Original Article

Cassia auriculata Flower Extract Articulate its Antidiabetic Effects by Regulating Antioxidant Levels in Plasma, Liver and Pancreas in T2DM Rats

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ABSTRACT

Objectives: The present study was designed to explore the protective effect of *Cassia auriculata* L. flower extract (CAE) in high fat diet and streptozotocin induced type 2 diabetic (T2DM) rats.
Methods: T2DM was induced by a combination of high fat diet and low dose streptozocin. Rats in different groups were treated with *Cassia auriculata* L. flower extract at two different doses viz. 300mg and 500mg/Kg body weight and the hypoglycemic potential as well as lipid lowering and antioxidant properties of CAE in liver and pancreas were evaluated.
Results: T2DM rats showed significantly elevated glucose and reduced c-peptide levels in serum. Also there was significant increase in serum marker enzymes of liver toxicity-alanine transaminase (SGPT), aspartate transaminase (SGOT) and alkaline phosphatase (ALP) along with significant reduction in liver glycogen and increase in lipid peroxidation levels. There was also deregulation in lipid levels in plasma and liver and significant reduction in antioxidant enzymes in plasma, liver and pancreas. Encouragingly, treatment with *Cassia auriculata* extract caused significant improvement in the glucose, insulin, lipid levels in plasma and the antioxidant status of liver and pancreas.
Conclusion: Out of the two doses of CAE used in this study, 500mg/kg b.w dose was found to be more effective in regulating the levels of antioxidants and lipid levels in plasma, liver and pancreas indicating its potential to ameliorate peripheral insulin resistance.

Keywords: Type 2 diabetes mellitus, *Cassia auriculata*, Streptozotocin, Antioxidants, Lipids.

INTRODUCTION

Diabetes mellitus is basically a metabolic disorder associated with excess accumulation of glucose in blood. The two main forms of diabetes are type 1 or insulin-dependent diabetes mellitus (IDDM) and type 2 diabetes or non-insulin-dependent diabetes mellitus (NIDDM)[1]. Both types are characterized by progressive β-cell failure. In type 1 diabetes, this is typically caused by an autoimmune assault against the β-cells inducing cell death. Type 2 diabetes (T2DM) occurs when the pancreatic β-cell fails to adapt to the increased insulin demand induced by peripheral insulin resistance in liver, muscle and adipose tissue that culminates in chronic hyperglycemia[2,3]. It is the loss of β-cell function and/or mass that eventually defines the disease. Chronic hyperglycemia can cause oxidative stress, leading to defective insulin secretion[4,5]. Changes in lifestyle such as consumption of high-calorie diet and lack of physical exercise have contributed to the increased global prevalence of obesity as well as T2DM. About 60-90% of cases of T2DM are related to obesity[6]. Increasing incidence of T2DM worldwide necessitates the implementation of alternative therapies based on herbal medicine as plant-based medicines are believed to be safe, economic, and effective[7]. Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety[8,9]. Some of the plants which are being used for the treatment of diabetes have received scientific scrutiny. One such traditional plant is *Cassia auriculata* commonly called Tanner's Cassia in English and in Tamil as "Avarai". *C. auriculata* (family: *Cesalpinaceae*) is an evergreen shrub that grows in many parts of India and in other parts of Asia. The flower, leaves, stem, root, and unripe fruit are profoundly used in Ayurvedic medicine as a remedy for diabetes, conjunctivitis, joint and muscle pain (rheumatism), opthalmia, jaundice, liver disease, and urinary tract disorders[10]. A number of constituents such as flavonoids, polysaccharides, anthracene derivatives, dimeric procyanidins, and β-sitosterol have been reported in various plant parts of *C. auriculata*[11].

The present investigation was designed to evaluate the hypoglycemic, hypolipidemic and antioxidant properties of ethanolic extract of *Cassia auriculata* flowers in High fat diet (HFD) and Streptozotocin (STZ) induced diabetic rats along with assessing metabolic changes in liver and pancreas. The effect was compared with Glibenclamide, a well known hypoglycemic agent.

MATERIALS AND METHODS

Streptozotocin was purchased from Sigma-Aldrich, All other chemicals and solvents were of the highest analytical grade.

Experimental animals

Male Wistar strain rats of age 8-10 weeks and with body weight 150-200g were purchased from Sri Ragavendra animal suppliers, Bangalore, India. The animals were maintained in the animal house facility of Pondicherry University, in accordance with the guidelines of Committee for the purpose of Control and supervision of Experiments on Animals (CPCSEA), Govt. of India. The animals were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature (22+2^{o}C) and humidity (55+5%) with 12:12 h lights and dark cycle. All rats were allowed for acclimatization for two weeks. The present work was carried out with the approval from Institutional

Animal Ethical Committee, Pondicherry University (1159/C/07/CPCSEA).

High fat diet

HFD was prepared with the following constituents – powdered NPD (365g), Lard (310g), casein (100g), Yolk(100g), Butter (100), vitamins (10g) mineral mix (10g), Methionine (3g), Yeast (1g) & NaC1 (1g).)

Cassia auriculata flowers were purchased from local market, Puducherry. The flowers were dried under shade and were finely powdered in a mixer.

Preparation of plant extract

Ethanolic extract of *Cassia auriculata* flowers (CAE) was prepared by mixing 50g of *Cassia auriculata* flower power with 250 ml of 95% ethanol and extracted in soxhlet apparatus for 6 hours. The residue was re-extracted twice under the same condition to ensure the complete extraction and the combined solvent was evaporated by rotary evaporator, lyophilized and stored at -70 ^{o} C and later used for determination of phytoconstituents and for feeding the experimental animals.

Induction of Diabetes in Rats

T2DM was induced in male wistar by feeding high fat diet (HFD) for four weeks followed by administration of streptozotocin (STZ) at a dose of 35mg/Kg body weight (BW) by a single intraperitonial injection (ip) in 0.1 M citrate buffer (pH 4.5) to overnight fasted rats. The animals were continued on HFD for next four weeks[12].

Quantitative determination of the phytocompounds

Total Flavonoid Content in CAE was determined by the aluminum chloride colorimetric assay method[13]. Total flavonoid content of the extract was expressed as percentage of quercetin equivalent per 100 g dry weight of sample. The total phenol content of CAE was measured at 765 nm by using Folin Ciocalteu reagent according to the method of Slinkard and Singleton[14] and the alkaloid content was determined by the method described by the method described by Harbone[15].

Experimental Design

The animals were divided into 5 groups of 6 rats each (n=6) and the experimental duration was for 8 weeks. Rats in Group 1 were given normal pellet diet (NPD) which served as control group; Group 2 rats were given HFD for first 4 weeks followed by single intraperitoneal administration STZ on 28th day at 35mg/Kg b.w and the animals were continued on HFD for the next 4 weeks; this group served as the T2DM model. Rats in Groups 3 and 4 were induced with T2DM as in Group 2 and were treated orally daily with 300mg and 500mg/Kg b.w of CAÉ respectively from the 3rd day after STZ administration for 4 weeks. Groups 5 animals were induced with T2DM as in Group 2 and treated orally with 5mg/ Kg b.w of Glibenclamide, a standard antidiabetic drug for 4 weeks. At the end of the experimental period, the rats were euthanized by cervical dislocation after an overnight fasting and immediately opened surgically. Blood was collected from heart and stored at 4^{0}C. Plasma and serum were separated.

Methods

Blood glucose was estimated by GOD-PAP method (AGGAPE-diagnostics). Serum GOT, GPT, ALP levels and the levels of Cholesterol, HDL and LDL in plasma and liver were determined by using diagnostic kits (AGGAPE-diagnostics). Serum C Peptide level was estimated by using ELISA kit (Dia Source). Liver glycogen level was quantified by the method of Plummer[16] and triglyceride level by GPO-

PAP method (AGGAPE-diagnostics). Major markers of oxidative stress including Lipid Peroxidation (LPO)[17], reduced glutathione (GSH)[18], superoxide dismutase (SOD)[19], catalase[20] and glutathione peroxidase[21] were analyzed in plasma, liver and pancreas tissue homogenates (10%) using standard procedures.

Statistical Analysis

Statistical analysis was performed by one way ANOVA followed by Tukey's test using SPSS software version 7.5.

RESULTS

Phytocompounds in CAE

Quantitative determination of major phytocompounds in ethanolic extract of *Cassia auriculata* (CA) flowers showed that the flavonoid, phenol and alkaloid content in 100g of flower as 21.1g, 9.2g and 6.66g respectively.

Serum levels of Glucose, c-peptide, and liver marker enzymes and liver glycogen levels

The diabetic group showed significant increase in blood glucose levels when compared to control group ($P \leq 0.05$). On the other hand, treatment with CAÉ caused significant reduction in the glucose levels at both the concentrations used in this study (Fig 1). CAÉ at the dose of 500mg/Kg b.w produced well pronounced hypoglycemic effect by restoring the glucose levels to normal range and its effect was comparable to that caused by the standard hypoglycemic agent-glibenclamide used in this study (Table-1). Serum C-peptide levels showed significant reduction ($P \leq 0.05$) in diabetic group while the treated groups showed significant elevation in c-peptide levels (Table-1) confirming the antidiabetic potential of *Cassia auriculata* flowers. Glibenclamide also produced a similar positive effect. The serum levels of liver marker enzymes alanine transaminase (SGPT), aspartate transaminase (SGOT) and alkaline phosphatase (ALP) were also significantly elevated in diabetic group as compared to control indicating diabetes associated liver damage. The total liver glycogen content also was found to be significantly decreased in diabetic group when compared to control group (Fig. 2). Grippingly, treatment with CAE regulated the levels of liver marker enzymes and caused significant increase ($P \leq 0.05$) in glycogen levels (Table-1).

Lipid levels in Serum and Liver

The cholesterol levels in T2DM rats (Fig.3) were found to be significantly elevated ($P \leq 0.05$) in serum (Fig.3a) as well as in liver (Fig.3b). Treatment with CAE at both 300 and 500 mg/kg b.w concentrations caused significant reduction in serum as well as liver cholesterol levels. Glibenclamide treatment also caused significant reduction in cholesterol levels. Of the two doses of CAE used, the dose at 500mg/kg b.w was found to be more effective and produced maximum reduction in cholesterol levels (Fig.3a & 3b). HDL levels in serum and liver are presented in Fig.4. Serum HDL levels were significantly reduced in diabetic group when compared to control group. Treatment with CAE caused significant improvement in serum HDL levels at both doses used in this study ($P \leq 0.05$). However, CAE at 500mg/kg b.w concentration caused remarkable increase in serum HDL levels (Fig. 4a). Glibenclamide treatment also showed significant increase in serum HDL levels. Liver HDL levels were found to be elevated in diabetic group when compared to control animals and CAE treatment regulated the liver HDL levels. Glibenclamide treatment also showed similar effects (Fig 4b). The serum and liver LDL levels are presented in Fig. 5 which

demonstrates significantly elevated LDL levels in diabetic group (P≤0.05) as compared to control group. The serum LDL levels were restored to control levels in groups treated with CAE and glibenclamide (Fig. 5a). Liver LDL levels were also regulated by CAE and glibenclamide (Fig. 5b). CAE at 500mg/kg b.w concentration was more effective and produced maximum reduction in serum & liver LDL levels. The triglyceride levels in serum and liver are presented in Fig. 6a and 6b respectively which indicates significantly elevated TG levels in diabetic group as compared to control group. Treatment with CAE at the dose of 500mg/kg b.w showed significant reduction in serum TG levels (P≤0.05) and restored liver TG levels to control levels. Glibenclamide treatment also produced pronounced reduction in liver TG levels (Fig 6b).

Lipid peroxidation (LPO) levels in Liver

Liver LPO levels were significantly elevated (P≤0.05) in T2DM rats as compared to that of control animals (Fig.7). Treatment with CAE resulted in significant reduction in liver LPO levels at both the concentrations used in this study (P≤0.05) and CAE at the dose of 500 mg/kg b.w produced more pronounced effect. Glibenclamide also induced significant reduction in LPO levels (Fig.7).

Antioxidant levels in liver, pancreas and plasma

Catalase

Catalase activity in plasma, liver and pancreas is shown in Fig 8. In diabetic animals the catalase activity was found to be significantly decreased as compared to control group (Fig 8a, 8b & 8c). Treatment with CAE induced significant enhancement in catalase activity in plasma and liver at both the doses used in this study (P≤0.05). CAE at the dose of 500 mg/kg b.w effectively enhanced the plasma catalase levels comparable to that of control values. Glibenclamide also produced similar effects (Fig 8a). Catalase activity in liver and pancreas was also found to be increased significantly (P≤0.05) in 500mg CAE treated group as well as in glibenclamide treated group (Fig 8b &8c).

Superoxide Dismutase (SOD)

SOD activity of in plasma, liver and pancreas is presented in Fig. 9. Significant reduction in SOD activity in plasma, liver and pancreas (P≤0.05) was observed in diabetic animals when compared to control rats (Fig. 9a 9b & 9c). CAE at the dose of 500 mg/kg b.w caused significant improvement in SOD activity in plasma, liver and pancreas and its effect was comparable to that of glibenclamide (Fig. 9a 9b & 9c).

Glutathione Peroxidase (GPx)

Glutathione peroxidase activity in plasma, liver and pancreas is presented in Fig. 10. Significant reduction in of GPx activity was observed in plasma, liver and pancreas in diabetic rats (P≤0.05) when compared to control animal (Fig. 10a, 10b &10c). Treatment with CAE significantly enhanced the activity of GPx in plasma and pancreas (Fig. 10a &10c) at both concentrations of CAE used in this study (P≤0.05) but did not produce any improvement in liver GPx activity. Glibenclamide restored the activity of GPx in plasma and pancreas to control values (Fig. 10a &10c). CAE at the dose of 500 mg/kg b.w caused also produced similar effect in plasma (Fig. 10a).

Reduced Glutathione (GSH)

Significant reduction in the levels of reduced glutathione was observed in the plasma, liver and pancreas of diabetic rats

when compared to control group (Fig 11). CAE at the dose of 500 mg/kg b.w and glibenclamide caused significant increase ($P \leq 0.05$) in the levels of GSH (Fig.11 a, 11b &11c).

DISCUSSION

Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in the metabolism of carbohydrate, fat and protein and an associated deficiency of insulin secretion. Over-nutrition and physical inactivity promote insulin resistance. Pancreatic beta cells are constantly stimulated to cope with this increased demand of insulin secretion. When beta cells fail to maintain sufficient insulin secretion frank diabetes ensues. Several mechanisms have been proposed for increased β-cell apoptosis in type 2 diabetes, including glucose toxicity[4], oxygen free radicals[5], free fatty acid toxicity[22] and ER stress[23]. Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. In the present study, a combination of high fat diet and low dose streptozotocin (STZ) administration (35mg/kg body weight) was used to induce Type 2 diabetes (T2DM) in Wistar rats that would mimic natural incidence of the disease in humans[12]. Many medicinal plants have been shown for potential anti-diabetic activity with the potential for lowering glucose as well as modifying lipid profile[24]. The antidiabetic and lipid lowering effects of different parts of *Cassia auriculata* plant has been shown in experimental animals by earlier workers[25-29.] The present study was an endeavor in the direction of assessing the protective effect of ethanol extract of *Cassia auriculata* flower (CAE) against the metabolic changes in liver and pancreas that occurred during the onset of Type 2 diabetes. CAE was used at two different concentrations in this study viz. 300mg and 500mg / kg b.w of animals and Glibenclamide was used as positive control.

Earlier reports have shown that the serum levels of ALP, AST and ALT levels normally will increase due to metabolic changes in the liver in conditions like administration of toxin, cirrhosis of the liver, hepatitis and also in diabetes[30]. In the present study also elevated serum levels of ALP, AST and ALT and reduced glycogen content in liver were observed in diabetic animals but this condition was reversed in animals treated with CAE with regulated levels of serum liver marker enzymes as well as enhancement of liver glycogen content suggesting the protective effect of CAE against T2DM induced liver damage. Our findings are in agreement with similar observations made by earlier workers diabetic rats treated with *Cassia auriculata* flower extract[31,32]. Of the two doses of CAE used in the present study, CAÉ at the dose of 500mg/Kg b.w was found to be more effective in regulating the blood parameters.

Abnormal high concentration of serum lipids in diabetic condition is mainly due to increased mobilization of free fatty acids from peripheral depots due to activation of hormone sensitive lipase during insulin insufficiency. Excess of fatty acids in serum promotes conversion of excess fatty acids to phospholipids and cholesterol in liver. Type 2 diabetes is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced HDL cholesterol, a predominance of small dense LDL particles, and elevated triglycerides[33]. These changes are also a feature of the insulin resistance syndrome (also known as the metabolic syndrome), which underlies many cases of type 2 diabetes. In fact, pre-diabetic individuals often exhibit an atherogenic pattern of risk factors that includes higher levels of total cholesterol, LDL cholesterol,

and triglycerides and lower levels of HDL cholesterol than individuals who do not develop diabetes[34]. Observations made in the present study of elevated levels of cholesterol, triglyceride and LDL in serum as well as in liver in diabetic group correlates with the above report. Interestingly, treatment with *Cassia auriculata* flower extract restored the lipid levels in plasma and liver particularly at the concentration level of 500mg/Kg BW. Similar observations on regulation of elevated levels of cholesterol and triglycerides in serum and peripheral tissues by *Cassia auriculata* flower extract have been reported in experimental diabetes [25,28,31]. In another report ethanolic extract of *Cassia auriculata* flowers significantly reduced the total cholesterol (TC), triglycerides (TG) and low-density lipoprotein-cholesterol (LDL) levels and significantly increased the high-density lipoprotein (HDL) level in Triton WR1339-induced hyperlipidemic rats[35].

An increasing body of evidence suggests that free radical formation and oxidative stress are involved in the pathogenesis of diabetes and the development of diabetic complications[36,37]. There are many ways by which hyperglycemia may increase free radical generation, such as glycoxidation, polyol pathway, prostanoid biosynthesis, and protein glycation[38]. Hyperglycemia can lead to both a rise in ROS (reactive oxygen species) production and to the attenuation of free radical scavenging compounds[39]. Under normal physiological conditions, a wide range of enzymatic and non-enzymatic antioxidants protect the body from the adverse effects of free radicals that are produced *In vivo*[40]. Lipid peroxidation (LPO) is a free radical mediated process that results in oxidative deterioration of polyunsaturated lipids but the levels of lipid peroxides in plasma and tissues are maintained at low concentrations under normal physiological conditions by natural cellular antioxidants. But free radicals are generated more in diabetes and insufficient antioxidant defense mechanisms have been reported in diabetes[41]. There is also ample evidence that elevation in glucose concentration may depress natural antioxidant defense such as GSH[42]. Glutathione is a significant component of the collective antioxidant defenses and a highly potent antioxidant and antitoxin. It is an important water phase antioxidant and essential cofactor for antioxidant enzymes. It is critical for cell function and cell survival. Without the presence of glutathione other antioxidants are depleted even more quickly[43]. The activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase which is low in islet cells when compared to other tissues becomes further worsened under diabetic conditions[44]. Earlier reports have shown apoptosis of β-cells by glycation mediated reactive oxygen species[45]. Increased LPO levels in liver along with decreased levels of glutathione and antioxidant enzymes like SOD, catalase and glutathione peroxidase in plasma, liver and pancreas observed in the present study in T2DM rats indicated oxidative stress in diabetic condition. The *In vivo* antioxidant potential of CAE was indicated by its ability to increase the levels of SOD, Catalase, GPx and reduced glutathione levels in plasma, liver and pancreas. Of the two different concentrations of *Cassia auriculata* extract used in this study 500mg treatment was more effective in restoring the antioxidant levels. Our findings are supported by previous reports on the antioxidant activity of extract of *C. auriculata* flowers[46,47]. Several authors have reported that flavonoids, sterols/terpenoids, phenolic acids are known to act as antidiabetic bioactive principles[48-50]. Flavonoids are

known to regenerate the damaged beta cells in the diabetic rats. Phenolics are found to be effective anti-hyperglycemic agents. In the present study also the phytochemical analysis of ethanol extract of *Cassia auriculata* flowers clearly indicated abundant presence of flavonoids and phenolic acids.

CONCLUSION

Data obtained in this study point to potent hypoglycemic, lipid lowering as well as antioxidant properties of *Cassia auriculata* flowers which may be attributed to the its rich flavonoid and polyphenol content. These observations indicate that inclusion of *Cassia auriculata* flowers in the diet may bring about beneficial effects by delaying the onset of T2DM as well as reducing the complications associated with it.

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Table 1. Serum levels of glucose, C-peptide and liver marker enzymes

Groups/ Parameters	Glucose (mg/dl)	C-Peptide pmol/ml	SGOT U/L	SGPT U/L	ALP U/L
Control	123.3 ± 13.8[a]	0.81±0.1[a]	40.13±2.46[a]	40.21± 2.46[a]	179.9±1.75[a]
T2DM	378.8 ± 0.42[b]	0.39±0.02[b]	101.9±6.29[b]	155± 2.46[b]	215.8±1.944[b]
300 mg CAE	215.6± 12.55[c]	0.51±0.09[c]	35.7±1.23[c]	67.18± 3.70[a]	195.25±3.88[c]
500 mg CAE	118.9 ± 7.53[a]	0.76±0.19[d]	14.8±1.23[d]	65.43± 1.23[a]	173.25±3.88[d]
Glibenclamide	103.2 ± 0.41[a]	0.83±0.08[a]	24.43±2.46[d]	65.44± 1.23[a]	166.375±1.944[e]

(Results are expressed as mean ± SD from 6 rats in each group. Values are at $p<0.05$ mean significant level. The values sharing common superscript do not differ significantly.

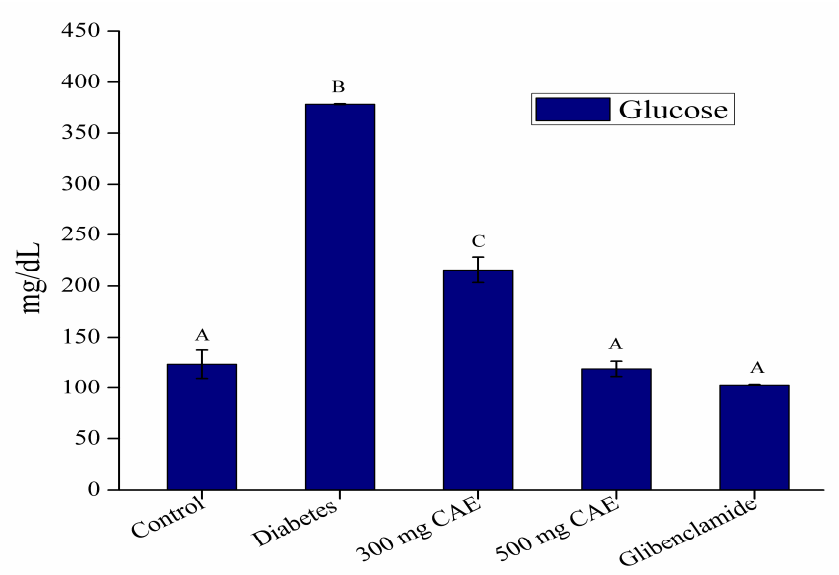

Figure 1. Glucose levels in serum

(Results are expressed as mean ± SD from 6 rats in each group. Values are at $p<0.05$ mean significant level. The values sharing common superscript do not differ significantly).

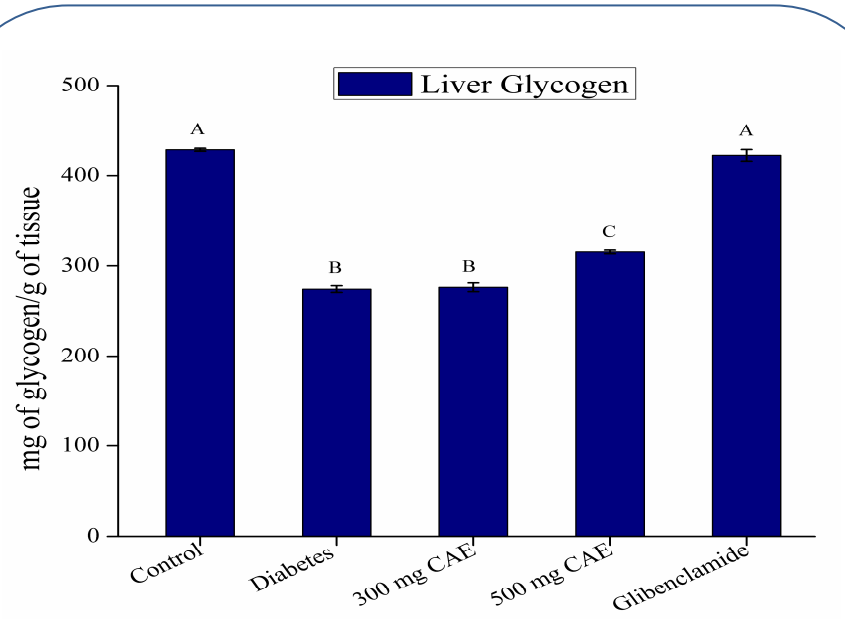

Figure 2. Liver glycogen levels

(Results are expressed as mean ± SD from 6 rats in each group. Values are at $p<0.05$ mean significant level. The values sharing common superscript do not differ significantly).

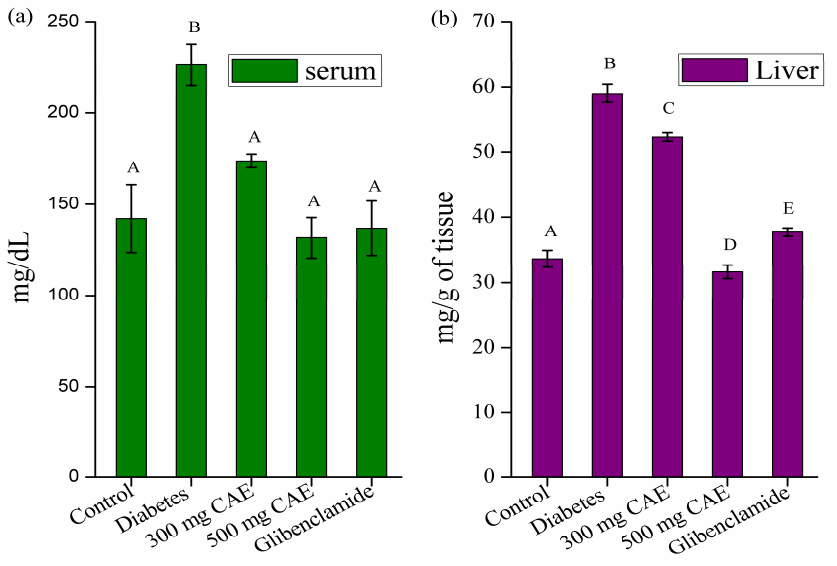

Figure 3. Cholesterol levels in serum and liver

(Results are expressed as mean ± SD from 6 rats in each group. Values are at p<0.05 mean significant level. The values sharing common superscript do not differ significantly).

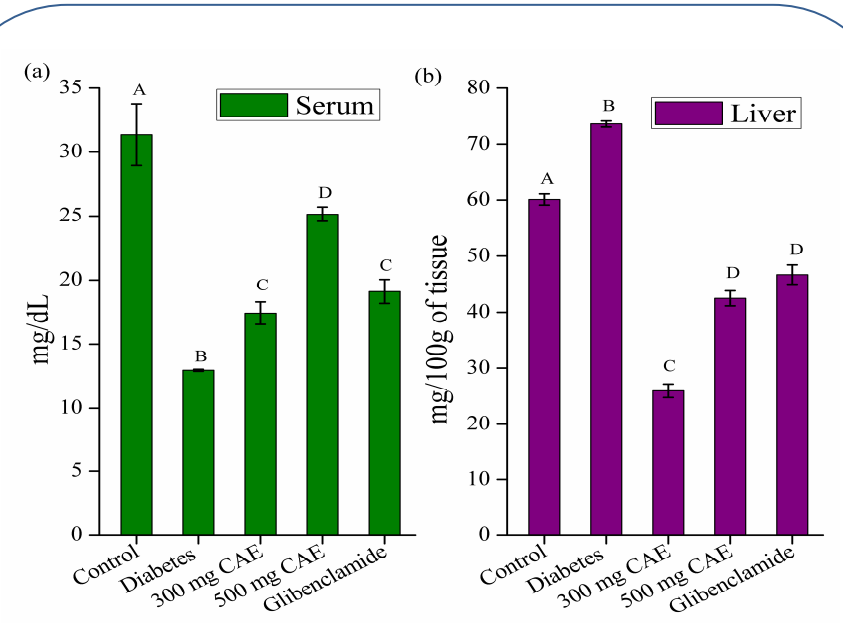

Figure 4. HDL levels in serum and liver

(Results are expressed as mean ± SD from 6 rats in each group. Values are at p<0.05 mean significant level. The values sharing common superscript do not differ significantly).

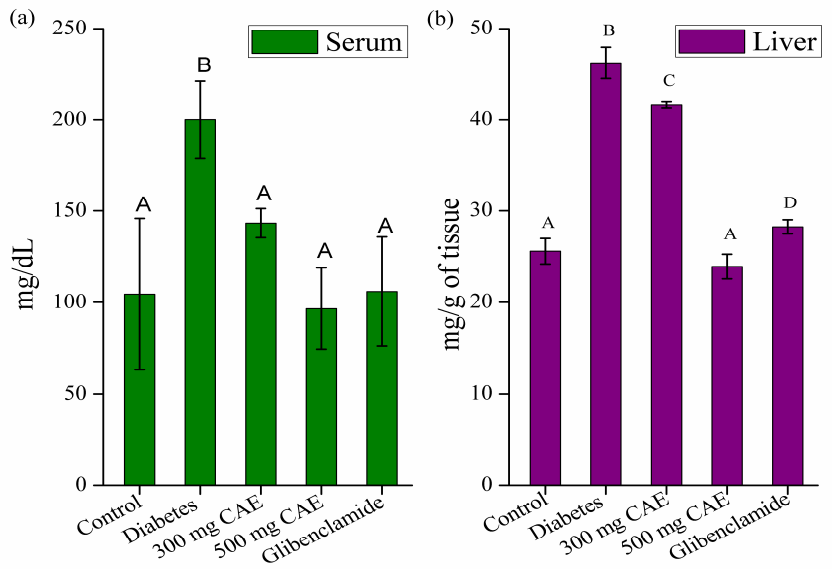

Figure 5. LDL levels in serum and liver

(Results are expressed as mean ± SD from 6 rats in each group. Values are at p<0.05 mean significant level. The values sharing common superscript do not differ significantly).

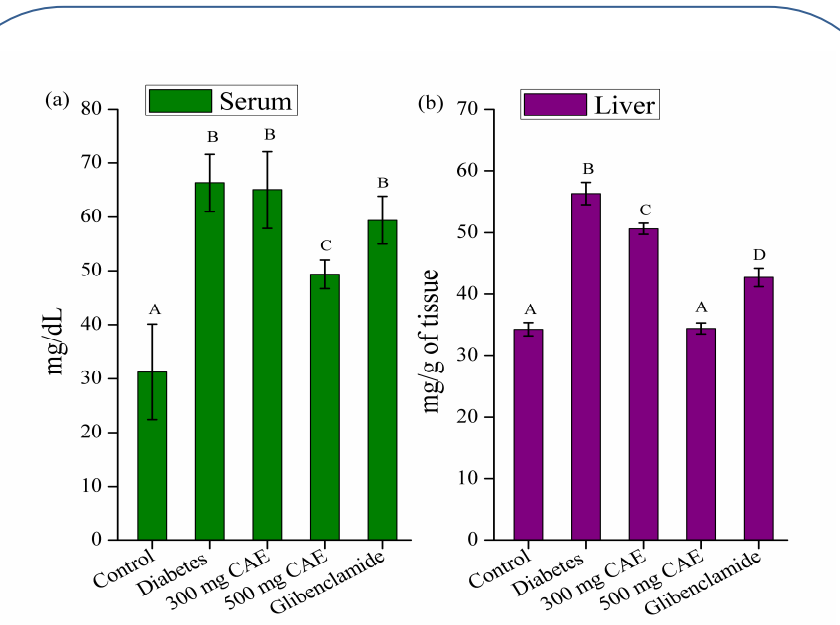

Figure 6. Triglyceride levels in serum and liver

(Results are expressed as mean ± SD from 6 rats in each group. Values are at p<0.05 mean significant level. The values sharing common superscript do not differ significantly).

1. The mean difference is significant at.05 level
2. Values sharing common superscript does not differ significantly

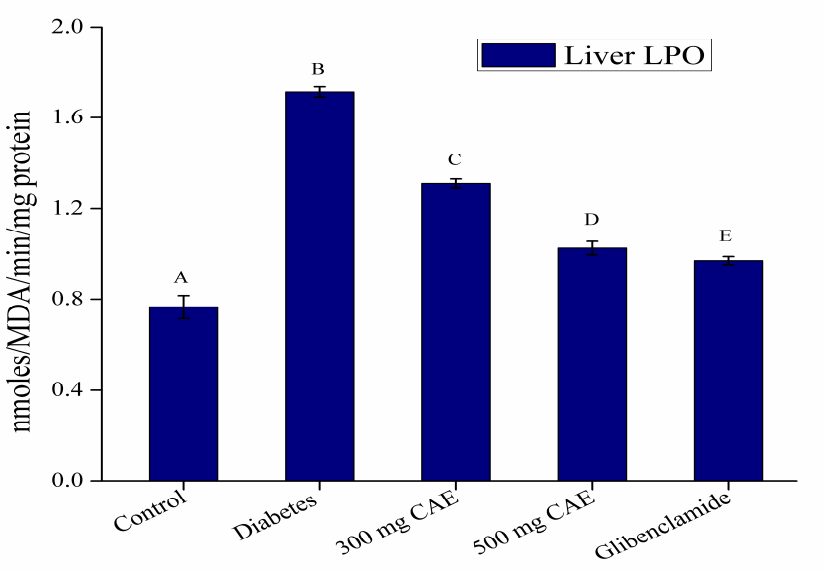

Figure 7. Lipid peroxidation (LPO) in Liver

(Results are expressed as mean ± SD from 6 rats in each group. Values are at $p<0.05$ mean significant level. The values sharing common superscript do not differ significantly).

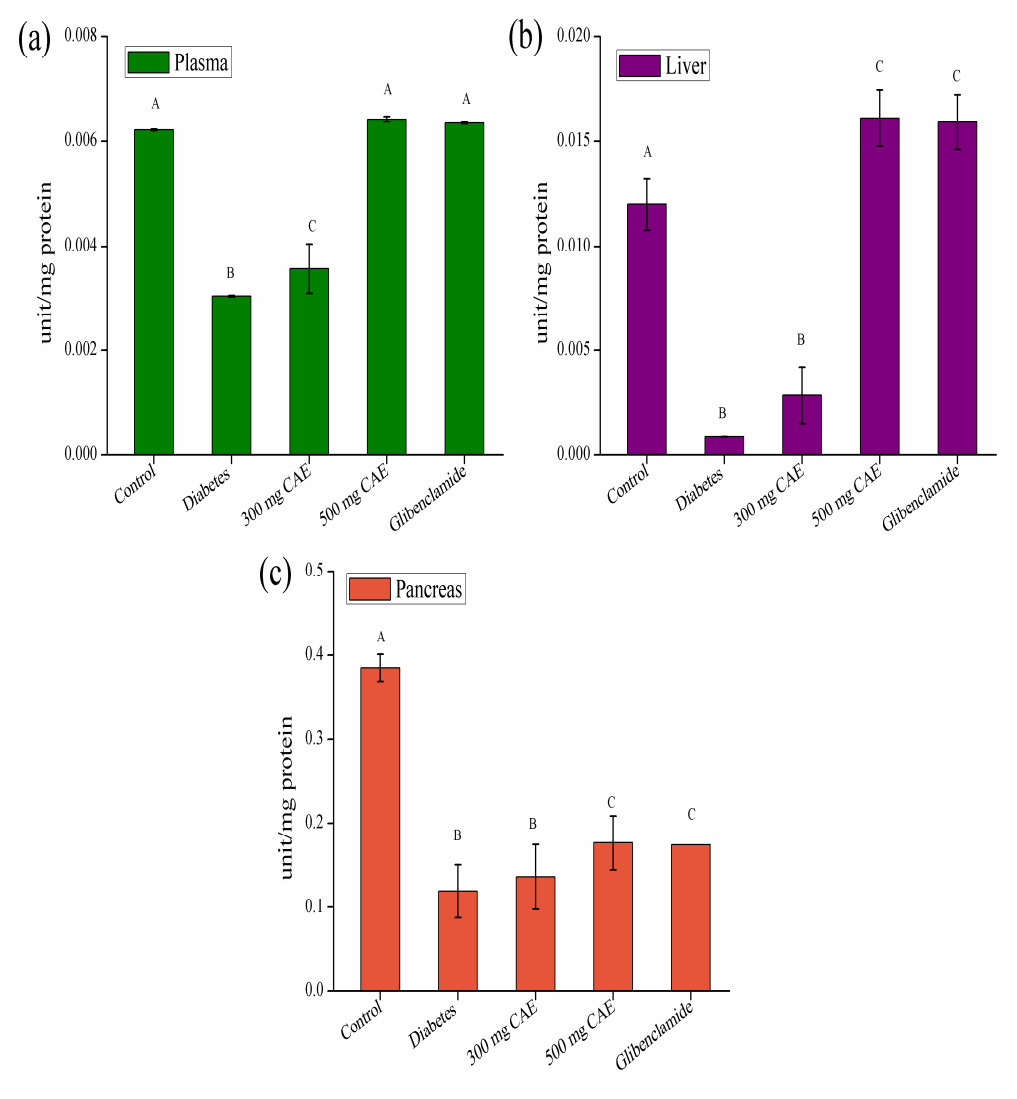

Figure 8. Activity of Catalase in plasma, liver and pancreas (Enzyme Unit = µmoles of H_2O_2 degraded/min)

(Results are expressed as mean ± SD from 6 rats in each group. Values are at $p<0.05$ mean significant level. The values sharing common superscript do not differ significantly).

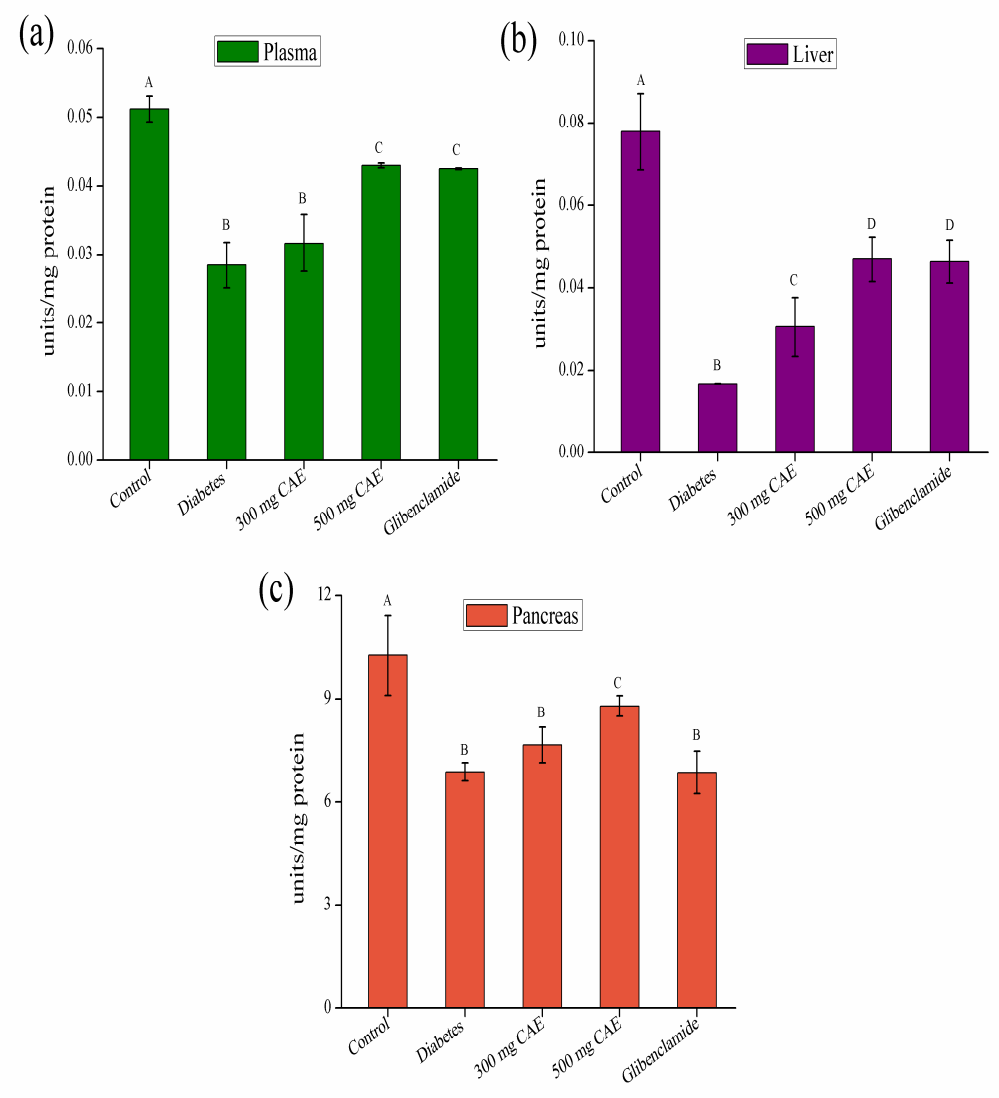

Figure 9. Superoxide dismutase activity in plasma liver and pancreas

(Enzyme Unit = mmoles of Pyrogallol oxidized/min)

(Results are expressed as mean ± SD from 6 rats in each group. Values are at $p<0.05$ mean significant level. The values sharing common superscript do not differ significantly).

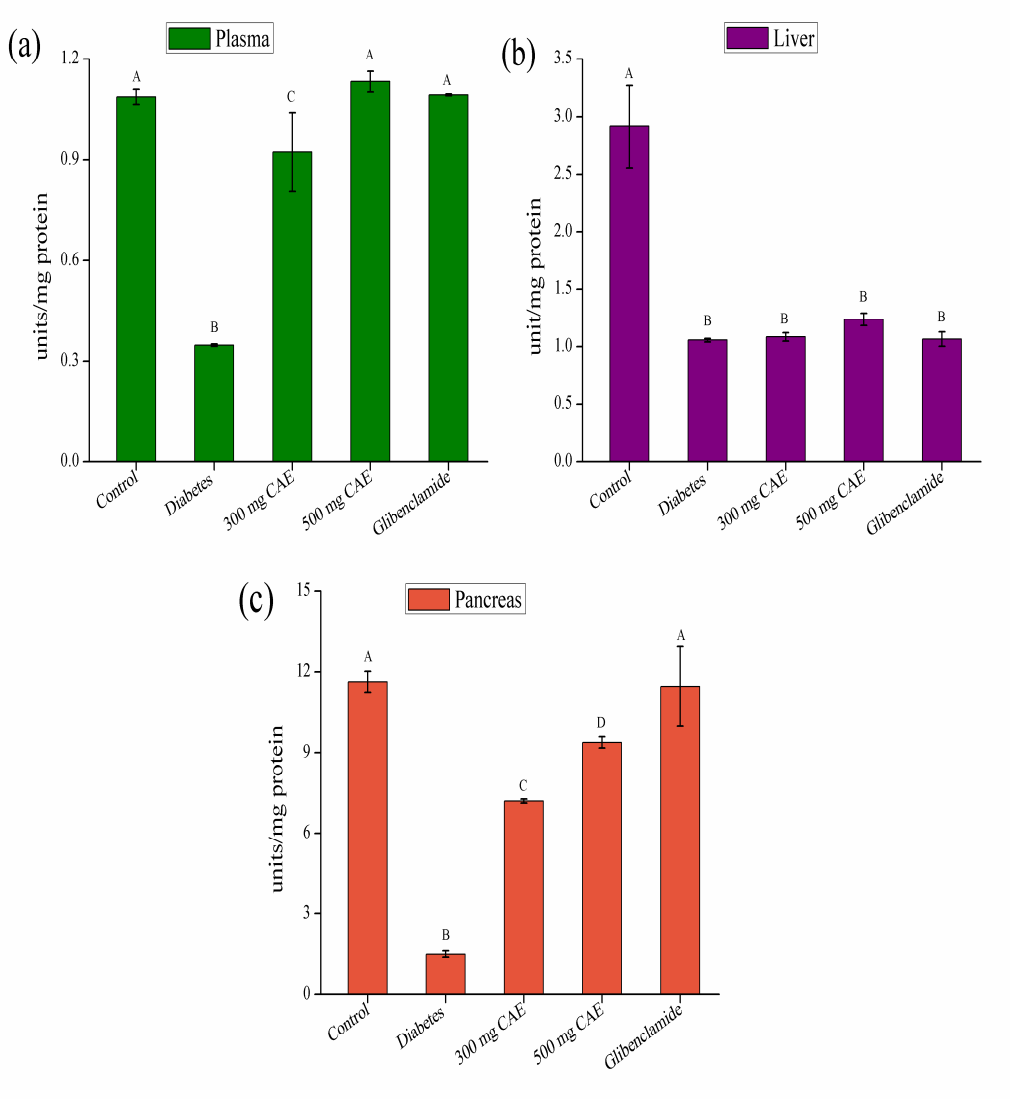

Figure 10. Glutathione peroxidase activity in plasma liver and pancreas

(Enzyme Unit = µg of glutathione utilized /min)

(Results are expressed as mean ± SD from 6 rats in each group. Values are at $p<0.05$ mean significant level. The values sharing common superscript do not differ significantly).

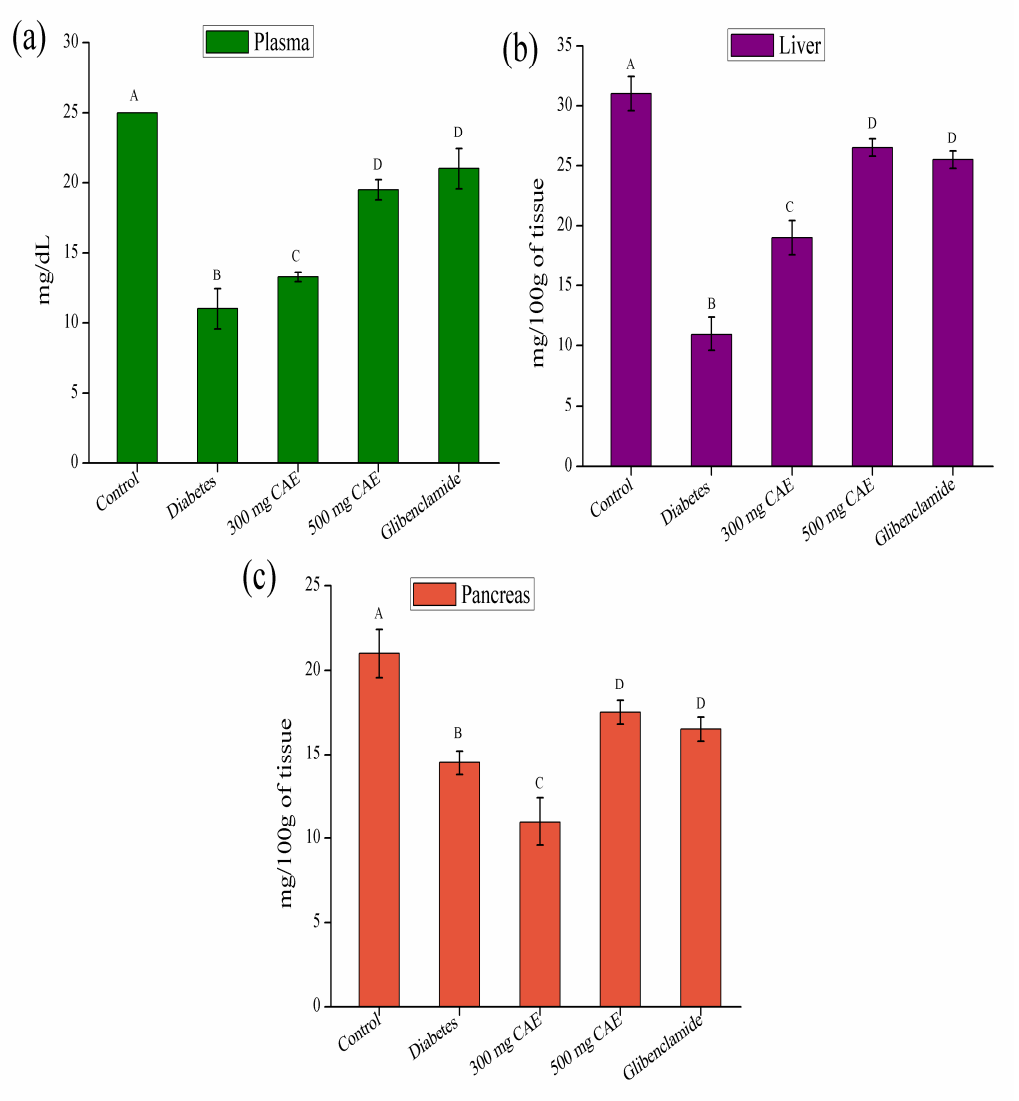

Figure 11. GSH levels in plasma liver and pancreas

(Results are expressed as mean ± SD from 6 rats in each group. Values are at $p<0.05$ mean significant level. The values sharing common superscript do not differ significantly).