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ANTIDIABETIC AND ANTIOXIDANT POTENTIALS OF YOYO BITTERS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Background: This study investigated the antidiabetic, anti-dyslipidemic and antioxidant activities of Yoyo bitters in streptozotocin- induced diabetic rats. **Materials and Methods:** Forty (40) Wistar rats weighing (180-200g) were used and diabetes was induced by single intraperitoneal injection of streptozotocin (50mg/kgbw). The animals were randomly divided into four groups (10rats/group). Group I: control, Group II: untreated diabetic, Group III & IV: diabetic rats treated with 0.5ml of Yoyo bitters and 2mg/kgbw Glimepiride respectively for 4 weeks. Food and water intake were recorded daily, body weight, and blood glucose levels were measured at weekly interval throughout the experiment. On the last day of the experiment, the animals were sacrificed and blood sample were collected for biochemical parameters estimation. **Results:** Administration of Yoyo bitters significantly ($p<0.05$) increased the body weight, feed intake with significant ($p<0.05$) reduced in water intake. The plasma blood glucose, triglycerides (TG), total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels were significantly ($p<0.05$) lower in Yoyo bitters treated group, whereas the high density lipoprotein cholesterol (HDL-C) level significantly ($p<0.05$) increased. Furthermore, Yoyo bitters administration significantly ($p<0.05$) reduce the malondialdehyde (MDA) level and significantly ($p<0.05$) increase total antioxidant capacity (TAC), reduced glutathione (GSH), superoxide dismutase (SOD), glutathione perioxidase (GPx) and Catalase (CAT) levels. Liver enzymes and kidney function parameters were also deccreased ($p<0.05$) significantly. **Conclusion:** The result of these findings demonstrated that Yoyo bitters possess

hypoglycemic, anti-dyslipidemic and antioxidant properties. Yoyo bitters can be a source of alternative therapy for diabetes against the synthetic drugs.

KEYWORDS: Yoyo bitters, Diabetes mellitus, Hyperglycemia, Dyslipidemia, Antioxidant.

INTRODUCTION

Diabetes mellitus and its associated complications are foremost chronic diseases threatening human life and globally reduce quality of life for patient.[1] Diabetes prevalence is rapidly increases and according to the International Diabetes Federation (IDF), nearly estimated 451 million individual were suffering from diabetes in 2017 worldwide and this figure is projected to reach 578 million by 2030, and 700 million by 2045 if the current diabetes growth rate persist.[2]

Diabetes mellitus (DM) is a common chronic metabolic disorder of several etiologies characterized by persistent hyperglycemia, resulting from alteration in carbohydrate, protein, and lipid metabolism due to dysfunction of pancreatic beta-cells insulin secretion, insulin action in peripheral tissues or both.[3] Chronic hyperglycemia, polyphagia, polyuria, polydipsia, glycosuria, coupled with excessive weight loss are the common clinical diagnosis symptoms of diabetes.[4]

Long-term uncontrolled hyperglycemia is associated with dyslipidemia, generation of reactive oxygen species, and declining antioxidant defence enzymes level.[5] Chronic hyperglycemia and diabetic dyslipidemia associated with elevate levels of free radical and the concurrent decline of antioxidant defense system lead to numerous long-term diabetic complications includes micro-vascular damage neuropathy, nephropathy, and retinopathy, and macro-vascular complications cardiovascular disease, stroke, and peripheral vascular disease.[6–8] Oxidative stress also plays a major role in the diabetes progression, pathogenesis of cellular damage, and innumerable diabetic complications.[9]

Till date, there's no proper effective therapeutic drug to combat and manage this particular disease. The existing synthetic oral hypoglycemic drugs for treatment and management of diabetes have many noxious effects and limit their uses.[10] These limitations of synthetic anti-diabetic drug necessitate the search for alternative therapy that may be effective without or less toxic for treating diabetes.

The uses of mono and polyherbal formulations with minimum side effects than the synthetic drugs have been reported to be effective for curable of different diseases.[11] They are mixture of several bioactive chemical compounds from different plant parts with therapeutic potential and are often characterized with the bitter taste.[12] Yoyo Bitters is a polyherbal formulation commonly used in Nigeria which contained *Aloe vera*, *Acinos arvensis*, *Citrus aurantifolia*, *Chenopodium murale* and *Cinamomum aromaticum*. Majority of Yoyo Bitters compounds acclaimed anti-cancer, anti-tumor, anti-ulcer, anti-viral, anti-hyperlipidermic, anti-diabetic, antioxidant, hepato-protective, immune-modulatory and detoxifying properties similar to other bitters due to its phyto-constituents mixture.[13-16] Therefore, this study investigated the hypoglycemic, anti-dyslipidemic and antioxidant potential of Yoyo Bitters in streptozotocin-induced diabetic male Wistar rats.

MATERIALS AND METHODS

Chemicals and Drugs: Yoyo Bitters, Glimepiride, Streptozotocin (STZ), Ketamin and Xylazine.

Experimental Animals

Forty (40) male Wistar albino rats weighing between 180-200g were used for the study. The animals were purchased from the Animal House of Physiology Department, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. The animals were housed in a plastic cage of 10rats/cage and acclimatized for two weeks under free contaminated environment at room temperature (25 ± 2^{o}C), relative humidity (45±5%), 12 hours light and 12 hours dark cycle with access to commercial rats pellets feed with water *add libitum* before the commencement of the experiment. All experimental protocols and handling of animals were in compliance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals for Biomedical Research and were approved by the Institutional Animals Care and Use Committee of Ladoke Akintola University of Technology.

Diabetes Induction

The animals were subjected to 12hrs fasting prior diabetes induction and a single dose of (50mg/kgb.w) of freshly prepared streptozotocin (STZ) dissolved in 0.01 M citrate buffer (pH4.5) was injected intraperitoneal to induced diabetes in overnight fasted rats.[17] The animals were allowed to drink a 20% glucose solution overnight to fend off initial drug induced hypoglycemic death. After 72hours of streptozotocin injection, fasting blood sample were obtained from the rats' tail tip to check the blood glucose level using one touch Accu-

Chek glucometer and animals with fasting blood glucose level higher than 200mg/dL were considered diabetics and selected for the experiment.

Experimental Design and Animals Treatment

The Forty (40) rats were divided into four categories of 10rats/group. Group I: Normal control (non-diabetic); Group II: Diabetic rats (untreated); Group III & IV: Diabetic rats treated with 0.5ml/kg Yoyo Bitters (YB) and 2mg/kgbwp.o Glimepiride (GMP) respectively. Details of the grouping were as follow.

Group I: Normal control (non-diabetic)

Group II: Diabetic rats (untreated)

Group III: Diabetic rats + 0.5 ml of Yoyo Bitters

Group IV: Diabetic rats + 2mg/kgbwp.o Glimepiride

Administration of Yoyo Bitters and Glimepiride was done orally with oral canular. Experimental treatment period lasted for four (4) weeks with feed and water *add libitum*. During the experimental treatment phase, food and water intake were measured on a daily basis.

Plasma Blood glucose level Determination and Body weight Measurement

Body weight and blood glucose level of the rats were recorded at the beginning of the study and every week throughout the experimental treatment period. Glucose oxidase/peroxidase (GOD-POD) method was used to determine the plasma blood glucose level using digital Acuu-chek glucometer and test stripes and body weight was measured with a digital weighing scale.

Sample Collection and Biochemical Assay

At the end of the experimental treatment period, the rats were fasted overnight, anesthetized through intraperitoneal injection of ketamine (75mg/kgb.w) and xylazine (20mg/kgb.w), and sacrificed by cervical dislocation. Fasting blood samples were collected from the rats' heart via cardiac puncture into heparinized tube, centrifuge at 1500rpm for 5mins and clear supernatant plasma was retrieved for biochemical parameters estimation.

Plasma total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-C) levels were determined by enzymes colorimetric method using a commercial Diagnostic Kit (Genzyme Diagnostics, MA, USA) according to manufacturer's instruction.

Level of plasma low density lipoprotein-cholesterol (LDL-C) were calculated using the Friedewald formula; LDL-C = TC - HDL-C - TG/5.[18]

Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant activities levels were measured by enzymelinked immunosorbent assay (ELISA) methods using Rat MDA, SOD, GPx and CAT commercial Elisa Kit (Elabscince, China) followed the protocol provided by the manufacturers. Glutathione reductase (GSH) was measured based on the method described by Gupta and Gupta.[19]

The Liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), in the plasma were determined spectrophotometrically using standard automated techniques based on the manufacturer's instruction. Plasma kidney function markers urea, uric acid and creatinine were estimated using commercially available assay kits (Siemens Health Care Diagnostics).

Statistical Analysis

Statistical Package for Social Sciences (SPSS, version 22.0) was used for data analysis. All of the data were presented as standard error of the mean (mean ± SEM) and statistical mean difference between groups were evaluated using one-way analysis of variance (ANOVA) followed by bonferoni post-hoc test. Statistical difference were considered significant at p-value less than 0.05($p<0.05$).

RESULTS

Effects of Yoyo Bitters on Body weight, Food and Water intake in Streptozotocin-Induced Diabetic Rats

Body weight and food intake of untreated diabetic rats were significantly ($p<0.05$) decreased with significant ($p<0.05$) increase in water intake compared with non-diabetic rats (normal control). Treatment of diabetic rats with Yoyo bitters and Glimepiride result in significant increase in body weight, food intake and reduction of water intake when compared with untreated diabetic rats. The increase in body weight of diabetic rats treated with Glimepiride was higher than that of Yoyo bitters treated group. However, both Yoyo bitters and Glimepiride treated groups showed succeeded similar effect on increased food intake near the non-diabetic group value (figure1. a, b, and c).

Effects of Yoyo Bitters on Blood glucose, Lipid profile and Total antioxidant capacity in Streptozotocin-Induced Diabetic Rats

There was significant ($p<0.05$) increase in blood glucose level of untreated diabetic rats when compared with non-diabetic rats (normal control). Administration of Yoyo bitters significantly reduced the plasma blood glucose level in comparison to the untreated diabetic rats (figure. 2a). Also, total cholesterol (TC), triglycerides (TG) and low density lipoprotein-cholesterol (LDL-C) levels in untreated diabetic rats significantly ($p<0.05$) increased with reduced high density lipoprotein-cholesterol (HDL-C) level compared to the non-diabetic rats. Supplementation of Yoyo bitters to the diabetic rats markedly lower the TC, TG, and LDL-C levels than Glimepiride and improved the HDL-C level respectively compared with untreated diabetic rats (figure.2b).

Total antioxidant capacity level of untreated diabetic rats significantly ($p<0.05$) lower than the control rats (non-diabetic). Treatment with Yoyo bitters elevate the total antioxidant capacity level compared to the untreated diabetic animals (figure. 2c).

Effects of Yoyo Bitters on Antioxidant Enzymes and Oxidative Stress Markers in Streptozotocin-Induced Diabetic Rats

There was a significant ($p<0.05$) decrease in the activities of reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase (CAT) in the untreated diabetic rats as compared to the control rats (non-diabetic). In contrast, malondialdehyde (MDA) level was significantly ($p<0.05$) increased in untreated diabetic group when compared to the control group. Antioxidant activities of GSH, SOD, GPx and CAT levels were significantly enhanced with depletes level of MDA in diabetic rats treated with Yoyo bitters and reference drug in comparison to the untreated diabetic group. Treatment of diabetic rats with Yoyo bitters remarkable lowering the MDA level and improving the SOD and GSH levels more than the glimepiride. However, glimepiride group exhibits higher levels of GPx and CAT than Yoyo bitter (table 1).

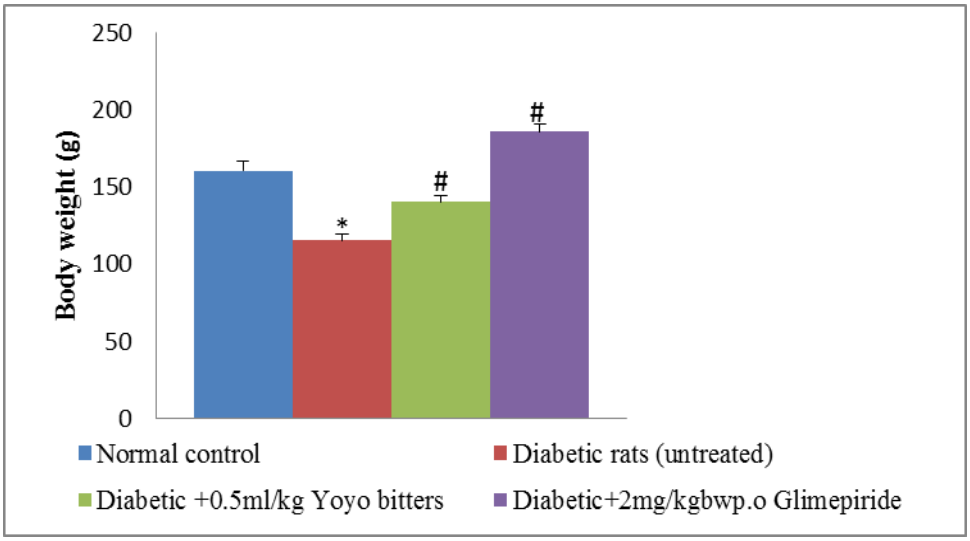
Effects of Yoyo Bitters on Markers of Liver Enzymes and Kidney Function in Streptozotocin-Induced Diabetic Rats

The untreated diabetic rats demonstrated a significant ($p<0.05$) elevation of hepatic enzymes activities aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) compared with the non-diabetic group (normal control).Administration of

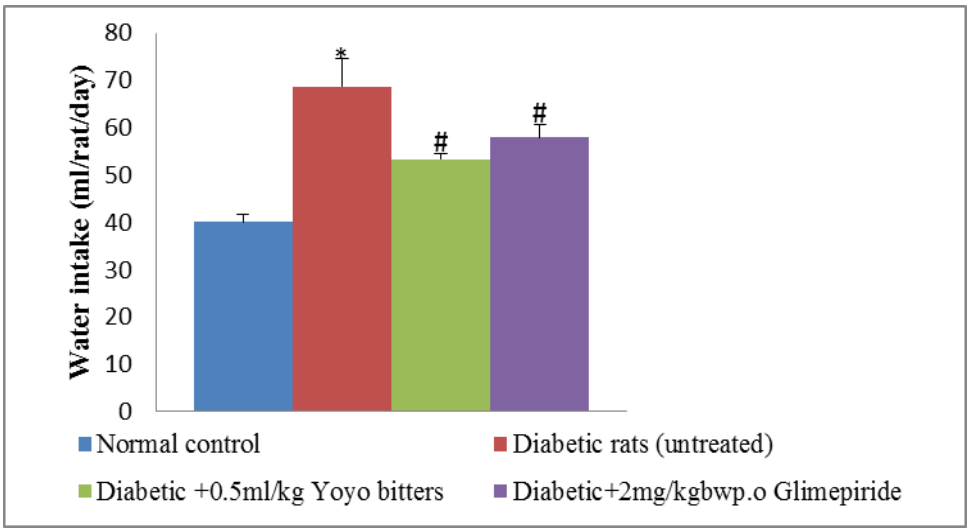
Yoyo bitters to the diabetic rats diminished the level of hepatic enzymes AST, ALT and ALP activities when compared to the untreated diabetic rats (table 2).

In addition, there was a significant ($p<0.05$) increase in plasma kidney function parameters urea, uric acid and creatinine in untreated diabetic rats compared respectively with non-diabetic group. The levels of urea, uric acid and creatinine were significantly reduced after treatment with Yoyo bitter compared to the untreated diabetic group (table 2).

a.



b.



c.

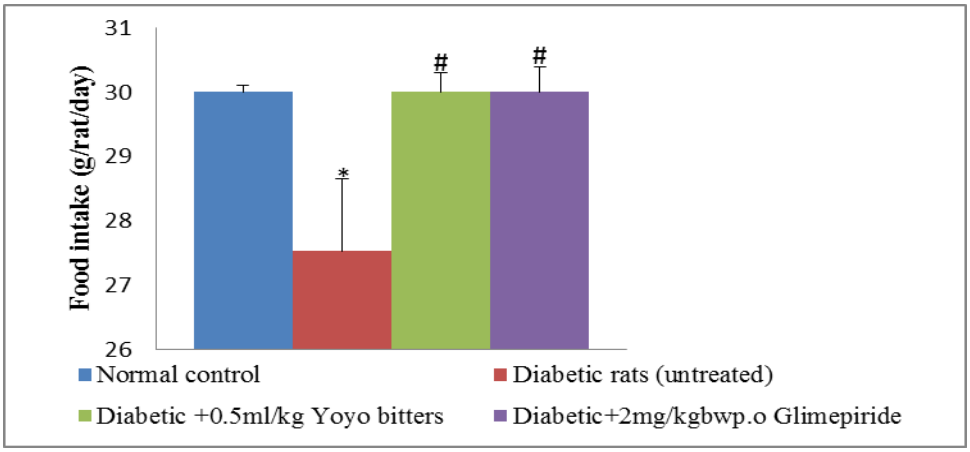
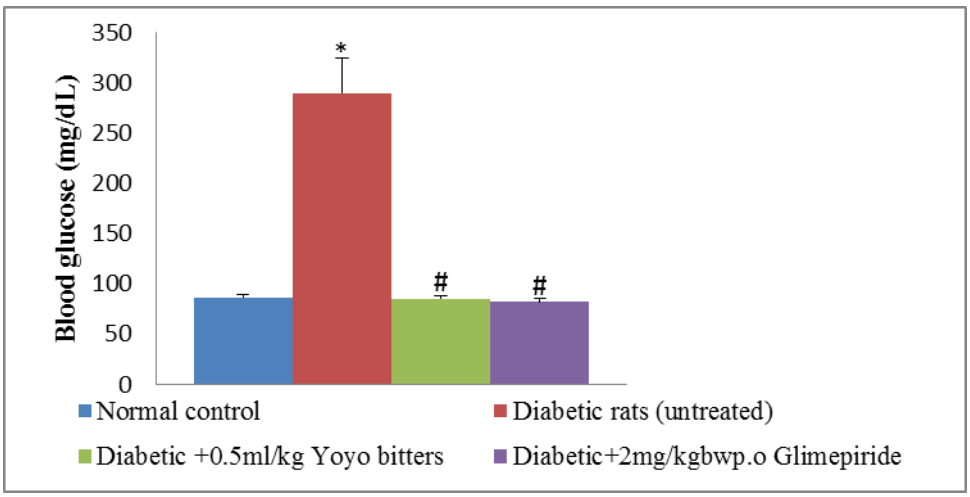


Figure 1: Effects of Yoyo bitters on (a) body weight (b) water intake (c) food intake in streptozotocin (STZ)-induced diabetic rats. ***Values are expressed as mean ± SEM (n=10). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.***

a.



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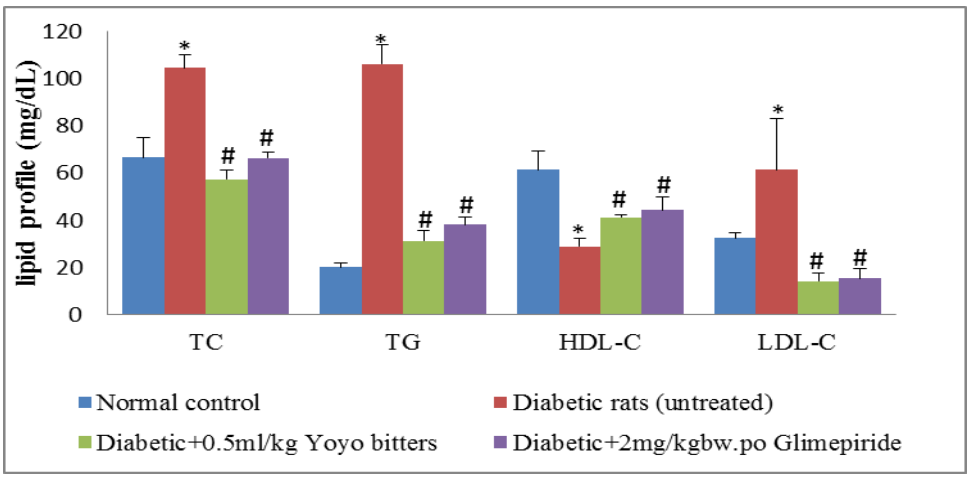

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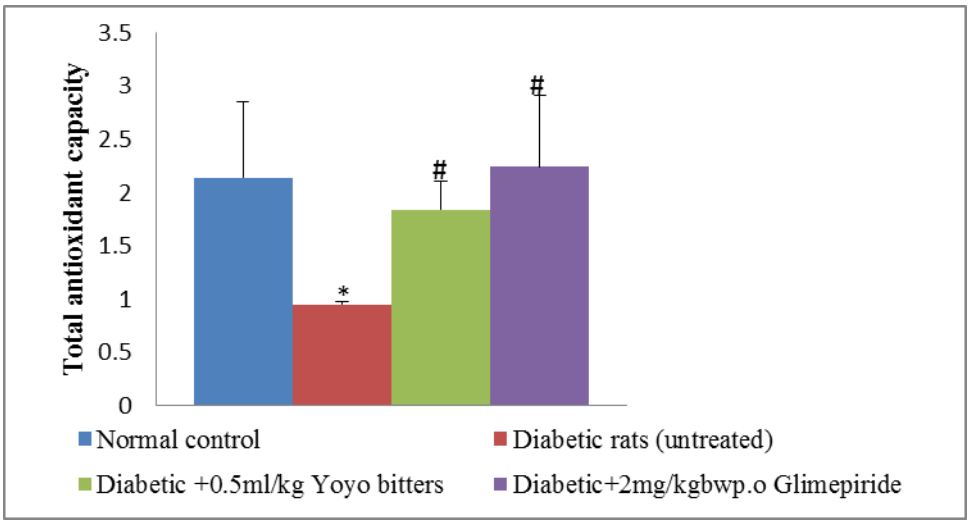

Figure 2: Effects of Yoyo bitters on (a) blood glucose level (b) lipid profile parameters (c) total antioxidant capacity in streptozotocin (STZ)-induced diabetic rats. ***Values are expressed as mean ± SEM (n=10). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.***

Table 1: Effects of Yoyo bitters on Oxidative Stress Parameter and Antioxidant Defence Enzymes Level in Streptozotocin (STZ)-Induced Diabetic Rats.

Parameters	Control	Diabetic (untreated)	Diabetic + 0.5ml/kg Yoyo bitters	Diabetic +2mg/kgbwp.o Glimepiride
MDA(µM)	4.06 ± 0.16	8.54 ± 0.95*	5.54 ± 0.62#	5.62 ± 1.18#
GPx(U/L)	9.00 ± 1.22	1.87 ± 0.27*	6.00 ± 0.53#	9.00 ± 0.79#
SOD(µ/ml)	1.21 ± 0.013	1.07 ± 0.02*	1.30 ± 0.04#	1.07 ± 0.03#
CAT(mol/ml/mins)	53.12 ± 2.83	28.35 ± 0.32*	49.41 ± 2.33#	53.39 ± 3.22#
GSH(mM)	3.95 ± 0.53	2.79 ± 0.13*	4.95 ± 0.33#	4.28 ± 0.25#

*Values are expressed as mean ± SEM (n=10). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.*

Table 2: Effects of Yoyo bitters on Markers of Liver Enzymes and Kidney Function in Streptozotocin (STZ)-Induced Diabetic Rats.

Parameters	Control	Diabetic (untreated)	Diabetic +0.5ml/kg Yoyo bitters	Diabetic + 2mg/kgbwp.o Glimepiride
Aspartate aminotransferase (AST) (U/L)	69.30 ± 2.64	108.50 ± 8.92*	76.47± 1.85#	72.67 ± 5.61#
Alanine aminotransferase (ALT) (U/L)	123.60 ± 14.87	226.20 ± 23.76*	82.27 ± 7.60#	88.90 ± 7.48#
Alkaline phosphatase (ALP) (mol/ml/mins)	137.00 ± 7.14	350.30 ± 33.55*	126.33 ± 4.05#	128.16 ± 5.41#

Urea (mg/dL)	20.19 ± 0.67	25.66 ± 1.23*	20.29 ± 0.58[#]	20.65 ± 0.61[#]
Uric acid (mg/dL)	3.55 ± 0.5	4.98 ± 0.44*	3.92 ± 0.45[#]	3.08 ± 0.38[#]
Creatinine (µmol)	69.21 ± 4.16	140.18 ± 8.28*	66.63 ± 3.62[#]	70.97 ± 6.17[#]

*Values are expressed as mean ± SEM (n=10). *significant at p<0.05 compared with control; [#]significant at p<0.05 compared with untreated diabetic group.*

DISCUSSION

Herbal medicines have contributes enormously as an alternative or complementary therapy in diseases alleviating. Several studies have investigated antidiabetic activities of various plants and have confirmed potentials for therapeutic efficacies.[20, 21] This study investigated anti-diabetic potential of Yoyo bitters.

In the present study, increase in water intake, and excessive body weight loss was observed in the diabetic group rats. The excessive water consumption by hyperglycemic rats is typical sign of diabetes. The present results are in line with the findings reported by Parmar in which water intake increase in hyperglycemic rats.[22] The increase in water intake may be the consequence of obligatory renal water loss combined with hyper-osmolarity in diabetes which tends to reduce intracellular water, triggering the osmo-receptor of the thirst centre of the brain and this result in polydipsia which leads to water intake.[23] The decrease in body weight in the diabetic rats might be the result of protein wasting due to insulin deficiency and decreased sensitivity of muscle tissue to insulin.[24-26] Also, decrease in food intake observed in this study might contribute to the body weight loss. Improvements in diabetic conditions were observed after administration of Yoyo bitters.

Diabetes mellitus is associated with hyperglycemia.[27] Hyperglycemia was observed in present study as the diabetic rats showed elevated fasting blood glucose level. These findings agree with Reham *et al*. who reported substantial increase in fasting blood glucose level of diabetic rats.[28] Treatments with Yoyo bitter lower the blood glucose level of the diabetic rats which indicate hypoglycemic activity of this herb which support the result of Arhewoh et al.[29] The possible mechanisms of blood glucose lowering effect Yoyo bitters could be due to potentiation of glucose induced insulin release and increasing peripheral uptake of glucose.

Dyslipidemia is a complication of diabetes and has been reported to result from excess mobilization of fat from the adipose tissues due to under-utilization of glucose or inhibition of the hormone sensitive lipase by insulin.[30, 31] The observed elevated plasma levels of total cholesterol (TC), triglyceride (TG) low-density lipoprotein cholesterol (LDL-c) and

decreased level of high density lipoprotein cholesterol (HDL-c) of diabetic rats in this current study are in accord with previous reports documented on elevated levels of TG, LDL and TC in diabetic subject.[32] However, on treatment of the diabetic groups with Yoyo bitters, there were significant reductions in TG,TC, and LDL levels while HDL-c was increasing. This observation is in consonance with the findings that most hypoglycemic plants have potentials of ameliorating diabetic lipid metabolism abnormality.[33] Several previous studies have reported alkaloids, flavonoids, saponins, and cardiac glycosides possess a hypolipidemic effect.[34] These phytochemicals were present in the Yoyo bitters.

Oxidative stress has been implicated in diabetes both type 1 and 2 by increasing level of lipid peroxidation which generate free radicals in many vital organs and subsequently decline in activities of endogenous antioxidant enzymes.[35-38] In the present study lipid peroxidation marker plasma malondialdehyde (MDA) levels were found to be higher accompanied by a decrease in plasma antioxidant enzymes reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities. These findings corroborate previous studies.[39] The changes of antioxidant parameters may be due to an increase in reactive oxygen species (ROS) involved in diabetes mellitus production and progression.[40] Yoyo bitters and glimepiride administrations decreased the MDA level and improved GSH, SOD, GPx and CAT levels in diabetic rats. This suggests that Yoyo bitters have antioxidants compound that can reverse the damage caused by diabetes.

In addition to the moderation of dyslipidemia, hypoglycemic and antioxidant effects, Yoyo bitters exhibited medicinal protective effect by reducing some markers of tissue injuries. Yoyo bitters decreases plasma liver enzymes (ALT, AST, and ALP) and Kidney function (creatinine, uric acid and urea) activities which were increased in diabetic rats in this study. The effect of Yoyo bitters on liver enzyme markers and kidney function parameters is in agreement with several other studies on effects of medicinal plant in diabetic rats.[41, 42]

CONCLUSION

These findings showed that Yoyo bitters exhibit potent hypoglycemic, anti-dyslipidemic and antioxidant activities. These therapeutic effects might stem from different phytochemicals compound of Yoyo bitters. Therefore, Yoyo bitters can be used as alternative therapy for diabetes and prevention of diabetes complications.

Declarations

Authors' Contributions

FO conceived the original idea, designed and supervised the research. MO performed the experiments with the support of FO. FO, MO collected the data. MO analyzed the data and prepared the manuscript. FO reviewed the manuscript. All authors' have read and approved the final manuscript.

Ethics Approval

All procedures were approved by the Animal care committee of the Ladoke Akintola University of Technology and conducted according to the "Principles of Laboratory Animal Care" and specific national laws where applicable.

Consent for Publication

All authors agreed to publish the article.

Availability of Data and Materials

All data generated and analyzed during this study are included in this article.

Competing Interests

No competing interests.

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REFERENCES

1. Raafat K, El-Haj R, Shoumar D, Alaaeddine R, Fakhro Y, Tawil N, Shaer F, Daher A, Awada N, Sabra A, Atwi K, Khaled M, Messi R, Abouzaher N, Houri M and Al Jallad S. **(2017).** Neuropathic Pain: Literature Review and Recommendations of Potential Phytotherapies. Pharmacognosy Journal, 9(4).
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwinb N, Colagiuric S, Guariguated L, Motalae AA, Ogurtsovaf K, Shawg JE, Bright D and Williams R. **(2019).** Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9th edition. Diabetes Res Clin Pract; 157: 107843.

3. American Diabetes Association, "Standards of medical care in diabetes—2017," *The Journal of Clinical and Applied Research and Education*, vol. 40, supplement 1, pp. S11–S24, 2017.
4. Pawar D, Thalkur P, Radhe B, Jadhar H and Behire V. **(2017)**. The Accuracy of polyuria, polydysia, polyphagia and Diabetes Risk score in Adults screened for Diabetes type –2. Medical Journal of Dr. D.Y. Palfil University, 10(3): 263 –526.
5. Hu XF, Zhang Q, Zhang PP, Sun LJ, Liang JC, Morris-Natschke SL, Chen Y and Lee KH. **(2018)**. Evaluation of in vitro/in vivo anti-diabetic effects and identification of compounds from Physalis alkekengi. Fitoterapia, 127: 129–137.
6. Afroz A, Zhang W, Wei AJ. Loh D, Jie Lee X and Billah B. **(2019)**. Macro- and micro-vascular complications and their determinants among people with type 2 diabetes in Bangladesh, Diabet. Metab. Syndr, 13(5): 2939–2946.
7. Forbes JM and Cooper ME. **(2013).** Mechanisms of diabetic complications, Physiol. Rev., 93(1): 137–188.
8. Massaro JD, Polli CD, Costa ESM, Alves CC, Passos GA Sakamoto-Hojo ET et al. **(2019).** Post-transcriptional markers associated with clinical complications in Type 1 and Type 2 diabetes mellitus, Mol. Cell. Endocrinol. 490: 1–14.
9. Shi GJ, Zheng J, Wu J, Qiao HQ, Chang Q, Niu Y, Sun T, Li YX and Yu JQ. **(2017)** Beneficial effects of Lycium barbarum polysaccharide on spermatogenesis by improving antioxidant activity and inhibiting apoptosis in streptozotocin-induced diabetic male mice. Food Funct. 8: 1215–1226.
10. Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C and del Cañizo-Gómez FJ. **(2016)**. Update on the treatment of type 2 diabetes mellitus. World J. Diabetes, 7: 354–395.
11. Ahmed S, Hasan MM and Mahmood ZA. **(2016)**. Antiurolithiatic plants: Formulations used in a. different countries and cultures. *Pak J Pharm Sci.*, 29(6): 2129-2139.
12. Bussmann RW, Glen A, Meyer K, Kuhlman A and Townsmith A. **(2010).** Herbal mixtures in traditional medicine in Northern Peru. Journal of Ethnobiology and Ethnomedicine, 6-10.
13. Anyasor GN, Ogunbiyi B and Akinlusi IA. **(2017)**. Antioxidant and anti-inflammatory properties of selected polyherbal preparations: Oroki herbal, Swedish bitters and Yoyo bitters. *Oxid Antioxid Med Sci.*, 5(2): 25-29.
14. Kale OE, Akinpelu OB, Bakare AA, Yusuf FO, Gomba R, Araka DC, Ogundare TO, Okolie AC, Adebawo O and Odutola O. **(2018)**. Five traditional Nigerian polyherbal

remedies protect against high fructose fed, streptozotocin-induced type 2 diabetes in male Wistar rats. *BMC Complement Altern Med.*, 18: 160-171.

15. Imaga NOA and Ogunnusi OT. **(2014)**. Biochemical assessment of the therapeutic properties of Yoyo(R) herbal bitters. *Int J Biol Pharm Allied Sci.*, 3(1): 8-20.
16. Anionye J and Onyeneke EC. **(2016)**. Composition and *in vitro a*ntioxidant capacity of Alomo bitters. *Eur J Biol Sci.*, 8(3): 116-123.
17. Furman BL. **(2015).** Streptozotocin-induced diabetic models in mice and rats, Curr. Protoc. Pharmacol, 70: 5.47.1-5..20.
18. Friedewald WT, Levy RI and Fredrickson DS. **(1972).** "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," *Clinical Chemistry*, 18(6): 499–502.
19. Gupta R, and Gupta RS. **(2009).** Protective Role of Pterocarpus marsupium in Diabetes-Induced Hyperlipidemic Condition. Journal of Complementary and Integrative Medicine, 6(1): 21.
20. Ezuruike UF and Prieto JM. **(2014).** The use of plants in the traditional management of diabetes in Nigeria: pharmacological and toxicological considerations. J Ethnopharmacol, 155(2): 857–924.
21. Gbolade AA. **(2009).** Inventory of antidiabetic plants in selected districts of Lagos state Nigeria. J Ethnopharmacology, 121(1): 135–9.
22. Parmar K, Patel S, Patel J, Patel B and Patel M. **(2011).** Effects of bittergourd (Momordica charantia) fruit juice on glucose tolerance and lipid profile in type-II diabetic rats. Int. J. Drug Dev. Res. 3: 139–146.
23. Goje LJ, Maisamari CA, Maigari FU, Ghamba PE, Goji ADT and Mshellia P. **(2014).** The Hypoglycemic and Hypolipidemic Effects of the Aqueous Extract of Vernonia Amygdalina Leaves on Alloxan Induced Diabetic Albino Rats. Int. J. Sci. 9, 5–11.
24. Teng BS, Wang CD, Zhang D, Wu JS, Pan D, Pan LF, Yang HJ and Zhou P. **(2012).** Hypoglycemic effect and mechanism of a proteoglycan from Ganoderma lucidum on streptozotocin-induced type 2 diabetic rats. Eur. Rev. Med. Pharmacol. Sci. 16, 166–175.
25. Subash Babu P, Prabuseenivasan S and Ignacimuthu S. **(2007).** Cinnamaldehyde—A potential antidiabetic agent. Phytomed. Int. J. Phytother. Phytophar. 14, 15–22.
26. Abdel-Sattar E, El-Maraghy SA, El-Dine RS Rizk SM. **(2016).** Russelioside b, a pregnane glycoside ameliorates hyperglycemia in streptozotocin induced diabetic rats by regulating key enzymes of glucose metabolism. Chem.-Biol. Interact. 252, 47–53.

27. Ajiboye BO, Oyinloye BE, Agboinghale PE, et al. **(2019).** Antihyperglycaemia and related gene expressions of aqueous extract of Gongronema latifolium leaf in alloxan induced diabetic rats. Pharm Bio, 57: 604–611.
28. Reham Arafat A, Sindi Heba A and Darwish Amnah M. **(2020).** "Hypoglycemic Effect of *Solanumnigrum* Leaf Extract on Male Diabetic Rats", International Journal of Pharmaceutical and Phytopharmacological Research, 10(2):10-18.
29. Matthew Arhewoh I, Eraga Sylvester O, Jonah Irabor and Iwuagwu Magnus A. **(2017).** A study on the interaction between metformin and constituents of a commercial herbal product'', *Trop J Pharm Res,* 16(7): 1703
30. Nimenibo-Uadia R. **(2003).** Effect of aqueous extract of Canavalia ensiformis seeds on hyperlipidaemia and hyperketonaemia in alloxan-induced diabetic rats. Afr. J. Biochem, 15: 7–15.
31. Sharma SB, Gupta S, Ac R, Singh UR, Rajpoot R and Shukla SK. **(2010).** Antidiabetogenic action of Morus rubra L. leaf extract in streptozotocin-induced diabetic rats. J. Pharm. Pharmacol. 62: 24755.
32. Soliman AM. **(2016).** Potential impact of Paracentrotus lividus extract on diabetic rat models induced by high fat/streptozotocin. J. Basic Appl. Zool. 77: 8–20.
33. Nwogor UA. **(2016).** Effects of Prolonged Administration of Aqueous Extract of Ocimum gratissimum (Scent Leaf) on Blood Glucose and Lipid Profile in Alloxan Induced Diabetic Albino Rats. Am. J. Biomed. Life Sci., 4: 30–34.
34. Ezekwe CI and Obidoa O. **(2001).** Biochemical effect of Vernonia amygdalina on rats liver microsome. Niger. J. Biochem. Mol. Biol. 16: 1745–1798.
35. Abdullah KM, Abul Qais F, Hasan H and Naseem I. **(2019).** Anti-diabetic study of vitamin B6 on hyperglycaemia induced protein carbonylation, DNA damage and ROS production in alloxan induced diabetic rats. Toxicology research, 8(4): 568–579.
36. Ulla A, Alam MA, Rahman M, Isha Olive Khan DM, Sikder B, Islam M, Rahman T, Rahman N, Reza HM, Jain P and Subhan N. **(2019).** Supplementation of Citrus maxima fruits peel powder improves glucose intolerance and prevents oxidative stress in liver of alloxan-induced diabetic rats. Mediterranean Journal of Nutrition and Metabolism, 12(1): 33–44.
37. Robertson RP and Harmon JS. **(2006).** Diabetes, glucose toxicity, and oxidative stress: a case of double jeopardy for the pancreatic islet β cell. Free Radical Biology and Medicine, 41(2): 177–184.

38. Jia G, Whaley-Connell A and Sowers JR. **(2018).** Diabetic cardiomyopathy: a hyperglycemia-and insulin-resistance-induced heart disease. Diabetologia, 61(1): 21–28.
39. Afify AMR, El-Beltagi HS, Fayed SA and El-Ansary AE. **(2018).** In vivo correlation of olive leaves extract on some oxidative stress markers in streptozotocin-induced diabetes mellitus in rats, Grasas y Aceites, 69(1): 243.
40. Moussa S. **(2008).** Oxidative stress in diabetes mellitus. Romanian J Biophys, 18(3): 225-236.
41. Ighodaro OM, Akinloye OA, Ugbaja RN and Omotainse SO. **(2017).** Sapium ellipticum (Hochst.) pax ethanol leaf extract maintains lipid homeostasis in streptozotocin-induced diabetic rats. International Scholarly Research Notices, 1-7.
42. Mollica A, Zengin G, Locatelli M, Stefanucci A, Mocan A, Macedonio G, Carradori S, Onaolapo O, Onaolapo A, Adegoke J, Olaniyan M, Aktumsek A, Novellino E. **(2017).** Anti-diabetic and anti-hyperlipidemic properties of Capparis spinosa L.: in vivo and in vitro evaluation of its nutraceutical potential. Journal of Functional Foods, 35: 32–42.