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ANTIDIABETIC EVALUATION OF VARIOUS POLAR & NON-POLAR EXTRACTS OF *CORCHORUS TRILOCULARIS* LINN. IN STREPTOZOTOCIN INDUCED DIABETIC ANIMALS

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ABSTRACT

Aim of Study*-The study was designed to investigate the antidiabetic activity of Corchorus trilocularis Linn. In Streptozotocin Induced Diabetic Animals.* ***Material & Methods****-The leaves were extracted by using petroleum ether, dichloromethane, ethyl acetate, ethanol, butanol and water. All the extracts were screened for the presence of various phytoconstituents i.e. fatty acids, alkaloids, flavonoids, terpenoids, steroids, glycosides, protein etc. Diabetes was induced by a single intraperitoneal injection of streptozotocin (40 mg/kg). The test extracts were given from day zero to 21st day and simultaneously blood glucose level was estimated. Oral glucose tolerance test was also performed in normal animals.* ***Results****- In 21-day study glibenclamide the standard drug restored the blood glucose highly significantly with the p<0.001 in 14 days whereas dichloromethane extract (200 & 400 mg/kg) reduced the glucose level moderately and highly significant with p<0.01 & p<0.001. In OGTT, Dichloromethane extracts at the dosage levels of 200 & 400mg/kg body weight shown highly significant effect (p<0.001) hypoglycemic effect in overnight fasted normal rats at 270 minutes.* ***Conclusion****- The antidiabetic effect Corchorus trilocularis may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration.*

KEY WORDS

Streptozotocin, Diabetic animals, Polar and Non-polar, Corchorus trilocularis Linn. OGTT

INTRODUCTION

Diabetes is defined as a state in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated as a consequence of a relative or absolute deficiency of insulin secretion, resistance to insulin action or both at one or more points in the complex pathways of hormone action. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis doesn't return to normalcy and continuous for a protracted period of time, it leads to hyperglycemia that is due course turns into a syndrome called diabetes mellitus (Sangal, 2011).

Various therapies are now available to enhance insulin secretion, decrease hepatic glucose output, and increase peripheral insulin sensitivity. However, they are associated with various disadvantages i.e. weight gain, hyponatremia and syndrome of inappropriate antidiuretic hormone (SIADH) are common side effect in 16 weeks treatment (Boavida *et al*., 2007, Sabu and Kuttan, 2004).

Traditional medicines readily available from medicinal plants offer great potential for the discovery of new antidiabetic drugs. As per the literature review, it has been observed that *Corchorus trilocularis* Linn. is listed among the various medicinal plants widely been used as

a antibacterial, demulcent, bitter tonic, laxative, carminative, refrigerant, and febrifuge, diuretic, useful in chronic cystitis, gonorrhea and cadiotonic, acute-chronic inflammatory conditions and in treatment of diabetes mellitus (Kritikar and Basu, 1935).

In the absence of any scientific evidence for their anti-diabetic activity in diabetic animals, there is a need in scientifically establishing the anti-diabetic activity in diabetic animals, so that we are able to come up with a more effective and potent bioactive extract or phytoconstituents with fewer side effects in comparison with existing synthetic drugs (Khan *et al*., 2006).

MATERIALS & METHODS

Collection and authentication of the plant leaves

The leaves of *Corchorus trilocularis* were collected from outfield Medicinal garden during the month of July that shows the green color with rough surface. The plant leaves were washed thoroughly in tap water, dried in shade, finely powdered and used for successive extraction methods. Plants were identified by senior botanist.

Successive Solvent Extraction Methods

The dried leaves of *Corchorus trilocularis* were powdered and a fixed quantity of powdered drug was defatted by using petroleum ether. Then dried mark was again extracted by using dichloromethane, ethyl acetate, ethanol, butanol and finally with water. All the extracts were dried & % Yield of the Petroleum ether, Dichloromethane, Ethyl acetate, Ethanol, Butanol, & Aqueous extract of *Corchorus trilocularis* was calculated (Mukherjee, 2002; Kokate, 1996).

Phytochemical Screening

All the extracts were screened for the presence of various phytoconstituents i.e. fatty acids, alkaloids, flavonoids, terpenoids, steroids, glycosides, protein etc (Kokate, 1996).

Evaluation of Antidiabetic Activity

Acute toxicity determination

Acute oral toxicity test was carried out according to the OECD guideline No. 423. A total of three animals (Wistar albino rats) were used, which received a single oral dose in 2000 mg/kg, body weight of different extracts. The animals were observed for a period of 24 hr for the changes in behavior, hypersensitivity reactions etc. Mortality, if any, was determined over a period of 2 weeks. Hence in our studies we selected 1/10 and 1/5th dose i.e. 200 and 400 mg/kg dose (OECD, 2001).

Preparation of Doses

Doses equivalent to 200 and 400 mg/kg of the crude drug body weight were calculated and suspended in 1% w/v Tween 80 solutions for the experiment.

Streptozotocin (STZ) induced diabetes in rats

After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40 mg/kg Streptozocin (Sigma, St. Louis, Mo, USA), freshly dissolved in citrate buffer (pH 4.5). After injection, the rats had free access to food and water. The diabetes was confirmed by estimating the blood glucose level after 3 days by glucometer based on glucose oxidation method. Rats having blood glucose level more than 250 mg/dl were selected for further study (Bhatia *et al*., 2011).

Treatment Schedule

All the animals were divided into fifteen groups. Group I & II served as normal and diabetic control, group XV served as standard drug treated and group III to XIV served as test extracted treated of *Corchorus trilocularis* in a dose of 200 & 400 mg/kg. The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes.

Oral Glucose Tolerance Test (OGTT) of extracts showing best activity in STZ induced diabetes

Experimental animals were divided into four groups. Group I served as control (0.5% w/v CMC, 5ml/kg BW), group II served as standard (glibenclamide 5 mg/kg BW) and group III & IV treated by dichloromethane extract of *Corchorus trilocularis* in dose of 200 & 400 mg/kg by oral route. Test samples and standard were given immediately after the collection of initial blood samples. The blood glucose levels had been determined in the subsequent model: 0 min. and 30 min to access the effect of test samples on normoglycaemic rats. The rats were then loaded orally with 2g/kg glucose and the glucose levels were determined at 120, 150 and 270 min after glucose load. Blood was collected from the tip of the end vein and fasting blood glucose level was calculated utilizing single touch glucometer which was designed based on the glucose oxidase technique (Badole *et al*., 2006; Choudhari *et al*., 2012).

Statistical analysis

The values are expressed in mean ± SEM. The results were analyzed by using one-way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. $P < 0.05$ was chosen as the level

of significance. Statistical analysis was performed using Graph Pad Prism Software 5.0 version.

RESULTS

Phytochemical Screening

Phytochemical screening of different extracts showed the presence of different phytochemical.

Acute Toxicity Studies of Plant Extracts

No toxic effects were observed at a higher dose of 2000 mg/kg body weight of Albino Wistar rats. Hence, 1/ 10th dose was selected as effective dose or therapeutic dose. The cut off value of 200 and 1/5 dose double of 400 mg/kg were selected for anti-diabetic activity.

Streptozotocin induced antidiabetic activity of *Corchorus trilocularis*

Effect on Blood glucose level

The induction of diabetes with streptozotocin increases the blood glucose level significantly ($p<0.001$) in group II rats as compared to normal rats. In 21-day study glibenclamide the standard drug restored the blood glucose highly significantly with the $p<0.001$ in 14 days whereas dichloromethane extract (200 & 400 mg/kg) reduced the glucose level moderately and highly significant with $p<0.01$ & $p<0.001$. Petroleum ether, ethyl acetate, ethanolic and butanolic extracts had moderately significant effects ($p<0.01$) on 14th and 21st days. However, aqueous extracts showed significant effect ($p<0.05$) in glucose levels. The effect of aqueous extract is very less as compared to other extracts. The results are shown in table no.1.

Table No. 1: Effect of different extracts on glucose level in streptozotocin induced diabetic rats

Group No	Group	Blood Sugar level				
		Long Term Study (Days)				
		Before inducing Diabetes	3	7	14	21
I	Normal control	80.3 ± 0.46	82.2 ± 0.17	81.4 ± 1.7	81.9 ± 0.57	80.11 ± 0.18
II	Diabetic control	82.4 ±0.81	241.7 ± 1.89	274.8 ± 1.43***	267.3 ±3.07***	290.1 ± 0.24***
III	Pet. Ether extract (200 mg/kg)	79.4 ± 0.92	241.6 ± 1.44	238.9 ± 2.11**	233.8 ± 2.98**	225.6 ± 0.20**
IV	Pet. Ether extract (400 mg/kg)	83.77 ± 1.08	243.4 ± 3.04	222.3 ± 2.89**	216.8 ± 3.09**	204.4 ± 0.49**
V	Dichloromethane extract (200 mg/kg)	84.27 ± 1.09	244.4 ± 3.05	216.2 ± 2.89***	202.8 ± 3.08***	195.2 ± 0.29***
VI	Dichloromethane extract (400 mg/kg)	87.78 ± 1.09	245.6 ± 3.09	208.2 ± 2.79***	192.6 ± 3.02***	172.3± 0.82***
VII	Ethyl acetate extract (200 mg/kg)	78.4 ± 0.89	243.6 ± 1.39	263.9 ± 2.09	254.6 ± 2.88*	255.6 ± 0.20*
VIII	Ethyl acetate extract (400 mg/kg)	79.4 ± 0.92	244.5 ± 1.38	259.9 ± 2.18	250.8 ± 2.94*	248.6 ± 0.20*
IX	Ethanolic extract (200 mg/kg)	79.4 ± 0.92	240.7 ± 1.69	225.3 ± 1.41	219.3 ±3.09**	217.1 ± 0.34**
X	Ethanolic extract (400 mg/kg)	80.3 ± 0.82	242.6 ± 1.42	222.9 ± 2.19	216.8 ± 2.88**	214.6 ± 2.30**
XI	Butanolic extract (200 mg/kg)	81.4 ± 0.84	243.6 ± 1.46	223.9 ± 2.19	216.8 ± 2.99**	214.6 ± 1.80**
XII	Butanolic extract (400 mg/kg)	79.4 ± 0.22	244.6 ± 1.39	221.2 ± 2.18	213.8 ± 2.88**	213.6 ± 3.20**
XIII	Aqueous extract (200 mg/kg)	82.4 ±0.91	240.7 ± 1.49	270.8 ± 1.33	265.3 ±3.12*	260.1 ± 0.34*
XIV	Aqueous extract (400 mg/kg)	83.4 ±0.81	241.7 ± 1.89	269.2± 1.33	260.3 ±3.11*	258.1 ± 1.35*
XV	Glibenclamide (5 mg/kg)	83.25 ± 0.97	244.8 ± 2.54	199.4 ± 3.49**	169.3±2.77***	160.8 ± 0.24***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups

Oral Glucose Tolerance Test of Dichloromethane extract showing best activity in STZ induced diabetes model

Dichloromethane extracts at the dosage levels of 200 & 400mg/kg body weight shown highly significant effect ($p<0.001$) hypoglycemic effect in overnight fasted normal rats at 270 minutes. Dichloromethane extracts also shown moderately significant effect ($p<0.01$) on glucose level at 150 minutes. Standard drug i.e. glibenclamide also shown moderately significant activity in treated rats as compared to normal rats. The results are summarized in Table No. 2.

Table No. 2: Effect of dichloromethane extracts on glucose tolerance test in normal rats

Gr.	Test sample (mg/kg)	Blood glucose levels (mg/dL)					
		0 min	30 min	60min (glucose load)	120min	150min	270min
I	Normal Control	81.43±3.70	81.80±1.31	81.38±2.96	184.10±4.12	134.05±3.49	87.26±3.60
II	Standard	82.54±3.17	70.21±3.90**	56.59±1.14***	140.18±36.20**	93.98±4.14***	66.28±1.15***
III	Dichloromethane extract of *Corchorus trilocularis* 200 mg/kg	80.23±2.70	71.20±2.11**	67.48±1.96***	160.30±4.32	110.04±4.49**	89.16±3.60***
IV	Dichloromethane extract of *Corchorus trilocularis* 400 mg/kg	81.33±5.70	64.30±1.61**	60.38±3.52***	158.20±4.12	101.05±2.43***	80.21±2.10***

DISCUSSION

Predictably, insulin dependent diabetes is treated with exogenous insulin and noninsulin dependent diabetes with synthetic oral hypoglycemic agents like sulphonylureas and biguanides. However, hormone fails as a curative agent for complications of diabetes and the major drawbacks of insulin therapy are the side effects like insulin allergy, lipodystrophy and lipoatropy, insulin antibodies, altered metabolic control, autoimmunity and other late complications like morphological changes in kidneys and severe vascular complications. Similarly, oral hypoglycemic drugs have many side effects such as nausea, vomiting, cholestatic jaundice, aplastic and hemolytic anemia's, generalized allergic reactions, dermatological reaction etc (Mallick *et al*., 2007; Pepato *et al*., 2005).

The research was envisaged for antidiabetic activity of different extracts of *Corchorus trilocularis* procured by successive extraction methods. All extracts has been evaluated by STZ induced diabetes. Preliminary phytochemical evaluation of different extracts illustrates that petroleum ether extract showed the existence of triterpenoids, steroids and fatty acids, dichloromethane extract showed presence of saponins, phytosterols, flavonoids, phenols, steroids, terpenoids ethanolic extract showed the presence of alkaloids, flavonoids and glycosides and aqueous extract showed the presence of carbohydrates, as phytoconstituents.

The toxicity studies was determined by OECD guidelines 423.Based on the LD50 value, 1/5th and 1/10th (200 & 400 mg/kg) of its value was chosen for pharmacological studies.

The levels of antioxidant defense structure are altered in streptozotocin-induced diabetic rats, which are in good correlation with the present observation. Non-protein thiols like glutathione are one of the important primary defenses that counteract the oxidative stress. Decreased levels of serum glutathione in streptozotocin diabetic rats, which is in consistent with earlier reports (Cai *et al*., 2005). The observed decrease may be due to utilization of non-protein thiols by increased oxygen-free radicals produced in hyperglycemic conditions associated with diabetes mellitus.

The dichloromethane extract of *Corchorus trilocularis* produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin-diabetic rats after 21 days treatment. The antidiabetic effect *Corchorus trilocularis* may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration.

Dichloromethane extract showed the presence of triterpenoids and steroidal compounds. From the previous reported literature, triterpenoids, flavonoids and phenolic compounds are responsible for anti-diabetic effect. So probably, antidiabetic effect of plants may be due to presence of terpenoids and steroidal **(**Stahl, 1969**)**.

The dichloromethane extract shows significant enhancement in glucose tolerance in glucose fed hyperglycemic normal rats. A single dosage of two levels of dichloromethane extract shows significant hypoglycemic action in streptozotocin- induced hyperglycemic rats. During our previous research work phytochemical investigations revealed presence of terpenoids and sterol that illustrates their strong antioxidant properties and this chemical composition of plant may also be the reason for its hypoglycemic activity. An immense reservoir of biologically active substances with different chemical structures and illness preventive properties is the one and only plant kingdom (Chandiran *et al*., 2014; Pacifici *et al*., 1992). For this reason, herbal drugs have actually gotten greater interest as an alternative to allopathic medication and the need for these herbal treatments features significantly increased recently.

Dichloromethane extract also showed a highly significant effect in glucose tolerance test and antidiabetic effect may be due to independent of insulin release.

The present findings are significant for the development of alternative, inexpensive and perhaps safer strategies for the treatment of diseases.

CONCLUSION

In present study, the dichloromethane extract of *Corchorus trilocularis* produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin-diabetic rats after 21 days treatment. The antidiabetic effect *Corchorus trilocularis* may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration.

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