Lafia Journal of Scientific & Industrial Research (LJSIR), Vol. 2(1), 2024
p-ISSN: 3026 – 9288 e-ISSN: 3027 – 1800 pages: 47 – 53
https://lafiascijournals.org.ng/index.php/ljsir/index ⇒ Published by the Faculty of Science,
Federal University of Lafia, Nasarawa State, Nigeria

The Effect of Post-treatment with Yellow Bitter Yam (*Dioscorea dumetorum*) on the Antioxidant Status of Streptozocin-induced Diabetic Rats

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Abstract

This study assesses the effects of supplemented diet of *Dioscorea dumetorum* on the antioxidant status of streptozotocin-induced diabetic rats. Twenty-five adults male Wistar rats were randomly assigned into five groups each containing five rats, group two to five were subjected to high-fat diet for four weeks and were thereafter induced with STZ intraperitoneally (45 mg/kg body weight) resulting to Type 2 diabetes. The body weight, glucose concentration, and the activities of the antioxidant enzymes, Glutathione peroxidase (GPx), Glutathione S-transferase (GST), Catalase (CAT), and Malondialdehyde (MDA) and Glutathione concentration in the kidney, heartand liver were assessed. The post-treatment with 10 and 20% Yellow Bitter Yam significantly reduced glucose and a healthy reduction in weight in treated groups, 206 and 197 g in groups treated with 10 and 20% supplemented diet of *D. dumetorum* after 21 days of dietary therapy. In diabetic kidneys, GSH and GST were like controls, while GPX, CAT, and MDA decreased significantly ($p>0.05$). Diabetic hearts showed increased CAT, GSH, and MDA, regulated in the treated group. In diabetic livers, GPx, GST, and MDA were consistent across groups, but GSH and CAT significantly ($p>0.05$) decreased in post-treated groups. The study suggests *D. dumetorum* supplementation regulates antioxidant enzymes, indicating potential hypoglycemic and antioxidative effects, particularly in kidney, heart, and liver enzyme activities associated with Type 2 diabetes.

Keywords: Antioxidant enzymes, *Diabetes mellitus, Dioscorea dumetorum, Glucose, Streptozotocin*

Article History
Submitted
December 14, 2023
Revised
March 19, 2024
First Published Online
April 19, 2024

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doi.org/10.62050/ljsir2024.v2n1.264

Introduction

Diabetes is one of the most common non-communicable diseases globally and a leading cause of death in many countries. Diabetes mellitus (DM) is a chronic, metabolic disease characterized by elevated levels of blood sugar (hyperglycemia) when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces [1]. Diabetes poses a serious threat to health worldwide due to its severe effects on the micro- and macrovascular systems. Specifically, diabetes can cause damage and/or dysfunction of multiple organs and tissues, especially the eyes, kidneys, nerves, heart, and blood vessels.The IDF Diabetes Atlas (2021) reports that 10.5% of the adult population (20-79 years) has diabetes, with almost half unaware that they are living with the condition. By 2045, IDF projections show that 1 in 8 adults, approximately 783 million, will be living with diabetes, an increase of 46%. [2], with a large portion of the cost of diabetes being connected with its complications [3]. According to the report of Adeloye *et al*., the overall mortality rate of diabetes in Nigeria was 30.2 (95% CI 14.6 to 45.8) per 100,000 population, with a case fatality rate of 22.0% (95 CI 8.0 to 36.0%) [4]. Due to the high prevalence of this disorder in our society research light is being beamed on the development of appropriate diets that can affect glycemic control and thus can be used to manage the disorder [4]. Pertinently noted that dietary management could help reduce the risk of diabetes complications and prolong life expectancy.

D. dumetorum is not only used for human consumption but also various pharmaceutical purposes [5]. Kumar *et al*. [6] reported that tubers are possible sources of antimicrobial and antioxidant agents due to richness of phenolic contents in the tubers [7]. Another Nigerian study also reported flavonoid content as well as the associated antioxidant activity in *D. dumetorum* [8]. Some novel bioactive compounds like dioscorine been detected in *D. dumetorum* [9-10], have been used advantageously as a hypoglycaemic agent to reduce the blood glucose level in situations of acute stress [11]. Due to its hypoglycemic effect, *D. dumetorum*, plays an active role in the treatment of diabetes. It is also reported that the aqueous extract of *D. dumetorum* tuber, known for its alkaloid (dioscoretine) content, which control hypercholesterolemia, hyperlipidemia, and hyperketonemia [12]. Egbuonu *et al*. [13] discussed its industrial applications as a source of the pharmaceutical agent. The minimal presence of phytates, oxalates, saponins, and tannins could guarantee its safe recommendation for food processing applications [14].

Bitter yam also contains important minerals such as iron, magnesium, phosphorus, calcium, and zinc. The yellow coloration of the yellow bitter yam is due to the presence of β-carotene. Studies have pointed out that β-carotene acts as a protector against various diseases, such as Type 2 diabetes mellitus, cardiovascular disease, obesity, and metabolic syndrome [15-16], This study aims to evaluate the effect of post-treatment with yellow bitter yam supplemented diet on the antioxidant status of Streptozotocin-induced diabetic rats, evaluating its antioxidative and hypoglycemic properties.

Materials and Methods

Materials

Yellow bitter yam variety locally called "Esuru" in the western part of Nigeria was obtained at a farm in Odo-Oro, Ikole Local government, Ekiti State. The sample was peeled, soaked overnight in lukewarm water, drained, and dried. The dried sample was then milled in a blender and stored after drying before being used for further analysis.

Experimental design

Twenty-five adult male albino rats were obtained from the College of Medicine's Animal House, Ekiti State University, Ado-Ekiti, Ekiti State. After two weeks of subjecting the rats to high-fat diet, diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of a freshly buffered (0.1 M citrate, pH 4.5) solution of STZ at a dosage of 45 mg/kg body weight leading to induction of Type 2 diabetes mellitus in the rats, Fasting blood glucose (FBG) level was assessed 72 h after the induction using a blood glucometer and glucometer strips. Only rats with hyperglycemia (glucose level over 250 mg/dL) were considered diabetic and used in the present study.

Animal grouping and feeding

The rats were randomly distributed into five groups, comprising five rats in each group, and were treated as follows:

Group 1 (Normal control): The rats in this group were fed with rat normal pellet diet and water *ad libitum* throughout the period of experiment, with no other treatment, and served as the baseline control rats.

Group 2 (Diabetic control): The rats in this group were made diabetic by a single intraperitoneal (i.p.) injection of STZ at a dosage of 45 mg/kg body weight during which they were fed with rat normal pellet diet and water ad libitum with no other treatment.

Group 3 (Diabetic rats given 10% YBY supplement): The rats in this group were also made diabetic by a single intraperitoneal (i.p.) injection of STZ at a dosage of 45 mg/kg body weight. The rats were fed with rat normal pellet diet and water *ad libitum* and supplemented with 10 g (10%) of yellow bitter yam.

Group 4 (Diabetic rats given 20% YBY supplement): The rats in this group were also made diabetic by a single intraperitoneal (i.p.) injection of STZ at a dosage of 45 mg/kg body weight. The rats were fed with rat normal pellet diet and water *ad libitum* and supplemented with 20% yellow bitter yam (YBY).

Group 5 (Diabetic rats treated with metformin): The rats in this group were also made diabetic by a single intraperitoneal (i.p.) injection of STZ at a dosage of 45 mg/kg body weight. The rats were also fed with rat normal pellet diet and water ad libitum. They were treated with metformin at 100 mg/kg body weight.

Assessment of blood glucose level

Blood samples were obtained from the vein in the rat tail and fasting blood sugar concentrations were checked at 7-day intervals an effective glucometer (On Call® Plus glucometer).

Preparation of tissue homogenate

Animals were sacrificed after 21 days of treatment and the blood sample was collected through cardiac puncture into EDTA bottles. The heart, liver, and kidney were isolated, rinsed in 1.15% KCI, blotted with filter paper, and weighed. The heart, liver, and kidney were minced separately with scissors into volumes of ice-cold 0.1 mol L-1 sucrose buffer (pH 6.9) using the Teflon homogenizer. The homogenates were later centrifuged at 10,000 rpm for 10 min to obtain a clear supernatant (post- mitochondrial fraction), which was stored at -4^{o}C and later used for biochemical assays.

Analysis for oxidative stress markers in the kidney, heart, and liver homogenates

Total Proteins were estimated by the method previously described by [17]. To different tubes, 4 minutes of Biuret reagents were dispensed. 50 µl of standard, controls, and test samples were added into the respective tubes. The preparation inside each tube was mixed well and allowed to incubate at room temperature for 20 min. The absorbance was measured in the spectrophotometer at 540 nm using 4 mls of biuret reagent as blank.

The concentration of total proteins in the homogenate was determined from the equation below as.

Protein (mg/dL) = O.D Reading of Test × Concentration of standard
O.D Reading of Test

Lipid Peroxidation was measured using the malondialdehyde (MDA) method as previously described by some scholars [18–19]. An aliquot of 400 µl of the sample was mixed with 1.6 ml of Tris-KCL buffer to which 0.5 m of 30% TCA was added. Then 50 µl of 0.75% TBA was added and removed, placed in ice, and centrifuged at 3,000 rpm. The clear supernatant was collected, and absorbance was measured against a reference blank of distilled water at 532 nm.

Concentration of reduced glutathione (GSH) An aliquot of the sample was deproteinated by the addition of an equal volume of 4% sulphursalicyclic acid. This was centrifuged at 4,000 rpm for 5 min. Thereafter, 0.5 ml [sp] of the supernatant was added to 4.5 ml of Elman's reagent. Glutathione (GSH) is proportional to the absorbance at 412 nm.

Catalase (CAT) activity was determined using the method described by Sinha. Tubes containing 0.1 ml of sample, 0.4 ml of H_2O. 22 mls of dichromate acetic acid reagents and 1 ml phosphate buffer were boiled for 10 min cooled, and readings were taken at 620 nm.

Statistical analysis

Data are expressed as means standard errors of the mean (SEMs). Differences between groups were evaluated by analysis of variance (ANOVA) with the Bonferroni post hoc test or by calculation of Spearman's rank correlation coefficient, as appropriate, using Prism 5.03 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was at $p< 0.05$.

Results and Discussion

Effect on biochemical parameters

A significant decrease in body weight ($p <0.05$) of non-treated diabetic rats were observed in the groups treated with yellow bitter yam and metformin. This decrease in weight of diabetic rats is an indication of physiopathology diabetes.

Administration of STZ partially damages pancreatic cells [20]. The induction of the diabetes with STZ in rat models shows typeII- like features as compared to the control groups of rats, thus developing a state of oxidative stress. Diabetic effect has been associated with weight loss and this was observed in streptozotocin-induced diabetic rats [21]. Hyperglycemia is associated with metabolic changes in the liver, kidney, heart, and other organs and parts of the body, especially loss of body weight and antioxidant imbalance [22]. Much evidence from both animal and human studies supports a key role for these imbalances alongside changes in enzyme activities in the onset and progression of diabetes. Medicinal plants, however, can reduce the effects of diabetes, mainly because of their hypoglycemic properties and capacity to counteract oxidative stress and regulate enzyme activities [23]. Despite its popularity, information is lacking on the efficiency of *D. dumentorum* as a treatment for diabetes. This study was therefore designed to evaluate the hypoglycemic effects of a supplemented diet of yellow bitter yam (YBY) on the antioxidant status of streptozotocin-induced diabetic rats by regulation of the activities of some related enzymes such as catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) in the kidney, heart, and liver of STZ-induced rats, to contribute to the current understanding of *D. dumentorum* as an antioxidative and hypoglycemic agent.

In Fig. 1 and Table 1, there is a significant decrease in the weight of the induced rats, this reduction could have been a result of disruption of the islet structure and β-cells, when this occurs, the body starts burning fats and muscles for energy, causing a reduction in overall body weight. Conversely, weight loss has been shown to improve glycaemic control in patients with Type 2 diabetes mellitus [24]. The reduction in body weight might also be due to the phenolic and flavonoid compounds that are believed to be present in the tuber which help maintain blood sugar levels and might also stimulate some enzymes in the synthetic pathway of *Dioscorea dumetorum* digestion enhancing satiety thereby reducing the intake of food [25]. Fig. 2 and Table 2 reveals the effect of yellow bitter yam on hyperglycemia which may be associated with the stimulation of an increase in glucose metabolism, a mechanism of action suggested by plant extracts that reverse hyperglycemia [26].

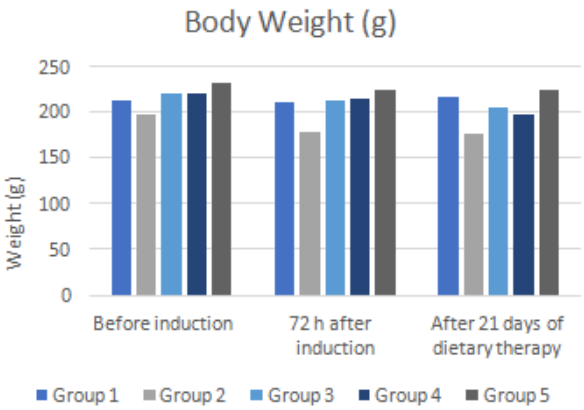

Figure 1: Effects of yellow bitter yam supplemented diet on the weight in grams of STZ-induced diabeticrats

Table 1: Effect of yellow bitter yam supplemented diet on weight in grams of induced diabetic rats

Groups	Weight (g)		
	Before Induction	After 3 days of induction	After 21 days of treatment
Group 1	212	211	217
Group 2	198	178	177
Group 3	220	212	206
Group 4	220	215	197
Group 5	232	225	224

Values are the mean

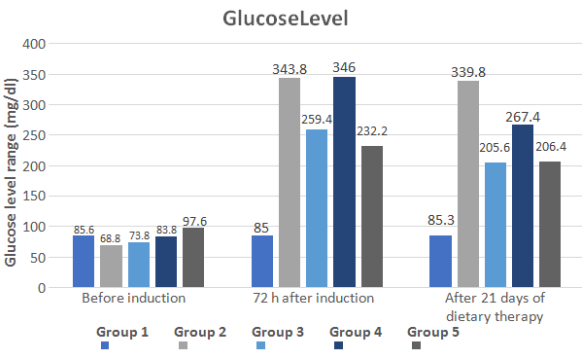

Figure 2: Effects of Yellow Bitter Yam on the glucose level of STZ-induced diabetic rats after 21 days of treatment

Table 2: Effect of yellow bitter yam supplemented diet on glucose levels in the blood sample of induced diabetic rats

Groups	Glucose level(mg/dl)		
	Before Induction	After induction	After dietary therapy
Group 1	85.7	84.9	85.3
Group 2	68.8	343.1	340.2
Group 3	73.8	259.2	205.6
Group 4	83.9	345.9	267.1
Group 5	97.9	231.5	206.3

Values are the mean

Oxidative stress has been associated with a decrease in tissue glucose uptake and insulin secretion in diabetic conditions. The concentration of GSH, GPx and GST activities were observed in the liver to have no significant difference across the studied group but an increase in GST activity is seen in Groups 3 treated with 10% YBY and Group 5 treated with metformin which might be due to an increase in toxins produced by dioscorine in YBY in the kidney (Fig. 3 and Table 3). This is similar to the report of Jiang *et al.* [27], who also found a significant increase in the activity of GST compared to normal rats. It was concluded that GST expression in diabetic kidneys is influenced by hyperglycemia or other forms of glucotoxicity, which is in correlation with this present study.

The level of GST activities observed in the kidney homogenate in this study would also suggest that it is not related to diabetic complications. This is similar to the result found by Noce *et al.* [28] who stated that the levels of GST were significantly higher in nephropathic patients without diabetes compared to nephropathic diabetic patients, in almost every stage of chronic kidney disease.

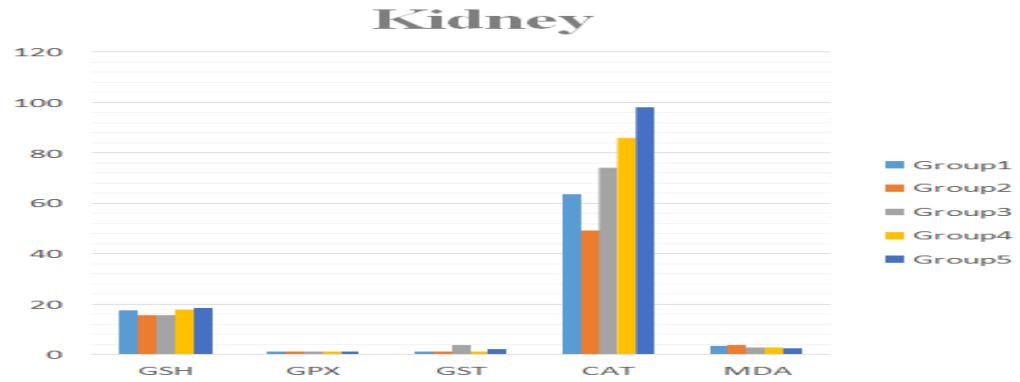

Figure 3: Effect of the administration of yellow bitter yam on antioxidant status on the Kidney of STZ-induced diabetic rats

Table 3: Effect of yellow bitter yam supplemented diet on GSH, GPX, GST, CAT, and MDA levels in the kidney of induced diabetic rats

Group	GSH	GPX	GST	CAT	MDA
1	16.50 ± 7.45^{ab}	0.22 ± 0.10^{a}	0.19 ± 0.20^{a}	62.57 ± 10.57^{ab}	2.38 ± 0.88^{ab}
2	14.50 ± 4.24^{a}	0.19 ± 0.01^{a}	0.16 ± 0.22^{a}	48.17 ± 12.58^{a}	2.89 ± 0.38^{b}
3	14.65 ± 6.83^{a}	0.15 ± 0.01^{a}	2.75 ± 6.03^{b}	73.17 ± 19.47^{b}	1.78 ± 0.22^{a}
4	16.65 ± 6.05^{a}	0.15 ± 0.01^{a}	0.34 ± 0.48^{a}	85.00 ± 10.18^{bc}	1.72 ± 0.04^{a}
5	17.48 ± 7.96^{ab}	0.16 ± 0.02^{a}	1.02 ± 2.17^{ab}	97.20 ± 38.28^{c}	1.55 ± 0.13^{a}

Values are the mean ± S.E.M

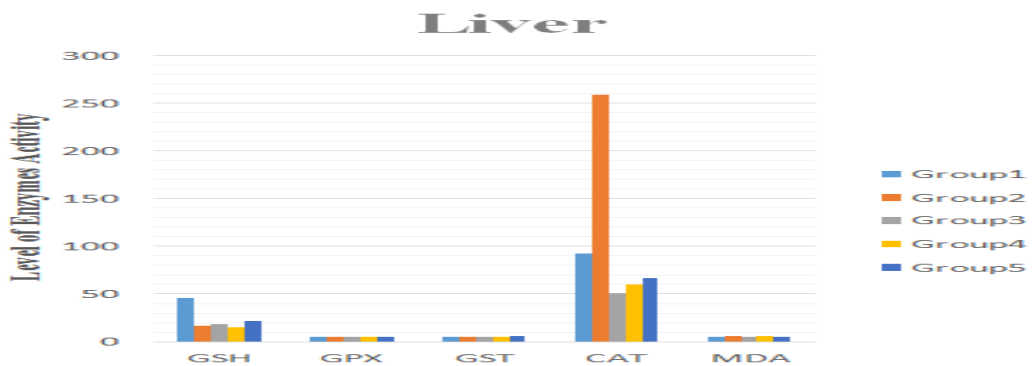

Figure 4: Effect of the administration of yellow bitter yam on antioxidant status on the liver of STZ-induced diabetic rats

Table 4: Effect of yellow bitter yam supplemented diet on GSH, GPX, GST, CAT, and MDA levels in the liver of induced diabetic rats

Group	GSH	GPX	GST	CAT	MDA
1	45.57 ± 22.65^{b}	0.09 ± 0.06^{a}	0.05 ± 0.09^{a}	92.17 ± 53.70^{a}	1.28 ± 0.41^{a}
2	16.37 ± 5.54^{a}	0.16 ± 0.08^{b}	0.14 ± 0.13^{a}	258.83 ± 171.59^{b}	1.65 ± 0.71^{ab}
3	18.53 ± 8.86^{a}	0.13 ± 0.02^{ab}	0.28 ± 0.40^{ab}	50.83 ± 35.66^{a}	2.58 ± 0.10^{c}
4	15.18 ± 3.69^{a}	0.14 ± 0.03^{ab}	0.18 ± 0.34^{ab}	59.83 ± 32.99^{a}	1.94 ± 0.48^{b}
5	21.46 ± 4.69^{a}	0.12 ± 0.02^{ab}	0.50 ± 0.58^{ab}	66.67 ± 18.05^{a}	1.41 ± 0.26^{ab}

Values are the mean ± S.E.M

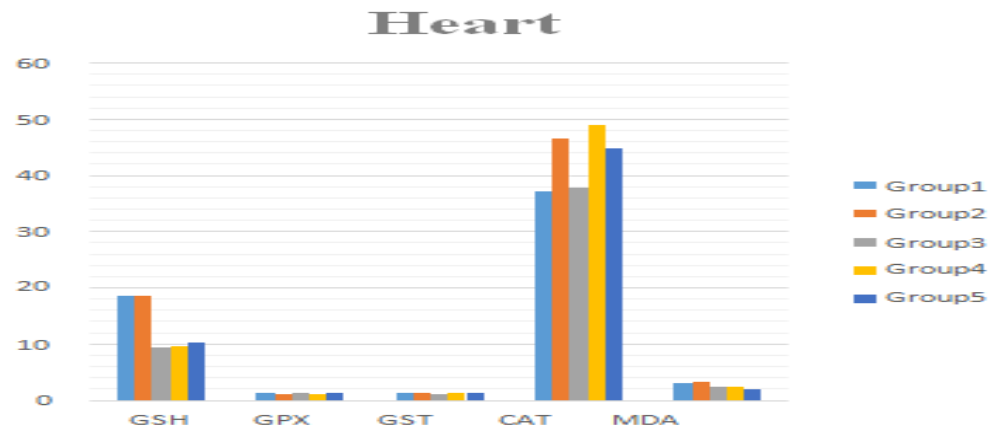

Figure 5: Effectoftheadministrationofyellowbitteryamonantioxidantstatus in theheartof STZ-induced diabetic rat

Table 5: Effect of the administration of yellow bitter yam on GSH, GPX, GST, CAT, and MDA levels in homogenized heart of STZ-induced diabetic rat. *Values are the mean ± S.E.M*

Group	GSH	GPX	GST	CAT	MDA
1	17.58 ± 3.32^{b}	0.27 ± 0.06^{a}	0.29 ± 0.36^{ab}	36.17 ± 5.23^{a}	2.18 ± 0.60^{ab}
2	17.52 ± 6.33^{b}	0.22 ± 0.03^{a}	0.37 ± 0.41^{ab}	45.50 ± 10.37^{c}	2.37 ± 0.41^{b}
3	8.38 ± 1.67^{a}	0.22 ± 0.02^{a}	0.08 ± 0.11^{a}	36.83 ± 5.95^{a}	1.44 ± 0.51^{ab}
4	8.72 ± 1.50^{a}	0.22 ± 0.03^{a}	0.37 ± 0.29^{a}	48.00 ± 10.56^{c}	1.39 ± 0.64^{ab}
5	9.38 ± 0.89^{ab}	0.22 ± 0.05^{a}	0.19 ± 0.28^{a}	43.80 ± 6.90^{b}	1.05 ± 0.41^{a}

Results are expressed as Mean ± Standard deviation of three replicates for each group of rats, n=35 (number of animals per group) using Analysis of Variance (ANOVA) followed by Duncan's (multiple range tests) Post Hoc test; values in the same row for each parameter with superscript different from control are significantly different at $p<0.05$.
GST, Glutathione S-transferase; GPX Glutathione peroxidase; CAT, Catalase; MDH, Malonialdehyde; Group 1- Normal control group, Group 2- Untreated Diabetic control group, Group 3-10% Yellow Bitter Yam treated group, Group 4-20% Yellow Bitter Yam treated group, Group 5- Standard Drugs treated group.

An elevated level of CAT activities was observed in the liver (Fig. 4 and Table 4) and heart (Fig. 5 and Table 5) with a decrease in its concentration in the kidney. This elevation is similar to the work done by Okutan *et al.* [29]. Increased levels of liver homogenate might be due to the hepatoprotective mechanism of the liver. However, CAT activity was attenuated in the groups given 10 and 20% *Discorea dumentorum* supplements. This result confirms the hepatoprotective ability of *D. dumentorum* to alleviate diabetes. CAT activity was significantly attenuated in the kidney of the diabetic untreated group relative to normal control. Whereas the groups supplemented with 10% *D. dumentorum* and Metformin showed elevated levels of CAT activities, with this elevation in the activity of catalase in Group 3 treated with 10% *D. dumentorum*, it is regarded as the most effective in the regulation of catalase activity towards the normal control. Also, in this study, there a significant increase in MDA concentration in streptozotocin-diabetic untreated rats was observed because the hyperglycemic environment inhibited radical scavenging activity and exposed the lipids to oxidation. In contrast, MDA formation was attenuated in the diabetic groups treated with *D. dumentorum* and Metformin which reduced the accumulation of glucose and contributed to the radical scavenging activity, and hence inhibited the oxidation of lipids.

Conclusion

Since diabetes, a metabolic disorder has resulted in mortality and requires continuous medication in its treatment; this current study is a contribution to the search for an anti-diabetic compound from medicinal plants that would play an effective role in alleviating the deleterious impact of the disease. These results portray the nutraceutical effect of *Discorea dumentorum* supplemented diet against diabetes revealing its hypoglycemic and antioxidative effects.

However, further research into the phytochemical compounds of *D. dumetorum* that caused the decreased glucose level, body weight, and the antioxidative constituents should be carried out, as this research only focuses on the nutraceutical effect of *D. dumetorum.*

Conflict of interest: The authors have no competing interest or conflict of interest.

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Citing this Article

Pius, E. M., Ajayi, O. B., Mohammed, A., Atinuke, T., Opeyemi, O. & Musa, A. L. (2024). The effect of post-treatment with yellow bitter yam (*Dioscorea dumetorum*) on the antioxidant status of streptozocin-induced diabetic rats. *Lafia Journal of Scientific and Industrial Research*, *2*(1), 47 – 53. https://doi.org/10.62050/ljsir2024.v2n1.264