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Research Article
Assessment of the Ameliorative Effect of Ruzu Herbal Bitters on the Biochemical and Antioxidant Abnormalities Induced by High Fat Diet in Wistar Rats

[1]Olubanke Olujoke Ogunlana, [2]Oluseyi Ebenezer Ogunlana, [1]Stanley Kelechukwu Ugochukwu and [1]Alaba Oladipupo Adeyemi

[1]Department of Biochemistry, Covenant University, Ota, Ogun, Nigeria
[2]Department of Biological Sciences, Crawford University, Igbesa, Ogun, Nigeria

Abstract

Background and Objective: Ruzu herbal bitters (RHB) is a poly-herbal preparation that is widely taken in Nigeria and it is used as an anti-obesity medicinal concoction. The concoction is an aqueous composition of different plant parts of *Curculigo pilosa*, *Uvaria chamae* and *Citrullus colocynthis* and so far, there has been no published scientific verification on the health enhancing claims of RHB's intake. This study is aimed at evaluating the anti-obesity and the biochemical and antioxidant effects of RHB's consumption, using an albino Wistar high-fat dietary model rats. **Materials and Methods:** A total of thirty-six (n = 36) rats were divided into six groups of six animals each. Group 1: The negative control animals (NEC), received the high-fat diet, group 2: The normal control animals (NC), was fed on standard rat chow and distilled water, groups 3-6 were placed on the high-fat diet and then dosed orally with the following: Pioglitazone (PIO) (30 mg kg^{-1} b.wt.,), RHB (0.3 mL kg^{-1}), vitamin E (Vit. E) (10 IU kg^{-1}) and a combination of PIO and Vit. E, respectively for 8 weeks. The animals were then sacrificed and antioxidant and biochemical tests on blood and other tissue samples were carried out by standard methods. Statistical analysis of the results was carried out by one-way analysis of variance with the SPSS. **Results:** The group 4 RHB administered animals had a significant reduction ($p<0.05$) in total body weight, in comparison with group 1 animals. As well as a significant ($p<0.05$) reduction the plasma activities of alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) and concentrations of LDL-cholesterol, triglyceride, fasting blood glucose, total and indirect bilirubin when compared with group 1. A significant ($p<0.05$) increase in the concentrations of plasma HDL-cholesterol and reduced glutathione in the brain, spleen and liver of rats were also observed in group 4 while a significant ($p<0.05$) increase was observed in the activity of peroxidase in the liver and brain of rats in the RHB group in comparison with group 1. **Conclusion:** These findings validate the anti-obesity and antioxidant claims of RHB and these activities were attributed to its plant's constituents.

Key words: Ruzu herbal bitters, anti-obesity activity, biochemical assessment, high-fat diet, antioxidant activity, oxidative stress, pioglitazone

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Corresponding Author: Olubanke Olujoke Ogunlana, Department of Biochemistry, Covenant University, Ota, Ogun, Nigeria Tel: +2348080454316

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dietary fat plays an important role in the physical well-being, energy needs, growth and the likelihood of developing cardiovascular diseases or being diagnosed with the onset of diabetes[1]. Obesity has been defined as an abnormal buildup of fat in the adipose tissue throughout the body[2]. It is the most common nutritional disorder in humans from wealthy regions of the world and a medical disorder caused by the accumulation of excess fat to the extent that it may have an adverse effect on health[3]. Overweight and obese weight categories are determined by body mass index (BMI) values of 25-29.9 and greater than 30 kg m^{-2}, respectively[2]. Excess body weight classifications have been associated with physical discomfort, psychological trauma and a complex health condition termed metabolic syndrome. The condition is characterized by a group of health disorders named diabetes, lipid disorders, sleep apnea, certain types of cancer, osteoarthritis and hypertension[3]. Certain types of medications, lack of physical exercise, excessive intake of dietary calories, genetic susceptibility and endocrine disorders are some of the common causes of obesity[3]. Obesity can be defined as a state of excess body fat, or body energy stores in excess of physiologic needs. Obesity has reached epidemic levels not only in developed nations but also in developing nations[4,5]. There is a strong association between diets rich in fat or ketones and development of obesity, high blood pressure, heart disease, diabetes, immunosuppression, atherosclerosis and chronic fatigue[6,7]. The prevalence of overweight and obesity in Nigeria ranged from 20.3-35.1 and 8.1-22.2%, respectively amongst the adult population[8]. In the late 1990s, it was estimated that in the United States alone, approximately 300,000 premature deaths yearly resulted from complications of obesity[9]. This premature mortality rate is second only to tobacco use[10]. For clinical convenience, the degree of obesity is most frequently estimated by body mass index (BMI), which correlates reasonably well with total body fat.

Ruzu herbal bitters (RHB) is a poly-herbal mixture prepared in Nigeria and widely used as an anti-obesity concoction. RHB is claimed for the management/treatment of obesity, diabetes, hypertension, arthritis and infertility among others. RHB is an aqueous preparation of *Curculigo pilosa* root (40%), *Uvaria chamae* stem (30%) and *Citrullus colocynthis* bark (40%). *C. pilosa* have been used traditionally to treat impotence, arthritis, gastrointestinal and heart diseases[11]. Phytochemical investigations have shown that *C. pilosa* have antioxidant, antiasthmatic, hepatoprotective and neuroprotective activity[11]. The saponin extract of *C. colocynthis* fruits at doses 10, 15 and 20 mg kg^{-1} significantly lowered the fasting glucose levels in alloxan-induced diabetic rabbits when given orally[12]. *Uvaria chamae* has been reported for treating severe abdominal pains, diarrhea, sickle cell anaemia, cough, urinary tract and cerebral infections and also possesses hepatoprotective activity[13]. Citrullus colocynthis plant is widely recognized for its wide range of medicinal applications and uses, as well as its pharmaceutical and nutraceutical potentials. It has been widely applied in the management of diabetes, leprosy, common cold, cough, asthma, bronchitis, jaundice, joint pain, cancer, toothache, wound, mastitis and gastrointestinal disorders[14].

Obesity-associated health problems are increasing annually all over the world. Treatment options include drug therapy, psychotherapy, surgery, lifestyle changes and herbal and homeopathic remedies. Some of these treatment options have grave consequences and many of them especially the herbal remedies are yet to have their claims verified. It is therefore essential to continually evaluate the treatment claims and safety of herbal products to ensure sustenance of the quality of life. There is no scientific report on the health claims of RHB. This study is, therefore, aimed at evaluating the biochemical and antioxidant effects of RHB on a high-fat diet fed rats for 8 weeks.

MATERIALS AND METHODS

Materials: Ruzu herbal bitters (RHB) was obtained from A2W Global Ltd., Lagos, Nigeria with NAFDAC Registration Number A7-1102L. Pioglitazone hydrochloride (>99% purity) was obtained from Tokyo Chemical Industry (Shanghai) Development Co. Ltd, Shanghai, China. Vitamin E capsule was obtained from Bactolac Pharmaceutical Inc, New York, USA. All other chemicals and reagents used in the study were of analytical grade.

Experimental animals and diet: Male Wistar rats (n = 36, 120-170 g) used for the study were procured from the Central Animal House of the Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Experimental animals were housed in standard rat cages and maintained under uniform laboratory conditions. The test animals were acclimatized for 2 weeks on the normal pelletised diet and water *ad libitum*. Animal management and experimental procedures were performed in accordance with the requirements of the National Research Council's Guide for the use of Laboratory Animals[15]. The diets used in the study

were purchased from Graceline Feeds, Ota, Ogun State, Nigeria. The high fat diet was formulated with lard as a major fat source. The high fat diet was composed of maize (9%), flour binder (15%), soybean (7%), groundnut cake (10%), fish (9%), lard (45%), wheat offal (1%), bone (1.4%), vitamins premix (2%), lysine (0.1%), salt (0.2%) and methionine (0.3%). While the normal diet was composed of maize (45%), flour binder (15%), soybean (7%), groundnut cake (10%), fish (9%), oil (5%), wheat offal (5%), bone (1.4), vitamins premix (2%), lysine (0.1%), salt (0.2%) and methionine (0.3%).

Experimental design: The test rats were weighed before the commencement of treatment and weekly throughout the duration of the study. Thereafter, the animals were randomly classified into six groups of six animals each and were given various treatment by gastric intubation for 8 consecutive weeks. The animals were subjected to the various experimental groupings below and treated daily throughout the duration of the experiment:

Group 1: Negative control (NEC): Fed high fat diet (HFD) and given distilled water
Group 2: Normal control (NC): Fed normal diet and given distilled water
Group 3: Pioglitazone (PIO): Fed HFD and given PIO (30 mg kg^{-1}/day)
Group 4: Ruzu herbal bitters (RHB): Fed HFD and given RHB (0.3 mL kg^{-1}/day)
Group 5: Vitamin E (Vit. E): Fed HFD and given Vit. E (10 IU kg^{-1}/day)
Group 6: Combined (Vit. E+PIO): Fed HFD and given PIO and Vit. E (ratio 1:1)

At the end of the duration of the experiment, animals were fasted overnight, fasting blood glucose was measured using the standard diagnostic kit (ACCU-CHECK Diagnostics, England), after which the animals were put under light di-ethyl ether anesthesia and sacrificed[16]. Blood samples were collected by cardiac puncture into heparin tubes and used for biochemical and lipid profile analysis. While the heart, intestine, kidney, liver and brain tissues were excised, washed in cold normal saline, weighed, stored at -20°C and used for antioxidant assessment.

Biochemical estimation: The blood samples in heparin tubes were centrifuged at 3000 rpm for 10 min to collect the blood plasma. Total protein content was determined by Lowry method as described by Noeman *et al.*[17]. Plasma concentrations of albumin, aspartate aminotransferase, alanine aminotransferase, total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), total bilirubin and direct bilirubin were measured as described to the manufacturers' protocol (Randox Laboratories Ltd, UK). Spectroscopic measurements were performed using UV/Visible spectrophotometer (Thermo Fisher Scientific (GENESYS) USA). Insulin rat specific ELISA kit (East Biopharm, Co. Ltd., Hangzhou, PRC) was used to measure plasma insulin, following manufacturers' instructions. Insulin resistance was calculated as reported by Gutch *et al.*[18]. Homeostasis model assessment index for the measurement of insulin resistance (HOMA-IR) was calculated using the formula below:

$$\text{HOMA-IR index} = \frac{\text{Fasting glucose (mmol L}^{-1}) \times \text{Fasting insulin (U mL}^{-1})}{22.5}$$

Antioxidant assays determination: Lipid peroxidation and antioxidant assessment were carried out in all excised organs. Ten percent of tissues (heart, intestine, kidney, liver and brain) homogenates were prepared in ice-cold homogenizing buffer (pH 7.4) comprising of 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl and 10 mM HEPES. The mixture was mechanically homogenized using a table mounted, high-torque, thristor-controlled electric motor (DAKO homogenizer, Hamburg, Germany). The homogenate was centrifuged at 12000 rpm for 10 min at 4°C temperature. The supernatant was collected and used for the determination of reduced glutathione[19], catalase activity[20], peroxidase activity[20] and the concentration of thiobarbituric acid reactive substances (TBARS)[20].

Statistical analysis: Data were expressed as mean±standard error of mean (SEM). Statistical analysis of the results was carried out by one-way analysis of variance with the Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM SPSS Inc.). Test for statistical significance was carried out at 95% confidence limit.

RESULTS

Effect on body and organ weights: Body weight of all the experimental rats increased progressively throughout the 8 week experimental period (Fig. 1). However, group 1, (NEC) recorded a higher percentage increase in body weight (Fig. 2) than group 2 (NC) and other test groups. Significant ($p<0.05$) increase in the relative weights of the liver, spleen, brain and heart of the experimental animals were not recorded for all

Table 1: Relative organ weight (g) of organs collected from the animals

Groups	Liver	Kidney	Heart	Spleen	Brain
1	0.0347±0.0018	0.0040±0.0001	0.0043±0.0003	0.0036±0.001	0.0060±0.001
2	0.0364±0.0016	0.0037±0.0001	0.0041±0.0001	0.0043±0.001	0.0061±0.001
3	0.0345±0.0012	0.0036±0.0001[a]	0.0041±0.0001	0.0042±0.001	0.0052±0.001
4	0.0334±0.0012	0.0035±0.0002[a]	0.0048±0.0004	0.0039±0.002	0.0063±0.002
5	0.0374±0.0009	0.0038±0.0002	0.0041±0.0002	0.0040±0.001	0.0055±0.001
6	0.0340±0.0019	0.0039±0.0001	0.0042±0.0002	0.0043±0.001	0.0053±0.002

Groups 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively. Values presented as Mean±SEM, (n = 6), a: Significant difference (p<0.05) when compared to negative control

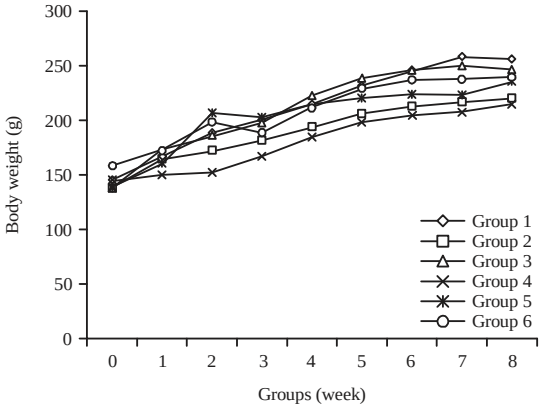

Fig. 1: Weekly weight (g) trend in all experimental groups during the treatment period of 8 weeks. Groups 1-6 represent animals' groups designated as Negative control, Normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively

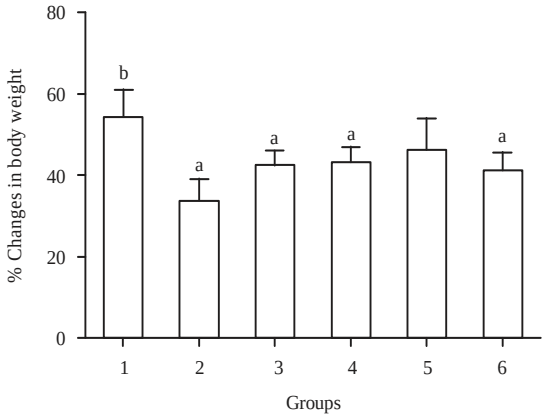

Fig. 2: Percentage change in mean body weight (g) of experimental animals after 8 weeks of experimental duration. Groups 1-6 represent animals' groups designated as Negative control, Normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively

Values presented as Mean±SEM, (n = 6), (a) shows significant difference (p<0.05) when compared to negative control while (b) shows significant difference (p<0.05) when compared to normal control

treatment groups. However, in comparison with groups 3 and 4 a significant decrease in the relative weight of kidney was recorded (Table 1).

Biochemical effect: Figure 3 shows that there was significant (p<0.05) decrease in the activities of ALT and AST in all high-fat diet treated groups (groups 3-6) in comparison with negative control group 1. The concentrations of total bilirubin (TB) and indirect bilirubin (IB) were significantly (p<0.05) reduced in all high-fat diet treated groups (groups 3-6) in comparison with untreated high fat diet, group 1. The concentrations of TB and IB significantly (p<0.05) increased for group 1 compared with group 2. No significant difference was recorded for the concentrations of total protein and albumin in all the test groups (Fig. 3). There was significant (p<0.05) difference in the concentrations of HDL-cholesterol, LDL-cholesterol and triglyceride in test animals administered RHB (group 4) when compared with animals in group 1 (Fig. 4). However, there was no significant change in the concentration of total cholesterol across all the groups except for group 6 test animals (Fig. 4).

The fasting blood glucose concentration (FBG), insulin level and HOMA-IR index were significantly elevated for group 1 animals; but were lower and comparable for groups 2-6 test animals (Fig. 5).

Oxidative stress in organs of experimental animals: Figure 6 illustrates the pattern of oxidative stress markers in the brain of the experimental animals. There was no significant difference in the concentrations of TBARS in the brain of experimental animals across the groups. However, a significant (p<0.05) increase in catalase activity was recorded for groups 3, 4, 5 and 6 animals. Similarly, peroxidase activity and concentration of reduced glutathione significantly increased for groups 4 (RHB) animals (Fig. 6).

Figure 7 shows the pattern of oxidative stress markers on the liver of experimental rats. There was no significant change in the concentration of TBARS in the liver of the experimental animals. Nevertheless, there was significant

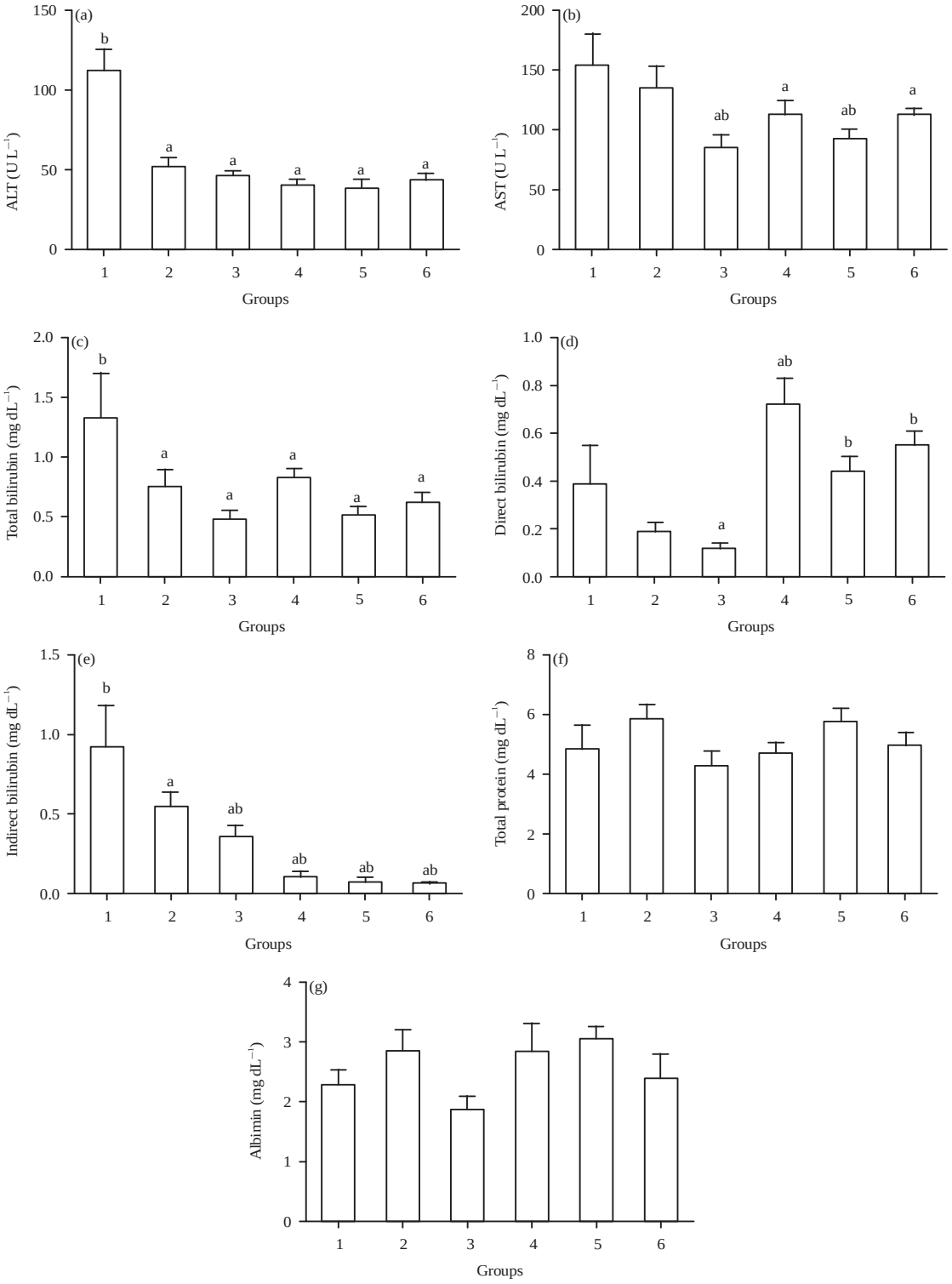

Fig. 3(a-g): Effect of high-fat diet on biochemical parameters of experimental animals. Results expressed as Mean±SEM of 6 replicates, (a) Activity of plasma ALT, (b) Activity of plasma AST, (c) Concentrations of plasma total bilirubin, (d) Concentrations of direct bilirubin, (e) Concentrations of indirect bilirubin, (f) Concentrations of total plasma protein and (g) Concentrations of albumin. Groups 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively

Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls (NC), respectively

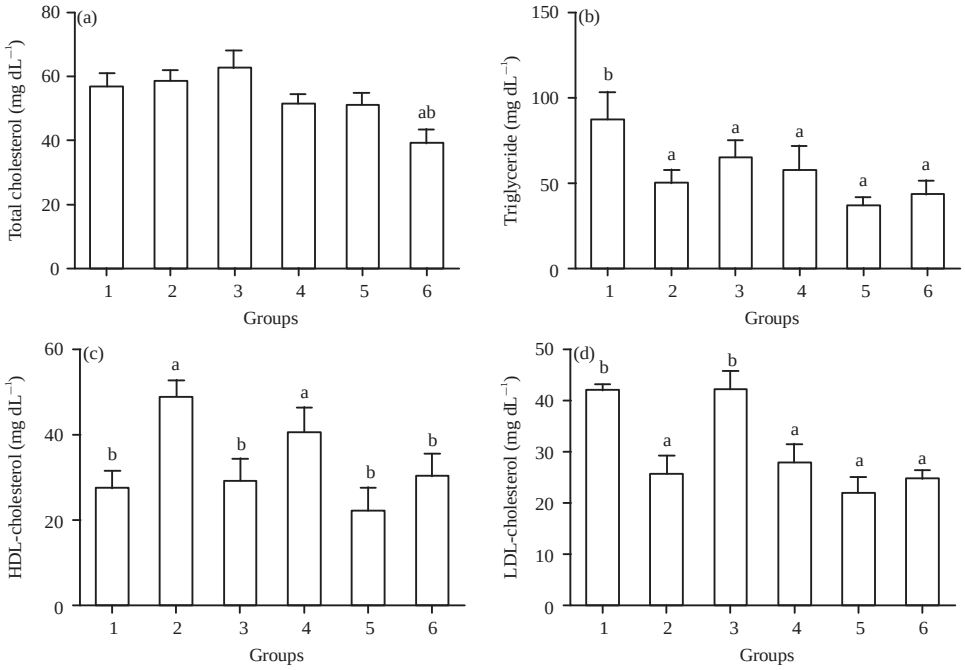

Fig. 4(a-d): Effect of high-fat diet on lipid profile of experimental animals. Results expressed as Mean±SEM of 6 replicates, (a) Concentrations of total cholesterol, (b) Concentration of plasma triglyceride, (c) Concentrations of plasma HDL-cholesterol and (d) Concentrations of LDL-cholesterol. Groups 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively
Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls, respectively

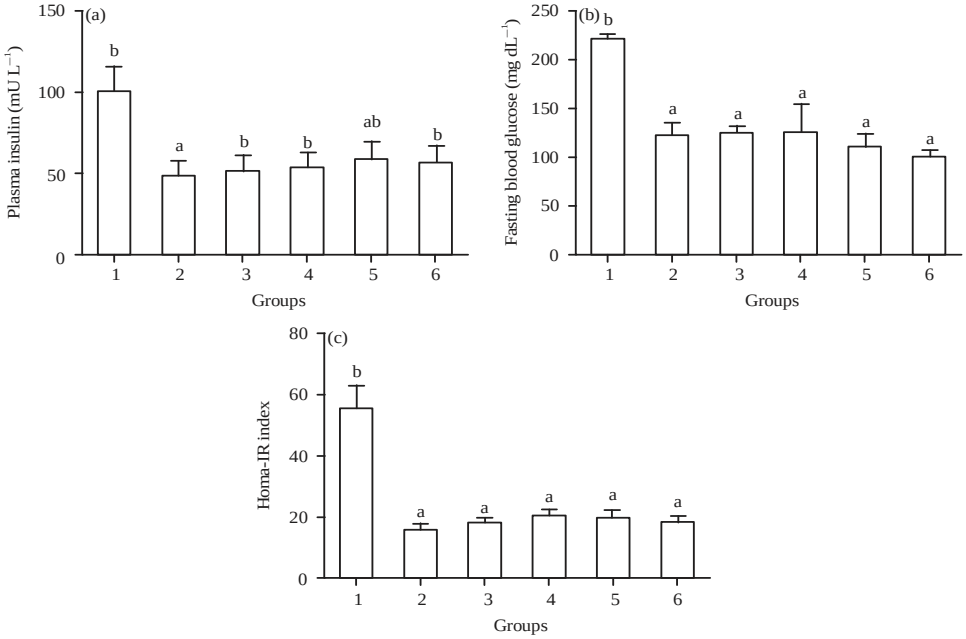

Fig. 5(a-c): Effect of high-fat diet on fasting blood glucose, plasma insulin and homeostasis model assessment index for insulin resistance (HOMA-IR) of experimental animals. Results expressed as Mean±SEM of 6 replicates study, (a) Concentrations of plasma insulin, (b) Concentration of fasting blood glucose and (c) Homeostasis model assessment index for insulin resistance (HOMA-IR). Groups 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively
Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls, respectively

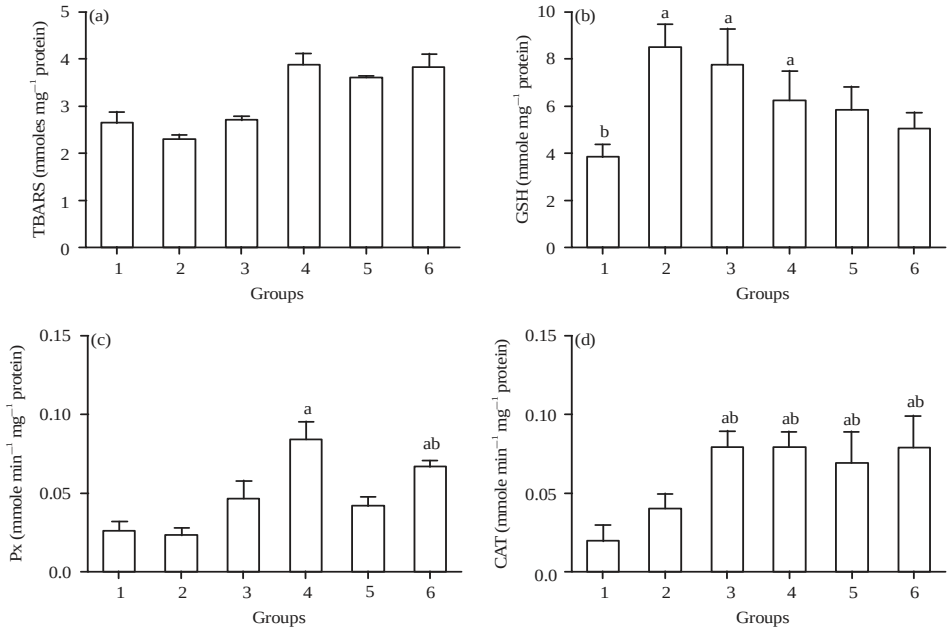

Fig. 6(a-d): Pattern of oxidative stress markers in the brain of experimental animals. Results expressed as Mean±SEM of 6 replicates, (a) Concentrations of thiobarbituric reactive substances (TBARS), (b) Concentration of reduced glutathione (GSH), (c) Activity of plasma peroxidase (Px) and (d) Activity of catalase (CAT). Group 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively

Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls, respectively

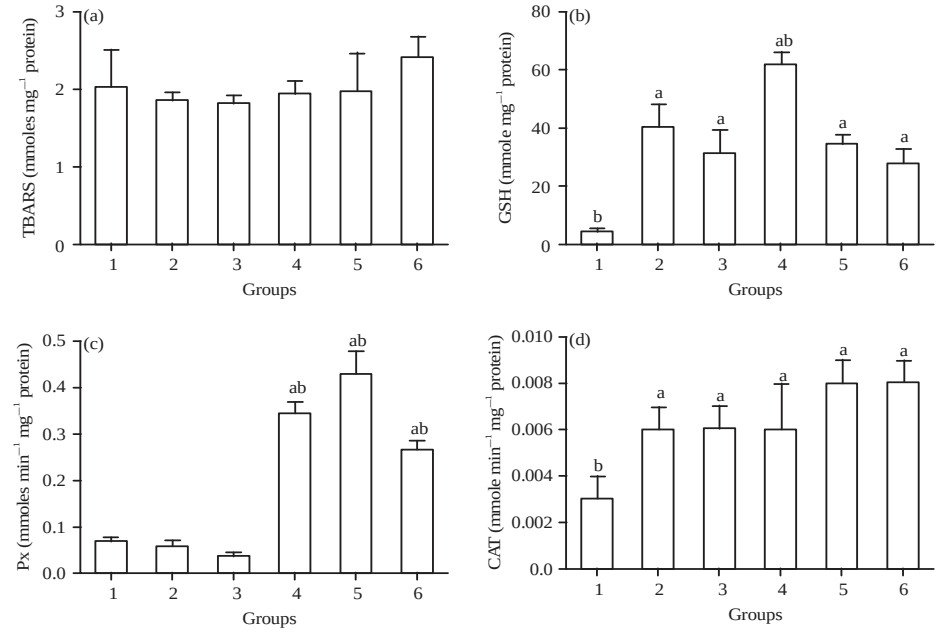

Fig. 7(a-d): Pattern of the oxidative stress markers in the liver of experimental animals. Results expressed as Mean±SEM of 6 replicates, (a) Concentrations of thiobarbituric reactive substances (TBARS), (b) Concentration of reduced glutathione (GSH), (c) Activity of plasma peroxidase (Px) and (d) Activity of catalase (CAT). Group 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively

Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls, respectively

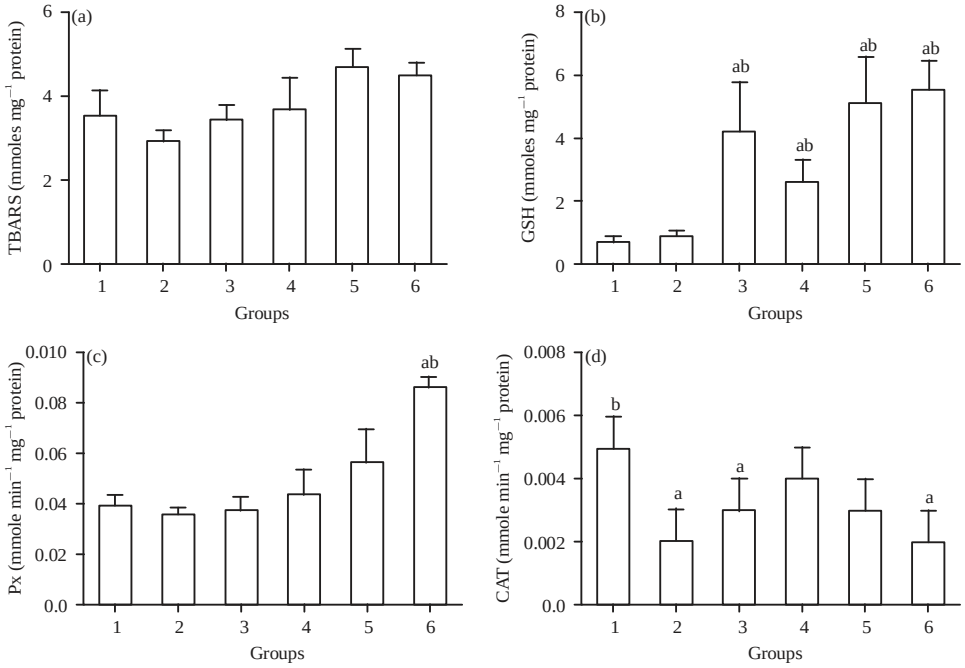

Fig. 8(a-d): Pattern of the oxidative stress markers in the spleen of experimental animals. Results expressed as Mean±SEM of 6 replicates, (a) Concentrations of thiobarbituric reactive substances (TBARS), (b) Concentration of reduced glutathione (GSH), (c) Activity of plasma peroxidase (Px) and (d) Activity of catalase (CAT). Group 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively

Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls, respectively

($p<0.05$) increase in reduced glutathione concentration and catalase activity in the liver of animals in groups 3, 4, 5 and 6 in comparison with animals in group 1. Conversely, only groups 4, 5 and 6 recorded significant increase in the activity of peroxidase in the liver of the test experimental rats.

Figure 8 shows the pattern of oxidative stress markers on the spleen of experimental rats. There was no significant alteration in the concentration of TBARS in the spleen of the experimental animals. However, a significant ($p<0.05$) increase in the concentration of reduced glutathione was observed in the spleen of rats in groups 3, 4, 5 and 6. While peroxidase activity significantly ($p<0.05$) increased only in group 6 and catalase showed a significant decrease in groups 2, 3 and 6.

Figure 9, 10 and 11, further illustrates the pattern of oxidative stress markers on the kidney, heart and intestine of experimental rats respectively. There was no significant difference in the concentration of TBARS in the kidney, heart and intestine of the experimental animals. However, a significant ($p<0.05$) increase was recorded for the peroxidase activity of the kidney in group 6, heart in group 4 and intestine in groups 4, 5 and 6 animals. Still, there was no significant increase in the concentration of reduced glutathione in the kidney, heart and intestine of the test groups 3, 4, 5 and 6.

DISCUSSION

According to the World Health Organization (WHO), 80% of the world's population use medicinal plants in the treatment of diseases. In African countries, this rate is much higher[21]. High fat or high caloric diet plays a major role in the development of obesity[17], which is a risk factor for the progression to dyslipidemia[22], type-2 diabetes[23], non-alcoholic fatty liver diseases[24], cardiovascular disorders and coronary heart disease[25]. Several scientific reports have shown that animals fed with high-fat diet (HFD) equally promote increased body weight, biochemical alterations to the hepatocytes membrane integrity and functionality and the development of insulin resistance hyperglycaemia[26,27]. The pathogenic mechanisms associated with the onset of these disorders is partly understood[28]. However, there is a strong association with hyperlipidaemia, oxidative stress and insulin resistance[17]. The safety of a pharmacological therapy for the management and attenuation of obesity is still largely a medical challenge[23].

The increased percentage change in body weight in animals given HFD could be attributed to increased fatty acid in diet without any form of intervention. Consequently,

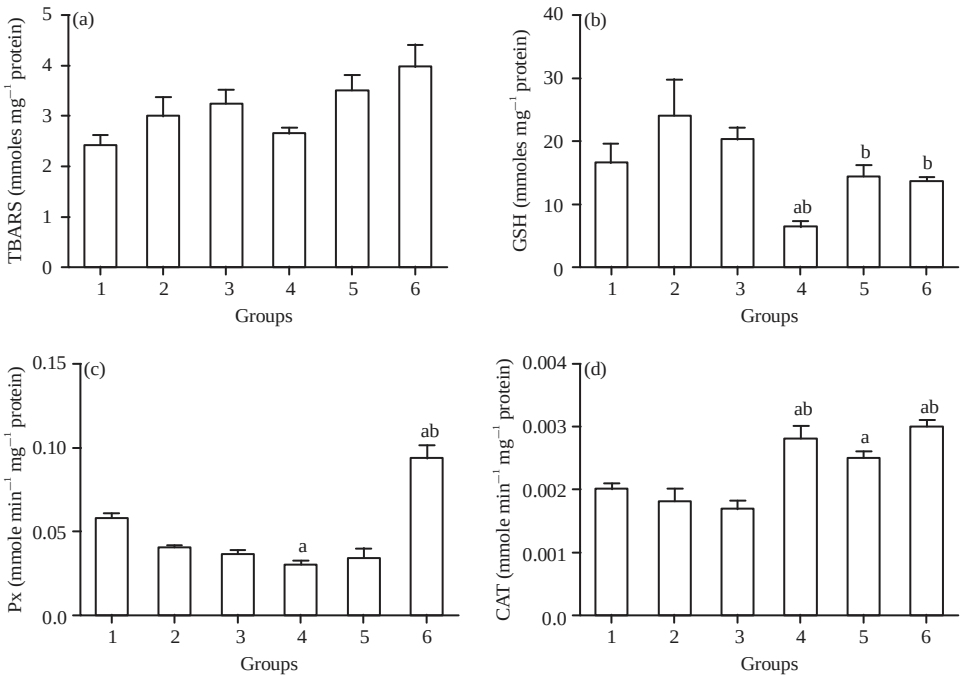

Fig. 9(a-d): Pattern of the oxidative stress markers in the kidney of experimental animals. Results expressed as Mean±SEM of 6 replicates, (a) Concentrations of thiobarbituric reactive substances (TBARS), (b) Concentration of reduced glutathione (GSH), (c) Activity of plasma peroxidase (Px) and (d) Activity of catalase (CAT). Group 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively

Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls, respectively

leading to dyslipidemic changes and other metabolic alterations. This report is corroborated with the result as previously described[17,28,29]. Significant reduction in the total body weight (Fig. 1 and 2) of the test animals given PIO, RHB, Vit. E and combination of PIO and Vit. E demonstrates the possible ameliorating effect of the treatment against weight gain. The present work revealed a significant increase in insulin resistance as indicated by increased HOMA-IR index in HFD fed rats. This result agrees with the studies of Elshazly[28] and Zaitone *et al.*[29]. This indicates that insulin resistance and obesity are induced in continuous intake of diet rich in fatty acids. Hence, insulin resistance may play a significant role in the development of metabolic syndrome associated with HFD and obesity[3]. The HOMA-IR index was significantly reduced in HFD fed animals treated with PIO, RHB and Vit. E, with a greater reduction in the combination of PIO and Vit. E. RHB is also able to cause a significant reduction in HOMA-IR index in HFD fed animals comparable with pioglitazone and vitamin E. PIO alleviates fat-induced insulin resistance in the peripheral tissues by increasing insulin sensitivity in the liver, muscle and adipose tissues and by improving glucose and lipid metabolism[28].

Liver as a major detoxification organ of mammals might be susceptible to the effects of high-fat diet after a continuous intake. Liver function markers such as ALT, AST, bilirubin and albumin are markers of membrane integrity and functionality of the metabolic organ. Bilirubin as an excretory product of haem is formed from the oxidation of biliverdin. It is used in the assessment of liver function[30]. The liver is involved in the conjugation of bilirubin with glucuronic acid to form a more water soluble derivative suitable for excretion through bile. The increase in indirect bilirubin (unconjugated bilirubin) is an indication of liver damage[31]. HFD fed rats showed significantly increased activities/concentrations of ALT, AST, total and indirect bilirubin indicating an alteration in the integrity and functionality of the hepatocytes. This report is in consonance with previous reports[28,29]. The treatment of HFD fed rats with PIO, RHB, Vit. E ameliorates hepatic injuries by significantly reducing the activities of ALT and AST and concentration of total and indirect bilirubin.

In addition, significantly ($p<0.05$) elevated levels of triglycerides and LDL-cholesterol, as well as decreased HDL-cholesterol level in circulation was observed in the HFD fed group 1 animals. This was in harmony with the results of

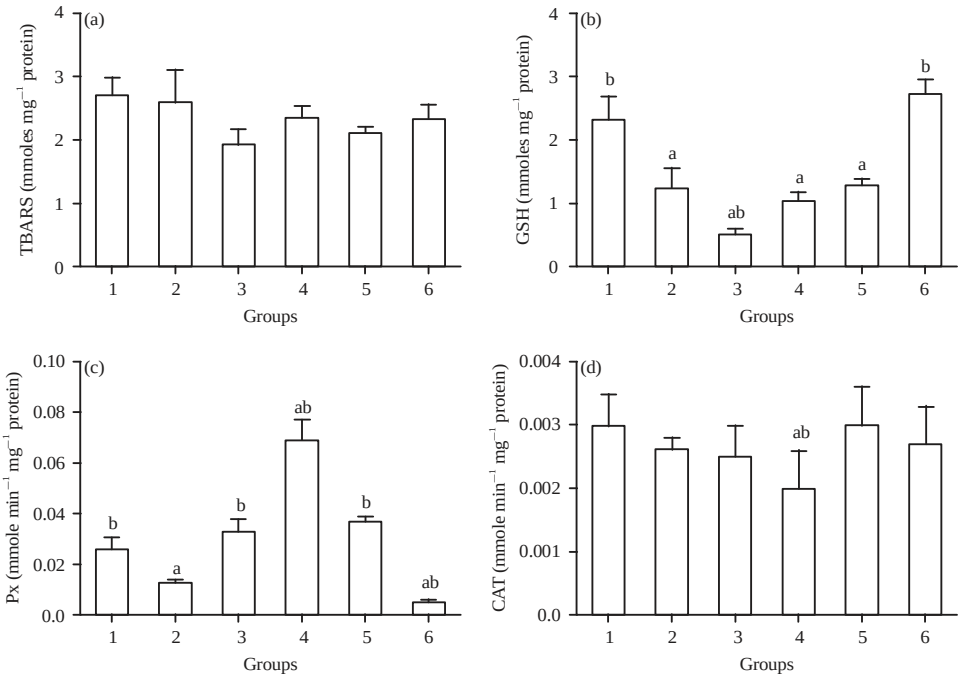

Fig. 10(a-d): Pattern of the oxidative stress markers in the heart of experimental animals. Results expressed as Mean±SEM of 6 replicates, (a) Concentrations of thiobarbituric reactive substances (TBARS), (b) Concentration of reduced glutathione (GSH), (c) Activity of plasma peroxidase (Px) and (d) Activity of catalase (CAT). Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls, respectively. Group 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively

Values presented as Mean±SEM, (n = 6), [a]$p<0.05$ shows significant difference when compared to negative control, [b]$p<0.05$ shows significant difference when compared to normal control

Noeman *et al.*[17], Elshazly[28], Zaitone *et al.*[29] and Zaitone *et al.*[32], who reported a significant increase in circulating triglycerides and total cholesterol in HFD fed rats. The high occurrence of dyslipidemia observed in HFD fed animals were significantly attenuated in PIO, RHB and Vit. E treated groups. Dyslipidemia changes which occur in obesity may be due to increase in the influx of lipids to the liver[17]. Increase in the circulation of triglyceride after over-nutrition with HFD could result in deposition of fat in non-adipocyte tissues. There is the limited capacity of the non-adipocyte tissues to store excess lipids and this accumulation could trigger cell dysfunction or cell death, a condition known as lipotoxicity[28]. Lipid alterations have been considered as contributory factors to oxidative stress, insulin resistance and hepatic dysfunction in obesity[17,28,32]. Increased production of reactive oxygen species as well as reduced antioxidant defense mechanisms has been suggested to play a role in both human and animal models of obesity[17,33].

In HFD fed state, prolonged hyperglycaemia and dyslipidemia provide additional substrates for auto-oxidation that generate free radicals that may impair antioxidant defense system resulting in the cellular injury of tissues[34]. Malondialdehyde (MDA), a measurement of lipid peroxidation[35] are reported as a measurable component of obesity-induced pathology[36]. The data presented in this study showed that concentrations of TBARS were not significantly affected in all studied organs. This was contrary to previously reported research involving HFD and obesity[17,28,29]. The inconsistency of this result with past report in this regard might be due to duration of the experiment, the genetic makeup and gender of the Wistar rats used in this present work. However, the reduction in the activities of antioxidant enzymes and concentrations of antioxidant molecule in the brain, liver, spleen and kidney of HFD fed rats in this study was attributed to increased oxidative stress in these organs, causing the depletion of antioxidant markers. The results obtained agree with previously reported researches[17,28,29,37], which showed that obesity-induced by HFD is an independent risk factor for decreasing cytoprotective enzymes. The observed hyperlipidaemia and hyperglycaemia as well as

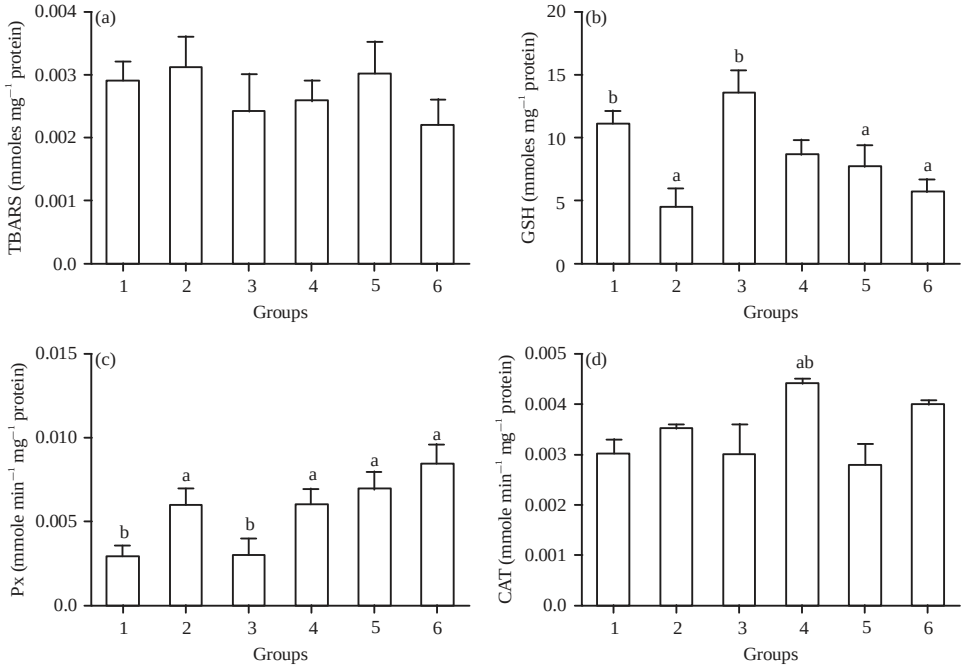

Fig. 11(a-d): Pattern of the oxidative stress markers in the intestine of experimental animals. Results expressed as Mean±SEM of 6 replicates, (a) Concentrations of thiobarbituric reactive substances (TBARS), (b) Reduced glutathione (GSH), (c) Activity of plasma peroxidase (Px) and (d) Activity of catalase (CAT). Group 1-6 represent animals' groups designated as negative control, normal control, Pioglitazone, Ruzu, Vitamin E and Pioglitazone with Vitamin E, respectively.

Values marked with (a) and (b) are significantly different at $p<0.05$ when compared with negative (NEC) and normal controls, respectively

insulin resistance as shown in this study might contribute to the alteration in the oxidant-antioxidant balance, hence depleting antioxidant defense enzymes and molecules in HFD fed rats. The reduction of antioxidant enzymes may be due to rapid consumption and diminution of stored enzymes by enzymatic scavenging process of free radicals generated during obesity development[17]. The reduction in the level of glutathione is an indication of its attrition rate in the process of antioxidant defense system, hence, compensating for the initiation and progression of free radical oxidation[37]. GSH is used in the redox-reactions as a supplier of SH-groups that protect the cell from reactive oxygen species[37]. It is known that glutathione can adjust the antioxidant response element of the cell and help in the maintenance or restoration of the antioxidant balance. Conversely, the imbalance in HFD fed rats was reversed by the administration of PIO, RHB and Vit. E. The decreased antioxidant mechanism of HFD fed rats recorded in the brain, liver and spleen was restored by the administration of RHB.

The present study disclosed that the administration of ruzu herbal bitters (RHB) alone and pioglitazone with vitamin E either alone or in combination efficiently ameliorated the deleterious effects of HFD. These effects were evident by the significant reduction in body and relative kidney weights, reduction in the activities of plasma ALT and AST and concentrations of total bilirubin, indirect bilirubin, triglyceride, LDL-cholesterol and HOMA-IR index. In addition, the administration of ruzu herbal bitters (RHB) alone and pioglitazone with vitamin E either alone or in combination exhibited protective effects by significantly increasing the concentrations of HDL-cholesterol and reducing glutathione. Furthermore, the activities of catalase and peroxidase in the brain, liver, spleen and kidney were also increased. This is the first report on the effect of RHB on HFD fed rats and in comparison, with other standard drugs. RHB is an aqueous preparation of *C. pilosa* root (40%), *U. chamae* stem (20%) and *C. colocynthis* bark (40%) popularly used in Nigeria with scientifically unverified biological claims. However, the antidiabetic and hypolipidemic activities of *U. chamae*[38] and *C. colocynthis*[39,40] have been previously reported, which support the report of this research. Nevertheless, there was no documentation on the hypolipidemic or antidiabetic activities of *C. pilosa*. The presence of *U. chamae* stem (20%) and *C. colocynthis* bark (40%) as major constituents of RHB might be responsible for the antioxidant and anti-obesity effect established in this study.

CONCLUSION

The antilipidemic, anti-oxidant and hepato-protective activities of RHB on HFD fed animals were established in this study. These activities were attributed to the presence of its two major plant constituents, *U. chamae* and *C. colocynthis*.

SIGNIFICANCE STATEMENTS

This study demonstrates the ameliorative effects of ruzu herbal bitters (RHB) intake on biochemical and antioxidant markers of high-fat diet fed Wistar rats. This beneficial and protective effects of RHB on high fat diet fed Wistar rat was used to investigate the potential role of RHB as anti-obesity plant concoction. This study will help to uncover the critical areas of anti-obesity and antioxidant claims of RHB that many researchers were not able to explore. Thus, a new theory on the antilipidemic, antioxidant and hepatoprotective activities of RHB may thus be enunciated.

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