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STUDY OF ANTIOXIDANT EFFICACY OF *ABELMOCHUS ESCULENTUS* (OKRA) FRUITS' VARIETIES ON RATS FED HIGH-FAT DIET

DH Mhya*[1], AA Nuhu[1], A. Mohammed[1], SI. Yau[1], MA. Milala[2], MK. Atiku[3] and AM. Wudil[3]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Abubakar Tafawa Balewa University Bauchi, Nigeria.

[2]Department of Biochemistry, Faculty of Sciences, University of Maiduguri, Nigeria.

[3]Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria.

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***Corresponding Author**
DH Mhya
Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Abubakar Tafawa Balewa University Bauchi, Nigeria.

ABSTRACT

Consumption of diet rich in fats have been reported to promotes the progression of oxidative stress. Literature survey shows that Okra fruits has the potentials to ameliorate oxidative stress. However, Okra plants are of different varieties, and whether their antioxidant efficacies may vary with varieties is not known. This has prompted the study to compare antioxidant activities of two varieties of Okra fruits (NHB-AI-B and Yar Kolon) that showed potent antihyperlidemic and rich phytochemicals content as reported by the authors' previous study. *NHB-AI-B* and *Yar Kolon* okra fruits were sliced, air dried and pulverized then extracted with methanol (80%) using Soxhlet extractor and concentrated at 30°C in a rotary evaporator then air dried. Nine groups of five rats were fed high-fat diet for 35 days followed by treatment with extracts for 21 days. Groups 1-3 (rats received varied dose of *NHB-AI-B* okra fruit variety), group 4-6 (rats received varied dose of *Yar kolon* okra fruit variety), group 7 (positive control), group 8: (normal control), and group 9 (negative control). Oxidative stress markers were assayed from serum of each rat. The results showed an elevated malondialdehyde (MDA) levels with low activities of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) in rats fed high-fat diet. Treatment with extracts of the okra fruits varieties lowered MDA levels and promotes SOD, CAT, GSH activities in the rats' groups. In conclusion, findings from the

study suggested both NHB-AI-B and Yar Kolon fruits varieties exert antioxidant activities likely to be in a similar manner.

KEYWORDS: Okra, varieties, *NHB-AI-B, Yar Kolon*, fruit-extracts, antioxidant, rats, high-fat diet.

1.0 INTRODUCTION

Oxidative stress is found to be associated in the pathogenesis of several human chronic diseases. It is essentially an imbalance between the production of free radicals and the ability of the body antioxidant defense system to counteract or detoxify their harmful effects. Due to the broad and profound biological effects of the free radicals, numerous experimental and clinical studies have focused on the understanding of the participation of oxidative stress as a key regulator in pathogenesis of many diseases.[1] To this effect, several recent studies targeted at finding novel therapeutic strategies that could ameliorate oxidative stress.[2,3]

Consumption of diet rich in fats have been reported to promotes the progression of oxidative stress.[4-6] It was found that, high-fat diet elevates oxidative stress by attenuating antioxidant enzyme system resulting in the increase lipid peroxidation products.[5,6] Literature shows that increase in oxidative stress due to high-fat diet precedes the development of several disorders including obesity and metabolic derangements.[7-8] Several medicinal plants have shown to possess some potent antioxidant compounds that aid in providing significant protection against chronic diseases. Some of these compounds were found to protect LDL cholesterol from oxidation, inhibiting cyclooxygenase and lipoxygenase enzymes, resulting in the suppression of lipid peroxidation.[9-11] Plants are reported to be good and cheap sources for the prevention and treatment of oxidative stress thus playing an important role in chemoprevention of diseases associated with oxidative stress.[12]

The plant *'Abelmoschus esculentus L*. (Moench)' known as ladies finger or Okra in English, is an important vegetable crop around the world.[13,14] In Nigeria, it is known as "*Kubewa"* (Hausa), "*O'okro*" (Igbo) and *L'laa*" (Yoruba). It has both food and medicinal values due to its rich phytochemicals like phenolic and flavonoids which are distributed around its different parts, where it was reported to possess vital pharmacological potentials including antioxidant properties.[15, 16] Recent study by the authors has reported significant antihyperlipidemic effects of NHB-AI-B and Yar Kolon okra fruits varieties in rats.[17]

Literature survey shows that Okra fruits has the potentials to ameliorate oxidative stress.[15,16] However, Okra plants are of different varieties, and whether their antioxidant efficacies may vary with varieties has not yet be ascertained. This has prompted the study to compare antioxidant activities of two varieties of Okra fruits (NHB-AI-B and Yar Kolon) which shows to possess potent antihyperlidemic properties and rich phytochemicals content as reported by the authors' previous study.[17]

2.0 MATERIALS AND METHODS

2.1 Chemicals

All chemicals used for the study were of analytical grade and were obtained from Sigma Aldrich, England and British Drug House (BDH), London.

2.2 Plant Collection/Identification

The Okra fruit varieties were obtained from the Teaching and Research Farm of the Faculty of Agricultural Science Technology, Abubakar Tafawa Balewa University, Bauchi. It was authenticated and identified with Voucher number: 1914.

2.3 Experimental Animals

Forty-five Wistar albino rats about 4 weeks old were obtained from the Animal House, Department of Biology, Bayero University Kano, Nigeria. They were kept in cages with free access to water and feed for two weeks for acclimatization. The principles of laboratory animal care[18] and ethical guidelines for investigation of experimental pain in conscious animals[19] were observed.

2.4 Plant Extraction

The *NHB-AI-B* and *Yar Kolon* Okra fruit varieties were each extracted following the method described by Doreddula *et al*[20] with modification in the extraction time (12 hours). The Okra fruits were sliced, air dried at 25^{o}C and then pulverized using pestle and mortar. The powdered *NHB-AI-B* (254g) and *Yar Kolon* (282g) were extracted with methanol (80%) using Soxhlet extractor for 12 hours at 25^{o}C and concentrated at 30^{0}C in a rotary evaporator then air dried. The dried extracts: *NHB-AI-B* (58.03g) and *Yar Kolon* (48.24g) was used.

2.6 Formulation of high fat diet

The high fat diet was formulated using Super starter animal feed composed of the following: maize (46%), soybean meal (18.5%), groundnut cake (15%), fishmeal (2%), wheat offal

(12.45%), bone meal (2%), oyster shell (3%), salt (0.25%), premix (0.25%), methionine (0.3%), and lysine (0.25) respectively. The basal diet was 100% super starter feed while the formulated high fat diet composed of 75% super starter feed, 5% egg yolk and 20% palm oil.

2.7 Experimental Design

The study was conducted with forty-five rats randomly divided into nine groups of five where eight groups were fed high-fat diet for 35 days period followed by administration of extracts (mg/kg body weight) for 21 days as follows:

Group I: Rats fed high fat diet + 250mg *NHB-AI-B* extract

Group II: Rats fed high fat diet + 500mg *NHB-AI-B* extract

Group III: Rats fed high fat diet + 750mg *NHB-AI-B* extract

Group IV: Rats fed high fat diet + 250mg *Yar kolon* extract

Group V: Rats fed high fat diet + 500mg *Yar kolon* extract

Group VI: Rats fed high fat diet + 750mg *Yar kolon* extract

Group VII: Rats fed high fat diet + 10mg Atorvastatin (positive control)

Group VIII: Rats fed normal diet with no treatment (Normal control)

Group IX: fed high fat diet with no treatment (Negative control)

2.8 Collection of Blood Sample

Animals were sacrificed after 21 days treatment, they were anaesthetized by putting in a plastic jar saturated with chloroform vapor. Blood collected and serum separated were used for analysis.

2.9 Determination of Antioxidant Parameters

Superoxide dismutase activity was determined by Misra and Fridovich[21] method. Catalase activity was ascertained by Claiborne[22] method. Reduced glutathione was estimated by Beutler *et al.*[23] method**.** Malondialdehyde was determined by Ohkawa *et al*[24] method.

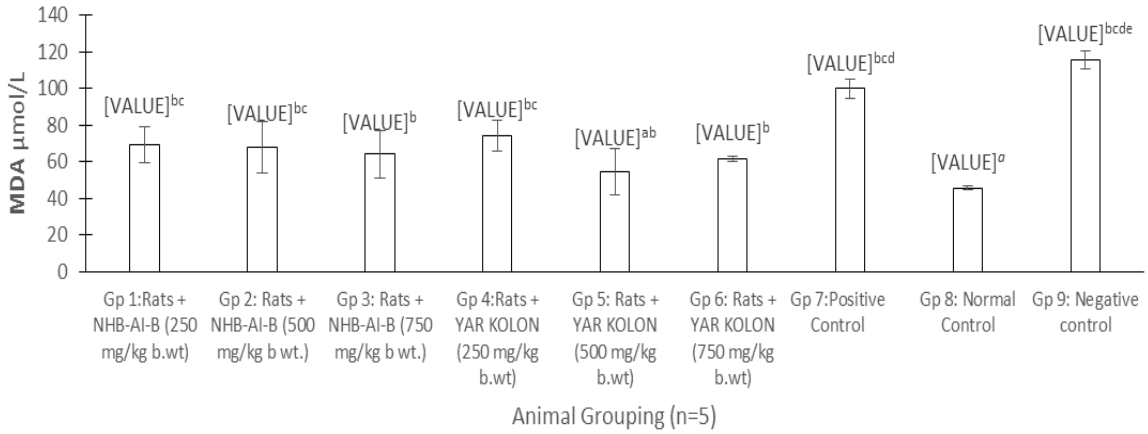
2.10 Statistical analysis

Data from the experiments were expressed as mean ± standard deviation (SD) and presented by a bar chart format. Means were analyzed using ANOVA and significant difference was accepted at $p<0.05$.

3.0 RESULTS

3.1 Effect of Okra Fruit Varieties on MDA in Rats Fed High-Fats Diet

The result of changes in malondialdehyde (MDA) level from the various groups is shown in Figure I. It showed that, the negative control rats had the highest increase in the MDA level compared to the treated rats' groups where both *NHB-AI-B* and *Yar Kolon* extracts at different doses showed a promising activities by lowering MDA toward normal value.

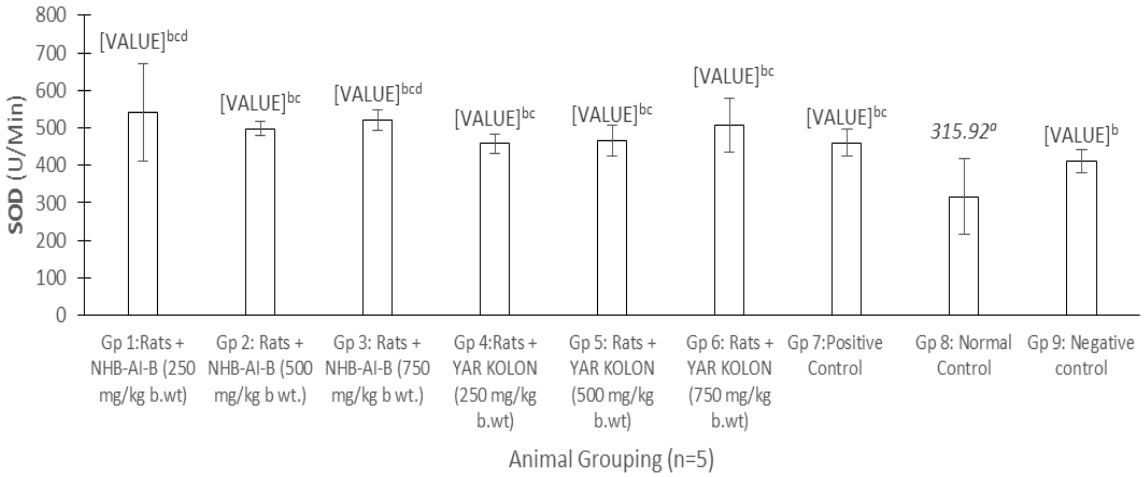


Values are Mean ± SD of 5 determinations. Bars with different superscript letter are significantly different at P<0.05

Fig. I: Effect of Oral Administration of Okra Fruits Varieties (*NHB-AI-B* and *Yar Kolon*) on Malondialdehyde Levels in Rats Fed-High Fats Diet.

3.2 Effect of Okra Fruit Varieties on SOD Activity in Rats Fed High-Fats Diet

Figure II present result of the activity of Superoxide dismutase (SOD) in treated and non-treated rats fed high fat diet. The study recorded a significant changes on the enzymes when compared the values from treated rats' groups with okra fruit varieties at different doses. The rats that were treated showed an increased activity of SOD compared to the negative and normal control rats.

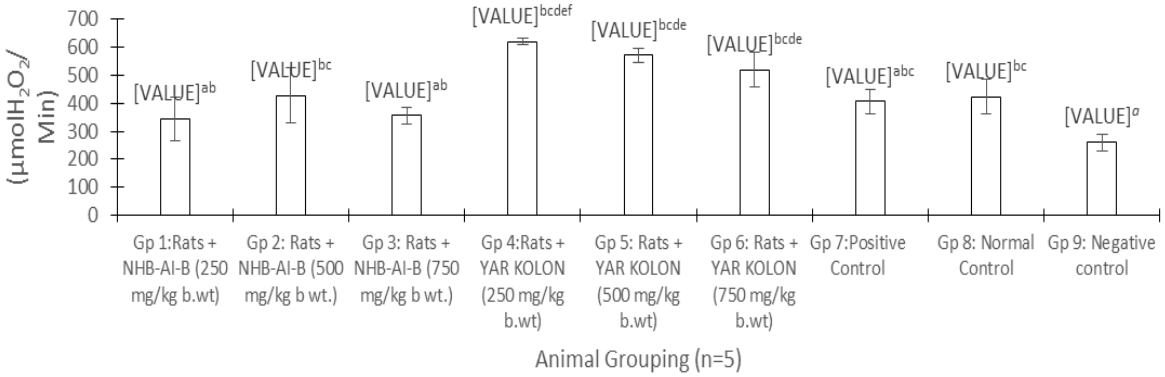


Values are Mean ± SD of 5 determinations. Bars with different superscript letter are significantly different at P<0.05

Fig. II: Effect of Oral Administration of Okra Fruits Varieties (*NHB-AI-B* and *Yar Kolon*) on Superoxide Dismutase Levels in Rats Fed-High Fats Diet.

3.3 Effect of Okra Fruit Varieties on Catalase Activity in Rats Fed High-Fats Diet

The result of catalase activity assayed is presented in figure III. The study recorded a significant changes in the enzyme's activity from rats' groups following treatment with varieties of okra fruits. High activity of the enzyme is recorded from rats treated with *Yar kolon* okra fruit variety while depressed activity was seen in the negative control rats as compared with normal control and extract treated rats.

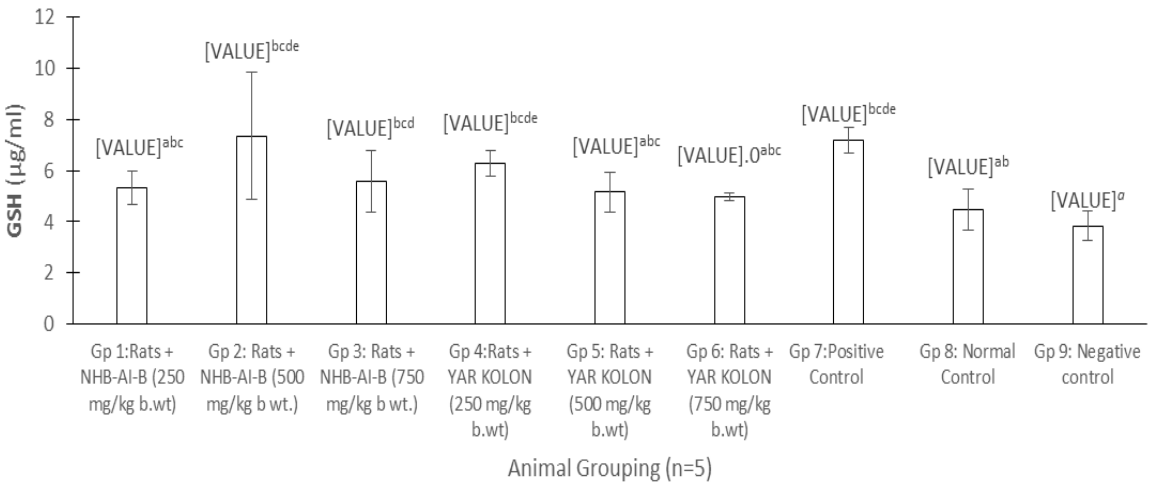


Values are Mean ± SD of 5 determinations. Bars with different superscript letter are significantly different at P<0.05

Fig. III: Effect of Oral Administration of Okra Fruits Varieties (*NHB-AI-B* and *Yar Kolon*) on Catalase Levels in Rats Fed-High Fats Diet.

3.4 Effect of Okra Fruit Varieties on GSH Activity in Rats Fed High-Fats Diet

The result for the effects of oral administration of okra fruit varieties (*NHB-AI-B and Yar Kolon*) on reduce glutathione activity in rats fed high fats diet is presented in Figure IV. Data from the result showed an increased activity of GSH in rats treated compared with those that are not treated.



Values are Mean ± SD of 5 determinations. Bars with different superscript letter are significantly different at P<0.05

Fig. IV: Effect of Oral Administration of Okra Fruits Varieties (*NHB-AI-B* and *Yar Kolon*) on Catalase Levels in Rats Fed-High Fats Diet.

4.0 DISCUSSION

Oxidative stress is a common factor involved in the pathological states of several human chronic diseases,[1] and is found to be elevated by consumption of diet rich in fats.[4-6] Okra was reported to ameliorate oxidative stress[15,16] but, there are of different varieties,[25] and whether the antioxidant efficacy varies with varieties is yet to be ascertained. This has prompted the study to compare antioxidant activities of two varieties of Okra fruits (NHB-AI-B and Yar Kolon) which shows to possess potent antihyperlidemic properties and rich phytochemicals content as reported by the authors' previous study.[17]

The study observed that feeding rats with high fat diet could induced oxidative stress as evidenced by elevated MDA levels and decreased activities of SOD, CAT, and GSH. When the various rats groups were treated with different varieties of okra fruits' (*NHB-AI-B* and *Yar kolo)* methanol extracts, activities of antioxidant enzymes; SOD, CAT, and GSH were

promoted where MDA levels was lowered in a vary degrees suggesting possible amelioration of oxidative stress.

A previous findings by the authors has reported some phytochemicals like phenols, flanovoids, saponin, tanins, and glycoside from both extracts of the okra fruit varieties.[17] Antioxidant activity of Okra had been suggested to be due to their phenolics and flavonoids contents.[16] Elevation in the activities of the antioxidant markers; SOD, CAT, GSH and decreased MDA level from the present study may suggest that methanol extracts of *NHB-AI-B* and *Yar kolon* okra fruit varieties possess components that exert antioxidant activities. This agrees with the findings reported by Sabitha *et al*[15] where fruit peel and seed powder of Okra significantly increased superoxide dismutase, catalase, glutathione peroxidase (GPx) and reduced glutathione (GSH). *In vitro* antioxidant effect of methanol extract of okra fruits was early reported by Atawodi *et al*[26] Based on these, one may suggest that presence of the phytochemicals identified in the two okra varieties might have contributed to their antioxidant activities. Where phenolics and flavonoids may be the key players as have been pointed out in a similar study.[16]

CONCLUSION

The study confirmed consumption of diet rich in fats may promote the progression of oxidative stress and treatment with extracts from NHB-AI-B and Yar Kolon okra fruits varieties reverses the alterations that may lead to the amelioration of oxidative stress.

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Authors' contribution

The work was done in collaboration among all authors. M.K. Atiku and A.M. Wudil designed and wrote the protocol of the study. Asmau Ahmad Nuhu and Daniel Hassan Mhya carried out the experiment and performed the statistical analysis. Mohammed Abdulrashid and Salamatu Ibrahim Yau wrote the first draft of the manuscript, Mohammed Adamu Milala read and adjust the manuscript fit for publication. All authors read and approved the final version of the manuscript.

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Ethical Consideration

Ethical issues including text plagiarism, data fabrication, falsification, manipulation, redundant publication as well as duplicate submission have been carefully observed.

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