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Original Research Article

Acute Toxicity Studies and *In Vivo* Antioxidant Potential of *Annona muricata* Leaf in Alloxan-Induced Diabetic Rats

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ABSTRACT

The acute toxicity effect and free radical scavenging abilities of ethanol leaf extract of *Annona muricata* were studied in diabetic rats. The acute toxicity effect of the leaf extract was determined using a standard method. Thirty Wistar rats were randomly divided into six (6) groups (Groups I-VI) of five (5) rats each. Diabetes was induced with 130 mg/kg of alloxan monohydrate intraperitoneally in II-V. Group I was non-diabetic and untreated and served as normal control (NC). Group II served as untreated diabetic control (DC). Group III (GL) received daily doses of glibenclamide (5 mg/kg), while groups IV (AM) and V (AM) received daily doses of a combination of glibenclamide (5mg/kg) and 200 mg/kg and 400mg/kg of *Annona muricata* leaf extract, respectively. Group VI received 400 mg/kg oral doses of *A. muricata* leaf extract only. The treatment continued for 14 days. The blood glucose concentration and antioxidant enzyme activities were determined using standard methods. The acute toxicity study revealed a median lethal dose (LD_{50}) of 3807.89 mg/kg. The antioxidant enzyme activities in groups I to VI for SOD, CAT, and GPx activity ranges from1.51E-01±1.07E-02 to 9.10E-02±5.55E-03 IU/L;3.87E-01±2.06E-02 to 6.38E-01±5.30E-02 IU/L and0.036 ±0.05 to1.35±0.152 IU/L, respectively. The extract exerted a concentration-dependent effect on antioxidant enzyme activities. The present studies suggest that ethanol leaf extract of *A. muricata* has effects in inducing antioxidant enzymes during oxidative stress.

***Keywords*:** *Annona muricata*, Acute Toxicity, Antioxidant enzymes, Superoxide dismutase

Introduction

Free radicals are typically created as metabolic by-products of healthy aerobic respiration as well as from the metabolism of medications and poisons, according to recent studies.[1, 2] Recently, free radical chemistry and reactive oxygen species (ROS) have become the cynosure of attention in scientific research, and a paradigm shift in the understanding of free radicals and reactive oxygen species (ROS) has resulted in a medical revolution that portends significant advancements in the management of health and disease. Ironically, oxygen, which is essential for life and vital in most cellular metabolism, can sometimes be claimed to affect the human body negatively. Oxidative stress is a major contributor to several pathological conditions, including cardiovascular disease, diabetes mellitus, cancer, rheumatoid arthritis, Alzheimer's disease, aging, atherosclerosis, etc.[2,3,4,5,6] It develops when ROS levels overwhelm endogenous antioxidant defense. Increased evidence from research investigations implicates oxidative stress as a significant pathogenic index for diabetes mellitus.[7] The amount of free radicals produced by these metabolic pathways is therefore disproportionate but yet results in the release of ROS in people with diabetes through non-enzymatic and oxidative breakdown of proteins, glucose oxidation, and lipid peroxidation, respectively.[8,9]

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The destruction of cellular organelles and enzymes may occur unintentionally when the quantity of free radicals is excessively elevated, and the antioxidant defense system is depleted. This will result in a high rate of lipid peroxidation and the subsequent emergence of insulin resistance. Consequently, oxidative stress may promote the emergence and presentation of problems from diabetes mellitus. Recent advances in clinical and experimental research have opened up new perspectives on stress contributions to diabetes progression, advocating a novel and useful path to an antioxidant therapy or regime to treat and significantly reduce oxidative stress-related conditions.

Free radicals are implicated in the pathophysiology of diabetes mellitus in both human and experimental models, according to research. The pancreatic beta-cells of experimental animals (rats) have been linked to severe necrosis after receiving alloxan.[10] Additionally, it has been proposed that alloxan triggers the creation of ROS like hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxides (HO^-), which initially cause cell damage before eventually leading to cell death.[8] Medicinal plants have been found to have high antioxidant levels due to the presence of potent phytoconstituents. These antioxidants may have both therapeutic and preventive benefits on a large number of diseases that are caused by oxidative stress, helping to modulate their effects in general.[3,11,12,13] The World Health Organization reports show that about 80% of sub-Saharan Africa's population depends on alternative medicine for their basic healthcare needs.[14] This is due to the benefits attributed to plants and demonstrated in some experimental studies on medicinal plants. This rising interest in medicinal plant use by researchers may be related to how readily available, inexpensive, and accessible plant extracts are to the general population, as well as how environmentally friendly they are.[15] As a result, varieties of compounds isolated from plants are now being seen as nature's remedy for aging, atherosclerosis, arthritis, ulcers, cancer, and many other diseases. Novel research has therefore focused on medicinal plants as sources of bioactive compounds utilized in ethnomedicine for the treatment of

many ailments.[12,16,17,18] Numerous biologically active components of these healing herbs, such as flavonoids, phenols, and tannins have been demonstrated to possess strong *in-vitro* and *in-vivo* antioxidative activity.[11,14,19, 20, 21]

One of these plants, *A. muricata*, has been linked to and used for various disease conditions in ethno-medicine. It is commonly known as sour sop (English), Shawa chop, Igbo (a corrupt form of sour sop in Igbo), Ebo (Yoruba), and Tuwon Biri (Hausa). It belongs to the Annonaceae family, which comprises 130 genera and over 70 species, and has been widely studied because of its alleged therapeutic properties .[22] Several *A. muricata* plant parts, including the root and stem barks, leaves, fruits, and seeds, have been linked to medicinal properties, necessitating their use in alternative medicine in Africa, Asia, and America for a variety of human illnesses, particularly for parasitic infections and cancer. [1,23,24]

Annona muricata has also been reportedly farmed in Southern and Northern America, the West Indies, and China.[24] It is primarily abundant in the Southeastern region of Nigeria, where it is used ethno-medically for the management of a plethora of disease conditions. The plant components also have other ethnomedicinal uses, such as sedative, antispasmodic, astringent, anticancer, hypotensive, insecticide, piscicide, and vermifuge effects, as well as for coughs, fevers, aches, and skin conditions.[1] The plant's blossoms, fruit pods, and leaf decoction are useful in the treatment and prophylactic management of colds, cystitis, diabetes mellitus, headaches, and sleeplessness, according to various studies.[1,24,25] The root-bark and stem-bark are also thought to act as antihelmintic and antiphlogistic agents. When taken orally, the leaf decoction has been ascribed to possess anti-inflammatory and neuralgic effects, as well as being useful in the treatment of intestinal malaise. When cooked, the leaves can also be applied topically to treat abscesses and rheumatism. For nerve shock, the leaves can be massaged; for anxiety episodes, the leaves or bark can be brewed into a decoction.[23] Colds, chest pain, and nerve problems can all be improved with a combination of flower bud tea and honey, while diarrhea and dysentery can be treated with the young fruit's bark. Additionally, to stop bleeding, *Annona muricata's* green bark is applied to wounds.[26]

The usefulness of alternative medicine, both in and outside of Africa, has been the subject of numerous research findings, and this has positively boosted and enhanced interest in pharmacology, particularly due to the perceived safety of these plant extracts in comparison to synthetic drugs. However, given the ongoing recorded and undocumented reports of adverse pharmacological reactions linked to herbal medications, pre-clinical toxicological investigations on these natural products must be conducted before usage.

Diabetes and its complications, possibly due to free radicals assault on the body, have continued to affect people's overall well-being. This has prompted numerous studies aimed at creating a therapeutic regimen that will help treat these medical conditions, but their occurrence continues to rise. There is a need to explore the *in vivo* antioxidant properties of *A. muricata* leaf ethanol extract. Despite wide claims of its usefulness in traditional medicine regimens, no recent studies have been carried out to that effect. The present study assessed the *in vivo* antioxidant potentials of ethanol leaf extracts of *Annona muricata* grown in Southeast Nigeria in an alloxan-induced diabetic Wistar rats model.

Materials and Methods

Leaf collection, identification, and preparation

Fresh leaves of *Annona muricata* were collected from a homestead in Ebikoro village,Uratta community, Owerri North Local Government Area, Imo State, Nigeria, located between Latitude 5° 29' 41" North and Longitude 7° 4' 23" East, on March 26, 2021. The samples were identified and given the herbarium number NAUH-004B by Mrs. Bibian Aziagba, a plant taxonomist at the Department of Botany, Nnamdi Azikiwe University, Awka. The mature leaves were detached from the stalk, cleaned under running water, and allowed to air dry in a warm environment. The leaves were dried to constant weight, ground to powder using a commercial blender, and kept in an airtight container until needed.

Animal studies

Thirty (30) healthy Wistar rats of both sexes weighing 150–220g were used for the study. The animals were purchased from the animal house of the Anatomy Department, Imo State University Owerri, and kept in steel cages under standard lighting (12 hours of light and 12 hours of darkness), temperature, and humidity. The animals were fed rat pellets (Vital finisher) and tap water at all times and were acclimatized for a week before the experiment. The care of the animals was carried out in compliance with the standards established by the Nnamdi Azikiwe University's Animal Research Ethics Committee, with Ethical Clearance Reference Number NAU/AREC/2023/00023.

Plant extraction

The powdered leaf sample (100g) was macerated macerated in 1 L of 70% ethanol at room temperature for 48 hrs. The extract was filtered with Whatmann No.1filter paper. The filtrate was concentrated by evaporation in a water bath at 60°C to obtain a semi-solid slurry of crude extracts, which was stored at 4°C in the refrigerator until needed for the studies.[27]

Assessment of Acute Toxicity

The LD_{50} of *A. muricata* leaf ethanol extract was evaluated in rats using Lorke's approach[28], as reported by Aroma and Enegide.[29] For this study, exactly twelve (12) Wistar rats were employed. The study was carried out in two phases. Phase involved nine (9) Wistar rats divided into three (3) groups of three (3) animals each. The groups received oral doses of 10, 100, and 1000 mg/kg b. wt. of the extract, respectively. The animals were closely monitored for 24 hours after extract administration to check for the onset of toxicity effects, the time it took for them to recover, and death. In the second phase, the extract was given to exactly three (3) rats, separated into three (3) groups, at oral doses of 1600, 2900, and 5000mg/kg, respectively. They were examined for potential toxicity signs and potential delayed manifestations of toxicity for 7 to 14 days. [28,29] The lethal dose was calculated as follows:

$$LD_{50} = \sqrt{(L_0 \times L_{100})}$$

Where L_o = highest dose that gave no mortality
L_{100} = lowest dose that produced mortality

Grouping of Animals

Six groups of five Wistar rats each were formed from the exact thirty Wistar rats acclimated for the study. Animals in groups I and VI were designated as normal control (NC) and extract control (A. M. only), fed rat pellets and water *ad libitum*, and administered 400 mg/kg b. wt of the extract respectively. Group II served as the diabetic control (DC) and was induced and untreated. Diabetes mellitus was induced in Groups designated II (DC), III (GL), IV (A.M. 200), and V (A. M. 400) by intraperitoneal injection of 130 mg/kg alloxan monohydrate. After diabetes was confirmed, the groups GL, A.M. 200, and A. M. 400 were treated with glibenclamide (5mg/kg), 200mg/kg, and 400 mg/kg *A. muricata* leaf extract daily for 14 days, respectively. Group VI received daily treatment of 400 mg/kg of the extract without induction of diabetes mellitus (A.M. only).

Induction of Diabetes Mellitus

The experimental rats were fasted for 16 hours and administered 0.3 mL intraperitoneal injection of alloxan monohydrate (130 mg/kg body weight) in normal saline for induction of diabetes mellitus.[30] Fasting blood glucose levels of the experimental rats were assessed using a glucometer and test strip (Accu-Chek, Germany), and blood samples for diabetes testing were collected from the tail tip by puncturing with a lancet. The blood glucose concentrations of 200 mg/dl after 72 hours of induction were considered diabetic, and these rats were thus included in the study.

Extract administration and collection of blood samples

Exactly 25 mL of distilled water was used to reconstitute precisely five grams (5 grams) of the leaf extract. The rats received oral administration of the extract at the needed concentration for the study for a total of 14 days at doses of 200 mg/kg b. wt. and 400 mg/kg b. wt. Every two (2) days, the experimental animals' body weights were measured, and then

the animals were euthanized with chloroform, and their blood was collected by cardiac puncture for biochemical testing. The blood samples were kept to clot before being centrifuged at 1500 rpm for 5 minutes. The clear serum was extracted using a small pipette into a sample bottle, stored in a refrigerator at 4ºC, and then used for biochemical analysis.

Antioxidant Enzyme Assay

Superoxide Dismutase activity determination

The Sun and Zigma approach, described by Lawal *et al*.,[2] was used for this study. The capacity of the enzyme to stop the chain-propagating autoxidation of epinephrine caused by free radicals served as a measure of superoxide dismutase activity. The assay is based on the autoxidative by-product of epinephrine known as adrenochrome adsorption, whose absorbance is measured at 480nm. [2, 31]

Glutathione Peroxidase (GP_X) Activity determination

Serum GPx activity was assessed as described by Tappei.[32] The reaction mixture was composed of 1ml of 0.3M phosphate buffer (pH 7.4),0.33ml of 10mM glutathione, 0.33ml of 15mM H_2O_2, 0.1ml of serum, and 1.37 mL of distilled water in a cuvette.After adding the serum, the mixture was agitated for 4 minutes, and the optical density (OD) was measured for 3 minutes at 340 nm.[32] The formula below was used to compute enzyme activity using a molar extinction coefficient of 1.622×10^{-3} $m^{-1}cm^{-1}$.

$$GP_x\ (IU/L) = \frac{\text{Average change in OD} \times \text{Volume of reaction mixture (ml)}}{\text{Extinction coefficient} \times \text{volume of sample (ml)}}$$

Catalase Activity determination

The Beers and Seizer method to estimate the serum catalase activity adapted by Usoh *et al.*[33] was used for this study. The serum activity of the catalase enzyme was assessed by monitoring the decline in optical density at 240 nm caused by the breakdown of H_2O_2 in an ultraviolet (UV) spectrophotometer. The assay was performed in a total volume of 3 mL, consisting of 2.9 mL of 30 mM H_2O_2in phosphate buffer (50 mM, pH 7.0) and 0.1 mL of serum. For two minutes, absorbance was read at 240 nm every 30 seconds. Decreased H_2O_2 per minute per mg of protein was used to express the specific catalase activity. [33] Using the formula and the extinction coefficient of $40.0 m^{-1}cm^{-1}$, the enzyme activity was determined;

$$Catalase\ (IU/L) = \frac{\text{Average change in OD} \times \text{Volume of reaction mixture}}{\text{Extinction coefficient} \times \text{volume of sample}}$$

Malondialdehyde (MDA) concentration determination

The technique outlined by Usoh *et al.*[33] was used to determine malodialdehyde (MDA), a biomarker of lipid peroxidation.[33] The test tubes were filled with precisely 1.6mL of 0.25N HCl, 0.5mL of 15.0% trichloroacetic acid (TCA), and 0.5mL of 0.375% thiobarbituric acid (TBA) and 0.4mL of serum. The reaction set-up was further boiled in a water bath at 100℃ for 20 minutes, then cooled at room temperature (25 ± 2℃). The supernatant was carefully aspirated after centrifugation at 3000 rpm for 10 minutes, and the optical density was measured spectrophotometrically at 532 nm against a reagent blank.[33] The MDA concentration was calculated using the molar extinction coefficient for the MDATBA-complex, which is 1.56×10^5 $m^{-1}cm^{-1}$.

$$MDA\ (\mu mol/ml) = \frac{OD \times Total\ volume\ of\ reaction\ mixture\ (ml)}{\text{Extinction coefficient} \times \text{volume of sample (ml)}}$$

Statistical Analysis

The results were presented as mean ± standard deviation of five determinations. Statistical analysis was performed using one-way analysis of variance (ANOVA) in the 2007 version of the Microsoft Excel statistical software, presuming equal variances. The statistical significance of mean value across groups was considered at $p < 0.05$ using turkey and homogeneity of variance test.

Results and Discussion

The result of the acute toxicity of *A. muricata* leaf ethanol extract on Wistar rats presented LD_{50} of 3807.89 mg/kg (Table 1). Hence, *A. muricata* leaf ethanol extracts are acutely poisonous, above 2900 mg/kg. This result contrasts with that reported by Arthur *et al*.,[34] which reported non-lethality in all dose levels up to the seventh day, and the estimated LD_{50} was 5000 mg/kg. Consequently, the present results agree with the report of Opara *et al*.,[14]. They reported that large doses of an aqueous leaf extract from *A. muricata* were toxic or fatal to animals. This might be ascribed to the presence of antinutrients and annonaceous acetogenins, both of which have been linked to harmful effects at high doses or concentrations, according to a study by Opara *et al*.,[14] Coria-Tellez *et al*.,[24] and some other works. [1, 14, 35, 36]

The effect of the extract on antioxidant enzyme activity showed that administration of the extracts caused no significant ($p>0.05$) changes in superoxide dismutase (Figure 1) and catalase enzyme activities (Figure 2) between the extract and glibenclamide-treated groups, respectively. Comparatively, the extract-treated groups were similar to the normoglycemic, diabetic (untreated) group; no statistically significant changes in activities occurred. SOD activity increased at 200mg/kg of the extract compared with the standard drugs-treated group and 400 mg/kg extract-treated groups, thereby suggesting the potency of the extract to elevate SOD activity in diabetes-induced stress.[37] There was an increase in the activity of SOD observed in animals administered with 200mg/kg of the extract, however, this trend was not seen in the higher doses of the extract. These may be suggestive of a dose-independent effect of the extract on the SOD activity. The statistically non-significant ($p>0.05$) changes in catalase activity between the extract and glibenclamide-administered groups are indicative of the increased production of peroxide radicals caused by diabetes-induced oxidative stress as previously reported by Adewole and Ojewole.[23]

Table 1: Acute toxicity studies of ethanol leaf extracts of *Annona muricata*

Groups	Dose of *A. muricata* leaf extract	No. of deaths recorded
Phase 1		
Dose 1	10	0/3
Dose 2	100	0/3
Dose 3	1000	0/3
Phase 2		
Dose 1	1200	0/3
Dose 2	1600	0/3
Dose 3	2900	0/3
Dose 4	5000	2/3

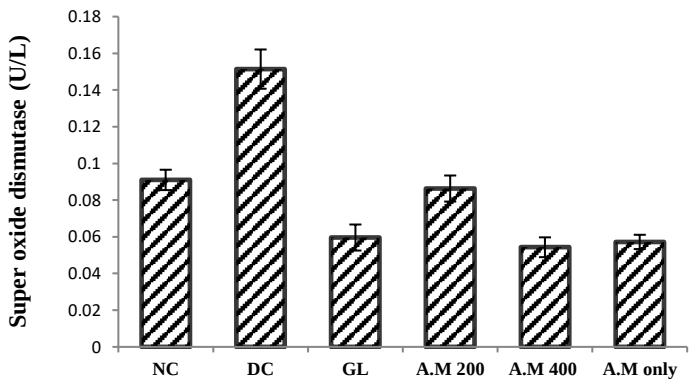

Figure 1: Serum superoxide dismutase activity of alloxan-induced diabetic rats treated with *A. muricata* leaf ethanol extract

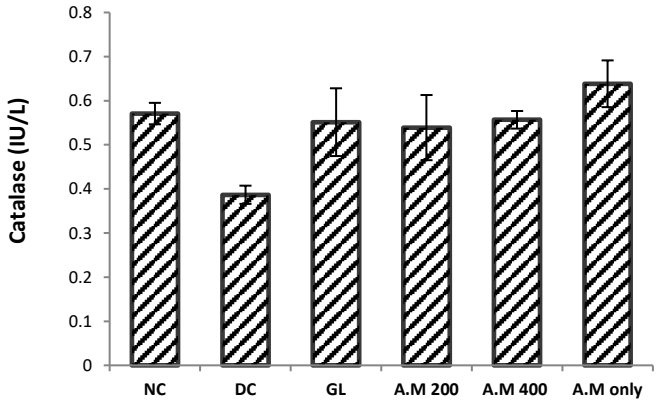

Figure 2: Serum catalase activity of alloxan-induced diabetic rats treated with *A. muricata* leaf ethanol extract
The bar chart represents the mean ± standard deviation of 5 determinations. Bars with different superscript letters are significantly different (p<0.05).

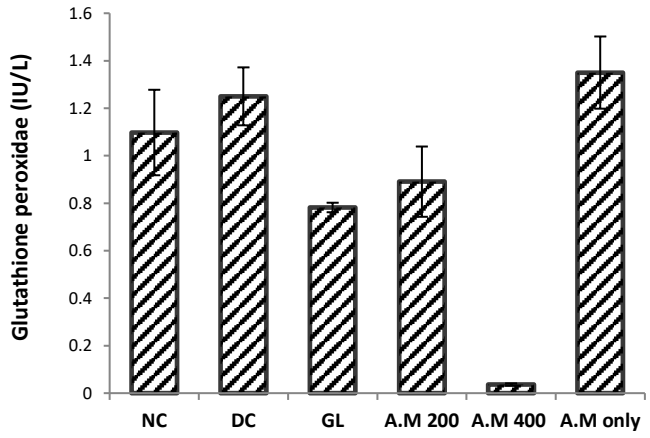

Figure 3: Serum glutathione peroxidase activity of alloxan-induced diabetic rats treated with *A. muricata* leaf ethanol extract

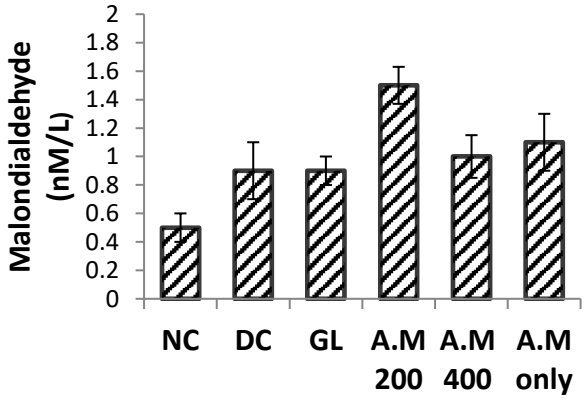

Figure 4: Serum malondialdehyde (MDA) concentration of alloxan-induced diabetic rats treated with *A. muricata* leaf ethanol extract
The bar chart represents the mean ± standard deviation of 5 determinations. Bars with different superscript letters are significantly different (p<0.05).

Notably, the result presented in Figure 3 indicates that *A. Muricata* leaf ethanol extract treatment resulted in significant (p<0.05) elevation of glutathione peroxidase activity in the glibenclamide treated group and the extract-treated groups, and there was also a significant (p<0.05) dose dependent increase between the extract treated groups. Glutathione peroxidase activity was significantly decreased in the A.M 400mg/kg group when aligned with those administered with 200mg/kg under normal physiological conditions. This implies that an increased dose of the extract increased the activity of Glutathione peroxidase under normal physiological conditions. In contrast, at 200mg/kg, the extract showed higher potency in increasing the glutathione peroxidase activity in diabetic rats than glibenclamide. This, according to Taheri *et al.*,[37] is a compensatory response to oxidative stress. [37]

Furthermore, *A. Muricata* leaf ethanol extract administration did not significantly (p>0.05) alter malondialdehyde (MDA) concentration in the diabetic Wistar rat models (Figure 4). The result revealed a significant (p<0.05) increase in malondialdehyde (MDA) level of the standard drug-treated group and the *A. muricata* extract-treated groups when aligned with the normoglycemic rats. However, no significant variation in MDA concentration occurred between the groups treated with the extract. According to Tiwari *et al.*,[38] high MDA levels in people with diabetes indicate that lipid peroxidation harm has resulted in the deterioration of the antioxidant defense.[38]

The present study corroborates the works of Adewole and Ojewole.[23] Opara *et al.*,[14], Lawal *et al.*[2],and Balderrama-Carmona *et al.*[39] suggest that the increase in antioxidant enzyme activity is attributed to the increased presence of phytoactive constituents in the plant extract.[2,14,39] Although from the study, it could be observed that the activity of the antioxidant enzymes was high in the negative control group (i.e., diabetic untreated); this, according to Taheri *et al.*,[37] suggests stimulation of antioxidant enzymes activity to counter ROS proliferation at the early stage of Type 1 diabetes mellitus. Enzymatic antioxidants such as CAT, SOD, and GPx are at equilibrium with circulating free radicals, but at the later and advanced stages of diabetes progression, antioxidant balance is impaired, thereby resulting in oxidative stress.[37] The present study's findings showed that *A. muricata* leaf ethanol extract increased MDA concentration; this agrees with the findings from the work of Lawal *et al.*,[2]. Their study suggested that certain plants used traditionally to treat diseases act as pro-oxidants *in vivo*.[2] The ethanol extract of *A. muricata* may be acting by stimulating the body's natural defense mechanisms to elicit an antioxidative effect. The bioactive component of the ethanol leaf extract may be implicated in inducing antioxidant enzyme activities and increased lipid peroxidation in normal rats.[2] The results, therefore, suggest that leaf extracts of *A. muricata* have antioxidant potentials and may ameliorate diabetes-induced oxidative stress and conditions in which free radicals are implicated.[13,16, 19,27]Although no specific mechanism of action has been proposed, earlier works have found plant secondary metabolites to be useful antioxidants.[14, 20,35]

A. muricata leaf contain bioactive phytoconstituents including but not limited to ellagic acid, tannins, flavonoids, triterpenoids, and sitosterol.[1,11,15,26,35,40] Therefore, it is appropriate to speculate that the plant's chemical components may be implicated in the antioxidative and other pharmacological benefits associated with the *Annona muricata* leaf ethanol extracts in this study.

Conclusion

In conclusion, alloxan-induced diabetes results in oxidative stress, and *A. muricata* leaf ethanol extract possesses antioxidant potentials capable of ameliorating alloxan-induced oxidative damage. According to the LD_{50} findings, the extract may be lethally poisonous when given in quantities of more than 3807.89 mg/kg. This may be ascribed to the presence of specific phytoconstituents like oxalate and annonaceous acetogenins.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Coria-Tellez AV, Montalvo-Gonzalez E, Yahia ME, Obiedo-Vazquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action, and toxicity. Arab.J.Chem. 2018;11(5): 662-691.
2. Lawal ZA, Hamid AA, Shehu AE, Ajibade OS, Subair OA, Ogheneovo P, Mukadam AA, Adebayo CT. Biochemical Properties, In-Vitro Antimicrobial, and Free Radical Scavenging Activities of the Leaves of *Annona muricata*. J. Appl. Sci. Environ. Manage. 2017; 21 (6) : 1197-1201.
3. Adeosun AM, Asejeje FO, Ighodaro OM, Oluwole BA, Akinloye OA. Hypoglycemic, Antidyslipidemic, and Antioxidant activities of methanol extract of *Struchium sparganophora* leaves in alloxan-induced oxidative stress-mediated diabetes in rats. Futur. J. Pharm. Sci. 2020; 6:59.
4. Yeldu MH, Arinola OG. Oxidative stress factors in Nigeria with rheumatoid arthritis. Int. J. Res. Med. Sci. 2017; 5(5):1948-1952.
5. Landmesser U, Drexler H. Towards Understanding Superoxide Dismutase Regulation in atherosclerosis. Atheroscler.Thromb. Vasc. Biol. 2002; 22:1367-1370.
6. Ojezele OJ, Ojezele MO, Adeosun AM. Comparative Phytochemistry and Antioxidant Activities of Water and Ethanol Extract of *Annona muricata* Linn Leaf, Seed and Fruit. Advan. Biol. Res., 2016; 10 (4): 230-235.
7. Lukacinova A, Mojzis J, Benacka R, Keller J, Maguth T, Kurila P, Vasko L, Racz O, Nistiar F. Preventive effects of flavonoids on Alloxan-induced Diabetes mellitus in Rats. Acta Vet.Brno. 2008; 77:175-182.
8. Pasupuleti VR, Arigela CS, Gan SH, Salam SKN, Krishnan KT, Abdul Rahman N, Jeffree MS. A Review on Oxidative Stress, Diabetic Complications, and the Roles of Honey Polyphenols. Oxid. Med. Cell. Longev, 2020; 2020:1-16.
9. Singh A, Kukreti R, Saso L, Kukreti S. Mechanistic Insight into Oxidative Stress-Triggered Signaling Pathways and Type 2 Diabetes. Molecules, 2022; 27(3): 950.
10. Lankin VZ, Korchin VI, Konovalova GG, Lisina MO, Tikhaze AK, Akmaev IG. Role of antioxidant enzymes and antioxidant compound probucol in antiradical protection of pancreatic beta-cells during alloxan-induced diabetes. Bull. Exp. Biol. Med., 2004;137: 20-23
11. Agu KC, Okolie PN. Proximate Composition, Phytochemical Analysis, and *In Vitro* Antioxidant Potentials of Extracts of *Annona muricata* (Soursop). Food Sci Nutr. 2017; 21 (2): 1–8.
12. Jafri SAA, Khalid ZM, Khan MR, Ashraf S, Ahmad N, Karami AM, Rafique E, Ouladsmane M, Al Suliman NMS, Aslam S. Evaluation of some essential traditional medicinal plants for their potential free scavenging and antioxidant properties. J. King Saud Univ. Sci., 2023; 35 (2023): 102562
13. Najmuddin SUFS, Romli MF, Hamid M, Alitheen NB, Abd-Rahman NMAN. Anti-cancer effect of *Annona muricata* Linn leaves crude extract (AMCE) on breast cancer cell line. BMC Complement Altern. Med., 2016;16:311- 330.
14. Opara PO, Enemor VHA, Eneh FU, Emengaha FC. Blood glucose-lowering potentials of *Anonna muricata* leaf extract in alloxan-induced diabetic rats. EJBIO. ,2021; 2(2):106-113.
15. Gotep JG, Agada GOA, Gbisi DS, Chollom S. Antibacterial Activity of Ethanolic extract of *Acalypha wilkesiana* leaves growing in Jos, Plateau State, Nigeria. Malays. J. Microbiol. 2010; 6(2):69-74.
16. Agu KC, Okolie NP, Falodun A, Engel-Lutz N. In vitro anticancer assessments of *Annona muricata* fractions and in vitro antioxidant profile of fractions and isolated acetogenin (15-acetyl guanacone). J. Cancer Res. Pract, 2018; 5(2): 53-66.
17. Kumar S. The Importance of Antioxidants and their Role in Pharmaceutical Science - A Review. Asian J. Res. Chem Pharm. Sci. 2014;1(1):24-44.
18. Keshari AK, Srivastava A, Upadhayaya M, Srivastava R. Antioxidants and free radicals scavenging activity of Medicinal Plants. J. Pharmacogn. Phytochem. 2018; 7(3): 1499-1504
19. Hasmila I, Natsir H, Soekamto NH. Phytochemical analysis and antioxidant activity of soursop leaf extract (*Annona muricata* Linn.), J. Phys.: Conf. Ser. 2019; 1341(3): 1-7.
20. Olude OM, Asaya VE , Uchenna HI. Phytochemical and Nutritional Composition of Crude Powder and Ethanol Extract of *Annona muricata* Leaves. Trop J. Nat Prod Res. 2020; 4(7):315-318.
21. Usoh IF, Akpan EF, Etim EO, Farombi EO. The antioxidant action of dried flower extracts of *Hibiscus sabdariffa* on Sodium Arsenite-n induced oxidative stress in rats. Pak. J. Nutr. 2005; 4: 135-141.
22. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA. *Annona muricata* (Annonaceae): A Review of its traditional uses, isolated Acetogenins and Biological Activities. Int. J. Mol. Sci. 2015; 16:15625-15658.
23. Adewole S, Ojewole J. Protective effects of *Annona muricata* Linn (*Annonaceae*) leaf aqueous extract on serum lipid profile and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. Afr J. Tradit Complement Altern Med. 2009; 6: 30-41.
24. Coria-Tellez AV, Montalvo-Gonzalez E, Yahia ME, Obiedo-Vazquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action, and toxicity. Arab.J.Chem. 2016;1:1-2.
25. Adewole SO, Caxton-Martins EA. Morphological changes and hypoglycemic effects of *Annona muricata* Linn (*Annonaceae*) leaf aqueous extract on pancreatic B-cells of streptozotocin-treated diabetic rats. Afr J Biomed Res. 2006; 9: 173-187
26. Olugbuyiro JAO, Omotosho OE, Taiwo OS, Ononiwu FO, Banwo AS, Akintokun OA, Obaseki OS, Ogunleye OM. Antimicrobial Activities and Phytochemical Properties of *Annona muricata* L. Leaf. Covenant J. of Physical & Life Sciences (CJPL). 2017; 5 (2): 40–49.
27. Usunobun U, Okolie NP, Anyanwu OG, Adegbeg AJ, Egharevba ME. Phytochemical Screening and Proximate Composition of *Annona muricata* leaves.EJBPSP. 2015;2(1):18-28.
28. Lorke D. A new Approach to Practical Acute Toxicity Testing. Archives of Toxicology, 1983; 54:275-287
29. Aroma D, Enegide C. The Importance of Toxicity Testing. J. Pharm. Bio.Sci. 2014; 4(2013): 146-148
30. Etuk EU. Animal Models for Studying Diabetes Mellitus. ABJNA. 2010; 1(2):130-134
31. Sun M, Zigman S. An Improved Spectrophotometric Assay for Superoxide Dismutase based on Epinephrine Autoxidation.Anal. Biochem. 1978; 90:81-89
32. Tappei Al. Glutathione Peroxidase and Hydroperoxides. Meth. Enzymol. 1978; 52:506-513.
33. Usoh IF, Akpan EF, Etim EO, Farombi EO. The antioxidant action of dried flower extracts of *Hibiscus sabdariffa* on Sodium Arsenite-n induced oxidative stress in rats. Pak. J. Nutr. 2005; 4: 135-141.
34. Arthur FKN, Woode E, Terlabi EO, Larbie C. Evaluation of acute and sub-chronic toxicity of *Annona muricata* (Linn) aqueous extract in animals. Eur. J. Exp. Biol. 2011; 1 (4): 115-124.

35. Egharevba E, Chukwuemeke-Nwani P, Eboh U, Okoye E, Bolanle IO, Oseghale IO, Imieje VO, Erharuyi O, Falodun A. Evaluation of the Antioxidant and Hypoglycaemic Potentials of the Leaf Extracts of *Stachytarphyta jamaicensis* (*Verbenaceae*). Trop J Nat Prod Res. 2019; 3(5):170-174.
36. Abdul Wahab SM, Jantan I, Haque MA, Arshad L. Exploring the Leaves of *Annona muricata* L. as a Source of Potential Anti-inflammatory and Anticancer Agents. Front. Pharmacol. 2018; 9:661
37. Taheri E, Djalali M, Saedisomeolia A, Moghadam AM, Djazayeri A, Qorbani M. The relationship between the activities of antioxidant enzymes in red blood cells and body mass index in Iranian type 2 diabetes and healthy subjects. J. Diabetes Metab. Disord. 2012; 11:3-17.
38. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI, Markers of Oxidative Stress during Diabetes Mellitus. J. Biomak. 2013; 20(13): 207-217.
39. Balderrama-Carmona AP, Silva-Beltrán NP, Gálvez-Ruiz JC, Ruiz-Cruz S, Chaidez-Quiroz C, Morán-Palacio EF. Antiviral, antioxidant and Antihemolytic Effect of *Annona muricata* L. Leaves Extracts. Plants,J. King Saud Univ. Sci.2020; 9(12):1650.
40. Ma CY, Lu JH, Li X, Liu X, Chen JW. Eight New Cytotoxic Annonaceous Acetogenins from the Seed of *Annona sqamosa*. Chin. J. Nat. Med. 2019; 17(4):291-297.