Cosmosil C18 column (150 mm x 4.6 mm, 5 μm particle) was used for the analysis. The mobile phase used was a mixture of acetonitrile:water 30:70 (v\v) delivered at a flow rate of 0.8 ml min-1, and run time of 5 min. The data was collected at wavelength of 275 nm. Peak of bergenin was identified by comparison with retention time of standard bergenin (3.20 min).

2.4.1. Sample Preparation

Crude alcoholic extract of C. digyna (30 mg) was taken in a volumetric flask and dissolved using HPLC grade methanol and volume was finally adjusted to 10 ml accurately. The mixture was sonicated for 30 min at room temperature and filtered through a 0.45 μm nylon filter to obtain a clear solution. Solution was injected (25 μl) into the HPLC system directly for the analysis.

2.4.2. Preparation of Standard Solutions for Calibration Curves

For quantification, an external standard method was utilized. Peak areas from the HPLC chromatogram were plotted against the known concentrations of stock solutions at varying concentrations. Equations generated by linear regression were used to establish concentrations of bergenin in alcoholic extract of C. digyna. About 10 mg of a standard bergenin weighed accurately was dissolved into a 10 ml volumetric flask in methanol to obtain stock solutions. For calibration curves, the stock solution was diluted with methanol to obtain the concentration in the range of (0.25-2.5 μg/ml).

2.5. Animals

Healthy male albino rats of Charles foster strain (150-220 g) were obtained from the Central Animal House, (Reg. No. 542/02/ab/CPCSEA) Banaras Hindu University, Varanasi, India, and were maintained under standard environment conditions (22-28 ℃, 60-70% relative humidity, 12h dark:light cycle) and were fed with standard rat feed (Mona Laboratory Animal Feeds) and water ad libitum. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. All the experimental procedures conducted after the approval of ethical committee (No. Dean/2009-10/579) and were in strict accordance with institutional animal ethical committee guidelines for the care and use of laboratory animals.

2.6. Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 425. Alcoholic extract of C. digyna (5 g/kg) was suspended in 0.5% w/v carboxy methyl cellulose (CMC) and given p.o. to overnight fasted rats and animals were observed individually for 48 h and their behavioral and neurological changes such as tremors, convulsions, salivation, diarrhea, sleep and lacrimation in drug treated rats were observed for sign of acute toxicity[9].

2.7. Experimental

2.7.1. Normoglycemic study of the ACD in overnight fasted rats

Rats were divided into five groups of six rats in each group. Group I animals were orally administered equal volume carboxy methyl cellulose (CMC) solution, which served as control. Group II rats were given the oral hypoglycemic, glibenclamide at a dose level 10 mg/kg; body weight; p.o. The rats in group III-V were given CMC suspension of ACD orally at dose levels of 250, 500, and 750 mg/kg body weight, respectively. Blood samples were collected just prior to and 1, 2, 4 and 6 h after administration of the extract from the retro-orbital plexus of eye. Plasma was separated and

glucose levels were estimated[10].

2.7.2. Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (18 h) normal rats[11]. Rats were divided into five groups (n = 6). Control animals received an equal volume of the vehicle (CMC solution). Group II rats were given glibenclamide orally at a dose level 10 mg/kg body weight. Rats in group III-V received orally ACD in doses of 250, 500 and 750 mg/kg body weight, respectively. Glucose (2 g/kg; p.o.) was fed 30 min after the administration of extract. Blood was withdrawn from the retro orbital plexus at 30, 60, 90 and 120 min of extract administration and plasma glucose level was estimated.

2.7.3. Induction of Type 2 diabetes in rats

The rats were fasted overnight and were then administrated with freshly prepared solution of streptozotocin (STZ) dissolved in citrate buffer pH 4.5 at a dose of 65 mg/kg intraperitoneal (i.p.), 15 min after i.p. administration of 110 mg/kg body weight nicotinamide[12]. Normal rats (n = 6) received 1 ml citrate buffer as vehicle. As administration of STZ can induce fatal hypoglycemia as a result of massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 h of STZ administration for the next 24 h to prevent hypoglycemia. After 72 h of streptozotocin injection the blood glucose level of each rat was assayed and further on day 7 of the injection. The rats with fasting blood glucose level above 200 mg/dl were considered to be diabetic and used in this investigation.

2.7.4. Experimental design

The diabetic animals were divided in six groups of six rats each. The animals of group I and II served as normal and diabetic control respectively and group III received glibenclamide (10 mg/kg; p.o). The animals in the normal and diabetic control groups were administered with CMC solution as vehicle (0.5%). The rats of group IV, V and VI received ACD 250, 500 and 750 mg/kg p.o. prepared in CMC solution (0.5%) respectively for 14 days. Blood samples were collected by retro-orbital plexus of eye under light ether anesthesia and fasting blood glucose levels were determined by glucose oxidase method on day 0th, 7th, and 14th with commercially available biochemical kit. The day of administration of ACD is considered as day 0. Body weight of rats was also taken on day 0th and 14th. On 14th day, plasma lipid profiles were estimated using biochemical kits (Span Diagnostics Ltd.) and liver glycogen levels were estimated using anthrone reagent, lipid peroxidation in liver was estimated by measuring thiobarbituric acid reactive substances (TBARS), Catalase (CAT) was estimated by the method of Sinha and the activity of superoxide dismutase (SOD) was assayed by the method of Kakkar[13-16]. Protein content in tissue homogenate was measured by the method of Lowry[17].

2.7.5. Effect of ACD on glucose utilization by isolated rat hemi-diaphragm

Glucose utilization by rat hemi-diaphragm was estimated by the method described by Hemalatha et al[18]. Group I served as a control which contained 2 ml of Tyrode solution with 2% glucose, Group II contained 2 ml Tyrode solution with 2% glucose and regular insulin (Biocon Ltd.) 1 ml of 0.25 IU/ml solution. Group III contained 2 ml Tyrode solution with 2% glucose and 1 ml of (25 mg/ml of ACD) and regular insulin (Biocon Ltd.) 1 ml of 0.25 IU/ml solution. Group IV contained 2 ml Tyrode solution with 2% glucose and 1 ml of (25 mg/ml of ACD). The volumes of all the test tubes were made up to 4 ml with distilled water to match the volume of the test tubes of group III. Albino rats were fasted overnight and killed by cervical dislocation. The diaphragms were dissected out quickly with minimal trauma and divided into two halves. Two diaphragms from the same animal were not used for the same set of experiment. The hemidiaphragms were placed in test tubes and incubated for 30 min at 37 ℃ in an atmosphere of 95% oxygen, 5% CO2 with shaking at 140 cycles/min. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium.

2.8. Statistical analysis

All the values of the experimental results were expressed as mean±standard error of mean (SEM). Two-ways ANOVA followed by Bonferroni post test was performed from normoglycemic, oral glucose tolerance test and evaluation of blood glucose of STZ-nicotinamide induced diabetic rats. One-way ANOVA followed by Tukey's Multiple Comparison test was applied for the statistical analysis of the rest of parameters. GraphPad Prism (version 4) software was used for all statistical analysis. A difference in the mean values of $P<0.05$ was considered to be statistically significant. For the study of glucose uptake by rat hemidiaphragm, results were analyzed by unpaired t-test.

3. Results

3.1. Phytochemical screening

The alcoholic root extract of C. digyna showed the presence of phenols, tannins, triterpenoids, phytosterols, amino acids and carbohydrates. The total phenolic content in the extract was found to be 470 mg/g of dry extract equivalent to gallic acid.

3.2. Quantification of bergenin in C. digyna alcoholic extract

From the standard plot of bergenin and from the linear regression equation ($Y = 22887X + 32677$, $R^2 = 0.986$). It was estimated that the content of bergenin in the crude alcoholic extracts of C. digyna was found to be 0.548% w/w.

3.3. Effect on blood glucose levels in fasted normal rats

Figure 1 illustrates the effect of ACD on overnight fasted rats. ACD extract in all the doses tested did not show any hypoglycemic effect on normal rats. Glibenclamide at dose 10 mg/kg body wt. orally significantly reduced the blood glucose level in normal rats when compared to control group.

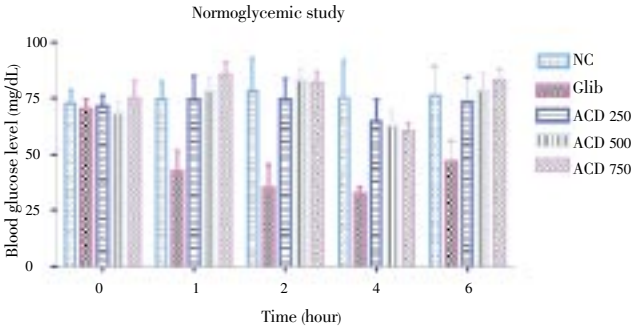

Figure 1. Effect of ACD on Normal rats.
Values are mean±SEM of 6 animals in each group. Two-way ANOVA revealed that there was a non significant difference between control group and treatment groups [$F(4,25)=11.74$, $P>0.05$]. $^{**}P<0.01$ treated with glibenclamide compared to normal control. (Two-way ANOVA followed by Bonferroni post test). NC: Normal control; Glib: Glibenclamide; ACD: Alcoholic extract of C. digyna.

3.4. Oral glucose tolerance test (OGTT)

The effect of ACD (250, 500, and 750 mg/kg); p.o. on OGTT was depicted in Figure 2. The administration of C. digyna significantly prevented the increase in blood glucose levels without causing a hypoglycemic state. Maximum effect of C. digyna was observed 30 and 60 min after the oral glucose administration. Animals treated with ACD and glibenclamide showed a significant decrease in blood glucose level when compared to vehicle control animals.

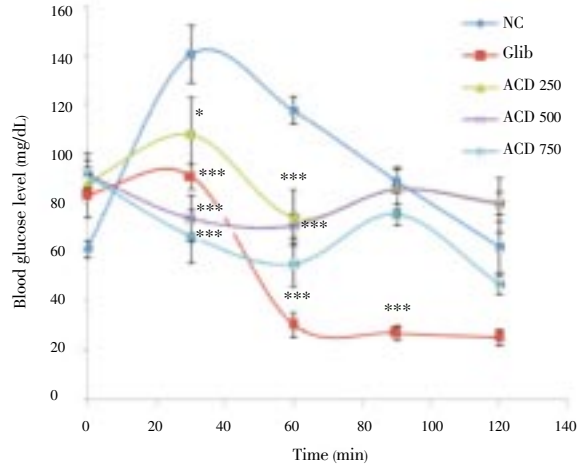

Figure 2. Effect ACD on oral glucose tolerance test in normal rats. Values are mean±SEM of 6 animals in each group. Two-way ANOVA indicated that there were significant differences between experimental groups [$F(4,25)=22.20$, $P<0.05$] after treatment. $^{*}P<0.05$, $^{***}P<0.001$compared to normal control. (Two-way ANOVA followed by Bonferroni post test). NC: Normal control; Glib: Glibenclamide; ACD: Alcoholic extract of C. digyna.

3.5. Effect on fasting blood glucose level of STZ-nicotinamide induced (Type 2) diabetic rats

The effect of ACD (250, 500 and 750 mg/kg; p.o) on the STZ-nicotinamide induced diabetic rats was shown in Figure 3. A significant increase in the level of blood glucose was observed in STZ-nicotinamide treated rats when compared to control rats. Administration of ACD (250, 500 and 750 mg/kg) p.o. of C. digyna significantly reduced the fasting blood glucose levels in dose-dependent manner on 7th and 14th day compared to diabetic control. Treatment of diabetic rats with glibenclamide also significantly reduced the increased blood glucose level.

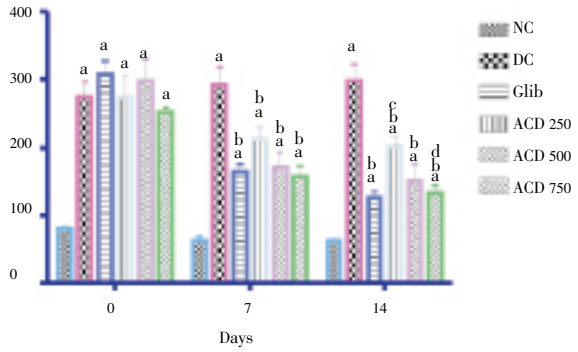

Figure 3. Effect of ACD on the blood glucose level of streptozotocin-nicotinamide induced diabetic rats.
Values are mean±SEM of 6 animals in each group. Two-way ANOVA reveals that there were significant differences in the experimental groups [$F(5,36)=46.99$, $P<0.05$]. a: compared to normal control; b: compared to diabetic control; c: compared to glibenclamide; d: compared to ACD 250; (Two-way ANOVA followed by Bonferroni post test). NC: Normal control; DC: Diabetic control; Glib: Glibenclamide; ACD: Alcoholic extract of C. digyna.

3.6. Effect on plasma lipid profile

The plasma lipid profile i.e. total cholesterol, triglycerides, and lipoproteins are shown in Table 1. The levels of plasma total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL-C) were significantly increased, whereas levels of high density lipoprotein (HDL-C) were significantly decreased, in diabetic rats as compared to control rats. Administration of ACD to diabetic rats, reversed plasma lipid profile near normal values in a dose-dependent manner showed that treatment with C. digyna significantly improved the lipid profile in diabetic animals. The effect of C. digyna (750 mg/kg; p.o.) was more significant than that of 250 and 500 mg/kg and was comparable with that of glibenclamide (10 mg/kg; p.o.).

3.7. Effect on body weight and liver glycogen

Effect of treatment of rats with ethanol extract of C. digyna on body weight and liver glycogen is shown in Table 2. The mean body weight of diabetic rats was higher when treated with ethanol extract of C. digyna (250 and 500 mg/kg; p.o.) but it was not statistically significant. However, the rats treated with 750 mg/kg; p.o. showed significant increase in body weight on 14th day which was comparable to that of glibenclamide treated rats. A significant decrease in liver glycogen content was observed in diabetic rats compared to normal control group. Rats treated with 250 mg/kg; p.o. did not show significant increase in liver glycogen level however, rats treated with 500 and 750 mg/kg; p.o. showed pronounced increases in liver glycogen level. In comparison, the results of higher dose of C. digyna (750 mg/kg) were more significant than 500 mg/kg. Glibenclamide treatment also

Table 1
Effect of ACD on lipid profile of streptozotocin-nicotinamide induced diabetic rats

Group (n=6)	Treatment (dose in mg/kg)	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
I	NC	63.14 ± 3.47	71.58 ± 6.95	40.05 ± 1.78	18.90 ± 5.86
II	DC	155.91 ± 9.37[a]	183.77 ± 25.84[a]	23.36 ± 1.77[a]	129.22 ± 4.93[a]
III	Glib (10)	74.71 ± 4.58[b]	86.70 ± 5.30[b]	46.16 ± 3.73[b]	25.59 ±4.87[b]
IV	ACD (250)	110.69 ± 19.55[a]	112.02 ± 12.68[b]	40.48 ± 1.94[b]	49.39 ±11.30[b]
V	ACD (500)	80.88 ± 10.30[b]	93.62 ± 12.80[b]	46.36 ± 4.62[b]	31.08 ± 8.51[b]
VI	ACD (750)	73.96 ± 7.60[b]	84.15 ± 5.06[b]	49.50 ± 6.55[b]	19.86 ±7.80[b]

Values are mean ± SEM of 6 animals in each group. One-way ANOVA showed a significant difference in drug treatment between the groups [F(5,36)=9923, P<0.05], [F(5,36)=7208, P<0.05] and [F(5,36)=527.8, P<0.05] respectively for total cholesterol, triglyceride and HDL-cholesterol (HDL-C). a: compared to normal control; b: compared to diabetic control; (One-way ANOVA followed by Tukey's Multiple Comparison test). (Abbreviation: NC-Normal control; DC-Diabetic control; Glib-Glibenclamide; ACD-Alcoholic extract of C. digyna).

Table 2
Effect of ACD on body weight and liver glycogen in streptozotocin-nicotinamide induced diabetic rats

Group (n=6)	Treatment (dose in mg/kg)	Body weight (g)		Liver glycogen (mg/g)
		0th Day	14th day	
I	NC	179.16 ± 6.75	184.33 ± 6.26	26.39 ± 1.36
II	DC	168.50 ± 6.18	127.50 ± 4.48[a]	10.67 ± 1.38[a]
III	Glib (10)	174.66 ± 5.72	170.16 ± 5.81[b]	21.37 ± 1.62[b]
IV	ACD (250)	162.33 ± 3.71	146.33 ± 6.30[a]	13.36 ± 0.32[ac]
V	ACD (500)	176.66 ± 9.27	151.33 ± 11.66	17.52 ± 0.79[ab]
VI	ACD (750)	174.16 ± 5.97	167.16 ± 6.28[b]	22.16 ± 1.42[bd]

Values are mean ± SEM of 6 animals in each group. One-way ANOVA reveals that there were significant differences among the experimental groups [F(5,36)=2443, p<0.05] and [F(5,36)=206.9, p<0.05] for body weight and liver glycogen respectively. a: compared to normal control; b: compared to diabetic control; c: compared to glibenclamide; d: compared to ACD 250; (One-way ANOVA followed by Tukey's Multiple Comparison test) (Abbreviation: NC-Normal control; DC-Diabetic control; Glib-Glibenclamide; ACD-Alcoholic extract of C. digyna)

Table 3
Effect of ACD on TBARS, SOD and CAT in streptozotocin-nicotinamide induced diabetic rats

Group (n=6)	Treatment (dose in mg/kg)	TBARS (n mol./mg protein)	SOD (U/mg protein)	CAT (μ mol. H_2O_2 consumed/min/mg protein)
I	NC	24.88 ± 1.62	0.81 ± 0.10	241.50 ± 8.78
II	DC	49.91 ± 5.55[a]	0.42 ± 0.03[a]	160.85 ± 9.45[a]
III	Glib (10)	29.79 ± 2.08[b]	0.78 ± 0.12[b]	239.86 ± 20.13[b]
IV	ACD (250)	40.25 ± 2.85[a]	0.54 ± 0.05	213.90 ± 8.30
V	ACD (500)	33.89 ± 3.96[b]	0.76 ± 0.05[b]	235.46 ± 15.95[b]
VI	ACD (750)	26.42 ± 3.11[b]	0.86 ± 0.05[b]	238.69 ± 12.59[b]

Values are mean ± SEM of 6 animals in each group. One-way ANOVA reveals that there were significant differences among the experimental groups [F(5,36)=540.4, P<0.05], [F(5,36)=0.1827 P<0.05] and [F(5,36)=5957 P<0.05] For TBARS, SOD and catalase respectively. a: compared to normal control; b: compared to diabetic control. (One-way ANOVA followed by Tukey's Multiple Comparison test).
(Abbreviation: NC-Normal control; DC-Diabetic control; Glib-Glibenclamide; ACD-Alcoholic extract of C. digyna).

Table 4
Effect of ACD on glucose utilization by isolated rat hemi-diaphragm

Group	Incubation medium	Glucose uptake (mg/g/30 min)
Control	Tyrode solution with glucose (2%)	18.66 ± 0.075
Insulin	Tyrode solution with glucose (2%) + Insulin (0.25 IU/ml)	23.43 ± 2.117**
Insulin + Root extract	Tyrode solution with glucose (2%) + Insulin (0.25 IU/ml) + root extract (25 mg/ml)	19.45 ± 0.439*
Root extract	Tyrode solution with glucose (2%) + root extract (25 mg/ml)	22.32 ± 0.473*

Values are mean ± SD from 3 experiments * P<0.05; ** P<0.01 compared to control group, results were Analyzed by unpaired t-test.

significantly increased liver glycogen levels compared to diabetic control rats.

3.8. Effect on anti oxidant enzymes

Table 3 represents the concentration of TBARS in liver of normal and experimental rats. There was a significant elevation in tissue TBARS during diabetes compared to the corresponding normal group. Administration of C. digyna extract significantly decreased the lipid peroxidation in diabetic rats. The effect of C. digyna at dose level 750 mg/kg was comparable to that of glibenclamide treated rats. SOD and catalase were significantly lower in diabetic rats as compared with their values in normal rats. Treatment with ACD in diabetic rats significantly restored the enzyme level when compared with untreated diabetes animals (Table 3).

3.9. Effect of ACD on glucose uptake by isolated rat hemi-

diaphragm

Alcoholic extract of C. digyna on isolated rat hemi-diaphragm has shown to possess significant glucose uptake when compared to glucose up take by rat hemi-diaphragm on control group (Tyrode solution with 2% glucose + rat hemi-diaphragm) only (Table 4).

4. Discussion

Streptozotocin is widely used as a diabetogenic agent in experimental animals which selectively destroy β-cell of pancreas and brings an increase in blood glucose levels. The administration of single high dose of streptozotocin (STZ) injection intraperitoneally can produce Type 1 diabetes by destroying the β-cells of the pancreas. Now a day's Type 2 diabetes can be induced by combination of STZ and nicotinamide administration in adult rats[12]. Nicotinamide has antioxidant property, exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic β-cell mass producing Type 2 diabetes[19]. In the present paper the Type 2 antidiabetic activity of standardized ACD was reported first time. Present study revealed that ACD in normoglycemic rats did not cause any reduction of blood glucose level indicating that the extract does not have any hypoglycemic activity. However, the ability of ACD to lower blood glucose level in the oral glucose tolerance test suggest that animals treated with extract have better glucose utilization capacity. Oral administration of ACD 750 mg/kg for 14 days caused a significant decrease in blood glucose levels in diabetic rats. Diabetes mellitus is a metabolic disorder characterized by elevated level of blood glucose. The fundamental mechanism underlying hyperglycemia involves over-production and decreased utilization of glucose by the tissues[20]. As the ACD did not cause hypoglycemia in normoglycemic rats but reduced blood glucose in OGTT and STZ-nicotinamide induced diabetic rats, ACD may act as antihyperglycemic, rather than a hypoglycemic. Rat hemi-diaphragm glucose utilization study reveals that ACD increases peripheral glucose uptake. Hyperlipidaemia is a recognized as a common complication of diabetes mellitus. A reduction in insulin secretion causes a variety of derangements in metabolic and regulatory mechanisms leading to accumulation of lipids[21]. The results of present study clearly show that ACD significantly reduced the triglyceride and total cholesterol in diabetic rats. Lipid-lowering effect of drugs in diabetes reduces the risk of vascular complications[22]. The characteristic loss of body weight associated with diabetes is due to increased muscle wasting or loss of muscle proteins due to hyperglycemia[23,24]. Rats treated with ACD (750 mg/kg p.o.) and glibenclamide (10 mg/kg p.o.) showed significant improvement in body weight as compared to the diabetic control rats suggesting a protective role of ACD on muscle wasting. The observed depletion of liver glycogen level in diabetic control rats was possibly due to loss of glycogen synthetase activation or increased activity of glycogen phosphorylase. Both ACD and glibenclamide treatment significantly elevated the reduced liver glycogen level in diabetic rats which suggest an improvement in the liver glycogen synthesis. Glycogen level, primary intracellular storable form of glucose in various tissues is a direct reflection of insulin activity as insulin promotes its deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase[25,26]. Hence, ACD interferes with glucose utilization and metabolism by storing excess carbohydrates as glycogen. This may be one of the important factors in the antidiabetic activity of ACD.

Chronic hyperglycemia has shown to play role in the development of diabetic microvascular and macrovascular complications. Four seemingly independent mechanisms are involved in the pathogenesis of diabetic complications: glucose induced activation of protein kinase C (PKC) isoforms, increased formation of glucose-derived advanced glycation endproducts, increased polyol pathway, and increased production of reactive oxygen species (ROS) [27]. The phytochemical screening of ACD revels the presence of tannin, phenolics, triterpenoids/sterols. Certain class of compounds viz flavonoids, triterpenoids/sterols, alkaloids and phenolics are known to be bioactive antidiabetic principles. Phenolics are found to be effective antihyperglycemic agents[28,29]. The antidiabetic effect of ACD may be due to the presence of more than one antihyperglycemic principles and their synergistic properties. Literature reveals that antioxidant activity of plant extract is mainly due to presence of phenolic compounds, which may exerts antioxidant effects as free radical scavengers, as hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators[30]. Lipid peroxide mediated tissue damage has been observed in the development of both Type 1 and Type 2 diabetes mellitus. Insulin secretion is impaired during diabetes and this may evoke lipid peroxidation in biological systems[31]. Enhanced levels of TBARS observed in the liver of diabetic rats indicate excessive formation of free radicals and activation of lipid peroxidative system. Present study shows that administration of ACD and glibenclamide inhibits production of liver peroxides. This indicates the anti-lipid peroxidative potential of ACD. SOD and CAT are the two major scavenging enzymes that remove toxic free radicals in vivo and are thought to play important role in protecting the cell against the potentially deleterious effects of reactive oxygen species. Reduced activity of SOD and CAT may result in a number of deleterious effects due to the accumulation of superoxide radicals (O^{2-}) and hydrogen peroxide[32]. Administration of ACD and glibenclamide results in the activation of SOD and CAT to near normal levels in diabetic rats. The result of the SOD and CAT activity clearly shows that extract of C. digyna has free radical scavenging activity, which could exert a beneficial action against pathological alterations caused by the presence of O^{2-} and OH^{-}.

In conclusion, Present study validated the traditional claims of use of roots of C. digyna in the treatment of diabetes. ACD significantly reversed STZ-nicotinamide induced raise in glucose levels indicating Type 2 antidiabetic activity. The antidiabetic activity may be due to improvement in glucose tolerance, restoration of liver glycogen and antioxidant activity of ACD thus, reducing the risk of secondary complications associated with diabetes. Further, detailed activity guided fractionation studies of C. digyna may unveil the constituent/s responsible for the

antidiabetic activity of ACD.

Conflict of interest statement

We declare that we have no conflict of interest.

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