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Evaluation of Antidiabetic Activity of Aqueous and Ethanolic Extracts of Leaves of *Chloroxylon Swietenia* in Streptozotocin (STZ) Induced Diabetes in Albino Rats

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ABSTRACT

To evaluate antidiabetic activity of *Chloroxylon swietenia* in STZ induced diabetes in albino rats. Forty two albino rats were randomly divided into seven groups (n=6). Diabetes was induced by intraperitoneal injection of streptozotocin (60mg/Kg). Distilled water, Tween 80, glibenclamide, *Chloroxylon swietenia* aqueous extract (CSAE), ethanolic extracts (CSEE) of 200 and 400mg/kg were given orally for 14 days to the normal control, diabetic control, standard group and test groups respectively. Glucose, TC (total cholesterol), TG (triglyceride), HDL (High density lipoprotein), AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), creatinine, direct bilirubin and indirect bilirubin levels were estimated. ANOVA followed by Student-Newman-Keuls test was used to analyze the data. CSAE of 200mg/kg showed a significant reduction in glucose, ALT, TB and ALP levels in diabetic rats. CSAE of 400mg/kg showed a significant decrease in glucose, AST, ALT, TB, DB and ALP levels in diabetic rats. CSEE of 200mg/kg showed a significant decrease in glucose, ALT, TB, ALP and creatinine levels in diabetic rats. CSEE of 400mg/kg showed a significant decrease in glucose, AST, ALT, TB, DB and ALP levels in diabetic rats. Both extracts show antidiabetic activity in STZ induced diabetes.

Keywords: Antidiabetic activity, *Chloroxylon swietenia*, streptozotocin, AST, ALT, ALP, creatinine and Bilirubin**.**

INTRODUCTION

Diabetes mellitus is an endocrine, metabolic disorders caused by relative or an absolute lack of insulin.[1] According to International Diabetes Federation (IDF), worldwide 382 million people were affected by diabetes in 2013 and it is expected to raise to 592 million by 2035. IDF estimates 65 million diabetic patients in India in 2013 and it is expected to cross 109 million by 2030.[2] In India diabetic patients are increasing day by day may be because of the change in food pattern, i.e. fast food diet intake and change in lifestyle.[3] Management of diabetes is a tough task. The medicines used in diabetic treatment are either too costlier or have adverse effects like hypoglycemic coma, insulin resistance, hypersensitivity and metallic taste etc.[4] Hence, in the recent years, herbal compounds are gaining popularity in both developed and developing countries because of their natural origin, low adverse effects.[5] Ethnobotanical information indicates that around 800 medicinal plants having hypoglycemic or antidiabetic potential.[6] Herbal plants are abundant in India. Hence the search for safer and effective

antidiabetic agents has become the current research area.[5]

An ethnobotanical study was carried on the medicinal plants often used for the management of diabetes in Warangal district, Andhra Pradesh by traditional healers. *Chloroxylon swietenia* is the one of the plants used by the traditional healers for diabetes.[7,8] Even though medicinal plants are widely used, the effective treatment of the disease has not been verified with scientific standards. Only a few plants used for diabetes in traditional medicine are scientifically audited in vivo.[9] *Chloroxylon swietenia* belongs to the Rutaceae family. Common name – satinwood, Telugu name – billu, bildu, billedu, Tamil name- porasu or vaaimaram. *Chloroxylon swietenia* has been reported to have anti-inflammatory activity[10], mosquitocidal activity [11-13], antioxidant activity[14], analgesic activity[14], anthelmintic activity[15], antimicrobial activity.[15-17] Antidiabetic activity was reported with this plant, but with different parts of stem, bark and whole plant.[18,19] *Invitro* antidiabetic activity was reported with the leaf extract of *Chloroxylon swietenia* in our previous report.[20] Based on the claims and available evidence, it was thought worthwhile to investigate *Chloroxylon swietenia* for diabetes in animal models.

MATERIALS AND METHODS

Plant material & extract Preparation

The leaves of *Chloroxylon swietenia* were collected locally and authenticated by Dr. Shiva Kumari, Department of Botany, Andhra Loyola College. After shade-dried (Temp<40°C.), plant material was grounded into a moderately coarse powder. The aqueous extract was made by maceration and the ethanolic extract was made by using soxhlet apparatus. The extract was allowed to dry. The dried extract was weighed. The % yield of each plant extract was calculated. The % of yield obtained was 8.96 and 9.16% for alcoholic and aqueous extracts respectively. Both the extracts were preserved in the refrigerator till further use.

Experimental Design

Both sexes of albino rats weighing 250-300g were used. Rats were fed with a standard pellet diet and water ad libitum. Animals were kept in a controlled environment (12 h/12 h light/night) and temperature (27±2°C). Before starting the experiment, rats were allowed to acclimatize to the laboratory conditions. All the animal experiments were approved by the institutional animal ethics committee (36/IAEC/NRIMC/2013-14) in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals.

Induction of Diabetes

STZ was freshly prepared by dissolving in citrate buffer (0.01M, PH-4.5) and kept on ice prior to practice. The overnight fasted rats were made diabetes with a single intraperitoneal injection of STZ (60 mg/kg). After 4hrs STZ administration 5% glucose was administered orally in drinking water for a day to overcome the early hypoglycemic phase. Rats were allowed to stabilize for three days. On the third day (72hrs) blood samples were drawn to estimate the blood glucose concentration to confirm the development of diabetes. Rats with plasma glucose above 250 mg/dL were considered as diabetic and used in the study. Both the test extracts and standard drug treatment were given orally for 14days. Blood was collected by the retro-orbital puncture under light ether anesthesia on 1, 7 & 14th day of treatment schedule for biochemical estimations. Rats were randomly allocated into 7groups (n=6) (Table-1).

Biochemical estimations

Serum was used to estimate the biochemical parameters like ALP, AST, ALT, TB, DB, creatinine, TG, HDL and TC using commercially available kits. LDL and VLDL values were calculated by using Friedewald's formula[21] as mentioned below

VLDL= TG/5

LDL= TC-HDL-VLDL

Atherogenic index (AI) values were calculated by using formula as given below[22]

AI = (TC-HDL) /HDL

Statistical Analysis

The data were expressed as mean ± standard error (SE). The Significance of differences among the groups were assessed by using ANOVA, followed by Student-Newman-Keuls test. $p < 0.05$ (5%) were considered as significant.

RESULTS

Effect on blood glucose

The effect of extracts on glucose level is illustrated in Graph-1. Statistical analysis at '0' (zero) day by One-way ANOVA revealed that there was no significant (P>0.05) difference among the groups. Further, statistical analysis on the 7th day of medication showed a significant (P<0.05) difference among the groups. There was a significant elevation of blood glucose level in diabetic control as compared to normal control rats. Student-Newman-Keuls test revealed that glibenclamide, $CSAE_1$, $CSAE_2$, $CSEE_1$ and $CSEE_2$ treated groups shows a significant reduction in blood glucose level as compared to the diabetic control. Similarly, statistical analysis at 14th day showed that there was significant (P<0.05) difference among the groups. Student-Newman-Keuls test revealed that glibenclamide, $CSAE_1$, $CSAE_2$, $CSEE_1$ and $CSEE_2$ treated groups shows a significant reduction in blood glucose level as compared to the diabetic control. Further analysis by ANOVA followed by Student-Newman-Keuls test revealed that glibenclamide, $CSAE_1$, $CSEE_1$ and $CSEE_2$ treated groups shows no significant difference between the groups when test groups are compared with standard (glibenclamide) control. This result indicates that test groups produced the effect almost equal to the standard group.

Effect on AST and ALT

The changes in the AST and ALT levels of all the groups are illustrated in Graph-4. Statistical analysis by One-way ANOVA revealed that there was a significant difference among the groups [p<0.05]. There was a significant elevation of AST and ALT level in diabetic control as compared to normal control rats. Glibenclamide, $CSAE_1$, $CSAE_2$, $CSEE_1$ and $CSEE_2$ treated groups show a significant reduction in ALT level as compared to the diabetic control. $CSAE_1$ and $CSEE_1$ reduced the AST level when compared to the diabetic control, but the reduction is not statistically significant (p>0.05). Both the extracts of 400mg/kg dose shows significant (p<0.05) reduction in the AST levels.

Effect on total bilirubin and direct bilirubin

The changes in the total bilirubin and direct bilirubin levels of all the groups are illustrated in Graph-5. There was a significant elevation of total bilirubin and direct bilirubin levels in diabetic control as compared to normal control rats. $CSAE_1$, $CSAE_2$, $CSEE_1$ and $CSEE_2$ treated groups shows a significant reduction in the total bilirubin level as compared to the diabetic control. CSEE of 400mg/kg dose shows the significant reduction in the direct bilirubin level when compared to the diabetic control.

Effect on ALP and creatinine

ALP and creatinine levels are significantly elevated in diabetic controls as compared to the normal control (Graph-4 and 5). Both extracts of all the doses significantly reduced the elevated ALP levels. CSEE of 200mg/kg showed a significant decrease in the creatinine levels.

Effect on lipid profile

There was a significant rise in TC, TG, VLDL and LDL levels in the diabetic control in comparison to the normal rats (Graphs-2). Both doses of CSAE showed a significant reduction in TC level. CSEE of 200mg/kg showed a significant reduction in the TG levels. CSAE (400mg/kg), CSEE (200mg/kg) and CSEE (400mg/kg) showed a significant elevation of HDL levels in comparison to the normal and diabetic control. Both extracts of all the doses significantly reduced the LDL levels in comparison to diabetic control.

AI and CRI values are significantly higher in diabetic control compared to the normal rats (Graphs-3). Standard drug and test extracts significantly reduce the AI and CRI values. Glibenclamide, $CSAE_1$, $CSAE_2$, $CSEE_1$ and $CSEE_2$ showed 86.5, 64.6, 89.3,94 and 94.5 % protection respectively (Graphs-3).

DISCUSSION

The development of safer medicines for diabetes is still a challenge for researchers working in this area.[23] The experimental data on herbal medication can offer new functional leads to reduce toxicity, time and money are the three main hurdles in drug development. It is correctly stated that 'laboratories to clinics' becomes 'clinics to laboratories' is a true reverse pharmacology approach. [24] The development of modern treatment

methods requires animal models that mimic the range of pathophysiological changes visualized in diabetic humans. [23]

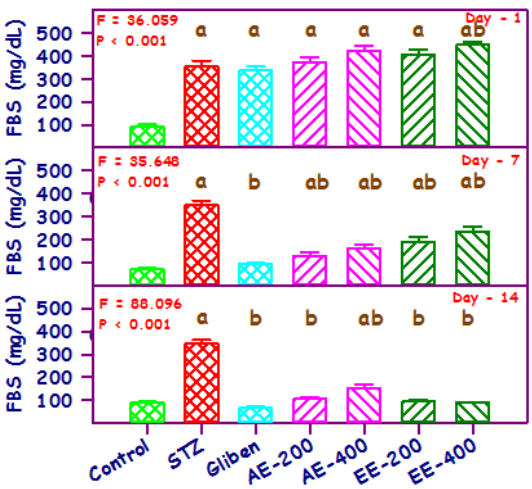
Streptozotocin is commonly using chemical to induce diabetes in rodents than the other chemical inducing agents like alloxan, gold thioglucose etc because of its less toxicity and specificity.[25] Streptozotocin (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is obtained from *Streptomycetes achromogenes* and is used to induce both type-1 and type-2 diabetes. STZ is taken up by pancreatic ²-cells *via* glucose transporter GLUT2. STZ alkylates the DNA leads to the ²-cell death. STZ is a nitric oxide (NO) donor and this NO destroys the pancreatic islet cells.[26]

Both doses of CSAE and CSEE significantly decreased the glucose level as compared to the diabetic control rats. This effect may be due the decrease in glucose absorption from the intestines or induction of glycogenic process along with decrease in glyconeogenesis and glycogenolysis. [27]

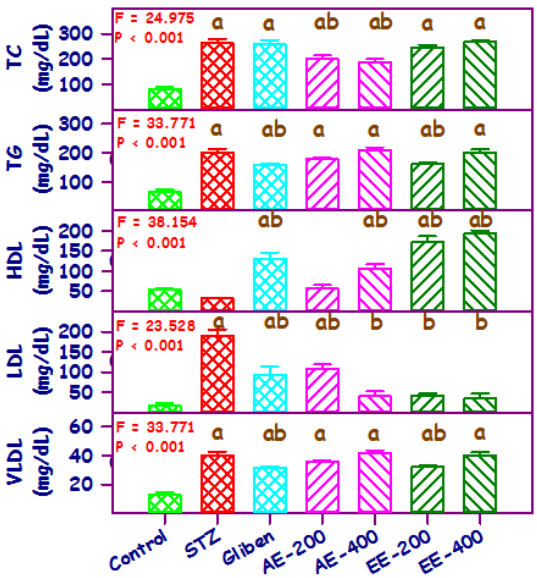
Table 1: Grouping of animals

S.No	Groups	Type of control	Dose
1.	Normal rats	Normal control (Non-diabetic)	Distilled water
2.	STZ induced Diabetic rats	Diabetic control	Tween 80
3.	Diabetic rats + Glibenclamide	Standard	5 mg/kg
4.	Diabetic rats + $CSAE_1$	Test	200 mg/kg
5.	Diabetic rats + $CSAE_2$	Test	400 mg/kg
6.	Diabetic rats + $CSEE_1$	Test	200 mg/kg
7.	Diabetic rats + $CSEE_2$	Test	400 mg/kg

CSAE= *Chloroxylon swietenia* aqueous extract, CSEE= *Chloroxylon swietenia* Ethanolic extract

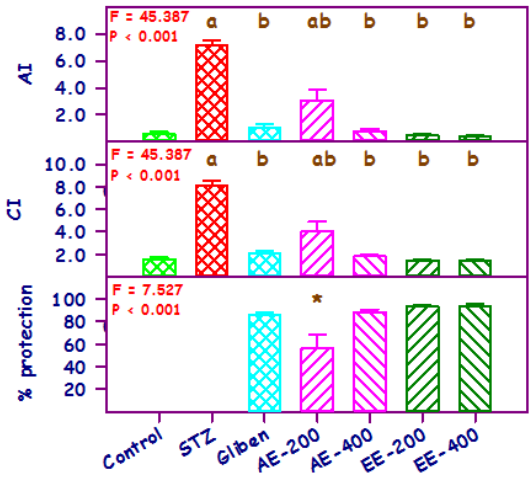


Graph 1: The effect of glibenclamide, CSAE and CSEE on plasma glucose (FBS) in diabetic rats. Mean ± SE (n = 6). a Statistically significant from control group. b Statistically significant from STZ group



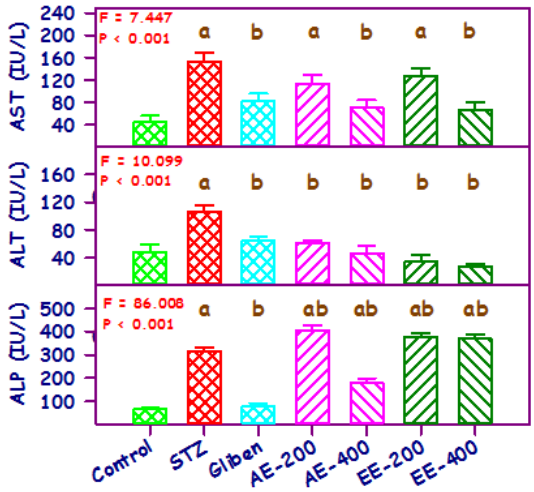
Graph 2: The effect of CSAE and CSEE on TC, TG, HDL, LDL and VLDL in diabetic rats. Mean ± SE (n = 6). a Statistically significant from control group. b Statistically significant from STZ group

Dyslipidemia is a most common complication observed in chemical induced diabetes and presents a serious risk of vascular disease.[6] In the present study, raise in TC and TG levels were observed in diabetic control rats. In diabetes the abnormal high levels of lipids are due to, an increase in the mobilization of free fatty acids from fat deposits due to the less utilization of glucose.[3] Hypertriglyceridemia is a most common abnormality in diabetes. [27]

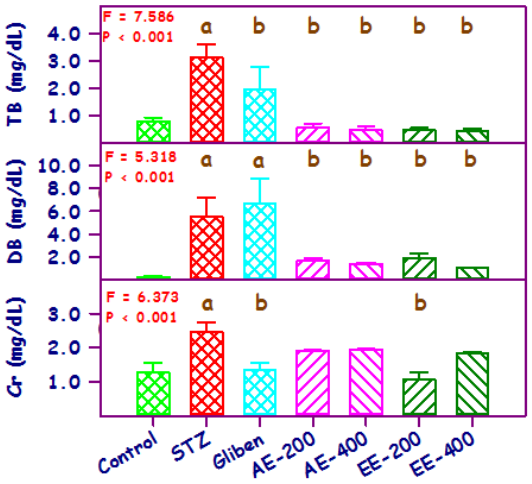


Graph 3: The effect of glibenclamide, CSAE and CSEE on AI, CRI and % protection in diabetic rats. Mean ± SE (n = 6). a Statistically significant from control group. b Statistically significant from STZ group. *Statistically significant from glibenclamide group

The serum lipid levels are generally high in diabetes; mapping a major risk factor for coronary heart disease.[6] Excess levels of TC and LDL are major coronary risk factors. The *C. swietenia* leaf extract reduced the TC, TG and LDL levels, where as it increased the cardioprotective lipid HDL levels significantly. It has been proved that raise in HDL levels is associated with a reduction in coronary risk.[28] In the present study, it has been observed that the *C.swietenia* leaf extract mitigated the raised TC and LDL levels in diabetic rats. Further, it has been indicated that TG itself is independently linked to coronary heart disease[29] and in the present study, the plant extracts lowered TG levels in diabetic rats. The atherogenic index and the coronary risk index were very high in the diabetic rats.[28] Standard drug and plant extracts significantly reduced the AI, CRI as to the normal rats. % protection was increased with the dose, CSEE has shown more protection than the CSAE.



Graph 4: The effect of glibenclamide, CSAE and CSEE on AST, ALT and ALP in diabetic rats. Mean ± SE (n = 6). a Statistically significant from control group. b Statistically significant from STZ group



Graph 5: The effect of glibenclamide, CSAE and CSEE on total bilirubin (TB), direct bilirubin (DB) and creatinine (Cr) in diabetic rats. Mean ± SE (n = 6). a Statistically significant from control group. b Statistically significant from STZ group

Diabetes is one of the common causes for a liver disease which includes abnormal liver enzymes, cirrhosis, hepatocellular carcinoma and acute liver failure. The AST, ALT, ALP , TB, and DB levels were raised in lever injury.[27] These enzymes are considered as a sensitive indicator of liver injury. *Chloroxylon swietenia* leaf extracts reduced the AST level, it shows the protective effect on the liver. The rise in ALP levels, indicates bone disease, bile tract obstruction or liver disease. *C. swietenia* extracts lowered the ALP levels, suggesting its protective effect on liver function.

Diabetes affects the kidney, result in the development of diabetic nephropathy. Serum creatinine levels reveal the kidney function.[30] CSEE (200mg/kg) significantly reduced the creatinine levels.

CONCLUSION

It is evident that C. swietenia leaf extracts contain antihyperglycemic agents capable of reducing the blood glucose level.

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