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Effects of "Lesser Known" Leafy Vegetables (*Vitex doniana* and *Corchorus oletorius*) on the Oxidative Stress Indices of Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author NN was involved in the conception, design and write up of the manuscript. Author HOA was involved in the conception, design and collection of data, while author EEJI provided necessary logistics. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To evaluate the effects of two "lesser known" leafy vegetables- *Vitex doniana* and *Corchorus oletorius* on the oxidative indices of Albino Rats.
Study Design: Forty eight rats, mean body weighty 351.83±1.39g were grouped into four to represent a control, and three diet groups. Dried powdered form of the vegetables was mixed with the normal rat chow in the ratio of 1:4 and pelleted before feeding to the rats. Feeding lasted for a total of three (3) months. The first analysis was done within two weeks, and thereafter repeated every two weeks throughout the study.
Place and Duration of Study: The research work was done at Biochemistry laboratory of the Federal University of Technology, Owerri and National Root Crop Research Institute, Umuahia. The study lasted for a period of three months and two weeks (104 days).
Methodology: Clean uninfected leaves of the samples were selected and sun dried to constant weight before grinding with a milling machine. The resulting powdered form was used to formulate the experimental diet with the normal rat chow in the ratio of 1:4 as shown in the text. Enzyme activities were determined according to standard methods as referenced in the text. Malonyladehyde and vitamin C contents were also determined

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according to standard methods.
Results: Values of malonyladehyde, Vitamin C and activities of catalase significantly ($P \geq 0.05$) increased when the sample vegetables were administered to the rats as compared with the control which received no vegetables. However, the activities of peroxidase decreased also significantly as compared with the control. However, only the increase in the values of indices determined were sustained throughout the period of study.
Conclusion: The studied vegetables may possess antioxidant components which may play important role in the management of diseases associated with oxidative stress.

Keywords: Vitex dononia; Corchorus oletorius; abino rats; oxidative stress; nutritional therapy; antioxidant.

1. INTRODUCTION

Leafy vegetable are plant leaves eaten as vegetable by man. Although, they may come from a very wide variety of plants, most share a great deal with other leafy vegetable in nutrition and cooking methods [1]. However the word "vegetable" in its modern usage is strictly a culinary term rather than botanical or scientific. The sample vegetables are not popular or common among the people. In fact, they are not cultivated, but are found in the wild farmland. In most cases, only poor individuals use them in food preparation. However, they are in no way inferior in nutrition content to other popular vegetables like, lettuce, pumpkin, etc. [1,2]. Leafy vegetables were typically low in calories, fats, but high in protein, dietary fibre; iron, calcium, and some phytochemcials like vitamin C, carotene [3,4]. Vegetables are known to play important role in nutrition and health because of the high content of minerals, vitamins, antioxidants, dietary fibre, and essential oils [3,5,6].

In normal cells there is an appropriate prooxidant – antioxidant balance. However, this balance can be shifted towards prooxidant level when the production of reactive oxygen species is increased or when the levels of antioxidants are diminished. The resulting state is called "Oxidative stress". This can lead to serious cell damage if the stress is massive or prolonged. Oxidative stress has been implicated in a number of diseases including atherosclerosis, cancer, neurodegenerative diseases, ageing, and chronic inflammatory diseases [7].

Antioxidant status may be increased by exogenous administration of antioxidants either from food sources including vegetables and fruits or by the use of pharmaceutical agents like synthetic vitamins. Clinically, relevant antioxidants are classified into two groups according to the mechanism of action;

(a) Preventive and
(b) Chain breaking antioxidants.

Preventive antioxidants e.g. catalase, metal binding proteins, glutathione peroxidase, prevent the initiation of radical chain reaction through reducing hydroperoxides to molecular species without formation of free radicals. Chain breaking antioxidants e.g. ascorbate, α-tocopherol, superoxide dismutase, trap free radicals directly, thereby stopping chain propagation reactions [8].

The occurrence of such diseases like atherosclerosis, diabetes, rheumatoid arthritis with direct implication from oxidative stress has been on the increase within the Nigerian population. One of the means to combat this development is the use of preventive antioxidants which are readily available in fruits and vegetables [9-11]. Therefore, in this study we incorporated two leafy vegetables into rat feed in order to evaluate possible effects of such vegetables on the oxidative stress status of the rats.

2. MATERIALS AND METHODS

2.1 Materials

Fresh leaves of *Vitex doniana* (Uchakiri) and *Corchorus oletorus* (Ahihiara) were collected from a farmland in Ehime Mbano, Imo State, Nigeria. They were scientifically indentified by a taxonomist from School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri.

2.2 Sample Preparation

Clean, uninfected leaves were selected, and sun dried to constant weight before grinding using a milling machine. The resulting powdered form was sieved with 1mm sieve and used to formulate the rat diet as shown below in Table 1.

Table 1. Content of formulated feed

Diet group	Normal Seed (g)	*V. doniana* (g)	*C. oletorus* (g)	Total composition (g)
A	1000.00	-	-	1000.00
B	800.00	200.00	-	1000.00
C	800.00	-	200.00	1000.00
D	800.00	100.00	100.00	1000.00

The mixture was compounded, pelleted and fed to the rats twice daily throughout the period of the study.

2.3 Animal and Treatment

A total of forty eight adult rat mean body weight, 351.83±1.39g were purchased from the Department of Veterinary Medicine of University of Nigeria, Nsukka. They were separated into four groups of twelve rats into cages and left to acclimatize for 2 weeks on water and normal rat ad.libitum. The four groups represented the sample and three different diet groups as shown before. Experimental diet was given for three months, while analysis was done once every two weeks for the whole period of study.

2.4 Preparation of Serum

Eighteen hours after the last feeding, two rats from each group were collected and sacrificed under light chloroform anaesthesia. A 5.0ml syringe was used to collect blood from cardiac puncture, after the heart was eviscerated. The blood was collected into a test tube containing heparin and allowed to stand for two hours for clear separation of serum. The serum was decanted and used for analysis.

2.5 Determination of Serum Vitamin C and Malonyladehyde Contents

Vitamin C was determined spectrophotometrically according to the methods of Toro and Ackermann, [12], while malonyladehyde was measured according to the methods of Das et al. [13].

2.6 Assay of Catalase and Lipid Peroxidase Activities

Catalase and peroxidase activities were assayed according to the methods of Hancock et al. [14] and Toro and Ackermann [12] respectively.

2.7 Statistical Analysis

Data were expressed as a mean ± SD 3 determinations. ANOVA was used to compared values with control and P≥0.05 was regarded as significant [15].

3. RESULTS AND DISCUSSION

Serum vitamin C content generally significantly (P≤0.05) increased when the diet compounded with the leafy vegetables were fed to the rats. This pattern of increase was sustained throughout the period of study. This is important because in some cases, such effects are not sustained [2]. Vitamin C is a well known antioxidant or free radical scavenger and therefore can prevent or delay the oxidation of substances like DNA, lipids or other important cellular components. It can also prevent carcinogenic nitrosamine formation in cancer, Infact, epidemiological studies have indicated an inverse association between vitamin C intake and the risk of cancer [16]. Vitamin C can act as a co-antioxidant by regenerating α-tocopherol radicals produced during scavenging of reactive oxygen molecules [17]. Vitamin C is found in high concentration in fresh fruits especially citrus and vegetables [18]. The high serum content of vitamin C recorded in this study, therefore, is possibly contributed from the leafy vegetables.

Malonyadehyde content also increased in the rat serum when the leafy vegetables were incorporated in the diet. Diet group C (Uchakiri alone) had the greatest increase effect followed by diet group D (Mixed vegetables). However, the increasing effect was not sustained throughout the period of study. Malonyladehyde is the major reacting aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids (PUFA) [19]. Malonyladehyde is also said to be product of normal metabolism and therefore present in a number of fat containing food items [19]. It can form adducts with DNA, Adenine and Cytosine which contribute to the carcinogenicity and mutagenicity in mammalian cells [20]. In this study, increase in the serum level of malonyladehyde could be due to response to normal metabolic effect, since such increase was not sustained throughout the period of study.

Catalase activity increased significantly (P≤0.05) both in the acute (Table 2.) and chronic (Figs. 1-4) phases of this study. The highest activity was determined in diet group C (Uchakiri alone) followed by diet group D. Catalases have been found in most cells as an antioxidant enzyme involved in the decomposition of hydrogen peroxide to water and oxygen. Catalase is known to act 10^4 times faster than peroxidase, and is found localized in the mitochondria and subcellular respiratory organs [21]. Most studies suggest that catalase functions as an antioxidant by promoting or transforming inhibitor in carcinogenesis. Infact

Glutathione and catalase were found to be important in the inactivation of many environmental mutagens [22]. It is possible that the sample vegetables may contain phytochemicals that activated catalase activity.

Lipid peroxidase activity was found to decrease significantly (P≤0.05), both in the acute (Table 2) and chronic (Figs. 1-4) phases of the study. The decrease in activity was highest in diet group C. Peroxidase like catalase is also an antioxidant enzyme involved in the decomposition of hydrogen peroxide. Peroxidase activity is one of the markers of oxidative stress in a biological system. Infact, the higher the activity, the greater the chances of an oxidative stress-induced diseases, or the aggravation and acceleration of an existing one [23]. Vegetables like the samples used in the study are known to be rich in antioxidant phytochemicals like vitamin C and phenolic compounds. Therefore, the decrease in activity of peroxidase may be due to this antioxidant activity as quenching agent on the propagation of the oxidative chain reaction mechanism or as an activator of peroxidase enzyme.

Table 2. The effect of lesser known leafy vegetables on oxidative status of rats

Diet group	Catalase activity (i.u/L).	Vit. C (mg/ml)	MDA (mg/ml)	Peroxidase activity (i.u/L)
A	44.0+0.30	0.25+0.20	0.73+0.01	77.93+1.00
B	45.0+0.10	1.14+0.444	0.73+0.01	77.54+1.20
C	46.0+0.20	1.13+0.32	0.76+0.03	75.15+1.01
D	47.0+0.76	1.11+1.05	0.73+0.01	76.14+1.85

A= Control Group; B= Ahihara Alone: C= Uchakiri Alone; and D= Mixture of A and B

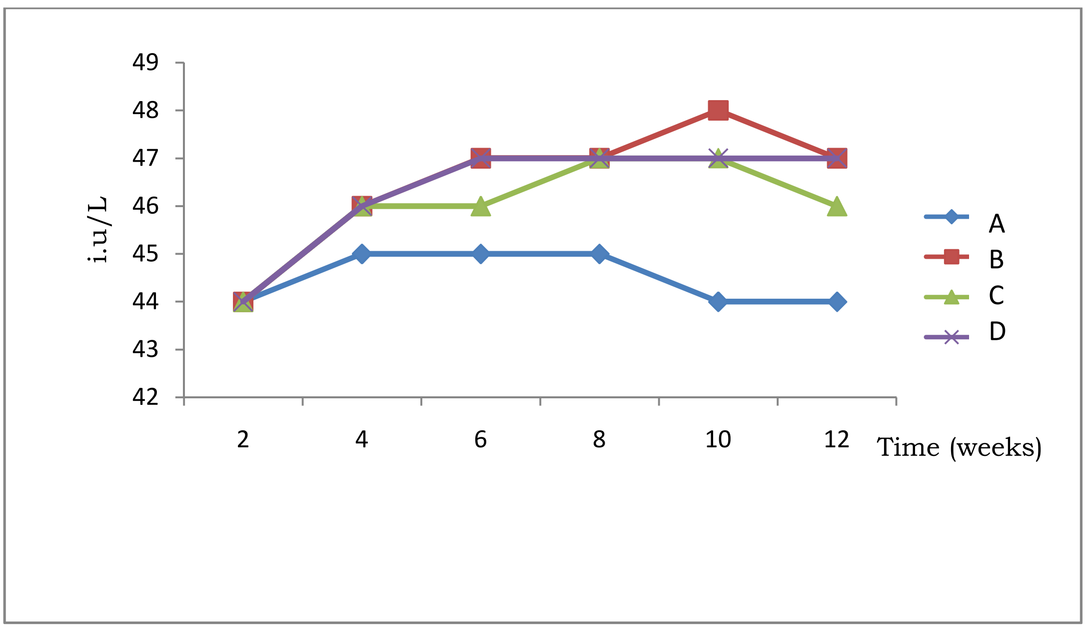

Fig. 1. Time dependent effect of diet formulated with lesser known leafy vegetables on Rat Serum Catalase activity

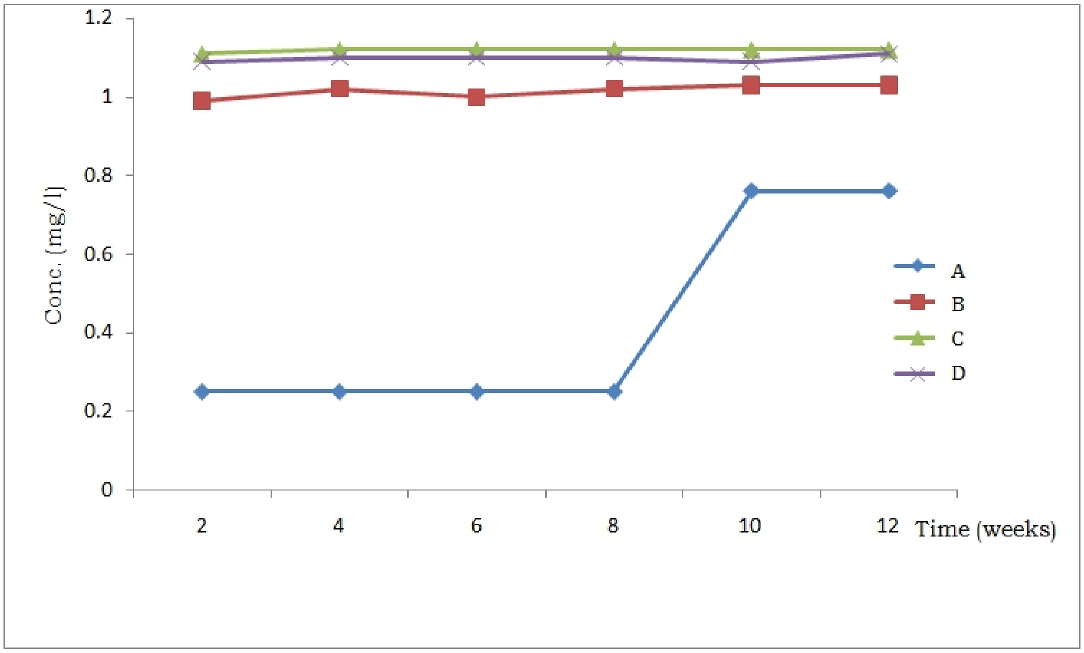

Fig. 2. Time dependent effect of diet formulated with lesser known leafy vegetables on Rat Serum Vitamin C content

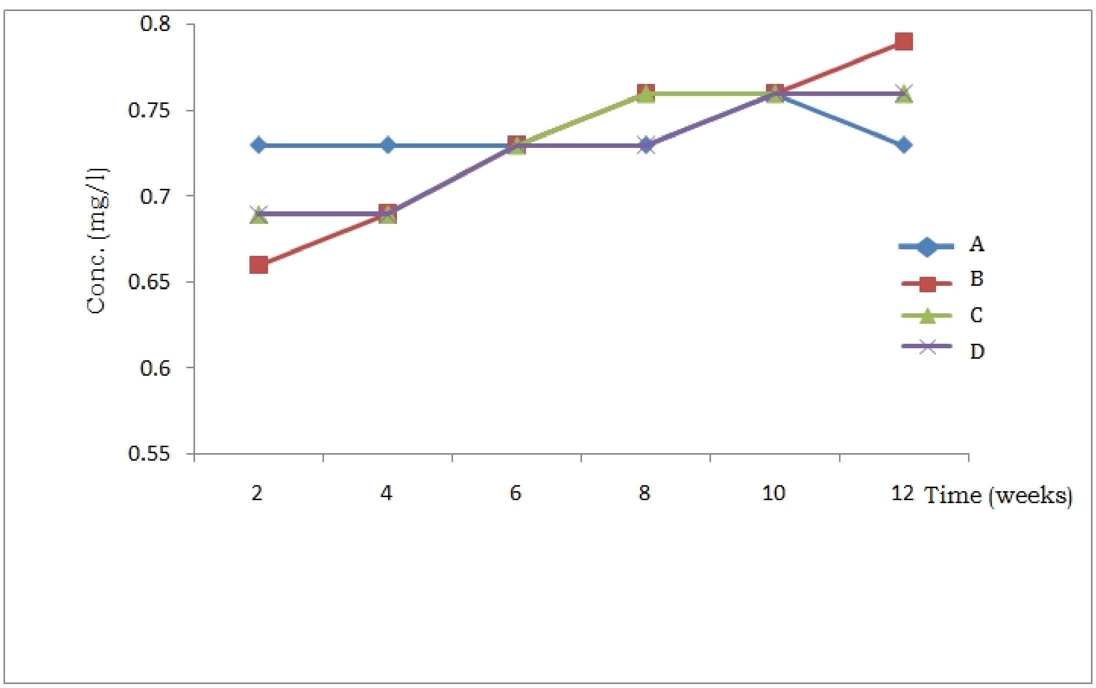

Fig. 3. Time dependent effect of diet formulated with lesser known leafy vegetables on Rat Serum malonyaldehyde content

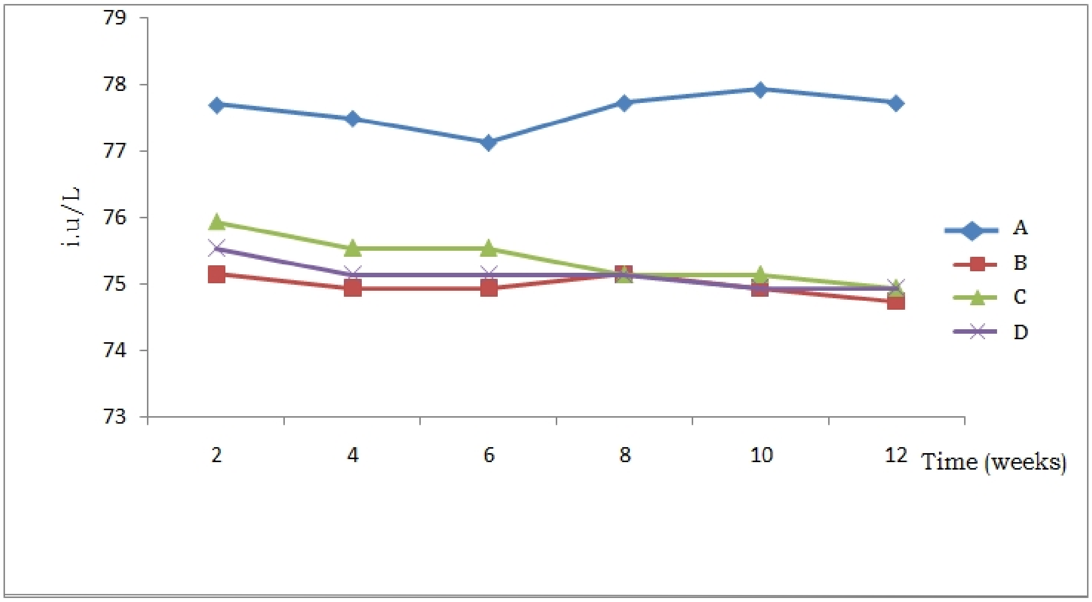

Fig. 4. Time dependent effect of diet formulated with lesser known leafy vegetables on Rat Serum peroxidase activity

A = Control (no sample), B = Ahihara alone, C = Uchakiri alone, D = A and B mixed in the ratio 1:1

4. CONCLUSION

In summary the serum values of antioxidant markers vitamin C and catalase activity increased when rat diet was formulated with V, doniana, and C. Oletorus, while the prooxidant marker, peroxidase activity decreased. Fortunately, the trend in the result was sustained both in the acute and chronic phases of the study. This generally support the use of the sample vegetables in the management of oxidative stress induced diseases if incorporated in human diet.

It is also important to note that the antioxidant effect of these vegetables is best achieved when added together in the diet.

CONSENT

Not applicable.

ETHICAL APPROVAL

Handling of animals in this study followed strictly approved guidelines both in the Federal University of Technology and the Research Institute.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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