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In vivo Antioxidant Effects of Coconut (*Cocos nucifera*) Water Extract in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author OJM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UIE, OAA, IEA and MKN managed the analyses of the study. Author OJM equally managed the literature searches. All Authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study was designed to investigate the invivo antioxidant effects of coconut (*Cocos nucifera*) water extract in wistar albino rats.
Methodology: Thirty (30) male wistar albino rats of mean weight 128 g were used for the study. The animals for the study were grouped into five (5) of six (6) rats each. Group 1 served as the normal control group that received feed and water only while groups 2, 3, 4, and 5 served as the test groups that were orally given 10 ml, 20 ml, 30 ml, and 40 ml of the coconut water extract for 28 days. The rats were sacrificed after 28 days and the blood samples were collected for biochemical analysis.

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Results: From the result obtained there is a significant increase ($p< 0.05$) between the normal control group (group 1) and the test group (group 2) that received 20 ml of the coconut water extract for MDA. There is a significant increase ($p< 0.05$) between the normal control group (group 1) and the test groups (groups 2 and 5) that received 10 ml and 50 ml of the coconut water extract for SOD. Also, there is a significant increase ($p< 0.05$) between the normal control group (group 1) and the test groups (groups 2, 3 and 4) that received 20 ml, 30 ml and 40 ml of the coconut water extract for Catalase. For GSH and Vitamin C, there is a significant increase ($p< 0.05$) between the normal control group (group 1) and the test groups (groups 2, 3, 4 and 5) that were orally given 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract.
Conclusion: The present investigation showed that the coconut water extract increased antioxidant properties in wistar albino rats and may also be used pharmacologically in the treatment of diseases implicated by free radicals.

Keywords: Coconut; antioxidant; catalase; malondialdehyde; muperoxide dismutase; vitamin C, reduced glutathione.

1. INTRODUCTION

For thousands of years, coconut (*Cocos nucifera*) products have held a respected and valuable place in local folk medicine [1]. Coconut water has a host of yet scientifically unproven but traditional uses in cultures all over the world [2]. From ancient times in Africa, reports support the position that about 85% of the world's population relies on coconut fruit in traditional medicine [3]. It is used to conquer irregular or painful menstruation and also taken during pregnancy to give the unborn babies strength and vitality [4]. It is also used to boost semen quality and induce libido [5]. Coconut water contains numerous antioxidant compounds that can scavenge free radicals in the body [6]. Furthermore, micronutrients such as inorganic ions present in coconut water play a vital role in aiding the human body antioxidant system. Kinetin was shown to act as a strong antioxidant both under in vitro and in vivo from oxidative damage mediated by the Fenton reaction. Kinetin inhibits the formation of 5- oxo- 2- deoxy guanosine, which is a common marker of oxidative damage in DNA. The oxidant properties of kinetin suggested that it may also present the oxidative damage of unsaturated fatty acids located within the cell membranes [7]. Coconut water or coconut juice is a sweet refreshing drink taken directly from the inner part of coconut fruits [2]. It differs from coconut milk, which is the oily white liquid extracted from the grated fresh kernel in most cases, coconut tree plantations more related to garden. As a consequence, the coconut water remains a traditional and water used resource which could thus be considered as an exotic beverage by most people living far from the coconut production area [8]. Coconut water is not only a tropical beverage but also traditional medicine. A microbiological growth medium and a ceremonial gift [9], and can be processed into vinegar or wine [10]. These various uses are possible thanks to the original biochemical composition of the juice. The particular mineral composition and reasonable total sugar content make coconut water a natural isotonic liquid. The characteristics of coconut water make it an ideal rehydrating and refreshing drink after physical exercise [11].

Consequently, in this present study, the *in vivo* effects of coconut (*Cocos nucifera*) water extract were studied in wistar albino rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

The coconut fruits (*Cocos nucifera*) were purchased from Umuahia market, Abia state, Nigeria and identified by Dr. Garuba Omosun of the Plant Science and Biotechnology Department, Michael Okpara Uiversity of Agriculture, Umudike.

2.2 Experimental Animals

Healthy male Wistar albino rats of mean weight (128 g) were obtained from Department of Veterinary Medicine, Michael Okpara University of Agriculture. The rats were freely allowed access to standard feed and water *ad libitum*. All experiments with the Laboratory Animals were conducted in accordance with National Institute of Health Guidelines revised in 1985 (NIH Publications No. 8- 23).

2.3 Chemicals and Reagents

Hydrochloric acid (Hcl), sulphuric acid solution, phosphate buffer, disodium hydrogen phosphate, potassium permangate, sodium dihydrogen carbonate (NaH_2CO_3) ethylene diamine tetracetate (EDTA), sodium citrate, sodium hydroxide, trichloroacetic acid (TCA), Thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (Steinhelm, Germany). All other chemicals were of analytical grade.

2.4 Preparation of Coconut Water Extract

The coconut fruits obtained were punctured at the holes using a sterilized nail. It was then placed over a container and the water was allowed to drain. The coconut water extract was obtained fresh and used immediately.

2.5 Experimental Design

Animals were grouped into six animals each

Group A -Vital feed and water only (normal control group)
Group B - 10 ml coconut water + 150 g vital feed
Group C - 20 ml coconut water + 150 g vital feed
Group D - 30 ml coconut water+ 150 g vital feed
Group E - 40 ml coconut water + 150 g vital feed

Treatment lasted for 28 days, after which the animals were sacrificed on day 29 under mild anesthesia (10% formasaline). Blood samples were collected in the plain bottle for the analyses of the effects of the coconut water extract on the antioxidant parameters in wistar albino rats.

2.6 Evaluation of the Various Parameters Studied

2.6.1 Determination of Reduced Glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of [12].

2.6.2 Determination of vitamin C

Determination of ascorbic acid was according to the method proposed by [13].

2.6.3 Catalase assay

Determination of catalase activity was according to [14].

2.6.4 Determination of Superoxide Dismutase (SOD)

This was determined using the method [15].

2.6.5 Determination of Malondialdehyde (MDA)

Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA) as described by [16].

2.7 Statistical Analysis

The data were expressed as Mean ± Standard error of Mean (Mean ± SEM) and presented as figures. Data were analyzed using statistical package for the social sciences (SPSS 22.0). Comparison was made between the test groups and the control group using one way Anova and $P < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effects of Coconut Water Extract on Malondialdehyde (MDA)

The result of the mean comparison of the control group (group 1) and the test groups (Group 3, 4, 5) that received 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract showed that there is a significant increase ($p < 0.05$) only between the normal control group and the test group (Group 2) that received 20 ml of the coconut water extract.

3.2 Effects of Coconut Water Extract on Superoxide Dismutase (SOD)

The result of the mean comparison of the normal control group and the test groups (Group 2, 3, 4,5) that received 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract showed that there is a significant increase ($p < 0.05$) between the normal control group and the test groups that received 10 and 40 ml of the extract.

3.3 Effects of Coconut Water Extract on Catalase

The result of the mean comparison of the normal control group (group 1) and the test groups (Group 2, 3, 4, 5) that received 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract showed that there is a significant increase ($p <$

0.05) between the normal control group and the test groups (Group 3, 4, and 5) that were orally given 20 ml, 30 ml and 40 ml of the coconut water extract.

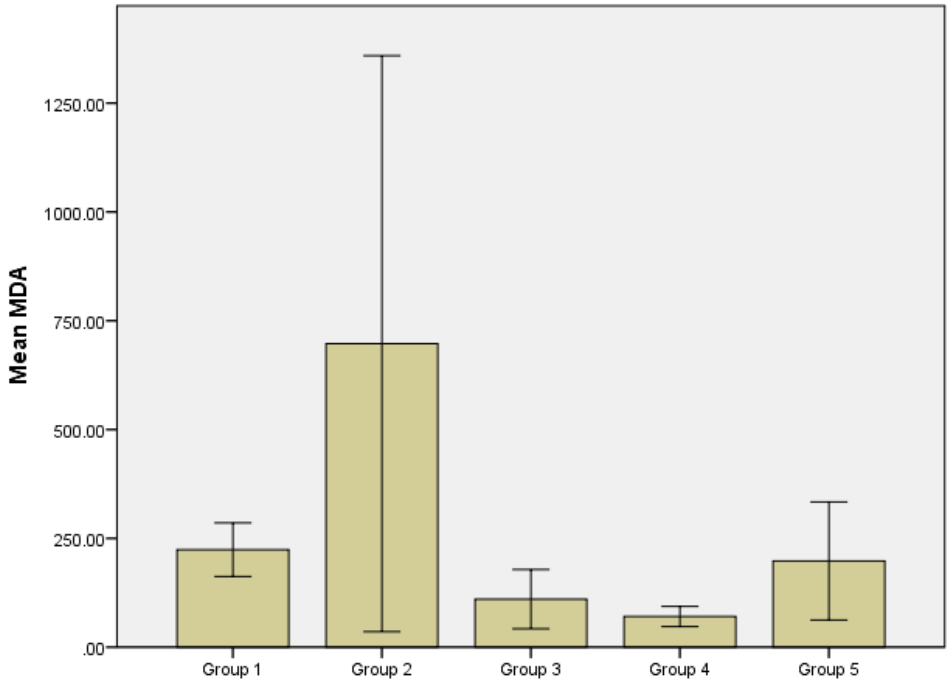

Fig. 1. Mean comparison of the normal control group (group 1) and the test groups (group 2, 3, 4 and 5) that received 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract for MDA

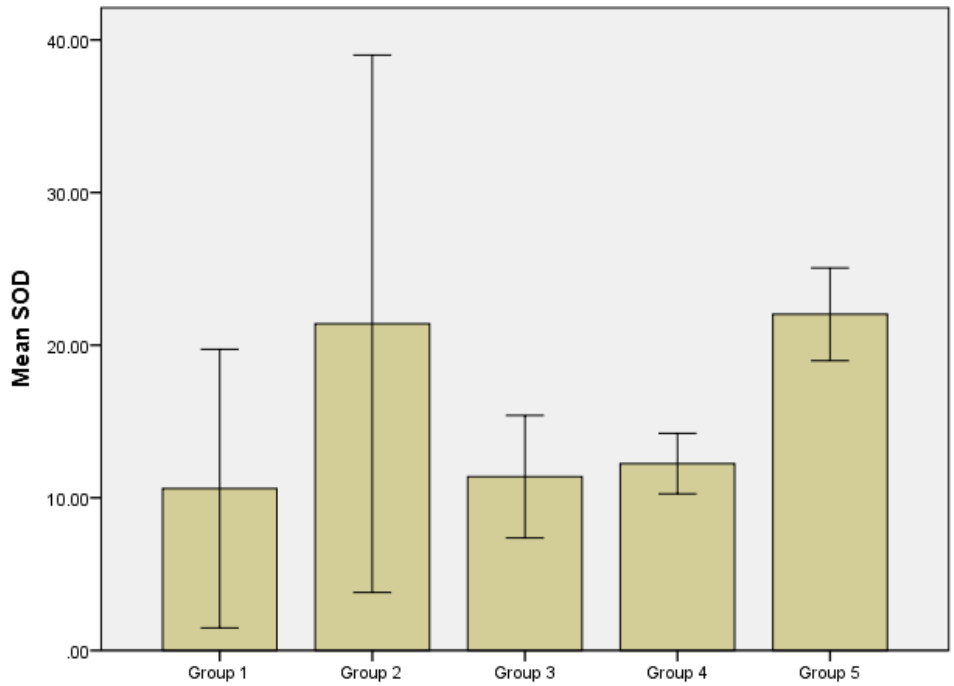

Fig. 2. Mean comparison of the normal control group (group 1) and the test groups (Group 2, 3, 4 and 5) that received 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract for SOD

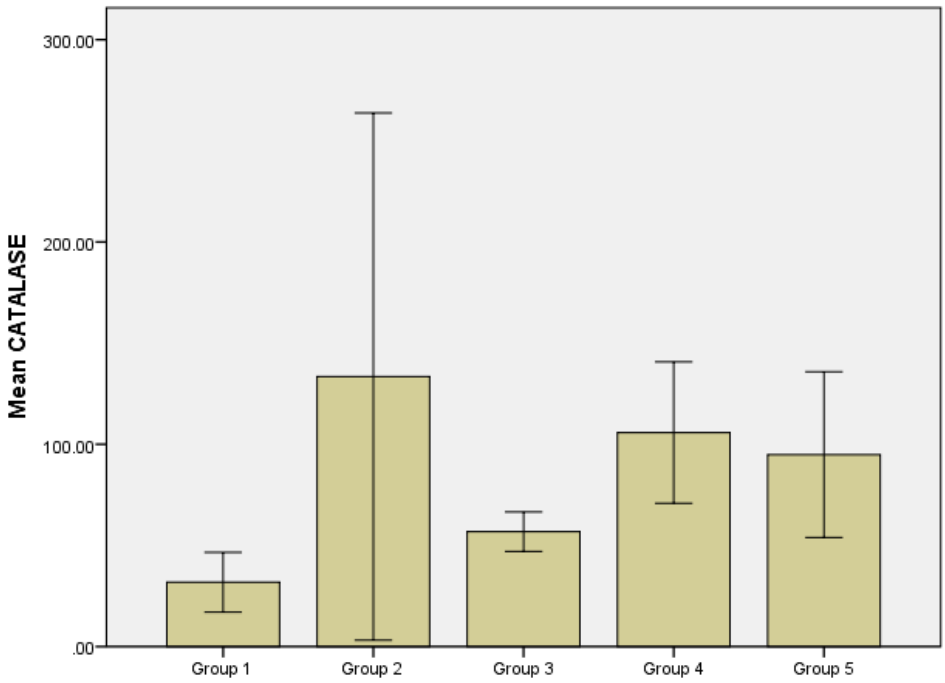

Fig. 3. Mean comparison of the normal control group (Group 1) and the test groups (Group 2, 3, 4, and 5) that were orally given 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract for Catalase (CAT)

3.4 Effects of Coconut Water Extract on Reduced Glutathione (GSH)

The result of the mean comparison of the control group (group 1) and the test groups (Group 2, 3, 4 and 5) that received 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract showed that there is a significant increase ($p < 0.05$) between the normal control group and the test groups.

3.5 Effects of Coconut Water Extract on Vitamin C

The result of the mean comparison of the normal control group (group 1) and the test groups (Group 2, 3, 4 and 5) that were orally given 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract showed that there is a significant increase ($p < 0.05$) between the normal control group and the test groups.

Antioxidant enzymes (made in the body) and antioxidant nutrients (found in foods) can scavenge and deactivate these reactive free radicals turning them to harmless particles [17]. Improving body antioxidant status is a way to fight against degenerative diseases. This could be achieved by a higher consumption of vegetables and fruits [18]. The positive effect attributable to antioxidant is due to the presence of carotenoids, flavonoids, lycopene, phenolics, vitamin C and B-carotene [19]. The effectiveness of the antioxidants usually increases with their concentrations [20].

The effect of the antioxidants usually increases with their concentrations [20]. The effect of the coconut water on some parameters was examined, for the test group that received 10 ml, 20 ml, 30 ml, and 40 ml of the coconut water extract in comparison with the mean difference of the control group, there was a significant increase ($p < 0.05$) between the control group and the test groups signifying that the level of these free radical scavengers (MDA, SOD, GSH Catalase, and Vitamin C) increased in the test groups in comparison with the control group, which indicates that the coconut water extract enhances antioxidant activities in wistar albino rats.

Malondialdehyde (MDA) is the organic compound with formula CH2 (CHO)2. MDA mainly exist in the enol form. MDA results from the lipid peroxidation of polyunsaturated extract)) fatty acids [21]. MDA is the end product of

lipid peroxidation and measures free radical generation. Also, there is no significant difference ($p< 0.05$) between the control group and the test group (group 4), for the MDA and SOD, but there is a significant increase ($p< 0.05$) between the control group and the test group for Catalase.

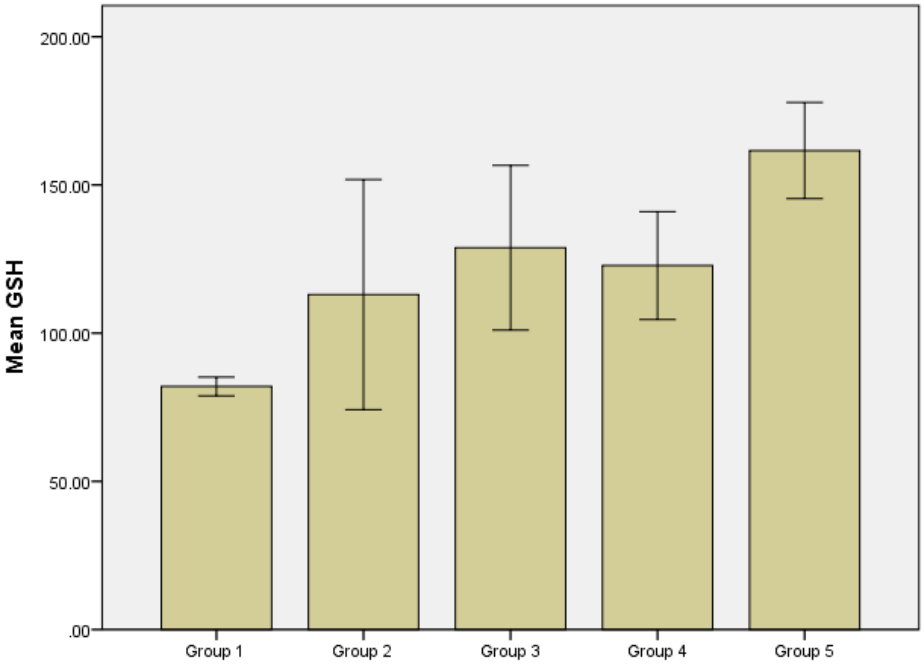

Fig. 4. Mean comparison of the normal control group (Group 1) and the test groups (Group 2, 3, 4 and 5) that were orally given 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract for reduced glutathione (GSH)

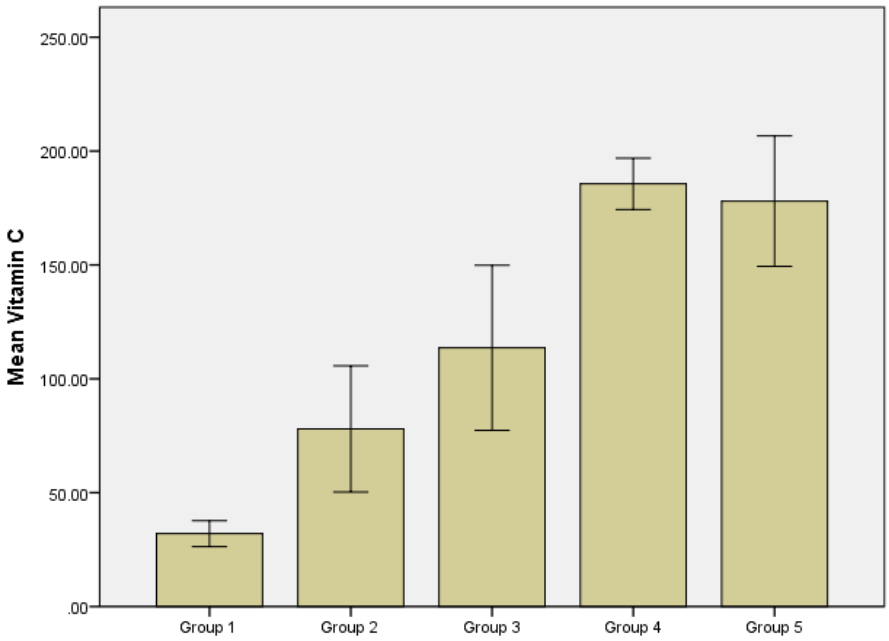

Fig. 5. Mean comparison of the normal control group (Group 1) and the test groups (Group 2, 3, 4 and 5) that were orally given 10 ml, 20 ml, 30 ml and 40 ml of the coconut water extract for vitamin C

SOD which are enzyme that alternately Catalase the dismutation of the superoxide (O2) radical into either ordinary molecular oxygen (O_2) of hydrogen peroxide (H_2O_2), hydrogen peroxide is also damaging, but less so, and is degraded by other enzymes such as Catalase. The ligands of copper and zinc which are the active sites of SOD or proteins are histidine and one aspartate side chain, one histidine is bound between the two metals. This shows that coconut water extract has some antioxidant properties that help to detoxifier the effect of some harmful substances such as nitrogen oxides or drugs [22]. Glutathione (GSH) is a tripeptide found in most cells and reacts with the free radicals to protect cells against hydroxyl radical, singlet oxygen and superoxide radical [6]. The activity of GSH increased in the test groups. This shows that the coconut water extract possesses antioxidant properties that help to stabilize the integrity of cell membrane and also prevent hepatic damage mediated by free radicals [23].

Vitamin C (ascorbic acid) activity in the study showed a dose-dependent increase in the test groups that received 10 ml, 20 ml, 30 ml and 40 ml. Again, indicating the ability of the coconut water extract to act as an antioxidant supplement.

4. CONCLUSION

The present investigation showed that coconut water extract possesses antioxidant properties. The plant should, therefore, be employed in the formulation of more effective antioxidant medicines that will improve human health.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 8-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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