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Research Article

Enzymatic protein hydrolysates from aduwa (*Balanities Aeqyptiaca* L) seed meal supplemented diet on α-amylase, α-glucosidase and anti-oxidants activity of Streptozotocin-induced diabetic wister albino rat

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Abstract

The *Aduwa* seeds were toasted, milled, and defatted. The defatted meal is used to produce protein isolate. The *Aduwa* enzymatic hydrolysed peptides were produced from Aduwa protein isolate using pancreatin, and pancreatic-pepsin enzymes combination. The work investigated the anti-diabetic and anti-oxidant effects of *Aduwa* enzymatic hydrolysed peptides supplemented diet in streptozotocin (STZ)-induced diabetic rats. The rats were given high fat diet (HFD) for two weeks and made diabetic by intra peritoneal administration of STZ (35 mg/kg b.w.) and fed diets containing 5% (APHpa) and 5% (APHpa + pe) for 14 days. The effects of the diet on blood glucose, α-amylase, α-glycosidase, and anti-oxidant activities were monitored. Marked increases in the blood glucose and thiobarbituric acid reactive substances (TBARS); with corresponding decrease in feed intake weight gain, fed conversion ratio, α-amylase and intestinal α-glucosidase inhibitory activities and anti-oxidant capacity were monitored in diabetic rats. These trends were however reversed in diabetic rats fed diets supplemented with *Aduwa* protein hydrolysates of APHpa and APHpa+pe for 14 days. The *Aduwa* Protein hydrolysates were able to attenuate starch digestion, absorption and anti-oxidant capacity in serum hydrolysate supplemented diet. Thus, these anti-diabetic properties and anti -oxidant capacities from Aduwa supplemented diet may be attributed to the influence of its constituent phytochemicals and peptides inherent in hydrolysates supplemented diet.

Keywords: Aduwa, Protein hydrolysate, α – amylase activities, α - glucosidase activities, Anti-oxidants capacity, Diabetes

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Introduction

The global prevalence of type 2 diabetes mellitus (T2DM) and obesity concurrently continue to abate synthetic drug therapists and this disease continue to increase exponentially with substantial efforts to promote public health message targeted at dietary/ lifestyle interventions in the early stages of the treatment of these metabolic diseases, the preventive ways often remain unproductive (Kakkar et al.,1995). Dietary protein derived from enzymatic hydrolysates of desert date (Aduwa), alternative approaches may favour the modulation of glycaemic control, anti-oxidant build up and appetite to reduce the incidence of disability and the associated risk of developing T2DM. However, Aduwa tree parts such as the leaves, seeds and fruits or pulps have been reported edible and could modulate glycaemic level (Myers et al., 2010). This led to an emerging interest in the development of protein/ Peptide of Aduwa based functional food ingredients to advantageously modulate glycaemic control and excessive weight gain and reduce the risk of metabolic deregulation associated with T2DM and obesity (Meda et al., 2005).

Proteins/peptides have been shown to stimulate incretin hormones such as GLP-1 and GIP in the gut and inhibit DPP-4 mediated incretin hormone degradation, which enhances post-prandial insulin response and improves glycaemic control (Cudennec et al., 2012); (Girgih et al., 2011); (Oyeleye et al., 2021).

The bioactivity of protein/peptides components is heavily dependent on the nature and structure of the intact protein (Obidah et al., 2009); (Aluko et al., 2005); (Girgih et al., 2014). One of the primary motivations for the utilization of plant -nut protein is based on the increasing desire for a sustainably source and quality of plant proteins. The high-quality dietary protein derived from underutilized Aduwa seed meal source could provide much-needed relief for rural people and means of economic revamping, while improving the dietary quality of the global population. The Aduwa protein hydrolysates have not been studied as anti-diabetic and anti-oxidants potentials remedy using animal model. Thus, the aim of the present study was to determine the effect of both hydrolysates from pancreatin and combined pancreatin and pepsin to see if these hydrolysates exert bioactivity to benefit diabetes as well as bodyweight, feed conversion, blood glucose and antioxidant capacities (Aluko et al., 2012).

MATERIALS AND METHODS

Materials

All the reagents such as, Triton-X 100, sodium phosphate, reduced glutathione (GSH), 95% methanol,Tris-HCl buffer, EDTA, NaOH, HCl, 1,10-phenanthroline, hydrogen peroxide, $FeSO_4$, $FeCl_3$, FeCl2, were of analytical grade and purchased from Sigma (St. Louis, MO, USA). Mature aduwa (*B. aegyptiaca L) cracked seed* was purchased from a Gashua market in Yobe State, Nigeria. The seeds were produced in the 2020/20201 harvesting season, were purchased from accredited sellers from same market winnowed and cleaned prior to utilization.

Seed processing and meal making of peptides

Aduwa seeds were preconditioned as appropriate and processed into defatted meals according to the methods described by (Gbadamosi et al., 2012) through unit operations involving toasting, milling into whole flours, milled, oil extracted by mechanical expulsion, cake dried to 10% moisture content, pulverised and then defatting the flours using acetone and followed by air drying the residue cakes overnight in a fume hood. The dried defatted aduwa meal cake residues were finally ground into defatted aduwa protein meals. The defatted meals were made into protein isolate. The isolate samples were hydrolysed using 4%-0.5% weights of enzymes from calculated protein content of the isolate sample. The amount of each and respective enzymes added during hydrolysis on magnetic stirrer at 45°C were on the basis of 4% pancreatic enzymes and 0.5% for combined enzymes using the calculated crude protein content of isolate samples. After precipitating and centrifuging the supernatants were then dried using freeze dryer to obtain respective hydrolysates. The hydrolysate samples were then packed in an air tight container and constantly kept under refrigeration throughout analysis periods at the Functional Food and Nutraceutical unit, Federal university of Technology Akure, Biochemistry Department Figures 1 and 2.

METHODS

Preparation of aduwa protein hydrolysates using pancreatine enzyme

Aduwa protein hydrolysate by pancreatin (APHpa) was prepared using pancreatin enzyme optimum reaction conditions acting on the isolate. Pancreatin with pH 7.5 at 40°C using the method of (Aluko & Monu, 2006) as shown in Figure 1. A 1:20 w/v aduwa seed protein isolate's slurry was adjusted to pH 7.5 and incubated at 40°C followed by addition of pancreatin (4% w/w, on the basis of protein content of okra seed protein isolate) or pH 7.5 in and incubation at 45°C followed by the addition of pancreatin enzyme (4% w/w, on the basis of protein content of Aduwa seed protein isolate for pancreatin respectively. The digestion was carried out for 4 H and the pH is maintained by adding 1 M NaOH or HCl when necessary. The digestion was terminated by adjusting the pH to 4.0 and then placing the mixtures in boiling water for 30 min to inactivate the enzymes which ensures complete denaturation of enzyme protein and coagulation of undigested proteins. The mixture was allowed to cool to room temperature and later centrifuged to get the supernatant and freeze dried, See (**Figure 1).**

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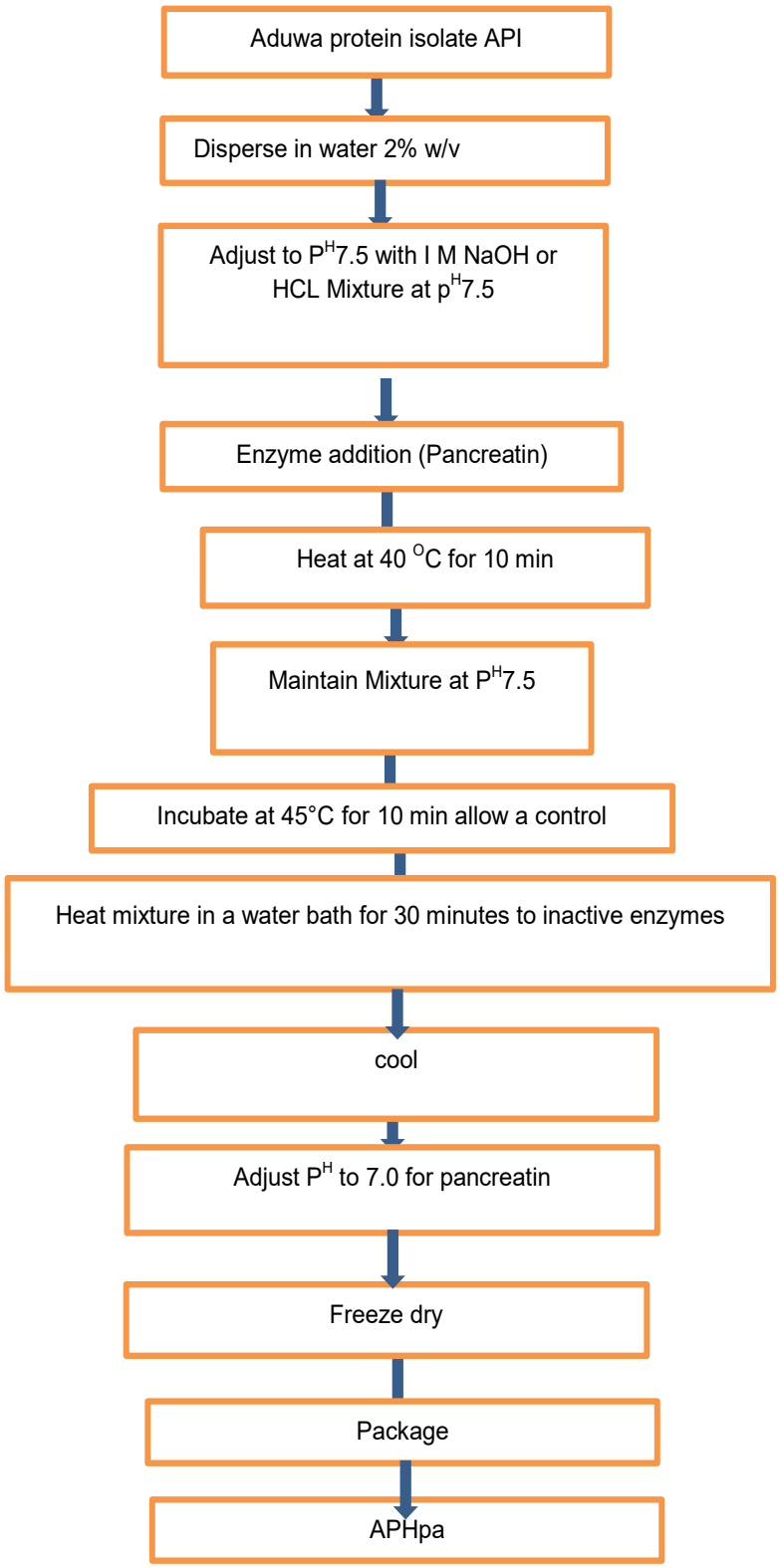

Figure 1: Production of Aduwa protein hydrolysate from pancreatin
Source: (Aluko & Monu, 2006).

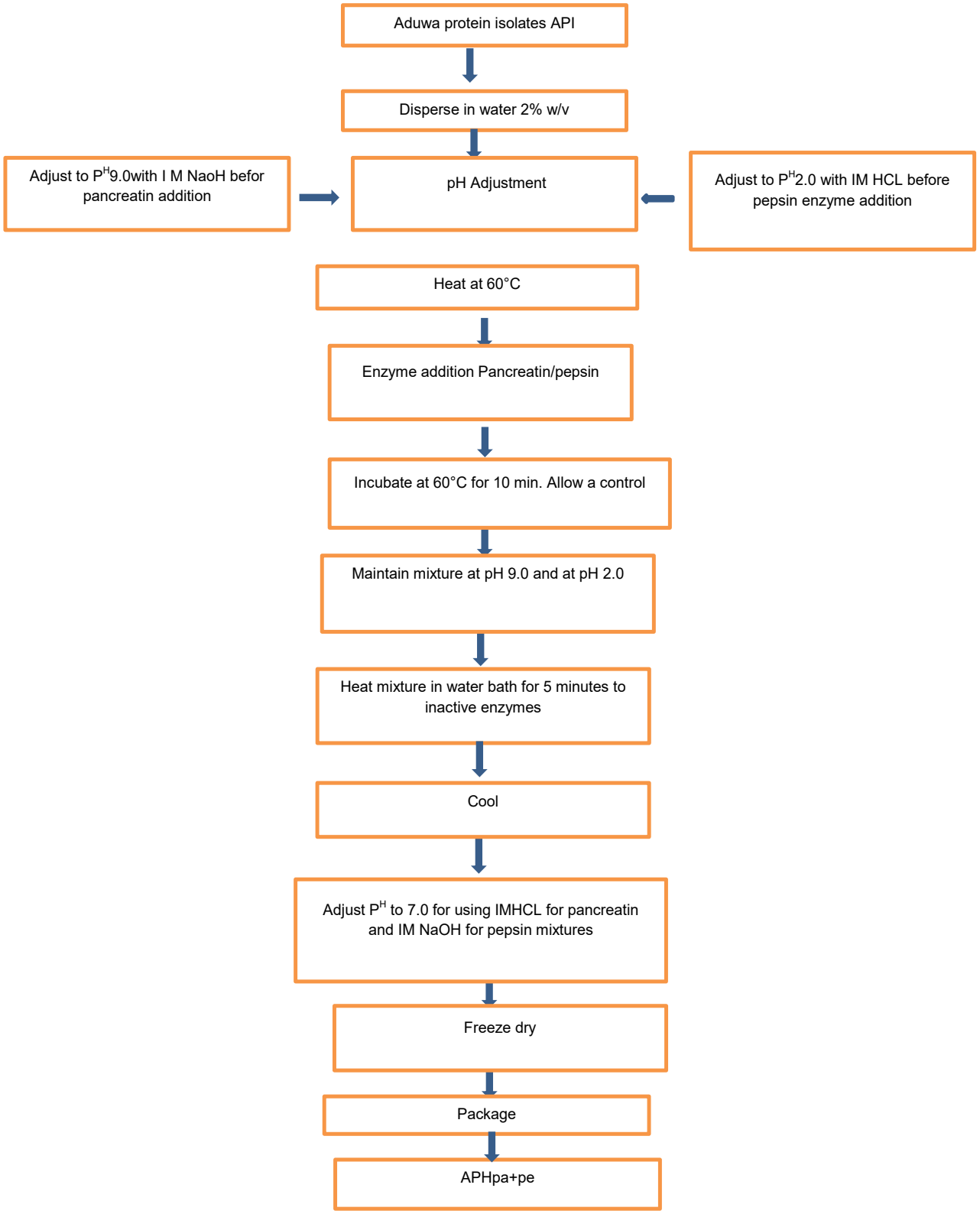

Figure 2: Production of Aduwa protein hydrolysate using combined enzymes.
Source: (Aluko and McIntosh, 2004) Modified by (Girgih et al., 2013)

Enzyme hydrolysis of API for making Aduwa Protein Hydrolysate using pancreatic and pepsin combined enzymes (APHpa + pe)

Enzymatic hydrolysis of API to produce APHpa +pe was carried out using the method of (Aluko & McIntosh, 2004) with slight modification by (Girgih et al., 2013). The API was dispersed in water (2%, w/v) and was adjusted to pH 9.0 using 1 M NaOH solution for pancreatin while pH 2.0 was used for pepsin digestion. The dispersion was heated to 60°C under continuous stirring on a hotplate equipped with an electronic thermometer. The enzymes (0.5% w/w) were added based on the protein content of the API and incubated at constant temperature of 60°C for 2.5 or 10 min. An un-hydrolysed control for each time was prepared by omitting the enzymes during thermal incubation of the AMPI. The reaction mixture was maintained at pH 9.0 using 1 M NaOH solution or pH 2.0 with 1 M HCl. At the end of the incubation period, the hydrolysates were transferred into a boiling water bath for 5 min to inactivate the enzymes. The hydrolysates were cooled to room temperature (22 ± 2°C) and adjusted to pH 7.0 with 1 M HCl solution (for pancreatin) or 1 M NaOH (for pepsin digest) and finally freeze-dried **(Figure 2).**

Diet formulation

The diet formulation followed the protocol of (Ademuliyi et al., 2015) as reported in (Oboh et al., 2018) The inclusion of (5%) Aduwa protein hydrolysates by pancreatin (APHpa) and combine pancreatin and pepsin protein hydrolysate (APH pa+pe) respectively were established based on previous toxicological and sensory evaluation and palatability of the formulated diets, which is in line with the safe consumption of aduwa seed extract by (Obidal et al., 2009).

Animal source and handling ethics

The male Wistar rats used were adults weighing between 261-285 g, procured from University of Ilorin, Animal breeding colony belonging to Biochemistry Department. The animals were kept in a room temperature, having control 12 H light/ dark cycle and unrestricted access to food and water for 14 days before the commencement of the experiments.

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.

Induction of diabetes using streptozotocin and animal grouping

In the first feeding experiment 30 male wisker rates (150-170 g) were housed in the Animal Facility at the Federal university of Technology Akure, Functional food and nutraceutical unit of biochemistry department under a 12 H day and night cycle at 30°C. The rats were acclimatized by feeding and libitum with a regular high fats diet (HFD) and tap water for two weeks and then allowed to fast overnight before intraperitoneal administration of 30 mg/kg body weight of STZ freshly dissolve in 0.1 M of citrate buffer (pH 4.5) to model T1DM (Hasanien & Shahidi, 2011). After 72 H blood glucose test was conducted, and rats with blood glucose 250 mg/dl were considered diabetic and were used for this study. They were thereafter, divided into five groups (similar average body weight) of 6 rats each that received similar feed but with addition of APHpa and APHpa+pe products to determine ability of each diet to attenuate α-glucosidase and α-amylase carbohydrate degrading enzymes.

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% APHpa diet and 5% APHpa + pe diet (see table 1 below). The rats were then fed their respective diets and tap water *ad libitum* for 14 days during which feed consumption and body weight were measured. At the end of the 14 days of feeding. The blood glucose was monitored on day 1, 4, 7, 10, and 14, respectively using automatic auto glucose analyser (Fine test Auto-coding). The experiment lasted for 14th days followed by fasting overnight and therefore, sacrificed via decapitation. The intestines and pancreases tissues were carefully isolated, and rinse with cold saline. The pancreas and intestine were homogenized with three volumes of 0.1 M of phosphate buffer (pH 7.4) (Oyeleye et al., 2021); (Adefegha et al., 2018) and subsequently centrifuged for clear supernatant that was used for the determination of biochemical assays, while the blood was collected into heparinized tubes. The blood was centrifuged at 1500xg for 10 min to obtain plasma or serum, which was then stored. Both homogenate and centrifuged blood were used for α-glucosidase, α-amylase, Gxp, GST, lipid peroxidation (TBARS) analysis (Hasanein & Shahidi, 2011).

Group I. Rats received citrate buffer (pH 4.5) and fed with basal diets and were designated as control rat.

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

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

Group IV. Rats were diabetic fed with 5% of Adwa protein hydrolysate pancreatin inclusive diet and STZ (APHpa) (STZ + 5% APHpa).

Group V. Rats were diabetic fed with 5% of Aduwa protein hydrolysate combined enzymes inclusive diet and administered and STZ (STZ + 5% APHpa+ pe) **(Table 1)**.

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
APHpa 5%				5%	
APH pa+pe 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: *Control (Group I), STZ= Streptozotocin (Group II), STZ+ACA= Streptozotocin and Acabose drug, (Group III), AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatin and pepsin (Group V)*

Note: *Skimmed milk = 32% protein; vitamin premix composed 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 antioxidant.*

Determination of α-amylase inhibitory activities

α-amylase activities were evaluated following the methods (Worthington, 1993) as described in the report of (Ademiluyi et al., 2015) with slight modifications. Fifty microliters of pancreas homogenates were prepared in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) and added to 50 µL of 1% starch solution. Reaction mixtures were incubated (25ºC/10 min), and 200 µL of dinitrosalicylic acid was added for colour development. Mixtures were then boiled in a water bath (5 min) to stop the reaction, cooled to room temperature, and then diluted with 2 mL of distilled water. The absorbance was measured at 540 nm in a ultraviolet-visible spectrophotometer.

Determination of α-glucosidase inhibitory activities

α-glucosidase activity was evaluated in the small intestine as described by (Apostolidis et al., 2007). Small intestine homogenates (15 µL) were prepared in 0.1 M phosphate buffer (pH 6.9) added to 15 µL of 3 mm GSH and 445 µL of 0.1 M phosphate buffer (pH 6.9), and incubated (37°C/10 min). Then, 40 µL of 5 mM *p*-nitro phenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to the mixture and the mixture was incubated at 37°C for 10 min. Sodium carbonate (2 mL) was then added and the absorbance was measured at 405 nm.

Statistical Analysis

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

RESULTS

The weekly average feed intake of the rats showed that there is no significant ($P < 0.05$) differences that existed for the weekly average feed intake of the rats in all group **(Table 2)**. There was decrease in feed consumption from the initial day compared to the control normal group. This decrease was significantly ($p < 0.05$) followed by APHpa +pe (groupV) and STZ (group III) but lower than APHpa (group IV) and STZ +ACA (group III).

The weekly average feed intake and feed conversion ratio (FCR) of *aduwa* protein hydrolysates fed wister albino rats showed that there was a significant ($P < 0.05$) differences that existed between the weekly average feed intake and feeds conversion ratio of the rats in all groups **(Table 3)**. However, there were no significant ($p < 0.05$) changes in final body weight gain between group III and IV when compared with control (group I). The body weight gain in STZ+ACA and AHPa groups is higher and the least body weight gain is seen in STZ (group II). The FCR in (Week 1) control (group I) is significantly $P < 0.05$ low, but higher than APHpa (group VI). Similar trend was observed in week 2 body weight gain and PCR results. However, the control (group I) is significantly higher than APH pa + pe (group V) on account of body weight gain but differs from STZ +ACA (group III) on account of feed conversion ratio.

The effect of aduwa protein hydrolysates supplemented diet on blood glucose levels in diabetic rats revealed significant ($P < 0.05$) increase in blood glucose of diabetic rats STZ (group II) when compared with normal Control rats (group I). Figure 3. However, Acarbose or drug treatment (group III), APHpa (group IV) and APHpa+pe (group V) significantly ($P < 0.05$) decreased the blood glucose levels in diabetic rats, bringing the blood glucose close to that observed in normal rats **(Figure 3)**.

In vivo α –amylase activities of *aduwa* protein Hydrolysates on Wister albino rats.

The pancreatic α-amylase activity in the diabetic rats revealed a significant ($P < 0.05$) increase in the enzyme activity in diabetic control rats (STZ group II) as compared to the normal control rats (group I) **(Figure 4).** However, treatment with protein hydrolysate from pancreatic APH pa

Table 2. Feed Intake Level of Aduwa Fed Wister Albino Rats g/rat/day/week.

Sample	Initial Quantity offeed /week	WEEK 1	WEEK2
Control (Group I)	420	420.00^{a} ± 0.00	390.00^{c} ± 0.00
STZ (Group II)	182	416.95b ± 0.63	414.65^{b} ± 0.49
STZ+ACA (Group III)	161	410.70c ± 0.00	420.0^{a} ±0.00
APHpa (Group IV)	183	309.60e ± 0.00	327.40e±0.00
APHpa+pe (Group V)	178	374.55d ± 0.63	292.85f±0.02
LSD		**0.878**	**0.869**

Mean are readings from triplicates determination; Means followed by the same alphabetic on the column are not significantly different at p<0.05

Key: *Control (Group I), STZ= Streptozotocin (Group II), STZ+ACA= Streptozotocin and Acabose drug, (Group III), AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatin and pepsin (Group V).*

Table 3. Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) of *Aduwa* Feed Wister Albino Rats.

Sample	BWG (Week 1)	FCR(wk1)	BWG(Week2)	FCR(wk2
CONTROL(Group I)	8.41^{b} ± 26.79	48.94^{d} ± 1.34	6.82^{b} ± 0.70	57.18^{d} ± 0.358
STZ(Group II)	1.92^{e} ± 26.79	217.16^{b} ± 0.01	1.87^{c} ± 1.23	221.74^{b} ± 0.33
STZ+ACA(Group III)	11.80^{a} ± 0.00	34.80^{f} ± 0.89	11.49^{a} ± 5.71	6.55^{g} ± 0.83
APH pa (Group IV)	11.66^{a} ± 14.17	26.55^{g} ± 0.45	21.38^{a} ± 2.25	546.44^{a} ± 2.43
APH pa+pe(Group V)	3.88^{c} ± 8.65	96.52^{c} ± 0.56	0.59^{d} ± 1.59	87.16^{c} ± 1.44
LSD	**30.57**	**26.90**	**151.8**	**51.4**

Mean are readings from triplicates determination; Means followed by the same alphabetic on the column are not significantly different at p<0.05

Key: *Control (Group I), STZ= Streptozotocin (Group II), STZ+ACA= Streptozotocin and Acabose drug, (Group III), AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatin and pepsin (Group V).*

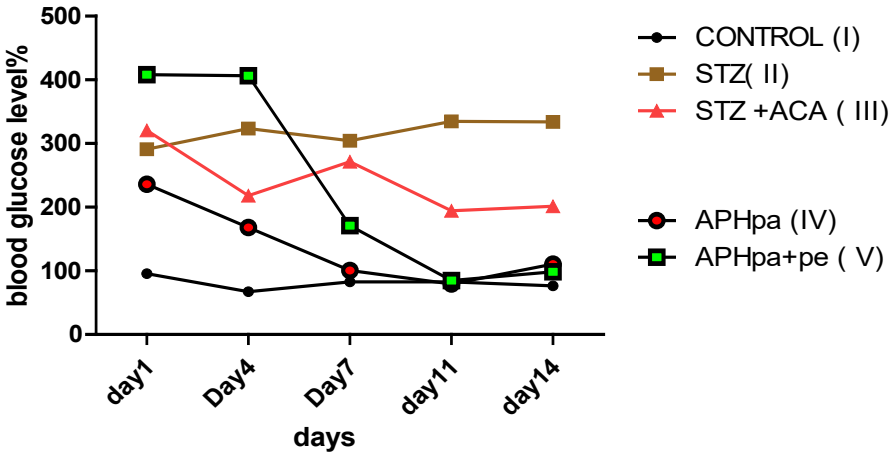

Figure 3: Blood glucose level of aduwa protein hydrolysates supplemented diet.

Key: *Control (Group I), STZ= Streptozotocin (Group II), STZ+ACA= Streptozotocin and Acabose drug (Group III), AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatic and pepsin (Group V).*

(group IV) and APH pa + pe (group V) resulted in a decrease in the α-amylase activity in the diabetic rats to near normal rat (group I) which are significantly ($p<0.05$) lower than STZ +ACA (group III) treatment.

In vivo α- glycosidase activities on *aduwa* protein and Hydrolysates on Wister albino rats.

In vivo α- glycosidase activities on *aduwa* protein hydrolysates on Wister albino rats are presented in **(Figure**

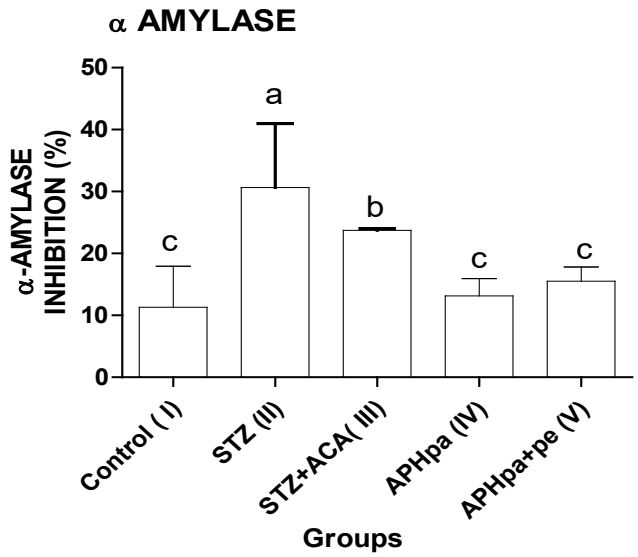

Figure 4: *In vivo* alpha -amylase activities of Aduwa protein hydrolysates supplemented diet on Wister albino rats.

Mean are readings from triplicates determinations; Means followed by the same alphabetic on the column are not significantly different at p<0.05

Key: *Control (Group I), STZ= Streptozotocin (Group II), STZ+ACA= Streptozotocin and Acabose drug (Group III) , AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatin and pepsin (Group V)*

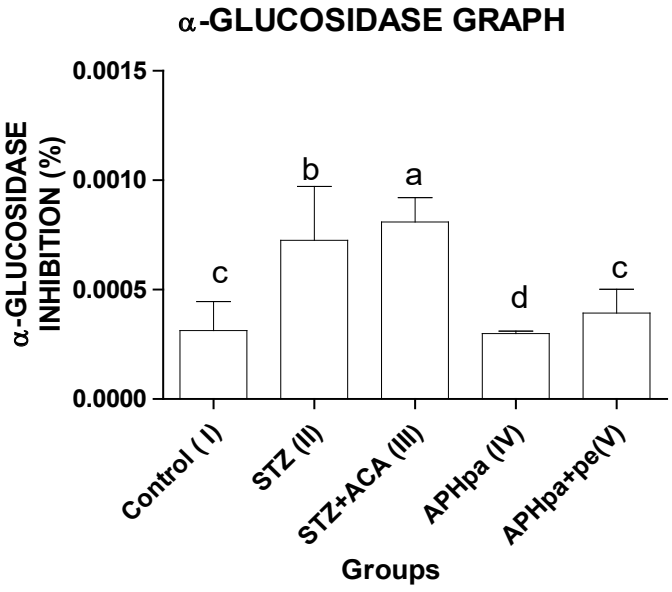

Figure 5: *In vivo* alpha- glucosidase activities of Aduwa protein hydrolysates supplemented diet on Wister albino rats.

Mean are readings from triplicate determinations; Means followed by the same alphabetic on the column are not significantly different at p<0.05

Key: *Control (Group I), STZ= Streptozotocin (Group II), STZ+ACA=Streptozotocin and Acabose drug, (Group III), AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatic and pepsin (Group V).*

5). Intestinal α-glycosidase activity increased significantly ($P < 0.05$) in the diabetic STZ rats (group II) and diabetes treated with drug, STZ + ACA (group III) when compared to the normal control rats (group I).But APHpa and APH pa+pe aduwa supplemented diet treatments caused a significant ($p < 0.05$) marked decrease in the activity of this enzyme in diabetic rats (group IV-V).

TBARS *In-vivo* Antioxidant Properties of Aduwa protein Hydrolysates in Serum

Thiobarbituric acid reactive substances (TBARS) are one of the oxidative stress markers that reveal the occurrence of oxidative stress (Kakkar et al., 1995). Diabetic patients have significant deficits in anti-oxidant capacity, and this deficiency enhances ROS generation (Ihara et al., 1999) TBARS level in serum examined are presented in (**Figures 6)**. TBARS level in control and diabetic STZ groups were significantly increased ($p < 0.05$), as compared with treated STZ +ACA (group III), APHpa+pe (group V) and APHpa (group IV). In addition, TBARS level in serum was significantly ($p < 0.05$) higher in APHpa+pe (group V) than in STZ + ACA (group III) and APHpa (group V). The protein hydrolysates by pancreatin APHpa (group IV) had better TBARS level when compared to the positive control (group I) and negative STZ (group II).

Serum glutathione peroxidase (GPX) Antioxidant Properties of Aduwa Protein hydrolysates

Serum glutathione peroxidase (GPx) antioxidant Properties of *Aduwa* Protein Hydrolysates under diabetic condition presented in **(Figure 7)**. GPx value is significantly ($p<0.05$) higher in the (control) non diabetic group and the diabetic group treated with combined hydrolysate APHpa + pe than the other groups under study. The diabetic treated group with drug STZ+ACA (group III) and APHpa (group IV) are not significantly different but higher than untreated group induced with STZ (group II).There is an observation that aduwa protein hydrolysates treated groups could reduce peroxidase possibly produced by the diabetic conditions in plasma cells.

Serum Glutathione transferase (GST) Antioxidant Properties of aduwa protein hydrolysates

Glutathione transferase (GST) Antioxidant Properties of *aduwa* protein hydrolysates are presented in **(Figure 8)**. The control group and hydrolysate group by combined enzymes APHpa+pe (group V) is higher in GST activities than STZ (group II) and STZ+ACA (group III) respectively with significant differences ($p<0.05$). Hydrolysates from combined enzyme as well as STZ + ACA (group III) can protect plasma and sequesters metal and ions in a diabetes situation. The GST from the non-diabetic control (group I) fed with normal basal diet showed a normal but high content of GST correspond significantly with the hydrolysate and drug- treated groups (Kalra et al., 2021).

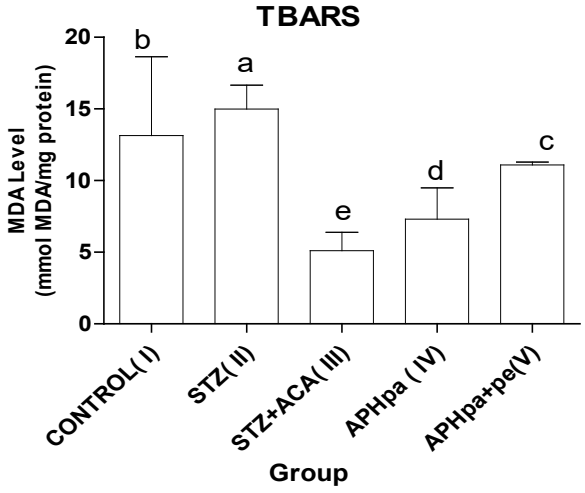

Figure 6: TBARS *In-vivo* Antioxidant Properties of Aduwa Protein.

Mean are readings from triplicate determinations; Means followed by the same alphabetic on the bar are not significantly different at p<0.05.

Key: *Contol (Group I), STZ= Streptozotocin (Group II), STZ+ACA= Streptozotocin and Acabose drug, (Group III), AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatin and pepsin (Group V)*

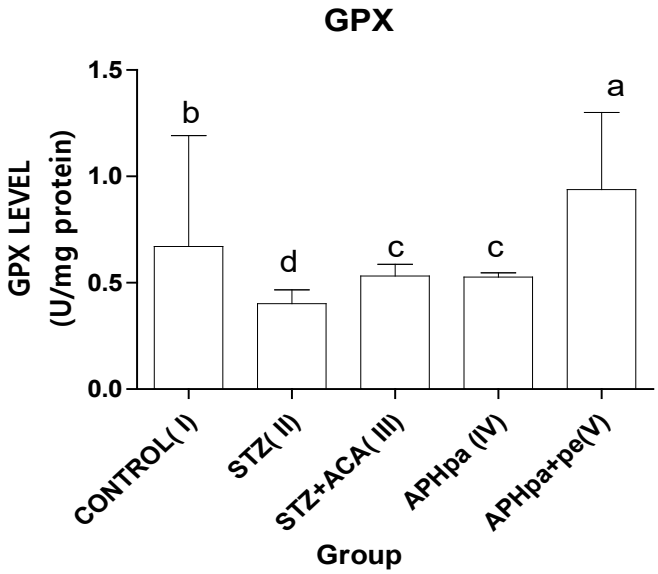

Figure 7: Glutathione peroxidase (GPX) Antioxidant Properties of aduwa protein hydrolysates in SERUM.

Mean are readings from triplicate determinations; Means followed by the same alphabetic on the bar are not significantly different at p<0.05.

Key: *Control (Group I), STZ= Streptozotocin (Group II), STZ+ACA= Streptozotocin and Acabose drug, (Group III), AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatic and pepsin (Group V).*

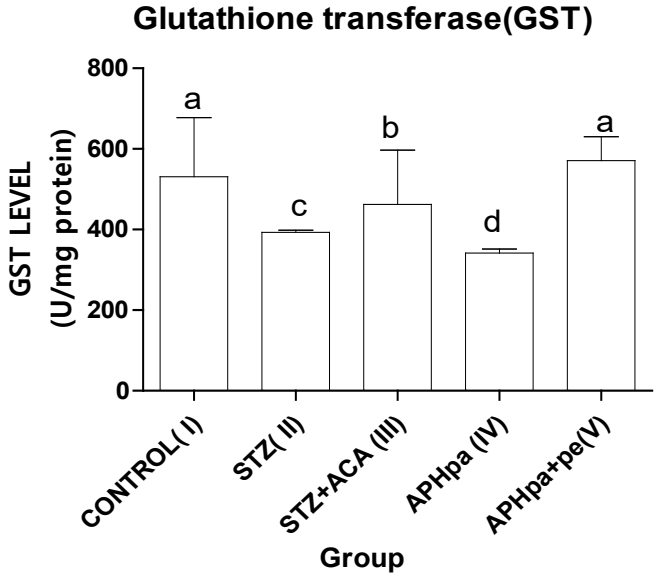

Figure 8: Glutathione transferase (GST) Antioxidant Properties of Aduwa protein hydrolysates in SERUM.

Mean are readings from triplicate determinations; Means followed by the same alphabetic on the bar are not significantly different at p<0.05.

Key: *Control (Group I), STZ= Streptozotocin (Group II), STZ+ACA= Streptozotocin and Acabose drug (Group III), AHPa= Aduwa protein Hydrolysates by pancreatic (Group IV), AHpa+pe= Aduwa protein hydrolysates by pancreatin and pepsin (Group V).*

Discussion

The results of this study showed that feed inclusion of *aduwa* hydrolysate from both APHpa and APHpa+pe once daily for 24 consecutive days resulted in a significant reduction in feed intake feed conversion ratio, weight gain and blood glucose level. This was accompanied by no significant change in body weight with synergistic effect on fed conversion process over the study duration, indicating that the ant diabetic effects were likely to be completely dependent on the alleviation of obesity. Similar, anti- diabetic effects of *aduwa* seed extracts such as leave extracted with water and leave extracted with organic solvent when were administered could both lower blood glucose and improved anti-oxidant capacity (Kakkar et al., 1995). Similarly with *aduwa* hydrolysates (Kaneto et al., 1996).

Leptin is important in weight loss (Myer et al., 2010) thus in this study there was no changes in body weight found in APHpa despite a significant reduction in energy intake (FCR) compared to the normal (group I). The reduced energy intake as revealed by low feed conversion ratio without a corresponding reduction in body weight suggests that the energy intake reduction in APHpa may be due to a leptin-independent mechanism, shown in feed intake level by the rats group. But this declined sparsely in STZ group and APHpa + pe (group V) compared to the control and APHpa groups. The drastic low feed intake observed in diabetes STZ and APHpa+pe group could be attributed to disease condition and bitter nature of protein hydrolysates. The APHpa feed intake may be high due to single enzymatic hydrolysis which may have not release much peptides leaving most biomaterial intact. The effects of food consumption on body weight gain has already been studied (Meda et al., 2005); (Obidah et al., 2009). In this present study there were treatment related changes observed in final body weight gain and FCR of the rats such as weight loss and low FCR, which may imply that consumption of the diet mixed with aduwa protein hydrolysates, had affected rat appetite.

One of the clinical tests used for determination of T2DM progression is blood glucose profile, projected into glucose tolerance test (GTT). Glucose intolerance is a characteristic of T2DM and often coupled with insulin insufficiency or resistance and impaired, APHpa (group IV) and fasting glucose (Ademiluyi et al., 2016).The drug treatment STZ + ACA (group III) APHpa+pe (group V) significantly decreased the blood glucose levels in the diabetic rats closed to the value observed in the normal rats. The observed decrease in the blood glucose levels in the diabetic rats treated with *aduwa* protein hydrolystaes suggests anti-hyperglycaemic properties of the *Aduwa* product. This fit could be through the enhancement of the peripheral sugar uptake and metabolizing mechanism or stimulation of insulin secretion from the surviving pancreatic cells by constituent *Aduwa* phytochemicals (Kwon et al., 2012); (Ruzaidi et al., 2008). The anti-hyperglycaemic effect of Aduwa samples could also be ascribed to the inhibitory effects of the total phenolic content present in the protein hydrolysates on α-amylase and α-glucosidase activities Figure 4, 5 in the gut of the rats, thus reducing the rate of carbohydrate or starch digestion, glucose liberation and absorption into blood circulation. Recent studies revealed that bioactive peptide could also inhibit the activities of both α-amylase and α-glucosidase (Yoshida et al., 1995). As well the function of dietary fibre (Oboh et al., 2015); (Oboh et al., 2018). These anti-hyperglycaemic effects from *aduwa* hydrolysate probably reflect the more potent insulin tropic response observed by APHpa and APHpa+pe in Figure 4 and 5 compared to drug treated STZ + ACA group. The *Aduwa* hydrolysate may also be assisting in promoting anti-diabetic actions by promoting insulin release (Akinyemi et al., 2015).

The reduction in the increased α-amylase activities in diabetic rats fed with protein hydrolysates supplemented diet could be attributed to the enzyme inhibitory properties of the *aduwa* protein hydrolysates (Yoshida et al., 1995);(Ademuluyi et al., 2015). Attributed α-amylase plasma glucose level inhibitory activities to bioactive present in such samples. Nevertheless, hydrolysates from *aduwa* meal sample could be of importance in the management of (T2D) diabetes. The treatment with protein hydrolysate from pancreatin APHpa (group IV) and APHpa+pe (group V) Figure 4 resulted in a decrease in the increased α-amylase activity in the diabetic rats to near normal control rat (group I) which is significantly lower than STZ + ACA(group III) (Ademiluyi et al., 2014).

Intestinal α-glycosidase activity increased significantly in the diabetic STZ rats (group II) and diabetes treated with Acarbose drug STZ +ACA (group III) when compared to the normal Control rats (group I). The APHpa and APHpa+pe *aduwa* supplemented diet treatments caused a marked decrease in the activity of this enzyme in diabetic rats (group IV-V).The enzyme inhibitory properties of the aduwa APHpa and APH pa+pe aduwa on intestinal α-glucosidase activity are favourable against acarbose drug treated group. This observation could be attributed to crude fibre and polyphenol contents (Oyeleye et al., 2021).

All treatment groups showed a positive impact upon anti -oxidant capacity Figure 6 and 7. The Gpx and GST capacity from treated samples and TBARS are also significantly reduced in activities. Thus the significant improvement in the GST and GPx anti- oxidant capacity found in this study could indicate wider-reaching beneficial effects on diabetes co-morbidities, along with improved blood glucose management. The reduction in energy intake with all the treatment groups was also interesting; however, this was not associated with any drastic change in bodyweight gain. Therefore, on examining the anti-oxidant effects of *aduwa* protein

Hydrolysate by pancreatin APHpa and combined enzymes APHpa+pe in STZ-induced diabetic and non-diabetic rats groups, it appeared that the TBARS levels in diabetic rats were significantly affected by *aduwa* treated samples. This implies that the levels of TBARS were significantly higher in non-diabetic group (control) and diabetic induced groups (STZ) without *Aduwa* protein hydrolysates treatment than in diabetic rats with *aduwa* treatment, APHpa and APHpa+pe (groups IV-V) respectively. TBARS levels are closely associated with lipid peroxidation (Opara, 2002). These results suggest that lipid peroxidation is reduced under the use of *aduwa* protein hydrolysate. Hence ,when used to make functional food or nutraceuticals, *aduwa* protein hydrolysate could reduce the activities of thiobarbituric acid reactive substances (TBARS) which may limit the loss of membrane fluidity and pancreatic function in diabeticism (Oboh et al., 2018).

Glutathione peroxidase (GPx) in serum decomposes hydrogen peroxide and phospholipid. This endogenous enzyme also catalyzes the reduction of both organic and inorganic hydro peroxides into their corresponding alcohols at the expense of GSH. The reduced GPx activity in the diabetic control STZ (group II) in Figure 6 suggest inactivation of this enzyme by reactive oxygen species (ROS) because of low or used up anti-oxidant effects. The use of protein hydrolysates by combined enzymes especially APHpa+pe can increase endogenous glutathione peroxidase from lipid and other metabolic mechanisms (Parthsarathy et al., 2018); (Ademiluyi et al., 2012).

Glutathione transferase (GST) is one of the three important endogenous antioxidant enzymes which have an important role as to protect cells from the reactive oxygen species. Glutathione-*S*-transferase decomposes lipid hydro peroxides, Sequesters ion and metal by chelating mechanism. The decrease in the GSH level in APHpa groups could be due to increased direct reaction of GSH with free radicals produced in hyperglycaemic diabetic state. This is in agreement with the covalent scavenging function of GSH (Ademiluyi et al., 2015). In addition it could due to possible less bioactivity from pancreatin hydrolysate treatment (APHpa) resulting from single enzymatic break down of protein to peptides. The *Aduwa* protein hydrolysates exert their bioactive effect through several mechanisms of action and it is likely that the results observed herein emerge from the beneficial synergistic effects arising from a number of peptides or amino acids and phenolic within the protein hydrolysates (Harnedy et al., 2017).

CONCLUSION

Both APHpa and APHpa+pe are effective for the management of diabetes in a Streptozotocin induced diabetes rats model. Blood glucose profile showed improvements with Aduwa protein hydrolysates treatment groups above acabose drug. It is believed that a synergistic effect arising from several peptides and possibly due to phytochemicals (phenolic) within the hydrolysates are associated with the effects observed in this study. Despite no effect on bodyweight, there was however a reduction in energy intake, indicating that these hydrolysates are potentially of interest for their effects on satiety if assessed in a more physiological situation such as high fat fed diet-induced obesity model or diabesity research. To the best of our knowledge, this is the first research to highlight the anti-diabetic potential of hydrolysates from Aduwa seed *in vivo* and these *in vivo* findings add further weight to the evidence supporting the anti-diabetic and anti-oxidant potentials of Aduwa enzymatic protein hydrolysates.

This data suggests that protein hydrolysates derived from Aduwa seed meal have potent anti-diabetic and anti-oxidant potentials and could be a suitable option for early management and prevention of T2DM. Exploitation of these raw materials as sources of high-value functional peptide ingredients presents an opportunity for desert dates food processors to add value to existing literature on nuts. Translation of these findings to human studies could emphasise the attractiveness of *aduwa* protein hydrolysate by enzymes as a high quality and sustainable protein source with potent bioactivity.

Author Contribution

Ogori A.F designed the experiments and performed the experiments and wrote the draft manuscript. (Girgih et al., 2014) supervised the work, read, edited the manuscript and wrote the draft manuscript. Famuwagun F A supervised the work and read and edited the manuscript and Adefega S A collected and analyzed the data (Ogori et al., 2019).

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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.

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