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Anti-Hyperglycemic Acitivity of Ethonalic Extract of Stem *Cissus Quadrangularis* in Streptozotocin Induced Experimental Diabetic Rats

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ABSTRACT

Introduction: *Cissus quadrangularis* is a popularly known traditionally used medicinal plant from ancient times. The common name of *cissus quadrangularis* in tamil 'pirantai'. This plant has been consumed as a vegetable and easily available plant. The plant is used to heal the many sorts of diseases including stroke, epilepsy, bone related diseases, skin infections, constipation, piles, anemia, burns, wounds and diabetes also. The present study was hypothesized to evaluate the effects of anti-hyperglycemic activity of stem *cissus quadrangularis* on Streptozotocin induced diabetic rats.

Materials and Methods: The animals are divided into 5 groups, each group contain 6 animals. Group I- control. Group II -STZ induced negative control. Group III- STZ induced rats treated with metformin. Group IV& V -STZ induced rats treated with (200 and 400 mg/kg) ethanol extract. After overnight fasting, a single dose of STZ (40 mg/kg) were given by intraperitoneally. After confirmation of diabetes the ethanolic extract of cissusquadrangularis 200 mg and 400 mg were given orally.

Results: This study reveals that cissusquadrangularis have more effects in reducing blood sugar level in diabetic rats. The results shows that 400mg/kg/bw CQ have significant effects then with 200 mg/kg/bw. The entire study concluded diabetic patients are prone to wound and related diseases.

Conclusion: Conception of *cissus quadrangularis* are more effective drug to reduce the blood sugar levels and also heal diabetic wounds.

Keywords: *Cissus quadrangularis*; Ethonal; Hyperglycemia; Streptozotocin; Diabetes, plasma.

Introduction

According to the diabetic atlas of the International diabetic federation, 366 million people were affected by diabetes throughout the world in 2011,and diabetes frequency is expected upto 522 million by 2030.[1] Diabetes mellitus (DM), commonly known as diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. Diabetes is the most common est and greatest healthcare problem in modern global world. Untreated diabetes can cause many disorders like diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long term complications include cardiovascular disorder, stroke, chronic kidney disease, foot ulcers, damage to nerves, damage to the eyes and congnitive impairments.[2] Prevalence of diabetes mellitus in children and adult is more of concern. Diabetes can cause huge number of medical complications including retinopathy, neuropathy, cardiovascular disorder and male infertility. Hyperglycemia mediated oxidative stress and inflammation plays a vital role in the adverse effects of diabetic complications. Pharmacotherapy are either too costly and have serious side effects, WHO recommended the effects of medicinal plants possessing high success rate in hypoglycemic activity which may provide a utilizable source of a new oral antidiabetic drug[3]. *Cissus quadrangularis* plant belongs to the *vitaceae* family, and is a widely distributed throughout, India. In Ayurveda medicinal system different parts of this plants have been used as a appetizer, many skin diseases, bone healer, anemia, asthma, piles, irregular menstrual cycle and wounds[4]. CQ have the ability to accelerate the healing of bone fracture and swelling[5]. Cissus quadrangularis extract have active phytoconstituents properties such as carbohydrates, proteins, flavonoids, saponins, tannins, glycosides, phenolics, steroids, alkaloids, and terpenoids. This phytoconstituents have more antioxidant and anti-inflammatory activitie[6]. The stem of this plant have been used as anticancer, antifungal, antiviral and anti-oxidant. Due to this more anti-oxidant property, this plant quickly to heal wounds and anti hyperglycemic property will reduce the blood glucose levels[7].

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Materials and Methods

Chemicals

Streptozotocin (STZ) were purchased from Himedia company and ponmani and co,coimboture. Totally 200 mg STZ were purchased and used for this study.

Collection of plant sample

Cissus quadrangularis stem were collected from in and around Tamilnadu. During the period of Auguest to November 2019. The plant is identified and authendicated by agricultural university,coimbatore. The voucher no. BSI/SRC/5/23/2020/Tech/799.

Extraction of *cissus quadrangularis*

Freshly collected stems were cut into small pieces and washed with saline water and air dried under the shade. The dried stems were grinded and powdered. The powdered materials was mixed with ethanol (5g/100ml) the mixer was incubated overnight at room temperature. The plant extract in ethanol was filtered through a whatsman filter paper no.1. the filtrate was evaporated to dryness at low temperature (>40°c) under reduced pressure in a rotary evaporator. During the experimental periods, the stored plant extract was used by dissolving in distilled water with the required amount. The dried extract were stored in glass container for this study purpose[8].

Experimental Animals

Adult male wister albino rats weighting between 250-350 gms were used for this study. 60 days old adult rats were used for this experiment. Totally 36 animals were divided into 6 groups. Each group contains 6 animals. All animals were housed in animal house at erode college of pharmacy, with proper temperature and humidity. All the animals care was taken as per guidelines of the Institutional Animals Ethics Committee (IAEC).[9] The ethical committee approved no. KSRCT/BT/IAEC/2018/37. Animal house contain proper facility to conducting the animal study.

Induction of Diabetes

A single intraperitoneal injection of streptozotocin (STZ) were administrated to rats. The STZ dosage is 40 mg/kg body weight in 0.1 m citrate buffer given. The animals were administrated orally 10% glucose solution overnight to overcome the drug-induced hypoglycemia[10]. After 72 hours, blood samples were collected from tail. The blood glucose levels were estimated by using accucheck, to confirm the diabetes. The blood glucose level ranging between 200-400 mg/dl were consider the diabetic model. This experimental diabetic model rats were used for this study.

Experimental Design

Group I - control with normal saline
Group II - STZ induced with saline
Group III - STZ induced treated with metformin
Group IV - STZ induced treated with CQ (200 mg/kg/bw)
Group V - STZ induced treated with CQ (400 mg/kg/bw)
Group VI - STZ induced treated with CQ (800 mg/kg/bw)

Twenty-four hours after induction of STZ the animals were feeded with standard pellet diet. From next day onwards the experimental diabetic groups (IV, V, VI) were received 200, 400 & 800 mgs of ethanol extracts of CQ by using oral cavange, once in a day. Group III were treated with metformin drug. Blood samples were taken 7, 14, 21, 28th days during the experimental period to check the glucose level. After 60 days of treatment the animals were sacrificed by euthanasia method. Blood and tissues were collected for various experimental trials.

Biochemical parameters

Blood were collected from experimental rats by retro orbital plexus technique. Heparinised capillary tubes were used to collect the blood samples. The blood samples were analyzed for glucose levels.

Results

Phytochemical Analysis

Ethanol extract of *cissus quadrangularis* shows various phytochemical constituents like aminoacids, proteins, glycosides, saponins, tannins, carbohydrates, quercetin, lignings, phenolics, flavanoids, etc. phytoconstituents present in ethanol extracts in *cissus quadrangularis* are shown in Table 1.

Table 1. Phytochemical study of ethanol extracts of *cissus quadrangularis*.

S.NO.	Phytoconstituents	Ethanol extract of CQ
1.	Alkaliods	+
2.	Proteins	+ +
3.	Carbohydrates	_
4.	Phenolics	+ + +
5.	Flavonoids	+ + +
6.	Tannins	+
7.	Saponins	+ +
8.	Lignins	+
9.	Quercetin	+ + +

Present: (+); Absent: (-)

Effect of eecq on blood glucose& body weight:

Body weight and blood glucose levels of all groups are shown in Tables 2 and 3 and Figures 1 and 2. Administration of ethanol stem extract of *cissus quadrangularis* in diabetic rats reduced the blood glucose levels and significant ($p < 0.05$) effect was

observed at dose 400, 800mg/kg compared with diabetic untreated rats (Table 3 and Figure 2). Diabetic rats treated with standard drug metformin also significantly ($p < 0.05$) decreased the elevated blood glucose levels. The significant effect was observed at dose 400 mg/kg with ethanol stem extract of *cissus quadrangularis* (Table 2 and Figure 1). Diabetic rats showed marked decrease in body weight, when compare with the rats treated with ethanol stem extract of *cissus quadrangualris* restored the decrease in body weight to normal.

Plasma insulin

After the experiment, the diabetic rats showed significant reduction in plasma insulin level (18.13±3.87) and compared with control and metformin treated rats (27.76±0.98). *Cissus quadrangularis* treated rats shows highly significant values in plasma insulin level at dose of 400mg/kg/bw (24.17±9.54), 800mg/kg/bw (27.33±8.52**) to near normal value (Figure 3).

Table 2. Effect of eecq on body weight.

Weeks	BODY WEIGHT (gm/rat)					
	Control	STZ Control	Metformin	EECQ 200ml/kg/body weight	EECQ 400mg/kg/body weight	EECQ 800mg/kg/body weight
1st week	188.67±87.09	211.98±87	220.67.98±80	163±15.09	193.44±98.70	193.98±98
2nd week	192.09±32	211.87±34	227.12±32	163.09±23.00	195.98±23.98	203.98±21
3nd week	200.54±10	250.98±9.65	230.32±65.91	179.71±00	208.33±7.98	228.63±60.92
4th week	225.67±5.76	199±67.32	239.90±76	200.89±98.00	239.87±90	249.87±00
5th week	245.09±00	188.01±7.54	241.54±11	212.98±99.87	254.76±00	249.98±98.00
6th week	267.76	181.67±7.09	278.98±12	226.87±76	267.23±76	265.87±09

STZ – Streptozotocin; EECQ - Ethanol Extract of *Cissus Quadrangularis*
All values from the table no. 1.2 are expressed as mean ± SEM for n= 6 animals. Significance was determined by one way ANOVA followed by Dunnett's test. *p <0.05 compared with control group, # p < 0.05 compared with STZ control.

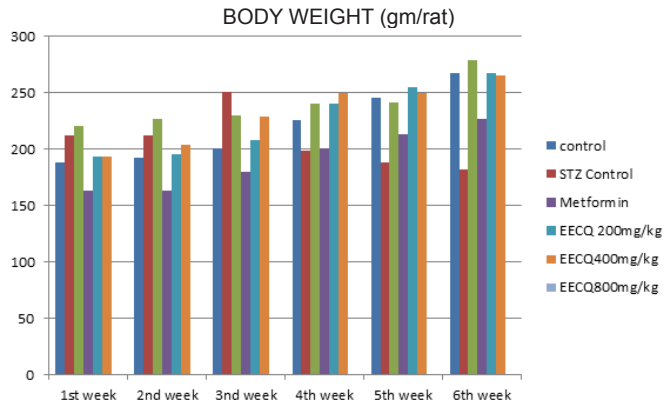

Figure 2. Fasting blood glucose levels of control, STZ- induced diabetic rats, metformin treated rats and extracts of CQ treated rats where shown in figure 1.5. CQ treated groups show significant values when compare with STZ- induced diabetic rats. The standard drug(matformin) treated group also show significant values. All values were analyzed by standard deviation by using one way ANNOVA methods.

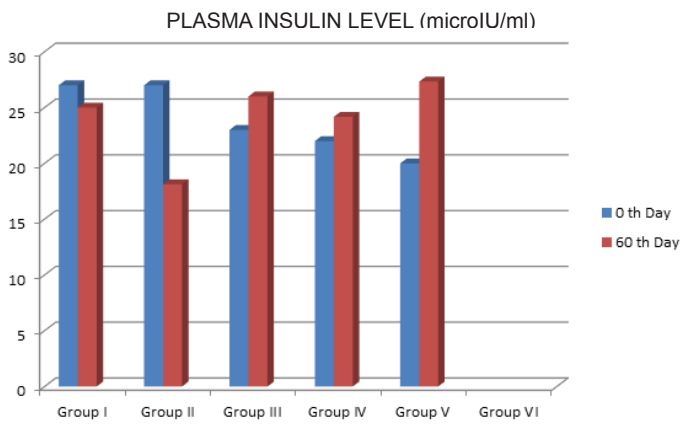

Figure 3. Effects of EECQ treated on plasma insulin values of control and diabetic and metformin treated rats, Mean± standard values of all groups were shown in figure. The plasma insulin values significantly high in EECQ to compare with untreated diabetic groups.

Table 3. Effects of eecq on blood glucose level.

Groups	Fasting blood glucose level ml/dl					
	Day 0th	Day 7th	Day 15th	Day 30th	Day 45th	Day60 th
I	91.83±24.95	97±14.44	97.67±10.11	99.67±12.09	98.5±15.42	98.5±15.32
II	397.33±87.567	405.47±69.43	421.00±76.21	436.81±41.76	451.32±078	465.32±09**
III	375.5±65.987	176.5±87.098	103.83±23.08	89.09±33.44	75.59±535	72.98±23.57
IV	282.42±75	267.98±54.567	234±23.56	209.86±30	198.43±74	178.23±778
V	294.90±24.23	259.79±42.78	232.89±89.09	199.67±89.09	167.12±87	141.33±98.9
VI	304.67±87.43	279.87±98.00	255.67±56.9	205.87±12.13	198.87±34.00	157.17±9.80

Data's are described as Mean ± S.D values of all groups, *P lesser than 0.01, **P lesser than 0.001, P greater than 0.05 ns- non significance; Ethanol extracts of *cissus quadrangularis* group were compare with drug treated group and control, 1st Group- control, 2nd Group – diabetic induced with streptozotocin (45mg/kg/body weight), 3nd Group – Diabetic treated with metformin (7.5 mg/kg/body weight), 4th Group- Diabetic treated with EECQ(200mg/kg/body weight), 5th group – Diabetic treated with (400 mg/kg bodyweight) of EECQ, 6th Group – Diabetic treated with (800 mg/kg/bodyweight) of EECQ.

Histopathology of pancreas

Figure 4 shows the morphology of the pancreas in the different experimental groups, in parafin sections stained with hematoxilin and eosin.

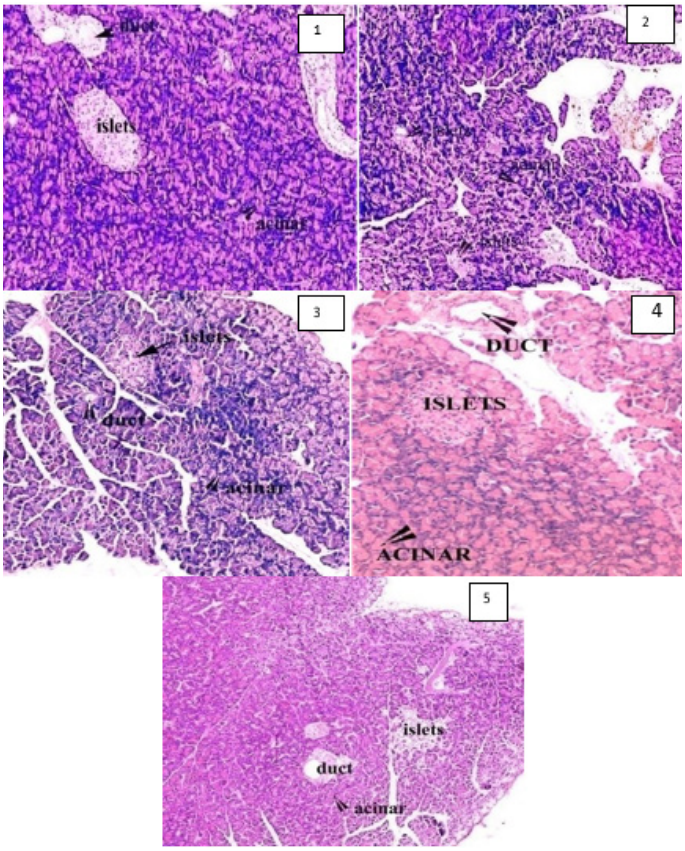

Figure 4. 60 Days treatment of Pancreatic tissues stained with H&E: 1 (control group) - shows normal; 2 (Diabetic group) - shows damaged islets with necrotic stage; 3 (Metformin treated group) - shows normal architecture of islets with nomal cellular density; 4 (*Cissus quadrangularis* 400 mg/kg treated group)- shows increased cellular density in islets; 5 (*Cissus quadrangularis* 800 mg/kg treated group) - shows islets shape altered with high cellular density.

Discussion

The *cissus quadrangularis* posses variable important phytoconstituents such as carbohydrates, proteins, flavonoids, glycosides, quercetin, lignings, polysaccharides, etc. polysaccharides are one of the most important compounds, which is responsible for the glucose and insulin lowering effects[11]. The plant extract contain huge amount of phenolic compounds variety of quercetin. This quercetin stimulates insulin secretion in beta cells by closure of K^+- ATP channels, membrane depolarization and stimulate the Ca^{2+} influx, its starting stage of insulin protection from β cells or from newly formed β cells(R.K. Lekshmi, *et al*,. 2015). According to Kianifard *et al*., 2011 – experimentally induced diabetic rats not able to produce β cells in the pancreatic tissues and do not lead to a complete lack of β cell population. Adminstration of *cissus quadrangularis* extracts significantly decrease the elevated fasting blood glucose levels in experimentally induced diabetic rats and also increase the plasma insulin level in blood[13], also restore the bodyweight of animals due to polypegia. According to srinivasa Rao the petroleum ether fraction of *cissus quadrangularis* showed no effects against the high blood glucose levels[14]. Histopathology of pancreas also shows huge distortion in pancreatic tissues in diabetic group compare with *cissus quadrangularis* treated group. Damaged islets were noted in diabetic group, *cissus quadrangularis* group shows normal architecture of islets. The present study revealed the ethanol extract of cissus quadranularis posses powerful phytoconstituents like quertein and flavoniod groups. These compounds are responsible for the reduced blood glucose level in STZ induced diabetic rats.

Conclusion

Our present study revealed that ethonalic extracts of *cissus quadrangularis* supplementation have anti hyperglycemic activity against stz induced diabetic rats. The weight of animals were also decreased in diabetic rats. Pronounced activity where noted at the doses of 400mg/kg and 800 mg/kg body weight. So this study concluded the EECQ may be effective in preventing hyperglycemia. Further study are necessary to findout the proper pathway of mechanism.

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