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Moringa oleifera Leaf Prevents Oxidative Stress in Wistar Rats

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Authors' contributions

Author DPO designed the study, wrote the protocol, performed the laboratory analysis of measured biochemical parameters and revised the final draft of the manuscript. Author ASA performed the statistical analysis, wrote the first draft of the manuscript and managed the literature searches Furthermore, all authors read and approved the final manuscript

Original Research Article

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ABSTRACT

Background: It is believed now in some group of people that *Moringa oleifera* is medicinal in nature. People take it in any form to cure various clinical conditions. A number of clinical conditions have been linked to oxidative stress injury. Therefore its mechanism of action may be to control oxidative stress (oxidant-antioxidant status).
Objective: It was designed to measured plasma malondialdehyde (MDA), total antioxidant status (TAS) and red blood cell glutathione peroxidase (GPx) of Wistar rat.
Materials and Methods: Seventy healthy Wistar rats of both sexes were recruited into the study. Fourty and 30 rats were fed with diet fortified with *Moringa oleifera* leaf and normal diet respectively for a period of four weeks. Serum (for the measurement of MDA and TAS) and haemolysate (for the measurement of GPx) were prepared from blood sample collected after rats were sacrificed. Variables gotten were analyzed using SPSS version 17, taking level of significance to be 0.05.
Results: Serum MDA was observed to be significantly lower in rats fed with *Moringa oleifera* than those with normal diet ($p<0.05$). Mean serum TAS as well as red blood cell GPx were found to be significantly higher ($p<0.01$) in rats fed with *Moringa oleifera* than those with normal diet. There were negative and positive correlations between 'MDA and TAS' and 'MDA and GPx' respectively in both study groups.
Conclusion: *Moringa oleifera* leaf may prevent oxidative stress in Wistar rats.

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Keywords: Moringa oleifera; oxidant; antioxidant; oxidative stress; lipid peroxidation.

1. INTRODUCTION

Among myriad of plants, *Moringa oleifera*, is one of the best known and most distributed species of Moringaceae family*. Moringa* is an important tropical plant that is used as human food supplement and medicine [1]. It is cultivated in tropical regions because of its good nutrient status and the belief that it is medicinal [2]. *Maringa* has been used in the traditional medicine passed down to centuries in many cultures around the world. The reasons why people take it to cure some clinical conditions have not been fully ascertained. However, the control of oxidative stress injury by supplying various forms of antioxidants is being suggested [3-5].

Most disorders in the body have been linked to oxidative stress [6-9]. Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. [10-11]. Oxidative stress may not only be associated with decreased antioxidant levels but also excessive production of oxidants (free radicals) like reactive oxygen species [10-11]. These free radicals, attack various cellular organelles to cause injury to the cell. Their attack on polyunsaturated membrane lipid leads to the formation of an intermediate product called malondialdehyde (MDA) [12,13]. Malondialdehyde is now widely measured as evidence of oxidant attack on membrane lipid. This dangerous effect in the phase of insufficient antioxidants may lead to cellular death [12,13]. Among the antioxidant markers are Glutathione peroxidase (GPx), superoxide dismutase (SOD) and antioxidant vitamins. Total antioxidant status (TAS) of an individual is a function of dietary and, enzymatic and other systemic antioxidants like vitamins.

Previous study has shown that *Moringa oleifera* leaves increased superoxide dismutase and catalase activities [14]. Moreover, other studies have documented increased antioxidants, especially β–carotene, Vitamin B, C and E. However, there are limited studies in our environment to ascertain above findings [15] . Moreover, an antioxidant content of *Moringa oleifera* has been observed to be different in some African regions (Ghana, Rwanda, Senega and Zambia) but not yet a study to show this in Nigeria [16]. This study was designed to determine to what extent *Moringa oleifera leaf* affects oxidant-antioxidant status of Wistar rat. It was designed to measured plasma malondialdehyde, total antioxidant status (TAS) and red blood cell glutathione peroxidase (GPx) of Wistar rat fed with *Moringa oleifera.*

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted at animal house of Department of Biomedical Sciences, Ladoke Akintola University of Technology Osogbo.

2.2 Source and Preparation of *Moringa oleifera* Leaf

Moringa Oleifera leaf was gotten in a local farm in Osogbo, Nigeria and its powder form was prepared as follows: after proper identification, the fresh leaves of *Moringa oleifera* were collected and dried in fresh air for seven days.The dried plants materials were grinded into fine powder

2.3 Experimental Animal

Seventy healthy Wistar rats of both sexes were recruited into the study. They were kept under standard conditions in the animal house. The Wistar rats were divided into two groups (control and test groups) using simple random sampling techniques. These two groups were placed in two different cages. Cage 1 contained 40 rats (test group) and cage 2 contained 30 rats (control group). The cage for the test rats was rectangular shaped and measured 165cm and 80cm in length and width respectively (floor area of about 13,200cm^2) while that of control rats also was rectangular shaped with 165cm by 55cm (floor area of 9,075cm^2) in dimension. The height of the two cages was 45cm and 43cm for test and control rats respectively. The floor of the cage was made of plastic material and major parts of the body were made of wire mesh giving minimal illumination for the test rats. Other parts were made of plastic material.

The rats were acclimatized for a period of two weeks during which they were allowed free access to their normal feed (growers mash) and deionized water. After acclimatization, the control animals (group 2) continued on normal diet and drinking water for a period of 4weeks. The test animals (group 1), dried *Moringa oleifera* leaf powder were included in their normal feed (growers mash) for a period of 4weeks. The *Moringa oleifera* leaf powder made up 15% of feed given to test group. The control and test groups were given 300ml of water but that of test group was mixed with 15 mls of freshly blended *Moringa* leaf daily (5% of their fluid).

The weights of the animals were measured at the beginning and at the end of the study using an electronic weighing balance.

2.4 Blood Sample Collection and Preservation

At the end of the 6th week, the rats (both the control and test groups) were sacrificed and blood was collected from the carotid artery into a plain and lithium heparin specimen bottles. The blood samples were centrifuged at 3500rpm for 5minutes at room temperature to separate the serum and plasma respectively. The serum and plasma samples were dispensed into a new, well labeled plain specimen bottle . Haemolysate was prepared from red blood cell sediment from lithium heparin specimen bottles. Red blood cell was washed three times with equal volume of normal saline solution and centrifuged at 3000g for 5minutes. Lysed erythrocytes were prepared by putting cells through three freeze-thaw cycles in dry ice and by the addition of four volumes of ice-cold distilled water. Cell membranes were removed by centrifugation and the supernatant was separated into another plain specimen bottle. Both serum and haemolysate were kept for a period of three days at -20ºC before analysis. Serum was used for the laboratory measurement of MDA and TAS while haemolysate was used for the measurement of GPx.

2.5 Laboratory Measurement of Biochemical Parameters

The method of Satoh et al. [17] was followed with certain modifications to determine serum malondialdehyde. This is based on the principle that MDA is heated with thiobarbituric acid (TBA) at a low PH, it gives a pink chromogen, allegedly a $(TBA)_2$-MDA complex, which is measured spectrophotometrically at a wavelength of 535nm. The method of Koracevic et al. [18] was employed for the measurement of TAS. A standardised solution of Fe-EDTA complex reacts with hydrogen peroxide to give hydroxyl radical. This reactive oxygen

species, hydroxyl radical degrades benzoate to produce thiobarbiturate reacting substances (TBARS). The antioxidant from added sample causes suppression of the production of TBARS. The decrease in the concentration of TBARS as a result of antioxidants is measure spectrophotometrically and serves as concentration of total antioxidants present in the serum sample added. Glutathione Peroxidase (GPX) catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH, the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm is measured [19].

Variables were analyzed using SPSS version 17, taking level of significance to be 0.05.

3. RESULTS

Table 1 showed weight of Wistar rat before and after feeding, before sacrificing. The weight of both test (p<0.0001) and control (p<0.0001) groups were statistical significantly higher after feeding than before feeding. The mean weight gained in test rats (77.93±10.11)gm was significantly higher than weight gained by the control rats (51.70±12.12)gm, P<0.0001.

Table 1. Comparison of weight of the study groups

	Weight before feeding/gm	Weight after feeding/gm	*P*-value
Test sample	124.70±23.43	200.63±22.00	0.000*
Control sample	120.97±18.71	172.47±30.24	0.000*

** Statistically significant (p<0.05)*

The mean of biochemical parameters were compared, this is as shown in Table 2. Serum MDA was observed to be significantly lower in rats (test) fed with *Moringa oleifera* than those (control) with normal diet (p<0.05). Mean serum TAS as well as red blood cell GPx were found to be significantly higher (p<0.01) in rats fed with *Moringa oleifera* than those with normal diet.

Table 2. Comparison of Mean±SD of Biochemical Variables

Variables	Test (n=40)	Control (n=30)	*P*-values
Malondialdehyde (MDA)µmol/l	1.00±0.13	1.48±0.09	0.039*
Total antioxidant status (TAS) mmol/l	3.74±0.41	2.10±0.37	0.008*
Glutathione peroxidase (GPx) U/gHb	82.00±5.25	58.10±5.37	0.006*

** Statistically significant (p<0.05)*

Correlations of MDA to TAS and GPx were observed in Table 3. There was significant negative correlation between MDA and TAS in both study groups. Positive correlation was observed between MDA and GPx in both study groups.

Table 3. Correlation of MDA with TAS and GPx in both Test and Control Samples

Variables	TAS mmol/l		GPx U/gHb	
	R	*P*	R	*P*
MDA for test sample (µmol/l)	-0.333	0.036*	0.215	0.151
MDA for control sample (µmol/l)	-0.472	0.008*	0.944	0.013

** Statistically Significant (P<0.05)*

4. DISCUSSION

As illustrated in Table 1. The significant weight gained in both test and control rats indicate adequate feeding of the experimental animal. However, test rats gained weight significantly than the control rats. Test rats had their food and water made up of 15% and 5% *Moringa* leaf extract respectively. However, it should be realized that experimental animals ordinarily gained weight with adequate feeding [20-21]. Peculiarity comes in our study with higher weight gained in rat fed with *Moringa oleifera* leaf. This finding is contrary to general belief that *Moringa oleifera* helps in weight reduction. However, evidence or reasons for this was not found in literature available to us.

The decreased mean serum malondiadehyde in test rats may indicates a lower level of free radical injury in them. Free radicals such as reactive oxygen species have dangerous attack on some cellular organelles like DNA [22], proteins [23], membrane [11] etc. Attack of free radicals on membrane polyunsaturated lipid generates series of reactions. The whole process of this is termed lipid peroxidation. In the course of these reactions, malondiadehyde is produced to show evidence of free radical injury on membrane lipid. Free radical injury may result in oxidative stress injury when production of free radicals (oxidant) overwhelms the available antioxidants. Alternatively, if free radicals are not generated but antioxidants are depleted probably because of inadequate dietary intake, oxidative stress may also results. What we suggested to be responsible for a decreased malondialdehyde in our test rats is an increase in antioxidant probably from *Moringa oleifera*. These antioxidants must have mumped up some free radicals and also prevented it effects on membrane lipid to stop further damage.

Total antioxidant status (TAS) accesses majority of antioxidant components in the system. The lower TAS observed in test rats than control rats may indicate the supply of antioxidant by *Moringa Oleifera.* Antioxidants like superoxide dismutase and glutathione peroxidase are jointly accesses and they are both known to counteract free radicals (reactive oxygen species). Superoxide dismutase enhances the breakdown of superoxide anion into oxygen and hydrogen peroxide. It should be noted that hydrogen peroxide also is a reactive oxygen species that can function as a free radical [24]. In the light of this, catalase or glutathione peroxidase, another known antioxidant enzymes has to come in place to convert hydrogen peroxide into water and oxygen. Furthermore, one may not be conclusively say that *Moringa oleifera* contains all the antioxidants being accessed by accessing total antioxidant status because elevation of any of the components may present with increase total antioxidant status. The need to measure individual antioxidant may be necessary.

Glutathione peroxidase is an important antioxidant enzyme present in virtually all tissues. The enzyme limits the generation of lipid peroxides and utilizes glutathione as its co-factor to convert lipid peroxide into relatively harmless hydroxylated fatty acids, water and glutathione disulfide. The higher level of glutathione peroxidase observed in our study is in agreement with the work of Ashok et al. [25]. Based on the report of this study, *Moringa oleifera* leaf contains antioxidants as observed with some other vegetables [1]. Consumption of *Moringa oleifera* and other vegetables are been advocated to improve individual antioxidant status [14]

Moringa oleifera is being promoted as a dietary supplement to address malnutrition [1]. *Moringa oleifera* has been use traditionally for some years now in some parts of the world. It is being used to treat some clinical conditions like skin infections, anaemia, anxiety, asthma, [26-27]. All parts of the tree are considered to posses medicinal properties. The root is

laxative, expectorant, diuretic, and good for inflammations, throat bronchitis, piles, cures stomatitis, urinary discharge and chronic asthma. The back of the stem is useful in heart complains, eye diseases. Majority of these clinical conditions have been linked to oxidative stress injury. The treatment this plant offers may be and is being proven to be as a result of its richness in antioxidant status.

Correlation among biochemical parameters shows negative correlation between MDA and TAS in both test and control rats and this is expected. It means that *Moringa oleifera* leaf must have supplied antioxidant to mump up free radical as well preventing it deleterious effects and its production. On the other hand, the positive correlation observed between MDA and GPx may be unusual. One may want to talk off, the compensatory mechanism in which production of free radical comes with compensatory accumulation of antioxidants. Furthermore, it can also be said that GPx as a component of TAS is not involved in the prevention of free radical attack originated in these rats. One may hypothesis that components of total antioxidant except glutathione peroxidase compensate with increasing free radical production in these rats.

5. CONCLUSION

We conclude that *Moringa oleifera* leaf may prevent oxidative stress in Wistar rats. It may improve oxidant-antioxidant status in faviour of antioxidants. The findings of this study support the hypothesis that *Moringa oleifera* has potential effect to prevent oxidative damage when consumed as dietary supplement

6. LIMITATION TO THE STUDY

Determination of individual antioxidant enzymes and vitamins would have helped to know which of the components contributed to the increased total antioxidant status apart from glutathione peroxidase.

CONSENT

Not applicable.

ETHICAL APPROVAL

Ethical approval for the use of laboratory animal was gotten from ethical committee of Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jed WF. *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. Trees for Life Journal. 2005;1:5.
2. Busani M, Patric JM, Amold Hugo, Voster M. Nutritional Characterization of *Moringa (Moringa oleifera* Lam) Leave. Afri Journ of Biotech. 2011;10(60):12925-12933.
3. Sinha M, Das DK, Bhattacharjee S, Majumdar S, Dey S. Leaf extract of *Moringa oleifera* prevents ionizing radiation-induced oxidative stress in mice. J Med Food. 2011;14(10):1167-72.
4. Gupta R, Dubey DK, Kannan GM, Flora SJ. Concomitant administration of *Moringa oleifera* seed powder in the remediation of arsenic-induced oxidative stress in mouse. Cell Biol Int. 2007;31(1):44-56.
5. Sreelatha S, Padma PR. Protective mechanisms of *Moringa oleifera* against CCl(4)-induced oxidative stress in precision-cut liver slices. Forsch Komplementmed. 2010;17(4):189-94.
6. Nur E, Biemond BJ, Otten HM, Brandjes DP and Schnog JJ. Oxidative stress in sickle cell disease; pathophysiology and potential implications for disease management. Am J Hematol. 2011;86(6):484-9.
7. Erica NC and Vincent P. Role of Oxidative Stress in the Pathogenesis of Sickle Cell Disease. IUBMB Life. 2012;64(1):72–80.
8. Shi GX, Liu CZ, Wang LP, Guan LP and Li SQ. Biomarkers of oxidative stress in vascular dementia patients. Can J Neurol Sci. 2012;39(1):65-8.
9. Bennett S, Grant MM, Aldred S. Oxidative stress in vascular dementia and Alzheimer's disease: a common pathology. J Alzheimers Dis. 2009;17(2):245-57.
10. Canaud B, Cristol J, Morena M, Leray-Moragues H, Bosc J, Vaussenat F. Imbalance of oxidants and antioxidants in haemodialysis patients. Blood Purif. 1999;17(2-3):99-106.
11. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol. 1997;82(2):291-5.
12. MacNee W. Oxidants/antioxidants and COPD. Chest. 2000;117:303S–317S
13. Halliwell B. Antioxidants in human health and disease. Annu Rev Nutr. 1996;16:33–50
14. Ashok KN, Pari L Antioxidant action of *Moringa oleifera* Lam. (drumstick) against antitubercular drugs induced lipid peroxidation in rats. J Med Food. 2003;6(3):255-9
15. Ranira G, Rimi H, Kaushik R, and Debajani G. Effect of *Moringa Oleifera* in Experimental Model of Alzheimer's disease: Role of antioxidants. Annals of Neurosciences. 2005;12(3):1-6.
16. Julius C. A study of the nutritional and medicinal values of *Moringa oleifera* leaves from sub-sahara Africa: Ghana, Rwanda, Senegal and Zambia. Thesis submitted to Rutgers, the State University of New Jersey; 2008.
17. Satoh K. Serum lipid peroxide in cardiovascular disorders determined by a new colourimetric method. Clin Chem Acta. 1978;90:37-42.
18. Koracevic D, Koracevic G, Djordjevic V. Method of the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001;54: 356-61.
19. Pippenger CE, Brown RW, Armstrong D. Regulatory antioxidant Enzymes. Methods Mol Biol. 1998;108:299-313.
20. Swithers SE, Ogden SB, Davidson TL. Fat substitutes promote weight gain in rats consuming high-fat diets. Behav Neurosci. 2011;125(4):512-8.
21. Leibowitz KL, Chang G, Pamy PS, Hill JO, Gayles EC, Leibowitz SF. Weight gain model in prepubertal rats: prediction and phenotyping of obesity-prone animals at normal body weight. International Journal of Obesity. 2007;1-12.
22. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2004;266(1-2):37-56.

23. Belén B, Antonia O, Emilio C and Salvador M' Oxidative stress and S-nitrosylation of proteins in cells. Br J Pharmacol. 2000;129(5):953–960.
24. Wu ML, Tsai KL, Wang SM, Wu JC, Wang BS and Lee YT. Mechanism of hydrogen peroxide and hydroxyl free radical-induced intracellular acidification in cultured rat cardiac myoblasts. Circ Res. 1996 Apr;78(4):564-72.
25. Ashok Kumar N, Pari L. Antioxidant action of *Moringa oleifera* Lam. (drumstick) against antitubercular drugs induced lipid peroxidation in rats. J Med Food. 2003;6(3):255-9.
26. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales PN, Phivthong-ngam L et al. The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of the water extract of *Moringa oleifera* Lam. leaves. J. of Ethnopharmacology. 2008;116:439-446.
27. Dahiru D, Obnubiyi JA., Umaru HA. Phytochemical screening an antiulcerogenic effect of Moringa. African Journal of Traditional, Complimentary and Alternatives Medicines. 2006;3(3):70-75.

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