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Determination of antihyperglycemic activity of ethanolic crude leaf extract of cassia auriculata in the streptozocin induced male wistar albino rats

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Abstract*---Cassia auriculata*** is used extensively in the indigenous system of medicine as an anti-diabetic agent. The current investigation focuses on determination of antihyperglycemic activity of ethanolic crude leaf extract of *cassia auriculata (CA)* in the streptozocin induced male wistar *albino* rats. A single dose of (45mg/kg of body weight/day) STZ was administered to induce diabetes and the effect of ethanolic leaf extract of *cassia auriculata* on STZ induced diabetes were assessed based on glucose, plasma insulin, creatinine and urea, serum lipid profile, serum protein, albumin, globulin, serum Transminases and Alkaline phosphatase (ALP)] analysis.The ethanol extract of CA leaf elicited significant reductions in the STZ induced elevation in the biochemical markers. From the above results, it is concluded that ethanolic leaf extract of CA possesses significant antihyperglycemic effects in STZ induced diabetic rats.

***Keywords*---**antidiabetic activity, cassia auriculata, stz induced diabetic rats.

Introduction

Diabetes mellitus (DM)

Diabetes Mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both (Sicreeet al., 2006). The high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Conventionally, diabetes has been divided into three types namely: Type 1 DM or insulin-dependent diabetes mellitus (IDDM) in which body fails to produce insulin, and presently requires the person to inject insulin or wear an insulin pump. This is also termed as "juvenile diabetes". Type 2 DM or non-insulin-dependent diabetes mellitus (NIDDM), results from insulin resistance, a condition in which cells fail to use insulin properly, with or without an absolute insulin deficiency. This type was previously referred to as or "adult-onset diabetes". The third main type is gestational diabetes which occurs when a women without previous history of diabetes develop high blood glucose level during her pregnancy. It may precede development of type 2 DM. Various herbs have been used for medicinal purposes including the treatment of diabetes mellitus. One such medicinal plant widely used to manage diabetes is *Cassia auriculata* commonly known as “Tanner’s cassia” widely used in Indian folk medicine treat of diabetes mellitus. (Gray *et al.,* 2000)

Material and Methods

Sample Collection and Identification

Fresh leaves of *Cassia auriculata* were collected from Adhiparasakthi Agricultural college, kalavai, Tamil Nadu, India and the plant material was identified and authenticated Reg No: BSI/SRC/5/23/2018/3282 by Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu, India.

Soxhlet extraction

The collected leaves were washed and shade dried under ambient conditions in a sterile location devoid of moisture. The shade-dried leaves were made into a fine powder using a mechanical mixer and stored at −20^{0}C in an airtight container until further, Use 25 g powdered sample was dissolved in 250 ml distilled water and the extract was prepared by the hot percolation method using a Soxhlet apparatus. The crude filtrate was wholly dried at room temperature and the yield of the extract was determined to be 5.656 and stored at −20^{0}C in an airtight container until further use.

Experimental animals

Male *Albino* rats (Wister stain) were procured from King Institute of Preventive Medicine and Research at 6 weeks of age and housed in stainless steel screen bottom cages under standard conditions (12 h light/dark cycle, ambient temperature of (25 ±2 °C))in the animal house at Adhiparasakthi college of arts

and science, Kalavai, Vellore, Tamilnadu, India. The rat was allowed free access to normal pellet diet (NPD; 4.1%fat, 22.2% protein and 12.1% carbohydrates, as a percentage of total kcal) and water *ad libitum*. The rat was acclimatized to the laboratory environment for one week.

Induction of experimental diabetes

Adult male rats were made diabetic by single intraperitoneal (i.p) injection of Streptozotocin (STZ), dissolved in sterile citrate buffer (0.1 m,pH 4.5) at a dose 45mg/kg body weight, after overnight fasting to induce hyperglycemia (Yuill et al., 2015). Diabetes was confirmed in STZ-induced rats by measuring the fasting blood glucose concentration, 72h after injection. Rats with blood glucose level above 250mg/dL was considered as diabetic and used for the experiment.

Experimental Design

The rats were divided into seven groups comprising of six animals in each group as follows:

- Group I: Control rats administered orally with distilled water for 28 days.
- Group II: Normal rats administered orally with crude leaf extract *Cassia auriculata* (200 mg/kg) orally for 28 days
- Group III: STZ- induced diabetic rats (STZ 45 mg/kg) administered interperitoneally (i.p) single dose rats served as diabetic control.
- Group IV: STZ- induced diabetic rats treated orally with ethanolic crude leaf extract of *C. auriculata, at* the dose of 50 mg/kg.b.wt orally once a day for continuous 28 days.
- Group V: STZ- induced diabetic rats treated orally with ethanolic crude leaf extract of*C. auriculata* at the dose of 100 mg/kg.b.wt orally once a day for continuous 28 days.
- Group VI: STZ- induced diabetic rats treated orally with ethanolic crude leaf extract of C*. auriculata at* the dose of 200mg/kg.b.wt orally once a day for continuous 28 days.
- Group VII: Diabetic rat treated with glibenclamide (600 µg/kg) orally once a day for continuous 28 days.

Statistical Analysis

The values were expressed as mean value (n=6) of +S.E.M, The in vivo experimental data were analyzed using one way ANOVA and the significant mean($p < 0.05$) value were calculated using SPSS software version IBM SPSS statistics 26.0 (32 bit)

Biochemical analysis

The animals were sacrificed at the end of the experimental period of 28 days by decapitation. Blood was collected, sera was separated by centrifugation at 3000g for 10 minutes. Serum glucose was measured bythe O-toluidine method (Sasaki, et al., 1972). Insulin level was assayed by Enzyme-Linked Immunosorbent Assay (ELISA) kit (Anderson , et al., 1993).Urea estimation was carried out by the

method of (Varley, et al., 1976); serum creatinine was estimated by the method of (Owen., Iggo ., et al 1954). Serum total cholesterol (TC) (Parekh , et al 1954), totaltriglycerides (TG) (Rice , et al 1970), low density lipoproteincholesterol (LDL-C), very low density lipoproteincholesterol (VLDL- C), high density lipoprotein cholesterol (HDL-C) (Warnick ., et al 1985) and phospholipids (Takayama. ., et al 1977) were analysed. Serum protein 23 and serum albumins was determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives theglobulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel (Reitman and Frankel.,et al 1952). Serum alkaline phosphatase(ALP) was measured by the method of King and(Armstrong King. Armstrong., et al 1934)were analyzed in the normal, diabetic induced and drug-treated rats.

Result

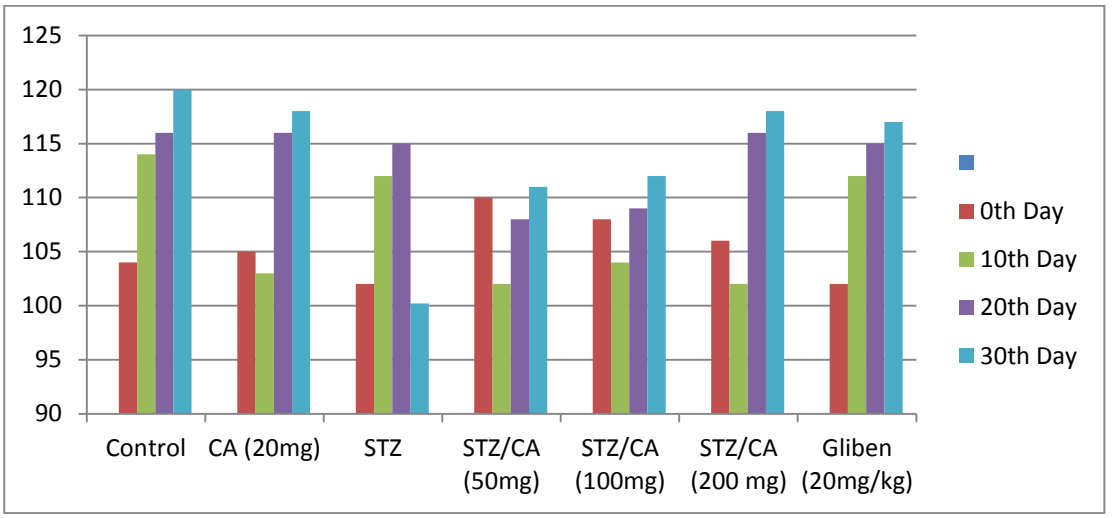

Figure 1. Effect of Ethanolic crude leaf Extract of CA on STZ induced Body Weight changes in Albino Rats

The figure shows the weight of the non-diabetic control group slightly increased throughout the study. In disease group animals, the initial body weight of the animals was decreased with the progression of the disease. On treatment with CA crude leaf extract, the rats gained their weight towards normal. The results disclose that the animals recovered from diabetes on treatment with CA

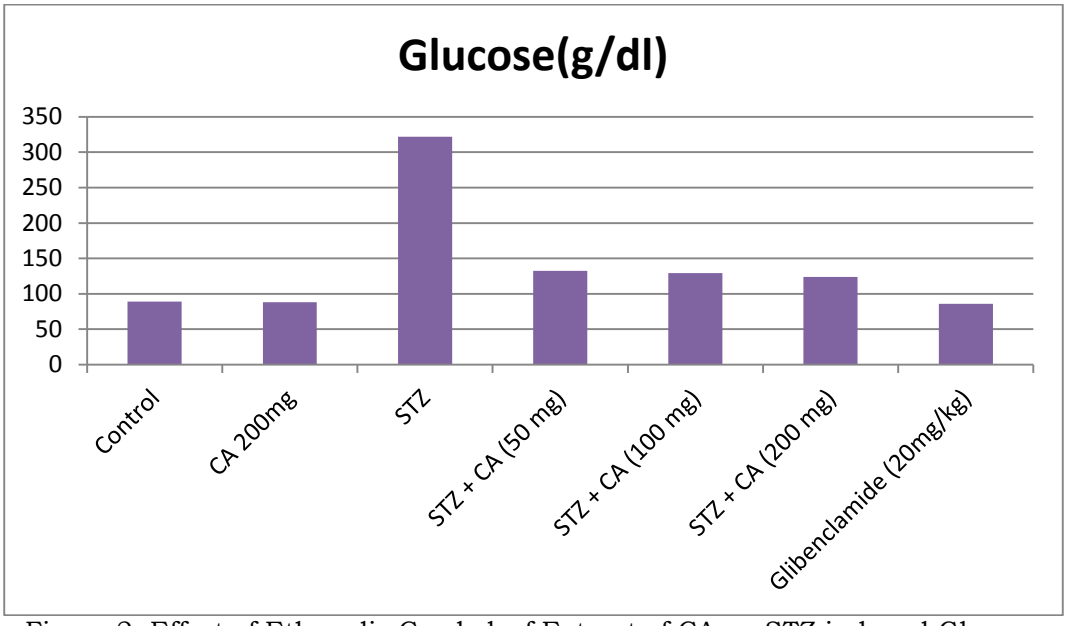

Figure 2. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Glucose changes in Albino Rats

The diabetic group showed significantly high blood glucose level compared to control group. On the other hand, treatment with crude leaf extract of CA significantly reduced the glucose level in treated rats.

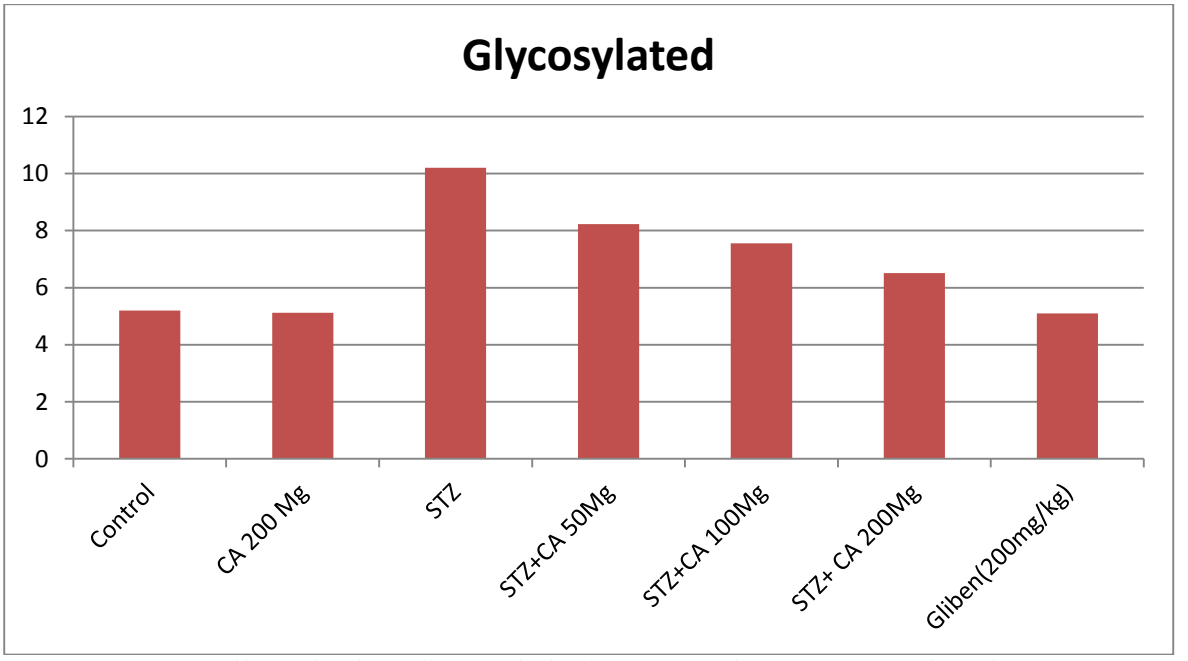

Figure 3. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Glycosylated Hb changes in Albino Rats

The diabetic group significantly reduced compared to the control group. On the other hand, treatment with crude leaf extract of CA significantly reduced the glycosylated haemoglobin level in treated rats.

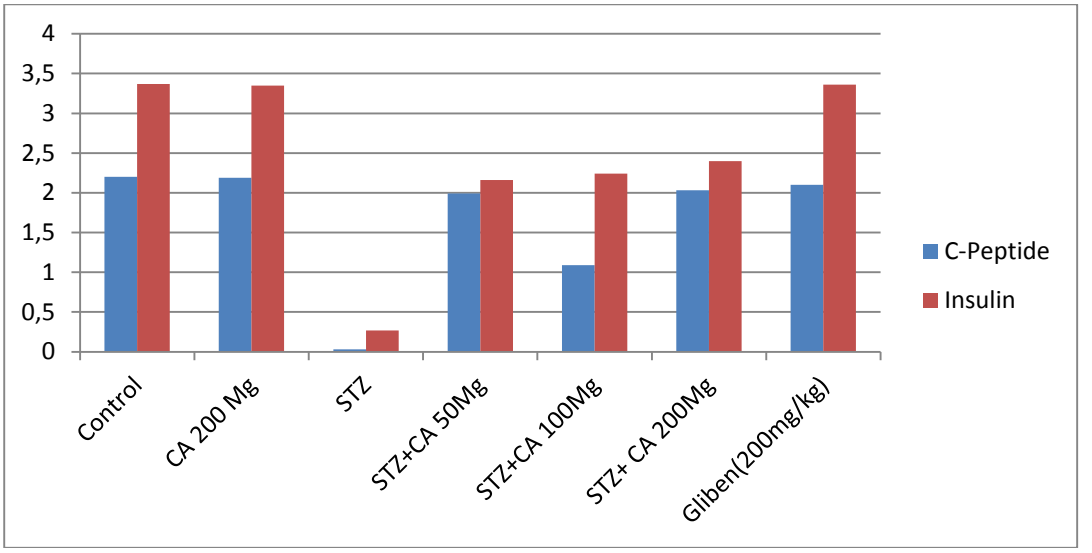

Figure 4. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Insulin and C-Peptide changes in Albino Rats

There was a significant elevation in blood glucose while the insulin level decreased significantly along with C-peptide in STZ induced diabetic rats compared with normal rats. Serum C-peptide levels showed a significant reduction in the diabetic group while the crude leaf extract *CA crude leaf extract* treated groups showed opposite response significantly in insulin & c-peptide levels confirming the antidiabetic potential of *CA*.

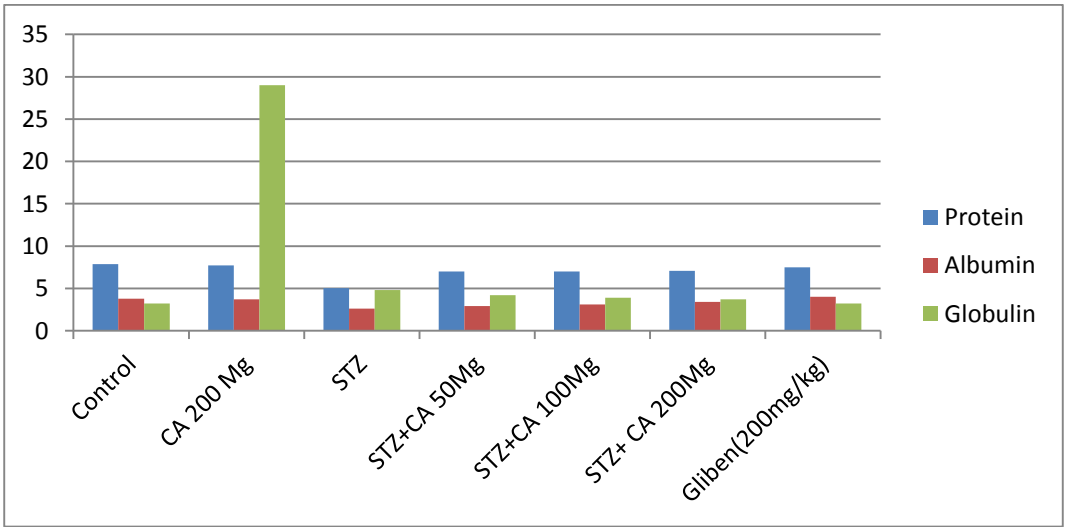

Figure 5. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Total Protein, Albumin and Globulin changes in Albino Rats

Administrated STZ decreased total protein along with Albumin and globulin and diabetic rats treated with crude leaf extract of CA showed an increase in protein, Albumin and Globulin levels return toward normal.

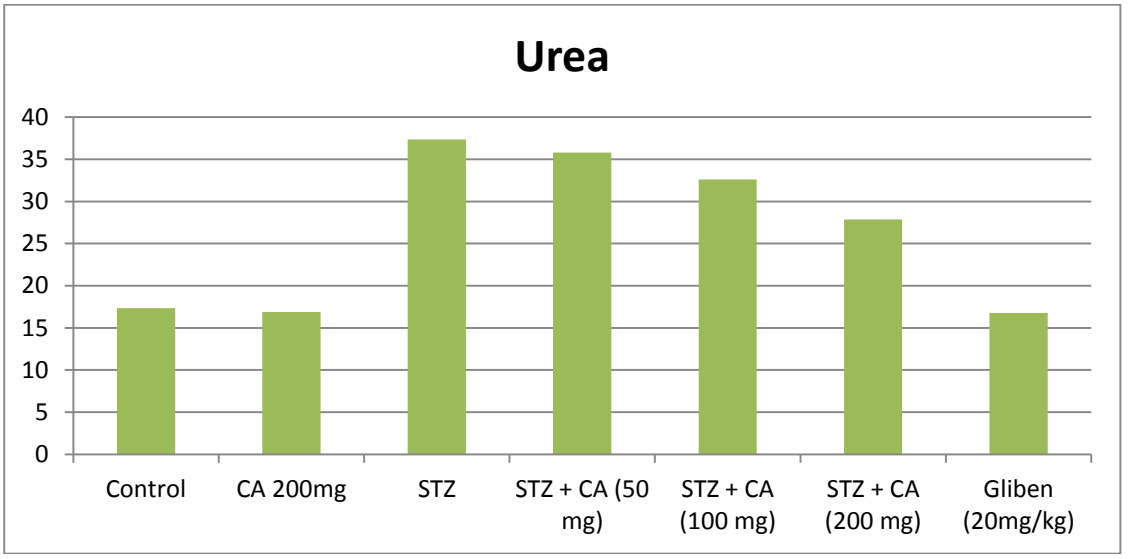

Figure 6. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Serum Urea changes in Albino Rats

There was a significant elevation in urea levels, while the level increased significantly in STZ induced diabetic rats are compared to normal rats whereas treatment crude leaf extract CA restored towards normal.

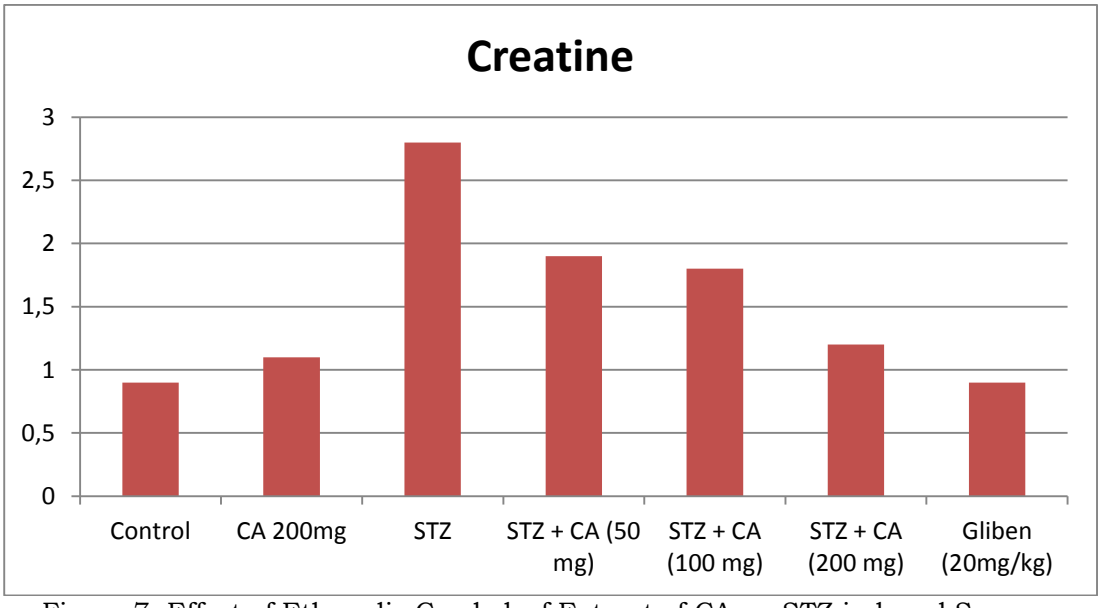

Figure 7. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Serum Creatinine changes in Albino Rats

There was a significant elevation in creatinine levels were the significantly increased in STZ induced diabetic rats are compared to normal rats whereas treatment crude leaf extract CA restore back to normal.

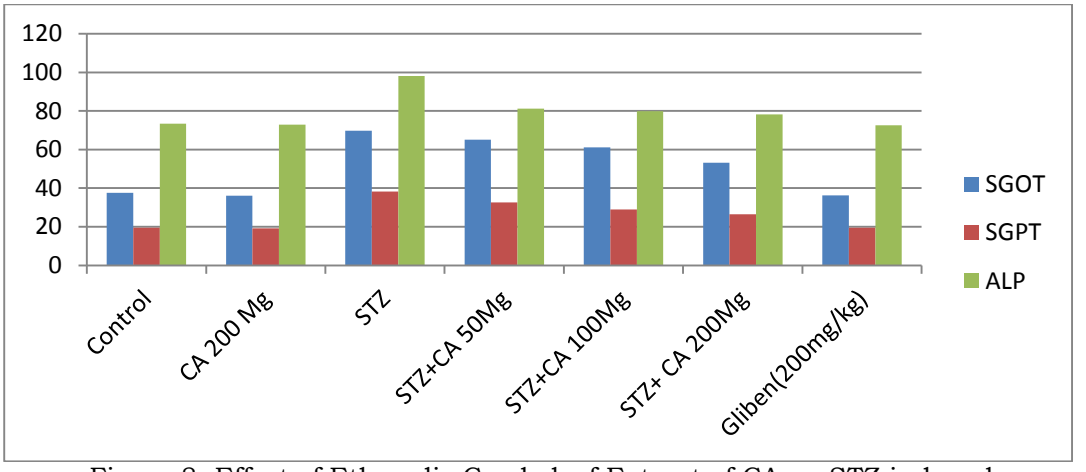

Figure 8. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Transaminases and ALP changes in Albino Rats

The animals treated with STZ developed hepatic damage which was evident from the increase in the enzyme activities. On the other hand treatment with ethanol extract of *c.auriculata* result in a decrease transaminase activities in STZ treated rats. The serum ALP, AST and ALT levels increased as a result of metabolic changes and the effect of ethanolic extract of CA evidenced by the decrease in the enzyme activities

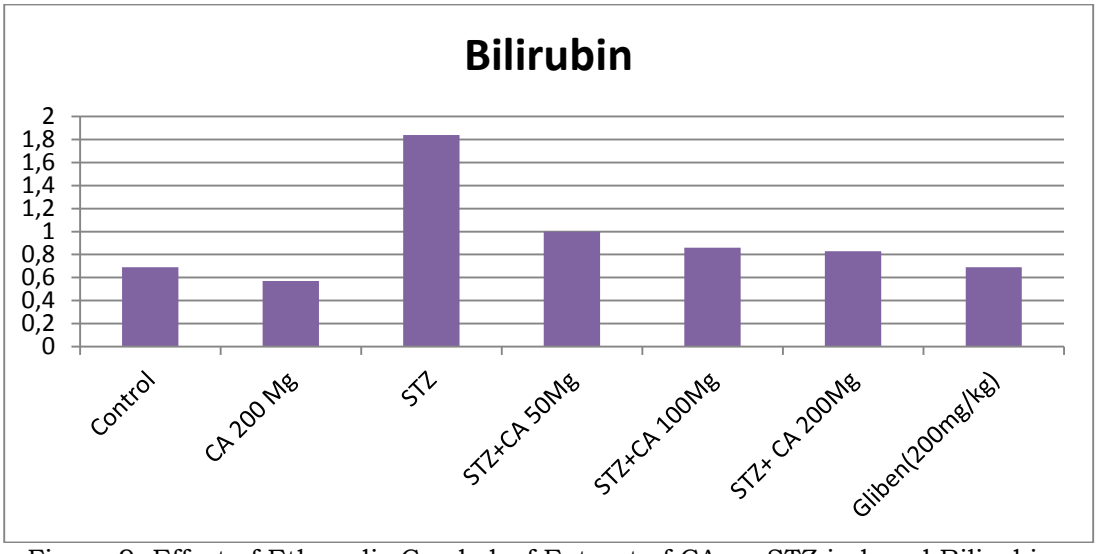

Figure 9. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Bilirubin changes in Albino Rats

The increased level of serum bilirubin in STZ induced diabetic rats whereas treatment with ethanolic extract of CA showed a marked reduction in the level of total bilirubin.

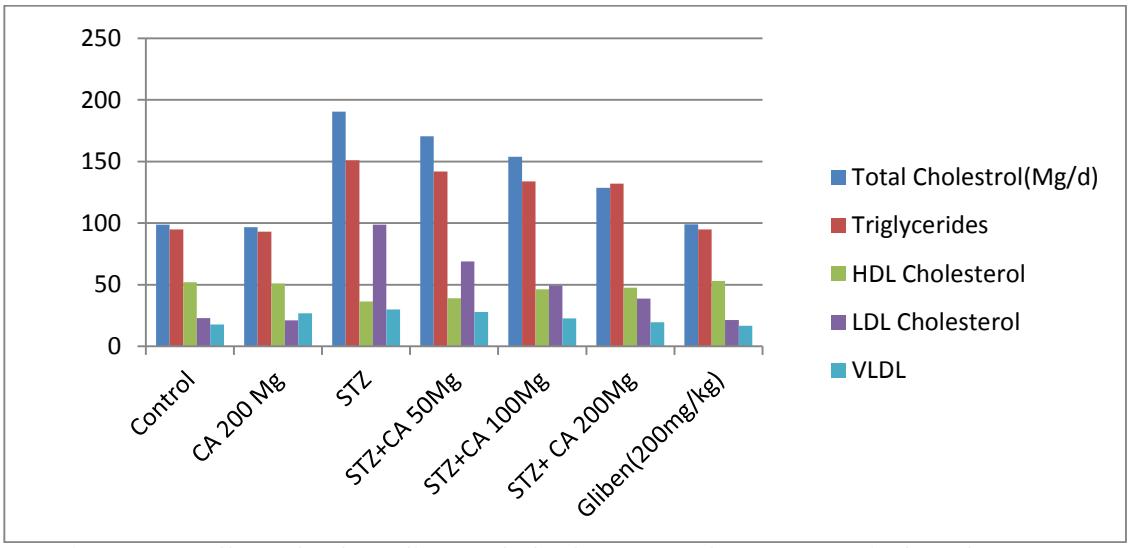

Figure 10. Effect of Ethanolic Crude leaf Extract of CA on STZ induced Serum Lipid profile changes in Albino Rats

In our study there was a significant increased level of serum total cholesterol (TC), Triglycerides (TG),LDL-C,VLDL but markedly decreased level of HDL-C in STZ induced diabetic rats whereas treatment with crude leaf extract to prevent the hypercholesterolemia and hypertriglyceridemia reducing cardiovascular risk factors.

Discussion

In this present study, diabetes was induced with a combination of a high fat diet and a low dose of streptozotocin (STZ) and the effect of the *CA* leaf extracts was evaluated. The change in the body weight of rats of different groups is presented in the diabetic group significantly reduced. In contrast, the treatment with the *CA* leaf extract could help regain the weight much better than the *Cassia auriculata* - treated group. In the STZ, a beta cytotoxin, destroys β- cells of islet of Langerhans of the pancreas resulting in a decrease in endogenous insulin secretion and paving the ways for the decreased utilization of glucose by the tissue (Omamoto and Ucgigata., 1981). It results in an elevation of blood glucose levels. Expression of elevated fasting blood glucose level confirmed induction of diabetes in STZ-induced experimental rats. This study indicates that administration of STZ increased the blood glucose level and oral administration of CA ethanolic leaf extract restored the glucose levels significantly to the normal similar to control rats.

Javari et al., 2008 reported earlier that treatment of Diabetic rats with ethanolic leaf extract of CA for 28 days brought down the elevated blood glucose levels towards normal range. The study shows that *Cassia auriculata*

is able to bring down blood glucose levels considerably; hence it was useful in the management of diabetes. This glucose lowering effect may be due to activation of β- cells and granulation returns to normal giving insulinogenic effect (Padmini and Chakrabarti., 2002). Perhaps CA bring about its hypoglycaemic action through stimulation of surviving pancreatic β- cells to release more insulin. This is clearly evidenced by the increased level of serum insulin in diabetic rats treated with CA.

In the current investigation it was also observed that a decrease in total haemoglobin during diabetes was attributed to increased formation of glycosylated haemoglobin. On the other hand Increase in the level of haemoglobin was noted upon the administration with ethanolic leaf extract of CA and simultaneously decreased blood glucose and glycosylated haemoglobin levels (Bopanna et al., 1997). In the present study, serum insulin and C-peptide level in STZ-induced diabetic animals were decreased at the same time the drop in the circulating insulin and C-peptide levels in STZ-induced diabetic animals restored back to normal levels after the treatment with CA leaf extract. Insulin is synthesized as a precursor molecule, proinsulin and in the β- cells of the pancreatic islets of Langerhans. Proinsulin is cleaved to give, comprised of 2polypeptide chains, A(21 amino acids) and B (30 amino acids), and C-peptide a single chain of 31 amino acids. When insulin is released in response to elevated glucose levels, equal amounts of C-peptide level is increased. In this respect, we can come into a conclusion that the damaged β- cells are regenerated back by the treatment of CA leaf extract.

Administration of STZ decreased protein level in the diabetic rats treated with CA leaf extract showed an increase in protein level, albumin and globulin level returns toward normal (Maruthupandian., Mohan .,et al 2011). In the present study, the administration of ethanolic leaf extract of CA on STZ induced diabetic rats reduced AST and ALT levels efficiently. In addition to the assessment of AST and ALT level during diabetes, the measurement of enzymatic activity of alkaline phosphatases (ALP) is of clinical and toxicological important as changes in their activities are indicative of tissue damage by toxicants (Somnath Singh *et al.,* 2001). In our study, ALP increased considerably in STZ-induced diabetic rats. Elevated level of the above enzymes in diabetes may be due to extensive damage to liver in the experimental animals by STZ. Treatment with *Cassia auriculata* Leaf extract in STZ-induced diabetic rats produce decline in these levels than the treated rats (Stanley *et al.,* 1999). From the present observation, it was evident that *Cassia auriculata* Leaf ethanol extract protects the adverse effect of STZ on liver.

Estimation of bilirubin, metabolic product of the breakdown of heme is one of the better liver function tests. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in hemolysis and defects of hepatic uptake and conjugation of bilirubin treatment such as Gilbert's disease. It is secreted by the liver and stored in gall bladder. In the present study, STZ induced diabetic animals showed a marked increase in the level of bilirubin; whereas the treatment with *Cassia auriculata* showed a marked reduction in the

levels of total bilirubin. It was due to hepatic healing effect of *Cassia auriculata*. (Maruthupandian., Mohan .,et al 2011).

Similarly, kidney dysfunction was also observed only in diabetic rats in this study as reported by previous studies. It showed significantly increased levels of urea and creatinine, indicating kidney damage in diabetic rats. The prolonged hyperglycemia in the diabetic condition is known to damage the kidney and leads to diabetic nephropathy. As an indication of kidney dysfunction, there was a significant increase in the plasma concentration of urea and creatinine in diabetic rats compared to control whereas treatment with *Cassia auriculata* leaf extract suppressed effect occurred due to STZ administration. (Nikkila, Kekki., et al 1973) Cholesterol is structural component of cell membrane and an important from in which lipoprotein are transported in the body. In our study, there was a significant increase in the level of serum total cholesterol, triglycerides and LDL-cholesterol but markedly decreased level of serum HDL-cholesterol in STZ induced diabetic rats. Increase in TC, LDL, VLDL Cholesterol and TG while decrease in HDL cholesterol has been reported in diabetes and all these factors contribute to the coronary artery disease (Arvind et al., 2003). Several studies propose the most of the drugs that decrease total cholesterol also decrease HDL cholesterol. In the present study, *Cassia auriculata* effectively reduced TG possibly by decreasing the non-esterified fatty acids in diabetic rats. The significant control on serum lipids of diabetic animals by *Cassia auriculata* leaf extract to prevent simultaneous coexistence of hypercholesterolemia and hypertriglyceridemia reducing cardiovascular risk factors and supported by the finding if Murali et al., 2002.

Conclusion

Medicinal plants have been reported to possess antihyperglycemic activity; *cassia auriculata* leaf is gaining much importance in diabetic control as it has been used as a traditional medicine for diabetes. The preliminary investigation on the antidiabetic efficacy of ethanolic leaf extract of *Cassia auriculata (CA)* proved and will be significant to proceed further in this path for the isolation of active principles components responsible for antidiabetic activity.

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