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Original Research Article

Inclusive diet of *Balanites aegyptiaca* Del defatted protein meal and protein concentrate on organ weight and diabetic conditioned Liver of Wister Albino rat

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Abstract: The study was on the In-vivo anti-oxidant influence of the Aduwa protein-inclusive diet on organ weight and anti-oxidant capability. Five albino rats were assigned to one of 4 treatment groups. Group 1 served as control; groups 2, 3, 4 and 5 were orally treated with 35 mg STZ/kg/ BW for 14 days and diet made of Aduwa meal and protein concentrate was introduced to groups 4 and 5. Results showed that DAM significantly attenuated TBARS, regenerated liver cells and kidneys, increased catalase activity, and enhanced feed intake levels. The endogenous anti-oxidant such as GST, GPx, SOD, and the level of reduced GSH in rat liver and kidney weight gain were enhanced when APC was supplemented in the group diet. APC diet influenced oxidative stress in rats' livers through free radical scavenging capability. DAM and APC diet samples could significantly potentiate liver cellular integrity and regeneration, as well as excellent ingredients in food processing and as pharmaceutical incipient.

Keywords: STZ-diabetes, Deffated Aduwa meal, concentrate, Body weights, Weight of kidney, Weight of Liver, anti-oxidant, Rats.

Significance: The study demonstrates Aduwa protein's potential to enhance liver function and antioxidant capacity in diabetic conditions, promoting organ regeneration.

INTRODUCTION

STZ is a diabetogenic agent of molecular weight 265 and empirical formula C_{14} $H_{27}N_5$ O_{12} [1,2]. When used in experimental animals, it induces toxic effects on islet beta cells, thereby triggering insulin-dependent diabetes mellitus and its complications [3,4]. The effects of STZ on organs have been studied with various biological effects, including the production of cellular injury, carcinogenesis, and mutagenesis [5], which are triggered by free radical build-up in the cell. STZ is given intravenously or intraperitoneally at a dose between 35- 45 mg/kg. The body weight of the animal produces hyperglycaemia [6]. Still, at a dose of 65 mg/kg body weight, STZ inducement could produce hyperglycaemia and mucosal ulcerations

[7]. It has been reported that organs such as the Liver's visceral lining and kidney could suffer lesions when exposed to STZ inducement [2]. These wounds and alterations of these organs in terms of functionalities could result from free radical, which degenerates into radical oxygen species (ROS). *Aduwa* (*Balanites aeqyptiaca* Del) derived products such as it meal, concentrate, and hydrolysates as anti-diabetics, anti-hypertensive, and anti-cancer agents have been opined [8].

In traditional forklore medicine, *Aduwa* seeds have been attributed to curing many diseases. Also, the seed has been reported as anti-helminthic, purgative, and of high food values [9,10]. The seed has a wide range of ethno-medicinal values and has been used with excellent nutritional qualities. The use of *Aduwa* protein as an *in-vivo* anti-diabetic agent in the serum of albino rats has been demonstrated [8] but not on the liver function or property. The effect of this *Aduwa*-derived protein meal and concentrate in conditioned diabetes rats on the Liver *in-vivo* anti-oxidant capacity and organ weight are significantly imperative to ascertain the effect of this bioactive in the liver lesion, hypertrophy, cell regeneration, and mutations.

MATERIAL AND METHODS

Material

One and a half kilograms (1.5 kg) of mature (*B. aegyptiaca* Del*) fruits* bought from the Gashua market in Yobe State of Nigeria were transported to the Federal University Gashua biochemistry laboratory. Half kilograms (0.5 kg) of cracked seed kernels *of Balanites aegptiaca Del* were dried using a sola cabinet dryer to ascertain 10% moisture value before it was given to toasting treatment under dry heat at 70 ^{0} C for 25 minutes and then allowed to cool.

Preparation of defatted *Aduwa* protein meal (DAM)

The defatted balanitis aeqyptiaca Del seed meal was prepared from toasted seed made from flour samples. The method of [11] Sathe (1994) defeated the *Aduwa* meal. The flour sample was defatted with cold (4 ◦C) acetone using a meal-to-solvent ratio of 1:5 w/v. The mixture was stirred over a magnetic stirred for 4 h. The slurry was then filtered through a Whatman No. 1 filter paper. The residue was re-extracted twice in a similar fashion. The defatted flour was dried in a fume hood at room temperature, and the dried meal was ground in a blender to obtain a homogeneous defatted *Aduwa* protein meal, which was stored in an air-tight plastic bottle for analysis.

Preparation of *Aduwa* protein concentrates (APC)

Aduwa protein concentrate (APC) was prepared using a modified method [12]. A known weight (200 g) of the defatted meal was dispersed in distilled water (2000 mL) to give a final flour-to-water ratio of 1:10. The dispersion was gently stirred on a magnetic stirrer for 10 min to form a suspension, after which the pH of the resultant slurry was adjusted with 0.1 M HCl to pH 4. The precipitation process will be allowed to proceed with gentle stirring for 2Hr keeping the pH constant. Soluble carbohydrates (oligosaccharides) and minerals will be removed by centrifugation at 3500g for 30 min using a centrifuge. The residue (concentrate) will be washed with distilled water to remove the residual minerals and soluble carbohydrates. The pH is later adjusted with 0.1 M NaOH to 7.0 for neutralization and then centrifuged at 3500xg for 10 min. The resultant precipitate (concentrate) was then collected and dried in an oven at 45 ◦C for 8 h and kept in an air-tight container for further analysis.

Method

Diet formulation

The diet formulation followed the protocol of [13] as reported in [14] (Table 1). The inclusion of (5%) *Aduwa* protein hydrolysate by pancreatin and combined pepsin and pancreatin protein hydrolysate, respectively, were established based on previous toxicological and sensory evaluation and palatability of the formulated diets, which is in line with the use of safe consumption of *Aduwa* seed extracts [14].

Animal Source and Handling Ethics

This study was performed in the Department of Biochemistry functional food and nutraceutical unit of the federal University of Technology Akure in Nigeria. STZ was obtained in powder form from Sigma Chemical Company, St.Louis, USA. Immediately before use, STZ was dissolved in 10 mM sodium citrate buffer, pH 4.5,). After obtaining approval from the ethical guidelines by the National Institute of Health (2011)

and Ethical Committee (reference number FUTA/SOS/1412) of the Federal University of Technology, Akure, on animal handling, A total of 30 adult male albino rats (body weight 100-250 gm) of white albino rate from the mice institute of the federal university of technology Akure were used in the study. All rats were housed in metabolic cages on a 12-h light/dark cycle at a temperature of 26°C [14].

Induction of diabetes using streptozotocin and animal grouping.

In the first feeding experiment, 30 male wicker rates (150-170/g) were housed in the Animal Facility at the Federal University of Technology Akure, Functional food and nutraceutical unit of the Biochemistry department under a 12 H day and night cycle at 30°C. The rats were acclimatized by feeding *ad libitum* with a regular high-fat diet (HFD) and tap water for two weeks. Then, the patient was allowed to fast overnight before intraperitoneal administration of 35 mg/kg of body weight of STZ freshly dissolved in 0.1 M of citrate buffer (pH 4.5) to model T1DM [15]. After a 72 H blood glucose test, rats with 250 mg/dl of blood glucose were considered diabetic and were used for this study. They were, after that, divided into 5 groups (similar average body weight) of 5 rats, each that received similar feed but with the addition of Dam and Apc products to determine the ability of each diet to attenuate diabetic effects in the liver cells. The diets were prepared as follows to contain skimmed milk as the main source of protein: 16% (w/w) (32% protein) skimmed milk (control diet), 5%DAM diet, and 5% APC diet (see Table 1 below). The rats were then fed their respective diets and tap water *ad libitum* for two weeks, during which feed consumption and body and organ weight were measured. At the end of the two days of feeding, the liver organs were carefully isolated and rinsed with cold saline. The livers were homogenized with three volumes of 0.1 M of phosphate buffer (pH 7.4) [16,17] Ojeleye *et al.* and Adefegha *et al*.) and subsequently centrifuged for clear supernatant that was used for the determination of *in-vivo* anti-oxidant assay. The homogenates were used for Gpx, GST, SOD, and lipid peroxidation (TBARS) analysis.

Group I. Rats receiving citrate buffer (pH 4.5) were fed basal diets and designated as standard control (NC) rats.

Group II. Rats were diabetic rats fed with basal diets and were designated as STZ-induced.

Group III. Rats were diabetic rats fed basal diets and administered with ACA orally (STZ + ACA).

Group IV. Rats were diabetic and were fed 5% of Adwa deffated meal (Dam), including a diet and STZ (APHpa) (STZ + 5% DAM).

Group V. Rats were diabetic, fed a 5% Aduwa protein concentrate (APC) inclusive diet, and were administered STZ (STZ + 5% APC).

Table 1: Table on Experimental Rate Formulation

Ingredients	Group I (Control) (g)	Group II	Group III	Group IV	Group V
Skimmed milk	40.63	40.63	40.63	35.63	35.63
Cornstarch	45.37	45.37	45.37	45.37	45.37
Dam 5%				5%	
Apc 5%					5%
Groundnut oil	10.00	10.00	10.00	10.0	10.00
Premix	4.00	4.00	4.00	4.00	4.00

Key: Dam= Deffated *Aduwa* meal APC = *Aduwa* protein concentrate,

Note: Skimmed milk = 32% protein; vitamin premix composed of the following: 3,200 IU vitamin A, 600 IU vitamin D3, 2.8 mg vitamin E, 0.6 mg vitamin K3, 0.8 mg vitamin B1, 1 mg vitamin B2, 6 mg niacin, 2.2 mg pantothenic acid, 0.8 mg vitamin B6, 0.004 mg vitamin B12, 0.2 mg folic acid, 0.1 mg biotin H2, 70 mg choline chloride, 0.08 mg cobalt, 1.2 mg copper, 0.4 mg iodine, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg anti-oxidant.

Source: [14]

Determination of Catalase (CAT) activity

CAT activity was evaluated using the method of [18] with modification. Briefly, 50 µL of tissue homogenate (Liver) was added to a mixture

containing 500 µL of 59 mM H_2O_2 and 950 µL of 50 mM phosphate buffer (pH 7.0). The reaction was conducted at 25°C, and absorbance was monitored at 570 nm for 3 min at 150 s intervals. One unit of enzyme activity was defined as the amount of enzyme catalyzing decomposition of 1 mmol H_2O_2 per minute at 25°C and pH 7.0.

Determination of superoxide dismutase (SOD) activity

SOD activity was determined according to the method of [19]. Approximately 40 µL aliquots of (liver) homogenate supernatants were pipetted into clean-sterilized test tubes containing 160 µL of distilled water to make a 1:4 (5-fold) dilution, and 2.5 mL of 0.05 M carbonate buffer (pH 10.2) was added. The reaction was initiated by adding 300 µL freshly prepared 0.3 mM adrenaline, mixed by inversion. The absorbance was measured at 480 nm for 150 s (at 30 s intervals) against the reagent blank (prepared by replacing the homogenate with distilled water).

Determination of glutathione-*S*-transferase (GST) activity

GST activity was carried out as described by [20]. Aliquots (30 µL) of tissue homogenate (Liver) were added to reaction mixtures containing 150 µL of 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 0.1 M phosphate buffer (pH 6.5), and incubated at 28°C. The absorbance was monitored for 3 min (every 15 s) at 340 nm. One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 unit of *S*-2,4-dinitrophenyl glutathione from CDNB and glutathione (GSH) per min at 28°C pH 6.5.

Determination of glutathione peroxidase (GPx) activity

Evaluation of GPx activity in tissue homogenate was carried out following the method of [21]. Exactly 500 µL of tissue homogenate (Liver) was added to 250 mL of 0.2 M phosphate buffer (pH 8.0), 50 µL sodium azide (10 mM), 100 mL GSH (4.0 mM), 50 µL H_2O_2 (2.5 mM), and 500 mL of distilled water. Reaction mixtures were incubated for 3 min at room temperature; 50 µL of 10% trichloroacetic acid was then added and mixed, and the mixture was centrifuged for 10 min at 3,000 rpm. The supernatant (50 µL) was then added 450 µL 0.3 M disodium hydrogen phosphate buffer (pH 7.4) and 500 mL of freshly prepared 0.6 mM dithiobisnitro-benzoate in 0.2 M sodium phosphate buffer, pH 8.0. The absorbance was read at 412 nm against blank (prepared by replacing homogenates with distilled water).

Determination of lipid peroxidation

Oxidative degradation of lipids in the Liver was determined by measuring the concentrations of thiobarbituric acid reactive substances (TBARS) in liver homogenates by following the method of [22]. Liver homogenate (100 µL) was mixed with 200 µL of 8.1% sodium dodecyl sulfate, 500 µL of 0.8% thiobarbituric acid, and 500 µL of acetic acid solution (2.5 M HCl, pH 3.4), and heated at 100°C for 1 h. The absorbance was read at 532 nm using a spectrophotometer.

Statistical Analysis

Except where indicated, in vivo data were collected in triplicate while the *in vivo* data are based on 5 rats/group. GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA, USA) was used for statistical analysis of experimental results. Data were expressed as mean standard.

RESULTS AND DISCUSSION

The feed intake level of *Aduwa* defatted meals and concentrate feed Wister albino rats g/rat/days are shown in Table 3. The STZ initial feeds were significantly higher at $p>0.05$ than the treatment and sample feed categories for initial intake levels. There was a significant difference between treatments for day 7 feed intake level; the control samples had a significantly higher feed intake level than the rest. The control and Dam samples had no significant difference and were higher than the Apc Apc-supplemented diet group. On day 14, the control was not significantly different from a supplemented diet, but it was lower than STZ and STZ +Aca. The drug-treated sample had significantly higher feed intake levels than the other sample, with APC having the lowest values. The lowest value in feed intake, when APC was supplemented could be due to the nature of the concentrate, unlike the dam feed sample, which has intact sugar moiety. During precipitation, the biomaterial, such as fat and sugar molecules, were detached using reacting solvents like acid and base.

Table 1: Feed intake level of *Aduwa* meal and concentrate feed wister abino rats' g/rat//week

Samples	Initial Quantity of Feed	Day 7	Day 14
Control	420a	420.00a ± 0.00	390.00c ± 0.00
STz	182c	416.95b ± 0.63	414.65b ± 0.49
STz + Acarbose	161d	410.70c ± 0.00	420.00a ± 0.00
DAM	193b	368.60d ± 59.96	362.00c ± 94.75
APC	161d	316.55e ± 14.77	309.70d ± 24.32

Mean are readings from duplicate determinations; Means followed by the same alphabetic on the column are not significantly different at p>0.05 Key:, Contol sample, STz= streptomycin, STz+Acarbose= Streptomzcin and Acabose drug, Dam =deffated *Aduwa* protein meal, Apc *Aduwa* protein concentrate.

Body weight gain (BWG) of *Aduwa meal* and concentrate feed Wister albino rat

The weekly average weight gain of *Aduwa* defeated meal and concentrate-fed Wister albino rats showed that there were significant (P < 0.05) differences that existed between the weekly average weight gain of the rats in all groups (Table 2). However, there were comparatively no significant (p < 0.05) changes in final body weight gain between the control group and the Dam group. The body weight gain in the STZ+ACA group is higher, and the least weight gain is seen in the STZ group. The BWG in (day 7) with STZ+Acarbose (group 3) was significantly P < 0.05 higher than all the groups. A dissimilar trend was observed in day 14 body weight gain results. However, the APC (group) was significantly higher than the STZ +ACA group on body weight gain. This observation could result from the APC protein feed diet, which may have muscle metabolism effect because certain proteins such as glutamate and leucine aid in muscle metabolism.

Table 2: Body weight gain (BWG) of *Aduwa meal* and concentrate feed Wister albino rats

Sample	BWG Day 7 (g)	BWG Day 14 (g)
Control	8.41b ± 26.79	6.82c ± 0.70
STz	1.92d ± 0.38	1.87d ± 1.23
STz + Acarbose	11.80a ± 0.00	11.49b ± 5.71
DAM	2.89c ± 9.96	7.76c ± 0.74
APC	2.89c ± 20.95	21.38a ± 0.43

Means are readings from duplicate determinations; Means followed by the same alphabet on the column are not significantly different at p>0.05. Key: Control sample, STz= streptomycin, STZ+Acarbose= Streptomzcin and Acabose druge, Dam =deffated *Aduwa* meal, Apc *Aduwa* protein concentrate.

Organ weight (g) of *Aduwa* meal and concentrate feed wister abino rats

The organ weight (g) of *Aduwa* feed Wister albino rats from defatted meal, concentrate, and hydrolysate are shown in Table 6. The kidney weigh of the rat treated with drugs was significantly p>0.05 higher than the *Aduwa* feed samples. The least kidney organ weigh was seen in the control samples with a low value of (0.70g). There was no significant difference at p>0.05 between the induced Stz group and the treated APC group for liver organ weight. The APC group was significantly different and higher than the drug-treated stz +Aca group.

The kidney is an organ that aid in liquid regulation and purifies blood. The kidney is made up of glumeruli which can regenerate and degenerate. ROS could lead to myriad of malfunction especially triggers of free radical influence by diabetic inducement or complications. The normal rat had good kidney weight but the disease and drug treated group revealed high weight gain which could be as a result of glomerulus hypertrophy. The *Aduwa* feed defatted and concentrate samples had similar value and

close to the normal controlled rat group. This reflected that *Aduwa* protein could stabilize diseased condition kidney with excellent glomerulus regeneration from Dam and Apc samples, however lower that the control group. Progressive feeding beyond day 14 could create excellent normalization of the disease kidney from STZ inducement. The observation in weigh difference seen in the Liver of the rat showed that Dam feed group could compete with drug treated sample. The variation in weight observed in APc group though there was no significance difference between Stz group and APC group and might be due to presence of fat, Fatty liver could increase the weight of liver organs in which STZ inducement could generate as diabetes progress. But for the test sample, APC group high weight gain could be due to emulsified surface protein or charges on the surface of the sample during isoelectric precipitations process.

Table 3: Organ weight (g) of *Aduwa* meal and concentrate feed wister abino rats

Sample	Kidney Weight (g)	Liver Weight (g)
Control	0.70 ± 0.65b	4.96 ± 4.30d
STz	1.32 ± 0.51a	8.08 ± 0.69a
STz + Acarbose	1.40 ± 0.33a	6.92 ± 2.87b
DAM	1.28 ± 0.35a	6.72 ± 2.10b
APC	1.28 ± 0.29a	8.32 ± 2.64a

Means are readings from duplicate determinations; Means followed by the same alphabet on the rows are not significantly different at p>0.05. Key:,Contol sample, STz= streptomzin , STz+Acarbose= Streptomzcin and Acabose druge,Dam =deffated *Aduwa* meal, Apc *Aduwa* protein concentrate.

TBARS *In-vivo* Anti-oxidant Properties of defatted *Aduwa* meal, Protein Concentrate in liver tissue.

TBARS levels in liver tissue examined are presented in Figures 1. TBARS level in diabetic STZ groups was significantly increased (p>0.05), as compared with non-diabetic control group and treated STZ +ACA, STZ+Apc and STZ+Dam groups. In addition, TBARS levels in the liver tissue were not significantly (p>0.05) different in Control and STZ +Apc but significantly lower than in the STZ group. The drug treated sample and defatted meal and concentrate groups had better TBARS levels like the positive control group. A high TBARS level elevates activities of anti-oxidant enzymes (SOD, glutathione peroxidase, and catalase) and triggers lipid peroxidation, thus increased the T-bars in STZ-induced diabetic rats [22,23]. Therefore, on examining the oxidative effects of *Aduwa* meal, protein concentrate and non-diabetic rats (control) groups, it was observed that high TBARS levels in diabetic rats were significantly reduced by *Aduwa* treated samples and did compete favourably with drug control Acarbose group. Thiobarbituric acid reactive substances (TBARS) level observed in STZ suggest the occurrence of oxidative stress [24]. The levels of TBARS were significantly higher in diabetic induced groups (STZ) without defatted *Aduwa* meal and protein concentrate compared to diabetic rats with *Aduwa* meal and protein concentrate treatment groups. Since TBARS levels are closely associated with lipid peroxidation these results suggest that lipid peroxidation is reduced under the use of *Aduwa* protein concentrate and defatted *Aduwa* meal better than the synthetic Acarbose drug. Lipid peroxidation trigger free radical and ROS which can damage bio molecules. It has been reported that TBAR levels decrease GSH levels in cells, exposing cells to oxidative damage. Reduced GSH levels enhance the toxic effect, because GSH Play certain roles in detoxifying ROS. Oxidative stress resulting from increased production of free radicals and ROS, and/or a decrease in anti-oxidant defence barriers, leads to damage of biological macromolecules and disruption of normal metabolism and physiology [25]. This work is agreement with TBARS Manawadi and Kaliwal work on liver oxidative stress enzyme activities of GST, SOD and CAT which were significantly decreased in mice treated with methomyl at different time intervals [26].

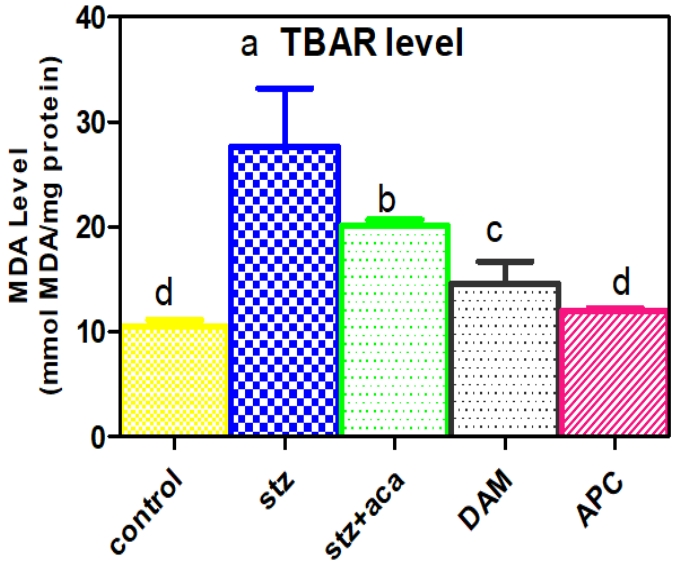

Figure 1: TBARS *In-vivo* Anti-oxidant Properties of *Aduwa* protein meal, defatted flour protein concentrates in Liver

Means are readings from duplicate determinations;Means followed by the same alphabet on the bar are not significantly different at p>0.05. Key:, Control sample, STZ= streptomzin , STZ+ACA= Streptomzcin and Acabose druge,DAM =deffated *Aduwa* meal, APC *Aduwa* protein concentrate.

LIVER Catalase *In-vivo* anti-oxidant Properties of *Aduwa* protein meal, Defatted *Aduwa* meal and protein concentrate

Fig 2 presents catalase in vivo, an anti-oxidant property of Aduwa protein meal, defatted Aduwa protein meal, protein concentrate, and hydrolysates in LIVER under T2D diabetic conditions. Catalase (CAT) is an in-*vivo* anti-oxidant enzyme systems which breaks H_2O_2 to water and oxygen [27]. CAT enzyme is distributed in all animal tissues and a potent anti-oxidant enzyme that defend cell reactive oxygen species ROS. According to [28], CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Therefore, inhibiting this enzyme may enhance free radical-induced cellular damage in liver cells and stop cellular degeneration and necrosis .CAT activity in drug treated group STZ +ACA is significantly (p>0.05) higher than other groups under study. The CAT activities in APC, non-diabetic (Control) and STZ groups were significantly (p<0.05) lower than Dam treated groups The least CAT values were found in APC and STZ groups. This study revealed the increase in CAT level in STZ+Aca and Dam (P > 0.05) after two weeks treatment with Acabose drug and defatted *Aduwa* meal The *in vivo* results indicated that defatted *Aduwa* meal could be an important source of natural CAT anti-oxidant in liver cells which may have resulted in the liver cell regeneration and barring liver cell necrosis.

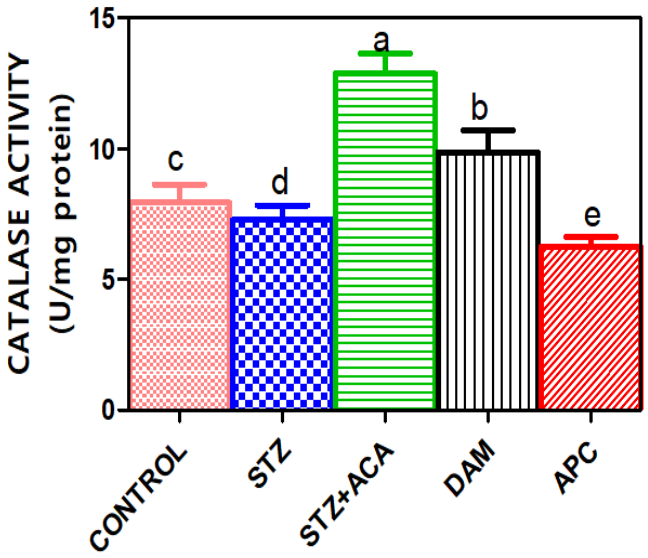

Figure 2: Catalase *In-vivo* Anti-oxidant Properties of *Aduwa* protein meal, defatted flour protein concentrate, isolate and hydrolysates in Liver

Means are readings from duplicate determinations;Means followed by the same alphabet on the bar are not significantly different at p>0.05. Key:,Contol sample, STZ= streptomzin , STZ+ACA= Streptomzcin and Acabose druge,DAPM =deffated *Aduwa* protein meal, APC *Aduwa* protein concentrate.

Liver SOD *In-vivo* Anti-oxidant Properties of *Aduwa* defatted meal and protein concentrate.

Liver SOD anti-oxidant properties of defatted *Aduwa* meal and protein under diabetic condition is presented in Fig 3. The SOD activity of STZ +ACA and APC are significantly higher (p>0.05) than diabetic and non-diabetic groups under this study. The treatment sample DAM, and APC groups are not significantly differently(p>0.05) but higher than the STZ diabetic group. This was also observed with control and Dam groups (p>0.05). ROS react with all biological substance; however the increased activities of SOD in liver tissue from the inclusion of 5% of Dam and APC test samples as observed in this study suggest that *Aduwa* protein materials compared to STZ diabetic group has an *in vivo* anti-oxidant activity and is capable of ameliorating the effect of ROS in biologic system The high activity of SOD in *Aduwa* APC samples could catalysing ROS to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive proximities in Liver tissue . SOD has anti-inflammatory and anti-fibrotic enzyme oxygen free radical scavenging effects and was observed to be supported by Apc sample under diabetic induced situation in this study.

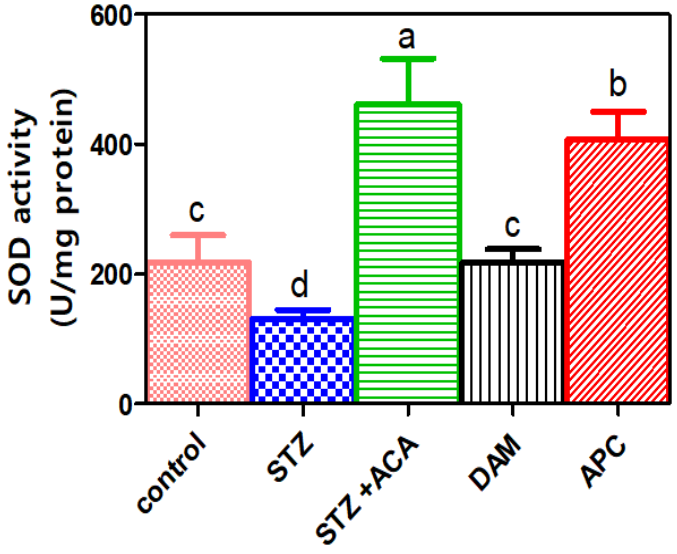

Figure 3: SOD *In-vivo* Anti-oxidant Properties of *Aduwa* protein meal, defatted flour protein concentrate, isolate and hydrolysates under diabetic condition in Liver

Mean are readings from duplicate determinations;Means followed by the same alphabetic on the bar are not significantly different at p>0.05, Key:,Contol sample, STZ= streptomzin , STZ+ACA= Streptomzcin and Acabose druge,DAPM =deffated *Aduwa* protein meal, APC *Aduwa* protein concentrate.

Liver Glutathione peroxidase (GPX) Anti-oxidant Properties of *Aduwa* Defatted meal and protein concentrate

Liver glutathione peroxidase (GPX) anti-oxidant properties of *Aduwa* Defatted *Aduwa* meal, and protein concentrate under diabetic condition is presented in Figure 4. GPx (glutathione peroxidase) (cellular) decomposes hydrogen peroxide and free fatty acid hydro peroxides both organic and inorganic hydro peroxides into their corresponding alcohol. GPx values are not significantly (p>0.05) different between the Control and STZ +Aca, but significantly higher than STZ and DAM groups, The *Aduwa* protein concentrate could reduce peroxidase possibly produced by diabetic condition in liver tissues Thiis work agreed with [29] work when *Lonicera caerulea* plant extract was feed to HFD mice with induce liver conditions. The use of protein concentrate can reduce endogenous peroxidase from free fatty acid. The reduction in GPx activity in the diabetic control STZ rats or group and DAM groups suggest in-activation of this enzyme by the presence of reactive oxygen species (ROS). DAM +Stz group could not scavenge ROS generated by diabetic condition in liver tissue.

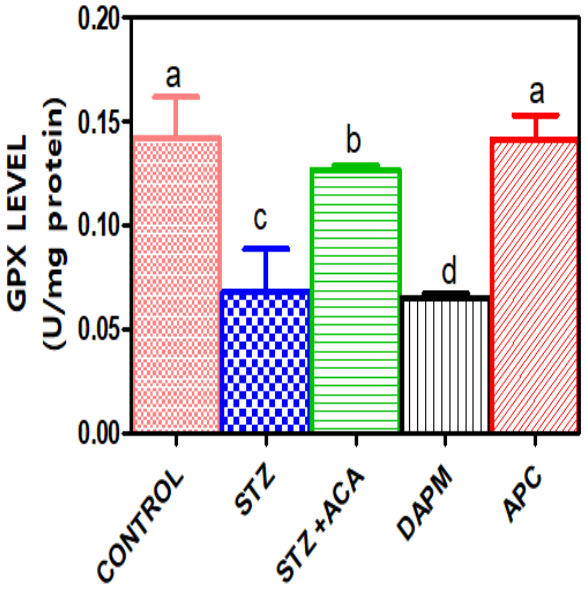

Figure 4: GPX In-vivo Anti-oxidant Properties of *Aduwa* protein meal, defatted *Aduwa* protein meal, protein concentrate, and hydrolysates in Liver

Means are readings from duplicate determinations;Means followed by the same alphabet on the bar are not significantly different at p>0.05. Key: Control sample, STZ= streptomzin , STZ+ACA= Streptomzcin and Acabose druge,DAPM =deffated *Aduwa* protein meal, APC *Aduwa* protein concentrate.

Liver Glutathione transferase (GST) Anti-oxidant Properties of *Aduwa* defatted mealand protein concentrate Under T2D condition

Glutathione transferase (GST) is one among the three important endogenous anti-oxidant enzymes tha have an important role as a protector of cells from the reactive oxygen species. Glutathione transferase (GST) invivo anti-oxidant properties of *Aduwa* defatted meal and *Aduwa* protein concentrate is presented in Figure 5.The STZ+Aca group is significantly (p>0.05) high in GST activities than the rest groups under this study .The control and STZ groups are not significant (p>0.05) different but lower in GST activities compared to Drug and diet groups .The least value in GST enzyme activity was observed in STZ and control groups , but are significantly not different. The defatted meal and protein concentrate can protect liver tissues and sequesters metal and ions like the synthetic drug acarbose under a diabetic situation in liver cell This might be due to *Aduwa* sample producing maximum GST enzymes to scavenge free radicals from ROS. Decrease in the GSH level observed in STZ group could be due to no GST enzymes reacting to scavenge free radicals produced in the hyperglycaemic state covalently induced [30-32].

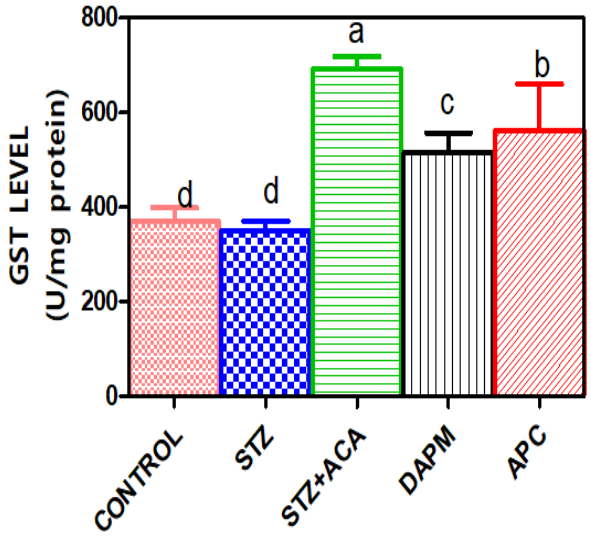

Figure 5: GST *In-vivo* Anti-oxidant Properties of *Aduwa* defatted meal and protein concentrate in Liver

Mean are readings from duplicate determinations;Means followed by the same alphabetic on the bar are not significantly different at p>0.05 Key:,Contol sample, STZ= streptomzin , STZ+ACA= Streptomzcin and Acabose druge,DAPM =deffated *Aduwa* meal, APC *Aduwa* protein concentrate.

CONCLUSION

Aduwa defatted meals and protein concentrate induced insights into anthropometric and biochemical changes in albino Livers under diabetic conditions. According to these results *Aduwa* meal and protein concentrate could aid kidney and liver regeneration, enhance Catalase enzyme and reduces TBARS propagation with increase feed intake level. APC sample supplementation was observed to enhance damaged Liver through anti-oxidant capacity through hypertrophy regeneration and end of cirrhosis with better body weight gain. Careful attention should therefore be given to *Aduwa* bioactive proteins to reduce or aid *in vivo* anti-oxidant capability of the liver organs, hence functional diet made of bioactive from *Aduwa* protein concentrate are significantly needed by occupationally exposed humans.

Recommendation

A histopathological study should be carried out on the liver samples of rats.

Author contributions

Ogori A.F designed the experiments and performed the experiments, collected and analyzed the data and wrote the draft manuscript. Abu Oneh and Eke O supervised the work and read and edited the manuscript.

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Ethics statement

The animal handling procedure adhered strictly to the prepared guideline by the National Institute of Health (2011) and Ethical Committee (reference number FUTA/SOS/1411) of the Federal University of Technology, Akure, on animal handling.

Conflict of interest

There are no conflicts of interest to declare among the researchers.

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