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Ameliorative Potential of Methanolic Extract of *Senna Siamea* on Fructose-Induced Prediabetes on the Liver and Pancreas of Adult Wistar Rats

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ABSTRACT
Consumption of fructose, widely used in food processing including soft drinks, has been associated with the occurrence of prediabetes and its complications. This has greatly contributed to the rapid increase in the number of people living with Diabetes Mellitus globally. *Senna siamea*, a tropical plant commonly called Casia and "Malga" in hausa have been reported to be useful in treatment of disease traditionally due to its phytochemical compound including anthraquinones, coumarins, alkaloids, flavonoids, glycosides, triterpenoids, sterols and other polyphenols. Therefore, the aim of this study was to investigate the ameliorative potential of methanol extract of *Senna siamea* on fructose- induced prediabetes on the liver and pancreas of adult Wistar rats. Twenty-five (25) adult male Wistar rats were used and divided into five groups according to substance of administration; distilled water, fructose, *Senna siamea*, 150 mg/kg b.w (SS 150), *Senna siamea*, 300mg/kb b.w (SS 300) and metformin. They were allowed to acclimatize for 2 weeks before the onset of administration which lasted for 14 days. All experimental procedures were conducted in accordance with the standard international guidelines on the use of animal for research. Approval for the study was obtained from the Departmental Ethics Committee and Health Research Ethics Committee on Animal Use, College of Medicine, University of Lagos, Nigeria. Body weight and blood-glucose level were monitored daily. After administrations, all animals were euthanized then sacrificed and preserved in accordance to various parameters assayed including oxidative stress markers (reduced glutathione- GSH, catalase- CAT, superoxide dismutase- SOD and lipid peroxidation- MDA), lipid profile and histology (Hematoxylin and Eosin stain). Fructose (p<0.0001) and SS 300 groups (p<0.001) showed a statistically significant increase in blood-glucose level compared to control group. SS 150 group showed no statistical significant changes in SOD and MDA level in both pancreas and liver compared to control group. Both SS 300 and SS 150 showed improved lipid profile compared to fructose diet group with mild occurrence of histopathological changes. Findings from this study elucidated that administration of SS 150 optimally ameliorate damages induced by the consumption of Fructose.

Keywords: Fructose, liver, oxidative stress, *Senna siamea*, Pancreas

INTRODUCTION
Non-genetic environmental factors including diet and food have been reported to induce multiple diseases[1,2]. Hence there is the need to carefully examine what we ingest. Fructose is an acceptable sweetener used widely in food processing including soft drinks[3,4], however, recent studies have associated the consumption of fructose with the occurrence of pre-diabetes and its complications[5,6]. Normal rats fed with fructose enriched diets have been demonstrated to develop hyper-glycemia, hypertension, hyperlipidemia, insulin resistance and hyper-triglycedemia[7].

The rapid increase in the number of people living with Diabetes Mellitus (DM) worldwide is quite alarming[8]. About 422 million people are living with diabetes globally [9] in which about 16 million of this population exist in Africa and approximately 1.7million in Nigeria [10]. Disturbances in glucose metabolism and changes in lipid profiles are important biochemical findings in fructose fed Rats[11, 12]The mechanism underlying fructose-induced metabolic syndrome still remain complex, however, studies have related this to its ability to increase in gluconeogenesis and lipogenesis since it by-passes the major regulatory steps of glycolysis[13].

Prediabetes (or intermediate hyperglycemia) already displays metabolic alterations and is a high risk state for developing T2DM. According to the American Diabetes Association, prediabetes is distinguished by having impaired fasting glucose (IFG) (100–125 mg/dL; 5.6 -7.0 mmol/L glucose), and glycated hemoglobin (HbA1c) levels between 5.7–6.4%. The prevalence of prediabetes is rapidly increasing with over 470 million people projected with prediabetes by 2030[14]. This likely anticipates increased morbidity, mortality, and healthcare costs in the near future with

DM management. Thus, preventing the progression of pre diabetes to T2DM is the most rational and effective way to combat the DM epidemic and lessen healthcare costs. However, before we can succeed, we need to unravel glucose and lipid metabolism at this stage of the disease.

Senna siamea Lam. is a tropical plant of family Caesalpiniaceae called "Malga [15,16].It is a native of Southeast Asia, introduced and now naturalized in Africa [16,17.] S. siamea is claimed traditionally to be useful in treatment of various medical conditions such as diabetes, insomnia, hypertension, asthma, constipation and dieresis (Hill, 1992) due to its phytochemical compound including anthraquinones, coumarins, alkaloids, flavonoids, glycosides, triterpenoids, sterols and other polyphenols[17,19].

Modern synthetic anti-hyperglycemic agents are reported to produce undesirable and unwanted side effects such as; weight gain, lactic acidosis and hypoglycemia[20] and are not readily affordable [21]. Thus, the need for alternative therapy and approach with less or no side effects are needed to manage DM. Many plants derived medicines were reported to demonstrate a bright future in therapy and management of DM due to their less toxic effects and are its availability [22]. Thus, this study was undertaken to evaluate theanti-hyperglycemic, anti-dyslipidaemia effect of methanol extracts of S. siamea in fructose fed rats as a way of validating its traditional usage.

MATERIALS AND METHODS

Animals: Twenty-five (25) adult male Wistar rats weighing between 150 and 175g were obtained from the animal house of the Department of Human Anatomy, College of Medicine university of Lagos, Idi-Araba, Lagos, Nigeria. The animals were cared for in accordance with guidelines for the care and use of experimental animals. They were given free access to rat feed from vital feed Company Ibadan, Nigeria and water ad-libitum except when fasting was required in the course of the experiment. The animals were allowed to acclimatize for 14 days before the commencement of administration. All administrations were carried out orally.

Plant collection and extraction: The leaves of S. siamea were harvested from gardens around Anyingba, Kogi state, Nigeria ,in the month of April, 2018. The study ended in November, 2018. Leaves were separately washed, wiped-dry, cut into small pieces and subsequently reduced to a coarse powder. About 100 g of the leaves and stem bark were separately extracted for 24 h with 90% methanol with intermittent vigorous shaking. The extracts were filtered, concentrated with a rotary evaporator and dried over a water bath at 45°C. The residue from the plant parts were used for experimental analysis.

Drugs and chemicals: Metformin (Merck, Germany) was purchased from a pharmaceutical store at Mushin, Lagos and was administered at 150 mg/kgbw/day. D-fructose were obtained (Sigma, France). All other chemicals used were of analytical grade.

Induction of hyperglycemia and lipidemia in albino rats: For the induction of hyperglycemia and lipidemia, rats were given 10% fructose solution as drinking water for 10 weeks according to the method of Ibrahim *et al.,* 2018.

Experimental design and treatment schedule: Male Wistar rats were randomly divided into five groups with five animals in each group. Substances of administration were administered to each group as follows:
Group I: control; received normal rat feed + distilled water

Group II: received normal rat feed + 10% fructose solution fructose drinking rats (FDR) Group III: received normal rat feed + 10% fructose solution (FDR) + SS 150 mg/kg Group IV: received normal rat feed + 10% fructose solution (FDR) +SS 300m g/kg Group V: received normal rat feed + 10% fructose solution (FDR) + Metformin 150 g/kg Determination of body weights of the Rats
Weights of the experimental animals were determined at the end of the study period using Adam weighing scale and body weights were recorded in grams (g).

Measurement of fasting blood sugar level: Blood samples were collected from the tail vein by the tail tip method [18]. Fasting blood sugar levels of animals were measured at the beginning and at the end of the study using Accu-check glucometer and Accu-chek active 50 test strips (Roche Diagnostic GmbH Mannheim, Germany). Results were expressed in mmol/l.

Collection of blood samples for biochemical analysis: Eight weeks after the commencement of the treatment, Animals were fasted overnight, sacrificed and blood samples were collected by cardiac puncture in centrifuge tubes as described by Ibrahim *et al.,* 2018. The serum was separated by centrifugation using BS 400 centrifuge at 3,000 × g for 15 minutes (Denley BS400; England). Plasma was collected into bottles using a Pasteur pipette to be used for biochemical analysis of lipid profiles.

Determination of serum lipid profiles; Total cholesterol (TC), Triglycerides(TGs), low density lipoproteins (LDL-C), and high density lipoproteins (HDLs) levels in plasma were determined using commercial diagnostic kits according to the instruction of the manufacturer (Asan Pharmaceutical, Seoul, Korea). Results were expressed in millimoles per liter (mmol/l).

Histopathology of selected organs: At the end of the

experiment, rats were sacrificed, dissected then the liver and pancreas were excised and fixed in 10% formol saline for histological studies. Hematoxylin and Eosin (H.E) stains and procedure were applied for histological demonstration.

Statistical analysis: Data from results of biochemical assay and oxidative stress markers were expressed as the means
± S.E.M. (standard errors of the mean) and analyzed with one-way and two-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test. The significance level of the analyses was set at $P<0.001$. Statistical program used was GraphPad Prism 7.0 Version for Windows, GraphPad Software (San Diego, CA, USA).

RESULTS

Effect of metformin and methanol extract of *Senna Siamea* on the Body weight

There is no statistically significant difference in body weight of animals in all treatment groups compared to control group both before and after administration period (Figure 1).

Effect of metformin and methanol extract of *Senna Siamea* on blood-glucose level

There is no statistically significant difference in blood-glucose level in all groups compared to control before administration. After 8 weeks of administration, fructose and SS 300 groupsshowed a statistically significant increase in blood-glucose level compared to control group (Figure 2).

Effect of metformin and methanol extract of *Senna Siamea* on the cytoarchitecture of the liver and pancreas of fructose-induced diabetes

Cytoarchitecture of the Liver of control group appeared normal. Hepatic lobules comprises of portal triad, hepatocytes sinusoid and central vein. Hepatocytes have rounded shape, euchromatin nucleus with acidophilic cytoplasm. Basement membrane of portal and central vein appeared preserved. Neither fatty changes nor inflammatory cells were observed. Alterations in cyto-architecture due to various degrees of pathological changes were observed in fructose, metformin, SS 300 and SS 150. Fructose diet group showed the most severe pathological changes including congestion of blood vessel, hyperplasia of inflammatory cells and multifocal Steatosis. Mild pathological changes were observed in both metformin and SS 150 treatment groups (Figure 3-7).

Pancreatic cyto-architecture of control group appeared normal. Islets of langerhans containing beta cells were located in between normal pancreatic acini of the exocrine gland. Neither necrosis of the beta cells nor atrophy of the islet of langerhans was observed. However, there were areas of hyperaemia within the blood vessels. Inversely, fructose diet group showed severe degree of pathological changes including destructed and ruptured islet of langerhans and loss of beta cell nuclei due to necrosis. SS 150 and Metformin treated group showed intense restoration potential by ameliorating the severe pathological changes induced by consumption of fructose. SS 300 showed slow progressive improvement of the pancreatic endocrine cell cyto morphology (figure 8-12).

Effect of metformin and methanol extract of *Senna Siamea* on oxidative stress level of the liver and pancreas of fructose-induced prediabetes

There was a statistically significant decrease in superoxide dismutase enzymes function in the liver and pancreas of fructose diet group compared to the control group. MDA level for Lipid peroxidation concentration showed a statistically significant increase in its level in fructoseadministered group compared to the control group in both organs. For Catalase enzyme, there was a statistically significant decrease in its activities in the pancreases but no statistically significant differences in the liver of Fructose administered group compared to control group. Metformin and methanol extract of *Senna Siamea* treatment groups showed no statistically significant changes in SOD, CAT and MDA compared to the control group in both the liver and pancreas. However SS 300 showed a statistically significant decrease in SOD activities in the pancreas compared to control group (Figure 13).

Effect of metformin and methanol extract of *Senna Siamea* on liver function test of fructose-induced diabetes

Fructose diet group showed a statistically significant increase in total cholesterol level compared to the control group. However, SS 150 and SS 300 treatment groups were more effective in reducing the increased total cholesterol level posed by fructose diet compared to metformin treatment group. There was a statistically significant increase in Triglyceride level in fructose diet group compared to control group. However, methanol extract of *Senna Siamea* at 150mg/bw and 300mg/bw showed poor potential ability in reducing the elevated triglycerides level induced by fructose diet compared to metformin treatment group.

Liver function test showed a statistically significant increase in HDL level in methanol extract of *Senna Siamea* at 150mg/bw and fructose groups compared to control group. Although, there was a statistically significant increase in LDL level in fructose diet group while methanol extract of *Senna Siamea* at 150mg/bw showed no statistically significant compared to control group. Also, methanol extract of *Senna Siamea* at 300mg/bw and Metformin treatment groups showed no statistically significant differences in both HDL and LDL level compared to control group (Figure 14).

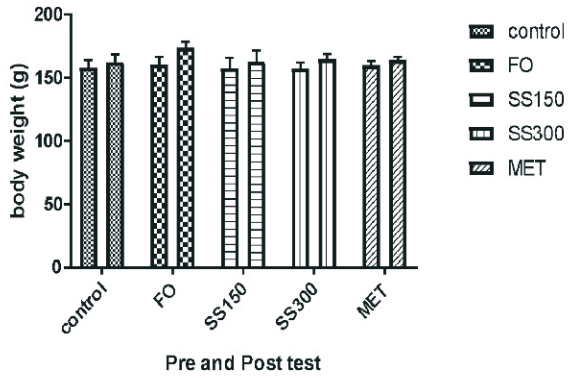

Figure 1: Body weight analysis of experimental and control groups

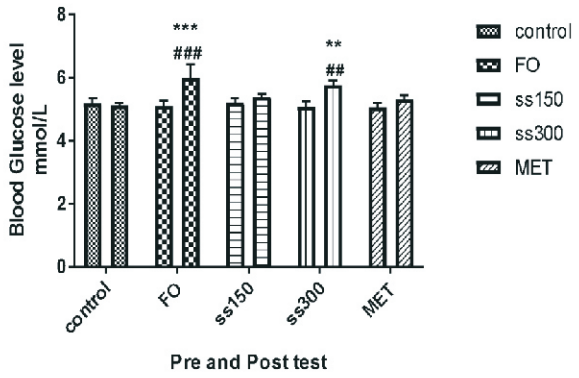

Figure 2: Blood-glucose level analysis of experimental and control groups

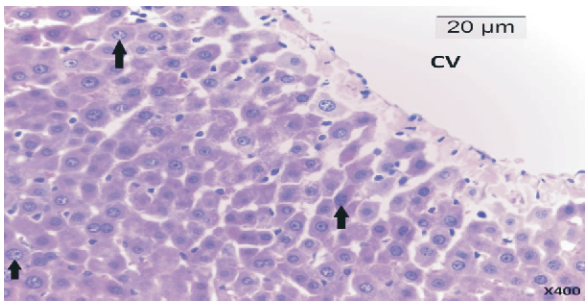

Figure 3: Photomicrograph of liver cytoarchitecture of control group appeared normal. The hepatocytes have rounded shape, euchromatin nucleus with acidophilic cytoplasm. Basement membrane of portal and central vein appeared preserved. Neither fatty changes nor inflammatory cells were observed (black arrow: hepatocytes, CV: central vein) (H&E x400)

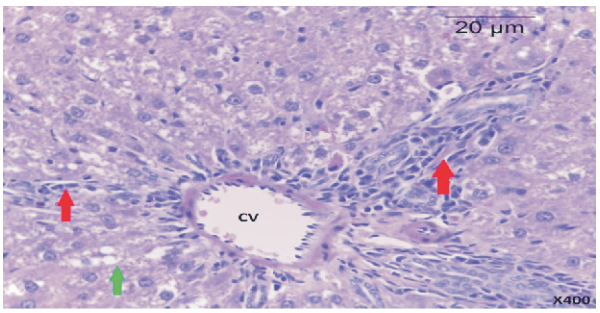

Figure 4: Photomicrograph of liver cytoarchitecture of fructose diet group showed areas of periportal inflammation and mild steatosis (red arrow: inflammatory cells, CV: central vein, green arrow: steatosis) (H&E x400).

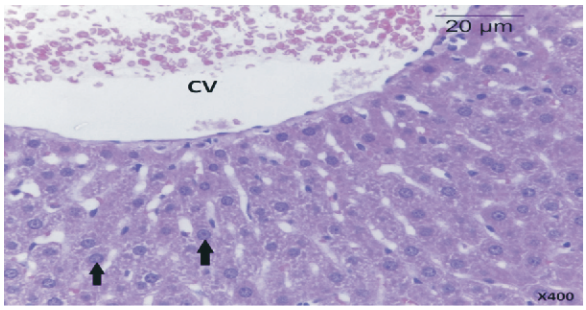

Figure 5: Photomicrograph of liver cytoarchitecture of metformin group appeared to be normal. Traces of vascular congestion and mild dilation of sinusoid were observed. Hepatic parenchyma, Basement membrane of portal and central vein appeared preserved. Neither fatty changes nor inflammatory cells were observed (black arrow: hepatocytes, CV: central vein) (H&E x400)

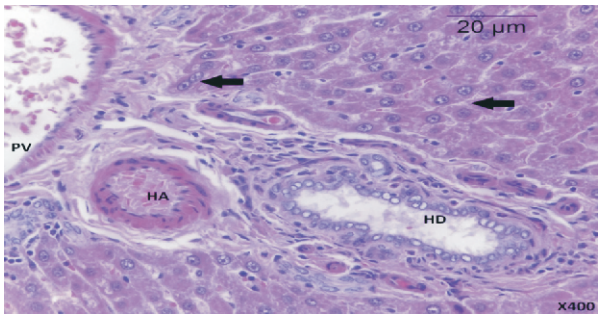

Figure 6: Photomicrograph of liver cytoarchitecture of aqueous extract of *Senna Siamea* at 300mg/bw group showed severe blood vessel congestion. Hepatic parenchyma appears preserved (PV: portal vein, HA: hepatic artery, HD: Hepatic duct, black arrow: hepatocytes) (H&E x400).

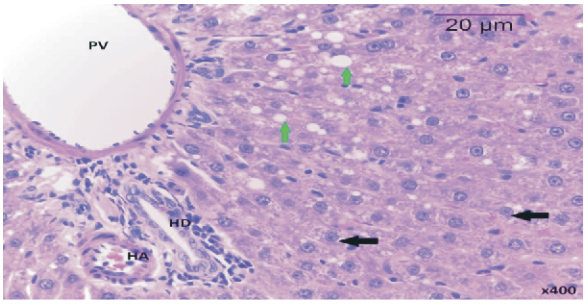

Figure 7: Photomicrograph of liver cytoarchitecture of aqueous extract of *Senna Siamea* 150mg/bw group showed little pathological changes. Hepatic parenchyma appears to be recovering due to the presence of mild areas of the hepatic lobules with mild hepatocyte necrosis. Congestion of blood vessel or hyperplasia inflammatory cells was not observed. (PV: portal vein, HA: hepatic artery, HD: Hepatic duct, black arrow: hepatocytes) (H&E x400)

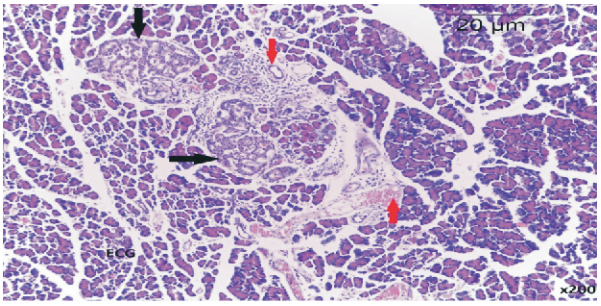

Figure 8: Photomicrograph of pancreatic cyto-architecture of control group appeared normal. Islets of langerhans containing beta cells were located in between normal pancreatic acini of the exocrine gland. Neither necrosis of the beta cells nor atrophy of the islet of langerhans was observed, however, there were areas of hyperaemia within the blood vessels (black arrow= Islet cells, red arrow= blood vessel, ECG= Exocrine gland. H&E x200)

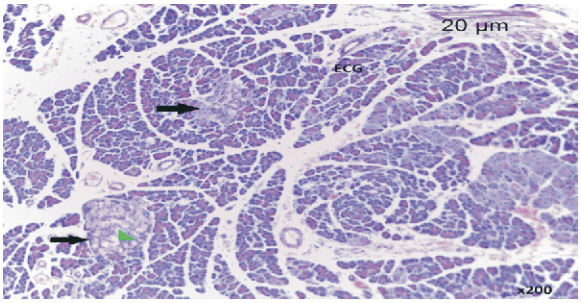

Figure 9: Photomicrograph of pancreatic Cyto-architecture of fructose diet group appeared disrupted and ruptured. Shrinkage of the islet of langerhans as well as loss of beta cell nuclei due to necrosis were observed (black arrow= Islet cell, green arrow head= loss of nuclei, ECG= Exocrine gland. H&E x200)

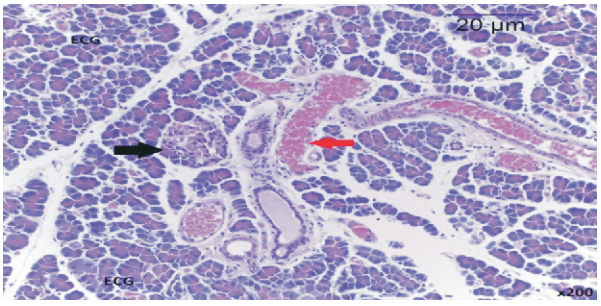

Figure 10: Photomicrograph of pancreatic Cyto - architecture of fructose/ Senna siamea, 300mg/kb b.w treated group shows mild disruption of the Islet of Langerhans and hyperaemia of blood vessel. Also, some of the beta cells showed steady progressive regeneration toward their normal configuration (black arrow= Islet cell, red arrow= blood vessel, ECG= Exocrine gland. H&E x200)

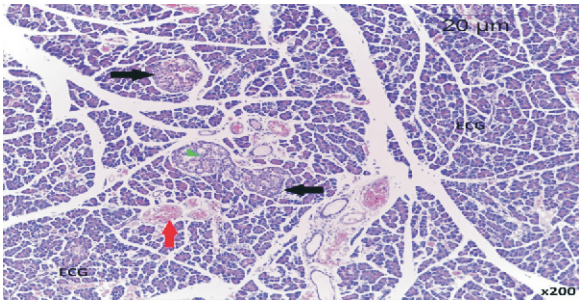

Figure 11: Photomicrograph of pancreatic Cyto-architecture of fructose/ Senna siamea, 150mg/kb b.w treated group showed intense restoration of pancreatic cyto -architecture to normal. Hypertrophy of Islet of langerhans, mild hyperaemia and immerse regeneration of beta cells toward their normal configuration was observed. However traces of nuclei necrosis were observed (black arrow= Islet cell, red arrow= blood vessel, green arrow head= loss of nuclei, ECG= Exocrine gland. H&E x200).

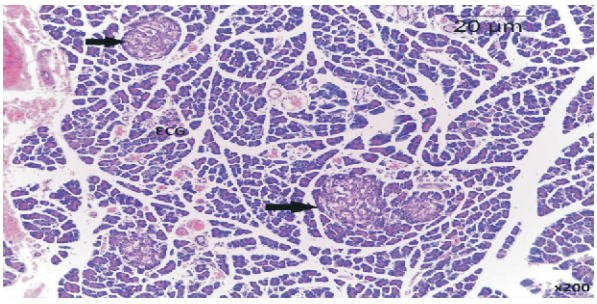

Figure 12: Photomicrograph of pancreatic cyto-architecture of metformin treated group appeared normal. Neither necrosis of the beta cells nor atrophy of the islet of langerhans was observed (black arrow= Islet cells, ECG= Exocrine gland. H&E x200)

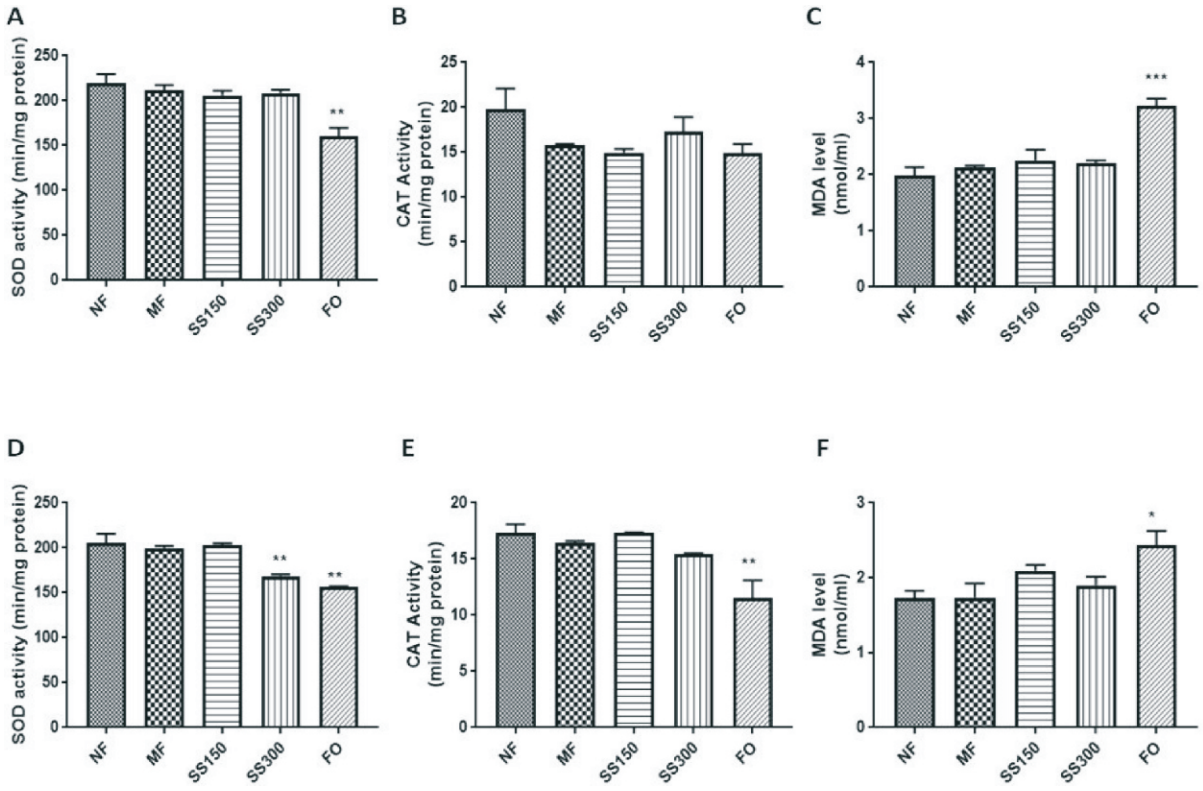

Figure 13: oxidative stress analysis of effect of metformin and SS on the liver (A-C) and pancreas (D-F) of fructose-induced diabetes. (NF= control, MF= metformin group, FO= fructose group SS= aqueous extract of *Senna Siamea* *p<0.01, ** p<0.001, *** p<0.0001 compared to control)

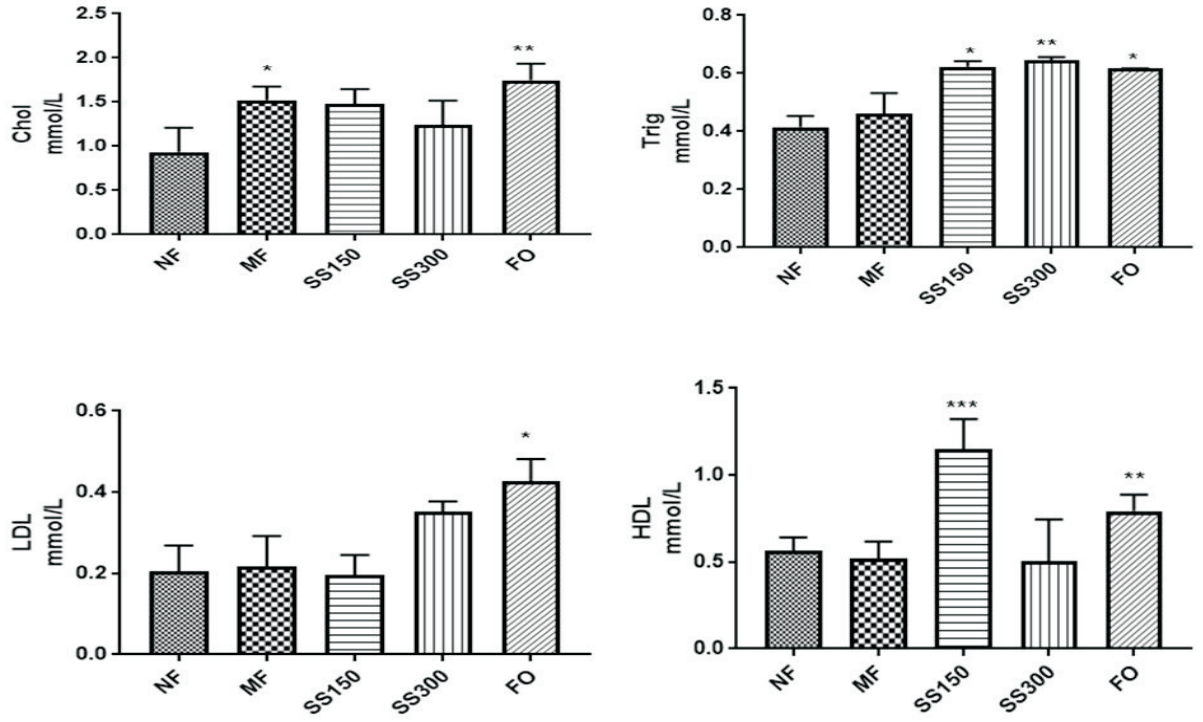

Figure 14: Effect of metformin and SS on liver function test. (Chol= total cholesterol, Trig= triglycerides, LDL= low density lipoprotein, HDL= high density lipoprotein, NF= control, MF= metformin group, FO= fructose group *p<0.01, ** p<0.001, *** p<0.0001 compared to control)

DISCUSSION

Over the years, Fructose intake from various sources including sugars in soft drinks, have been documented to induce fatty liver in animals and epidemiologically attributed to causes of nonalcoholic fatty liver disease in humans. However, studies have been on the task ofunderstanding the mechanism in which fructose causes its detrimental effects and a possible means of optimum amelioration of threat posed by consumption of fructose. Findings from this study will contribute earnestly to the existing knowledge and also elucidate the mechanism methanol extract of *Senna Siamea* uses to possibly ameliorate damages induced by fructose consumption.

Hyperglycemia is one of the leading complications of pre-diabetes and diabetes. Findings from this study showed that animals subjected to high fructose diet showed a symptom of pre diabetes due to increase in blood-glucose level (5.6m- 7.0mmol) which could be as a result of progressive glucose intolerance and insensitivity of pancreatic islet cells. This is similar to the report by Coudray *et al.,* 2019[23] that consumption of fructose causes glucose intolerance. He added that most of the changes started to surface at the onset of 8th week of consumption[23]. Increase in Leptin has also been associated with hyperglycemia and increased body weight induced by fructose consumption which could have possibly accounted for glucose intolerance and mild increase in body weight of animals subjected to fructose diet[24]. However the use of methanol extract of *Senna Siamea* at 150mg/kgb.w showed no symptom of glucose intolerance similar to those treated with metformin and the control. This potency of these substances might be due to their ability to maintain leptin level and/ or improve sensitivity of islet cells. Also, researches on chemical structures of flavonoids in extracts of Cassia genus have been documented to show anti-diabetic function most especially in reducing blood glucose level and improving glycogen content in both type I and II diabetes[25,26].

Increase in triglycerides, total cholesterol and other fatty products due to alterations in liver functions have been associated with complication of both pre-diabetes and diabetes. In this study, liver function test carried on fructose diet group out showed poor metabolic functional ability of the liver due to increase in total cholesterol, triglycerides and low density lipoprotein. Progressive degree of Pathological changes in hepatic parenchyma and cells due to consumption of fructose might have accounted for the poor liver function test exhibited by the group subjected to fructose diet [26]. Though, there was a statistically significant increase inHigh density lipoprotein level in fructose diet group. This insinuates the occurrence and progression of pre diabetes to diabetes. Interestingly, aqueous extract of *Senna Siamea* at 150mg/b.w and 300mg/b.w expounded progressive potentials of improving metabolic function of the liver due to its capability of progressive improvement of liver metabolic function test.

Imbalance in antioxidant function and reactive oxygen species due to oxidative stress have been reported to be a possible mechanism for the occurrence and progression of pre-diabetes and its complications[28,29,30]. Fructose diet group of this study showed a significant increase in oxidative stress marker - MDA and decrease in SOD markers. Excessive production of lipid peroxides and super oxides in the liver and pancreas of fructose diet group due to decrease activities of superoxide dismutase enzymes might have accounted for decrease in insulin secretion from pancreatic β cells resulting to increase in glucose intolerance level and poor liver metabolic function test[31]. Administration of methanol extract of *Senna Siamea* at 150mg/bw and 300mg/bw demonstrated its antioxidant strength in improving oxidative stress complications of fructose induced pre diabetes which is similar to study by[32] on In-vitro antioxidant and in-vivo anti-inflammatory activities of aerial parts of Cassia species[32]. In correlation with Tanty *et al.,* 2018 administration of *Senna Siamea* at 150mg/bw proved to be more effective in antioxidant and anti-diabetes function[33]

Incidences of oxidative stress have been a major pointer towards the occurrence of pathological changes in the cyto-architecture of body organs. Histological observation of the liver and pancreas demonstrated multiple areas of pathological changes including congestion of blood vessels, shrinkage of Islet cells, inflammation in hepatic parenchyma and fatty changes in fructose diet group. This finding agreed with previous result reported by Bagul *et al.,* 2012 on Attenuation of insulin resistance, metabolic syndrome and hepatic oxidative stress by resveratrol in fructose-fed rats, he reported that fructose diet for 8 week showed some discernible pathological changes including shrunken central vein and mild inflammations. Our study showed that while fructose induces pathological changes on both pancreas and liver, methanol extract of*Senna Siamea* at 150 mg/bw and 300mg/bw as well as metformin showed quality capability of progressively ameliorate these pathological changes caused by the consumption of fructose diet. This protective potency of metformin and methanol extract of *Senna Siamea* on the cyto -architecture of the liver and pancreas might be credited to their antioxidant function [34]

CONCLUSION

Results from biochemical assay, blood-glucose level, liver function test and histology observations of the liver and pancreas demonstrated the capability of methanol extract of *Senna Siamea* and metformin to ameliorate pre diabetes complications induced by consumption of fructose diet through balancing free radical, maintaining free radical scavenger system and additional defense system for liver and pancreas

architecture for effective functioning. However, methanol extract of *Senna Siamea* at 150mg/bw proved to be more effective in combating pre diabetes complications induced by fructose consumption than aqueous extract of *Senna Siamea* at 300mg/bw.

CONFLICT OF INTEREST

I declare that there was no conflict of interest.

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