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Original Research Article

Antioxidant Property and Lipid Profile Effects of Aqueous and Ethanol Root Extracts of *Gnetum africanum* Welw

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ABSTRACT

The root of *Gmetum africanum* Welw, which serves as food among some locals, has been under study for its nutritional quality. This research is aimed at determining its *in vitro* and *in vivo* antioxidant property, and its effect on lipid profile. The aqueous and ethanol root extracts of *Gnetum africanum* Welw were obtained following established protocols. The extracts were assessed for their *in vitro*, *in vivo* antioxidant potentials, and effect on the lipid profile of male Wistar rats. *In vitro* antioxidant indices were determined using only the ethanol extracts and, the results revealed that the concentration of the extract above 1000 µg/mL had a significant ($p<0.05$) nitric oxide scavenging power than tannic acid (standard). The results of the *in vivo* study involving thirty-five male rats aged 14 weeks, and weighing 118 ± 18 g, showed that at low concentrations (250, 500 mg/kg body weight) the aqueous and ethanol extracts caused a significant ($p<0.05$) increase in the activities of the antioxidant enzymes. A high concentration of MDA level ($p<0.05$) at 1000 mg/kg per body weight was noted, which pointed to lipid peroxidation and, a measure of free radical generation. Total cholesterol decreased ($p<0.05$) noted across the treatment groups (except the 1000 mg ethanol extract group) is indicative of the extract's potential to enhance cholesterol metabolism. The findings from the study suggest that a low concentration of the aqueous and ethanol root extracts of *G. africanum* Welw could be effective against oxidative damage while promoting cholesterol metabolism.

Keywords: *Gnetum africanum* Welw, Antioxidant enzymes, Lipid peroxidation, Oxidative stress, Lipid profile.

Introduction

Recently, medicinal plants have found increasing use as alternative remedies. The active phytochemical components of medicinal plants have conferred on them these potentials. Interests are now being shifted to plant sources for the development of such drugs as oral hypolipidemic substances.[1] There are rising interests in non-pharmaceutical neutraceutical remedies for hypercholesterolaemia[2] and other health-related conditions. Plant parts, which are rich in such antioxidants as vitamin C, Vitamin E, and glutathione, have found usefulness in this regard; and more so, in the treatment of oxidative stress conditions. More so, medicinal plants are also rich in phytochemicals and phytolexins which impact systemic tissues by a mechanism that includes reduction of oxidative stress in cells[3] and hence, the continuing research on medicinal plants for "phytoremedies".

G. africanum Welw belongs to the family *Gnataceae* which is highly valued across Central Africa, the Democratic Republic of Congo (DRC), Camaroun, and Gabon for their nutritional and therapeutic potential.[4] The leaf of *G. africanum* Welw is commonly called "ukazi" by Ndi-Igbo, and "Afang" by the Efiks/Ibibios. The leaf is eaten raw and is also widely utilized in the southeastern parts of Nigeria in the preparation of soups and stew.[5]

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The study of Ndomou *et al*.[6] revealed that the leaves of *G. africanum* Welw possess a significant amount of proteins, fiber, minerals, essential fatty acids, and amino acids. Phytochemical tests showed high contents of tannins, saponin, flavonoids, alkaloids, and glycosides in the leaves.[4] It grows all seasons through rhizomes or new shoots. The edible tuber,[4] could enhance food sustainability and security in regions of the world threatened by the challenges of starvation while serving a therapeutic purpose at the same time. This research assessed the effect of ethanol and aqueous root extracts of *G. africanum* Welw on selected *in vitro*, *in vivo* oxidative stress markers, and on the lipid profile of apparently healthy male Wistar rats.

Materials and Methods

Plant collection and preparation

The fresh root of *G. africanum* Welw used in this study was obtained from cultivated farmland in Awka, Anambra State, Nigeria on 16th April 2021. It was authenticated by Mr. Iwueze Francis of the Botany Department, University of Portharcourt, Rivers State, Nigeria with the voucher no UPH/V/1,138. The root parts were cut into pieces, washed, and air-dried at the ambient temperature of 28 ± 2℃ for ten days. The dried plant materials were ground into powder and kept in an airtight container.

Extraction

Equal portions of the pulverized roots of *G. africanum* Welw were immersed in aqueous and ethanol solutions respectively, in the ratio of 1:4 (1.0 g of plant sample to 4.0 mL of the solvents). The mixtures were placed on an electronic shaker to agitate for three (3) days and then filtered with No 1 Whatman filter paper. The filtrates were subjected to soxhlet extraction to remove the solvents used for the extraction of the active components. The crude extracts were transferred into a container and then placed in a water bath set at 50℃

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for complete evaporation of the solvents. The resulting grey sticky viscous extracts (21% yield) were placed in an air-tight container, wrapped in aluminum foil, and stored at 4°C for further use.

Assessment of the free radical scavenging activity

The *in vitro* free radical scavenging activity was investigated on the ethanol root extract. The activity of the ethanol extracts on Hydrogen peroxide was assessed using the method of Wettashinge and Shaidi,[5] while the scavenging activity on nitric oxide was carried out using the method of Marcocci *et al*.[6] The scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was also carried out using the procedure of Velazquez *et al*.[7] while the reducing power (Fe^{3+}/Fe^{2+} transformation) of the ethanol extract was assessed using the approach of Oyaizu.[8] At the same time, the Hydroxyl Radical-scavenging property was determined by adopting the standard Phenanthroline-Fe (II) Oxidation Assay as described by Jin *et al* .[9]

In vivo studies

Animals

Thirty-five (35) male Wistar rats aged 14 weeks and, weighing 118 ± 18 g were obtained from Chris Animal Breeding Farm in Awka, Anambra State, Nigeria. The animals were housed for 12 hrs light/ 12 hrs darkness at the room temperature of 28 ± 2℃ in a clean and well-ventilated animal house and fed with water and top feed produced by premier feed hills Company Limited, Ibadan, Nigeria. The animals were allowed for two weeks to get acclimatized to the environment before the introduction of the extracts. Treatment lasted for 14 days before sacrifice.

Ethical consideration

The guidelines for animal use and care were followed and ethical approval was obtained from Research Ethical Committee of the School of Biological Sciences (SOBS), FUTO (Reference number: FUTO/SOBS/EC/A2021001).

Experimental design

Fourteen-week old male Wistar rats were grouped into seven groups (A-G) comprising five rats of close weight range in each group. Group (A) received normal saline (1 mL) and served as normal control. Groups B, C, and D received 250 mg, 500 mg, 1000 mg of ethanol extract per kilogram bodyweight of animal, respectively, while Groups E, F, and G received 250 mg, 500 mg, 1000 mg of aqueous extract per kilogram bodyweight of animal, respectively. The extracts were administered daily to each animal orally. Treatment lasted for two (2) weeks within which the animals had access to food and water *ad libitum.*

Collection of blood samples

After the treatment, the animals were fasted overnight (12 hours) and sacrificed by cervical dislocation following anesthesia using dichloromethane (CH_2Cl_2) vapor for a few minutes. Blood collection was by cardiac puncture. About 5 mL of the blood was collected from each animal and dispensed into a plain vial, and immediately sent to the laboratory for analysis.

Biochemical analysis

The activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase were determined according to the method of Weydert and Cullen.[10] Determination of lipid peroxidation followed the method of Gutteridge and Wilkins.[11] The method of Allain *et al*.[12] was adopted in the determination of serum cholesterol concentration. In the same vein, serum HDL-cholesterol concentration was assessed according to the method of Lopes-virella,[13] while the serum triglyceride concentration was assayed according to the method of Fossati and Prencipe.[14]

Statistical analysis

The values obtained in triplicates were presented as mean ± standard errors of the mean (SEM). One-way analysis of variance (ANOVA) was performed using Graph Pad Prism software, version 5.3. $P < 0.05$ was considered significant.

Results and Discussion

The DPPH free radical scavenging ability of the ethanol extract is presented in Figure 1. Accordingly, the extract manifested an inhibitory potential against DPPH free radical and the DPPH scavenging capacity of the extracts was observed to be dose-dependent. The inhibitory percentage of the extract varied from 20 to 70%. The results collaborated with the findings of Igwe *et al*[15] who reported that plant extracts DPPH scavenging activity is proportional to extract concentrations. Hydroxyl radical scavenging (Figure 2) property at the reduced extract concentration of 250 µg/mL was observed at 25%. Increasing extract concentrations were observed to have resulted in increasing inhibitory percentage so much that, at the highest extract concentration of 2000 µg/mL, the highest inhibition was noted at 65%. The scavenging capacity of the extracts may be related to their phenolic composition since phenolics have been shown by Igwe *et al*[15] to pose radical scavenging capacity.

Hydrogen peroxide (Figure 3) was observed to exhibit the lowest activity of 12% at 500 µg/mL and the highest of 40% at 1000 µg/mL.

At a reduced extract concentration of 250 µg/mL, the activity of *Gnetum africanum* Welw against nitric oxide radical (Figure 4) was visible at 15% and, it produced the same effect as the Tannic acid (standard) at the concentration of 1000 µg/mL.

The reducing power of the extracts at 700 nm ranged from 0.25 to 0.9 (Figure 5). It showed that the reducing power of the *G. africanum* Welw root extract increased slightly in a dose-dependent manner.

The *in vivo* studies revealed the effects of aqueous and ethanol root extracts of *G. africanum*Welw (GA) at doses 250 – 1000 mg/kg on superoxide dismutase, catalase, and glutathione activities as well as the formation of malondialdehyde (an index of lipid peroxidation) in adult male Wistar rats.

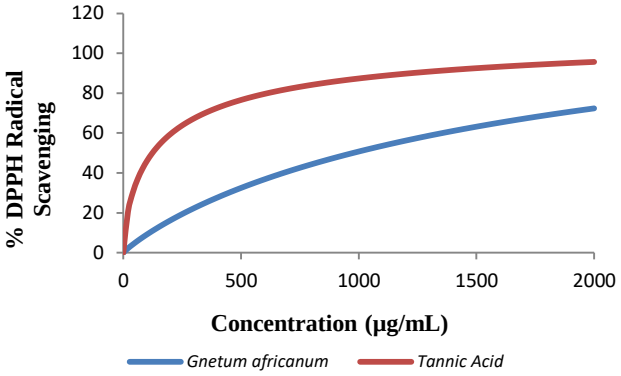

Figure 1: DPPH radical scavenging activity of *Gnetum africanum*Welw and tannic acid

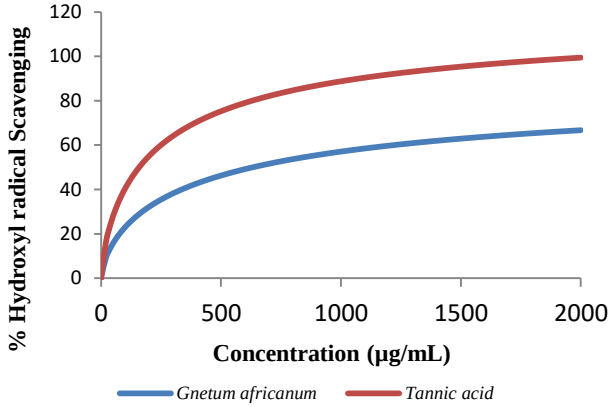

Figure 2: Hydroxyl radical scavenging activity of *Gnetum africanum* Welw and tannic acid

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When compared to the control, a significant ($p < 0.05$) increase was observed in the SOD activity of animals fed with 250 mg of aqueous extract as well as 250 mg and 500 mg of ethanol extracts of GA, these increases were not significantly different from each other (Figure 6). No significant ($p>0.05$) effect was seen in the SOD activity of animals that received higher doses (500 mg and 1000 mg) of aqueous extract as well as 1000 mg of ethanol extract of GA.

Figure 7 showed that the catalase activity of the animals treated with aqueous extract of *G. africanum* Welw decreased in a dose-dependent manner. In comparison to the control, the catalase activity of the groups fed with 500 and 1000 mg/kg of aqueous extract as well as those that received 250 and 1000 mg/kg of ethanol extract of GA was not statistically significant ($p<0.05$).

There was a significant ($p<0.05$) increase in the GPX activity at doses 250 and 500 mg/kg of aqueous extract of GA in comparison to the control, but a significant reduction in enzyme activity was observed in the animals that received 1000 mg/kg (Figure 8). Significant ($p<0.05$) increase was observed in the activity of GPX at 500 mg/kg of ethanol, while the reverse was the case with the groups that received 250 mg/kg and 1000 mg/kg of ethanol extract.

Significant ($p<0.05$) reduction in the degree of peroxidation was observed in the tested groups that received 250 and 500 mg/kg of the aqueous extracts when compared to the control (Figure 9). However, an elevated level of malonaldehyde (MDA) concentration was observed at 1000 mg/kg of aqueous extract. At 250 mg/kg of ethanol extract, MDA formation increased significantly, at 500 mg/kg and above it reduced to a level comparable to control. Lipid profile studies of the treated rats showed a significant reduction in serum cholesterol concentration of all the treated groups except the group treated with 1000 mg/kg bwt of the ethanol extract when compared to the control (Figure 10). Treatments with 500 mg/kg bwt of aqueous extract, 1000 mg/kg bwt aqueous extract, and 1000 mg/kg bwt of ethanol extract of *G. africanum* Welw caused a significant elevation of the serum Triglyceride concentration (Figure 11) of the Wistar rats compared to the control group. No significant difference was observed in HDL cholesterol concentration (Figure 12) of all study groups. Figure 13 and Figure 14 showed the effects of the treatments on the serum LDC and VLDL concentrations of the Wistar rats respectively. Only treatment with 500 mg/kg bwt of ethanol extract significantly reduced the serum LDL cholesterol concentration of the rats compared to the control group. Conversely, treatment with 500 mg/kg bwt of the aqueous extract caused a significant elevation of the serum VLDL cholesterol concentration of the rats compared to the control.

The first lines of defense against free radicals are the antioxidant enzymes systems: SOD, CAT, and GPx which are assisted by such micro-nutrients as copper, zinc, and selenium, serving as cofactors.[16] This study also investigated the in vivo effects of ethanol and aqueous root extracts of *G. africanum* Welw on some oxidative stress markers and lipid profile indices. The results of the investigations showed no reductions in SOD activity of the treatment groups compared to the control (Figure 6) indicating that the daily administration of the aqueous and ethanol extracts of *G. africanum* Welw orally, did not generate significant superoxide radical capable of causing inflammation or oxidative stress. Increased activity of SOD, and catalase at low concentration (250 mg) of aqueous crude extract of GA showed that this extract is capable of boosting enzyme activity, in other words, increasing enzyme protein expression at low dosage, which suggests antioxidant capacity.[17] Ethanol extract administration resulted to a significant increase in the SOD activity of all the experimental animals indicating that ethanol solvent extracted more bioactive agents capable of inducing SOD activity. The stable activity of catalase is a confirmation of non-significant generation of superoxide radical as well as hydrogen peroxide within the experimental period. The reduced SOD and catalase activities at 1000 mg of the ethanol extract compared to the control, showed that continuous administration of the extract beyond 500 mg/kg may induce oxidative stress. Also, the decrease in glutathione peroxidase activity observed at high concentration (1000 mg) of both aqueous and ethanol extracts of GA (Figure 8) is an indication of an increased scavenging activity which may be due to oxidative stress or physiological changes following extract-tissue interaction.

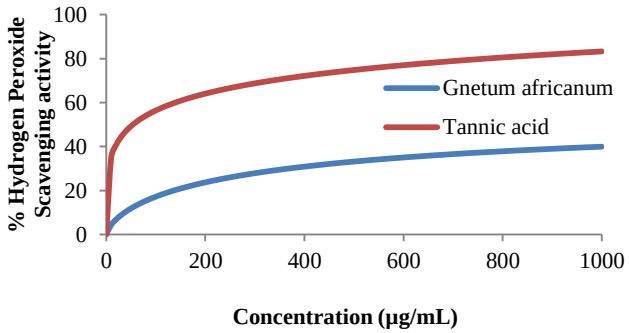

Figure 3: Hydrogen peroxide scavenging activity of *Gnetum africanum* Welw and tannic acid

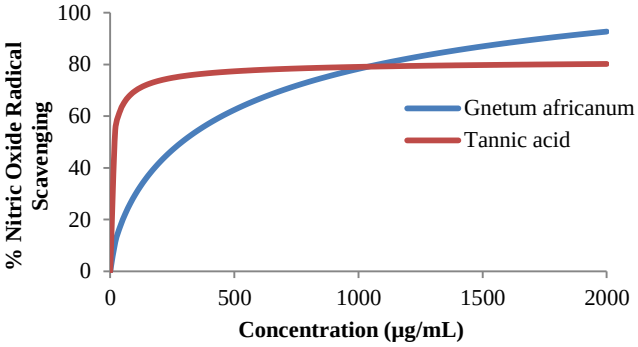

Figure 4: Nitric oxide radical scavenging activity of *Gnetum africanum*Welw and tannic acid

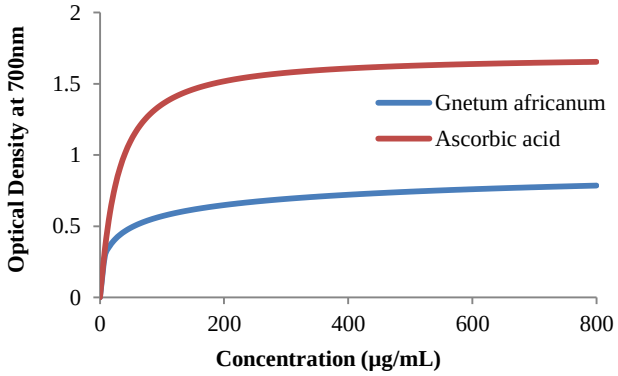

Figure 5: Reducing power of *Gnetum africanum* Welw root extract and tannic acid

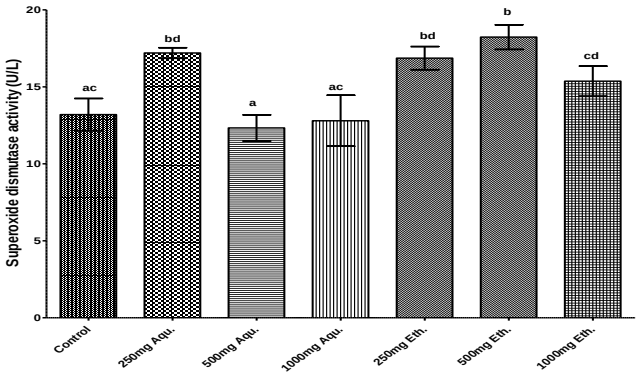

Figure 6: Superoxide dismutase activity of rats treated with aqueous (Aqu.) and ethanol (Eth.) extracts of GA. *****Bars are mean ± standard deviation. Each Bar with the variable letter(s)indicates statistical significance ($p < 0.05$).

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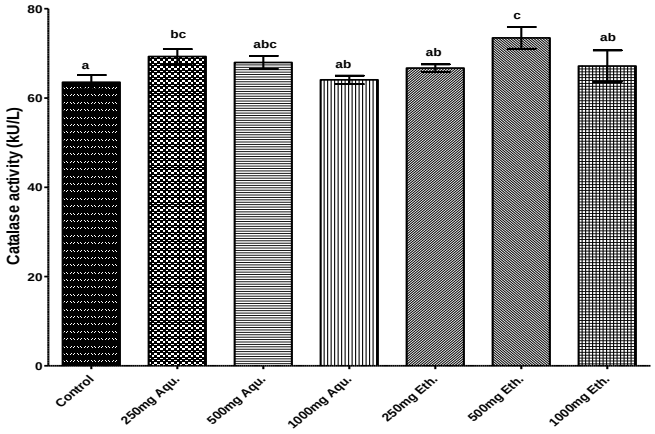

Figure 7: Catalase activity of rats treated with aqueous (Aqu.) and ethanol (Eth.) extracts of GA. *Bars are mean ± standard deviation. Each Bar with the variable letter(s) indicates statistical significance ($p < 0.05$).

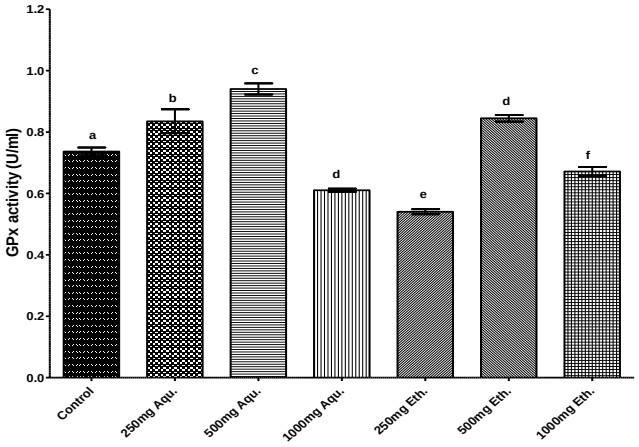

Figure 8: GPx activity of rats treated with aqueous (Aqu.) and ethanol (Eth.) extracts of GA. *Bars are mean ± standard deviation. Each Bar with the variable letter(s) indicates statistical significance ($p < 0.05$).

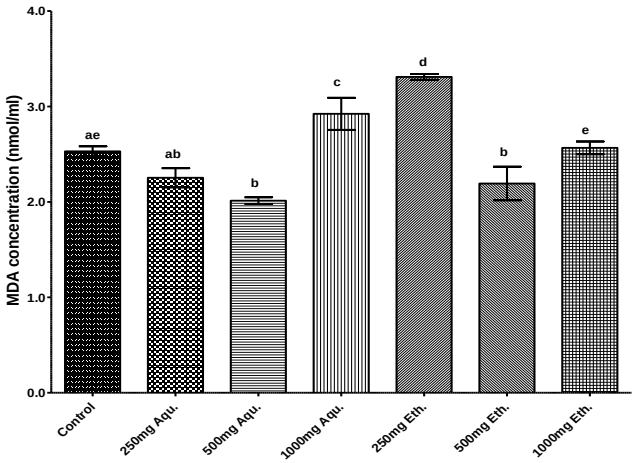

Figure 9: MDA concentration in rats treated with aqueous (Aqu.) and ethanol (Eth.) extracts of GA. *Bars are mean ± standard deviation. Each Bar with the variable letter(s) indicates statistical significance ($p < 0.05$)..

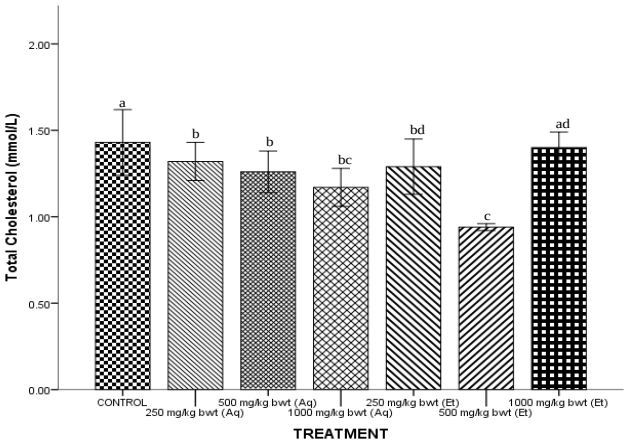

Figure 10: Total cholesterol concentration (mmol/L) in rats treated with aqueous (Aq.) and ethanol (Et.) extracts of GA. *Bars are mean ± standard deviation. Each Bar with the variable letter(s) indicates statistical significance ($p < 0.05$).

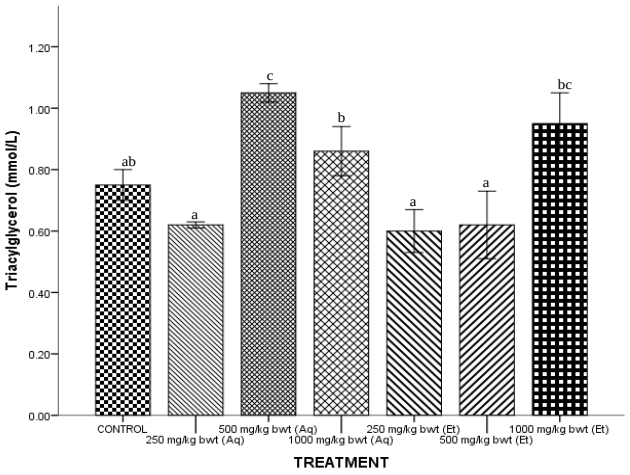

Figure 11: Triacylglycerol concentration in rats treated with aqueous (Aq.) and ethanol (Et.) extracts of GA. *Bars are mean ± standard deviation. Each Bar with the variable letter(s) indicates statistical significance ($p < 0.05$).

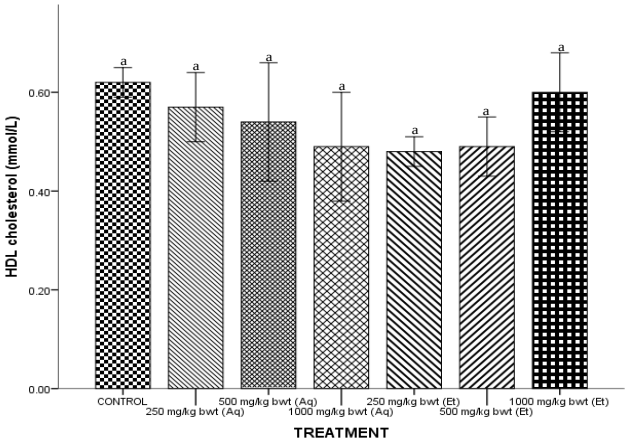

Figure 12: HDL cholesterol concentration in rats treated with aqueous (Aq.) and ethanol (Et.) extracts of GA. *Bars are mean ± standard deviation. Each Bar with the variable letter(s) indicates statistical significance ($p < 0.05$).

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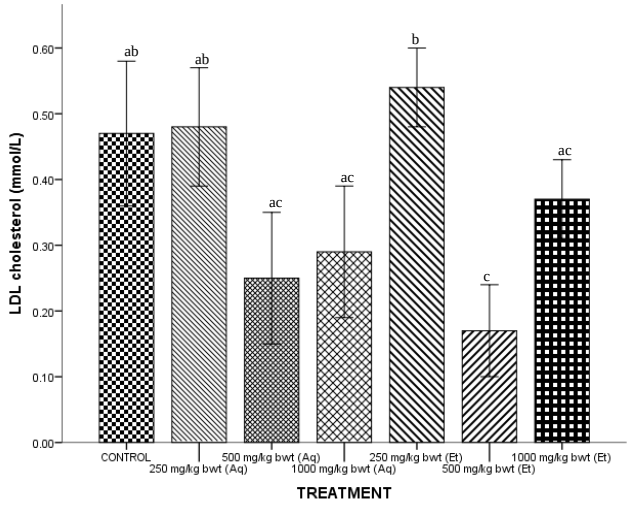

Figure 13: LDL cholesterol concentration in rats treated with aqueous (Aq.) and ethanol (Et.) extracts of GA. *Bars are mean ± standard deviation. Each Bar with the variable letter(s) indicates statistical significance ($p < 0.05$).

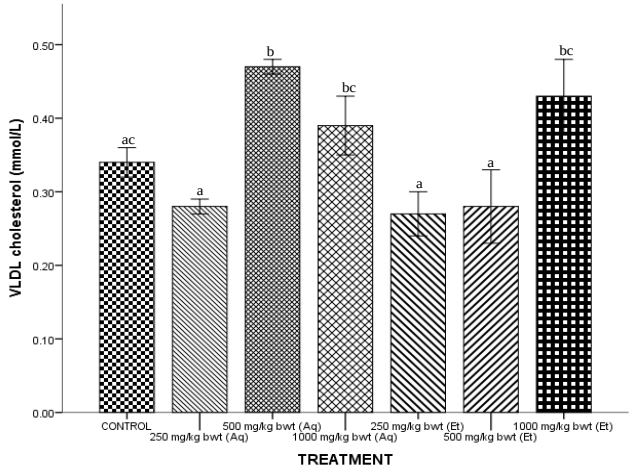

Figure 14: VLDL cholesterol concentration in rats treated with aqueous (Aq.) and ethanol (Et.) extracts of GA. *Bars are mean ± standard deviation. Each Bar with the variable letter(s) indicates statistical significance ($p < 0.05$).

The reduction in GPX activity may be a means of protecting the cellular system against the toxic effect of lipid peroxidation,[18] considering the concomitant increase in malondialdehyde level at the same concentration of 1000 mg/kg body weight. Malondialdehyde (MDA) is the end product and major reactive aldehyde of non-enzymatic degradation of polyunsaturated fatty acids (PUFAS), thus an important indicator of lipid peroxidation or tissue damage20 and a measure of free radical generation.[19]

The reduced MDA level in the groups that received 250 mg of aqueous extract and 500 mg of ethanol extract with the corresponding boost in SOD, CAT, and GPx activities showed that *G. africanum* Welw root possesses antioxidant properties that prevented oxidative stress and as well reduced membrane lipid peroxidation. The findings in this study demonstrated the interplay of antioxidant enzymes, which suggests that *Gnetum africanum* Welw root extract could be effective against oxidative damage and would also serve as a nutriceutical.

Our findings revealed a significant decrease in the serum total cholesterol concentrations of all treated groups (except the group that received 1000 mg/kg bwt of ethanol extract). Cholesterol is the major blood lipid implicated in the formation of atherosclerosis. The reduction of serum total cholesterol concentrations following the treatments could be indicative of enhanced lipid metabolism as a result of the treatment. The reduced MDA level in the groups that received 250 mg of aqueous extract and 500 mg of ethanol extract showed that *G. africanum* Welw truly enhanced the overall cholesterol metabolism in the Wistar rats. The decreased serum LDL-concentration observed in the study implies the reduced formation of ox-LDL-C in the blood, thus a reduced risk of oxidative stress condition and formation of atherosclerosis

Conclusion

Induction of SOD, CAT, and GPx activities, as well as the reduction in the formation of malondialdehyde, observed at low concentrations of aqueous and ethanol extracts of *G. africanum* Welw root clearly showed that this plant part is rich in antioxidants with the potential to protect the system against possible pathological conditions resulting from the presence of such free radicals like hydrogen peroxide, hydroxyl radical and other oxygen species. *G. africanum* Welw root is quite understudied; further research on the isolation of bioactive compounds will be required.

Conflict of Interest

The authors declare no conflict of interest.

Author's 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.

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