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EVALUATION OF ANTI-DIABETIC ACTIVITY OF *CORCHORUS TRILOCULARIS* LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT: The leaves of *Corchorus trilocularis* possesses several bioactivities and is used in traditional medicinal systems. However, its anti-diabetic activity has not been scientifically investigated so far. The present study was carried out to evaluate the anti-diabetic activity of chloroform extract extract of *Corchorus trilocularis* (family: Tiliaceae) leaves in Streptozotocin induced diabetic rats. Chloroform extract showed presence of saponins, phytosterols, flavonoids, phenols, steroids and terpenoids. From the toxicity study it was observed that chloroform extract of *Corchorus trilocularis* (CECT) was nontoxic up to the dose of 2000 mg/kg body weight. In this study, animals received continuous oral administration of CECT for a period of 21 days at the doses of 200 mg/kg and 400 mg/kg body weight. The effect of CECT was compared with oral dose of 5mg/kg Glibenclamide. The results showed that the CECT significantly lowered the blood sugar level of hyperglycemic rats in a dose dependent manner. CECT reduced glycosylated hemoglobin, lactate dehydrogenase and creatinine kinase levels in streptozotocin treated animals. The extract also ameliorated oxidative stress Parameters -TBARS, catalase and superoxide dismutase activity and glutathione content. In conclusion, the chloroform extract of *Corchorus trilocularis* leaves showed a significant anti-diabetic activity in streptozotocin induced diabetic rats possibly through increased secretion of insulin and the effect may be attributed to the presence of flavonoids and phenolic compounds present in extract.

INTRODUCTION: Diabetes mellitus is a major and growing public health problem throughout the world. According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetic patients worldwide. Recent estimates project that the number of patients diagnosed with Type II diabetes will more than double to 300 million before 2025.

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India has more than 30 million people with diabetics. It is estimated that by 2025, the number of diabetics will rise to 57 million in India, the highest number of diabetics in the world.[1, 2]

To control type II diabetes very low fat and lower glycemic index diet, regular exercise and weight control has evident potential but major proportion of population suffering from diabetes continue to eat whatever they want, avoiding exercise and stay fatty. Treatment of insulin dependent diabetes mellitus with insulin and non insulin dependent diabetes mellitus with synthetic oral hypoglycemic agents such as Sulphonylurea, Biguanides, thiazolidinediones *etc.* is currently available. However, many of these oral antidiabetic agents

have a number of serious adverse health effects. Therefore, the search for more effective and safer hypoglycemic with greatest effect on postprandial hyperglycemia including α-amylase, insulin and α-glucosidase has continued to be an important area for investigation.

Traditional medicines readily available from medicinal plants offer great potential for the discovery of new antidiabetic drug. Diabetes mellitus was known to ancient Indian physicians as 'madumeha'.[3] 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.[4]

MATERIALS AND METHODS:

Chemicals: Free sample of Glibenclamide was procured from Nicholas Piramal, Mumbai and Streptozotocin (STZ) (purchased from Sigma, St Loius, MO, USA). All other chemicals used were of analytical grade.

Plant material: The leaves of *Corchorus trilocularis* were collected from outfield near Tirunveli, Tamil Nadu, India, during the month of July 2013. Plant was identified by the Dr V. Chelladurai, Retd. Research Officer, Botany (Scientist C) at Central Council for Research in Ayurveda, Government of India.

Preparation of plant extract: The collected plant leaves were washed thoroughly in tap water, dried in shade at room temperature and reduced to coarse powder using a mechanical grinder. The dried powder material was extracted with chloroform by hot continuous extraction in a Soxhlet's apparatus for 48 h. The extract was concentrated under reduced pressure and stored in an air tight container.

Phytochemical screening: The chloroform extract was screened for the presence of various phytoconstituents like steroids, alkaloids, tannins, flavonoids, and glycosides by employing standard phytochemical tests.[5, 6]

Animal: Wistar Albino rats of either sex (150 to 200 g) were purchased from DRDE, Gwalior. They were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity (50 ± 15%) and normal photoperiod (12-hour light-dark cycle) were used for the experiment. Commercial pellet diet MFD, by Amrut trade corporation, Gwalior were given to the experimental animals throughout the study. The Experimental Protocol was duly approved by IAEC having proposal No-IPS/COP/IAEC/02.

Toxicity study: Acute oral toxicity test was carried out according to the OECD guidelines No. 423.[7] Wistar albino rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose in 2000 mg/kg, body weight of chloroform extracts. The animals were observed for a period of 24 hr for the changes in behavior, hypersensitivity reactions etc. Mortality was determined over a period of 2 weeks. The test was repeated in another three rats to confirm the acute toxic class of LD_{50} determination.

Induction of diabetes: After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40 mg/kg Streptozotocin (Sigma, St. Louis, Mo, USA), freshly dissolved in citrate buffer (pH 4.5). After injection, the rats had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats was observed by moderate polydipsia and marked polyuria. 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.

Experimental protocol: To assess the anti-diabetic activity, the animals were divided in five groups (n=6), as shown below:

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 chloroform extract of *Corchorus trilocularis* (200 mg/kg body weight)

Group 4: Treated with chloroform extract of *Corchorus trilocularis* (400 mg/kg body weight)

Group 5: Standard drug, Glibenclamide-treated rats (5 mg/kg body weight)

The test drug and standard drug was administered orally for a period of 21 days from starting day of diabetes.

Blood collection and biochemical estimations in blood/serum: On 22nd day, fasting blood samples were collected from the tail vein of all the groups of rats. Whole blood was collected for estimation of blood glucose by using the glucometer (Easy Gluco, morepen laboratories Ltd.; New Delhi), glycosylated hemoglobin (HbA1C) and glutathione levels. Serum was separated for estimation of specific serum marker enzymes, namely, lactate dehydrogenase (LDH) and creatine kinase (CK).[9] Streptozocin-induced oxidative stress in diabetes is also a predictor of cardiac damage. Since LDH and CK are specific cardiac marker enzymes, increased serum LDH and CK levels were considered as marker of oxidative stress-induced cardiac damage.

Biochemical estimation in pancreatic tissue: After blood collection, all the animals were sacrificed and pancreas was dissected out. Tissue was washed with ice cold saline, weighed and minced; 10% homogenate was prepared in 0.15M ice-cold KCl for TBARS (thiobarbituric acid-reactive substances), a marker for lipid per oxidation and protein estimation; in 0.02M EDTA for glutathione estimation; and in phosphate buffer (pH 7.4) for superoxide dismutase (SOD) and catalase estimations using a Teflon tissue homogenizer.[10] Decrease in levels of endogenous antioxidants with rise in TBARS levels was considered as oxidative stress.

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 between the control and treatment groups. $p< 0.05$ was chosen as the level of significance. Statistical analysis was performed using Graph Pad Prism Software 5.0 version.

RESULTS:

Phytochemical Screening: The preliminary phytochemical studies of chloroform extract showed presence of saponins, phytosterols, flavonoids, phenols, steroids and terpenoids in chloroform extract of *Corchorus trilocularis leaves*.

Acute toxicity studies: In this study, chloroform extract at the dose of 2000mg/kg indexed neither visible signs of toxicity nor mortality and observations did not point out any proofs of substance related toxicity. There is no observed adverse-effect level (NOAEL) was noticed at the dose of 2000 mg/kg. The toxicity studies was determined by OECD guidelines 423. Based on the LD_{50} value, 1/5th and 1/10th (200 & 400 mg/kg) of its value was chosen for pharmacological studies.

Anti-diabetic activity:

Effect of chloroform extracts on blood glucose level in streptozotocin induced diabetic rats: Blood glucose level was measured on 3, 7, 14 and 21 day of post induction. The blood glucose level of Group III (CECT 200 mg/kg) and Group IV (CECT 400 mg/kg) on 14 day of post induction were 204.8±3.08mg/dL and 195.6±3.02mg/dL respectively. The Blood glucose level of glibenclamide (5 mg/kg) treated animal was 169.3 ± 2.77mg/dL on 14 day of post induction. The blood glucose level of Group III (CECT 200 mg/kg) and Group IV (CECT 400 mg/kg) on 21 day of post induction were 199.2 ± 0.29mg/dL and mg/dL 179.3 ±0.82 respectively. The blood glucose level of glibenclamide (5 mg/kg) treated animal was 160.8±0.24 mg/dL on 21 day of post induction. The blood glucose level in STZ treated rats were significantly high ($P<0.001$) when compared to the non diabetic control. Oral administration of CECT at the dose of 200 mg/kg body weight showed a significant decrease in blood glucose level (290.1 ± 0.24to 199.2 ± 0.29 mg/dL, $P<0.01$), as compared with the diabetic control group.

Oral administration of CECT at the dose of 400 mg/kg body weight showed a significant decrease in blood glucose level 290.1 ±0.24 (to 179.3±0.82mg/dL, $P<0.001$), as compared with the diabetic control group. Glibenclamide treated group showed a significant decrease in the blood glucose levels (290.1±0.24 to 160.8±0.24 mg/dL,

P<0.001), as compared to the diabetic control. The continuous oral administration of CECT for a period of 21 days at the doses of 200 mg/kg and 400 mg/kg body weight showed a significant decrease in blood sugar level, which was dose dependant and also comparable to that of the standard drug glibenclamide.

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 chloroform extract (200 & 400 mg/kg) reduced the glucose level moderately and highly significant with p<0.01 & p<0.001.

Effect of chloroform extracts on glycosylated haemoglobin, blood glutathione, serum creatine kinase, serum lactate dehydrogenase: The dose of streptozotocin significantly elevated the level of glycosylated haemoglobin in group II diabetic control rats. After treatment with chloroform extract of *Corchorus trilocularis*, the level of glycosylated hemoglobin was significantly lowered in both doses. The levels of blood glutathione in diabetic rats (group II) were significantly lowered (p< 0.001) when compared with those in normal control rats of group I. Treatment with chloroform extract (200 mg/kg and 400 mg/kg) for 21 days significantly restored the blood glutathione (GSH) levels as compared to group II rats. Glibenclamide treatment showed highly significant (p< 0.01) increase in blood GSH levels when compared to group II.

Furthermore, the levels of CK and LDH were significantly increased in group II diabetic rats. However, the test drug treatment for 21 days significantly reduced the levels of CK (p< 0.01 with 200 mg/kg and 400 mg/kg) when compared to diabetic rats. The administration of chloroform extract moderately significantly reduced (p<0.01) the serum LDH levels in both doses when compared to diabetic control rats and the results were comparable to glibenclamide treatment.

Effect of chloroform extracts on antioxidant enzymes: chloroform extract had significant effects on antioxidant enzymes. Extent of TBARS formed was significantly higher (p<0.001) in STZ treated group (group II). In both group of chloroform extract (200 mg/kg and 400 mg/kg) treatment level of TBARS decreased significantly (p< 0.001). Significant reduction (p< 0.001) in the activity of SOD in pancreas of diabetic animals (group II) was observed in comparison to normal rats, that is, group I. Diabetic rats treated with chloroform extract (200 mg/kg and 400 mg/kg) showed significant increase (p< 0.01) in level of SOD. Total glutathione activity was reduced highly significantly in pancreatic tissue of diabetic rats as compared to normal control animals. The levels were significantly (p<0 .01, p<0 .001) increased with chloroform extract (200 mg/kg and 400 mg/kg).

DISCUSSION: Any drug that is effective in diabetes will have the ability to control the rise in glucose level by different mechanisms and the ability of the extracts to prevent hyperglycaemia could be determined by hyperglycaemic animal model. In animals, diabetes can be induced by partial pancreatectomy or by the administration diabetogenic drugs such as streptozotocin, alloxan, ditizona and anti-insulin serum. Streptozotocin is a naturally occurring nitrosourea product of streptomyces achromogenes, and it is widely used to induce diabetes in rats. A pancreatic mode of action is feasible because in mild diabetes not all the beta cells of the pancreas are destroyed by STZ. Surviving beta cells retain the capacity to synthesize and secrete insulin. [11, 12] In conclusion, the chloroform extract of *Corchorus trilocularis* leaves showed a significant anti-diabetic activity in streptozotocin induced diabetic rats. The phytosterols present in chloroform extract may be possibly responsible for the anti-diabetic activity. The antidiabetic effect *Corchorus trilocularis* may be due to increased release of insulin from the existing β-cells of pancreas (**Table 1**). Furthermore, there was a significant attenuation of serum LDH and creatine kinase levels with the test drug treatment indicating the cardio protective effect of chloroform extract of *Corchorus trilocularis*. The treatment showed normal pancreatic β-cells. The protection might have been mediated through a *Corchorus trilocularis* induced increase in basal pancreatic SOD and catalase activity (**Table 2 - 3**).

Treatment of diabetic rats with the chloroform extract of *Corchorus trilocularis* significantly increased the levels of non protein thiols in serum as well as in pancreatic tissues of rats. In present study, it was also observed that the level of blood glutathione significantly ($p< .05$) increased, as well as pancreatic levels of glutathione in diabetic rats when treated with chloroform extract of *Corchorus trilocularis*. Further, the activities of SOD and CAT were also increased in the pancreatic tissues of test drug-treated diabetic animals. The antioxidant activity of the test drug might have been due to the inhibition of glycation of the antioxidant enzymes SOD and CAT (**Table 3**). However, we suggest that further work should be carried out at cellular and molecular levels to find out the absolute mechanism of action of the plant in experimental diabetes. The present investigation has also opened avenues for further research to the development of potent phytomedicine for diabetes mellitus from the *Corchorus trilocularis*.

TABLE 1: EFFECT OF CHLOROFORM EXTRACTS ON BLOOD GLUCOSE LEVEL IN STREPTOZOTOCIN INDUCED DIABETIC RATS

S. 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	Chloroform extract (200 mg/kg)	84.27 ± 1.09	244.4 ± 3.05	218.2 ± 2.89***	204.8 ± 3.08***	199.2 ± 0.29***
IV	Chloroform extract (400 mg/kg)	87.78 ± 1.09	245.6 ± 3.09	210.2 ± 2.79***	195.6 ± 3.02***	179.3 ± 0.82***
V	Glibernclamide (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

TABLE 2: EFFECT OF CHLOROFORM EXTRACTS ON GLYCOSYLATED HAEMOGLOBIN, BLOOD GLUTATHIONE, SERUM CREATINE KINASE, SERUM LACTATE DEHYDROGENASE

S.no	Group	Whole blood HbA_1C (%)	Blood GSH (mg/dL)	Serum Creatinine Kinase (CK), (IU/L)	Serum LDH (IU/L)
I	Control	5.12 ± 0.22	3.19 ± 0.15	69.14 ± 2.88	190.12 ± 5.40
II	Diabetic control	15.33 ± 0.42***	1.10 ± 0.10*	153.32 ± 3.91**	311.44± 8.11***
III	Chloroform extract (200 mg/kg)	9.22 ± 0.14**	1.97 ± 0.21**	132.18 ± 0.65**	262.31 ± 8.23**
IV	Chloroform extract (400 mg/kg)	7.34 ± 0.17***	2.30 ± 0.26***	121.18± 0.64***	253.21± 8.30***
V	Glibenclamide (5 mg/kg)	6.17 ± 0.43***	3.79 ± 0.22***	89.62 ± 2.67***	233.76± 9.34***

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

TABLE 3: EFFECT OF CHLOROFORM EXTRACTS ON LIPID PEROXIDES, CATALASE, SUPEROXIDE DISMUTASE AND GLUTATHIONE LEVELS

S.no	Group	TBARS (Nmol MDA/mg protein)	CAT (nmol H_2O_2-consumed/min/mg protein)	SOD IU/mg protein	GSH level of phosphorous liberated/ min/mg protein
I	Control	0.489± 0.037	4.33± 0.089	3.59± 0.069	52.4±0.945
II	Diabetic control	4.921± 0.562***	0.79± 0.020***	0.262±0.025***	11.58±1.034***
III	Chloroform extract (200 mg/kg)	2.853± 0.451*	2.10± 0.132**	3.44± 0.272**	40.24±1.446***
IV	Chloroform extract (400 mg/kg)	0.893± 0.013***	2.92± 0.079**	3.68± 0.093***	43.52±1.123***
V	Glibenclamide (5 mg/kg)	1.223± 0.041***	3.39± 0.156***	3.78± 0.087***	44.65±1.656***

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

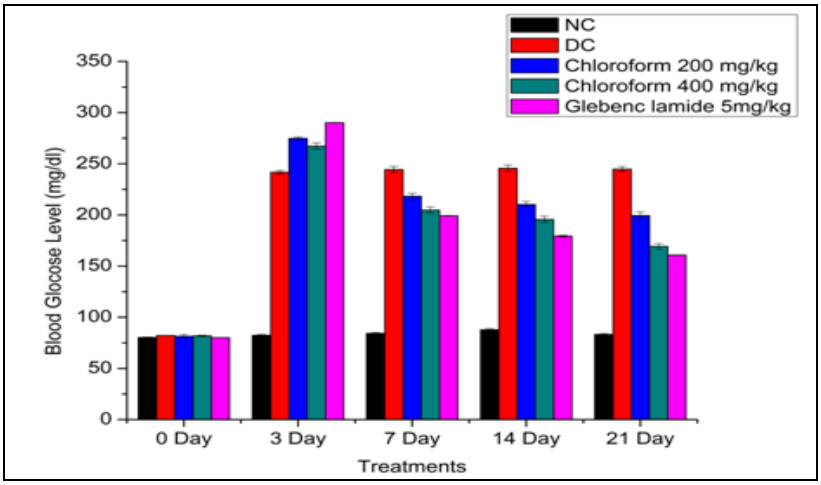

FIG. 1: EFFECT OF CHLOROFORM EXTRACT ON BLOOD GLUCOSE LEVEL IN DIABETIC RATS

CONCLUSION: In conclusion, chloroform extract of *Corchorus trilocularis* leaves exhibited antidiabetic effect against diabetes and also ameliorated the oxidative damage in pancreas. The effects may be attributed to presence of antioxidant phytochemical present in *Corchorus trilocularis*. Further mechanistic studies are required to suggest the appropriate mechanism for the antidiabetic effect of the plant.

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CONFLICT OF INTEREST: Authors declare that there is no conflict of interest.

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