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Research Article

Oral Glucose Tolerance and Protective Effect of Aqueous Extract of *Xylopia Aethiopica* Seed on 2,2-Azobis(2-Amidinopropane) Dihydrochloride (AAPH) Induced Oxidative Stress

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ABSTRACT

In Nigeria*, Xylopia aethiopica* (Annonaceae) is locally used in tea and beverages, and in treating constipation. This study evaluated the oral glucose tolerance and protective effect of *X. aethiopica* seed extract on 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress in red blood cells (RBCs). Twelve experimental mice were randomly distributed into four groups of three mice administered orally with distilled water, metformin, and *X. aethopica* aqueous seed extract. Glucose was administered orally to 12 hour fasted mice, and plasma glucose was evaluated after 0-4 hours. Antioxidant activity was evaluated using prepared RBCs from mice after pre-treatment with AAPH. Significant increase ($p < 0.05$) was observed in glucose level of all treated mice after 30 minutes, which gradually decreased to 11.41%, 16.52%, and 14.19% for aqueous extract of *X. aethiopica* seed (250 and 500 mg/kg b. wt) and metformin (14.5 mg/kg b.wt) respectively. The antioxidant activity indicated that the extract possessed higher hydroxyl radical scavenging activity compared with vitamin C. More so, aqueous extract of *X. aethiopica* seed protected cells against AAPH-induced oxidative stress in cells by decreasing the activity of catalase generated and malondialdehyde concentration after 40 minutes of incubation at 2.5 mg/mL compared with vitamin C. No significant difference ($p > 0.05$) was observed in superoxide dismutase activity *in vitro*. The total tannin and phenolic contents of *X. aethiopica* seed were 509.16±192.13 mgQE/g and 1.59±0.63 mgGAE/g, respectively. The study concludes that aqueous extract of *X. aethiopica* seed possesses glucose-lowering and antioxidant detoxifying properties against 2,2-azobis(2-amidinopropane) dihydrochloride-induced oxidative stress *in vitro*.

Keywords: *Antioxidant; Detoxification; Oxidative stress; Seed; Xylopia aethiopica*

INTRODUCTION

Diabetes is a chronic metabolic syndrome characterized by problems of insulin action, and insulin secretion, as well as elevated blood sugar, and high triglycerides (Jing *et al.*, 2021). It has a negative impact on people's quality of life and lifespan since it is associated with long-term tissue damage, and impaired metabolism of carbohydrate, fat and protein. Currently, synthetic drugs are the main intervention for managing diabetes. However, they present several detrimental effects that warrant the search for alternative therapy in natural products such as plants (Xuan *et al.*, 2014). Lifestyles and diets are other strategies employed by diabetic people to regulate their increased blood glucose (Gray and Threlkeld, 2015; Okoduwa and Abdulwaliyu, 2023).

Numerous data indicate that frequent eating of plant-based diet considerably enhances diabetic patients' immune system and the level of blood sugar. Several plants are known to be rich sources of phytochemicals with anti-diabetic properties. These plants are less toxic when used as an alternative strategy to synthetic antidiabetic agents (Bommakanti *et al.*, 2023). Some of these plants that have been scientifically proven to display antidiabetic activity are *Moringa oleifera* (Sarode *et al.*, 2023), Angelica (Enoki *et al.*, 2007; Halid and Rahmawati, 2023), *Xylopia aethiopica* L. (Liu *et al.*, 2023) and Chamomile (Parveen *et al.*, 2023).

In West African countries, *X. aethiopica* has been widely used as a food condiment, as well as traditional medicine for diabetes, malaria, fungal infections, uterine fibroid, female infertility, and other conditions (Liu *et al.*, 2023). According to recent pharmacological studies, *X. aethiopica* fruit extract can delay the digestion of carbohydrates, reduce oxidative stress in a type 2 diabetic rat model as well as enhance enzymes related to glucose metabolism (Adeyeoluwa *et al.*, 2020). In addition to the above properties, it was discovered that the plant exerted hypolipidemic effect by lowering the level of lipids in serum of experimental animals (Adefegha *et al.*, 2018). In a previous work, the aqueous seed extract of *X. aethiopica* contained significant quantities of secondary metabolites and exerted potent *in vitro* antioxidant property (Tijjani *et al.*, 2022). In the same study, it was also shown that the seed, pod, and whole seed extracts of *X. aethiopica* L. prevented the accumulation of conjugate dienes, and thrombolytic activities, and maintained membrane stability. Significant quantities of essential oils from the plant were found to have antioxidant properties, and its aqueous extract protect haemoglobin (Hb) from oxidation and subsequent red blood cell (RBC) lysis (Tijjani *et al.*, 2022).

Given the notable antioxidant and anti-diabetic properties of *X. aethiopica* seed extract, the goal of this study is to assess oral glucose tolerance ability and the protective effect of *X. aethiopica* seed extract on 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress in RBCs.

MATERIALS AND METHODS

Chemicals and Reagents

Hydrogen peroxide (H_2O_2), phosphomolybdenum, Vitamin C and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were of analytical grades and prepared using distilled water.

Plant material

Xylopia aethiopica whole seed was purchased from Azare market, Bauchi State, Nigeria and authenticated at the Biological Sciences Department, Sa'adu Zungur University, Gadau, Nigeria. A voucher sample was deposited at the herbarium with the Federal College of Forestry, Jos, Plateau State.

Preparation of plant material

The aqueous extract of *Xylopia aethiopica* seed was prepared by soaking fifty grams (50 g) of the powdered seed in 250 mL distilled water for 24 hours in a tightly stoppered glass container. After which the sample was filtered using Whatman No. 1 filter paper. The filtrates were concentrated separately using a rotary evaporator at 40^{o}C, to generate the crude extract used for the study.

Animal studies

Twenty-five (25) albino mice of both sexes were purchased from the Pharmacology Animal Unit, Department of Pharmacology, Sa'adu Zungur University, Gadau, Nigeria. They were allowed free access to standard mice feed and water *ad libitum*. Thirteen (13) animals were sacrificed in batches under diethyl ether anesthesia to obtain serum, plasma and blood cells. Others were used for Oral Glucose Tolerance Test. The University of Jos Ethical Review Committee approved the research procedures with the registration number UJ/FPS/F17-00379

Oral glucose tolerance test (OGTT)

Twelve (12) non-diabetic mice were randomly distributed into four (4) groups after checking their baseline glucose levels using a glucometer (Accu-Chek Active). A standard oral dose of glucose (2 g/kg bwt.) was administered orally to each mouse after overnight fasting for 12 hours. The blood glucose levels were monitored at regular intervals to evaluate the glucose tolerance ability following treatment with metformin, and *X. aethiopica* aqueous seed extract at 250 and 500 mg/kg bwt. Blood glucose levels were measured from tail veins after 30 minutes, 1, 2 and 4 hours of glucose administration.

In vitro antioxidant assays

Total tannins (TTC) and total phenolic contents (TPC) were determined in the *X. aethiopica* extract using the methods described by Belguidoum *et al.* (2015). The *in vitro* hydroxyl scavenging activity in blood cells after treatment with *X. aethiopica* extract was determined according to the method described by Smirnoff and Cumbes (1989). Total protein was measured according to the method of Bradford (1976), while malondialdehyde (MDA) concentrations, superoxide dismutase (SOD) and catalase (CAT) activities were determined according to the methods of Varshney and Kale (1990), Misra and Fridovich (1972) and Sinha (1972) respectively.

Statistical analysis

Data were subjected to statistical analysis using one-way analysis of variance followed by Duncan multiple range test (SPSS version 20, SPSS Inc., Chicago. IL, USA). Data (where applicable) are presented as mean ± standard deviation (SD). Significance levels were considered at $p < 0.05$ while the graphs were generated using GraphPad Prism 6 software (GraphPad Software, California, USA).

RESULTS AND DISCUSSION

Results

Anti-hyperglycemic activity of the aqueous seed extract in oral glucose-loaded mice shows no significant difference (p>0.05) in baseline blood glucose level across groups just before the administration of metformin, *Xylopia aethiopica* seed extract and glucose. Between groups, analysis showed that administration of metformin at the dose of 14.2mg/kg body weight was able to lower the blood glucose levels of the mice. However, four hours after treatment, aqueous extract of *X. aethiopica* seed at 250 mg/kg body weight and 500 mg/ kg body weight were able to significantly lower ($p < 0.05$) the blood glucose respectively compared with the control group (Figure 1).

The total tannin content and total phenolic content of the aqueous seed extract of *Xylopia aethiopica* are presented in Figure 2. The aqueous extract contains more total tannins (509.16 ± 192.13 mg QE/g) than total phenol (1.59 ± 0.63 mg QAE/g).

The antioxidant activity of aqueous seed extract of *X. aethiopica* indicated that the extract demonstrated high hydroxyl radical scavenging activities compared with vitamin C used as standard compound (Figure 3).

Furthermore, the aqueous seed extract increased protein secretion in cells against AAPH-induced oxidative stress *in vitro* (Figure 4). Moreover, *X. aethiopica* seed extract protected cells against AAPH-induced oxidative stress *in situ* by decreasing the activity of catalase generated (Figure 5). However, there were no significant differences in superoxide dismutase even with differences in concentration and duration (Figure 6). *X. aethiopica* seed extract protected cells indicated against AAPH-induced oxidative stress *in vitro* by decreasing the concentration of malondialdehyde generated (Figure 7).

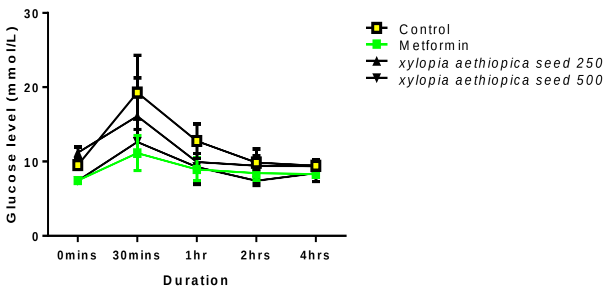

Figure 1. Effect of Aqueous Extract of *Xylopia aethiopica* Seed on Glucose Tolerance of Mice.

Values are mean ± SD, n = 3, Dosages are in mg/kg body weight

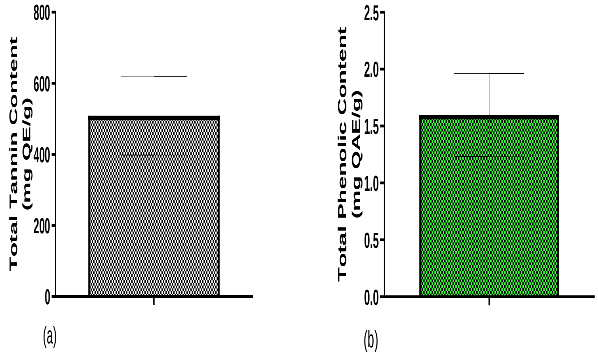

Figure 2. (a) Total Tannin and (b) Total Phenolic Contents of Aqueous Extract of *Xylopia aethiopica* Seed.

Values are mean ± SD, n = 5

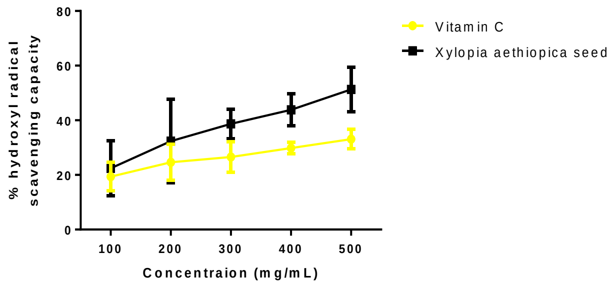

Figure 3. Hydroxyl Radical Scavenging Activities of Aqueous Extract of *Xylopia aethiopica* Seed.

Values are means ± SD, n = 5

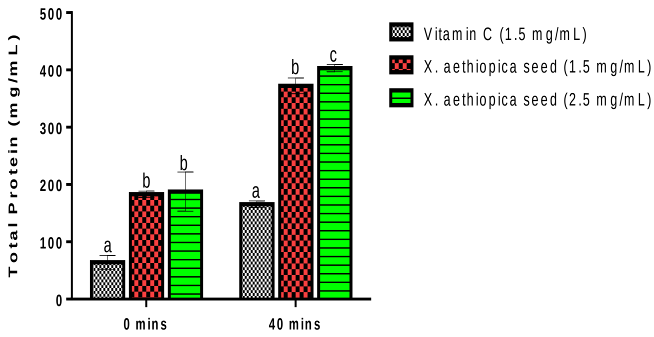

Figure 4. Total Protein Concentration in AAPH Induced Oxidative Stress Cells Treated with Aqueous Extract of *Xylopia aethiopica* Seed. Values are mean ± SD, n = 5, values with different superscript are significantly different at $p < 0.05$.

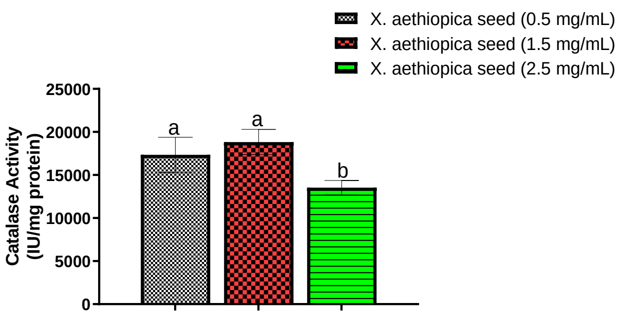

Figure 5. Catalase Activity in AAPH Induced Oxidative Stress Cells Treated with Aqueous Extract of *Xylopia aethiopica* Seed. Values are mean ± SD, n = 5, Values with different superscript are significantly ($p < 0.05$) different

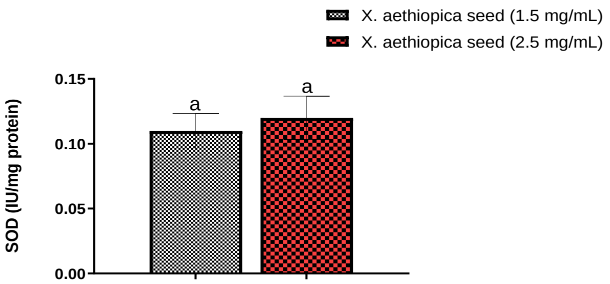

Figure 6. Superoxide Dismutase Activities in AAPH Induced Oxidative Stress Cells Treated with Aqueous Extract of *Xylopia aethiopica* Seed. Values are mean ± SD, n = 5

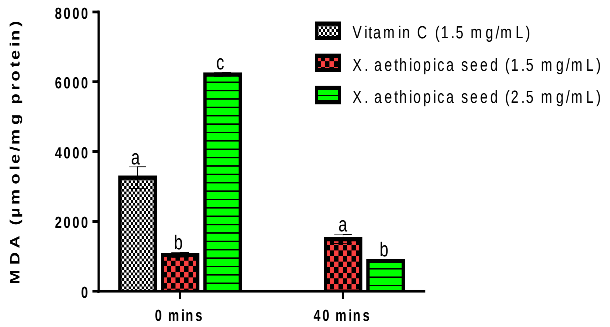

Figure 7. Malondialdehyde Concentration in AAPH Induced Oxidative Stress Cells Treated with Aqueous Extract of *Xylopia aethiopica* Seed. Values are mean ± SD, n = 5, values with different superscript are significantly different at $p < 0.05$.

Discussion

In the present study, aqueous extract of *X. aethiopica* seed demonstrated blood glucose-lowering potential in hyperglycemic-loaded mice. This is an indication of the seeds efficacy in reducing postprandial blood glucose. Increase in postprandial blood glucose is a serious metabolic condition that affects the whole-body system and an important marker for coronary heart disease (Jiang *et al.*, 2017). Tannins and phenolics compounds are well-established for their anti-diabetic potential (Manivannan and Johnson, 2020). From this study, the plant contained an appreciable amount of total tannin and total phenolic. However, the total tannin was higher compared to the total phenolic. The blood glucose-lowering potential of aqueous extract of *X. aethiopica* seed after postprandial could be attributed to the presence of tannins and phenolics contained in the plant (Bristy *et al.*, 2022). Apart from its postprandial blood glucose-lowering activity, the aqueous seed extract inhibited hydroxyl radical radicals, and this was greater than the reference compound (vitamin C). Hence, the antihyperglycemic action of *X. aethiopica* seed in postprandial state could be linked to its antioxidant activity and medicinal plants have been documented to show high radical scavenging activities (Giacco and Brownlee, 2010; Tijjani *et al.*, 2017; Saliu *et al.*, 2020). Furthermore, most of the therapeutic agents with blood glucose-lowering potentials are found to possess antioxidant properties (Tijjani *et al.*, 2018). Hypoglycemic substances derived through synthetic or natural product, that tend to decrease blood glucose also reduce the risk of diabetes-associated cardiovascular complications (Ogbonnia *et al.*, 2009; Folorunso *et al.*, 2013).

Antioxidant functions in blood and biological fluids are based on the reflective total antioxidant status (Silvestrini *et al.*, 2023) in the body system. Protein oxidation, malondialdehyde (MDA), superoxide dismutase (SOD), and catalase are important markers of oxidative stress in cells. In addition, SOD is a cellular defense enzyme that protect tissues from lipid peroxidation, oxidative damage and oxidative stress-related diseases such as diabetes (Pandey *et al.*, 2010). MDAs are products of lipids peroxidation and are used as markers to estimate this metabolic process (Qasim and Mahmood, 2015). Catalase (CAT) neutralise hydrogen peroxide by decomposing it and ensuring an optimal level of the molecules for essential cellular signalling in the cells (Nandi *et al.*, 2019). Concomitant increase in these enzymes (SOD and CAT as well as glutathione peroxidase) and MDA have been reported in increased reactive oxygen species production (Awang Daud *et al.*, 2022).

The use of 2, 2'-azobis(2-amidinopropane) dihydrochloride (AAPH) as oxidative model has been established and used in different studies (Qasim and Mahmood, 2015; Tijjani *et al.*, 2022). Mechanism of AAPH involves its decomposition to give nitrogen atom and initiating peroxyl radical which prompts oxidative stress (Sato *et al.*, 1995). Hence, the AAPH can be used as an oxidative stress marker for *in vitro* model. In this study, the aqueous extract of *X. aethiopica* seed increased protein secretion in cells against AAPH-induced oxidative stress. In support of this antioxidant activity, *X. aethiopica* seed extract protected cells against AAPH-induced oxidative stress in vitro by decreasing the concentration of generated malondialdehyde, however, no significant effect was recorded on SOD by seed extract of the plant.

In our previous research, it was shown that significant amounts of essential oils found in *X. aethiopica* display antioxidant properties, and its aqueous extract protected Hb from oxidation by preventing RBC lysis (Tijjani *et al.*, 2022).

The plant extract protects against inflammatory disorders, promotes the healing of wounds, and is used in the treatment of post-natal pains (Ezekwesili *et al.*, 2010). All these activities were associated with the antioxidant activity of *X. aethiopica*. Therefore, aqueous extract of *X. aethiopica* seed possesses blood glucose-lowering potential in postprandial state and this could be associated with its radical quenching activities.

CONCLUSION

The study concluded that the aqueous extract of *X. aethiopica* seed at 250 and 500 mg/kg bwt possesses glucose-lowering effect in mice and antioxidant properties against 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) induced oxidative stress in vitro, which could be associated with the tannin and total phenolic contents of the seed.

AUTHORS' CONTRIBUTIONS

HT, AO, OAS designed the study, AMA, FAM, GMB, HT carried out the laboratory investigations, HT, GMB, SA, OAI, AM drafted the manuscript, all authors proofread and approved the manuscript.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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