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Chrysophyllum albidum (African star apple) Fruit-Skin Attenuates Hyperglycemia-Mediated Oxidative Stress in Experimental Diabetic Rats

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Abstract: Background: Diabetes mellitus, a chronic metabolic disturbance characterized by hyperglycemia has become a health problem affecting millions of people worldwide. Hyperglycemia mediated oxidative stress performs a key role in diabetic complications. The fruit-skin of *Chrysophyllum albidum* (African star apple) has been reported to have anti-hyperglycemic, hypolipidemic and *in vitro* antioxidant properties, but its effect on oxidative damage in diabetic animals has not been well investigated.

Method: This study investigated the effect of *Chrysophyllum albidum* fruit-skin (CAFS) in mitigating hyperglycemia induced oxidative stress in diabetic rats. Oxidative stress markers included superoxide dismutase (SOD) and catalase (CAT), lipidperoxidation status using malondialdehyde and non-enzymatic antioxidants in serum, hepatic and pancreatic tissues. Non-diabetic and streptozotocin-diabetic rats were compared after 4 weeks of treatment.

Results: The diabetic untreated group showed significant ($p< 0.05$) elevation of malondialdehyde and depression of non-enzymatic antioxidants levels, while suppressing SOD and CAT activities in serum, hepatic and pancreatic tissues when compared to normal rats. CAFS supplemented diet treatment ameliorated these metabolic disturbances and increased the activities of hepatic and pancreatic SOD from 0.28±0.25 to 0.61±0.13 Unit/mg protein and 0.08±0.01 to 0.15±0.01 Unit/mg protein respectively and CAT from 0.21±0.06 to 0.81±0.17 Unit/mg protein and 0.07±0.01 to 0.50±0.15 Unit/mg protein respectively.

Conclusion: The findings suggest that CAFS treatment in diabetic rats exerts a protective effect via attenuation of oxidative stress mediated by hyperglycemia and improves the antioxidant status.

Keywords: Oxidative stress, lipid peroxidation, *Chrysophyllum albidum*, fruit-skin, hyperglycemia.

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1. Background

Diabetes Mellitus (DM) is a disorder of multiple aetiologies characterized by high blood glucose or hyperglycemia and disturbances in the metabolism of protein, fat and carbohydrate respectively. It is associated with total or relative deficiencies in insulin action or secretion (Ojo *et al.,* 2023; Sapra and Bhandari, 2021; Noriega-Cisneros *et al.,* 2012; Ramachandran *et al.,* 2012; Kumar *et al*., 2006). According to the International Diabetes Federation (IDF), global prevalence of DM in 2017 among adults (20-79 years of age) was 425 million and is expected to rise to 629 million by the year 2045 making it to be one of the precedence causes of disability and death throughout the world.

Studies have shown that, persistent hyperglycemia could mediates diabetic complications through the production of pro-oxidant in excess of anti-oxidant metabolites (Ojo *et al.,* 2023; Hung *et al*., 2020; Basu, 2010; Rahimi *et al.,* 2005; Rosen *et al*., 2001), resulting in depletion of the activity of defense system against oxidative damage and enhancing generation of free radicals (Gandhi *et al*., 2012). At a chronic stage of hyperglycemia, the production of glucose in the liver cells is inhibited and consequently leading to an acute increase in blood glucose level and a raised expression of phosphoenolpyruvate carboxykinase gene , which is a key enzyme in gluconeogenesis regulation (Sapra and Bhandari, 2021; Shao *et al*., 2005).

Free radicals are formed disproportionately in diabetes through the process of activation of the polyol pathway, and advanced glycosylation products formation, protein kinase C activation as well as hexosamine pathways (Ali *et al.,* 2023, Yusuf *et al.,* 2020; Brownlee, 2005). Increased levels of reactive oxygen species (ROS) can induce damage in protein, lipid and DNA, and also promote inflammation by stimulating inflammatory factor secretion from activated inflammatory cells (Ali *et al.,* 2023; Rahimi *et al.,* 2005; Babusíková *et al*., 2004). Because of the relatively low expression of antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) in diabetes, β-cells of the pancreas and other tissues are vulnerable to attack by reactive oxygen species (ROS) when the system is under oxidative stress.

Furthermore, increased levels of free radicals, due to weak antioxidant defense system, may cause cellular function distruption hence, increasing the membrane susceptibility to lipid peroxidation (LPO), Lipids can undergo modification due to their interaction with reactive oxygen species (Augustine *et al.,* 2021; Messarah *et al*., 2010; Haidara *et al*., 2006).

Antioxidants decrease diabetic complications by attenuation of free radical production associated damage (Argaev-Frenkel *et al.,* 2023; Aminu, 2023; Yusuf *et al.,* 2020; Huang *et al.,* 2011). These antioxidants may exist as molecules neutralizing the reactive radicals by removing the unpaired electrons of free radicals through their electrons accepting or donating potentials. Antioxidants can regulate ROS-related enzymes by increasing the activities and expressions of antioxidant enzymes [catalase, superoxide dismutase and glutathione peroxidase (GPX)] or by hindering the activities of free radical producing enzymes [NAD(P)H oxidase (NOX) and xanthine oxidase (XO)] (Argaev-

Frenkel *et al.,* 2023; Yusuf *et al.,* 2020; Shih *et al.,* 2007; Panchatcharam *et al.,* 2006). Antioxidant vitamins such as vitamins C and E have been regarded as one of the treatments against oxidative stress due to vitamin E's potential in lowering the risk of lipid peroxidation (Marian *et al*., 2007) and its regeneration from vitamin C.

Synthetic antioxidants such as butylated hydroxylanisole and butylated hydroxyltoluene are reported to be toxic with low margin of safety. Because natural antioxidants are safe and non-toxic, much attention are being drawn by researchers towards identification of plant food materials with significant antioxidant potential for human consumption as a means to prevent damages occasioned by oxidative stress in diabetes mellitus (Ajayi *et al.,* 2021; Kancheva, 2009).

Scientific literatures reported that vegetables, seeds, fruits and fruit peels, which are rich sources of phenolic phytochemicals with dietary fiber, possess hypoglycemic potential (Aminu, 2023; Scalzo *et al*., 2005). The fruit skin of *Chrysophyllum albidum* is one of such sources due to its bioactive compounds attributed by high levels of flavonoids, phenols and fiber constituents (Aminu, 2023; Ajayi *et al*., 2020). *C. albidum,* commonly called African star apple is a low land rain species of forest tree (Ehiagbonare *et al.,* 2008), belonging to Sapotaceae family and commonly distributed throughout the tropical West, Central and East regions of Africa as well as some other parts of the world (Amusa *et al.,* 2003). It is locally called 'agbalumo' in Yoruba,'Agwaluma' in Hausa, 'udara'in Ibo, 'Utieagadava'in Urhobo, Efik and Ibibio, '*Ehya'*in Igala tribes of Nigeria (Bello and Henry, 2015; Amusa *et al.,* 2003). *C. albidum* fruit is a seasonal plant found between December and April of every year. It is widely consumed in Southern part of Nigeria when ripe. The skin is orange to golden yellow in colour when ripe is chewy like gum with sweet/sour taste (Amusa *et al.,* 2003).

Diverse parts of *C. albidum* tree have been investigated *in vitro* and *in vivo* for their various pharmacological activities. Various research studies have described the antioxidant potential of its leaves (Morakinyo *et al.,* 2023; Adebayo *et al.,* 2011a), fruits (Ajayi *et al.,* 2020; Arueya and Ugwu, 2017; Dandare *et al*., 2017; Oloyede and Oloyede, 2014; Imaga and Urua, 2013), fruit juice (Omotosho *et al.,* 2013), stem-bark (Yusuf *et al.,* 2020; Idowu *et al*., 2016) and eleagnine, which is an alkaloid obtained from cotyledon of *C. albidum* seed (Ajayi *et al.,* 2020; Idowu *et al.,* 2006). Other reseachers reported the hypoglycemic effect of its leaves (Adebayo *et al.,* 2010) and seed cotyledon (Olorunnisola *et al.,* 2008). In addition, the antifertility effect of *C. albidum* root bark (Onyeka *et al.,* 2012), antiplatelet and hepatoprotective effects of its leaves (Adebayo *et al.,* 2010, 2011b respectively) have been reported. Similarly, the antimicrobial effect of its leaves (George *et al.,* 2018; Kamba and Hassan 2011; Okoli and Okere, 2010; Duyilemi and Lawal, 2009), fruits (George *et al.,* 2018), seed cotyledon **(**Okoli and Okere, 2010; Idowu *et al.,* 2003), root (Okoli and Okere, 2010) and stem bark (Idowu *et al*., 2016; Kamba and Hassan 2011; Adewoye *et al.,* 2010;) have been documented.

Earlier studies on *C. albidum*-fruit skin have confirmed the free radical scavenging potential of its methanolic extract by *in vitro* model (Ibrahim *et al*., 2019a). Furthermore, the antidiabetic and lipid

lowering properties of *Chrysophyllum albidum*-fruit (CAFS) supplemented diet in streptozotocin (STZ)-induced experimental diabetic rats has also been reported (Ibrahim *et al*., 2019b). Thus, this present study explores the *in vivo* antioxidant effect of CAFS based food supplement in STZ-induced diabetic rats as a means of assessing the potential antioxidant property of CAFS on hyperglycemia-induced oxidative stress in experimental rats.

Figure 1: *Chrysophyllum albidum* fruit-skin (a) fresh sample, (b) lyophilized sample

2. Materials and Methods

The fresh ripe fruits of *Chrysopyllum albidum* were purchased in Moniya market, located in Akinyele local government area of Oyo State, South-Western Nigeria. Identification and authentication of the fruits was done by a taxonomist, Esimekhuai, D.P.O. at the herbarium section of the department of Botany, University of Ibadan (UI), Oyo State, Nigeria and a voucher specimen was deposited with registration No. UIH/20I6/22502. The purchased fruit of *C. albidum* was washed thoroughly and its fruit-skin was removed, cut into small pieces and lyophilized (Figure 1b) for a period of 54 hours at International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria, using Millorock Bench-Top Freeze Dryer, Germany and stored at -20ºC until commencement of the experiment.

2.1 Feed formulation

Feed formulation was carried out according to the stipulations given by the National Academy of Sciences (1995) governing dietary nutritional requirement for maintenance and growth of laboratory rats. The two feed diets compounded were identically formulated using corn meal, casein, soya bean oil as the sources of carbohydrate, protein and fat respectively in both normal control group (NC) and test (CAFS) group diets. However, the fiber content was varied for the two diets. Rice bran served as the sole source of the 10% fiber composition in NC diet while 0.7% CAFS and 0.3% rice bran served as the two sources of the 10% fiber composition in the test (CAFS) group diet to produce CAFS based food supplement. The chemical compositions of the two formulated diets were conducted as previously reported (Ibrahim *et al*., 2019b).

2.2 Experimental animals

Forty (n = 40) male Wistar (Sprague Dawley) rats weighing 160 ± 20g were employed for this study. The rats were maintained in the research animal section of Babcock University, located at Ilishan-Remo, Ogun State, Nigeria and were housed under standard laboratory conditions as we previously reported (Ibrahim *et al*., 2019b). All the procedures of experiment were carried out according to internationally accepted guidelines specify for the use and care of experimental laboratory animals. The protocol used in this study was approved by Health Research Ethics Committee (BUHREC) - BU/BUHREC029/15, of Babcock University according to the Institute for Laboratory Animal Research Guides for the maintenance and Use of Laboratory research Animals (Institute for Laboratory Animal Research (ILAR), 2011; National Institute of Health (NIH), 1985). All rats were initially fed a standard chow diet during the two weeks of acclimatization and water *ad libitum*.

2.3 Induction of diabetes for experimental rats

Diabetes induction was carried out by a single intraperitoneal injection of streptozotocin (STZ) that was prepared freshly at a dose of 50 mg/kg bw. Streptozotocin was prepared in 0.1 M citrate buffer at pH 4.5, and administered to rats that have been fasted overnight according to Thirumalai *et al*. (2011) and Prasath *et al*. (2014) with small modifications of the procedure. 5% glucose solution was provided for the rats after six hours of STZ administration for the next 12 hours to overcome hypoglycemia occasioned by induction of streptozotocin. The hyperglycemic state was confirmed after 3 days of STZ induction, rats with marked hyperglycemia consisting of fasting blood glucose (FBG) $\geq$ 250 mg/dL and postprandial glucose (PPG) $\geq$ 350 mg/dL were employed in the study.

2.4 Experimental design

The experimental rats were divided into four (4) groups consisting of ten (10) rats each as represented in Figure 2. Group 1 consisting of normal (non-diabetic) rats, received 1ml normal saline as vehicle, fed normal control (NC) diet for four (4) weeks and labeled normal control. Group 2 consisted of diabetic rats that received vehicle, fed with a NC diet for 4 weeks and served as diabetic control (diabetic untreated). Group 3 consisted diabetic rats, received glibenclamide at a dose of 2.5 mg/kg bw dissolved in vehicle, fed NC diet for four weeks and were labeled glibenclamide

treated diabetic group. Group 4 consisted of diabetic rats that received vehicle, they were fed 70g/kg of freeze-dried CAFS supplemented diet serving as CAFS-treated (test) group.

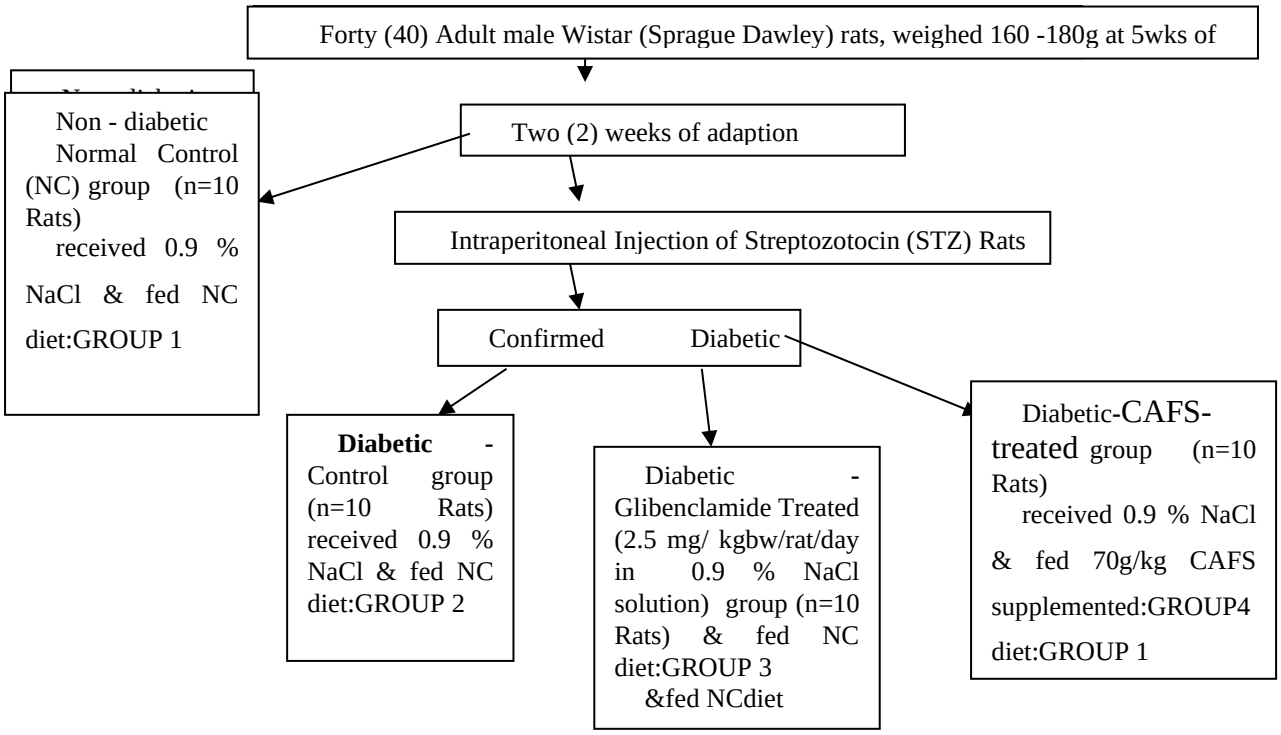

Figure 2: Experimental animal design

2.5 Blood sample collection and serum preparation

After 28 days of rats treatment, the rats were fasted overnight and sacrifice was done by cervical dislocation. The blood samples was collected by cardiac puncture using sterilized needle and syringe into specimen bottles, allowed to clot and centrifuged at 3,000 × *g* for the period of 10 minutes. The serum was decanted, stored at -80°C and then used for oxidative stress biomarkers assay.

2.6 Tissue homogenate preparation

The sacrificed rats were dissected to remove the liver and pancreas. A portion of liver and pancreas was rinsed in 0.25 M ice cold sucrose solution and subsequently homogenized in phosphate buffer (0.05M, pH 7.4) to obtain a final solution of 10% w/v homogenates using Ultra Turrax homogenizer. The centrifugation of homogenates was at 10,000 × g for 30 min at 4^{o}C and the supernatants were employed for the assay of oxidative stress biomarkers as well as total protein concentration.

2.7 Evaluation of oxidative stress biomarkers

2.7.1 Determination of Malondialdehyde (MDA)

The concentration of lipid peroxidation products that are present in the serum and supernatants of liver and pancreatic tissue homogenates (samples) were determined using thiobarbituric acid (TBA) method, measuring the malondialdehyde (MDA) reactive products in the method described by

Ohkawa *et al.* (1979) which is based on the measurement of the pink color produced by the reaction of a chromogenic reagent called 2-thiobarbituric acid, with malondialdehyde at 25°C using spectrophotometer. 2.0 molecules of 2-thiobarbituric acid react with One molecule of MDA to yield a chromophore, which absorbs at 532 nm. Extinction coefficient of MDA, $\varepsilon 532 = 1.53 \times 10^5\ M^{-1}cm^{-1}$. The amount of MDA is expressed in nmol/mg protein.

2.7.2 Superoxide dismutase (SOD) assay

The SOD activity was assayed in serum and supernatants of liver and pancreatic tissue homogenates using the method of Mccord and Fridovich (1969) and as described by Attar *et al*. (2006). This method is based solely on the inhibitory effects of superoxide dismutase on the pyrogallol autooxidation initial rate. Superoxide dismutase enzyme catalyzes the dismutation of the superoxide anion to give hydrogen peroxide (H_2O_2) as well as oxygen molecule. 50% inhibition of the rate of auto-oxidation of pyrogallol is 1unit of the enzyme activity, and this is measured at the absorbance of 420 nm per min. SOD activity was measured in U/mg protein.

2.7.3 Catalase (CAT) assay

The activity of CAT in serum and supernatants of liver and pancreatic tissue homogenates were measured by adopting Aebi (1984) method of assay as described by Li and Schellhorn (2007). Catalase decomposes H_2O_2 to give water (H_2O) and oxygen (O_2) and oxidation of hydrogen donors. H_2O_2 shows a continuous increase in absorption with reducing wavelength in ultraviolet range. H_2O_2 decomposition can be directly followed by the decrease in absorbance at the wavelength of 240 nm. Absorbance difference per unit time is a measure of catalase enzyme activity using the molar extinction coefficient of $H_2O_2 = 43.6\ M\ cm^{-1}$. One mmol of H_2O_2 consumed / min/ mg of protein is equal to one unit of activity and this is expressed as U/mg protein.

2.7.4 Determination of Reduced glutathione (GSH) content

The GSH concentration was measured in the serum and supernatants of liver and pancreatic tissue homogenates by the methods of Ellman (1959); Beutler and Kelly (1963).The thiol reagent, 5-5'-dithiobis [2-nitrobenzoic acid] (DTNB, Ellman's Reagent) reacts with free sulphydryl groups like glutathione to form 5-thionitrobenzoic acid (TNB) and GS-TNB complex. The relatively stable yellow complex formed can be measured at 412 nm and the concentration of reduced glutathione in the sample is proportional to the colour intensity. The concentration of reduced glutathione is measured in milligram/100 gram (mg/100g) wet tissue or in serum as mg/dl.

2.7.5 Determination of Vitamin C (ascorbic acid) content

Vitamin C estimation in serum and supernatants of liver and pancreatic tissue homogenates were carried out using the procedure of Omaye *et al.* (1979). The assay procedure is based on the colorimetric measurement, which involves precipitation with tricarboxylic acid followed by its

estimation in 2, 4 dinitrophenyl hydrazine: thiourea: copper sulphate for colour development. Absorbance of the solution was read at wavelength of 520 nm and pure ascorbic acid was used to prepare the standard curve. The value of the ascorbic acid was measured in mg/100g wet tissue and in serum as mg/dl.

2.7.6 Vitamin E (α-Tocopherol) content determination.

Serum Vitamin E content and that of supernatants of liver and pancreatic tissue homogenates was determined using the method of Desai *et al.* (1984). This method is a colorimetric measurement procedure, which involves saponification and extraction of the content with ethanol ascorbate and KOH followed by its estimation. The absorbance was read at 536 nm. The value of Vitamin E was measured in milligram/100 gram (mg/100g) wet tissue or in serum as mg/ dl.

2.7.7 Protein Assay

Total protein concentration was determined in the Supernatants of liver and pancreatic tissues using the colorimetric procedure of Lowry *et al.* (1951) and as portrayed by Holme and Peck (1998). The principle of the procedure is based on the peptide nitrogens (s) reactivity with copper (II) ions in alkaline medium and Folin-Ciocalteau phosphomolybdic phosphotungstic acid and subsequent reduction to give heteropolymolybdenum blue by oxidation of aromatic acids. Lowry method of assay maintained the pH of 10-10.5 because of its sensitivity to pH change. The absorbance of the solution was read at 550nm wavelength with bovine serum albumin (BSA) as standard.

2.8 Statistical analysis

All obtained data were analyzed with standard Statistical Software Package (Graphpad prism Version 5.01) and expressed as Mean ± SEM. One way Analysis of Variance (ANOVA) was used to compare relative expression levels for different experimental groups followed by Turkey post hoc test. Level of significance was taken at ($p< 0.05$) for Six (6) rats per group.

3.0 Results

3.1 Serum, hepatic and pancreatic Malondialdehyde concentrations

Diabetic untreated (diabetic control) rats exhibited a significant elevation of malondialdehyde (MDA) levels in serum, liver and pancreatic tissues when compared with the normal control, but markedly reduced on treatments with CAFS based food supplement (Figure 3).

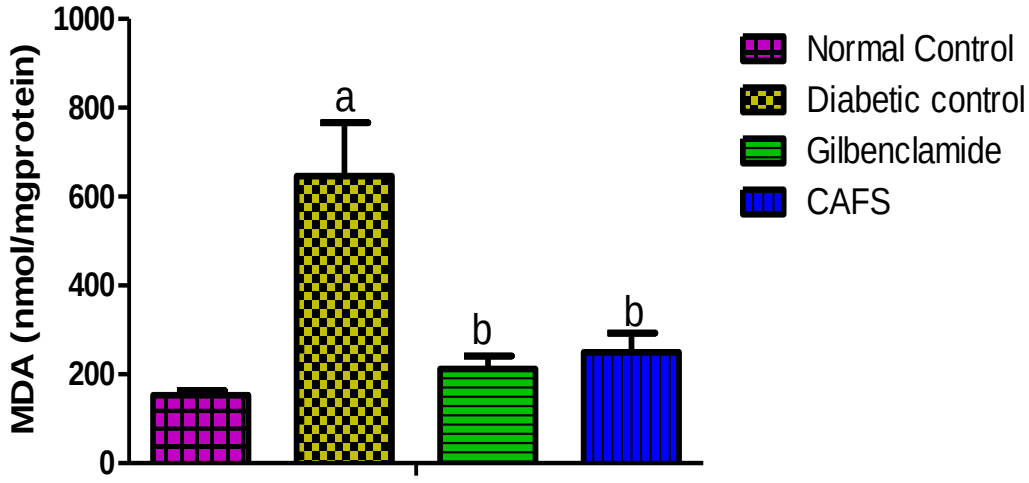

Figure 3.1a: MDA concentration in serum of various treatment groups

Level of significance was taken at $p<0.05$ for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

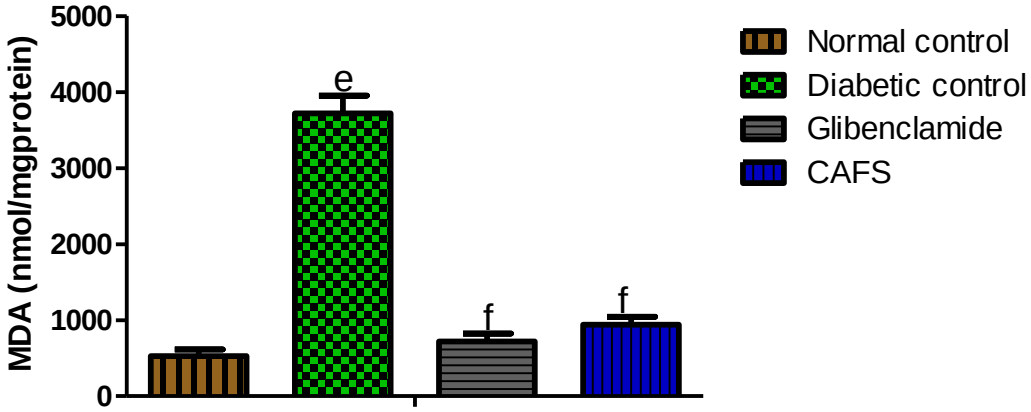

Figure 3.1b: MDA concentration in liver of various treatment groups

Level of significance was taken at $p<0.05$ for 6 rats per group
'e' is significantly different from normal control
'f' is significantly different from Diabetic control

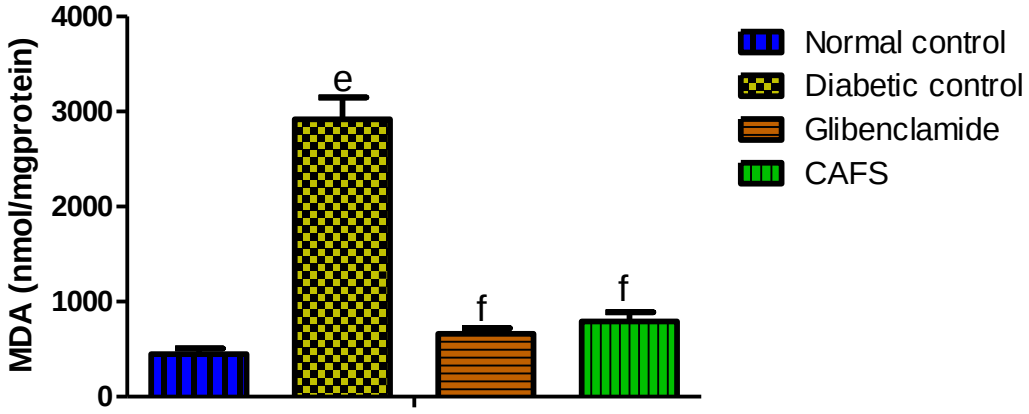

Figure 3.1c: MDA concentration in pancreatic tissue of various treatment groups

Level of significance was taken at $p<0.05$ for 6 rats per group
'e' is significantly different from normal control
'f' is significantly different from Diabetic control

3.2 Serum hepatic, pancreatic, superoxide dismutase (SOD) and catalase (CAT) activities

The activities of SOD (Figure 4) and CAT (Figure 5) in diabetic untreated rats were observed to be significantly ($p<0.05$) decreased in serum, liver and pancreatic tissues when compared with normal control rats. However, CAFS supplementary diet treated diabetic rats showed enhanced SOD and CAT activities in serum, liver as well as pancreas, which compared favourably with glibenclamide treated group (Figures 4 and 5).

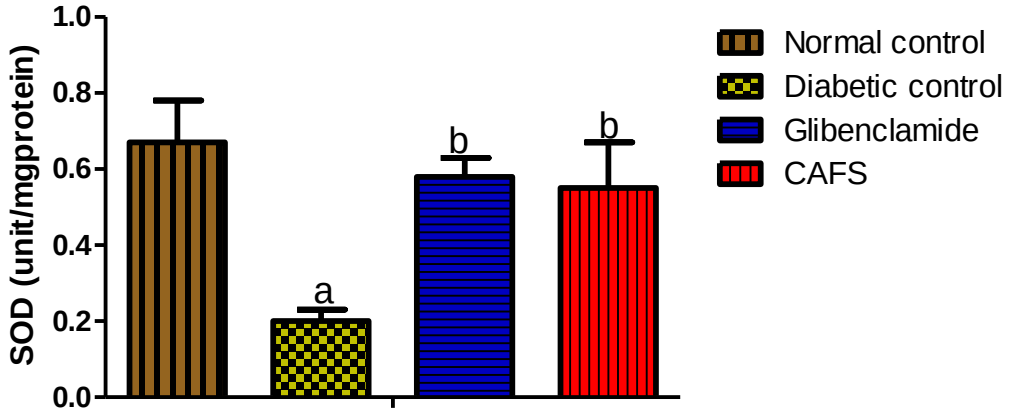

Figure 3.2a: SOD activity in serum of various treatment groups

Level of significance was taken at $p<0.05$ for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

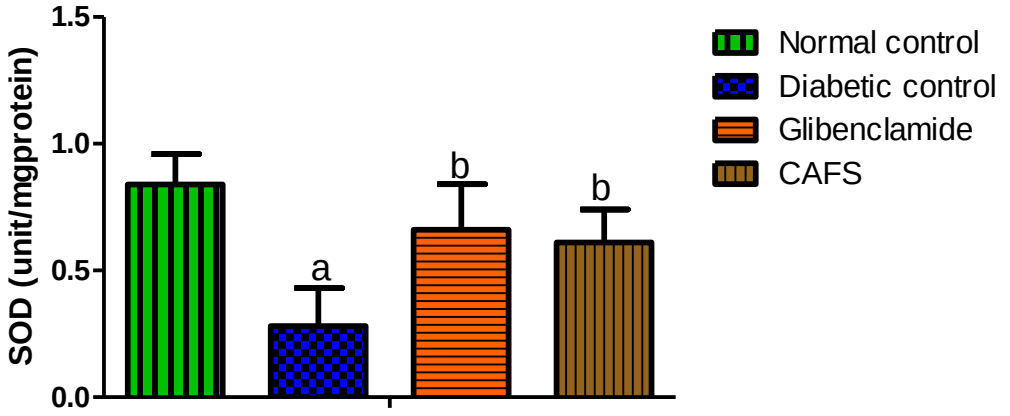

Figure 3.2b: SOD activity in Liver of various treatment groups

Level of significance was taken at p<0.05 for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

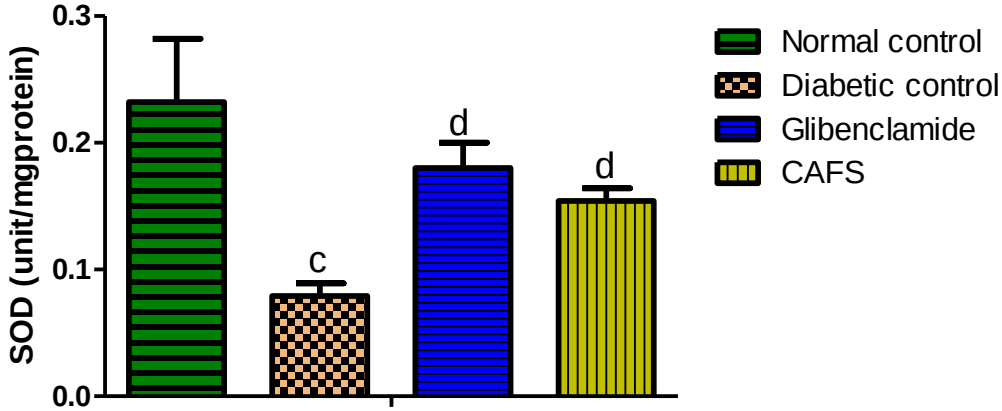

Figure 3.2c: SOD activity in pancreatic tissue of various treatment groups

Level of significance was taken at p<0.05 for 6 rats per group
'c' is significantly different from normal control
'd' is significantly different from Diabetic control

3.3 Effect of CAFS supplementary diet on serum, liver and pancreatic tissues catalase (CAT) activities

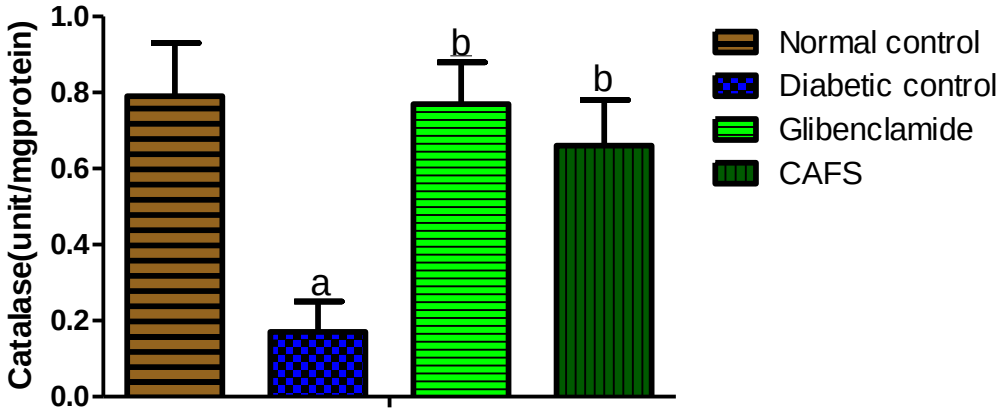

Figure 3.3a: Catalase activity in the serum of various treatment group

Level of significance was taken at p<0.05 for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

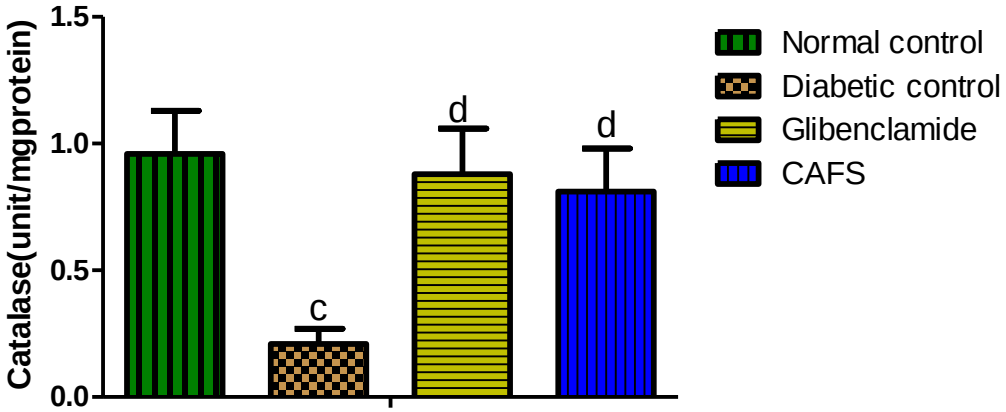

Figure 3.3b: Catalase activity in liver of various treatment groups

Level of significance was taken at p<0.05 for 6 rats per group
'c' is significantly different from normal control
'd' is significantly different from Diabetic control

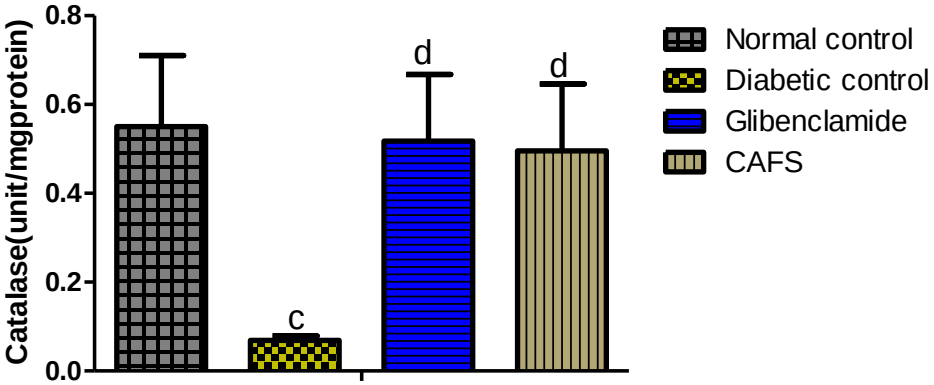

Figure 3.3c: Catalase activity in pancreatic tissue of various treatment groups

Level of significance was taken at (P<0.05) for 6 rats per group
'c' is significantly different from normal control
'd' is significantly different from Diabetic control

3.4 Serum, hepatic and pancreatic reduced glutathione

The concentration of reduced glutathione (GSH) in rats induced with streptozotocin was significantly ($p<0.05$) decreased in serum and pancreas as well as liver of the diabetic untreated rats when they were compared with the normal control rats. CAFS supplemented diet treated rats however, expressed significant ($p<0.05$) protection by increasing the concentrations of serum, liver and pancreatic GSH.

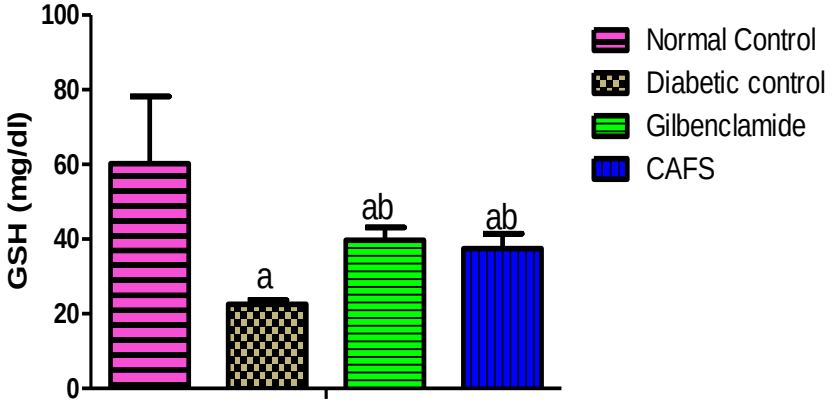

Figure 3.4a: GSH concentration in serum of various treatment groups

Level of significance was taken at p<0.05 for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

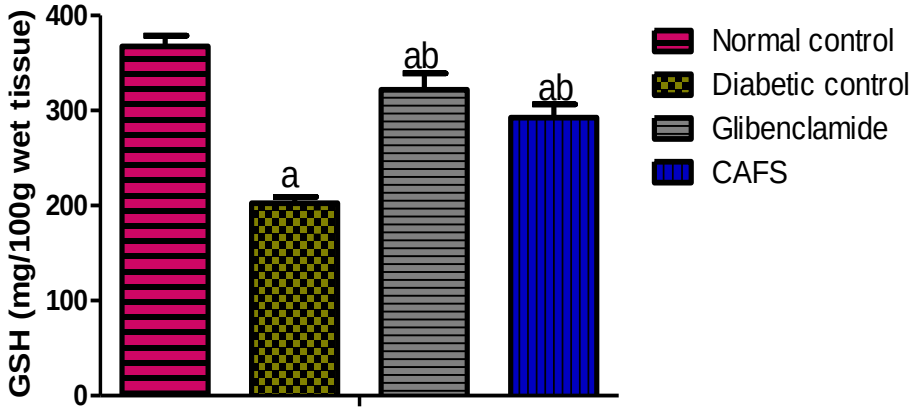

Figure 3.4b :GSH Concentration in Liver of various Treatment groups

Level of significance was taken at p<0.05 for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

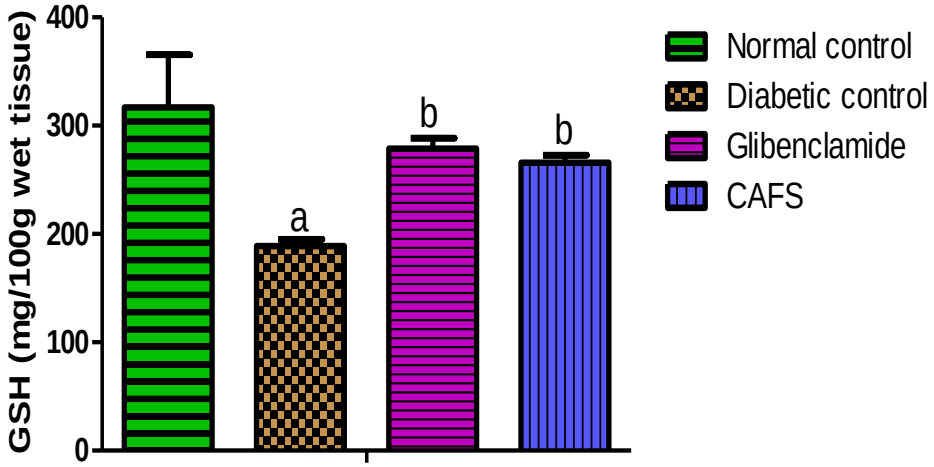

Figure 3.4c: GSH Concentration in pancreatic tissue of various Treatment groups

Level of significance was taken at p<0.05 for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

3.5 Serum, hepatic and pancreatic vitamin C concentrations

As shown in Figure 3.5(a, b, c) diabetic rats that were not treated significantly (p< 0.05) reduced serum, liver and pancreatic concentrations of vitamins C compared to those in normal control. However, following treatment with CAFS supplementation for four weeks, the observed decrease in serum, liver and pancreatic tissues vitamins C were reversed to near normal values.

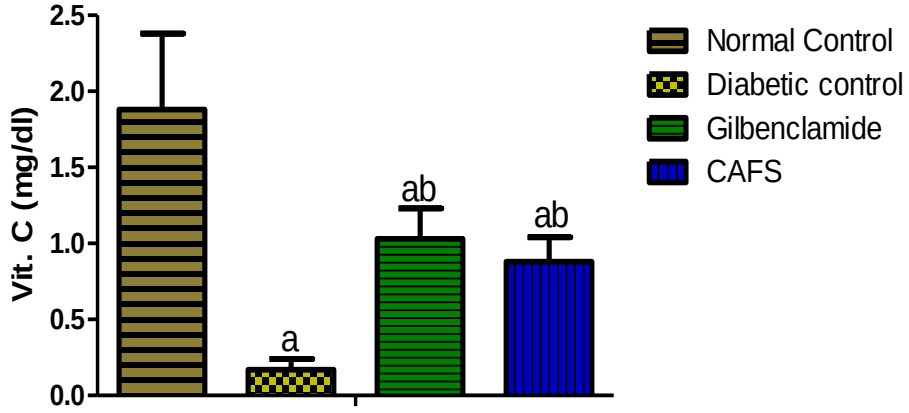

Figure 3.5a: Vitamin C concentration in serum of various treatment groups

Level of significance was taken at p<0.05 for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

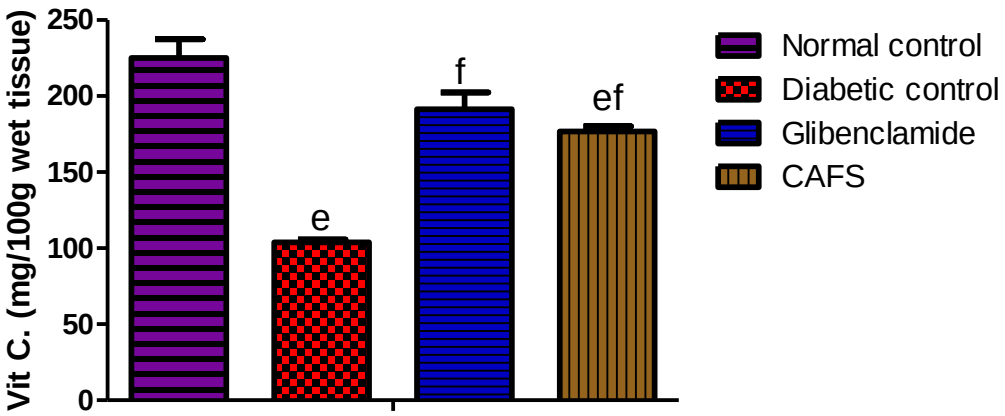

Figure 3.5b: Vitamin C concentration in liver of various treatment groups

Level of significance was taken at p<0.05 for 6 rats per group
'e' is significantly different from normal control
'f' is significantly different from Diabetic control

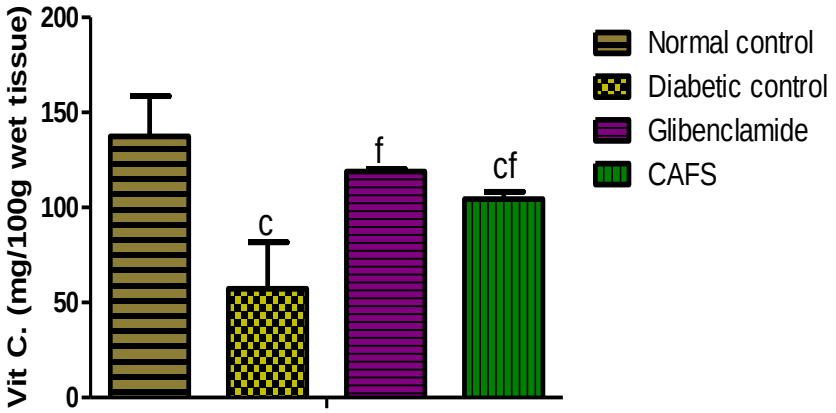

Figure 3.5c : Vitamin c concentration in pancreatic tisue of various treatment groups

Level of significance was taken at (P<0.05) for 6 rats per group
'c' is significantly different from normal control
'f' is significantly different from Diabetic control

3.6 Serum, hepatic and pancreatic vitamin E concentrations

As shown in Figures 3.6(a, b, c), diabetic rats that were not treated significantly (p< 0.05) reduced serum, liver and pancreatic concentrations of vitamins E compared to those in normal control. However, following treatment with CAFS supplementation for four weeks, the observed decrease in serum, liver and pancreatic tissues vitamins E were reversed to near normal values.

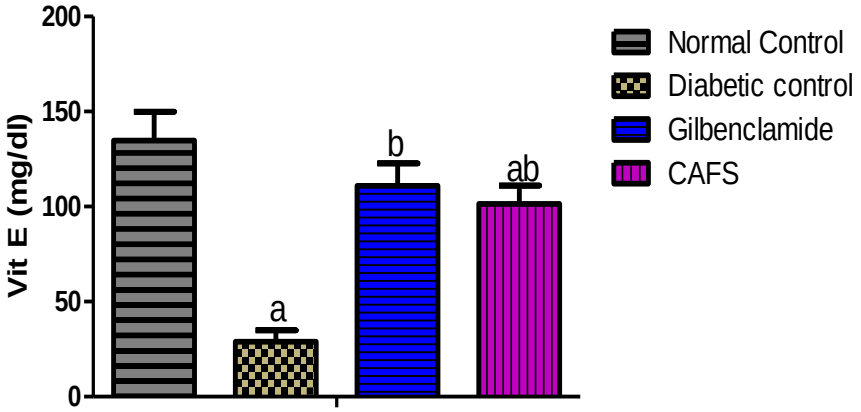

Figure 3.6a : Vit. E concentration in serum of various treatment groups

Level of significance was taken at $p<0.05$ for 6 rats per group
'a' is significantly different from normal control
'b' is significantly different from Diabetic control

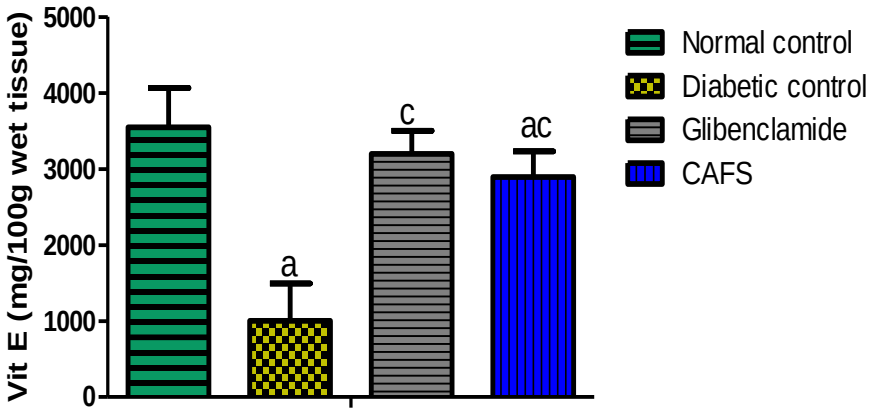

Figure 3.6b: Vitamin E concentration in liver of various treatment groups

Level of significance was taken at $p<0.05$ for 6 rats per group
'a' is significantly different from normal control
'c' is significantly different from Diabetic control

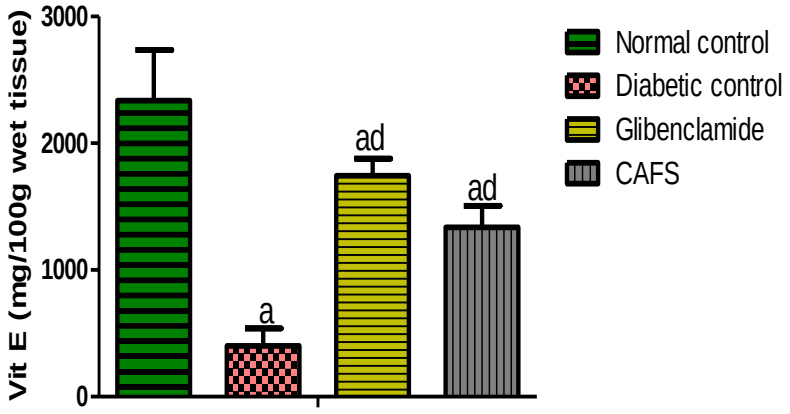

Figure 3.6c : Vitamin E concentration in pancreatic tissue of various treatment groups

Level of significance was taken at $p<0.05$ for 6 rats per group
'a' is significantly different from normal control
'd' is significantly different from Diabetic control

4.0 Discussion

Diabetes mellitus and oxidative stress have been reported to be well associated and this leads to decrease in non-enzymatic and enzymatic antioxidant defense system. Medicinal plants may act as an alternative resources or adjunctive treatment option in the treatment of diabetes and its cardiovascular complications (Aminu, 2023, Azemi *et al.,* 2022). The *in vivo* antioxidant defense parameters investigated in this study are reduced glutathione (GSH), Vitamin C, Vitamin E, malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). Plants with antioxidant properties can scavenge free radicals, offer protection to the islet of the pancreas against cellular toxicity occasioned by streptozotocin and regenerate oxidative damaged cells.

The present study investigated on the serum, hepatic and pancreatic lipid peroxidation status by measuring malondialdehyde (MDA) levels. The observed elevation of MDA in diabetic untreated in the blood and tissues studied revealed the degree of cellular lipid peroxidation and is regarded as the biomarker of cellular injury (Sentthilkumar *et al.,* 2021; Albano, 2008). This finding is in line with the reports of Youssef and McCullough (2002), Yusuf *et al.*, 2020 and Seena *et al.* (2017) who presented the increased levels of lipid peroxides in diabetic experimental rats models of liver and pancreatic tissues respectively. Padalkar *et al*. (2012) suggested that increased tissues MDA is a sign of oxidative stress in degenerative diseases such as diabetes mellitus. Shodehinde and Oboh (2013), described MDA as the major end product of peroxidation of lipids and an indicator of damage to the tissue by chain reactions. According to Evans *et al*. (2002), oxidative stress secondary products are lipid peroxides and are unleashed due to the harmful effect of reactive oxygen species (ROS) generated during peroxidation of lipids in diabetes. Food supplementation with CAFS to diabetic-induced rats in the present studies notably declined the levels of serum, hepatic as well as pancreatic lipid peroxides. This observation is consistent with Ochuko *et al*. (2012) in which reduction in MDA

in tissues of the brain of diabetic rats was reported after treatment with a certain plant fiber and also Setyawati and Hidayati (2021), who reported decreased in MDA concentration after treating rat induced by cigarette smoke with orange peel (Citrus sinensis) extract. This effect could be traced to the free radical scavenging and antioxidant potentials of CAFS.

Decreased activities of antioxidant enzymes like Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and non-enzymatic antioxidant such as reduced glutathione (GSH) concentration have been reported in diabetic experimental animals (Arif *et al.,* 2022). Superoxide dismutase (SOD) and catalase (CAT) are directly involved in the elimination of oxygen species. Superoxide dismutase catalysis anion superoxide ($O^{2\bullet}$) conversion to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) while CAT completing detoxification process initiated by SOD with the decomposition of H_2O_2 and gives protection to tissues against hydroxyl radicals damage (Chelikani *et al.,* 2004; Faraci and Didion, 2004). The observed reduced SOD and CAT activities in diabetic untreated group, agree with the reports of the studies of Indradevi *et al*. (2010); Alpsoy *et al*. (2014); Jemai and Sayadi (2015) and might probably be due to the elevation of enzymatic antioxidant consumption to compensate the increased generation of ROS. CAFS based food supplement was observed to elevate the activities of SOD and CAT and resulting in reduction of oxidative damage. This could possibly be due to a decline in oxidants because of high contents of heterogeneous phyto-constituents such as flavonoids, phenol and fatty acid antioxidant esters in CAFS (Ibrahim *et al*., 2017, 2019a). The potential of any compound to hinder the superoxide radical's formation, which are toxic species, can be considered as a good marker to quantitate antioxidant activities.

Reduced glutathione is regarded as a major non-protein thiol in living cells playing a key role of coordinating the antioxidants defence process of the body. It is involved in defense system of the cell against xenobiotics and other naturally occurring harmful substances like free radicals (Averill-Bates, 2023, Bandyopadhyay *et al*., 1999). The present studies observed a decrease in GSH level of diabetes untreated, which is an indication of its raised consumption to neutralize the generated free radicals. Several studies in the literature have demonstrated the decline in GSH level in the blood and tissues of experimental diabetic animals (Indradevi *et al.,* 2010; Kumarappan *et al*., 2012 and Waly *et al*., 2015). In diabetic conditions, the plasma and tissues GSH concentrations decrease due to increased utilization caused by oxidative stress (Tsai *et al.,* 2012). This depletion can be attributed basically to the STZ injection into the experimental rats, acting as a diabetes inducer and xenobiotic. The reduction in glutathione concentration could lead to a drastic reduction in the whole antioxidant status of the rats because reduced glutathione helps in recycling antioxidants of the cell, inhibits damage by free radical, and plays an important role in the detoxification of toxic compounds (Robert *et al*., 2000). The results obtained in the present studies is consistent with what had earlier been reported by Eleazu *et al*. (2010) and Polidori *et al*., (2000) on reduction of antioxidant capacity in plasma of uncontrolled diabetes.

According to Said *et al.* (2021), high dose of Vitamin A plus Vitamin E supplementation combined with zinc as adjunct could improve glycemic control, Beta-cell function and insulin secretion in adult patient with type 2 diabetes mellitus. Carr *et al.* (2022), also reported that Type 1 and Type 2 Diabetes are associated with low Vitamin C status and a high prevalence of hypovitaminosis C in those with Type 2 diabetes mellitus relative to Type 1 diabetes mellitus. Vitamin C, is a water-soluble, non-enzymic antioxidant intercepts oxidants in the aqueous phase and prevents oxidative damage (Beter, 1994). Vitamin E, a radical scavenging antioxidant reacts with lipid peroxyl radical and interrupts the chain reaction of lipid peroxidation. Under normal conditions, propagation of lipid peroxides are being suppressed by Vitamin E. Vitamin E with vitamin C inhibit the formation of hydroperoxide, some lipid peroxidation reactions involve metal complexing agents binding transition metals and these inhibit Fenton- and Haber-Weiss-type reactions generating hydroxyl radicals ($OH^{\cdot}$) from hydrogen peroxide (H_2O_2) as well as superoxide anion (O^{2-}) (Chow, 1991; Laight *et al*., 2000).

In the present studies, the reduction in vitamin C level of diabetic untreated rats could be associated with its increased utilization in scavenging reactive oxygen species. Reduction in GSH concentration, can also lead to a decrease of vitamin C since GSH is employed in recycling of vitamin C (Averill-Bates, 2023, Inofers and Sies, 1988). Furthermore, the observed reduction in vitamin E concentration of untreated diabetic rats could be linked to the declined vitamin C concentration, because literatures have established the synergy between vitamin C and E (Hossain and Asada, 1985; Beter, 1994). Our results agree with the earlier reports by Wittenstein *et al*. (2002); Varvarovska *et al*. (2004) as well as studies of Ehimen *et al.* (2012) who showed significant reduction in the levels of endogenous antioxidants vitamins C and E in diabetes patients. However, diabetic rats treated with CAFS supplementation showed significant elevation in the concentrations of liver, serum as well as pancreatic Vitamins C and E. This implies that CAFS based food supplement could possess an ameliorative effect on the changed antioxidant status of rats induced diabetes. This could be traced to the high contents of antioxidant compounds and active phytochemicals such as flavonoids, phenols, tannins and saponins in CAFS. Flavonoids possess many pharmacological activities like antihyperlipidemic, antidiabetic as well as hypoglycemic potentials. Lipid absorption can be inhibited by the presence of tannins and saponins present in the medicinal plants. In addition, phenolic compounds possess strong antioxidant activity that can offer protection to cells against free radicals mediated oxidative damage (Kirakosyan *et al.*, 2003).

5. Conclusion

Our results have clearly demonstrated that CAFS based food supplement exhibited significant protective effect against hyperglycemia-mediated oxidative damage in the hepatic and pancreatic tissues *via* decreasing lipid peroxidation, increasing the activities of SOD and CAT which are antioxidant defense system enzymes and raised the levels of GSH, vitamins C and Vitamin E in

experimental diabetic rats. Thus, CAFS could be used as prevention of tissues oxidative damage in experimental diabetes. Studies are in progress to further elucidate possible mechanisms employed by CAFS for its modulatory effects.

6. Abbreviations

CAFS, *Chrysophyllum albidum* fruit skin, CAT, Catalase, SOD, Superoxide dismutase, DM, Diabetes Mellitus, LPO, Lipid peroxidation, XO, Xanthine oxidase, NADPH, Nicotinamide adenine dinucleotide phosphate, NOX, Nitrogen oxides (NO and NO_2), ROS, reactive oxygen species, UIH, University of Ibadan herbarium, NC, Normal control, STZ, streptozotocin, MDA, Malondialdehyde.

7. Competing interest

The authors declare that they have no competing interests.

8. Consent for publication

Not applicable

9. Ethics approval and consent to participate

Ethics approval for the care of laboratory animals gotten.

10. Authors' contribution

HI and OO designed, coordinated the research and drafted the manuscript, HI carried out the experiment, KO, EO and MA carried out data analyses and interpretation of results, HO and OO conceived the study and participated in the study.

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