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THE GLYCEMIC IMPACT OF SYZYGIUM CERASOIDEUM IN DIABETIC RATS

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Abstract

This study aimed to evaluate the glycemic impact of *Syzygium cerasoideum* in normal as well as streptozotocin (STZ) induced diabetic rat model. The glycemic impact of CESC & MESC extracts were evaluated 4h post-administration in normoglycemic rats, and STZ induced diabetic animals over a period of 21 days. Variation of administered doses of CESC and MESC extracts of *Syzygium cerasoideum* impacted the blood glucose level (mg/dl) of normoglycemic rodents with observable hypoglycemic effects at 2h. Glibenclamide (600µg/kg) showed 32.56% lowering of blood glucose level, while CESC showed 29.33% lowering at a dose of 400mg/kg, and 25.61% lowering was observed in case of 200mg/kg administered dose of MESC. STZ-induced diabetic animals treated with 200mg/kg, 400mg/kg of both extracts demonstrated noteworthy decrease of blood glucose levels during a treatment span of day 21 (CESC200mg/kg:45.56%, CESC400mg/kg: 41.25%, MESC200mg/kg: 46.22%, MESC400mg/kg: 43.24%, respectively).Maximum reduction in blood glucose was observed in CESC and MESC extract dosage of 400mg/kg. However, commercially available, and clinically approved glibenclamide lowered blood glucose by 63.66%. After treatment with the extracts the liver enzyme were diminished in the diabetic rats in comparision to untreated diabetic rats. Untreated diabetic mice exhibited reduced activity of antioxidant enzymes. The level of antioxidant enzymes were normalized in the test animals after treatment with CESC and MESC, with levels at par with glibenclamide treated rats. The diabetic treated experimental group demonstrated a increase in body weight in comparison to the diabetic control group. Combinatorial administration of both extracts at 200mg/kg, 400mg/kg dosages caused critical increment in glycogen level within the liver. Conclusion: Our results show the potential application of CESC and MESC extracts of *Syzygium cerasoideum* as antidiabetic agents..

Keywords: *Hypoglycemic, Streptozotocin, Glibenclamide, Diabetes*

Introduction

Diabetes mellitus is a chronic metabolic disorder with increasing global incidence effecting individuals worldwide. Currently an abundant number of clinically approved synthetic drugs to control diabetes are readily available in the market, yet a substation number of diabetic patients rely on herbal formulations. Projected statistics predict the number of adults affected with diabetes will increase to 135 million by 2025[01]. Diabetes patients exhibit major anomaly in the metabolism of carbohydrate, lipid, and protein which leads to an increased glucose concentration in the blood plasma triggering a spike of free radical species, and non-enzymatic responses of proteins and autoxidation of glucose [02]. The raising global prevalence of diabetics has prompted the World Health Organization (WHO) to encourage research exploring phyto-compounds for the treatment of diabetes (1980). In the quest for exploring plant-based antidiabetic agents we explored the glycemic impact of *Syzygium cerasoideum* in a diabetic rat model. *Syzygium cerasoideum* is an Indian plant species, used traditionally for its anti-rheumatic, and analgesic activity.

Methods

In vivo diabetic model

Adult albino rats (Wistar) of either sex weighing 150-200g were procured from Hylasco, Hyderabad, India.

All experimental protocols involving the experimental animals were approved by the Institutional Animal Ethics Committee (IAEC) vide project number DSU/ Ph.D./IAEC/13/2017-18, and all experiments were conducted as per guidelines and principles of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The animals were housed in appropriately sized polypropylene cages with provision for water bottle holder and feed hopper. Shredded corn cobs was used as the bedding material, and individual cages were maintained under standard air-conditioned laboratory conditions with temperature of 24 °C ± 1°C, relative humidity of 63% to 48% with 12 h light followed by 12 h dark cycle. Maximum and minimum temperature and relative humidity in the experimental room was recorded daily. Ad libitum pelleted rodent feed (Teklad global, 14% protein, maintenance feed) was provided to maintain the animals on a high-chicken-fat diet, and water from deep bore well purified and sterilized using charcoal filters, and UV rays, respectively was provided to the animals in polypropylene water bottles

Screening of extracts for hypoglycemic activity

Experimental procedure

This study was performed using the method described by Kar*et al.*, (2006).The terminal point of the fasting time-frame, is considered to be the time T_0 and at this point blood sample was collected from the retro-orbital plexus of the test animal under mild ether sedation. Blood serum was isolated by centrifugation of the collected blood samples which was followed by glucose estimation by glucose oxidase-peroxidase method [03].

The experimental animals were randomly assigned to six groups with six animals assigned to each group.

Group 1	Control group (0.5% sodium CMC 1ml/kg/bw/p.o.)
Group2	Glibenclamide (600µg/kg/bw/p.o)
Group 3	Normal rats + chloroform extract (200mg/kg b.w/p.o.)
Group 4	Normal rats + chloroform extract (400mg/kg b.w/p.o.)
Group 5	Normal rats +methanolic extract (200mg/kg b.w/p.o.)
Group 6	Normal rats + methanolic extract(400mg/kg b.w/p.o.)

Measurement of blood glucose level was performed by glucose oxidase-peroxidase method at different time intervals, at0, 30, 60, 120 and 240 mins whereby blood was collected by retro-orbital plexus puncture.

Screening of extracts for hyperglycemic activity

Streptozotocin induced diabetes

Streptozotocin(2-deoxy-2-(3-(methyl-3-nitrosoreido)-D-glucopyranose) is produced by *Streptomyces achromogenes*. After intraperitoneal or intravascularly administration, the glucose transporter (GLUT-2) carrier transports into the pancreatic β- cells, and results in alkylation of the DNA [04].This causes initiation and activation of Poly (ADP-ribose) polymerase(PARP) which

prompts NAD+ exhaustion, reducing the cellular ATP which hinders the biosynthesis of insulin (Sandler and Swenne, 1983)[05]. The STZ is a free radical that damages the DNA, and prevents the cell from replicating. STZ will in general be managed as a solitary high portion or as various low dosages.

Diabetes was induced infusion of streptozotocin intraperitoneally (55mg/kg), dissolved in ice cold citrate, at pH 4.5. Control animals only received the vehicular control (citrate). Following 5 days of streptozotocin infusion, animals with fasting blood glucose above 250mg/dL were considered as diabetic and selected for the study.

2.3.1. 1. Experimental Procedure

Group 1	Normal + distilled water.
Group 2	Diabetic + distilled water
Group 3	Diabetic+ glibenclamide 10mg/kg/bw/p.o.
Group 4	Diabetic + chloroform extract 200mg/kg b.w/p.o.
Group 5	Diabetic + chloroform extract400mg/kg b.w/p.o.
Group 6	Diabetic + methanol extract 200mg/kg b.w/p.o.
Group 7	Diabetic + methanol extract 400mg/kg b.w/p.o.

The formulations were orally administered to the animals daily up to 21 days. Blood glucose levels as well as the body weight were monitored weekly and animals were kept under overnight starvation. Towards the end of the testing period, the animals were starved for a short-time and blood was drawn through the retro-orbital plexus using a glass capillary and collected in EDTA-coated tubes for different biochemical examinations. The animals were euthanized by cervical dislocation. Organs like pancreas and liver were collected immediately after euthanasia and washed with ice cold saline and stored appropriately for successive biochemical estimations [6].

Biochemical Analysis.

Body weight and food and water intake

The body weight of the normal and diabetic rats at the beginning and end of the experiment time period was measured and the food and water intake was recorded.

Blood glucose level estimation

Glucose oxidase-peroxidase technique was used to estimate the blood glucoseusing the glucose examination unit (Accu-check glucometer, Roche Diagnostics, Switzerland) weekly until the end of the study [7].

Estimation of liver tissue glycogen

The liver samples were weighed (200mg) and homogenized with 5% TCA (20ml) in a homogenizer. The precipitate protein was filtered and used for liver glycogen analysis. In a test tube, 2ml of liver extract was taken and 2ml of 10N KOH was added followed by boiling in a water bath for 1h. After cooling, the solution of acetic acid (1ml) was added to neutralize the alkali and the volume was adjusted to 20ml using water. This was followed by gradual addition of 2ml of above mixture to 4ml of anthrone reagent into a test tube, which was placed in cold water. The reactants were mixed by gradual shaking, followed by exposure to boiling water for 10min. The solution was cooled and the optical density was recorded at 650nm against a reagent blank [8].

***In vivo* antioxidant studies**

Glutathione Peroxidase (GPX) estimation

The GPX estimation method followed in this paper was first reported by Rotruck *et al.*, (1973). Quickly response blend contained 0.2 mL of 0.4 MTris–Hydrochloride cradle pH 7.0, then 0.1 mL of 10 mM(NaN_3) sodium azide, 0.2 mL of previously prepared homogenate mixture -homogenized in0.4 M, Tris–Hydrochloride support, pH 7.0, 0.2 mL Glutathione, and 0.1 mL of 0.2 mM Hydrogen Peroxide(H_2O_2). The substance is brooded at 37℃ for 10 minutes. The response is captured by 0.4 mL of 10% Trichloroacetic acid as well as centrifuged. A supernatant layer is examined for glutathione substance by utilizing Ellmans reagent (19.8 mg of 5, 5′-Dithiobisnitro Benzoic corrosive (DTNB) in100 mL of 0.1% Sodium nitrate). The results were expressed as U/mg protein[09].

Estimation of catalase activity

Catalase activity assay was performed based on the method described by Sinha *et al.*, (1972). The assay mix consists of 4 mL of H_2O_2 and 5 mL of

phosphate buffered saline, pH 7.0 taken in a 10 mL level base glass container. 1 mL of debilitated plasma test immediately mixes with the reaction mix by means of a carefully spinning development at room high temperature. The reaction mixture (1 mL) was added to 2 mL of ($K_2Cr_2O_7$) dichromate/acidic destructive reagent and allowed to react for 60s, followed by measurement of optical density using spectrophotometer (Spectrum Lab 752S UV) at wavelength 570 nm. Catalase development is impaired when 1mMof H_2O_2 crumbled per min[10].

Estimation of Superoxide Dismutase (SOD)

The estimation of superoxide dismutase was performed based in the technique reported by Kakkar*et al.* (1984). Supernatant of the centrifuged tissue homogenate was test tube (0.5mL). To this 1.5 mL of (HCO_3) carbonate support (pH10.2), 0.5 mL of Ethylene diamine tetra-acetic destructive (EDTA) with 0.4 mL of epinephrine be incorporated and the optical density was measured at 480 nm. Epinephrine was incorporated to each well prior to taking the OD[11].

Estimation of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT)

Serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase were detected by method described by Reitman and Frankel (1957). During this calorimetric estimation, 0.5 mL of substrate (sodium pyruvate) was heated in a water bath for 3 minat 37°C. To the substrate 0.1 mL of serum was mixed vigorously and agitated in a shaker for60 min. The chambers are expelled as of the shower and quickly, 0.5 mL of (DNPH) Deniltrophenol be included and mix well. DNPH was subjected to react in every cylinder for 20 minutes at room temperature and a short time later 0.4 N (5 mL) sodium hydroxide game plan was incorporated, mixed well and left used for an additional 10 min [12].

Total Protein Estimation

The protein extraction was performed using the lysis buffer composed of: 50 mMTris-hydrochloride pH 8, 150 mM sodium chloride, 0.1% sodium lauryl sulfate, 0.5% DeoxycholicAcid, 5 mM magnesium chloride, 1% Triton, 1× Protease Cocktail Inhibitor, 0.5 mM phenyl methane sulfonyl fluoride, 5 mM sodium orthovanadate (Na_3VO_4), 10 mM (NaF) sodium fluoride, 0.5 mM sodium pyrophosphate ($Na_4P_2O_7$) and 1 mMDithiothreitol (DTT). While performing the phosphoprotein assessment a modified cell lysis buffer was used which was composed of: 4-(2, hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) 50 mM pH 7.5, 10% glycerol, 150 mM NaCl, 1% Triton X-100, 1.5 mM $MgCl_2$, 10 mM NaF, 1 mM Ethylene glycol tetra acidic destructive (EGTA), 10 mM sodium pyrophosphate, 1 mM Na_3VO_4, 1×Protease Cocktail Inhibitor. All the chemical reagents were of highest purity and were obtained from Sigma-Aldrich (St. Louis, MO, USA). Cell lyses was performed on ice for 15min and the lysates were centrifuged at maximum centrifugation speed for 25 min at 4°C. BicinchoninicAcid (BCA) protein assay kit was used to measure the protein (Thermo Fisher Scientific Inc., Rockford, USA).

Estimation of serum insulin levels by RIA assay (Radioimmunoassay Method)

Insulin neutralizing antibody is used to isolate free insulin which was precipitated using 80% ethanol. This strategy is quick, simple and minimized the error usually introduced in a two-fold neutralizing method. This improved technique allowed the elimination of Cl, NO_3, HCOO, $CHgCOO^-$, and I and increases the insulin-immune response rate and can estimate the amount of agent/s bound to the insulin. The other residual components detected include: K^+, Na^+, NH^{4+} , $S04^-$, $HP04^-$, BO_3, citrate, and oxalate.

The ethanol precipitation in the immunoassay method was utilized in our study since it is straightforward and simple. However, in order to compare we also utilized an immunoassay strategy dependent on the two-fold antibody [13-14].

Histopathological studies of liver

On the day of blood withdrawal, two animals from each experimental group were euthanized and the liver was excised. The collected tissue tests were submerged in 10% neutral buffered formalin and kept overnight for tissue fixation 24 h to fix the tissue. The fixed tissue was paraffin embedded. This was followed by microtome based parffin sectioningand staining with Hematoxylin-Eosin. The tissue sections were studied under a light

microscope to discern any histopathological changes[15]

Results

Streptozotocin is generally used to induce diabetes in test animals and can lead to pancreatic islet β-cell cytotoxicity induced by influx of nitric oxide (NO), leading to decrease in pyridine nucleotide fixation in the pancreatic islet and β cell put refection. STZ also produces SOD anions in the mitochondria which results in diabetic complications [16].In light of these factors, we designed our study to assess the hypoglycemic activity of *Syzygium cerasoideum* in normoglycemic rats and STZ induced diabetes [17]. Glibenclamide is a known sulphonyl urea commonly employed as a standard antidiabetic agent to treat experimental test animals with STZ induced diabetes, to evaluate the efficacy of a variety of antihyperglycemic therapeutics [18].

The results of the effect of evaluated dosages of chloroform and methanol concentrates of *Syzygium cerasoideum* on blood glucose level (mg/dl) in ordinary solid rodents has been presented in Figure 1. In normoglycemic rodents the concentrates demonstrated hypoglycemic effects as early as at 2h. Decreased blood glucose decrease was seen in all the test animals. The impact of oral administration of the CESC and MESC extracts on the blood glucose levels in STZ-initiated diabetic rodents has been shown in Figure 2, with statically significant effects at200mg/kg, 400mg/kg doses of the two concentrates in STZ induced diabetic rodents($P<0.001$) with a marked decrease of plasma glucose during the entire treatment period. The maximum drop in the blood glucose level was observed at day 21.There was absence of hypoglycemic condition was observed in the treated diabetic rodents during the entire treatment period. Medications which have capacity to maintain a stable glycemic profile without causing hypoglycemia, would make attractive targets for diabetes [19].

Diabetes mellitus hinders the ability of the liver to breakdown glycogen. A chemical synthase phosphatase actuates glycogen synthase bringing about glycogenesis these outcomes to be flawed in diabetes [20]. A significant site of insulin simulated glucose uptake is the skeletal muscle [21]. In this study the hepatic glycogen was decreased in the diabetic control and on treatment with CESC & MESC at dosages of 200mg/kg, 400mg/kg, respectively for 21 days a significant increase in glycogen was observed which clearly indicates that the defective glycogen storage of the non-metabolized glucose was partially ameliorated by the phyto therapeutic agent.

Liver is a crucial organ processing and detoxifying xenobiotics along with their particular metabolites. SGOT, SGPT, ALP are strong markers of liver health. In diabetic rodents induced with STZ the liver can get totally necrotized. A development into the actions of SGPT, SGOT as well as ALP in plasma in view of spillage of these mixes from the liver cytosol into the flow framework which offers a hint of the hepatotoxic result of STZ[22]. In this study the SGOT and SGPT levels were stabilized. After treatment with the plant extracts the SGOT, SGPT levels were decreased in the diabetic test rats which were in stark contrast to that of the diabetic untreated rats which is intuitive since, the physiological functioning of the diabetic liver is impaired. A part of the key malignant growth avoidance operator impetuses like CAT, SOD and GPX which helps in looking through the toxic intermediates of lacking oxidation. A statistically significant decline in the activities of SOD CAT, and GPX, was observed in diabetic rodents ($P<0.001$). After the treatment the levels were stabilized comparable to glibenclamide treatment. An increase in the bodyweight of diabetic test animals may be the result of the glycemic control [23].Decrease in the protein content was seen in liver tissue of the diabetic rodents which may be assigned to dynamic proteinuria [24].Diabetic control kept on getting in shape till the end of the examination while the diabetic treated test animals showed a significant improvement ($P<0.001$) in body weight profile when compared to diabetic control. The excessive protein catabolism resulted in gluconeogenesis during insulin inadequacy which resulted in reduction of muscle mass and weight reduction in diabetic untreated rodents.

Histopathological investigations of liver in CESC and MESC treated groups exhibited improved lobular architecture and focal vein in the liver asobserved by histopathology. The animal groups

administered with CESC and MESC showed preservation of the islets and a smaller degree of necrotic change when compared to the untreated STZ diabetic rodents. In conclusion the current investigation demonstrates that CESC and MESC have antihyperglycemic effects which indirectly function in improving insulin release and may assist in improved starch assimilation

Discussion With the outcomes of the present study, we can conclude that CESC and MESC at a high dose possesses antidiabetic property which could be lead to potential development of a new antidiabetic therapeutic

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References

1. Liu, H., Liu, X., Lee, J., Liu, Y., Yang, H., Wang, G., Insulin therapy restores impaired function and expression of P-glycoprotein in blood–brain barrier of experimental diabetes. Biochemical Pharmacology.2008; 75:1649–1658.

2. Dewanjee, S., Das, A.K., Sahu, R., Gangopadhyay, M., 2009. Antidiabetic activity of *Diospyrosperegrina* fruit: effect on hyperglycemia, hyperlipidemia and augmented oxidative stress in experimental type 2 diabetes. Food and Chemical Toxicology. 2009; 47: 2679–2685.

3. Kar, D., Maharana, L., Pattnaik, S., Dash, G., Studies on hypoglycemic activity of *Solanum xanthocarpum* Schard and wendl. Fruit extract in rats. Journal of Ethano pharmacology. 2006; 108: 251-256.

4. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001; 50: 537–546.

5. Sandler S, Swenne I. Streptozotocin, but not alloxan, induces DNA repair synthesis in mouse pancreatic islets in vitro. Diabetologia 1983; 25: 444–447.

6. Arulselvan, P., Subramanian S. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra-structural changes of pancreatic cells in experimental diabetes in rats. Chemico Biological Interactions 2007; 165: 155–164.

7. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann Clin Biochem 1969; 6: 24-25.

8. Vries JV. Two methods for the determination of glycogen in liver. Biochem J 1954; 57:410-416.

9. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swason, A.B. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588–590.

10. Sinha, K.A. Colorimetric assay of catalase. Analytical Biochemistry 1972; 47:389–394.

11. Kakkar, P., Dos, B., Viswanathan, P.N., A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry and Biophysics 1984; 21: 130–132.

12. Reitman, S., Frankel, S., For the determination of SGPT (ALAT) activity in serum. Americal Journal of Clinical Pathology 1957; 28: 53–56.

13. V. Herbert, K. Lau , C. W. Gottlieb and S. J. Bleicher. — Coated Charcoal Immunoassay of Insulin. Clin.Endocrin.Metabolism.1965; 25: 1375.

14. S. Genuth , L. A. Frohma N and H. E. Lebowitz. — A Radioimmunological Assay Method for Insulin using 125I Insulin and Gel Filtration. J. Clin. Endocrin. Metabolism. 1965; 25 : 1043

15. Singh, G.K., Kumar, K. Acute and sub-chronic toxicity study of standardized extract of *Fumaria indica* in rodents. Journal of Ethno pharmacology 2011; 134: 992–995.

16. Papaccio. G, Pisanthi, F.A., Latronico, M.Y., Ammendola, E., Galdieri, M., Multiple low –dose and single high dose treatments with streptozotocin do not generate nitric oxide. Journal of Cellular Biochemistry.2000; 77: 82-91.

17. Sarulmozhia.S.,Mazumderb, P.M., Lohidasanc, S., Thakurdesai, P., Antidiabetic and antihyperglycemic activity of leaves of *Alstonia scholaris* Linn. R.Br. European Journal of Integrative Medicine. 2010; 2: 23-32.

18. Porksen, N., Therapy targeting β-cell survival and function in type 2 diabetes mellitus. Diabetes Res. Clin. Pr. 2006; 74(2): S63-S69.

19. Grover, J., Vats, V., Yadav, S., Effect of feeding aqueous extract of *Pterocarpus marsupium* on glycogen content of tissues and the key enzymes of carbohydrates metabolism. Molecular Cellular Biochemistry. 2002; 241: 53-59.

20. Bouche, C., Serdy, S., Kahn, R., Goldfine, A., The cellular fate of glucose and its revelance in Type 2 diabetes. Endocrine Review. 2004; 25: 807-830.

21. Ohaeri, O.C., Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. Bioscience Reports. 2001; 21:19-24.

22. Ramesh, B.K., Maddirala, D.R., Vinay, K.K., Shaik, S.F., Tiruvenkata, K.E.G., Swapna, S., Ramesh, B., Rao, C.A., Antihyperglycemic and antihyperlipidemic activities of methanol: water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. Food and Chemical Toxicology. 2010; 48: 1078–1084.

23. Eliza, J., Daisy, P., Ignacimuthu, S., Duraipandiyan, V., Antidiabetic and antilipidemic effect of eremanthin from *Costus speciosus* (Koen.) Sm., in STZ induced diabetic rats. Chemico Biological Interactions.2009; 182: 67–72.

24. Latha, R.C.R., Daisy, P., Insulin secretagogue, antihyperlipidemic and other protective effects of gallic acid isolated from *Terminalia bellerica* Roxb. In streptozotocin induced diabetic rats. Chemico Biological Interactions.2011; 189: 112–118.

Table 1: Blood glucose level (mg/dL) in normal rats after administration of chloroform and methanol extracts of *Syzygiumcerasoideum*.

Experimental Group	0 min	30 min	60 min	120 min	240 min
Normal Control	81.36±2.85	80.28±2.29$^{\#}$	74.93±2.30$^{\#}$	74.94±1.77$^{\#}$	77.4±1.71$^{\#}$
Glibenclamide600µg/kg	83.75±2.11	67.2±1.13^{a}	56.66±2.35^{b}	59.95±1.93^{b}	63.45±2.33^{b}
CESC 200mg/kg	81.91±3.88	64.49±1.52^{a}	55.28±1.71^{a}	56.24±1.16^{a}	62.67±1.06^{a}
CESC 400mg/kg	83.37±2.87	55.93±1.41^{b}	50.6±1.18^{a}	50.39±1.64^{a}	62.7±1.29^{b}
MESC 200mg/kg	83.83±5.06	67.76±1.01^{b}	56.05±3.11^{a}	52.5±1.27^{a}	60.2±0.73^{b}
MESC 400mg/kg	82.23±4.35	58.84±2.39^{a}	52.63±1.35^{a}	55.47±2.66^{a}	61.23±2.37^{b}

Experimental values have been expressed as mean ± SEM (n=6,in each experimental group). $^{\#}$P>0.01 was not considered statistically significant, and was reported in between groups at T_0 (One–way ANOVA, followed by post hoc Tukey's Honest Significant Difference test. ^{a}P<0.01 while groups compareby means of their respective '0' minutes (One–way ANOVA, followed by Tukeys test) ^{b}P<0.001when groups compared with their respective '0' minutes (One–way ANOVA, followed by Tukeys test)

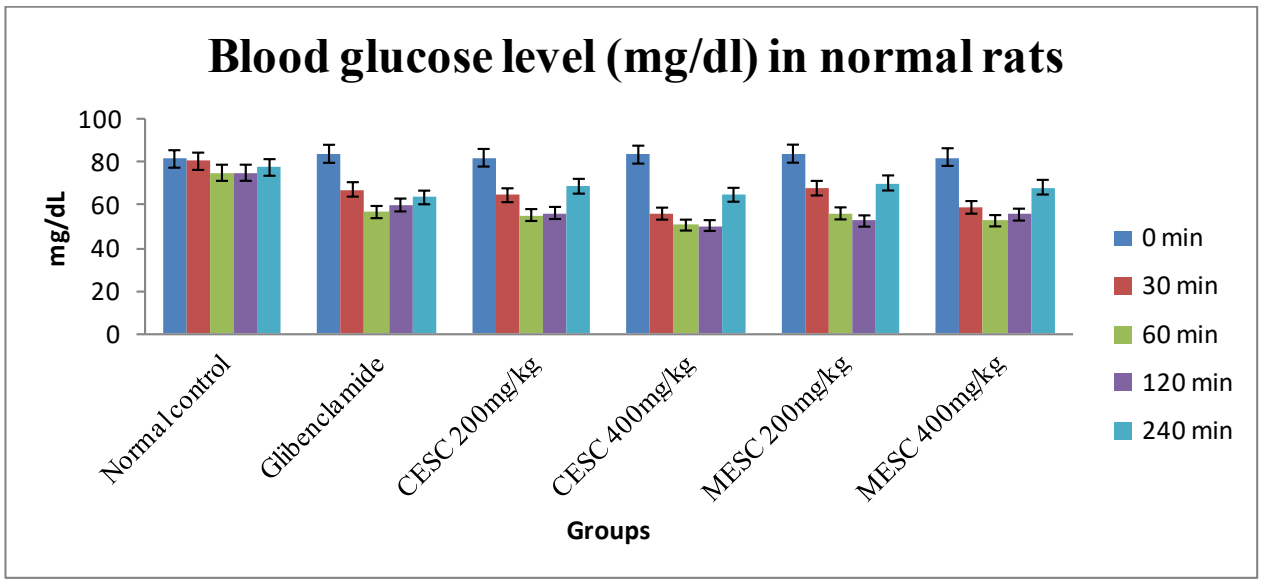


Figure 1. Results of graded doses of chloroform and methanol extracts of *Syzygium cerasoideum*on blood glucose level (mg/dL) in normal rats. In normoglycemic rodents the *Syzygium cerasoideum*extracts induced a hypoglycemic impact at 2h. Blood glucose levels dropped in rats administered with Glibenclamide by 32.56% (P<0.001) followed by 29.33% for CESC 400mg/kg (P<0.001), and 25.61% for MESC 200mg/kg (P<0.001).

Table 2: Blood glucose level (mg/dL) in streptozotocin induced diabetic ratsafter administration of chloroform and methanol extracts of *Syzygiumcerasoideum*.

Experimental Groups	Day 01	Day 07	Day 14	Day 21
Normal control	88.05 ±1.84	87.19±2.16***	90.35±3.00***	90.5±2.02***
Diabetic Control	278.2±4.68	295 ±4.32	307.8±2.25	286.2±3.13
Glibenclamide600µg/kg	275.8±3.68	209.9±2.10***	198.3±3.16***	175.7±4.80***
CESC 200mg/kg	275.7±5.93	258.3±3.07***	217.8±1.75***	202.7±2.20***
CESC 400mg/kg	275.8±3.06	222.3±3.09***	209.5±1.94***	190.5±5.56***
MESC 200mg/kg	276.3±5.78	249±4.21***	243.3±2.88***	218.3±2.61***
MESC 400mg/kg	274±5.82	250.7±3.12***	225.3±2.36***	212.7±2.65***

Experimental values have been expressed as mean ± SEM (n=6, ineach group). *** P<0.001 (a) whilecompareby means of respect to diabetic Control (One–way ANOVA, followed by Tukeys test)

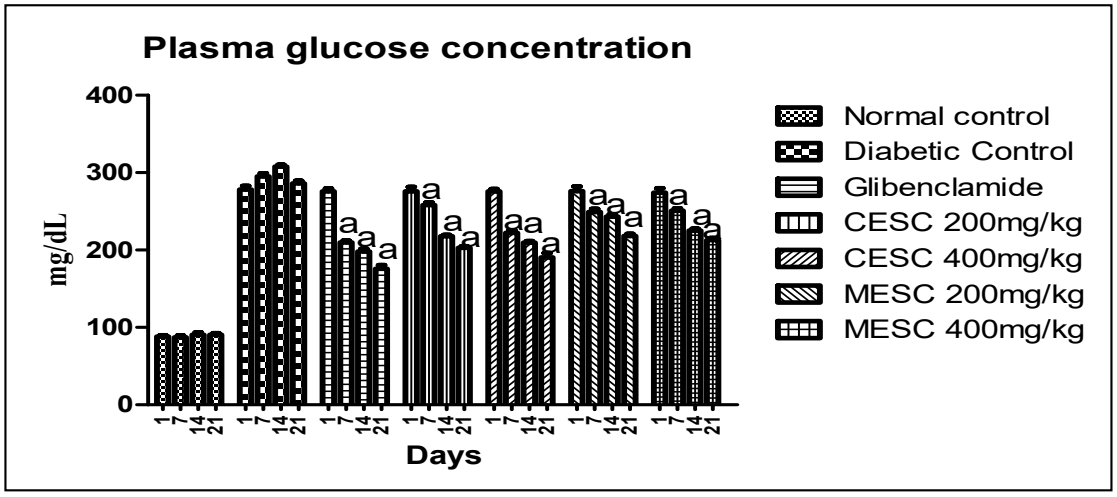


Figure 2: The effect of repeated oral administration of CESC& MESC extracts on blood glucose levels in STZ-induced diabetic rats with different doses (200mg/kg, 400mg/kg of both extracts). STZ induced diabetic rodents demonstrated statistically significant (P<0.001) decrease in blood glucose focus level identified over the treatment duration of 21 days. Maximum decrease was observed on day 21 (45.56%, 41.25%, 46.22% and 43.24% respectively). CESC 400mg/kg followed by MESC 400mg/kg induced maximum glucose lowering impact when compared to lower doses. The Glibenclamide treated group exhibit 63.66% decline in blood glucoselevel at the end of the study when compared to the diabetic control.

Table:3. Effect of chloroform and methanol extracts of *Syzygium cerasoideum* on different enzymes after 21 days of treatment in normal as well as diabetic rats.

Experimental Groups	SGOT	SGPT
Normal Control	31.92 ±1.41***	51.08±1.43***
Diabetic Control	63.03±2.41	89.52 ±2.60
Glibenclamide600µg/kg	41.2±1.22***	53.07 ±1.30***
CESC 200mg/kg	50.22 ±1.61***	80.06 ±2.73*
CESC 400mg/kg	48.84 ±2.20***	66.89±2.03***
MESC 200mg/kg	45.44 ±2.23***	77.87 ±2.83**
MESC 400mg/kg	42.56±2.03***	66.98 ±1.39***

Experimental Groups	CAT	SOD	GPX	Total Protein
Normal Control	60.88±3.21***	5.42±0.32***	44.51±2.05***	8.165±0.21***
Diabetic Control	33.87 ±1.71	2.968±0.19	24.22±1.11	5.035±0.08
Glibenclamide600µg/kg	51.71±1.15***	4.76±0.14***	40.77±0.70***	9.993±0.32***
CESC 200mg/kg	41.11±2.06***	3.627±0.17	34.09±1.43***	5.525±0.19
CESC 400mg/kg	55.63±3.32***	4.357±0.15***	37.62±1.42***	6.795±0.19***
MESC 200mg/kg	57.02±2.38***	4.023±0.09**	34.77±1.60***	7.832±0.39***
MESC 400mg/kg	57.8±1.56***	4.548±0.17***	40.43±1.40***	8.712±0.39***

Experimental values have been expressed as mean ± SEM (n=6 in each group) *** P<0.001 (a) whilecompareby respect of diabetic Control(One–way ANOVA, follow by Tukeys test)

Figure 3

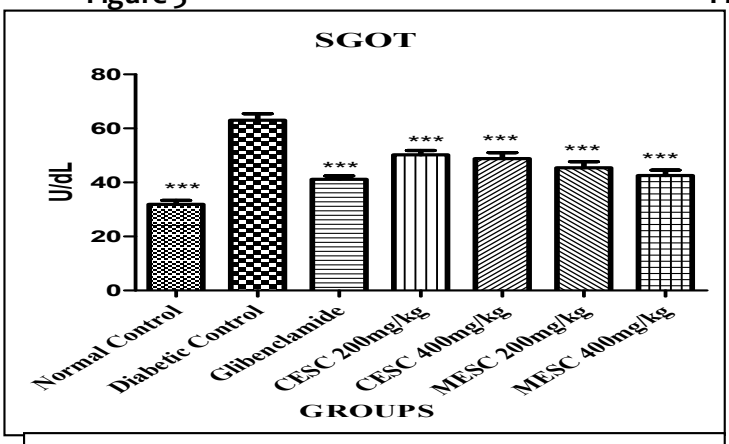
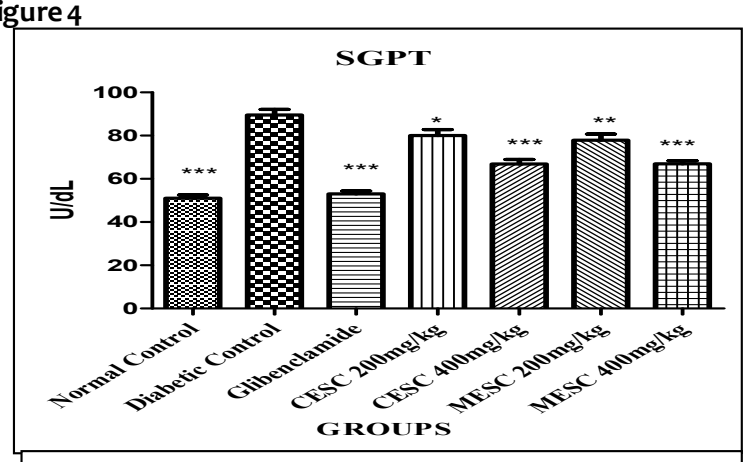
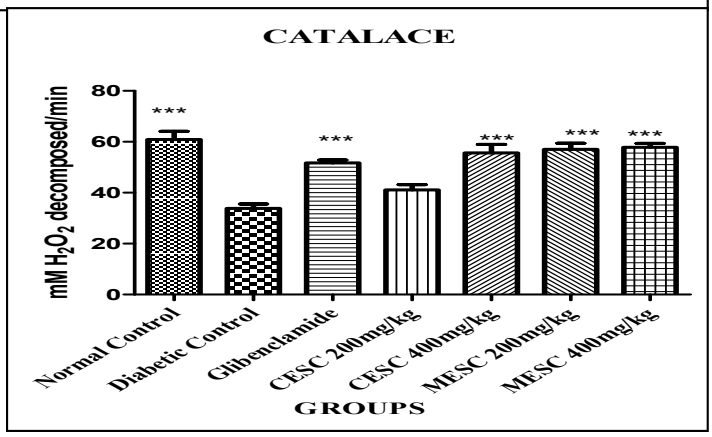


Figure 4





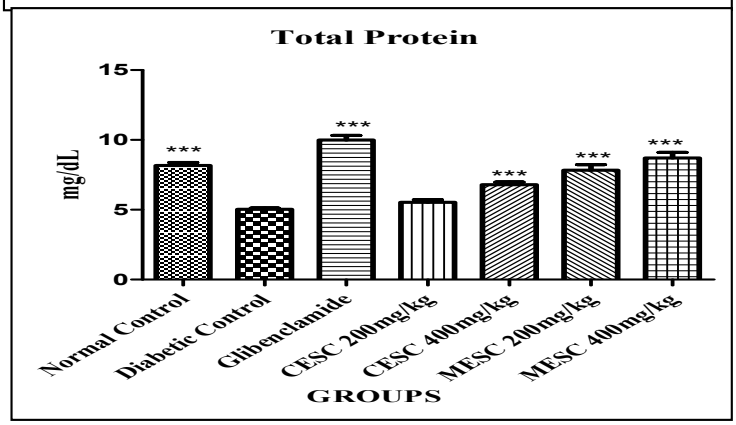


Figure 7

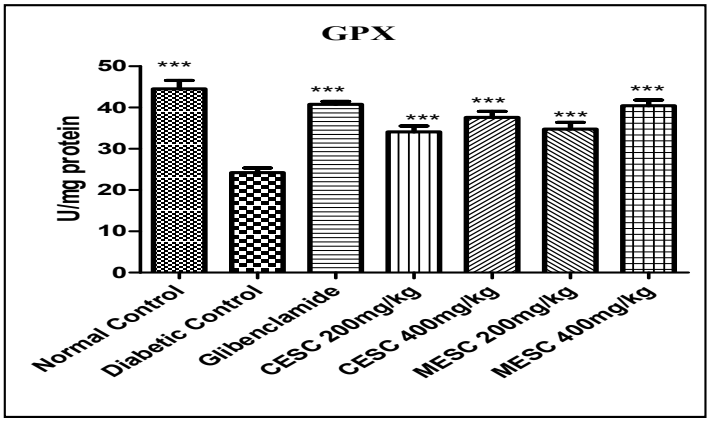
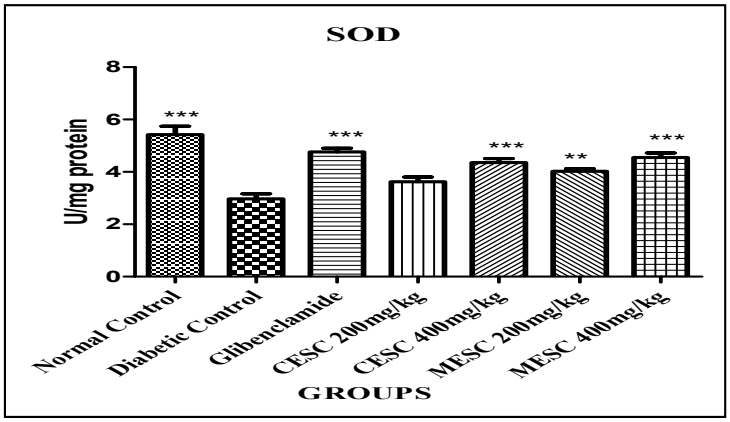


Figure 8



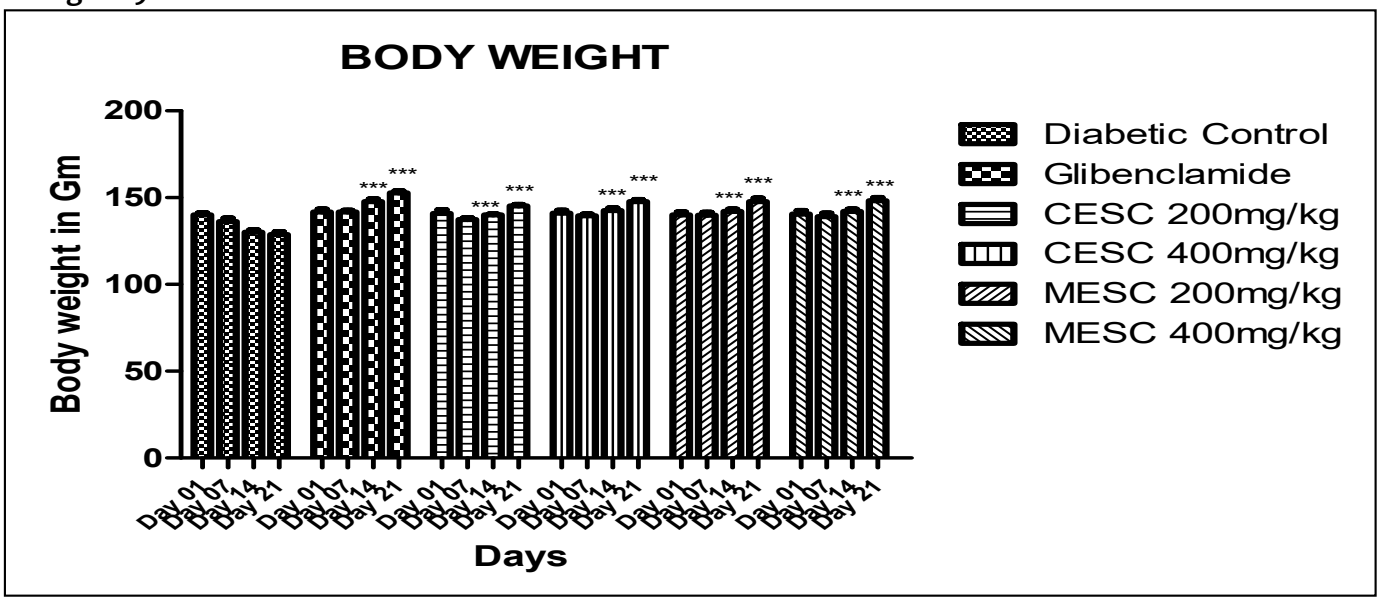
The activities of hepatic function markers (SGOT, SGPT) in STZ induced diabetic rats across different experimental group of rats have been tabulated in Table 3, Figure 3 and 4, respectively. The activities of hepatic markers were higher in diabetic untreated experimental groups when compared to the normal control group, After treatment with the plant based concentrates the SGOT, SGPT levels were diminished in the diabetic rodents in comparison to the diabetic untreated rodents. Figures 5,6 and 7 show the activity of the CAT, SOD,GPX and their levels in diabetic rodents. A critical ($P<0.001$) decrease withinthe activities of SOD, CAT, and GPX, an elevated level be see in diabetic rats. After treatment the values are normalized to levels similar to those in glibenclamide treated group.

Table4: Body weight profile in streptozotocin induced diabetic rats after treatment with chloroform and methanol extracts of *Syzygium cerasoideum*.

Experimental Groups	Day 01	Day 07	Day 14	Day 21
Diabetic Control	140.1±1.12	136.3±1.89	130±1.41	128.8±1.66
Glibenclamide600µg/kg	141.7±1.69	141.7±0.96	147.7±1.61***	152.7±1.28***
CESC 200mg/kg	140.9±2.25	137.4±1.00	140±0.53***	145.3±0.85***
CESC 400mg/kg	141.1±1.61	139.5±1.02	142.5±1.58***	147.8±1.19***
MESC 200mg/kg	140±1.75	139.9±1.63	142±1.36***	147.8±1.90***
MESC 400mg/kg	140.5±2.01	139±2.08	142±1.41***	148.3±1.85***

Experimental values have been expressed as mean ± SEM (n=6 within each group) *** P<0.001 (a) whilecompareby respect todiabetic control (One–way ANOVA, follow by Tukeys test).

Figure 9



The initial and the final body weight of normal and diabetic rodents are provided in Table4. A huge abatement in the body weight of diabetic control was observed when compared to normal untreated test animals. Diabetic control kept on losing weight till the termination of the study, while the diabetic treated rodents demonstrated statistically significant increase in body weight (P<0.001) in with respect to diabetic control animals.

Table5: Blood insulin and profile of glycogen content in the liver in streptozotocin induced diabetic rats after treatment with chloroform and methanol.

Experimental Groups	Serum insulin levels		Glycogen content in liver
	Day 01	Day 21	
Normal Control	21.43±0.95	22.1±0.86***	49.54±1.57***
Diabetic Control	10.3 ±0.65	7.895 ±0.29	17.74±0.82
Glibenclamide	9.705±0.44	18.86±0.48***	44.03±2.20***
CESC 200mg/kg	10.11±0.37	14.67±0.86***	34.9±1.06***
CESC 400mg/kg	9.965±0.32	15.53±0.60***	39.04±1.04***
MESC 200mg/kg	10.13±0.32	15.19±0.69***	34.95±1.68***
MESC 400mg/kg	10.17±0.37	16.52±1.03***	40.26±1.20***

Experimental values have been expressed as mean ± SEM (n=6 in each test group) *** P<0.001 (a) whilecompareby thediabetic control(One–way ANOVA, follow by Tukeys test).

Figure 10

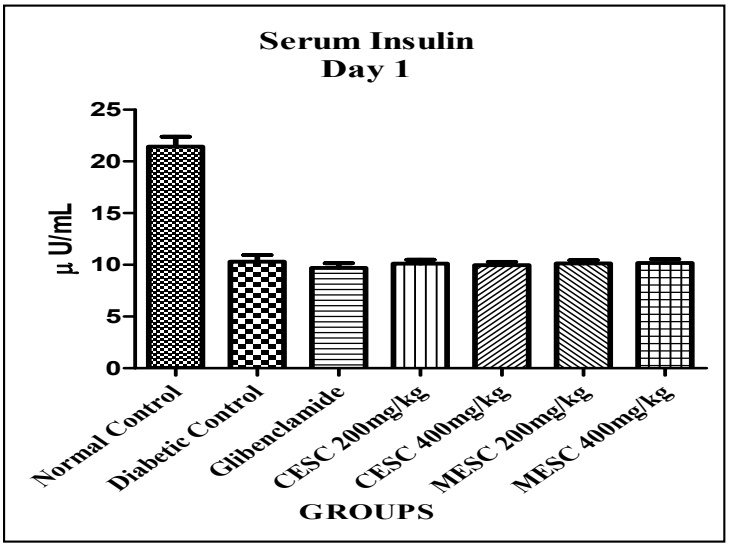
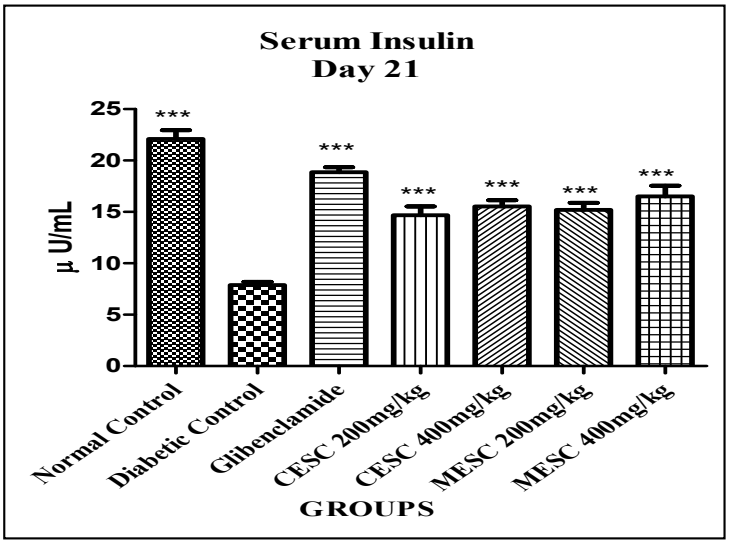
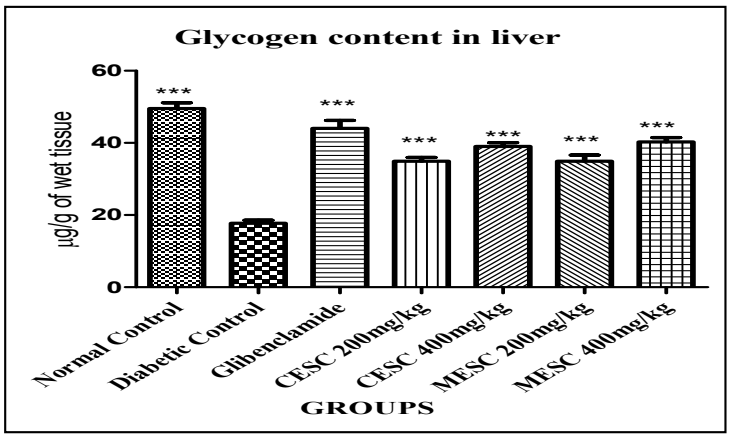


Figure 11





STZ induced diabetics in rats exhibited reduced insulin. Administration of CESC and MESC at 200mg/kg, 400mg/kg dosages caused noteworthy (P<0.001) increment in insulin levels toward send of the study period of 21 days. CESC 200mg/kg induced maximum increase in insulin comparable to that induced by glibenclamide (Table5, Figures 10, 11). The liver glycogen content in liver diminished significantly (P<0.001) in diabetic control when compared to normal control animal group. Administration of CESC and MESC of 200mg/kg, 400mg/kg dosages caused statistically significant increase in glycogen levels in the liver tissue (P<0.001). However, the glycogen values were normalized when the administration of the plant extracts was ceased.

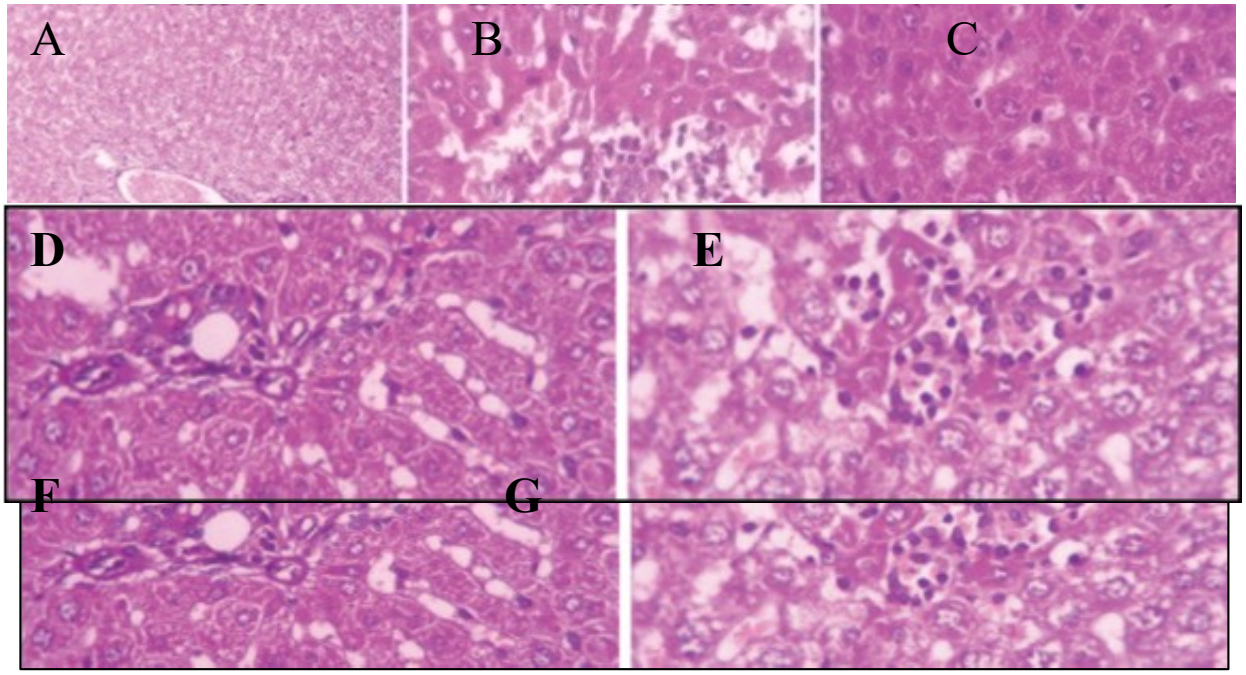


Figure 13: Histopathology of liver as observed in:
(A) Normal Control –Presence of normal pancreatic islet cells
(B) Diabetic Control- Presence of degranulated and dilated islet cells
(C) Diabetic+ Glibenclamide (10mg/kg)-granulated, non-appearance of dilation and hyper plasticity of islets
(D) Diabetic+ CESC 200mg/kg- Pancreas showing islets with endocrine cellsshowing more cytoplasm
(E) Diabetic+ CESC 400mg/kg- Granulated Pancreas showing islets
(F) Diabetic+ MESC 200mg/kg- Pancreas demonstrating islet shows Endocrine cells indicating typical cytoplasm
(G) Diabetic+ MESC 400mg/kg- Granulated Pancreas showing islets