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Evaluation of Conophor Nut (*Tetracarpidium conophorum*) Protein Isolates, Hydrolysate and Ethanolic Extract Ameliorating Potential on Hepatological and Renal Dysfunction of Streptozocin-induced Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OYO, OSI and IBO contributed to the study's conception, executed and designed the study. Author OYO analyzed and interpreted the hepatic, renal function and In vivo antioxidant indices of liver and kidney of diabetic wistar rats. Authors OSI and IBO did the hematological indices and histopathological examination. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed at extracting bioactive components from conophor nut as a probable nutraceutic in the treatment of diabetes.
Place and Duration: Food Chemistry Laboratory, Department of Food Science and Technology, and Biochemistry Laboratory of the Federal University of Technology, Akure, from June 2023 to September 2023.
Methodology: Protein isolate (CNPI) was produced via alkaline solubilisation of defatted conophor nut flour using NaOH. Hydrolysate was prepared by fermentation using *Lactobacillus fermentum BGT10* for 24, 48 and 72 hours (CNPH24, CNPH48, CNPH72) respectively while the ethanolic extract (CNE) was prepared by steeping defatted conophor nut flour in 95% ethanol. Diabetic model rats were subjected to hyperlipidemic diet for 4 weeks and then induced by single intraperitoneal injection of freshly prepared solution of streptozotocin (35 mg/kg). The rats were randomly divided into 18 groups of five (5) rats each; negative control group, diabetic control group, reference (positive) control group as well as the treatment group; CNE, CNPH24, CNPH48, CNPH72 and CNPI administered dose dependently namely, 250, 500 and 1000 mg/kg body weight. Oxidative stress was evaluated in liver and kidney by antioxidant markers, and also kidney functions were determined in diabetic control and treated rats.
Results: When compared with diabetic rats, oral administration of treatments at a concentration of 1000 mg/kg daily for 28 days showed significant reduction in biochemical parameters of liver and kidney for CNE, CNPH24, CNPH48, CNPH72 and CNPI respectively. Furthermore, the treatment resulted in significant increase in SOD, GSH, GST and CAT and decrease in MDA in the liver and kidney respectively.
Conclusion: Results from the study suggest that CNE, CNPH24, CNPH48, CNPH72 and CNPI may effectively normalize dysfunctional antioxidant status in streptozotocin-induced diabetics in a dose-dependent manner.

Keywords: Dietary; hyperlipidemic; dysfunctional; scavenging; administered.

1. INTRODUCTION

Africa traditionalists have used different plants in the treatment of diabetics' mellitus [1] a chronic disease which is one of the leading causes of mortality worldwide [2]. The World health Organization (WHO) has recommended the use of some medicinal plants for the treatment of diabetics' mellitus [3] and this has paved ways for the discovery and development of novel therapeutic control of diabetes. Some plants such as Karela (*Momordica charantia*) [4], Gudhal (*Hibiscus rosa sinensis*) [5] and *Mangifera indica L*. [6] have shown reduction in the blood sugar of the experimental animals.

Conophor nut (*Tetracarpidium conophorum*) is a perennial woody plant widely consumed by the Africans when cooked [7]. Conophor nut like other plants grown in Africa, it has been reported to possess nutritive, agricultural, decorative, medicinal and industrial values. It possesses bioactive components like ascorbic, phytate, oxalate, alkaloid, saponin and tannins [8]. It is also rich in protein, carbohydrate, vitamins, minerals, and fat and oils [9]. The extracts of Conophor nut leaves have been reported to have antibacterial properties against Gram positive bacteria [10]. The barks and leaves of the Conophor nut are used in traditional medicine to cure dysentery and other diseases. Conophor nut possesses fat composition capable of lowering cholesterol level. It has also reported that Conophor nut is rich in dietary omega-3-fatty acids that prevents body disorders including depression as well as dementia [11].

"Functional foods such as Conophor nut provides synthesizes complex organic molecules (phytochemicals) beneficial for human health. People consuming diets rich in functional or bioactive components are at lower risk of chronic illnesses, such as cardiovascular diseases, diabetes mellitus and the likes thus, reducing the risk of mortality" [12]. "Diabetes mellitus, a chronic endocrine disorder that affects the metabolism of carbohydrates, protein, fat, electrolytes and water which is characterized with hyperglycemia is a major health problem with increasing prevalence over the entire world". [13] "The synthetic treatment of diabetes mellitus through the use of chemical drugs in recent times has shown severe drawbacks. Consumers are turning massively to food supplements to

improve health due to the risk of toxicity or adverse effect of drug usage" [14]. "People have depended mostly on plants for nutritional and medicinal needs and research have focused on searching for anti-diabetic compounds from natural materials which could act as an anti-catalyst to compete with α-amylase and α-glucosidase that can prevent hyperglycemia without any side effects" [15].

Despite the medicinal uses of Conophor nut, only few studies are available on the Conophor nut protein isolates, hydrolysate and ethanolic extract on the hepathological and renal dysfunction of Streptozocin-induced Diabetic male Wistar Rats. Therefore, this study is aimed at investigating the biochemical ameliorating potential of Conophor nut protein isolates, hydrolysate and ethanolic extract on the hepathological and renal dysfunction of streptozocin-induced diabetic male wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Conophor nuts were purchased from a local farm in Ibadan, Oyo State, Nigeria. The plant was identified and authenticated at the Crop, Soil and Pest Management Department, Federal University of Technology, Akure, Nigeria.

2.2 Sample Preparation

2.2.1 Production of defatted conophor nut flour

Conophor nuts were washed thoroughly to remove all dirt and shelled manually by removing the kernels from the seeds. The removed seeds were washed and sliced (2-3mm) for easy drying. The shredded walnut seeds were oven dried at 70ºC for 24hours and the milled into coarse flour with a milling machine according to the method of [16]. Defatted Conophor nut flour was obtained by solvent extraction method to extract oil from the Conophor nut using Soxhlet Extraction method.

2.2.2 Production of conophor nut protein isolate

Conophor nut protein Isolate was produced using the method described by Gbadamosi [17]. The defatted flour (25±50g) was added to distilled water at flour to solvent ratio of 1:5 (w/v). The mixture was stirred with a magnetic stirrer for 10 min and the pH of the solution was adjusted to 9.0 using 1 M HCl or NaOH and stirring was continued for another 30 min at room temperature. Each extract was separated by centrifugation at 3500 ×g for 30 min. The residue was re-extracted two more times with the same solvent under the similar conditions. The extracts was then combined and proteins precipitated by adjusting the pH to 4.0 with 1 M HCl, followed separation by centrifugation at 3500 ×g for 30 min. The precipitate was re-dispersed in 100ml distilled water at pH 9.0 and re-precipitated at pH 4.0. After separation of proteins by centrifugation, the precipitate was washed twice with distilled water b(R= 1:2). The precipitated protein was re-suspended in distilled water on a magnetic stirrer and stirred for 10min while the pH was adjusted to 7.0 with 1 M NaOH prior to lyophilization. The dried protein isolate was stored in an air tight container for further analyses.

2.2.3 Production of conophor nut protein hydrolysate

Conophor nut protein hydrolysate was produced using the method described by Wang [18] with slight modification. The protein hydrolysate flour was produced using *Lactobacillus fermentum BGT10* which was obtained from the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria. The mixtures of protein isolate and lactic acid bacteria (7log cfu/g in flour after inoculation) were dispersed in distilled water and were thoroughly mixed in a food processor DW-25, Better Boiler Ltd., Shanghai, China). After mixing, the mixtures were placed in incubator at 37ºC for 24, 48 and 72 h respectively ; and thereafter, lactic acid bacteria was inactivated at 98 °C for 10 min. After inactivation, the mixture was centrifuged at 3500 x g for 30 min and filtered through a 0.45 µm membrane filter. The filtrates were then freeze-dried and stored at 4 °C before further analysis.

2.2.4 Production conophor ethanolic extract

Conophor nut extract was obtained by method of [19] with slight modification. 100g of defatted conophor nut flour was measured into a bottle and was extracted with 200ml Ethanol and placed in a shaker for 6 hours. The supernatant was filtered and spread out on a tray for the ethanol to escape and then the dried extract was scraped and grounded for further analyses.

2.2.5 Animal experimental design

Ninety (90) healthy male Wistar weaning rats of two weeks of age were purchased from the

animal house of Department of Biochemistry, Federal University of Technology, Akure, Ondo state, Nigeria. The rats were divided into 18 groups of 5 animals each per group based on their weight (140 -150g) and then allowed to acclimatize to the new environment for seven days. During the 7 days' acclimatization, the rats were fed on commercial feed and water *ad libitum*. After seven days, the rats were induced with type-2-diabetes.

2.2.6 Induction of type 2 diabetes mellitus

"Experimental type-2 diabetes mellitus in form of Streptozotocin (STZ) was induced by subjecting the rats to high fat diet (HFD) or hyperlipidemic diet) which contained 20% fat, 45% carbohydrate, 22% protein, for 4 weeks" [20]. "The streptozotocin (STZ) was prepared by dissolving appropriate amount in freshly prepared 5 mmol/L citrate buffer, pH 4.5. The intraperitoneal injection of single low dose of steptozotocin (STZ) (35 mg/kg, i.p.) was conducted on the fourth week on overnight fasted rats. After STZ injection, the rats had free access to glucose solution (5%) for 24 h to avoid and/or attenuate subsequent inevitable hyperinsulinemia and hypoglycemic shock. 48 hours post-STZ injection, animals were fasted overnight and a drop of blood samples were analyzed for glucose levels (mg/dL) by using strips on glucometer (ACCU-CHEK ACTIVE, Roche, Germany). Animals with glucose levels above 250 mg/dL were considered as diabetic and used in the therapeutic study" [20].

2.2.7 Animal grouping

Group 1: Diabetic-Negative control (NDBT) and water ad libitum
Group 2: HFD/STZ (35mg/kg BWT)-induction (DBT) without treatment
Group 3: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg body weight of Metformin (DBT-Metformin); Diabetic-Positive control
Group 4: HFD/STZ (35mg/kg BWT)-induction and 250 mg/kg body weight of CNE (DBT-CNE 250)
Group 5: HFD/STZ (35mg/kg BWT)-induction and 500 mg/kg body weight of CNE (DBT-CNE 500)
Group 6: HFD/STZ (35mg/kg BWT)-induction and 1000 mg/kg body weight of CNE (DBT-CNE 1000)
Group 7: HFD/STZ (35mg/kg BWT)-induction and 250 mg/kg body weight of CNPH24 (DBT-CNPH24 250)
Group 8: HFD/STZ (35mg/kg BWT)-induction and 500 mg/kg body weight of CNPH24 (DBT-CNPH24 500)
Group 9: HFD/STZ (35mg/kg BWT)-induction and 1000 mg/kg body weight of CNPH24 (DBT-CNPH24 1000)
Group 10: HFD/STZ (35mg/kg BWT)-induction and 250 mg/kg body weight of CNPH48 (DBT-CNPH48 250)
Group 11: HFD/STZ (35mg/kg BWT)-induction and 500 mg/kg body weight of CNPH48 (DBT-CNPH48 500)
Group 12: HFD/STZ (35mg/kg BWT)-induction and 1000 mg/kg body weight of CNPH48 (DBT-CNPH48 1000)
Group 13: HFD/STZ (35mg/kg BWT)-induction and 250 mg/kg body weight of CNPH72 (DBT-CNPH72 250)
Group 14: HFD/STZ (35mg/kg BWT)-induction and 500 mg/kg body weight of CNPH72 (DBT-CNPH72 500)
Group 15: HFD/STZ (35mg/kg BWT)-induction and 1000 mg/kg body weight of CNPH72 (DBT-CNPH72 1000)
Group 16: HFD/STZ (35mg/kg BWT)-induction and 250 mg/kg body weight of CNPI (DBT-CNPI 250)
Group 17: HFD/STZ (35mg/kg BWT)-induction and 500 mg/kg body weight of CNPI (DBT-CNPI 500)
Group 18: HFD/STZ (35mg/kg BWT)-induction and 1000 mg/kg body weight of CNPI (DBT-CNPI 1000).

After induction, rats in each group received their respective treatments by oral administration (gavage).

2.2.8 Necropsy, blood collection and preparation

The rats were sacrificed by cervical dislocation at exactly 18 hours after the last administration. Blood samples were collected by cardiac puncture into non-anticoagulant serum tubes and allowed to stand for 1h. The clotted blood was centrifuged for 10min at 3000rpm. The serum was transferred into clean tubes for the evaluation of biochemical indices.

2.2.9 Preparation of tissue homogenates

The liver were quickly rinsed in ice-cold 1.15% potassium chloride solution, blotted and weighed. The liver was subsequently homogenized in cold 0.1 M potassium phosphate buffer pH 7.4. The homogenates were cold-centrifuged for 10 min at

3000 rpm and supernatants were collected and stored at 4°C prior to biochemical assays.

2.3 Evaluation of Hepatic Function

2.3.1 Aspartate amino transferase (AST) activity

"Activity of AST was evaluated using manufacturer protocol of Randox AST Kit based on the principle of" [21]. "Diluted sample (0.1ml) was mixed with 0.5 ml of R1 (phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), and α-oxoglutarate (2 mmol/L)) and the mixture incubated for 30 min at 37 °C after which 0.5 ml of R2 (2, 4-dinitrophenylhydrazine (2 mmol/L)) was added to the reaction mixture and allowed to stand for another 20 min at 25 °C. Then, 5.0 ml of NaOH (0.4M/L) was added and the absorbance was read against the reagent blank after 5 min at 546 nm. The activity of AST in homogenate was obtained following the extrapolation of absorbance value on AST standard curve". [21]

2.3.2 Alanine amino transferase (ALT) activity

"Assay of alanine amino transferase ALT activity was carried out using the manufacturer protocol of Randox ALT Kit based on the principle" described by Reitman [21]. "Reagent1 (0.5 ml) containing Phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l) and α- oxoglutarate (2.0 mol/l) was added to a test tube already containing 0.1 ml of serum sample and the mixture was incubated at 37°C for 30 min. Then, 0.5ml of Reagent2 containing 2, 4-dinitrophenylhydrazine (2.0mmol/l) was added and the mixture incubated again at 20 °C for 20 min. Finally, 5 ml of NaOH was added. The mixture was allowed to stand for 5 min at room temperature and the absorbance was read at 546nm. The activity of ALT in the homogenate was obtained from a standard curve" [21].

2.3.3 Alkaline phosphatase (ALP) activity

"Assay of alkaline phosphatase (ALP) activity was carried out according to the procedure provided by RANDOX KIT Manufacturer. ALP activity was measured by monitoring the concentration of p-nitrophenol formed when ALP reacted with p-nitrophenyl phosphate. Exactly 1.0 ml of the reagent (1 mol/l, pH 9.8 Diethanolamine buffer, 0.5 mmol/l $MgCl_2$; substrate: 10 mmol/l p-nitrophenylphosphate) was added to 0.02 ml of sample and then mixed. The absorbance was read for 3 min at intervals of 1 min at a wavelength of 405nm". [21]

2.3.4 Calculation

ALP activity was determined using the formula:
U/l = 2760 x A405 nm/min.

2.3.5 Evaluation of gamma-glutamyl transferase (GGT) activity

The assay was conducted using a Randox kit manual. Using a water-bathe set at 37°C for the incubation of the reaction, the reaction is constituted of 100µl of the sample extract 1000µl of the reagent at a pH of 8.25 using tris-buffer of 100mmol/l concentration. The reagent contains glycylglycine (100mmol/l) and L-γ-glutamyl-3-carboxy-4-nitroanilide (2.9 mmol/l). The absorbance of the mixture was read at 405nm.

Calculation:

To calculate the GGT activity;
U/L = 1158 x A 405 nm/min

2.4 Evaluation of Kidney Function

2.4.1 Determination of creatinine

Creatinine level was determined using commercially available kits (Randox Laboratories, Antrim, UK). Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration. Procedure Exactly 2.0 ml of working reagent (picric acid + sodium hydroxide) was added to 0.2 ml of sample and mixed. The absorbance A1 of standard and sample was read after 30 s and 2 min later, absorbance A2 of standard and sample was read again at 492 nm.

2.4.2 Calculation

$$\text{creatinine}\left(\frac{\text{mg}}{dl}\right) = \frac{\Delta\text{A sample x standard concentration}}{\Delta\text{A standard}}\left(\frac{\text{mg}}{dl}\right)$$

Where:

ΔA sample or ΔA standard = $A_2 - A_1$
ΔA = Change in absorbance per minute,
A_1 = initial absorbance,
A_2 = final absorbance.

2.4.3 Blood urea nitrogen concentration

The Urea Nitrogen assay is a modification of a totally enzymatic procedure first described by

Talke [22]. One hundred microliter (100µL) of diluted serum (serum 1:4 in either normal saline or PBS) supernatant was added to the microplate wells; 150µL of Urease Mix solution was then added to it. Plate was tapped gently 3-4 times to mix sample and enzyme and incubated for 15 minutes at room temperature.100µL of Alkaline Hypochlorite was added to each well and incubate for 10 minutes at room temperature. The absorbance of each sample is measured in duplicate at 620 nm.

The urea concentration (dilution factor = 5) in the well can be determined using the equation:

Blood Urea Nitrogen concentration=(Dilution factor x (Average absorbance –y-intercept)/ slope)

2.4.4 *In vivo* antioxidant indices of liver and kidney

Catalase (CAT), malondialdehyde (MDA), superoxide oxidase (SOD), reduced glutathione (GSH), and glutathione transferase (GST) were evaluated using standard methods.

2.4.5 Evaluation of haematological indices

"Hematocrit (PCV) was determined using high-speed centrifugation of blood-filled hematocrit tubes with a Zipocrit Hematocrit Centrifuge (Thermo Fisher Scientific, Philadelphia, PA). All white blood cell (WBC) count estimates were performed by the same technics, at a location on the slide where the cells were one layer thick, adjacent to one another (membranes touching), evenly distributed, and showed no signs of morphological changes" [23]. "White blood cell estimates were made by using a 100X objective lens with immersion oil, counting the number of white blood cells in 10 fields, calculating the average, and then multiplying the number of cells by 2,000" [24]. "The absolute cell count for each type of cell was calculated by multiplying the percentage of the type of cell by the overall WBC estimate" [23]. "Total erythrocyte count (TEC) and total leukocyte count (TLC) were determined by Haemocytometric method" [25]. "With the use of Natt and Harricks diluting fluid, RBCs were viewed with transparent cytoplasm and pale staining nucleus. Immediately after the collection of blood, smears were prepared for differentiating and counting each type of leukocytes. Differential count of leukocytes was determined by using Giemsa stain. The smears were stained approximately 2-10 min after methyl alcohol fixation. Two hundred leukocytes, including granulocytes and agranulocytes were counted in different fields of the smears and expressed in percentage (%)". [21] The Mean Corpuscular Hemoglobin (MCH) value is determined by the equation:

MCH = (HGB/RBC) x 100.

The MCHC is classically determined by the equation:

MCHC = (HGB/HCT) x 100.

The MCV is classically determined by the equation:

MCV = (PCV/RBC).

2.4.6 Histopathological examination

Post-mortem examination was carried out on all the animals sacrificed. Both the liver and kidney were selected for histopathological study. After blood rinsing in normal saline, sections were taken from each selected vital organ, this was followed by preparing a 1 cm wide strip of a section of the Liver and Kidney and placed into formalin. It was then processed into 4-5 µm thick sections stained with haematoxylin-eosin and observed under a microscope.

2.5 Statistical Analysis

The results were pooled and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple tests was used for the post hoc (DMRT). Statistical package for Social Science (SPSS) 21.0 for Windows was used for the analysis. The significance level was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Influence of Conophor Nut Protein Isolate, Hydrolysates and Extract on the Haematological Properties of Streptozocin-induced Diabetic Wistar Rats

Haematological properties assessment is used in evaluating the extent of the deterioration of blood constituents of an animal [26]. It can also play a role in physiological, nutritional and pathological state of an organism [27]. The haematological

property of treated diabetic wistar rats is presented in Table 1. The Pack Cell Volume (PCV) ranged between 27.50% in Diabetic untreated group (DBT) to 41.50% in Negative control group (NDBT). Red blood cell (RBC), White blood cell (WBC) and haemoglobin (Hb) ranged between 97.87-239.59 $x10^3$ rbc/mm^3, 127.67-204.39 x50wbc/mm and 6.44-26.50g/100min DBT and NDBT rats respectively. The samples especially at high dose (1000 mg) were able to increase the PCV, RBC, WBC and Hb in the diabetic rats treated with conophor nut protein isolate, hydrolysates and extract compared to the metformin treated rats. This suggests the nutritional quality of conophor nut proteins and extract. Diets with poor protein composition have been reported to result in poor haemoglobin production and immunity [28] therefore resulting in anaemia [29]. Glycosylation of haemoglobin peculiar in diabetic states [30] may contribute to the low Hb concentration as obvious in the DBT. Red blood cell indices are used to evaluate erythropoiesis. Changes in the process may be in the direction of a decrease, indicated by anaemia or an increase, indicated by polycythemia [31]. The increase in RBC in treated diabetic rats with CNE, CNPH24, CNPH48, CNPH72 and CNPI indicates ameliorative effect on the streptozocin induced anaemia observed in the DBT group. The observed increase red cell indices in diabetic rats following the treatments with CNE, CNPH24, CNPH48, CNPH72 and CNPI suggest enhanced erythropoiesis. Some of the chemical constituents of conophor nut such as flavonoids stimulate the production of erythropoietin, the key hormonal stimulant of erythropoiesis, and prevent free radicals-induced haemolysis of RBCs [32,33]. Also, it was observed that treatment of diabetic rats with high dose of CNE, CNPH24, CNPH48, CNPH72 and CNPI up to 1000 mg was able to increase the lymphocyte index compared to the metformin treated diabetic group (DBT-Met). This could be due to the mature red blood cell that was not destroyed by the isolate, hydrolysates and extract [34]. The decrease in lymphocytes in the DBT group may be a response to the stress associated with diabetes since they are essential for maintenance of the body's defense /immunity [35].

Similarly, the treatments were able to increase the concentration of Neutrophil in diabetic rats from 33.21-38.43% in CNE, 33.10- 39.63% in CNPH24, 33.06 – 38.62% in CNPH48, 33.63 - 40.75% in CNPH72, and 32.99 – 40.06% in CNPI compared to the 40.32% in the metformin treated diabetic rats. The increase in neutrophils may be due to involvement of cells in the phagocytic processes against different antigens especially as the risk of infection is higher in diabetics [36].

3.2 Effects of Conophor nut Protein Isolate, Hydrolysates and Extract on Hepatic and Renal Endogenous Antioxidants Activities

Oxidative stress assessment was performed by recording the activities of anti-oxidative enzymes i.e. catalase (CAT), Glutathione transferase Activity (GTA), superoxide dismutase (SOD), reduced glutathione (GSH) and lipid peroxidation (MDA) on liver and kidney of streptozocin-induced male wistar diabetic rats. Increased oxidative stress is one of the major causes of diabetes in experimental animals [37] and it is linked to auto-oxidation of glucose, protein glycation, lipid peroxidation, dyslipidemia and lowered activities of enzymic and non-enzymic antioxidants [38].

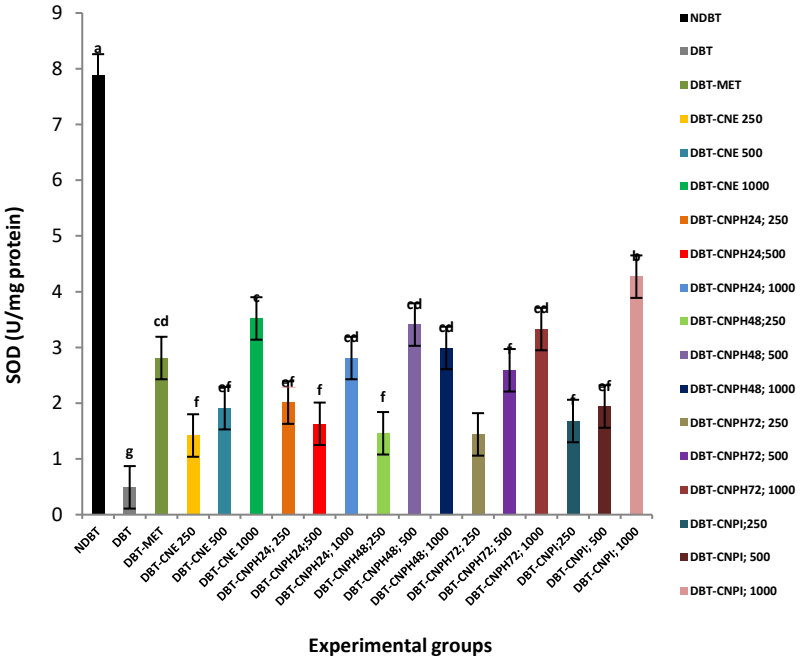
Catalase (CAT) is an enzyme that efficiently converts H_2O_2 into oxygen and water. Contrary to glutathione, the affinity of the catalase for hydrogen peroxide is increased only when H_2O_2 levels are high [39]. According to [40], CAT protects the cell from oxidative damage by reactive oxygen species (ROS). The hepatic and renal catalase activity is illustrated in Figs. 1 and 2. The non-diabetic rats (NDBT) had the highest hepatic and renal catalase activity (13.33 and 8.38 mmol H_2O_2/mg of protein) respectively while the DBT had significantly lower catalase activity (2.31 and 1.19 mmol H_2O_2/mg of protein) respectively ($p<0.05$). The activity of catalase in diabetic rat was dose dependent. Diabetic rats treated with 1000 mg/kg CNPH72, CNE and CNPI had significantly higher enzyme activity in the order CNPH72 > CNE > CNPI than CNPH24 and CNPH48 ($p<0.05$) therefore reflecting a decrease in ROS level in the cell. CNPH72, CNE and CNPI caused increased activity of the hepatic and renal enzyme than the drug treated diabetic rat (DBT-MET) and may be a better replacement for synthetic drugs like metformin in the treatment of diabetes.

Superoxide dismutase (SOD) is an enzyme that has a great effect on the biological defense mechanism by the disparity of endogenous cytotoxic superoxide radicals into hydrogen peroxide (H_2O_2) [41]. The hepatic and renal SOD

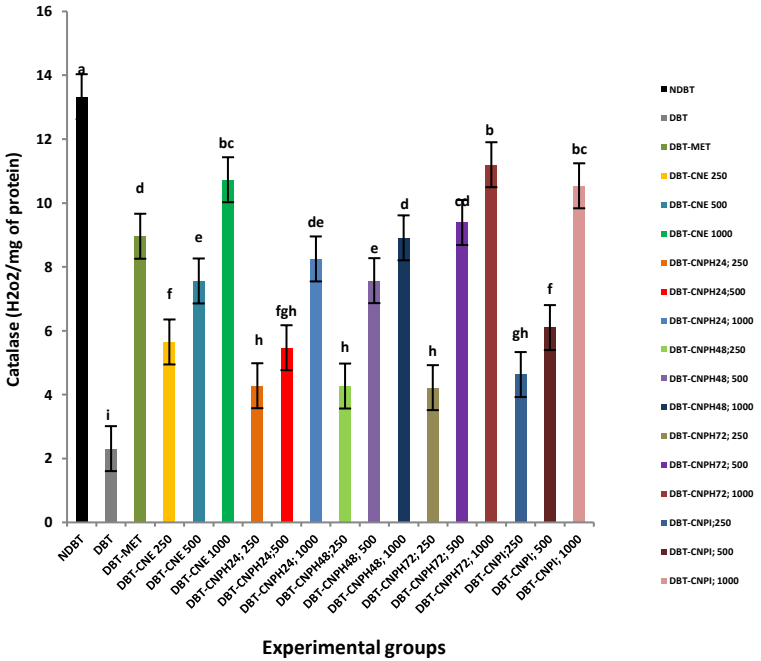
Table 1. Influence of Conophor Nut Protein Isolate, Hydrolysates and Extract on the Haematological properties of Streptozocin-induced Diabetic Wistar Rats

Samples	PCV (%)	HB (g/100 ml)	WBC (x 50 wbc/ mm)	RBC (x 10^3 rbc/mm^3)	Lymphovcyte (%)	Neutrophil (%)	Serum Total Protein (mg/dl)
NDBT	41.50±0.70^{a}	26.50±0.71^{a}	204.39±24.55^{bc}	239.59±2.99^{cd}	15.91±1.87^{ab}	48.64±1.74^{a}	44.91±2.20^{a}
DBT	27.50±4.95^{c}	6.44±0.51^{j}	127.67±5.64^{j}	97.87±1.19^{h}	9.92±0.44^{h}	23.21±1.15^{f}	17.57±2.37^{h}
DBT-MET	38.00±1.41^{ab}	18.00±2.68^{cde}	181.75±11.28^{defg}	239.44±4.32^{cd}	14.12±0.88^{cdef}	40.32±0.91^{bc}	33.57±1.50
DBT-CNE 250	35.50±0.71^{b}	12.60±0.28^{hi}	173.91±9.74^{fghi}	153.31±19.02^{g}	13.52±0.76^{defg}	33.21±1.28^{e}	21.65±1.67^{g}
DBT-CNE 500	36.50±0.71^{b}	14.05±0.78^{gh}	181.97±9.54^{defg}	214.28±12.85^{de}	14.14±0.74^{cdef}	37.87±1.58^{cd}	25.16±1.92^{ef}
DBT-CNE 1000	37.50±2.12^{ab}	17.70±0.99^{cdef}	187.18±7.43^{cdef}	299.62±3.85^{b}	14.50±0.58^{abcd}	38.43±1.53^{cd}	34.26±3.42^{bc}
DBT-CNPH24; 250	35.5±0.71^{b}	10.50±0.78^{i}	156.19±3.22^{i}	149.83±17.74^{g}	12.53±0.30^{fg}	33.10±0.79^{e}	22.50±1.28^{fg}
DBT-CNPH24;500	38.00±1.41^{ab}	13.40±1.98^{gh}	174.09±2.49^{fghi}	207.25±16.50^{ef}	14.31±0.91^{bcde}	37.80±0.51^{cd}	23.02±2.13^{fg}
DBT-CNPH24; 1000	37.50±0.71^{ab}	17.15±0.78^{def}	196.01±2.65^{bcde}	249.87±19.07^{c}	14.99±0.56^{abcd}	39.63±1.49^{bcd}	33.81±1.03^{bcd}
DBT-CNPH48;250	36.00±2.83^{b}	10.75±0.92^{i}	167.99±0.97^{ghi}	158.08±17.95^{g}	12.51±0.07^{fg}	33.06±0.19^{e}	25.73±2.61^{ef}
DBT-CNPH48; 500	37.00±1.41^{b}	15.00±0.71^{fgh}	184.79±2.74^{defg}	288.39±2.04^{b}	14.05±0.11^{cdefg}	37.13±0.28^{d}	31.30±2.58^{cd}
DBT-CNPH48; 1000	38.00±1.41^{ab}	19.20±0.57^{cd}	197.06±2.91^{bcde}	308.46±14.66^{b}	14.61±0.11^{abcd}	38.62±0.30^{cd}	30.64±1.35^{d}
DBT-CNPH72; 250	38.00±0.00^{ab}	14.40±0.42^{gh}	177.78±3.04^{efgh}	179.46±13.53^{g}	12.73±0.89^{efg}	33.63±2.36^{e}	17.63±1.56^{h}
DBT-CNPH72; 500	38.50±0.71^{ab}	17.60±1.98^{cdef}	213.64±0.87^{ab}	298.19±9.16^{b}	15.43±0.48^{abc}	40.75±1.22^{bc}	27.03±3.83^{e}
DBT-CNPH72; 1000	39.00±1.41^{ab}	22.05±0.35^{b}	227.21±6.06^{a}	346.13±24.02^{a}	16.10±0.62^{a}	42.55±1.66^{b}	34.55±2.93^{bc}
DBT-CNPI;250	35.50±2.12^{b}	15.70±1.41^{efg}	165.67±2.32^{hi}	162.60±6.51^{g}	12.49±0.73^{g}	32.99±1.92^{e}	17.89±1.80^{h}
DBT-CNPI; 500	36.00±1.41^{b}	17.95±1.06^{cde}	196.02±2.69^{bcde}	170.01±5.04^{g}	14.38±0.99^{bcd}	37.99±0.26^{cd}	20.46±1.09^{gh}
DBT-CNPI; 1000	38.50±0.71^{ab}	20.10±0.99^{bc}	211.39±2.94^{ab}	370.65±15.28^{a}	15.16±0.31^{abcd}	40.06±0.81^{bcd}	35.35±1.73^{b}

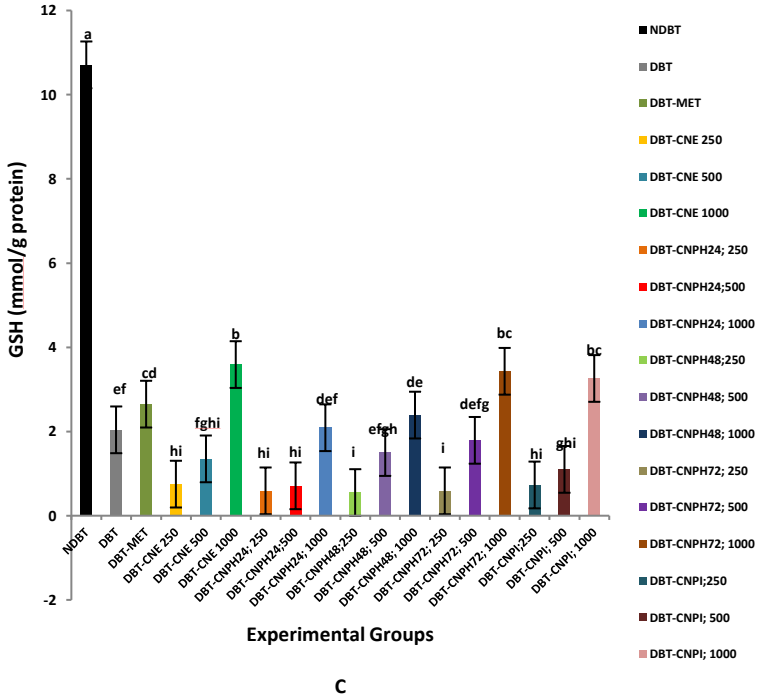
Means (±SEM) with different alphabetical superscripts in the same column are significantly different at P<0.05.
KEY: NDBT: Negative control;
DBT: HFD/STZ (35mg/kg BWT)-induction positive control;
DBT-METFORMIN: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg body weight of METFORMIN;
DBT-CNE: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Extract;
DBT-CNPH24 : HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 24h;
DBT-CNPH48: HFD/STZ (35mg/kg BWT) induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 48;
DBT-CNPH72: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented 72;
DBT-CNPI: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Protein Isolate


SOD (U/mg protein)
Experimental groups
NDBT
DBT
DBT-MET
DBT-CNE 250
DBT-CNE 500
DBT-CNE 1000
DBT-CNPH24; 250
DBT-CNPH24;500
DBT-CNPH24; 1000
DBT-CNPH48;250
DBT-CNPH48; 500
DBT-CNPH48; 1000
DBT-CNPH72; 250
DBT-CNPH72; 500
DBT-CNPH72; 1000
DBT-CNPI;250
DBT-CNPI; 500
DBT-CNPI; 1000

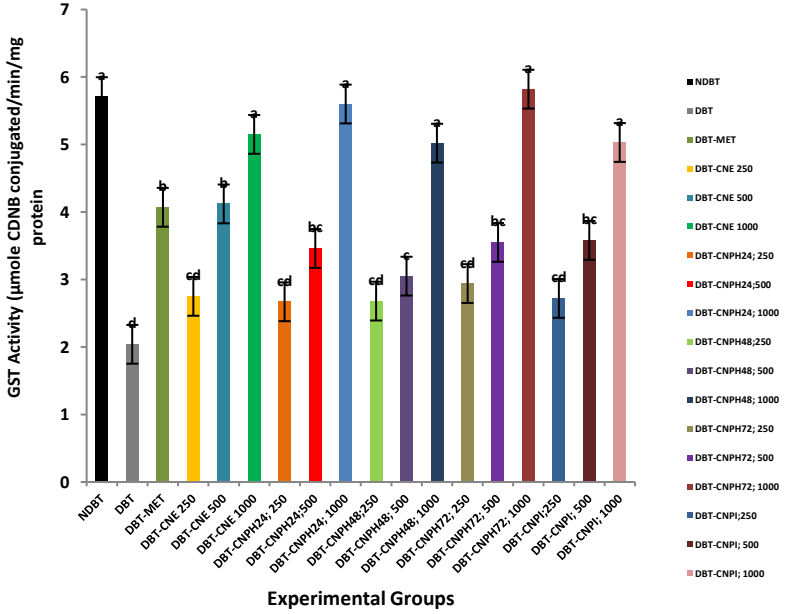
A


Catalase (H2o2/mg of protein)
Experimental groups
NDBT
DBT
DBT-MET
DBT-CNE 250
DBT-CNE 500
DBT-CNE 1000
DBT-CNPH24; 250
DBT-CNPH24;500
DBT-CNPH24; 1000
DBT-CNPH48;250
DBT-CNPH48; 500
DBT-CNPH48; 1000
DBT-CNPH72; 250
DBT-CNPH72; 500
DBT-CNPH72; 1000
DBT-CNPI;250
DBT-CNPI; 500
DBT-CNPI; 1000

B


GSH (mmol/g protein)
Experimental Groups
NDBT
DBT
DBT-MET
DBT-CNE 250
DBT-CNE 500
DBT-CNE 1000
DBT-CNPH24; 250
DBT-CNPH24;500
DBT-CNPH24; 1000
DBT-CNPH48;250
DBT-CNPH48; 500
DBT-CNPH48; 1000
DBT-CNPH72; 250
DBT-CNPH72; 500
DBT-CNPH72; 1000
DBT-CNPI;250
DBT-CNPI; 500
DBT-CNPI; 1000

C


GST Activity (µmole CDNB conjugated/min/mg protein
Experimental Groups
NDBT
DBT
DBT-MET
DBT-CNE 250
DBT-CNE 500
DBT-CNE 1000
DBT-CNPH24; 250
DBT-CNPH24;500
DBT-CNPH24; 1000
DBT-CNPH48;250
DBT-CNPH48; 500
DBT-CNPH48; 1000
DBT-CNPH72; 250
DBT-CNPH72; 500
DBT-CNPH72; 1000
DBT-CNPI;250
DBT-CNPI; 500
DBT-CNPI; 1000

D

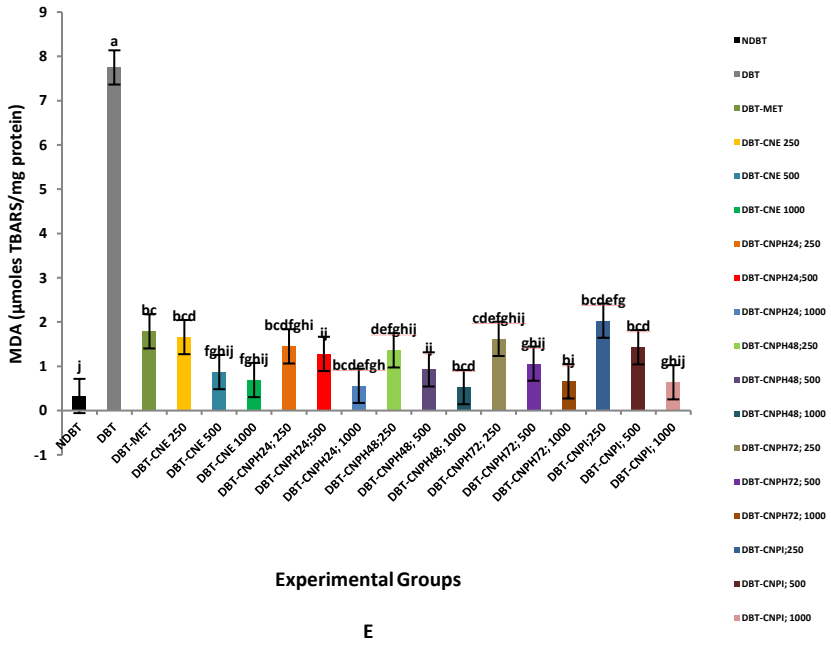
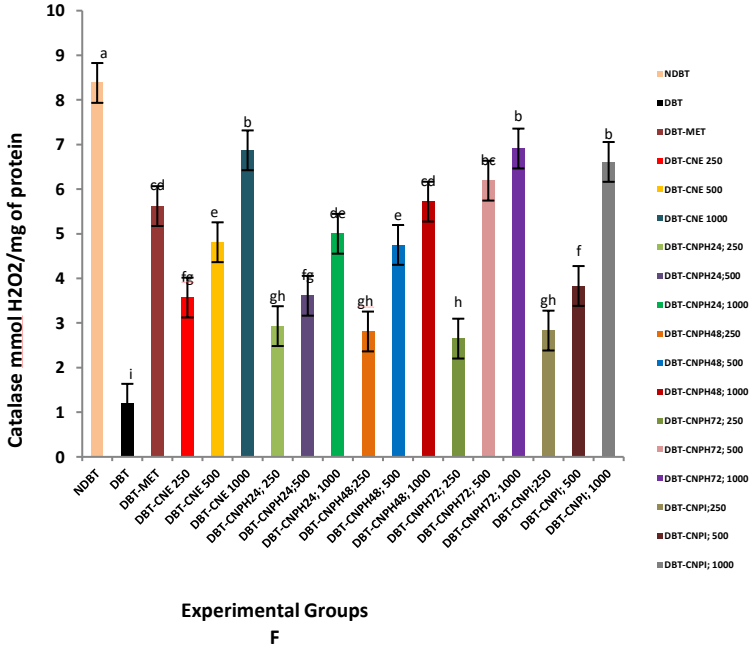
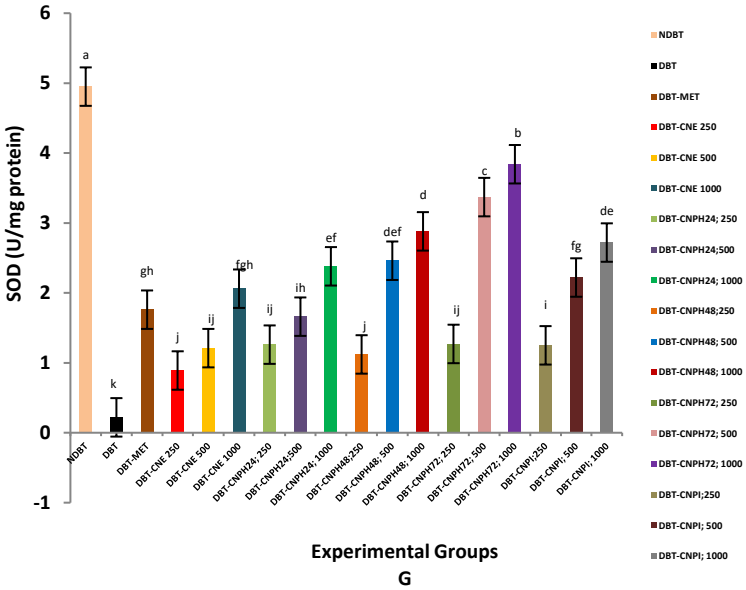


Fig. 1. Effects of Conophor nut Protein Isolate, Hydrolysates and Extract on Hepatic superoxide dismutase (A), Catalase (B), Reduce glutathione (C), glutathione transferase(D) and malondialdehyde (E)

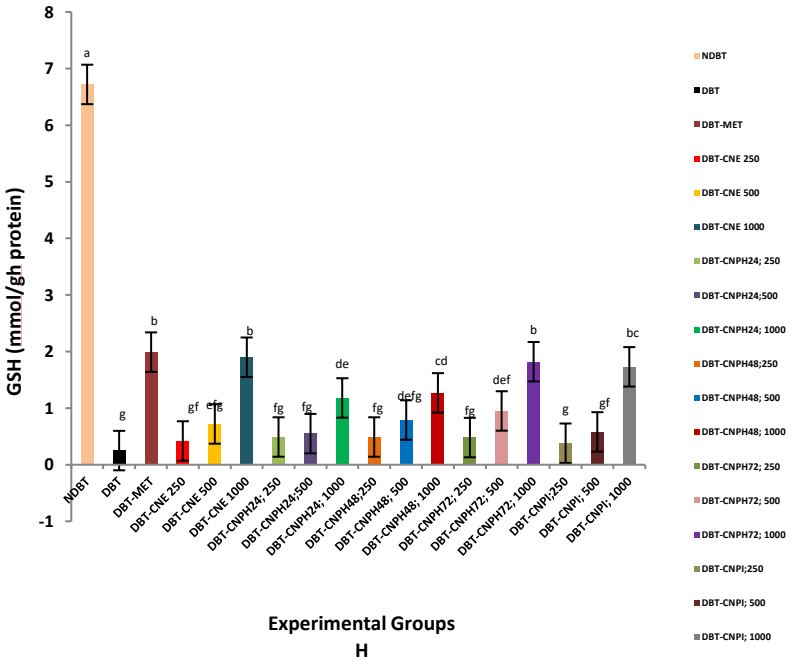
Bars with different alphabetical superscripts are significantly different at P<0.05. KEY: NDBT: Negative control; DBT: HFD/STZ (35mg/kg BWT)-induction positive control; DBT-METFORMIN: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg body weight of METFORMIN; DBT-CNE: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Extract; DBT-CNPH24 : HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 24h; DBT-CNPH48: HFD/STZ (35mg/kg BWT) induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 48; DBT-CNPH72: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented 72; DBT-CNPI: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Protein Isolate


Catalase mmol H2O2/mg of protein
Experimental Groups
NDBT
DBT
DBT-MET
DBT-CNE 250
DBT-CNE 500
DBT-CNE 1000
DBT-CNPH24; 250
DBT-CNPH24;500
DBT-CNPH24; 1000
DBT-CNPH48;250
DBT-CNPH48; 500
DBT-CNPH48; 1000
DBT-CNPH72; 250
DBT-CNPH72; 500
DBT-CNPH72; 1000
DBT-CNPI;250
DBT-CNPI; 500
DBT-CNPI; 1000

F


SOD (U/mg protein)
Experimental Groups
NDBT
DBT
DBT-MET
DBT-CNE 250
DBT-CNE 500
DBT-CNE 1000
DBT-CNPH24; 250
DBT-CNPH24;500
DBT-CNPH24; 1000
DBT-CNPH48;250
DBT-CNPH48; 500
DBT-CNPH48; 1000
DBT-CNPH72; 250
DBT-CNPH72; 500
DBT-CNPH72; 1000
DBT-CNPI;250
DBT-CNPI; 500
DBT-CNPI; 1000

G


GSH (mmol/gh protein)
Experimental Groups
H
NDBT
DBT
DBT-MET
DBT-CNE 250
DBT-CNE 500
DBT-CNE 1000
DBT-CNPH24; 250
DBT-CNPH24;500
DBT-CNPH24; 1000
DBT-CNPH48;250
DBT-CNPH48; 500
DBT-CNPH48; 1000
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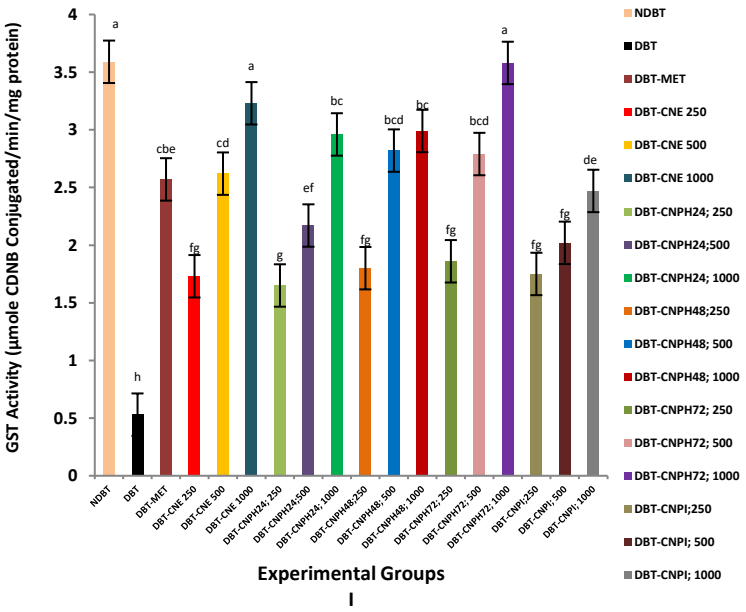
DBT-CNPI;250
DBT-CNPI; 500
DBT-CNPI; 1000


GST Activity (µmole CDNB Conjugated/min/mg protein)
Experimental Groups
I
NDBT
DBT
DBT-MET
DBT-CNE 250
DBT-CNE 500
DBT-CNE 1000
DBT-CNPH24; 250
DBT-CNPH24;500
DBT-CNPH24; 1000
DBT-CNPH48;250
DBT-CNPH48; 500
DBT-CNPH48; 1000
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DBT-CNPI; 500
DBT-CNPI; 1000

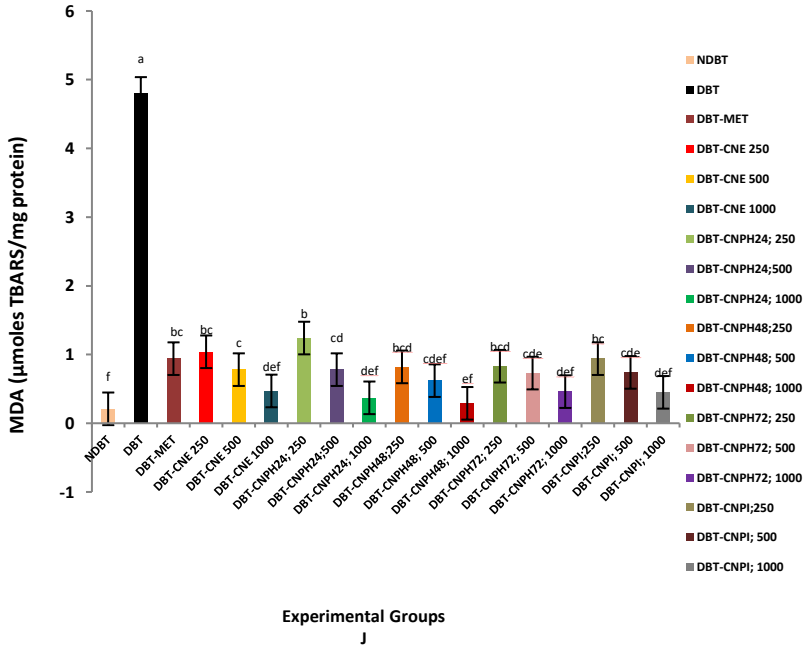


Fig. 2. Effects of Conophor nut Protein Isolate, Hydrolysates and Extract on Renal Hepatic Catalase (F), superoxide dismutase (F), Reduce glutathione (H), glutathione transferase (I) and malondialdehyde (J)

Bars with different alphabetical superscripts are significantly different at P<0.05. KEY: NDBT: Negative control; DBT: HFD/STZ (35mg/kg BWT)-induction positive control; DBT-METFORMIN: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg body weight of METFORMIN; DBT-CNE: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Extract; DBT-CNPH24 : HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 24h; DBT-CNPH48: HFD/STZ (35mg/kg BWT) induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 48; DBT-CNPH72: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented 72; DBT-CNPI: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Protein Isolate

activity of non-diabetic rats (NDBT) was significantly higher (7.88 and 4.95 µ/mg protein) than diabetic rats (DBT) (0.49 and 0.22 µ/mg protein) liver and kidney SOD respectively. The enzymatic hepatic and renal SOD activity of DBT group was very low due to the diabetic condition. However, 1000mg/kg of treatment sample CNE, CNPI, CNPH24, CNPH48 and CNPH72 had a significant effect on the oxidative defense mechanism of the body by increasing SOD activity considerably especially CNPH72 (3.84 U/mg protein) and CNPI (4.27 U/mg protein) in the kidney and liver respectively.

Glutathione (GSH) participates in many biological processes acting as an intra and extracellular antioxidant. It reduces hydrogen peroxide, thereby lowering the lipid peroxidation levels [42]. GSH also plays a fundamental role in cell defense against free oxygen radicals and other oxidative species [43]. The hepatic and renal GSH activity of non-diabetic rats (NDBT) was significantly higher (10.71 and 6.72 µ/mg protein) than diabetic rats (DBT) (2.04 and 0.25µ/mg protein) liver and kidney GSH respectively. In this study, hepatic and renal GSH levels increased in all doses. 1000mg/kg of the treatment was able to boost the GSH level in diabetic rats but not comparable to the level of GSH in the NDBT group in the liver and kidney. Sample treatment CNE, CNPH72 and CNPI had the highest level of GSH in diabetic rat in the liver and kidney respectively. This increase reflects the strong potential of Conophor nut extract, hydrolysate fermented for 72 h and protein isolate in the defense of the cell against free radicals and reactive oxygen species.

“Glutathione-S-transferase (GST) performs an important role in the detoxification and metabolism of many xenobiotic and endobiotic compounds” [44]. “GST also has peroxidase and isomerase activity. It binds covalently with reactive metabolites formed from xenobiotics and non-covalently with lipophilic molecules, thereby offering protection against oxidative stress” [45]. “Induction of diabetes by STZ in this study caused reduction in GST activity and the observed decrease may be due to suppression of the mRNA of GST by excessive free radicals” [46]. Metformin was able to increase the level of GST in diabetic rats by 50.12% in the liver and 20.62% in the kidney. There was no significant difference in the activity of the treatment samples and normal control (NDBT) group in increasing the level of GST in the liver. In the kidney, there was no significant difference between treatment sample, CNE, CNPH72 and the normal control group (NDBT) and also, no significant difference was observed in the level of CNPI, CNPH24 and CNPH48 at 1000mg/kg respectively in increasing the activity of GST in diabetic rats.

MDA is the final product of the lipid peroxidation and its Measurement reflects the degree of oxidative stress [47]. The reduction of MDA is explained by the decrease in lipid peroxidation induced by increased production of antioxidant enzymes. In the present work, the administration of treatment samples; CNE, CNPH24, CNPH48, CNPH72 and CNPI were able to decrease the level of MDA in diabetic rats by 8.90%, 7.23%, 6.84%, 8.52%, 8.26% in the liver and 9.79%, 7.71%, 6.04%, 9.58%, 9.38% in the kidney in contrast to 23.10% and 19.58% in the metformin treated group for the liver and kidney respectively. The treatment samples have the ability to block lipid peroxidation and therefore strengthen the antioxidant defense system. Because, induced hyperlipidemia leads to increased production of oxygen free radicals, thus causing lipid peroxidation [48].

3.3 Kidney Function

Renal dysfunction can be assessed by measuring serum creatinine and serum urea. Increase in serum creatinine and serum urea is an indication of renal dysfunction [49,50]. The creatinine and urea concentration (indices of kidney functionality) of the rats treated with conophor nut protein isolate (CNPI), hydrolysates (CNPH24, CNPH48, CNPH72) and extract (CNE) is presented in Table 2. The values for creatinine and urea concentration ranked from 3.72 mg/dl in NDBT, 77.29 mg/dl in DBT, 18.91 mg/dl in DBT- Met group and 13.42 mg/dl in NDBT, 139.42 mg/dl in DBT and 63.49 mg/dl in DBT-MET respectively. Metformin was able to crash the level of plasma creatinine and urea concentration in diabetic rats by 24.47 % and 45.54 % respectively. The increased level of plasma creatinine and urea in diabetic control rats (DBT) implies impaired renal function in the diabetic animals which may indicate the inefficiency of the kidney to filter waste products from the blood and excrete them in the urine. [51] reported that alteration in serum creatinine concentration is one of the basic markers for kidney dysfunction. Treatment with conophor nut protein isolate, hydrolysates and extract at all doses produced significant decrease in the level of plasma creatinine and urea in diabetic rats especially the hydrolysates whose activities were

Table 2. Influence of conophor nut protein isolate, hydrolysates and extract on the haematological properties of streptozocin-induced diabetic wistar rats s of diabetic rats

Samples	BUN (mg/dl)	CREATININE (mg/dl)	Urea/Creatinine
NDBT	13.42±1.90^{j}	3.72±.52^{i}	3.34^{f}
DBT	139.42±28.65^{a}	77.29±12.36^{a}	1.80^{j}
DBT-MET	63.49±3.95^{b}	18.91±2.43^{bc}	3.36^{f}
DBT-CNE 250	60.22±13.86^{bc}	18.78±1.36^{bc}	3.21^{g}
DBT-CNE 500	43.77±5.35^{ef}	13.49±2.03^{cdef}	3.24^{g}
DBT-CNE 1000	33.58±3.41^{fghi}	7.94±2.25^{ghi}	4.23^{a}
DBT-CNPH24; 250	58.67±3.13^{bcd}	21.07±1.45^{b}	2.78^{i}
DBT-CNPH24;500	47.05±4.78^{def}	16.71±2.11^{bcd}	2.82^{hi}
DBT-CNPH24; 1000	39.63±2.29^{efgh}	10.95±2.09^{efgh}	3.62^{c}
DBT-CNPH48;250	46.20±5.67^{def}	16.68±1.31^{bcd}	2.77^{i}
DBT-CNPH48; 500	42.39±5.35^{efg}	12.27±1.58^{defg}	3.45^{e}
DBT-CNPH48; 1000	29.24±4.44^{ghi}	8.53±1.32^{fghi}	3.43^{e}
DBT-CNPH72; 250	48.82±3.95^{cde}	13.63±1.13^{cdef}	3.58^{d}
DBT-CNPH72; 500	41.98±2.09^{efg}	11.44±0.60^{efgh}	3.67^{c}
DBT-CNPH72; 1000	25.84±4.72^{i}	6.34±0.83^{hi}	4.08^{ab}
DBT-CNPI;250	43.79±2.41^{ef}	14.52±3.06^{cde}	3.02^{h}
DBT-CNPI; 500	36.65±4.69^{efghi}	11.08±1.30^{efgh}	3.31^{f}
DBT-CNPI; 1000	28.18±4.30^{hi}	7.14±2.05^{ghi}	3.95^{b}

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at P<0.05.

KEY:

NDBT: Negative control; DBT: HFD/STZ (35mg/kg BWT)-induction;

DBT-METFORMIN: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg body weight of METFORMIN;

DBT-CNE: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Extract;

DBT-CNPH24 : HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 24h;

DBT-CNPH48: HFD/STZ (35mg/kg BWT) induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 48;

DBT-CNPH72: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented 72;

DBT-CNPI: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Protein Isolate.;

BUN: Blood urea nitrogen

Table 3. Influence of conophor nut protein isolate, hydrolysates and extract on the haematological properties of streptozocin-induced diabetic wistar rats s of diabetic rats

Samples	ALT (U/l)	ALP (U/l)	GGT (U/l)	AST (Ul)	AST/ALT ratio
NDBT	21.34±3.04^{g}	6.03±0.41^{g}	82.91±6.67^{cd}	11.86±1.38^{k}	0.56^{gh}
DBT	95.74±5.10^{a}	123.93±9.19^{a}	243.44±11.81^{a}	129.75±11.33^{a}	1.36^{a}
DBT-MET	48.23±3.89^{d}	27.52±1.27^{c}	90.29±5.11^{bc}	42.24±3.14^{f}	0.88^{c}
DBT-CNE 250	71.18±7.34^{b}	37.54±8.02^{b}	95.02±8.43^{b}	62.39±2.42^{c}	0.88^{c}
DBT-CNE 500	54.57±4.07^{cd}	27.30±1.57^{c}	80.43±5.62^{de}	46.65±3.89^{ef}	0.85^{d}
DBT-CNE 1000	28.02±2.68^{fg}	14.14±2.07^{ef}	38.35±2.49^{i}	26.28±1.39^{hi}	0.94^{b}
DBT-CNPH24; 250	74.34±7.83^{b}	28.11±3.63^{c}	83.08±3.97^{cd}	81.16±3.18^{b}	1.09^{ab}
DBT-CNPH24;500	47.81±2.88^{d}	20.71±2.71^{d}	66.19±5.71^{f}	42.87±2.74^{f}	0.90^{b}
DBT-CNPH24; 1000	24.20±4.69^{fg}	11.46±1.74^{fg}	47.67±4.70^{gh}	20.08±7.18^{j}	0.83^{d}
DBT-CNPH48;250	70.54±6.98^{b}	19.46±1.06^{def}	86.76±4.56^{bcd}	53.19±2.65^{d}	0.75^{e}
DBT-CNPH48; 500	57.14±3.59^{c}	15.31±4.56^{def}	63.81±10.83^{f}	50.91±2.44^{de}	0.89^{c}
DBT-CNPH48; 1000	36.66±3.48^{e}	7.76±2.91^{g}	42.51±1.74^{h}	29.51±2.29^{gh}	0.80^{d}
DBT-CNPH72; 250	74.41±5.96^{b}	20.76±1.90^{d}	69.43±5.31^{f}	44.34±2.60^{f}	0.60^{g}
DBT-CNPH72; 500	46.88±2.53^{d}	15.45±2.14^{def}	52.17±4.43^{g}	32.63±1.25^{g}	0.70^{f}
DBT-CNPH72; 1000	37.92±7.61^{e}	8.06±2.16^{g}	33.46±3.66^{i}	22.53±1.69^{ij}	0.59^{g}
DBT-CNPI;250	60.09±4.66^{c}	17.84±1.56^{de}	72.58±7.97^{ef}	42.45±3.58^{f}	0.71^{f}
DBT-CNPI; 500	48.03±5.62^{d}	10.84±1.99^{fg}	48.83±3.51^{gh}	33.18±1.98^{g}	0.69^{f}
DBT-CNPI; 1000	30.88±4.18^{e}	8.35±1.22^{g}	34.45±1.64^{i}	22.71±1.49^{ij}	0.74^{e}
*NR					<1.00

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at P<0.05.

KEY:

NDBT: Negative control;

DBT: HFD/STZ (35mg/kg BWT)-induction;

DBT-METFORMIN: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg body weight of METFORMIN;

DBT-CNE : HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Extract;

DBT-CNPH24 : HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 24h;

DBT-CNPH48: HFD/STZ (35mg/kg BWT) induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented for 48;

DBT-CNPH72: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Hydrolysate fermented 72;

DBT-CNPI: HFD/STZ (35mg/kg BWT)-induction and 250, 500 & 1000 mg/kg body weight of Conphor nut Protein Isolate.

AST: Aspartate Aminotransferase, ALT: Alkaline Aminotransferase, ALP: Alkaline Phospahate; GGT: Gamma-glutamyl transferase

closely related and more effective compared to the metformin treatment. This finding is a likely indication that the test treatments may be beneficial in diabetic nephrology.

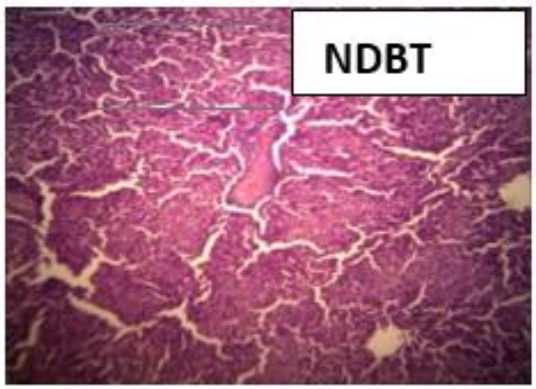
3.4 Liver Function

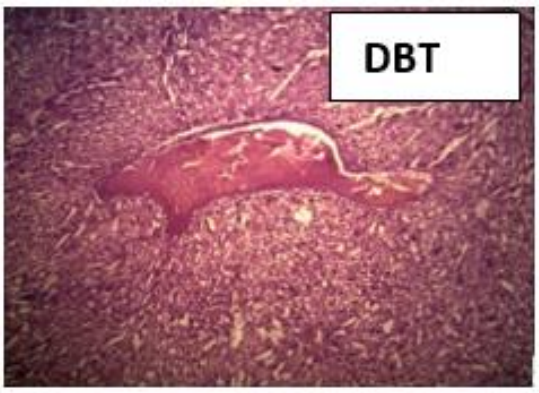
The influence of treatment samples on the functionality of the liver through enzymes activities of alkaline phosphate (ALP), aspartate amino transferease (AST), alanine amino transferease (ALT) and Gamma glutamyl transferase (GGT) of Wistar rats are presented in Table 3. The activities of ALP, AST, ALT and GGT were high in diabetic rat (DBT) and low in non-diabetic rats (NDBT). The level of plasma ALT, AST, and ALP and GGT of diabetic control rats were significantly ($p < 0.05$) increased than normal control rats. According to [52], High concentration of AST/ALT in the blood is an indication of liver damage. However, treatment of diabetic rats with 250, 500 and 1000 mg dose of CNE, CNPH24, CNPH48, CNPH72 and CNPI, significantly reduced the level of this parameter with respect to diabetic control rats. Thus, this study indicates that CNE, CNPH24, CNPH48, CNPH72 and CNPI reversed the effect of diabetes on level of plasma ALT, AST, ALP and GGT but the effect was dose dependent. Plasma and tissue aminotransferases (ALT and AST) were monitored in both treated and untreated diabetic rats. According to [53], Tissue damage is usually associated with the release of enzymes specific to the affected tissue or organ which could result in the increase in the activity of such enzymes in the blood. ALT is a cytoplasmic enzyme found in high amounts in the liver and an increase in ALT in the blood indicates liver damage, while AST is less specific than ALT as an indicator of liver damage [54]. The significant reduction in the level of ALT, AST,ALP and GGT in diabetic rats treated with CNE, CNPH24, CNPH48, CNPH72 and CNPI shows that conophor nut protein isolate, hydrolysates and extract are not hepatotoxic. The significant increase in aminotransferases in the liver (ALT) and the corresponding increase in the plasma (AST) observed in diabetic rats is an indication of increased de novo synthesis of the enzymes [55] bringing about the need for alternative source of energy through gluconeogenesis which could lead to increased catabolism of amino acids in the liver. ALT and AST reactions are extremely important for synthesis and degradation of amino acids. [56] previously reported increased activity of aminotransferases in the liver of diabetic rats. The reversal of the effect of diabetes on the activity of the aminotransferases (ALT most especially) in the plasma and liver of diabetic rats at a dose of 1000 mg CNE, CNPH24, CNPH48, CNPH72 and CNPI of is an indication of their hepatoprotective effect in diabetic rats compared to the treatment with metformin.

3.5 Histological Plates and Summary

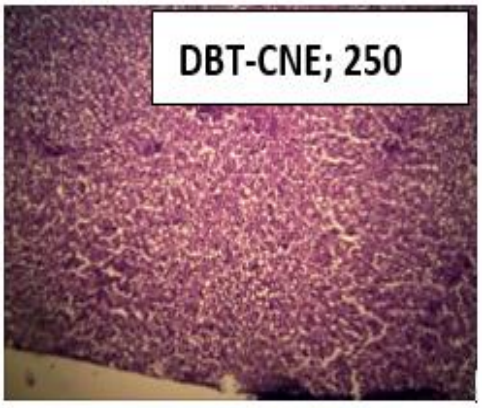
Histopathological studies are often used to analyse the hepatic and renal structural abnormalities. In the present study, histopathological analysis of the liver and kidney of diabetic treated rats using haematoxylin-eosin staining is revealed in Plate 1 and 2 respectively.

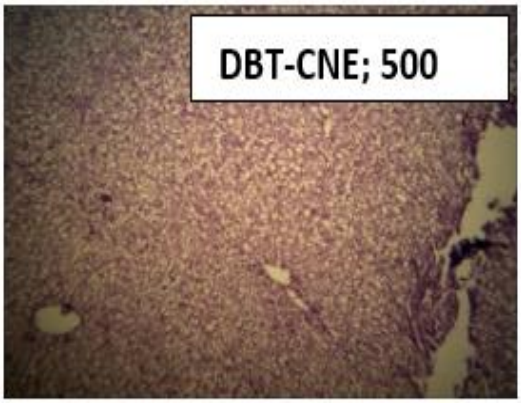
3.6 Liver Histology

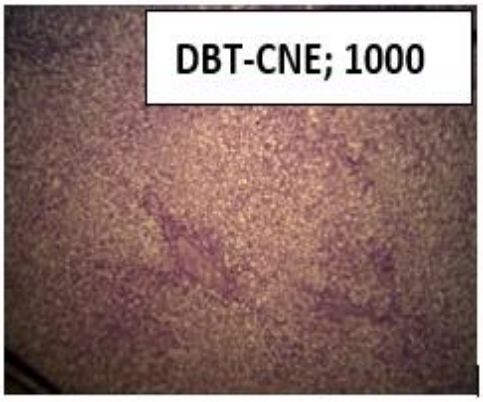


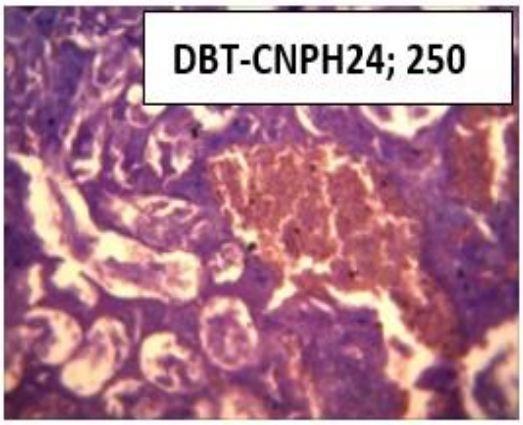


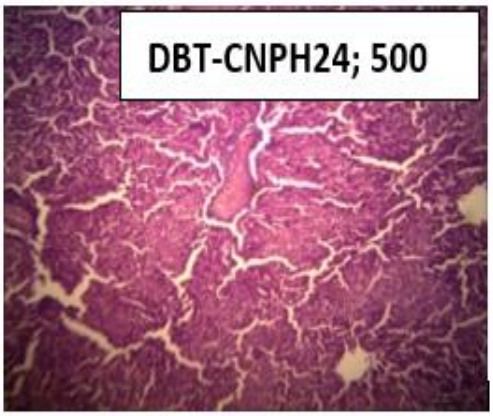

DBT-METFORMIN

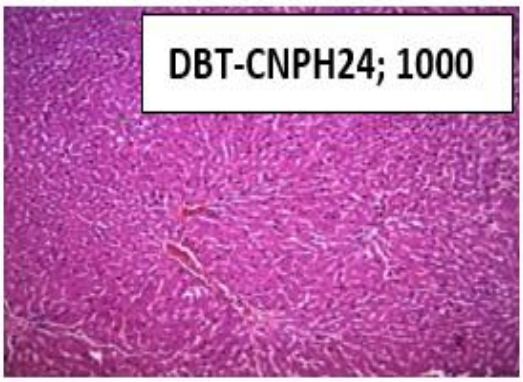

DBT-CNE; 250

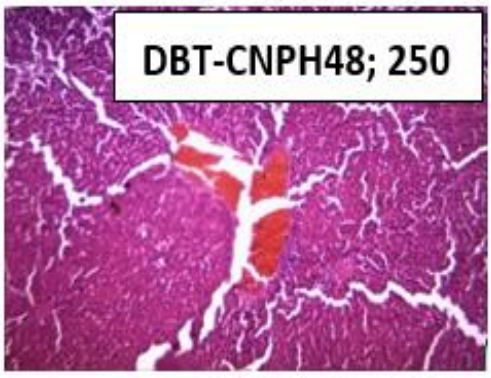

DBT-CNE; 500

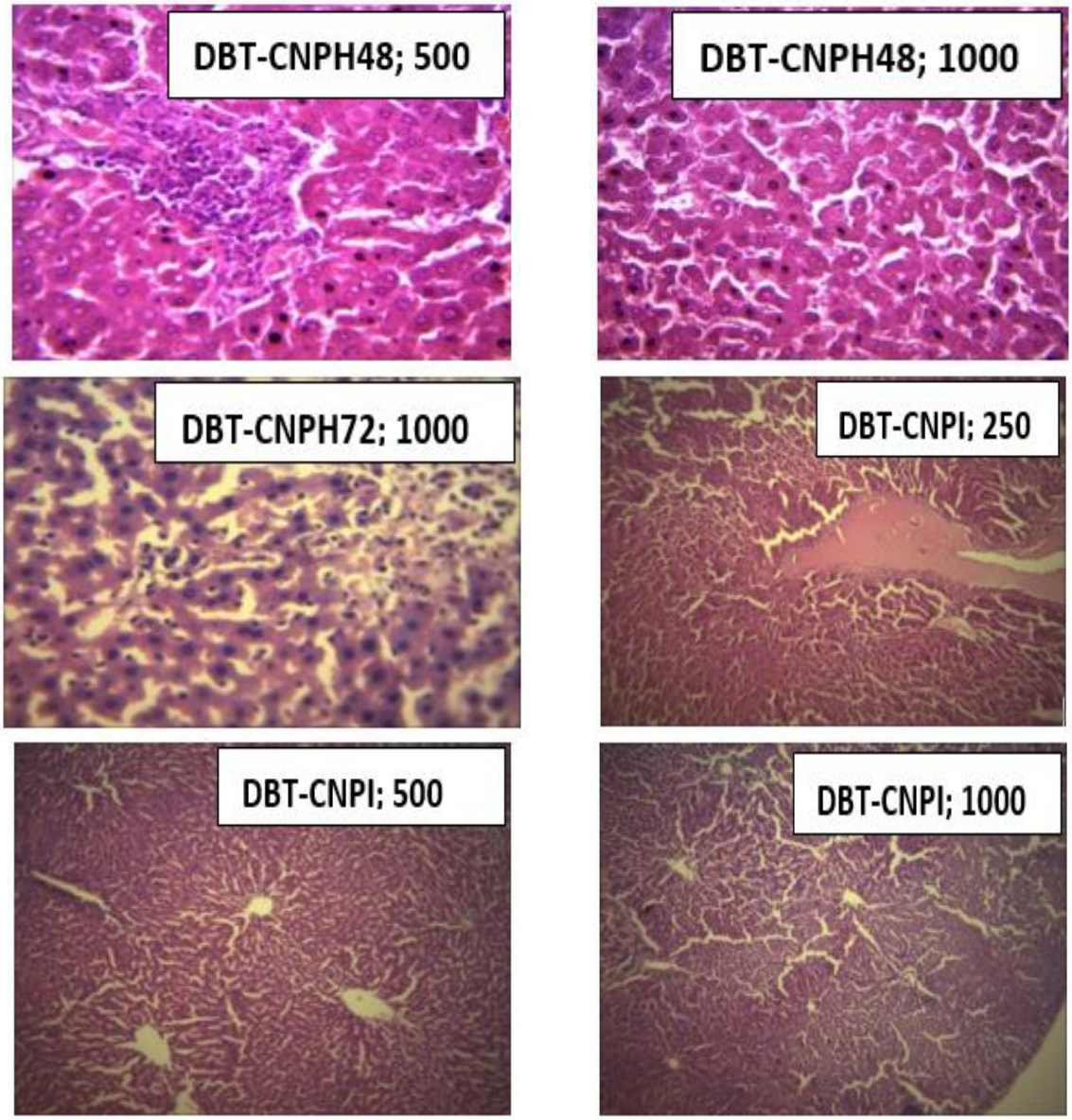

DBT-CNE; 1000


DBT-CNPH24; 250


DBT-CNPH24; 500

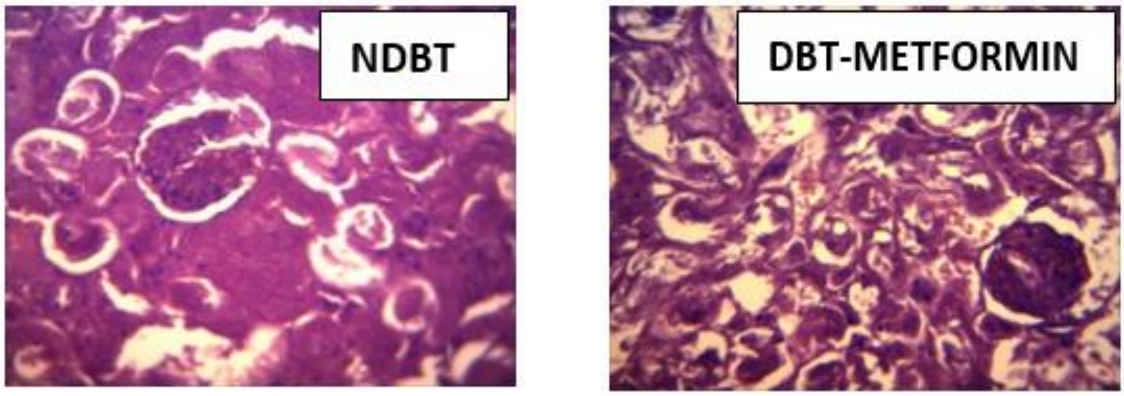

DBT-CNPH24; 1000

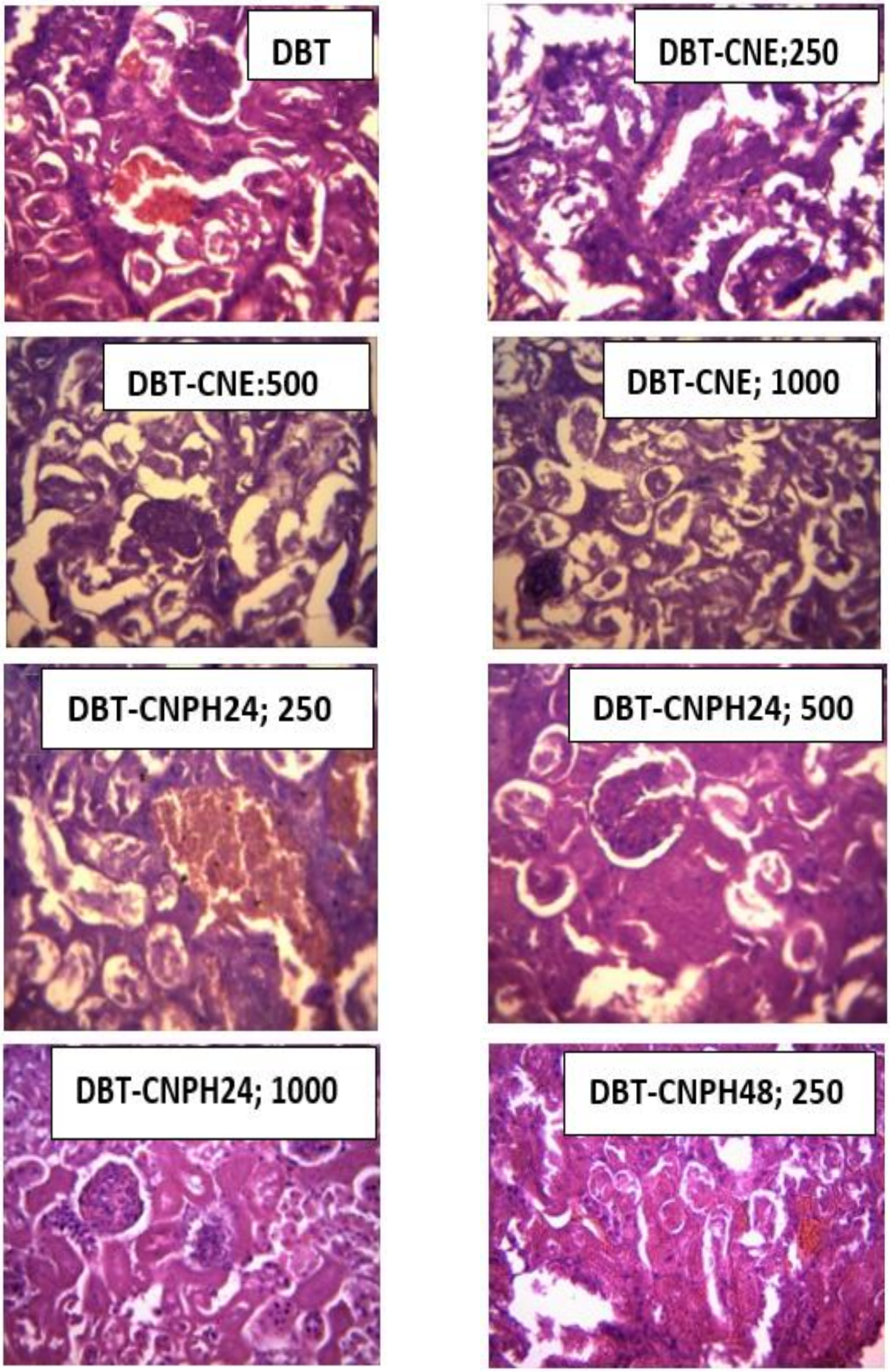

DBT-CNPH48; 250

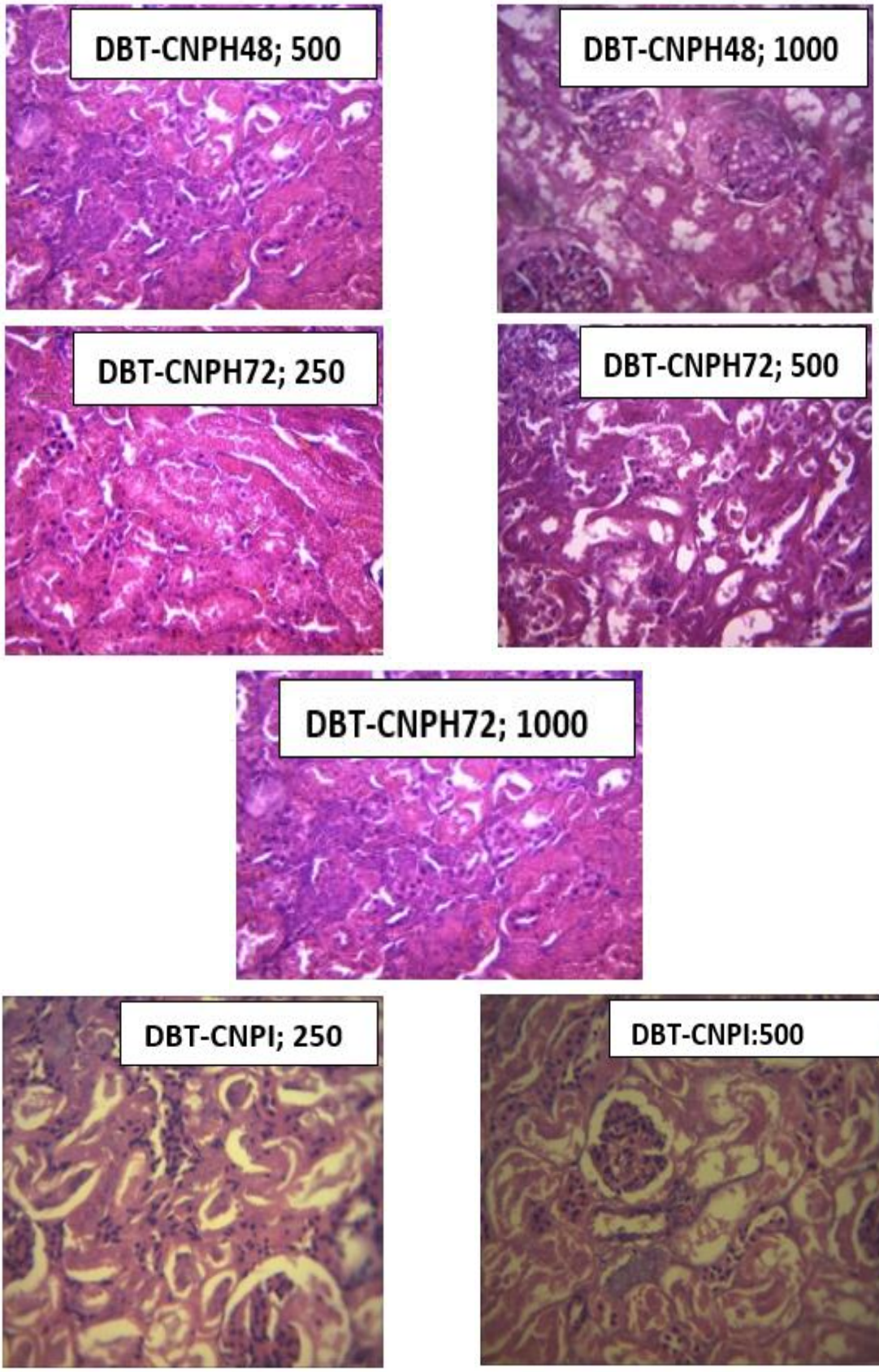


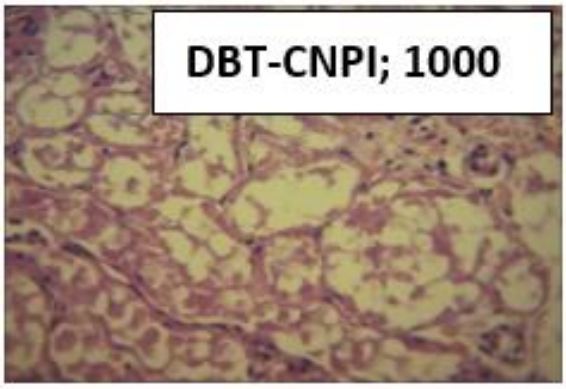
Plates 1. Liver Histology of animals induced with Streptozocin

3.7 Kidney Histology




DBT
DBT-CNE;250
DBT-CNE:500
DBT-CNE; 1000
DBT-CNPH24; 250
DBT-CNPH24; 500
DBT-CNPH24; 1000
DBT-CNPH48; 250


DBT-CNPH48; 500
DBT-CNPH48; 1000
DBT-CNPH72; 250
DBT-CNPH72; 500
DBT-CNPH72; 1000
DBT-CNPI; 250
DBT-CNPI:500



Plates 2. Kidney Histology of animals induced with Streptozocin

Table 4. Index terms and their summary

Groups	Summary
Liver	
NDBT	There is a moderate diffuse vacuolar degeneration of hepatocytes
DBT	There is a severe portal congestion. There is also a diffuse vacuolar degeneration of the hepatocytes
DBT-METFORMIN	There is a moderate diffuse vacuolar degeneration of hepatocytes
DBT-CNE 250	There is a severe diffuse vacuolar degeneration and necrosis of hepatocytes
DBT-CNE 500	There is a severe diffuse vacuolar degeneration and necrosis of hepatocytes
DBT-CNE 1000	There is a moderate portal congestion with a severe diffuse vacuolar degeneration and necrosis of hepatocytes
DBT-CNPH24 250	There is a severe portal congestion with a severe diffuse vacuolar degeneration of hepatocytes
DBT-CNPH24 500	There is a moderate diffuse vacuolar degeneration of hepatocytes
DBT-CNPH24 1000	There is a mild to moderate portal and sinusoidal congestion
DBT-CNPH48 250	No visible lesions seen
DBT-CNPH48 500	There are multiple foci of hepatic necrosis with cellular aggregation by mononuclear cells
DBT-CNPH48 1000	There is a moderate portal congestion
DBT-CNPH72 250	The sinusoids appear expanded/ congested
DBT-CNPH72 500	There are multiple foci of cellular infiltration <very mild> by mononuclear cells
DBT-CNPH72 1000	There are multiple foci of hepatic necrosis , with cellular aggregation around the same foci
DBT-CNPI 250	No visible lesions seen.
DBT-CNPI 500	No visible lesions seen.
Kidney	
NDBT	No visible lesions seen
DBT	There is a mild congestion of the renal interstitial vessels
DBT-METFORMIN	No visible lesions seen
DBT-CNE 250	No visible lesions seen
DBT-CNE 500	No visible lesions seen
DBT-CNE 1000	No visible lesions seen
DBT-CNPH24 250	There is a moderate to severe congestion of the renal interstitial vessels
DBT-CNPH24 500	No visible lesions seen
DBT-CNPH24 1000	No visible lesions seen
DBT-CNPH48 250	No visible lesions seen
DBT-CNPH24 500	Some renal tubules have protein casts in the lumen

Groups	Summary
DBT-CNPH24 1000	Some renal tubules have protein casts in the lumen
DBT-CNPH48 250	No visible lesions seen
DBT-CNPH48 500	There is a mild to moderate interstitial congestion
DBT-CNPI 250	Few renal tubules appear degenerated and slightly eroded.
DBT-CNPI 500	No visible lesions seen
DBT-CNPI 1000	No visible lesions seen

4. CONCLUSION

The study shows that Conophor nut proteins and ethanolic extract possesses high and potent antioxidant activity which may be responsible for their hypoglycemic property and as a result could be used as a nutraceutic.

5. RECOMMENDATION

In view of the findings from this study, the following recommendations are therefore proposed that;

1) Further investigations may be conducted on the optimum fermentation hours required for hydrolysis in the production of protein hydrolysates.
2) Studies should be carried out on the encapsulation of conophor nut proteins and extract for further use as a nutraceutic.
3) Further investigations may be carried out on possible changes in the micobiome due to the ingestion of the proteins and extract.
4) Furthermore, clinical study should be conducted to ascertain potency of Conophor nut proteins and extract as a possible replacement for synthetic drugs in the management of diabetes in human subject.

ETHICAL APPROVAL

The animal experiment adhered to the laws and regulations governing animal usage and was sanctioned by the Ethical Committee of the School of Agriculture and Agricultural Technology, Federal University of Technology Akure, Nigeria (approval number FUTA/SAAT/2018/021).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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