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Open Access **Research Article**

Evaluation of anti-diabetic activity of different extracts of *Corchorus trilocularis* in STZ induced diabetes in rats

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Abstract

Aim- The main aim of the present study is to evaluate the anti-diabetic study of different extracts of *Corchorus trilocularis* in STZ induced diabetes in rats. **Material and Methods-** Powdered drug 500 gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Complete defatting was ensured by placing a drop form the thimble on a filter paper give any oily spot. The mark was dried in air to remove traces of petroleum ether. Defatted mark was subjected to extraction with ethyl acetate in soxhlet apparatus and then again extracted by using ethanol, butanol and finally by using water. Acute oral toxicity test was carried out according to the OECD guideline No. 423. After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40 mg/kg Streptozocin, freshly dissolved in citrate buffer (pH 4.5). Then serum samples were also used to analyze for serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) and atherogenic index (AI). **Results-** Petroleum ether, 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. **Conclusion-** The present investigation comprises of the phytochemical and pharmacological investigations of leaves of *Corchorus trilocularis* for the antidiabetic activity.

Keywords- Anti-diabetic, Different extracts, *Corchorus trilocularis,* STZ induced diabetes, (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C)

INTRODUCTION

Diabetes mellitus was known to ancient Indian physicians as 'madumeha' (Ali *et al*., 2009). 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).

In 2005 WHO and International diabetic federation met in Geneva and published the new WHO guidelines. Diabetes is diagnosed when the fasting plasma glucose exceeds 7.0 mmol/l (126 mg/dl) and /or the value 2 hours after the glucose load of 75gm exceeds11.1mmol/l (200/dl). The WHO criteria recognize an intermediate zone of abnormal blood glucose levels called "impaired glucose tolerance (IGT)" – often regarded as kind of borderline diabetes. IGT is diagnosed when the 2- hour glucose value is 7.8-11.1 mmol/l (140-200mg/dl).

In the absence of any scientific evidence of *Corchorus trilocularis* 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 with fewer side effects in comparison with existing synthetic drugs (Khan *et al*., 2006).

MATERIAL AND METHODS

Collection and authentication of the plant leaves:

The leaves of *Corchorus trilocularis* were collected from outfield Medicinal garden of local area, India, during the month of August and September 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.

Successive extraction methods:

The plant leaves is dried in shade at room temperature & after 4-5 days, it is formed in powder by mixer grinder. Powdered drug 500 gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Complete defatting was ensured by placing a drop form the thimble on a filter paper give any oily spot. The mark was dried in air to remove traces of petroleum ether. Defatted mark was subjected to extraction with ethyl acetate in soxhlet apparatus and then again extracted by using ethanol, butanol and finally by using water. The extraction was completed in

17-18 cycles. All the extracts were dried at room temperature & stored at dark place in air tight container.

The % Yield of the Petroleum ether, Ethyl acetate, Ethanol, Butanol, & Aqueous extract of *Corchorus trilocularis* were calculated by using the following formula.

$$\% \text{ Yield} = \frac{\text{Net weight of extract in gram}}{\text{Total weight of leaf powder in gram taken for extraction}} \times 100$$

Evaluation of Anti-diabetic activity of different extracts

A. *Experimental Animals*

Wistar Albino rats of either sex (150 to 200 g) were purchased from the CPCSEA approved vendor New Delhi. They were maintained under standard laboratory conditions at 25 ± 2°C and normal 12-hour light-dark cycle were used for the experiment. Commercial pellet diet (MFD, by Nav Maharashtra Chakan Oil Mills ltd., New Delhi, India) and water were provided *ad libitum* throughout the course of study.

B. *Selection of Dose (Acute Toxicity Studies)*

Acute oral toxicity test was carried out according to the OECD guideline No. 423. A total of three animals 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.

C. *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.

D. *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 (Koyuturk *et al*., 2005).

E. *Experimental Design*

In order to assess the anti-diabetic activity of *Corchorus trilocularis* the animals were divided in thirteen groups. Each group contains six animals.

- ✓ Group 1: Normal control, 0.9% NaCl-treated animals
- ✓ Group 2: Diabetic control, STZ -treated rats (40 mg/kg body weight)
- ✓ Group 3: Treated with Pet. Ether extract of *Corchorus trilocularis* (200 mg/kg body weight)
- ✓ Group 4: Treated with Pet. Ether extract of *Corchorus trilocularis* (400 mg/kg body weight)
- ✓ Group 5: Treated with ethyl acetate extract of *Corchorus trilocularis* (200 mg/kg body weight)
- ✓ Group 6: Treated with ethyl acetate extract of *Corchorus trilocularis* (400 mg/kg body weight)
- ✓ Group 7: Treated with ethanolic extract of *Corchorus trilocularis* (200 mg/kg body weight)
- ✓ Group 8: Treated with ethanolic extract of *Corchorus trilocularis* (400 mg/kg body weight)
- ✓ Group 9: Treated with butanolic extract of *Corchorus trilocularis* (200 mg/kg body weight)
- ✓ Group 10: Treated with butanolic extract of *Corchorus trilocularis* (400 mg/kg body weight)
- ✓ Group 11: Treated with aqueous extract of *Corchorus trilocularis* (200 mg/kg body weight)
- ✓ Group 12: Treated with aqueous extract of *Corchorus trilocularis* (400 mg/kg body weight)
- ✓ Group 13: Standard drug, Glibenclamide-treated rats (5 mg/kg body weight)

The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes.

At the end of the experimental period, after an overnight fasting, the rats were anaesthetized with ketamine hydrochloride (30 mg/kg b.w.) and sacrificed by cervical decapitation. Blood samples were collected in heparinized tubes, after 60min rest in the supine position, and then centrifuged at 160×g for 10min at 20°C. Liver, kidney and pancreas were quickly dissected out after euthanasia, which were then carefully cleaned, weighed accurately and stored at 80°C until used.

Then serum samples were also used to analyze for serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) and atherogenic index (AI).

Evaluation of Biochemical Parameters

A. *Estimation of plasma glucose*

Glucose content was estimated by the method of Trinder (1969) using a diagnostic Kit (Sigma Diagnostics Pvt. Ltd., Baroda, India). 0.01mL of plasma, standard and distilled water (blank) were taken in three separate tubes, 1mL of the enzyme reagent was added to each tube, mixed and kept at 37°C for 15min. The colour developed was read at 510 nm against a reagent blank. Values are expressed in mg/dL plasma.

B. *Estimation of Serum total cholesterol (TC)*

This method was used for the estimation of serum cholesterol. In this method the following were pipette into the reaction vessel using a micro pipette. Test samples (T): 0.02 ml serum, 2.00 ml reaction solution; the standard sample (S): 0.02ml standard and 2.00 ml reaction solution, while for the blank sample (B): 0.02 ml DW and 2.00ml reaction solution. The mixture was mixed well and incubated for 10 minutes at +20 to 25c or 5 minutes at 37c.The absorbance was read at 505/670 nm against the reagent blank

C. *Estimation of serum triglycerides (TG)*

GPO-PAP method was used to estimate the serum triglycerides. For this 0.01 ml of serum was taken in a test tube (T) in which 1ml reaction solution was added. In an another test tube (S) 0.01ml standard and 1ml reaction solution were added. The solution was mixed well and incubated at +20 to 25C for 10 min. The absorbance of standard and test against reagent blank was read at 505 (500-540 nm).

D. *Estimation of HDL-cholesterol*

CHOD-PAP method was used to estimate the serum HDL cholesterol level. CHOD-PAP method (Henry, 1974) was used to estimate the serum HDL cholesterol level. For this 2ml if

serum was taken in a test tube and 0.5 ml of precipitation reagent was added. The mixture was shaken thoroughly and left to stand for 10min at +15 to 25c and then centrifuged for 15min at 4000rpm. Within 2hr after centrifugation, the clear supernatant was used for the determination of HDL-C. One ml of the supernatant was taken in a test tube (T) and 1 ml of reaction solution was added to it. In an another test tube 0.1 ml DW was taken and 1ml reaction solution (B) was added. The mixtures were mixed thoroughly, incubated for 10min at 15-25 c or for 5min at 37c and measured the absorbance of the sample against reagent blank at 546 nm.

E. *Estimation of LDL cholesterol*

LDL cholesterol was estimated by using Friedwald's (1972) formula as follows:

$$\text{LDL in mg \%} = \text{total cholesterol-HDL-C-Triglyceride}$$

$$5$$

F. *Estimation of VLDL cholesterol*

VLDL cholesterol was estimated by using following formula

$$\text{VLDL in mg \%} = \frac{\text{Triglyceride}}{5}$$

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 & DISCUSSION

Acute Toxicity Studies of Plant Extracts

The cut off value of 200 and 1/5 dose double of 400 mg/kg were selected for anti-diabetic activity.

Table No. 1: Acute toxicity studies of plant extracts

S. No.	Treatment	Dose (mg/kg)	Number of animals	Mortality			Toxicity Profile
				After 24 hrs	After 7 days	After 14 days	
1	Petroleum ether extract	2000 Mg/kg	5	0	0	0	Safe
	Ethyl acetate extract	2000 Mg/kg	5	0	0	0	Safe
	Ethanolic extract	2000 Mg/kg	5	0	0	0	Safe
	Butanolic extract	2000 Mg/kg	5	0	0	0	Safe
	Water extract	2000 Mg/kg	5	0	0	0	Safe

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 ethyl acetate extract (200 & 400 mg/kg) reduced the glucose level moderately and highly significant with $p<0.01$ & $p<0.001$. Petroleum ether, 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 2.

Table No. 2: 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 ± 0.24	82.2 ± 0.44	81.4 ± 1.72	81.9 ± 0.33	80.11 ± 0.33
II	Diabetic control	82.4 ±0.22	241.7 ± 1.11	274.8 ± 1.55***	267.3 ±3.22 ***	290.1 ± 0.45***
III	Pet. Ether extract (200 mg/kg)	79.4 ± 0.45	241.6 ± 1.46	238.9 ± 2.11**	233.8 ± 2.44**	225.6 ± 0.67**
IV	Pet. Ether extract (400 mg/kg)	83.77 ± 1.18	243.4 ± 3.22	222.3 ± 2.45**	216.8 ± 3.22**	204.4 ± 0.11**
V	Ethyl acetate extract (200mg/kg)	84.27 ± 1.22	244.4 ± 3.11	216.2 ± 2.23***	202.8 ± 3.56***	195.2 ± 0.78***
VI	Ethyl acetate extract (400mg/kg)	87.78 ± 1.56	245.6 ± 3.45	208.2 ± 2.57***	192.6 ± 3.67***	172.3± 0.55***
VII	Ethanolic extract (200 mg/kg)	79.4 ± 0.22	240.7 ± 1.67	225.3 ± 1.34	219.3 ±3.29**	217.1 ± 0.33**

VIII	Ethanolic extract (400 mg/kg)	80.3 ± 0.68	242.6 ± 1.22	222.9 ± 2.66	216.8 ± 2.67**	214.6 ± 2.38**
IX	Butanolic extract (200 mg/kg)	81.4 ± 0.22	243.6 ± 1.76	223.9 ± 2.78	216.8 ± 2.34**	214.6 ± 1.23**
X	Butanolic extract (400 mg/kg)	79.4 ± 0.67	244.6 ± 1.78	221.2 ± 2.22	213.8 ± 2.68**	213.6 ± 3.78**
XI	Aqueous extract (200 mg/kg)	82.4 ±0.98	240.7 ± 1.88	270.8 ± 1.56	265.3 ±3.22*	260.1 ± 0.64*
XII	Aqueous extract (400 mg/kg)	83.4 ±0.43	241.7 ± 1.22	269.2± 1.78	260.3 ±3.11*	258.1 ± 1.78*
XIII	Glibernclamide (5 mg/kg)	83.25 ± 0.55	244.8 ± 2.65	199.4 ± 3.52**	169.3 ± 2.39***	160.8 ± 0.99***

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

Effect of ethyl acetate extract on different lipid level

Untreated diabetic rats showed significant hypercholesterolemia, hyper triglyceridemia, elevated LDL-Cholesterol, VLDL-Cholesterol and decrease in HDL – Cholesterol in comparison to that of normal group. Both compounds α-amyrin and Rosmarinic acid showed a very good effect on lipid profile. α-amyrin at the dose of 5 and 10 mg/kg showed highly significant (p<0.001) and moderately significant (p<0.01) effect on lipid profile in comparison to that of diabetic group. Rosmarinic acid also showed a highly significant effect on various lipids and also increased HDL level as compared to disease group or diabetic animals.

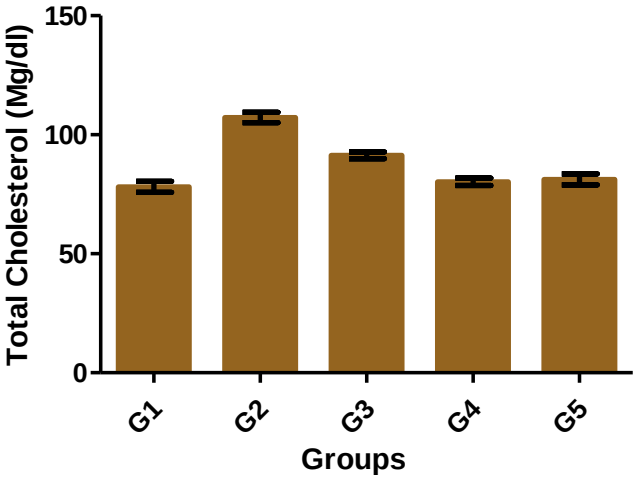

Figure 1: Effect of ethyl acetate extract on Total Cholesterol level

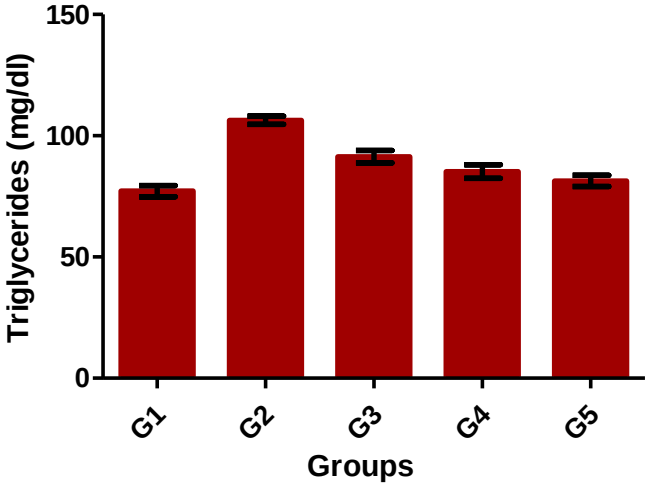

Figure 2: Effect of ethyl acetate extract on Triglyceride level

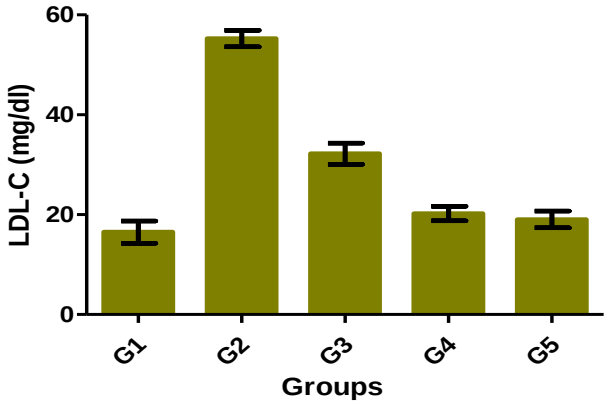

Figure 3: Effect of ethyl acetate extract on LDL-C level

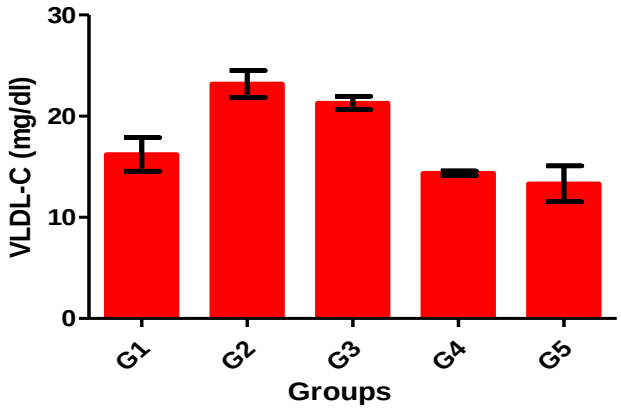

Figure 4: Effect of ethyl acetate extract on VLDL-C level

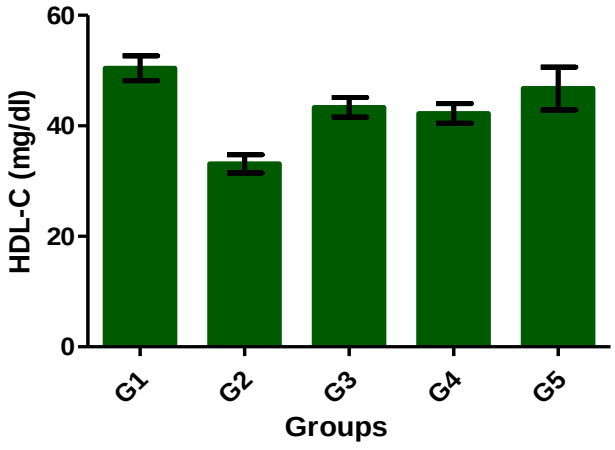

Figure 5: Effect of ethyl acetate extract on HDL-C level

In diabetes, the level of free radicals was reported to increase in alloxan and streptozocin treated rats an elevated level of free radicals was detected in several tissues including the kidneys (Shabeer *et al*., 2009).

Preliminary phytochemical evaluation of plant report illustrates that petroleum ether extract showed the existence of triterpenoids, steroids and fatty acids, ethyl acetate 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 no-observed- adverse-effect level was noticed at the dose of 2000mg/kg. 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 islet β-cells are vulnerable to damage caused by oxygen free radicals (Prince and Menon, 1998; Cai *et al*., 2005) since the antioxidant defense system is weak under diabetic condition. The levels of antioxidant defense structure are altered in streptozotocin-induced diabetic rats, which are in good correlation with the present observation.

The ethyl acetate 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.

Ethyl acetate extract showed the presence of triterpenoids, flavonoids, phenolic compounds and steroidal compounds. From the previous reported literature, triterpenoids, flavonoids and phenolic compounds are responsible for anti-diabetic effect. So probably, antidiabetic effect of plant may be due to presence of terpenoids and flavonoids.

The ethyl acetate shows significant enhancement in glucose tolerance in glucose fed hyperglycemic normal rats. A single dosage of two levels of ethyl acetate extract shows significant hypoglycemic action in streptozotocin induced hyperglycemic rats. During our previous research work phytochemical investigations revealed presence of flavonoids, terpenoids and sterols 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). Ethyl acetate extract also showed a highly significant effect in glucose tolerance test and antidiabetic effect may be due to independent of insulin release.

STZ-diabetic rats showed increase in plasma cholesterol and triglyceride concentrations (Sachdewa and Khemani, 2003), which may contribute to the development and progression of micro and macro-vascular complications (Tan *et al.,* 2005). Since insulin inhibits the sensitive lipases, the latter becomes active in the absence of insulin. Hence, attenuation of hyperglycemia or glycation of lipoproteins, enzymes and receptors involved in lipid metabolism can decrease the risk of cardiovascular death in diabetic patients.

Normally circulating LDL-C undergoes reuptake in the liver via specific receptors and gets cleared from the circulation (Lusis, 2000). HDL-C is protective by reversing cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogenic effects of oxidized LDL-C. The increased levels of LDL-C and VLDL-C decreases HDL-C as there is a reciprocal relationship between the concentration of VLDL-C and LDL-C. In diabetic rats treated with ethyl acetate extract showed an elevation in HDL-C and reduction in LDL-C and VLDL C. As there is a close relationship between the total cholesterol level of elevated plasma and the occurrence of atherosclerosis, the ability of ethyl acetate extract is reflected in the selective reduction of total cholesterol through the reduction of VLDL and LDL components. It could be beneficial in preventing atherosclerotic conditions, thereby reducing the possibility of coronary heart disease. It is therefore noteworthy that the effect of ethyl acetate extract on plasma HDL, clearly shows that the level of this lipoprotein fraction increased with α-amyrin and rosmarinic acid administration.

Cholesterol is a powerful risk factor for many coronary heart diseases (CHD). The degree of hypercholesterolemia is directly proportional to severity in diabetes. In the present study, we have observed higher levels of cholesterol in tissues of diabetic rats. The increased level of cholesterol in tissues could be due to the decreased level of HDL-cholesterol. Administration of ethyl acetate extract to STZ diabetic rats normalizes plasma levels of cholesterol due to the decrease in cholesterol absorption from the intestine, by binding with bile acids in the intestine and increasing bile acids excretion.

The increased concentration of free fatty acids was observed in liver and kidney of diabetic rats and this may be due to lipid breakdown and this may cause over production of NADPH, which results in the activation of NADPH dependent microsomal lipid peroxidation. Administration of ethyl acetate extract decreased the free fatty acid in the tissues of diabetic rats.

Several studies have reported that the increased phospholipid levels were seen in the tissues of diabetic rats. In diabetic rats, the elevated level of phospholipids may be due to the elevated levels of free fatty acids and the total cholesterol (Frayn, 1993). The levels of glycemic control and elevated levels of HDL cholesterol and decreased levels of triglycerides in the blood are significantly correlated with the phospholipids levels. The restoration of phospholipids by the administration of ethyl acetate extract may be due to controlled mobilization of plasma triglycerides; controlling the tissue metabolism and improving the level of insulin secretion and action presumably mediating cholesterol and phospholipids.

Several studies on different experimental models and on human cataract have now suggested that the level of various antioxidants in lens has important role in cataract development. But, a number of unresolved issues require further investigation.

CONCLUSION

The present investigation comprises of the phytochemical and pharmacological investigations of leaves of *Corchorus trilocularis* for the antidiabetic activity.

After performing the study we can summarize that the ethyl acetate and ethanolic extracts having potential antioxidant property and can restore the transparency of the crystalline lens by increasing the anti-oxidative enzyme levels. Ethyl acetate extracts exhibited potent anti-hyperglycemic, antioxidant effects in STZ induced type 2 diabetic rats. Ethyl acetate and ethanolic extracts decreases the increased glucose level probably by the insulin secretion, enhances insulin stimulated glucose uptake by glucose transporter and modulates the lipid and carbohydrate metabolism.

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