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LIVER FUNCTION AND BIOMARKERS OF OXIDATIVE STRESS LEVELS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS TREATED WITH LEAVES EXTRACT OF CARICA PAPAYA

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ABSTRACT

Diabetes mellitus is defined an in adequate insulin secretion or activity. Oxidative stress brought on by both experimental and human diabetes can harm multiple organs, including the liver. The present study investigated the liver ameliorating and antioxidant effects of hydroethanol extract of matured (yellow) *Carica papaya* leaf on streptozotocin-induced diabetes. We randomly assigned a total of 48 adult male Wistar rats into six groups of eight rats each. Normal control, diabetic untreated, diabetic + metformin, diabetic + 250 mg/kg body weight extract, diabetic + 500 mg/kg, and diabetic + 750 mg/kg body weight extract. A type I model of diabetes was induced in the rats via intraperitoneal injection of 60 mg/kg body weight streptozotocin. Treatment lasted for 14 days. Biochemical assays such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin, albumin, total protein and globulin were analyzed for liver function. Biomarkers of oxidative stress activity such as catalase, superoxide dimutase, glutathione reductase, glutathione peroxidase, and malondialdehyde were conducted using standard methods. Histopathological evaluation of the rats' liver was also carried out. The results obtained revealed a significant improvement in the liver function and likewise the antioxidant levels of the diabetic rats treated with the plant extracts, which was further confirmed by the histology evaluation. This study suggests that hydroethanol extract of the mature (yellow) leaves of *Carica papaya* has liver damage ameliorating potentials and can enhance the antioxidant defense system in the liver of streptozotocin-induced diabetic rats.

Keywords: *Carica papaya,* Diabetes, Liver function, Matured leaves, Oxidative stress, Streptozotocin

INTRODUCTION

Diabetes mellitus is defined by an insufficient secretion of insulin or its activity in the pancreatic beta-cells. A deficiency in insulin can lead to abnormalities in the metabolism of nutrients such as proteins, carbohydrates, and fats (Kharroubi & Darwish, 2015). Although there are various synthetic oral antidiabetic medications and insulin preparations available, the long-term negative effects and development of resistance to these medications make the search and development of novel anti-diabetic medications imperative. Conversely, scientists, researchers, and pharmaceutical companies worldwide are increasingly turning to herbal sources in an effort to find prospective bio-active components for the development of novel pharmacological interventions for diabetes while having the fewest side effects possible compared to standard anti-diabetic medications (Jugran *et al*., 2020 ; Alam *et al*., 2022).

The liver is a vital organ that plays a number of important metabolic functions in the human body. The liver's unique configuration of hepatocytes, hepatic artery, hepatic sinusoids, hepatic artery, portal vein, and the central vein is essential to its operation. Owing to its special place in the body, the liver interacts with parts of circulation that are intended concerning the remaining bodily parts. As a result, it is subjected to a wide range of exogenous agents, including pathogens, metabolites of diets, and substances taken up by intestines that include drugs and alcohol (Acharya *et al*., 2021). The liver is one of the most important organs in the body because the liver is involved in the metabolism and detoxification of xenobiotics (Eluehike *et al*., 2022). It also plays an important role in controlling normal glucose homeostasis (Mohamed *et al*., 2016).

Diabetes has an impact on the liver as well as other bodily systems. Insulin resistance is the primary cause of hyperglycemia, which impairs protein, carbohydrate, and lipid metabolism. It can also result in non-alcoholic fatty liver disease, which can subsequently advance to non-alcoholic steatohepatitis, liver cirrhosis, and hepatocellular carcinomas (Mohamed *et al*., 2016). Later stages of diabetes can cause liver damage because of abnormalities in lipid metabolism as well as elevated gluconeogenesis and ketogenesis. Furthermore, oxidative stress brought on by both experimental and human diabetes can harm multiple organs, including the liver (Omonkhua *et al*., 2014). Among the world's greatest causes of death are liver illnesses; any disease or damage to the liver can have catastrophic effects (Spengler *et al*., 2012). The fundamental mechanisms by which diabetes causes liver damage are both elevated oxidative stress levels and an abnormal inflammatory response; this damages hepatocytes and triggers the transcription of pro-apoptotic genes. Pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor have a significant role in aggravating the buildup of oxidative damage products in the liver, which includes conjugated dienes, fluorescent pigments, and malondialdehyde (Mohamed *et al*., 2016; Jugran *et al*., 2020).

Oxidative stress is established by an imbalance in the oxidant-to-antioxidant ratio that results in the production of free radicals. Hepatic injuries have been linked to the formation of oxidants by activated kupffer cells, including hydrogen peroxide, hydroxyl radicals, and ROS-like superoxide anions. Kupffer cells aid in preserving the structural integrity of liver cells. (Mohamed *et al*., 2016). To counteract free radicals and shield liver cells from oxidative damage, the liver has been equipped with powerful antioxidants, including superoxide dimutase, catalase, and the glutathione enzyme family, which includes glutathione-S-transferases (GSTs) and glutathione peroxidases (GPXs) (Alata *et al*., 2023). Research has indicated that a reduction in superoxide dimutase and catalase activities during a hyperglycemic condition results in a rise in

reactive oxygen species (ROS), which ultimately plays a role in oxidative liver damage (Abu *et al*., 2022).

Streptozotocin (STZ) is the most popular diabetogenic substance for causing diabetes in test animal models; it is a glucosamine N-nitroso derivative gotten from Streptomyces acromogenes. STZ causes selective toxicity to the pancreatic β-cell, inducing not reversible necrosis of the beta -cells. Currently, STZ is the most used diabetogenic agent for evaluating insulin and novel anti-diabetic medications in animals; the substance is harmful and disruptive to organs other than the pancreas that is essential to maintaining the body's normal glucose homeostasis (Qinna and Badwan, 2015).

Traditional medicine has utilized a wide variety of plants to treat liver problems (Eluehike *et al*., 2022). *Carica papaya* Linn belonging to the family Caricaceae is known for its nutritional and medicinal properties. Both the young and mature leaves of the plant have been reported to possess a lot of bioactive compounds with medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, and wound healing (Yogiraj *et al*., 2014; Igbashio *et al*., 2023). This study was designed to evaluate the ameliorating effects of hydroethanol extract of matured (yellow) *Carica papaya* leaves on STZ-induced diabetes.

MATERIALS AND METHODS

Plant Collection

The mature (yellow) leaves of *Carica papaya* were collected around University of Benin environs and were authenticated by a plant biologist, Prof. H.A. Akinnibosun, in the Department of Plant Biology and Biotechnology (PBB). Voucher number UBH-C505/2023 (*Carica papaya Linn*.) was obtained.

Preparation and Extraction of Plant

The leaves were then washed, shade-dried, and ground. A binary solvent (hydro-ethanol) containing 70% ethanol and 30% water was used for the extraction. Exactly 1.1 kg of the grounded plant was dissolved in 5000 ml of the solvent and was allowed to stay for three days with continued stirring. After which it was filtered and freeze-dried.

Experimental Animals

Fourty-eight Wistar rats (48), weighing between 160 - 200 grams, were acquired from the animal house situated at the Department of Anatomy, University of Benin. They were allowed to acclimatize for two weeks with unrestricted access to food and water. Ethical approval was given by the ethics committee, College of Medical Sciences, University of Benin, with reference number CMS/REC/2023/490.

Experimental design

The rats were placed in six groups of eight rats.

Group I: Normal control; received only food and water.

Group II: Diabetic untreated.

Group III: Diabetic rats administered 100 mg/kg body weight metformin.

Group IV: Diabetic rats administered 250 mg/kg body weight extract

Group V: Diabetic rats administered 500 mg/kg body weight extract

Group VI: Diabetic rats administered 750 mg/kg extract body weight

Induction of Diabetes

Streptozotocin was dissolved in acidified (pH 4.5) normal saline and administered to the rats intraperitoneally at a dose of 60 mg/kg body weight after a 12-hour fast. Diabetes was confirmed after four (4) days of streptozotocin administration by measuring fasting blood sugar using a fine-test glucometer, and only rats with a fasting blood glucose level of 200 mg/dl were considered diabetic and were used for this study. After diabetes was established, treatment of rats commenced and lasted for 14 days.

Procedure for Animal Sacrifice and Sample Collection

At the end of the 14 days of treatment, the rats were sacrificed. Prior to the sacrifice, the star rats were subjected to fasting overnight. Fasting blood sugar level and body weight of the rats were taken before the sacrifice. The anesthesia method of sacrifice was used. The rats were anesthetized for 2 minutes using chloroform, after which they were placed on a dissection table. Abdomino-thoracic incision was carried out to expose the viscera, and blood samples were collected from the inferior venacava using a syringe and needle. A blood sample for the liver function test was collected in a lithium heparin bottle. The liver samples were collected for histopathological analysis and placed in 10% buffered formalin, while the liver tissues for the determination of oxidative stress marker activity were collected in a plain container containing normal saline and were placed in an ice pack before homogenization. The blood samples in the lithium heparin bottles were centrifuged at 3000 rpm for 5 minutes. The blood plasma was collected, transferred into a plain container, and refrigerated for further investigation.

Tissue Processing for Histology

The fixed liver tissues in ten percent phosphate buffered formalin were dehydrated using alcohol of different grades, followed by clearing with acetone to remove the formalin and alcohol, and embedded in paraffin blocks to harden the tissues and sectioned into 5-mm-thick. Sections were stained with hematoxylin and eosin and examined under a light microscope.

Tissue Processing for Oxidative Stress Makers Assay

A small portion of the liver tissues for all the groups weighing 1 g were homogenized in 1 ml of phosphate buffer (pH 7.4) using a pestle and mortar. This was then centrifuged at 3000 rpm for 5 minutes, after which the supernatants were collected and refrigerated at 40 °C for further analysis.

Biochemical assays

Liver Function Tests

Reitman and Frankel (1957) method was used to analyse Alanine transaminase (ALT) and aspartate transaminase (AST) activities. Gornall *et al*. (1949) procedures was used for alkaline phosphatase (ALP), Biuret method was also employed to evaluate protein concentrations, Doumas and Biggs (1972) procedure was used for albumin levels. The Jendrassik and Grof (1938) method was used to assay for total and conjugated bilirubin levels.

Biomarkers of Oxidative Stress Activity

Superoxide dimutase (SOD) was determined according to the methods of Masra and Fridorich (1972). The catalase (CAT) assay was based on the reaction proposed by Cohen *et al*. (1970). Ellman's technique was used to determine the activity of glutathione reductase (GSH) (Ellman, 1959). Glutathione peroxidase (GPx) was estimated according to the method described by Nyman (1959). Malondialdehyde (MDA) was estimated by the method (Buege and Aust, 1978).

Statistical Analysis

Statistical package for social sciences (SPSS) version 25 (IBM Corporation, NY) was used to analyze the data obtained from the study. Results obtained were expressed as mean ± SEM (standard error of mean). Differences among the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant at $P<0.05$. The LSD, Duncan, and SNK Post Hoc tests were used to determine where the significance lay.

RESULTS AND DISCUSSION

The fasting blood sugars levels of the experimental rats are shown on table 1. A normal fasting blood sugar level was recorded for all the rats in various groups (Group I-VI) before the induction of diabetes. After induction of diabetes with streptozotocin, a threshold of fasting blood sugar was recorded in all groups with the exception of Group I rats; the normal control group which were not induced. The increase in fasting blood sugar levels in streptozotocin-induced diabetic rats was due to the cytotoxic and disruptive nature of streptozotocin on the pancreas, which is involved in preserving the body's normal sugar homeostasis (Igbashio *et al*., 2024). Insulin secretion rates are less responsive to changes in glucose levels in diabetic patients compared to normal subjects. This is an indication of the loss of normal glucose homeostasis in diabetic patients (Quina & Badwan, 2015).

Table 1: Fasting Blood Sugar Levels of Rats Before and After Induction with Streptozotocin (STZ)

	Group I	Group II	Group III	Group IV	Group V	Group VI
Before Induction (mg/dl)	91.0±3.10^{a}	86.8±3.33^{a}	91.4±2.86^{a}	92.8±5.00^{a}	88.0±2.46^{a}	91.00±4.49^{a}
After Induction (mg/dl)	89.9±2.99^{a}	466±21.65^{b}	531±11.28^{b}	494.17±33.66^{b}	450.1±25.43^{b}	498.33±25.59^{b}

Data are fasting blood sugar levels parameters of rats before and after induction and are expressed as means ±SEM (n=5) at (p ≤0.05). Alphabets represent levels of statistical difference.

Effects of treatments on liver parameter

The activities of the liver enzymes ALP, AST, and ALT are shown in Table 2. STZ induced significant elevation in ALP, AST, and ALT levels when compared with the normal control. An increase in the levels of liver enzymes AST, ALT, and ALP is a common sign of liver disease. The increase in aminotransferase levels may be due to the cellular damage in the liver caused by STZ induction (Qinna & Badwan, 2015).Treatment with different concentrations of the plant extracts and metformin showed a significant decrease in the concentrations of these liver enzymes when compared with the diabetic control rats. Eluehike *et al*. (2022) reported that induction of diabetes with STZ resulted in necrosis of the liver of rats. Hence, the elevated activities of AST and ALT may result from the leakage of these aminotransferase enzymes from the cytosol of the liver into the blood, which therefore indicates the hepatotoxic impact of STZ. Alkaline phosphatase is a membrane-bound glycoprotein enzyme. A high amount of this enzyme is present in the sinusoids and in the endothelium of the central and periportal veins. An increase in the serum concentration of this enzyme activity is an indication of a liver damage (Elisa *et al*., 2009).

Cell damage to the liver causes the liver enzymes to spill into the blood stream (Spiers *et a*l., 2021). The result of this study is in agreement with the reports of other researchers who observed similar elevations in the activities of liver enzymes following STZ induction (Eluehike *et al.,* 2022; Omonkhua *et al*., 2014; Salih *et al*., 2014). Treatment with metformin and the extracts of mature (yellow) *carica papaya* leaf resulted in a significant decrease in the activities of liver enzymes (AST, ALT, and ALP). Whereas treatment with 500 mg/kg of extract for Group E rats gave the highest percentage decrease in serum enzyme activities. The reduction in liver enzyme activities may be due to the hepatoprotective effects of *Carica papaya,* which was earlier reported by several researchers.

A significant increase in total bilirubin, conjugated bilirubin, and total protein was observed in the diabetic control rats as shown on Table 2. Treatment with extracts resulted in a profound decrease in total bilirubin and conjugated bilirubin when compared with the diabetic control. Whereas rats treated with 100 mg/kg metformin showed a non-significant reduction in direct bilirubin. In this study, a significant reduction of total protein was recorded only in a group of rats treated with 500 mg/kg body weight. Data from albumin levels showed a significant drop in the diabetic animals, treated with the plant extract significantly drop the levels of albumin when compared to the diabetic untreated group (Group II). A non-significant change ($p>0.05$) was recorded for globulin levels.

Table 2: Liver Function Indices of Diabetic Rats Treated with Leaves Extract of Matured (Yellow) Carica papaya

	Group I	Group II	Group III	Group IV	Group V	Group VI
AST (U/L)	171±8.77	223±3.09^{a}	204±2.46^{ab}	199±11.4^{ab}	179±5.36^{b}	211±4.47^{ab}
ALT (U/L)	97±1.38	261±28.2^{a}	208±2.69^{ab}	188±4.7^{ab}	108±1.11^{ab}	179±4.13^{ab}
ALP (U/L)	131±5.06	461±23.5^{a}	435±10.8^{ab}	315±1.70^{ab}	204±5.89^{ab}	335±4.29^{ab}
T.Bilirubin (mg/dl)	0.19±0.05	0.41±0.05^{a}	0.29±0.03^{ab}	0.29±.003^{ab}	0.44±0.25^{ab}	0.29±.003^{ab}
D.Bilirubin (mg/dL)	0.08±0.03	0.188±.00^{c}	0.185±.006^{c}	0.42±0.25^{ab}	0.085±0.25^{ab}	0.43±0.26^{ab}
T.protein (mg/dL)	9.32±0.09	9.88±0.05^{a}	9.70±0.06^{ab}	9.77±0.06^{ab}	9.30±0.04^{ab}	9.70±0.06^{ab}
Albumin (mg/dL	2.4±0.04	3.22±0.05^{a}	3.08±0.05^{b}	2.93±0.048^{ab}	2.42±0.05^{c}	2.87±0.048^{b}
Globulins mg/dL)	6.93±0.05	6.65±0.0^{c}	6.77±0.07^{c}	6.87±0.06^{c}	6.87±0.03^{c}	6.90±0.04^{c}

Data are liver function parameters of rats treated with extracts for 14 days and are expressed as means ±SEM (n=5) at (p ≤0.05). Alphabets represent levels of statistical difference.

Effects of treatments on Biomarkers of Oxidative Stress

Oxidative stress has been demonstrated to participate in the progression of diabetes, which plays an important role during diabetes, including impairment of insulin action and elevation of complication incidence. Antioxidants have already been shown to be prospective in the treatment of diabetes (Caturano *et al*., 2023). A study conducted by Kumar *et al*. (2024) on the antioxidant potential and mineral elemental

profiling of young and mature fruit and leaf of *Carica papaya* revealed that the mature leaves of *Carica papaya* exhibit antioxidant potential in combating diseases related to oxidative stress.

Effects of treatment with extracts of mature yellow *Carica papaya* leaves (MYCPL) on SOD levels are shown in Figure 1. The results revealed that, induction of diabetes with STZ, significantly decreased the levels of SOD in liver tissues of all the diabetic rats when compared to normal control rats. The decrease in the levels of SOD is an indication that the diabetogenic agent; STZ has induced oxidative stress in the cells. Treatment with extracts of mature yellow *Carica papaya* leaves significantly raised the levels of the antioxidant enzyme (SOD) when compared to the diabetic untreated group.

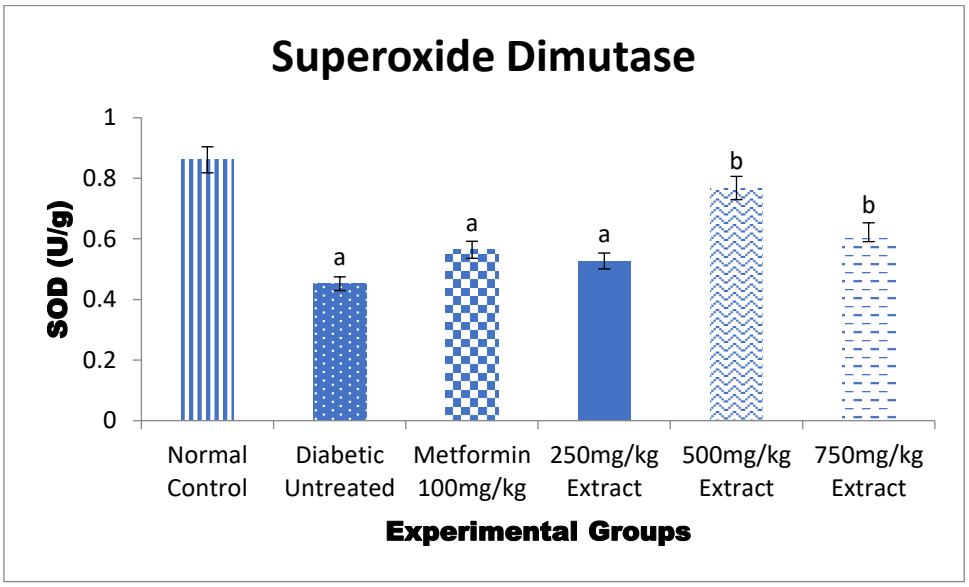

Figure 1: Effect of Hydroethanol Extracts of Mature (yellow) Carica papaya Leaves on SOD levels

Effects of treatment with extracts of mature hydroethanol extracts of mature (yellow) Carica papaya Leaves (MYCPL) on CAT are shown in Figure 2. The result revealed that induction of diabetes with STZ significantly decreased the levels of CAT in the liver tissues of all the diabetic rats when compared to normal control rats. The decrease in the levels of CAT indicates high levels of free radicals in the cell. Treatment with extracts of MYCPL significantly raised the levels of the antioxidant enzyme when compared to the diabetic untreated group. Catalase (CAT) is an antioxidant enzyme known to catalyse hydrogen peroxide into water and oxygen in an energy-efficient manner in the cells exposed to environmental stress (Bratovcic, 2020). An increase in the levels of this enzyme signifies that the extracts of mature hydroethanol extracts of mature (yellow) Carica papaya Leaves possesses bioactive components with antioxidant potentials.

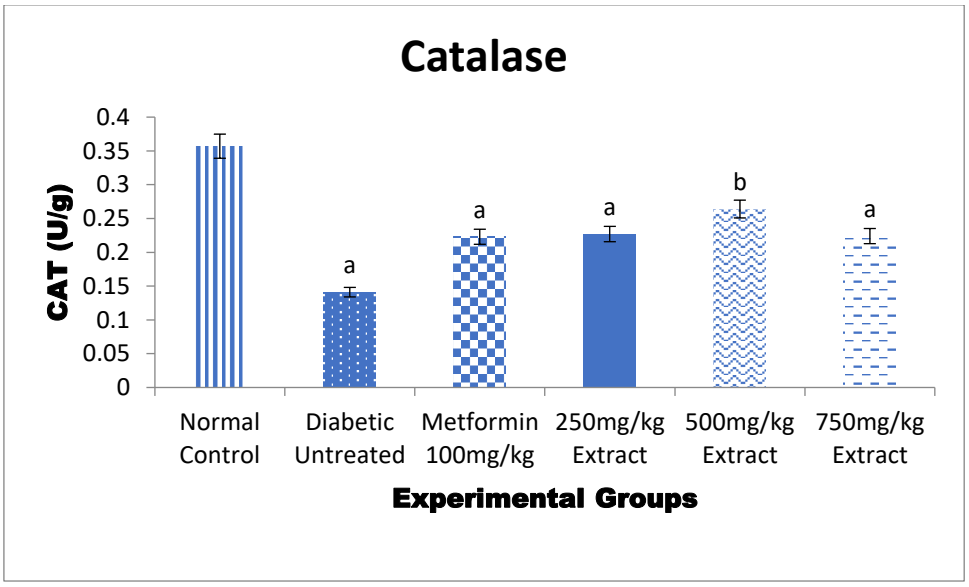

Figure 2: Effect of Hydroethanol Extracts of Mature (yellow) Carica papaya Leaves on CAT levels

Effects of treatment with extracts of MYCPL on glutathione reductase (GSH(are shown in figure 3. The result revealed that, on induction of diabetes with STZ, significantly decreased the levels of GSH in liver tissues of all the diabetic rats when compared to normal control rats. Treatment with extracts of MYCPL significantly raised the levels of the antioxidant enzyme when compared to the diabetic untreated group.

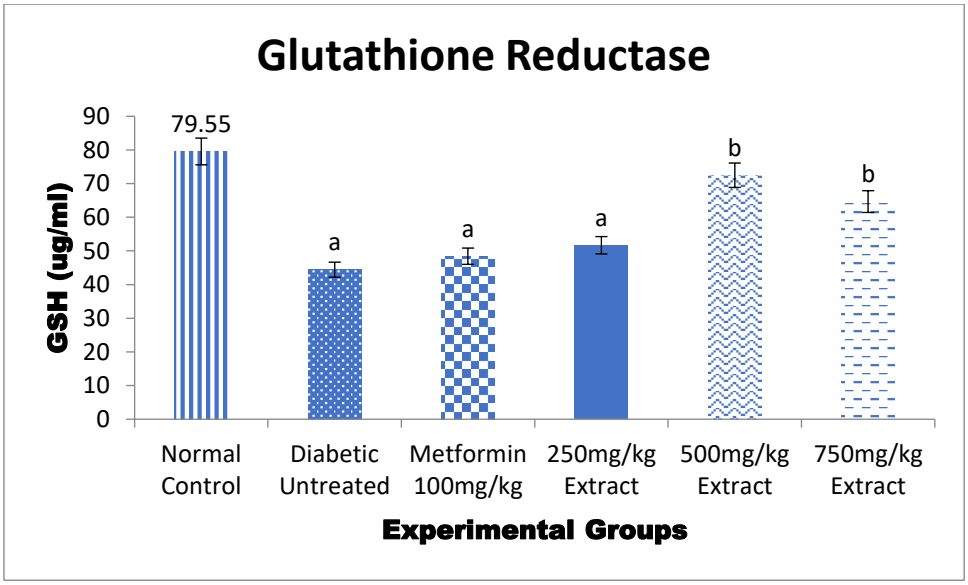

Figure 3: Effect of Hydroethanol Extracts of Mature (yellow) Carica papaya Leaves on GSH levels

Effects of treatment with extracts of mature yellow *Carica papaya* leaves (MYCPL) on glutathione peroxidase (GPx) are shown in Figure 4. The result revealed that, on induction of diabetes with STZ, significantly decreased the levels of GPx in the liver tissues of all the diabetic rats when compared to normal control rats. Treatment with extracts of MYCPL significantly raised the levels of the antioxidant enzyme when compared to the diabetic untreated group.

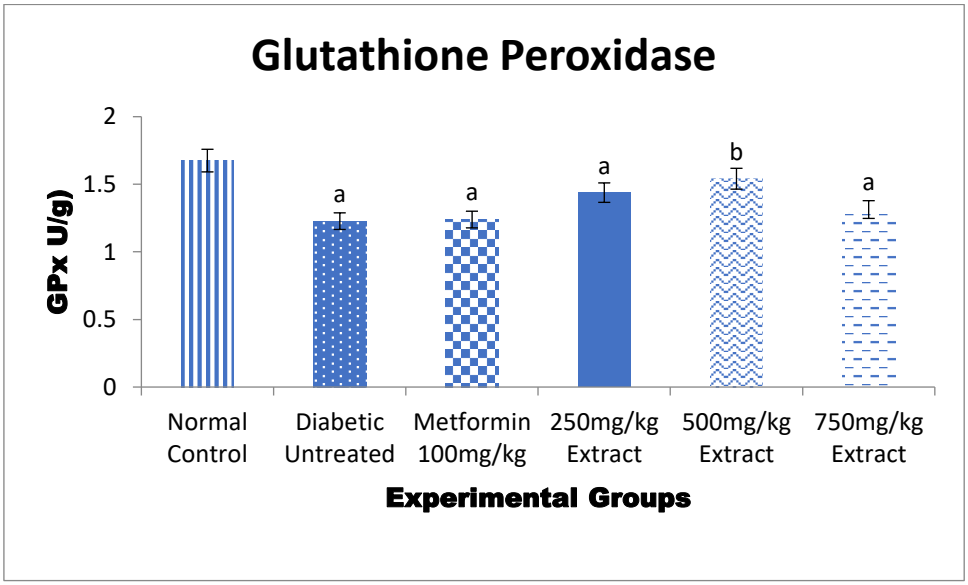

Figure 4: Effect of Hydroethanol Extracts of Mature (yellow) Carica papaya Leaves on GPx levels

Effects of treatment with extracts of mature yellow *Carica papaya* leaves (MYCPL) on malonyladehye (MDA) are shown in figure 5. When oxidative stress is high, the MDA level is frequently used as a gauge of lipid peroxidation. The result revealed that induction of diabetes with STZ significantly raised the levels of MDA in liver tissues of all the diabetic rats when compared to normal control rats. Treatment with 500mg/kg body weight of extracts of MYCPL significantly decreased the levels of the MDA when compared to the diabetic untreated group.

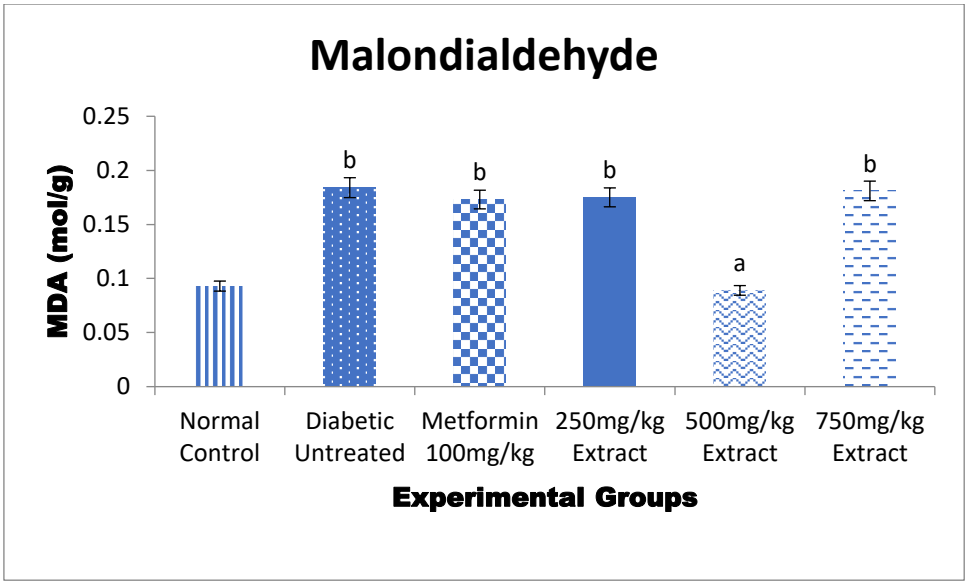

Figure 5: Effect of Hydroethanol Extracts of Mature (yellow) Carica papaya Leaves on MDA levels

Liver Histology of Diabetic Rats Treated with Hydroethanol Extracts of Mature (yellow) *Carica papaya* Leaves

The biochemical results were further confirmed with histopathological evaluation, as shown in plate 1-6. The histology of the diabetic rats liver revealed loss of hepatic architecture, degenerating hepatocytes with kypnotic nuclei, severe vascular congestion, and periportal infiltrates of inflammatory cells and lipid droplets in their cytoplasm when compared to the normal control rats. Mahfoh and Gawish (2022) reported that this could be due to increased adipogenesis and hepatocyte apoptosis, as well as inflammation as a result of diabetes. Eluehike *et al*. (2022) also reported that histological examination of the rat's liver induced with streptozotocin resulted in characteristic periportal infiltrates of inflammatory cells, portal vascular congestion and oedema, as well as portal vascular ulceration of the hepatocytes, which is an analogue to the result obtained from this study. Treatment of the diabetic Wistar rats with the crude extracts of mature (yellow) *Carica papaya* leaf revealed a characteristic normal hepatocyte when compared to the normal control groups, with the exception of the group treated with 100 mg/kg metformin and 750 mg/kg of the extracts, as shown in (plate 4) and (figure 6).

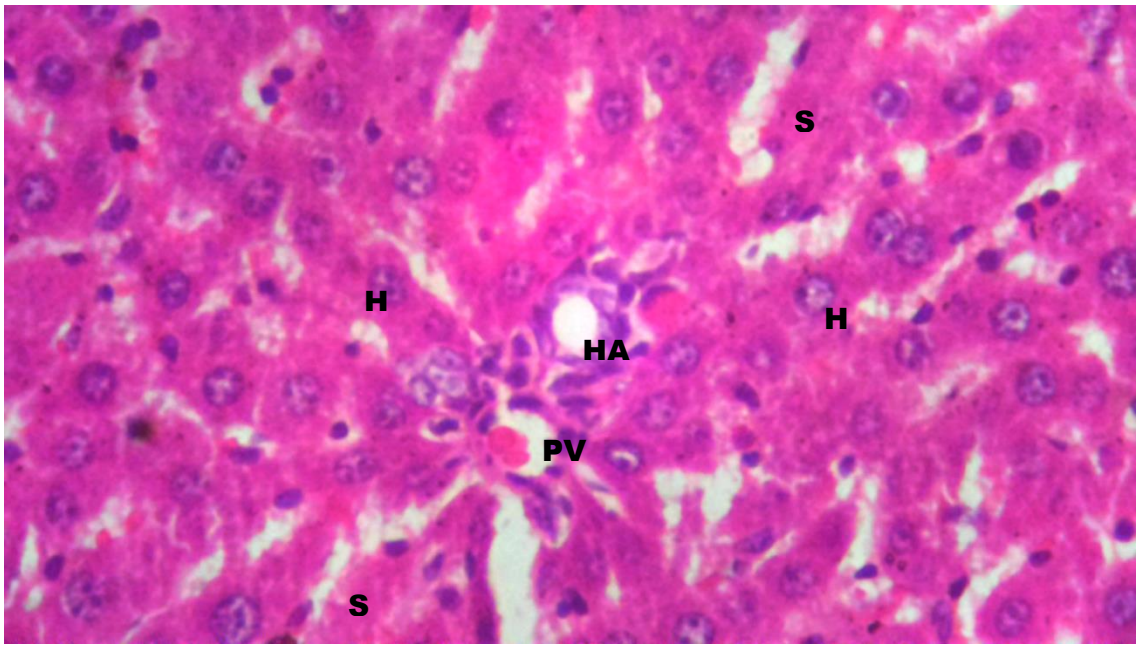

Figure 6: Photomicrograph of a section of liver of the group I; normal control rats showing normal histological features with portal vein (PV), hepatic artery (HA) and radiating hepatocytes (H) with large round nucleus and sinusoids (S) (H&E; 400X)

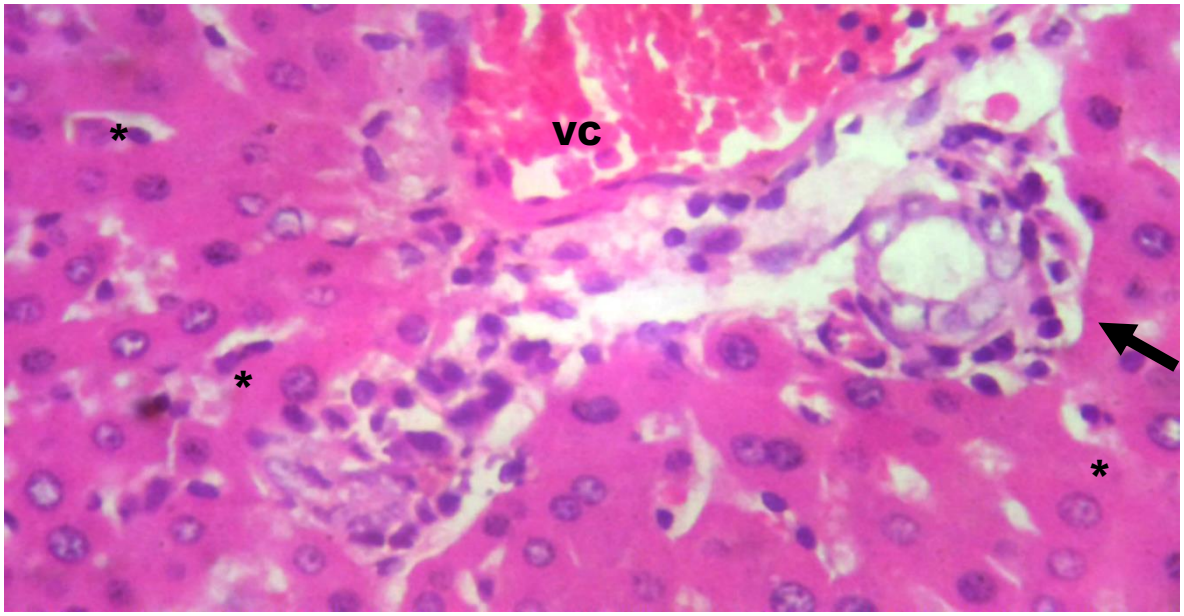

Figure 7: Photomicrograph of a section of liver of the group II administered only STZ showing degenerating hepatocytes with kypnotic nuclei (*) severe vascular congestion (VC) and periportal infiltrates of inflammatory cells (arrow) (H&E; 400X)

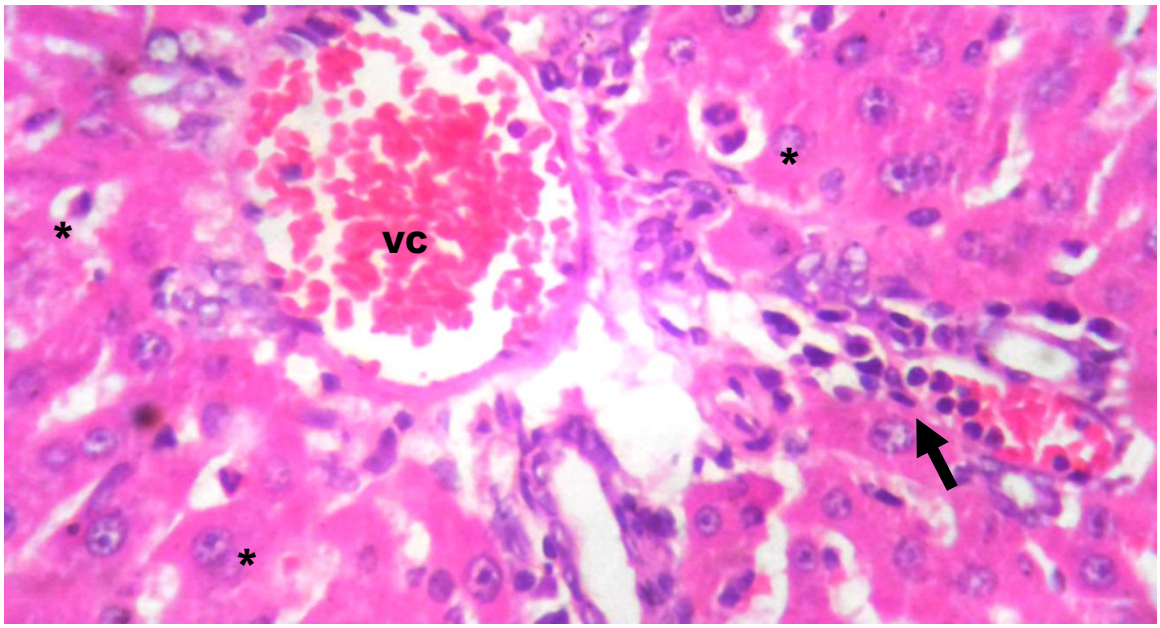

Figure 8: Photomicrograph of a section of liver of the group III administered 100mg/kg metformin showing degenerating hepatocytes with kypnotic nuclei (*) severe vascular congestion (VC) and periportal infiltrates of inflammatory cells (arrow) (H&E; 400X)

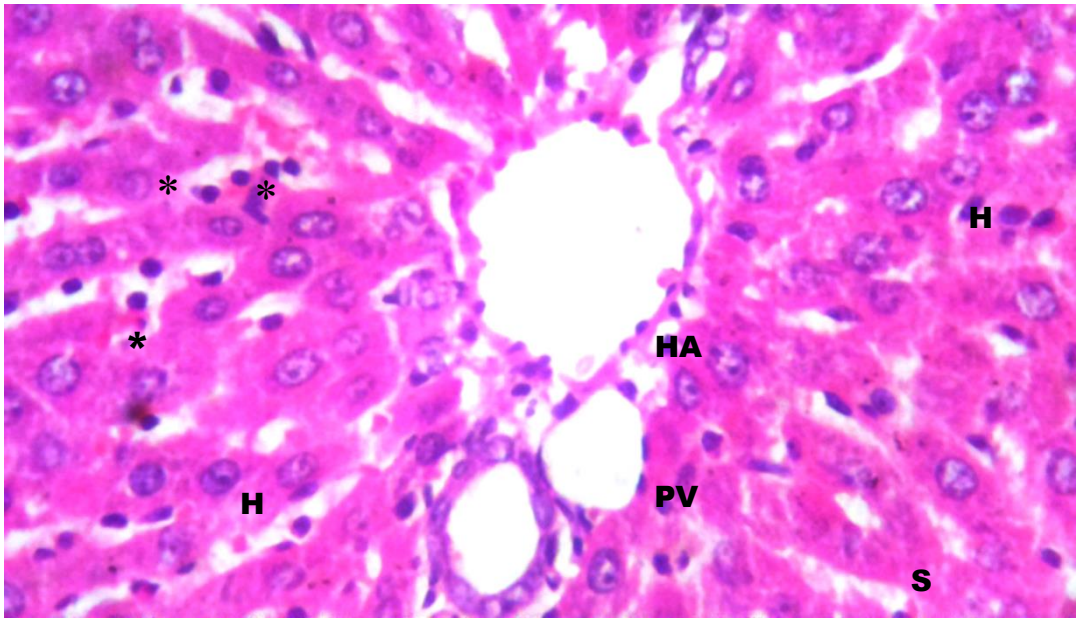

Figure 9: Photomicrograph of a section of liver of the group IV treated with 250 mg/kg of extract showing normal histological features with mild Kupffer cell activation (*), portal vein (PV), hepatic artery (HA) and radiating hepatocytes (H) with large round nucleus and sinusoids (S) (H&E; 400X)

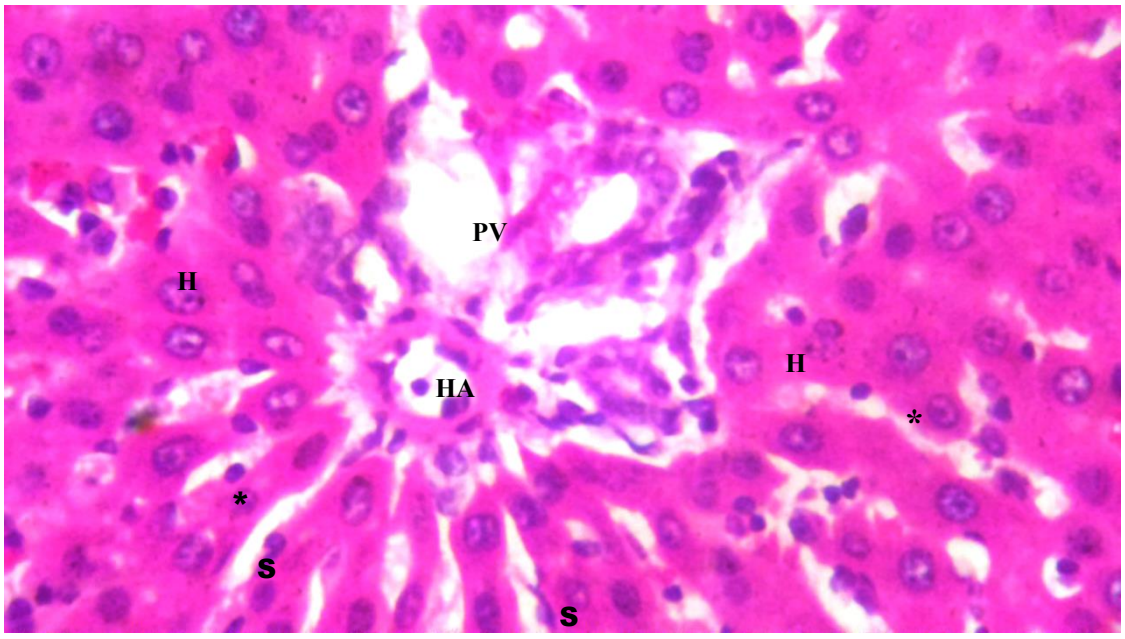

Figure 10: Photomicrograph of a section of liver of the group V treated with STZ and 500 mg/kg of extract showing normal histological features with mild Kupffer cell activation (*), portal vein (PV), hepatic artery (HA) and radiating hepatocytes (H) with large round nucleus and sinusoids (S) (H&E; 400X)

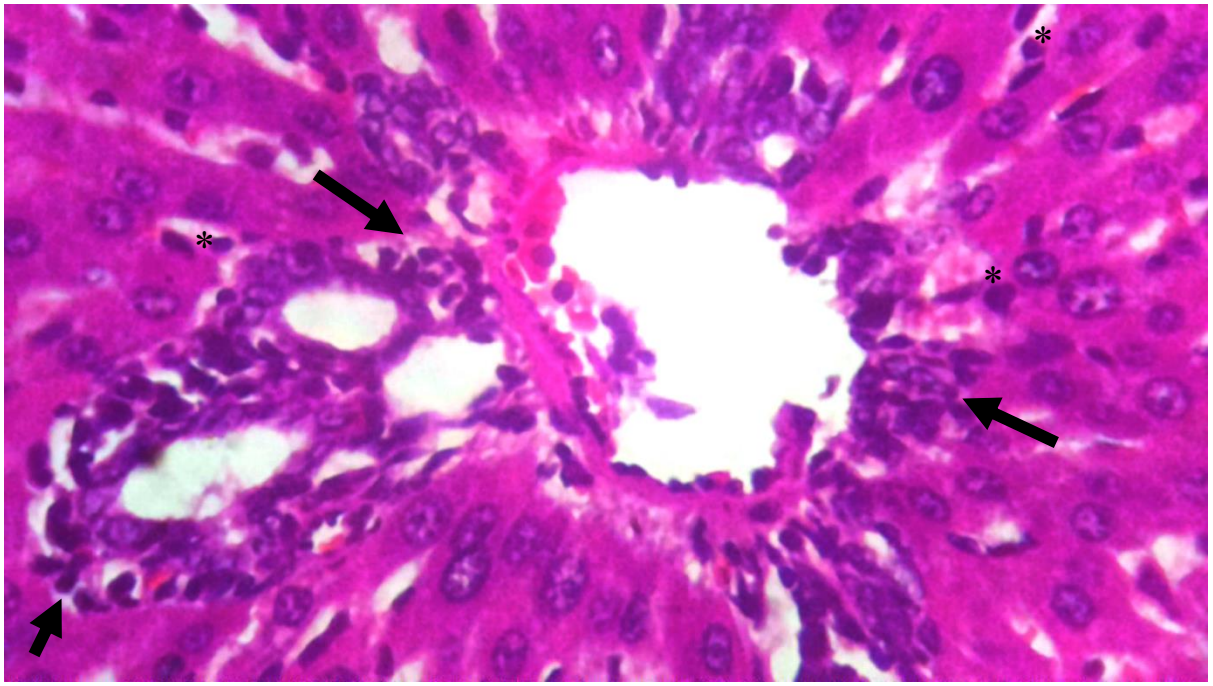

Figure 11: Photomicrograph of a section of liver of the group VI treated with STZ and 750 mg/kg of extract showing with severe periportal infiltrates of inflammatory cell (arrows) and Kupffer cell activation (*)(H&E; 400X)

CONCLUSION

The findings from this study revealed that the extract of mature (yellow) *Carica papaya* leaves has free radical scavenging potentials to repair damaged hepatocytes, which may be caused by oxidative stress; the pathophysiology of diabetes and its related complications.

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