Effect Of Cassia Glauca LAMK, Leaves Extract On Cardiovascular And Renal Complications Associated With Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Objective: The present study was designed to evaluate the cardio-protective and nephro-protective potential of ethanolic (CGE) and water extract (CGW) of *Cassia glauca* leaves.

Methods: Type 1 diabetes was induced by single intravenous injection of 45 mg/kg of Streptozotocin (STZ) in male Wistar rats. Rats with fasting blood glucose levels>200mg/dl after seven days of STZ administration were randomized into different groups and were treated with CGE and CGW in graded doses for 56 days. On 52nd day, Intra peritoneal glucose tolerance (IPGTT) test and serum insulin were performed and blood glucose, lipid profiles, cardiac and renal parameters were estimated. In addition, enzymatic and non-enzymatic liver antioxidant levels were also estimated.

Results: Treatment with extracts increases the glucose uptake in diabetic rats suggest that the mode of action is like Rosiglitazone. The insulin levels after glucose load and HOMA values suggest that there is no insulin resistance in diabetic rats indicates that rats are under severe diabetic condition (Type1). Supplementation with both doses of CGE and CGW improved glucose tolerance, and insulin tolerance suggesting that, there is an improvement in STZ-induced deleterious effects. In addition, CGE and CGW supplementation decreased oxidative stress by improving endogenous antioxidant levels. Furthermore, administration of CGE and CGW improved diabetic cardio-toxicity and nephro-toxicity.

Conclusion: Treatment with CGE and CGW improved cardiac and renal profile. Taken together, these results suggest that CGE and CGW have potent anti-diabetic, cardio-protective, nephro-protective, hypolipidemic and antioxidant activity.

Key words: *Cassia glauca*, anti-diabetic activity, cardio-toxicity, nephro-toxicity.

INTRODUCTION

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the “diabetes capital of the world”. Persistence of diabetes leads to development of long term and short term complication. Long-term complications of diabetes includes retinopathy with potential loss of vision, nephropathy leading to renal failure; peripheral neuropathy causing higher risk of foot ulcer, amputation, and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms. Short term complication includes Symptoms of marked life-threatening hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome which may leads to coma and death.[1] The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in women compare to men. According to recent reports of WHO nearly 2.37 millions of populations suffer from diabetes mellitus among which most of them are suffered from type II diabetes mellitus[2].As on date there are no drugs available that can cure as well as can inhibit diabetes & related complication. So the world health organization has also recommended the evaluation of plants for treatments of diabetes. This has led to an increase in demand of research on anti-diabetic natural products. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diabetes mellitus the indigenous plant like Andrographis paniculata, Aloe vera, Momordica chirantia, Gymnemma sylvestra, Eugenia jambolana and others are widely used for the treatment of diabetes.[3,4]

METHODOLOGY

Plant Material

Leaves of Cassia glauca (LAMK.) were collected from in and around Dharwad district, Karnataka, and authentication of the plant was done by Dr. G. R. Hegde, H.O.D., Department of Botany, Karnatak University, Dharwad. A herbarium specimen of the plant was kept in Department of Pharmacognosy

(SETCPD/Ph.cog/herb/12/2009), SET's College of Pharmacy, Dharwad, Karnataka, India. The collected plant material was washed with running water. The leaves were dried under shade. Dried leaves were coarsely powdered and used for extraction.

Preparation of Extracts

Authenticated leaves of *Cassia glauca* (LAMK.) were shade dried and pulverized in to coarse material. Coarse plant material was cleaned by passing the powder material through 120 mesh sieve to remove any fine dust or powder, and coarse powder was used for extraction. Dried powder of leaf was exhaustively extracted using Ethanol (95%) (CGE) in a Soxhlet apparatus. Aqueous fraction was prepared by macerating the drug in Chloroform water (CGW). Both the extracts were concentrated by rotary flash evaporator, under reduced pressure and controlled temperature, followed by freeze drying and stored in a descicator.

Animals

Albino wistar male rats weighing 150-200g were used for the present study. The animals were purchased from Sri Venkateshwara Enterprises. They were maintained in the animal house of SET's College of Pharmacy, Dharwad for experimental purpose. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET's College of Pharmacy, Dharwad, Karnataka (REG.No.112/1999/CPCSEA). According to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. We selected male animals for all our studies, since females are shown to be protected from changes in lipid–induced insulin action.[5]

Pharmacological Evaluation

Preparation of extract dose:

Weighed quantity of ethanol (CGE) and water (CGW) extracts of Cassia glauca were suspended in water using 0.5% tragacanth and administered orally to experimental animals. Suspension of extract was prepared freshly. The extracts were administered at a constant volume of 10 ml/kg for each animal.

Testing the extracts of CGE and CGW in standardized STZ-induced diabetic rats

Induction of Diabetes mellitus

Diabetic condition was induced in male Wistar rats by single intravenous injection of STZ (45mg/kg) [Chosen optimum dose] after overnight fasting for 12 h.[6] Rats showing SG level > 200 mg/dl seven days after STZ administration were considered diabetic and included in the study. Diabetic rats were randomized into different groups based on their SG levels.

Experimental design for 8 week study[6]

The experimental rats were divided into seven groups of six rats each and treated as follows.

Group 1: *Normal control (NC) received 0.5% tragacanth (10 ml/kg, p.o.)*

Group 2: *Diabetic control (DC) received 0.5% tragacanth (10 ml/kg, p.o.)*

Group 3: *DC rats treated with CGE (200 mg/kg, p.o.)*

Group 4: *DC rats treated with CGE (400 mg/kg, p.o.)*

Group 5: *DC rats treated with CGW (200 mg/kg, p.o.)*

Group 6: *DC rats treated with CGW (400 mg/kg, p.o.)*

Group 7: *DC rats treated with Rosiglitazone (3mg/kg, p.o.)[7]*

Intra peritoneal glucose tolerance test (IPGTT)[8]

On 52nd day, glucose tolerance of various groups was estimated by a simple IPGTT. Glucose (2 g/kg) was administered to 12 h-fasted rats and blood samples were collected from the retro-orbital plexus at 0 (before glucose load), 30, 60 and 120 mins after glucose administration. SG was estimated by the enzymatic glucose oxidase method. The results were expressed as integrated area under curve for glucose ($AUC_{glucose}$), which was calculated by trapezoid rule,

$$AUC_{glucose} = \frac{(C_1 + C_2)}{2} X (t_2 - t_1)$$

Also serum insulin was estimated 0 (before glucose load), 30 and 60 mins after glucose administration. Serum insulin (SI) was estimated by radioimmunoassay method using the kit from Bhabha Atomic Research Centre,

Mumbai, India. The results were expressed as integrated area under curve for insulin ($AUC_{insulin}$), which was calculated by trapezoid rule.

Insulin tolerance test (ITT)

On 54th day, insulin (2 U/kg, i.p) was administered to six h-fasted rats. Blood samples were collected from the retro-orbital plexus at 0 (just before insulin load), 10, 20 and 30 mins after insulin injection. SG was estimated by the enzymatic glucose oxidase method. The results were expressed as integrated area under curve for glucose ($AUC_{glucose}$), which was calculated by trapezoid rule using formula as given above.

Estimation of Cardiovascular parameters[6]

At the end of the treatment schedule, blood samples were collected from retro-orbital plexus. Serum was separated and analyzed spectrophotometrically for Creatinine Kinase-MB (CK-MB) and LDH using diagnostic reagent kit ERBA diagnostics Mannheim GMBH, Germany.

- **Measurement of ECG**

At the end of the treatment schedule, remaining animals from each group will be used for the evaluation of ECG. The rats were anaesthetized with light anesthetic ether and ECG was recorded using NIVIQURE computerized data acquisition system version 6.1 (Mfg. by INCO instruments and chemicals Pvt. Ltd)

Estimation of Renal parameters[9,10]

At the end of treatment schedule, blood samples were collected from retro-orbital plexus. Serum was separated and analyzed spectrophotometrically for Serum urea nitrogen, Serum albumin, Serum Total Protein and Serum Creatinine using diagnostic reagent kit ERBA diagnostics Mannheim GMBH, Germany. Urine glucose and ketone bodies were estimated by qualitative method using UroColor 2K (urine analysis strips) Mfg. by Standard Diagnostics, Korea.

Estimation of biochemical parameters

Estimation of lipid profile

At the end of the treatment schedule, blood samples were collected from retro-orbital plexus. Serum was separated and analyzed spectrophotometrically for triglyceride (STG), total cholesterol (STC), HDL-cholesterol (HDL-c), using diagnostic reagent kit ERBA diagnostics Mannheim GMBH, Germany. Serum insulin (SI) was estimated by radioimmunoassay method using the kit from Bhabha Atomic Research Centre, Mumbai, India. Homeostatic Model Assessment (HOMA) as a measure of insulin resistance was calculated by the formula

$$HOMA = \frac{\text{insulin } \mu U/ml \text{ X glucose mmol/L}}{22.5}$$

VLDL-cholesterol (VLDL-c) and LDL-cholesterol (LDL-c) in serum were calculated as per Friedewald's equation.

$$VLDL - c = \frac{\text{Triglyceride}}{5}$$

$$LDL - c = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - HDL - c$$

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The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were also calculated.[11]

Total thiols

The assay is based on the formation of a relatively stable yellow product when sulphydryl groups react with DTNB.[64] Briefly, 0.2 ml of liver homogenate was mixed with phosphate buffer (pH=8), 40 µl of 10mM DTNB

and 3.16 ml of methanol. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The total thiol content was calculated by using $\varepsilon=13.6 \times 10^3\ cm^{-1}\ M^{-1}$.[12]

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) in the homogenate were estimated by using standard protocol. Briefly, the homogenate was incubated with 15% TCA, 0.375% TBA and 5N HCl at 95°C for 15 min, the mixture was cooled, centrifuged and the absorbance of the supernatant measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using the formula $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as TBARS (n moles) per g of tissue.[13]

Statistical evaluation

The data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test using GraphPad Prism version 4.0, USA.

RESULT AND DISCUSSION

Pharmacological Evaluation

Testing of CGE and CGW in STZ-induced diabetic rats

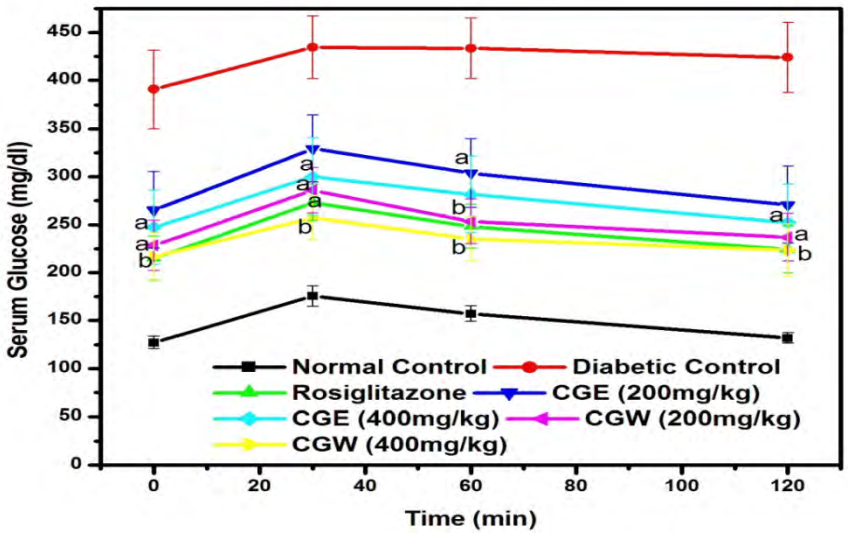
Intra peritoneal glucose tolerance test (IPGTT)

Intra peritoneal administration of glucose (2g/kg) on 52nd day did not produce significant change in SG level of normal control rats and AUC for the 120 min interval was not altered. The diabetic rats exhibited significant elevation in fasting SG (at time zero) and showed significant impairment in glucose tolerance to exogenously administered glucose compared to Normal rats (Fig 1 A). Treatment with CGW (200mg/kg) and CGE (200 and 400mg/kg) significantly ($P<0.05$) improved the glucose tolerance and reduced SG level whereas, CGW (400mg/kg) and Rosiglitazone (3mg/kg) showed significantly $P<0.01$ improvement in glucose tolerance and reduction in SG level (Fig 1 A).

Integrated areas under the glucose curve over 120 min ($AUC_{glucose}$) of diabetic group was significantly higher ($P<0.001$) compared to normal control. Treatment with tested extracts CGW (400mg/kg) and Rosiglitazone produced a significantly ($P<0.05$) decreased $AUC_{glucose}$ further, CGE (400mg/kg) and CGW (200mg/kg) produced significant ($P<0.01$) decreased $AUC_{glucose}$ compared to diabetic control (Fig 1 B).

Administration of glucose (2g/kg) stimulated the release of higher levels of insulin in normal control rats, whereas glucose load was ineffective in stimulating the release of insulin in diabetic rats, suggesting that these diabetic rats resembled severe diabetic (type I) condition in which a maximum pancreatic damage occurred. Whereas, treatment of CGE and CGW to diabetic rats enhanced the glucose stimulated insulin release from pancreatic β-cells and this response was comparable with Rosiglitazone treated diabetic rats (Fig 2 A). Integrated areas under the insulin curve over 60 min ($AUC_{insulin}$) of diabetic group was significantly lower ($P<0.001$) compared to normal control. Treatment with Rosiglitazone (3mg/kg) and CGE (400mg/kg) produced a significantly ($P<0.001$) increased $AUC_{insulin}$ compared to diabetic control (Fig 2 B) whereas CGW (400mg/kg) produced significant *P<0.01* increase in $AUC_{insulin}$ compared to diabetic control (Fig 5.2 B) Further, the integrated $AUC_{insulin}$ for the treatment of CGE (400 mg/kg), CGW (400 mg/kg) and Rosiglitazone (10mg/kg) was found to be 1075.0±44.4, 965±148.58 and 1220±86.75 respectively compared to untreated diabetic rats (400.0±42.72) (Fig 1 B)

[A]



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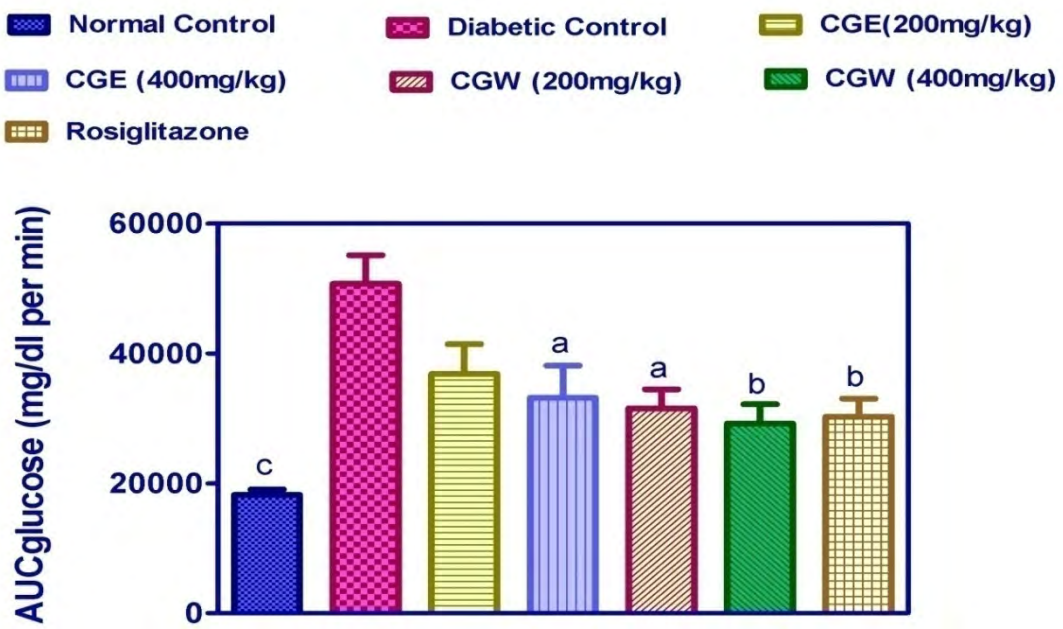


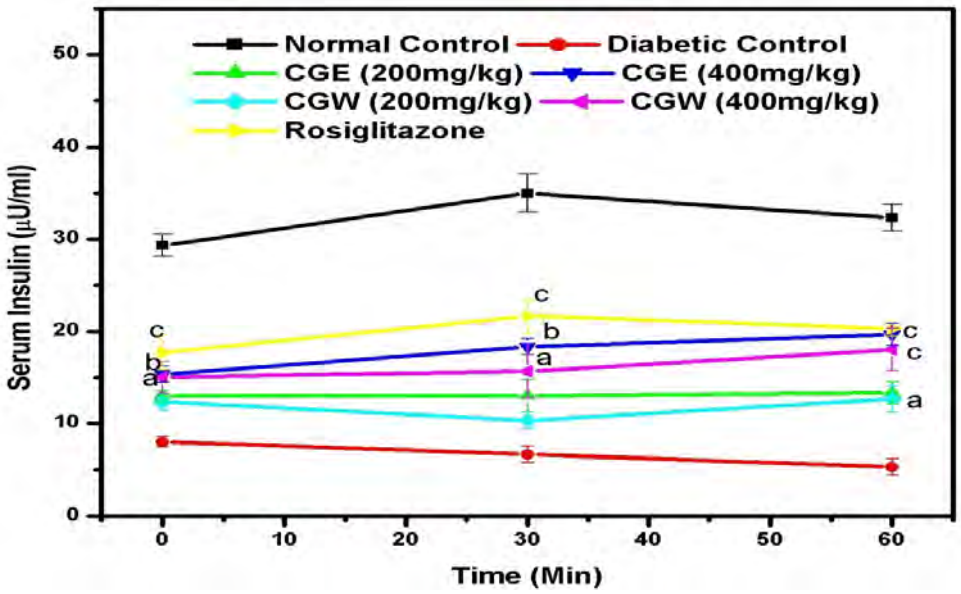
Fig 1: Effect of CGE and CGW on glucose tolerance in fasted diabetic rats.

[A] SG levels were measured prior to, and after i.p administration of glucose alone (2g/kg body weight), or in combination with CGE, CGW or Rosiglitazone.

[B] Area under curve for glucose ($AUC_{glucose}$) values for 0-120 min post glucose load.

Data represent the mean ± S.E.M., for 5 rats. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$ as compared with normal rats.

[A]



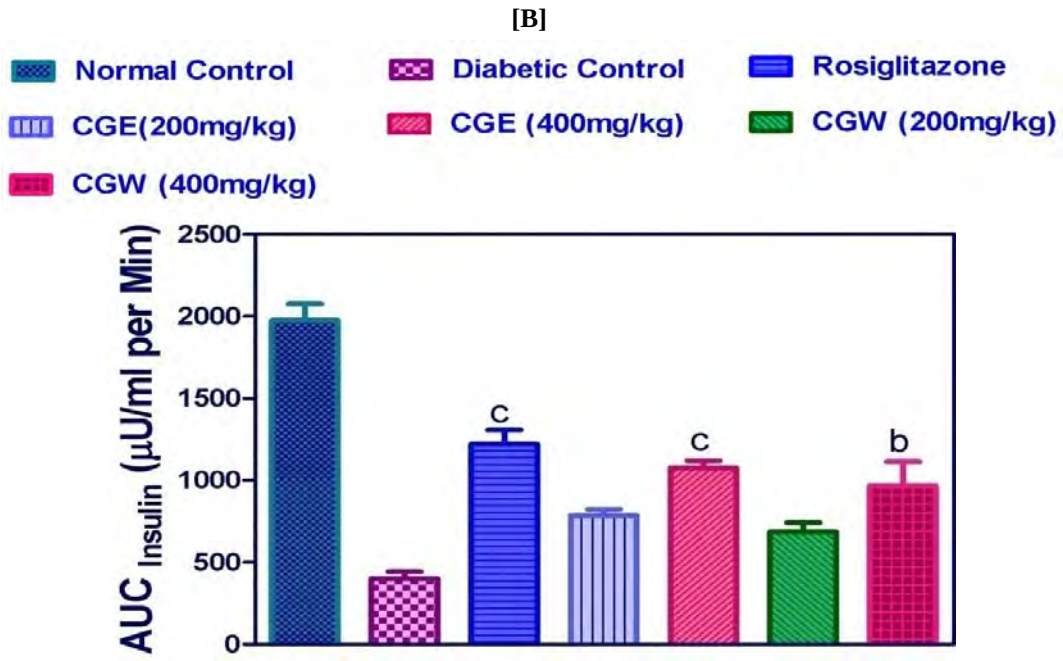


Fig 2: Serum insulin (SI) levels of diabetic rats treated with CGE and CGW.

[A] Post glucose (2g/kg body weight) challenge performed on fifty second day

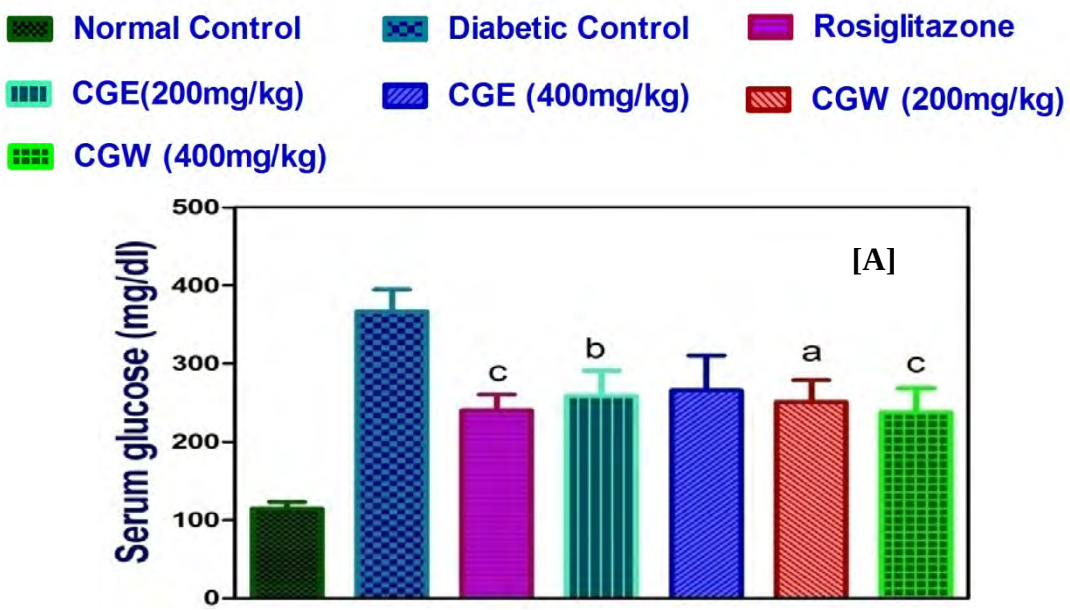
[B] Incremental AUC $_{insulin}$ values for 0-60 min.

Data represent the mean ± S.E.M., for 3 rats. ^{a} P < 0.05; ^{b}P < 0.01; ^{c} P < 0.001 as compared with normal rats.

Blood glucose, serum insulin and HOMA

Diabetic rats exhibited significant (P< 0.001) hyperglycemia (390.66±40.73) and hypoinsulinemia (8.0±0.58) as compared to normal control rats (Fig 3 A). The degree of insulin resistance as calculated by HOMA values were less in diabetic than normal control rats suggested that, the diabetic rats were not under insulin resistance condition (Type 1 DM) i.e. peripheral utilization of glucose was not compromised (Fig 3 C).

Oral administration of CGW (400mg/kg) and Rosiglitazone to diabetic rats, significantly (P< 0.001) decreased SG and increased SI levels (P<0.05 and P<0.01 respectively). However, HOMA values of extracts were in between the range of Diabetic and Normal rats. (Fig 3 C).



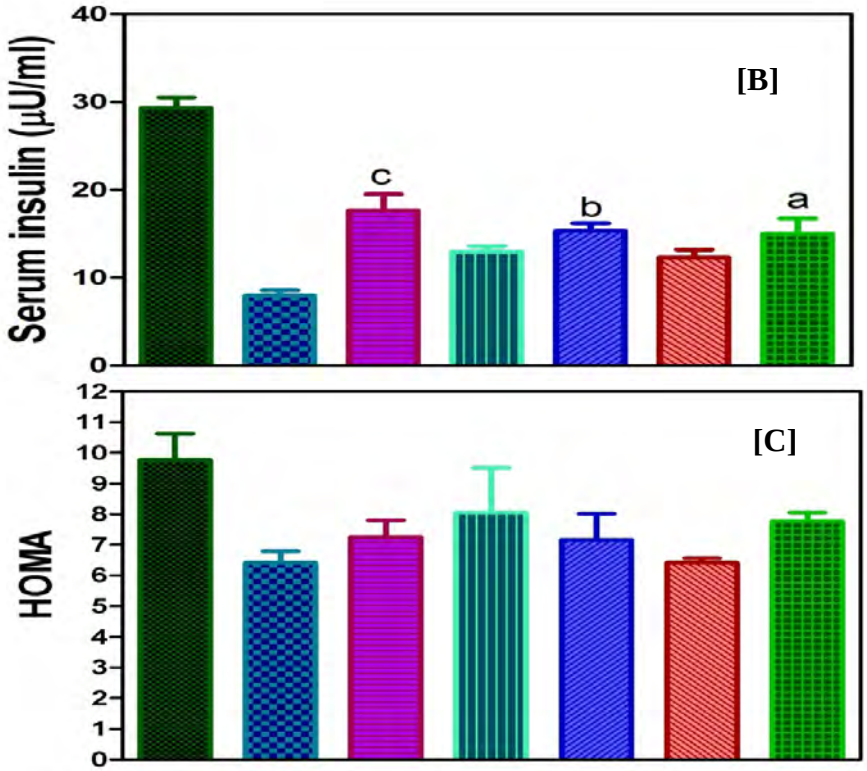


Fig 3: Effect of CGE and CGW on

[A] Serum glucose (SGL)

[B] Serum insulin (SI)

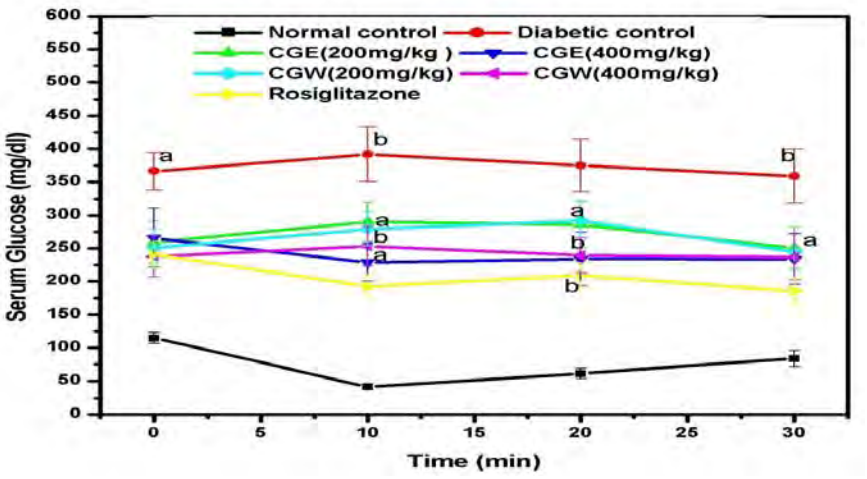
[C] Homeostatic model assessment (HOMA) levels in diabetic rats.

Insulin tolerance test (ITT)

Insulin tolerance test is a measure of the extent of peripheral utilization of glucose. On 54th day of study SG levels were measured following insulin challenge (2 U/kg, i.p). STZ induced severe (type I) diabetic rats subjected to insulin challenge did not exhibit a marked fall in SG levels suggested that, these diabetic rats were not able to utilize the exogenously administered insulin to reduce the SG levels (Fig 4 A).This observation may be due to the marginal loss of insulin sensitivity in diabetic rats (Type 2 DM), even though these diabetic rats were in type I diabetic condition.

Whereas, the blood-glucose levels and AUC $_{glucose}$ in diabetic rats treated with higher dose of CGE and CGW were significantly ($P<0.05$) lower glucose whereas Rosiglitazone showed significantly ($P<0.01$) lower glucose at 10, 20 and 30 min compared to the glucose levels at the corresponding time points in the diabetic rats receiving the vehicle (Fig 4 B)

[A]



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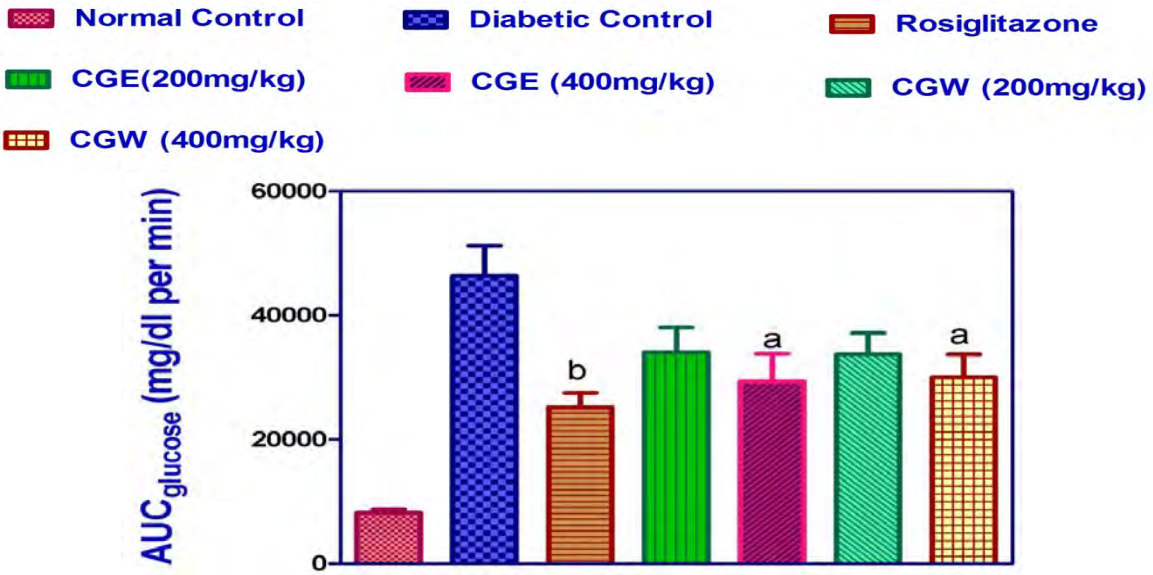


Fig 4: Effect of CGE and CGW on insulin tolerance in 6hr fasted diabetic rats.

[A] SG levels were measured prior to, and after i.p. administration of insulin alone (2U/kg body weight), or in combination with CGE, CGW or Rosiglitazone

[B] Area under curve for glucose ($AUC_{glucose}$) values for 0-30 min post insulin injection. Data represent the mean ± S.E.M., for 5 rats. [a] $P < 0.05$; [b] $P < 0.01$ as compared with normal rats.

Estimation of Cardiac parameters

ECG parameters

At the end of study ECG was measured. Normal control, CGE (400 mg/kg) and Rosiglitazone treated rats showed a normal ECG pattern; whereas diabetic animals showed significant elevation in ST segment, prolongation in QRS complex and R-R interval. In addition there was a decrease in heart rate as compared to normal control rats. Treatment with both CGE and CGW (200 mg/kg) for 8 weeks exhibited near to normal ECG pattern with a slight elevation in ST segment. Furthermore, it also elevated the prolongation of QRS complex and R-R interval, moreover, heart rate was increased than diabetic rats. (Table.1)

Heart weight:

Diabetic rats exhibited significantly increased heart weights in comparison with normal rats. Rats treated with CGE, CGW (400mg/kg) and Rosiglitazone (3mg/kg) exhibited the heart weight near to normal. Rats treated with CGE and CGW (200mg/kg) exhibited decrease in heart weight. (Table 1)

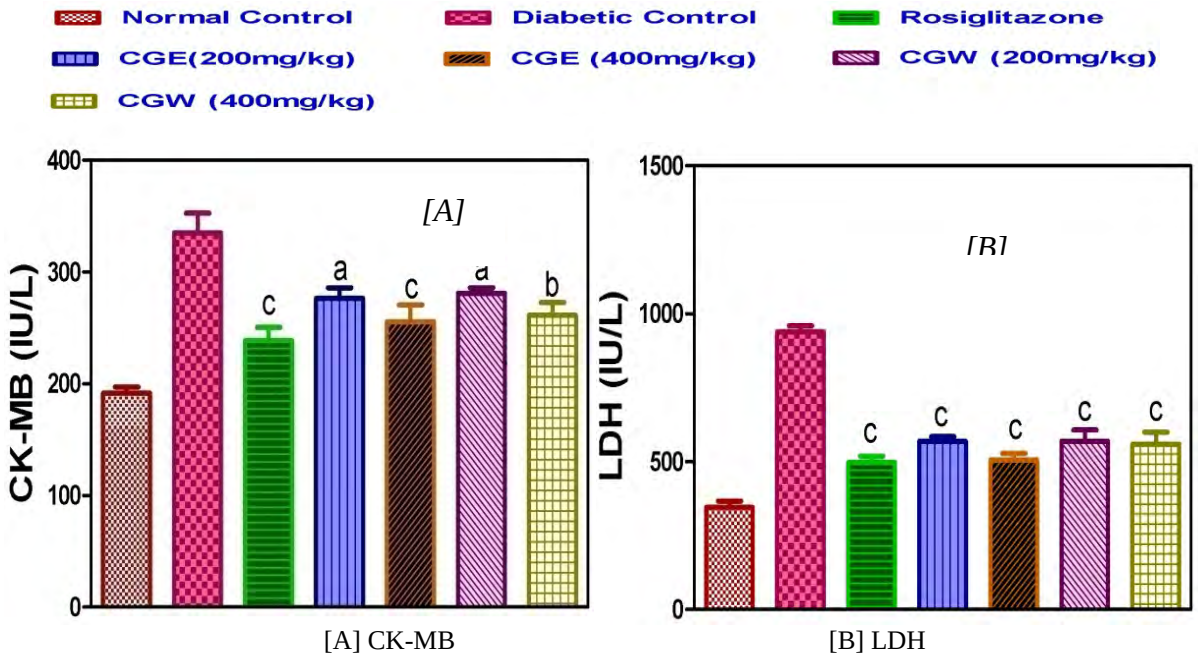
Table 1: Effect of CGE and CGW on Cardiac profile in STZ induced diabetic rats.

Treatment	*LDH (IU/L)*	*CKMB (IU/L)*	*Heart Rate (beat/min)*	*Heart Weight (gm)*
Normal control	*346.04±21.43*	*191.75±5.34*	*456.0 ± 24.03*	*0.59 ± 0.007*
Diabetic control	*938.64±20.76*	*334.97±17.69*	*355.4 ± 18.99*	*0.71± 0.028*
CGE 200mg/kg	*568.80±15.94****	*276.67±9.42**	*396.2 ± 6.317*	*0.65 ±0.010*
CGE 400mg/kg	*506.94±21.56****	*255.67±15.06****	*433.9 ± 22.85*	*0.60 ± 0.030*
CGW 200mg/kg	*569.90±37.14****	*281.04±5.13**	*370.8 ± 8.390*	*0.63±0.024*
CGW 400mg/kg	*559.84±40.09****	*261.34±11.29***	*441.8 ± 10.78*	*0.61 ± 0.020*
Rosiglitazone 3mg/kg	*497.90±20.80****	*238.54±11.94****	*466.2 ± 6.317*	*0.59± 0.01*

Each value represent Mean± S.E.M n=5 *P<0.05,**P<0.01,P<0.001 compared to Diabetic control. One way ANOVA followed by Tukey's post test.***

Estimation of serum enzyme markers:-

Diabetic rats exhibited a significantly increase in the levels of CK-MB and LDH as compared to Normal rats (Table 1). Rats treated with both doses of CGE, CGW (200 and 400mg/kg) and Rosiglitazone showed significant decrease (P< 0.001) in the level of LDH as compared to diabetic rats whereas, CGE, CGW (200mg/kg) and CGE, CGW (400mg/kg) exhibited significantly (P<0.001 and P<0.05) decrease in CK-MB level respectively (Table 1) (Fig.6).



[A] CK-MB [B] LDH

Fig 6: Effect of treatment with CGE and CGW on *in STZ-induced diabetic rats.*

Each bar represent the Mean ± S.E.M. (n = 5). [a] P < 0.05; [b] P < 0.01;[c] P < 0.001 compared with diabetic control.

Estimation of Renal parameters

Estimation of serum enzyme markers:-

At the end of study renal parameters like Serum urea, albumin, total protein and creatinine were measured. Diabetic rats exhibited a significantly increase in the levels of Serum urea and creatinine while decreased level of albumin and total protein as compared to Normal rats (Table 2). Rats treated with different doses of CGE, CGW (200 and 400mg/kg) and Rosiglitazone shows significant decrease (P<0.05, P<0.01 and P< 0.001) in the level of Serum urea and creatinine respectively and showed significantly (P<0.05, P<0.01 and P< 0.001) increased total protein and serum albumin as compared to diabetic rats. (Table 2).

Table.2: Effect of CGE and CGW extract on Renal profile in STZ induced diabetic rats.

Treatment	*Serum urea (mg/dl)*	*Albumin (g/dl)*	*Total protein (g/dl)*	*Creatinine (mg/dl)*	*Kidney weight (gm)*
Normal control	*58.61±1.97*	*6.3±0.13*	*9.4±0.9*	*0.30±0.01*	*1.23±0.033*
Diabetic control	*126.67±17.32*	*2.77±0.14*	*4.8±0.24*	*0.59±0.05*	*1.41±0.016*
CGE 200mg/kg	*88.96±4.77**	*4.3±0.13****	*7.61±0.47***	*0.52±0.02**	*1.25±0.016*
CGE 400mg/kg	*77.27±4.63****	*5.15±0.17****	*8.5±0.48****	*0.47±0.03*	*1.20±0.057*
CGW 200mg/kg	*76.58±6.03****	*4.21±0.1****	*7.07±0.19**	*0.52±0.01*	*1.27±0.057*
CGW 400mg/kg	*68.62±3.64****	*4.86±0.10****	*7.3±0.44**	*0.44±0.02***	*1.24 ± 0.013*
Rosiglitazone 3mg/kg	*61.88±3.20****	*4.4±0.2****	*8.9±0.34****	*0.35±0.02****	*1.21±0.049*

Each value represent Mean± S.E.M n=5 *P<0.05,**P<0.01,P<0.001 compared to Diabetic control.***

Urine estimation:

At the end of study urine parameters like urine glucose and urine ketone bodies were measured by qualitative method using urine analysis strips. Diabetic rats showed high level of urine glucose +++ and urine ketone bodies showed +++ as compared to normal rats glucose ± and ketone bodies ±. Whereas higher dose of CGE and CGW exhibited lower values as glucose ± and ketone bodies ± compared to diabetic rats. While lower dose of CGE and CGW showed glucose ++ and ketone bodies + lower than diabetic rats. (Table 3)

Table.3: Effect of CGE and CGW on urine analysis in STZ induced diabetic rats.

Treatment	*Urine Glucose*	*Urine ketone bodies*
Normal control	±	±
Diabetic control	+++	+++
CGE 200mg/kg	++	+
CGE 400mg/kg	±	±
CGW 200mg/kg	++	+
$CGW 400mg/kg	+	+
Rosiglitazone 3mg/kg	+	±

n=5

For glucose : ± (100), +(250), ++(500), +++(1000)

For keton bodies: ± (5) +(10), ++(50), +++(100)

Estimation of Lipid parameters

At the end lipid parameters such as STG, STC, VLDL-c, LDL-c, HDL-c, TC/HDL-c and LDL-c/HDL-c ratios were measured. STG, STC, VLDL-c and LDL-c levels were significantly (P<0.001) increased whereas HDL-c was decreased in diabetic rats compared to normal rats.(Table 4) . The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were significantly elevated in the diabetic group. Both the doses of CGE and CGW exhibited significant reduction (P<0.001) in all the tested lipid parameters. (Table 4)

Table.4. Effect of CGE and CGW on Lipid Profile in STZ-induced model

Serum parameter	***Normal control***	***Diabetic control***	***CGE 200mg/kg***	***CGE 400mg/kg***	***CGW 200mg/kg***	***CGW 400mg/kg***	***Rosiglitazone 3mg/kg***
STG (mg/dl)	*63.3±3.5*	*182.7±9.3*	*129.7±8.2***	*101.7±3.7***	*131.3±9.4***	*109.14±11.6***	*75.1±3.8***
STC (mg/dl)	*61.3±2.7*	*125.3±2.5*	*8.4±4.4****	*73.4±1.2***	*81.8±3.6***	*76.3±2.2***	*61.9±4.1***
HDL-c (mg/dl)	*33.8±1.5*	*16.8±2.3*	*22.7±2.3*	*26.1±3.4*	*23.6±3.4*	*24.6±0.9***	*25.9±1.3*
VLDL-c (mg/dl)	*14.8±0.7*	*36.5±1.8*	*25.9±1.6****	*20.33±0.7****	*26.26±1.9****	*21.8±2.3****	*15.0±0.7***
LDL-c (mg/dl)	*16.3±3.0*	*71.9±5.3*	*31.6±6.0****	*26.8±2.4***	*32.4±5.9***	*24.7±3.6****	*21.0±2.9***
TC/HDL-c ratio	*1.8±0.1*	*8.08±1.2*	*3.6±0.3*****	*3.0±0.3*****	*4.0±0.8*****	*2.6±0.03*****	*2.4±0.08***
LDL-c/HDL-c ratio	*0.5±0.1*	*4.75±0.9*	*1.44±0.3*****	*1.1±0.2*****	*1.7±0.6*****	*0.8±0.1*****	*0.8±0.1*****

Each value represent Mean± S.E.M n=5 [a]P<0.05,[b]P<0.01,[c]P<0.001 compared to Diabetic control. One way ANOVA followed by Tukey's post test.

Total thiols

Basal total thiol levels in normal rats were found to be 4.09±0.14 μmoles/mg of protein. STZ- induced diabetic rats exhibited significantly decreased (p< 0.001) levels of total thiols (0.68±0.06 μmoles/mg of protein) in comparison to normal rats. Moreover, treatment with both doses of CGE, CGW and Rosiglitazone (3mg/kg) showed significantly (p< 0.001) increased levels.

Lipid peroxidation

Normal control rats showed basal TBARS levels of about 11.6±0.52 nmoles/g of liver tissue and diabetic rats showed significantly increase (p< 0.001) in TBARS levels (37.67±1.1 nmoles/g of tissue). Treatment with both the extracts CGE, CGW (200 and 400 mg/kg) and Rosiglitazone significantly (p< 0.001) abolished the increase in TBARS levels induced by STZ.

DISCUSSION

The present study was also designed to evaluate the effect of ethanolic and water extract of *cassia glauca* on late complications such as cardio-toxicity and nephro-toxicity of diabetic rats. It is well known that, STZ induced diabetes leads to cardiotoxicity which was manifested by increase in serum CK-MB and LDH level. It is already been reported that diabetic cardiotoxicity was associated with marked elevation of serum lipid profiles. In addition it has been reported that lipid lowering is an important factor in preventing diabetic cardiotoxicity. Our results showed that treatment with CGE and CGW (200 and 400mg/kg) to diabetic rats showed significant reduction in the elevated levels of lipid profiles such as triglyceride, total cholesterol, VLDL-c, LDL-c and dyslipidemic markers.

Electrocardiographic (ECG) abnormalities are the criteria used for the diagnosis of myocardial injury. Our study showed significant alteration of ECG patterns in diabetic rats as compared with normal rats such as reduction in P-wave, QRS complex and decrease in heart rate. These alterations could be due to the consecutive loss of cell membrane damage due to oxidative stress in myocardium. Treatment with CGE and CGW showed a protective effect against diabetic rats altered ECG pattern. This resulted in elimination of acute fatal complication by protecting the cell membrane damage.

Observed diabetic cardio-toxicity is mainly due to the formation of ROS and might have resulted from high susceptibility, high oxidative metabolisms and lesser antioxidants defense mechanisms in heart as compared to other organs. Lipid peroxidation is one of the most upshots of free radical mediated tissue injury and is an indicator for oxidative damage. Treatment of diabetic rats with different doses of CGE and CGW showed reduction in lipid peroxidation and enhanced levels of antioxidant enzymes. The present study showed treatment with CGE and CGW protected diabetic nephrotoxicity. This was evidenced by decreased serum creatinine, lipid profile and urea level, also by increasing albumin and total protein levels. Urine glucose and ketone body levels were reduced.

CONCLUSION

We conclude that the ethanol (CGE) and water (CGW) extract of *Cassia glauca* has potent antidiabetic, cardio-protective, nephro-protective, hypolipidaemic and antioxidant activity in STZ induced diabetic rats. Also collectively, these findings indicate that the lipid lowering and antioxidant effects of extracts may be an important contributor to its cardio-protective and nephro-protective potential. The present investigation has also opened avenues for further research especially with reference to the development of potent phytomedicine for treatment of diabetes mellitus and its complication from the title plant.

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