



Alterations in the activities of some antioxidant enzymes, vitamins and malondialdehyde in rats fed the *Jatropha tanjorensis* leaves supplement

E.S.Omoregie^{1*}, A.U.Osagie²

Department of Biochemistry, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, (NIGERIA)

Tel.: +2348023397020

E-mail: ehimoregie@yahoo.co.uk

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ABSTRACT

We determined the effect of *Jatropha tanjorensis* leaves supplements on the activities of superoxide dismutase (SOD), catalase (CAT), vitamin E, vitamin C and malondialdehyde (MDA) in rats. Male albino rats of the Wistar strain were divided into six groups and maintained on normal diet (ND); normal diet supplemented with the leaves (ND+J) and fesolate® (iron) tablet (ND+F); low protein diet (LPD); low protein diet supplemented with the leaves (LPD+J) and fesolate tablet (LPD+F) for six weeks. The result showed significant reduction ($P < 0.05$) in malondialdehyde level in the rats fed the leaves supplemented diet but with significant increase ($P < 0.05$) in the concentrations of vitamin C and vitamin E relative to controls. The catalase and superoxide dismutase activities were significantly reduced ($p < 0.05$) in the rats fed the low protein diet while these were increased ($p < 0.05$) in the rats fed the leaves supplements as compared with control values. The study therefore suggests that *Jatropha tanjorensis* leaf may have antioxidative potential against reactive oxygen species (ROS) that are produced in protein energy malnutrition.

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KEYWORDS

Jatropha tanjorensis leaves;
Antioxidants;
Low protein diet;
Reactive oxygen species;
Superoxide dismutase;
Catalase;
Vitamin C;
Vitamin E;
Lipid peroxidation.

INTRODUCTION

Over the years the epidemiology of various degenerative diseases has been linked to cellular injury arising from reactive oxygen species (ROS) production. ROS can oxidize a large number of cellular components especially proteins, lipids, and nucleic acids resulting in degenerative disorders in human such as diabetes, cystic fibrosis, hepatitis, central nervous system injury, atherosclerosis, arthritis and acquired immunodeficiency syndrome (AIDs)^[3,28,29].

Oxidative stress may occur whenever there is an imbalance between oxidants and antioxidants in favour

of the oxidants, potentially leading to oxidative overload and production of ROS that are detrimental to cells, tissues and organs. For instance, in nutritional oxidative stress, there is an imbalance between the prooxidant load and the antioxidant defense as a result of inadequate supply of nutrients^[28]. Likewise, many of the clinical and pathological manifestations of PEM have been linked to an imbalance between free radical defenses and free radical production^[16,27]. In PEM, the free radical defense has been reported to be depressed, there are alterations in the activities of free radical defense enzymes and free radical production is often increased^[16,27].

Nevertheless, there is a multilayered strategy of defense against oxidative damage including enzymatic and non-enzymatic antioxidants as well as adaptive responses. Epidemiological studies have shown that high dietary intake of fruits and vegetables can predispose towards diminished risk of developing degenerative diseases. It is widely claimed that most fruits and vegetables contain dietary antioxidants which are thought to be responsible for their beneficial effects^[20,22,28]. The dietary antioxidants are plants bioactive compounds which contributes to the antioxidant capacity of these fruits and vegetables^[2,17] and they comprise tocopherols, ascorbate, carotenoids, thiols, tannins, flavonoids, alkaloids, nitrogen containing compounds and micronutrients^[14,17]. Thus high dietary levels of antioxidants may scavenge biological toxic ROS^[9,28].

In this study, a once popularly consumed vegetable leaves *Jatropha tanjorensis*, commonly called catholic vegetable, 'hospital too far' (a member of the *Euphorbiaceae* family) was evaluated for antioxidant activity in rats exposed to nutritional oxidative stress using a low protein diet. The leaves of the plant are mostly used in making soup in the south-western region of Nigeria. In our previous study the plant leaves were reported to possess anti-anemic properties in anemic rats^[8,24].

EXPERIMENTAL

Plant material and preparation of feed

Jatropha tanjorensis leaves were obtained from a private farm in Benin City. The selection of the plant was based on information obtained from traditional medicine practitioners in Benin City, Nigeria. The plant was authenticated at the Department of Botany, University of Benin, Benin City, Nigeria. The fresh leaves were thoroughly washed in distilled water and air dried at room temperature. The dried leaves were then blended into powder form and used for supplementation of the experimental feed. The low protein diet and the normal diet were prepared as previously described^[8,24].

Animal feeding experiment

36 inbred male albino rats of the Wister strain weighing between (100-110) g were distributed into six groups

(six rats per cage) with the average weight difference between groups less than 0.2g. The low protein diet was initially fed to three of the groups for six weeks while the remaining groups received the normal diet. Thereafter, their diets were supplemented with the various leaves for another four weeks with the group 1 animals fed with the normal diet and served as the control (ND), group 2 animals were fed the normal diet supplemented with 8% of *Jatropha tanjorensis* (ND+J) leaves, group 3 animals received low protein diet only (LPD), the group 4 animals were fed the low protein diet along with 8% of the leaves supplement (LPD+J), groups 5 and 6 rats were given fesoate (iron) tablet supplemented in the low protein diet (LPD+F) and normal diet (ND+F) respectively.

The animals were kept in highly sanitized metabolic cages and were initially allowed a 5-day adaptation period with their respective diets before the commencement of the study which lasted for six weeks. The animals were allowed free access to food and water throughout the duration of the experiment. Their body weight, faecal output and quantity of food consumed were monitored weekly. At the end of the feeding period, the animals were fasted overnight and sacrificed through cervical dislocation. Blood was collected immediately from the heart into heparinized tubes and the organs removed (at once) blotted dry, weighed and stored for subsequent analysis.

Preparation of liver and kidney homogenates

1.0g of the liver and kidney tissues were homogenized in 10ml of ice-cold 0.9% normal saline to obtain 10% (W/V) homogenates. The homogenates were centrifuged at 5000g for 10 minutes and the supernatant obtained were used for determination of superoxide dismutase (SOD), catalase (CAT), vitamin C, vitamin E and lipid peroxidation. The heparinized blood was also centrifuged; the plasma collected was used for determination of similar parameters as the kidney and liver homogenates.

Biochemical analysis

The superoxide dismutase (SOD) activity was assayed in the tissues according to the method of Misra and Fridovich^[18], based on the rapid auto-oxidation of adrenaline due to the presence of superoxide anions

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TABLE 1: Weight gain, faecal output and food intake

Parameters	ND	ND+J	ND+F	LPD	LPD+J	LPD+F
Food intake	18.4±0.11 ^a	20.3±0.52 ^c	17.1±0.32 ^a	9.2±0.04 ^b	14.3±0.07 ^d	13.8±0.10 ^d
Weight gain	4.5±0.02 ^a	5.7±0.02 ^c	4.8±0.07 ^a	-1.2±0.05 ^b	3.5±0.03 ^d	1.3±0.04 ^e
Faecal output	12.3±0.2 ^a	15.4±0.2 ^b	13.0±0.19 ^a	8.0±0.1 ^b	10.7±0.5 ^c	11.6±0.3 ^c

Values are expressed as mean ±SEM. N=6 determinations. Weight, food intake and faecal output are in grammes. Means of the same row with different superscript letter differs significantly (P<0.05). ND=normal diet; LPD=low protein diet; ND+J=normal diet with the leaves supplement; LPD+J=low protein diet with the leaf supplement; ND+F=normal diet with iron supplement; LPD+F=low protein diet with iron supplement

TABLE 2: Effect of *Jatropha tanjorensis* leaves supplement on vitamin C level in rats

Tissues	ND	ND+J	ND+F	LPD	LPD+J	LPD+F
Liver	12.5±0.25 ^a	11.3±0.31 ^a	11.9±0.62 ^a	33.4±0.25 ^b	20.5±0.12 ^c	30.6±0.47 ^b
Kidney	14.7±0.02 ^a	15.4±0.04 ^a	14.3±0.03 ^a	25.3±0.03 ^b	18.2±0.02 ^c	22.3±0.04 ^b
Plasma	3.29±0.09 ^a	3.17±0.04 ^a	3.59±0.01 ^a	9.14±0.02 ^b	6.45±0.03 ^c	9.33±0.06 ^b

Values are expressed as mean ±SEM. n=6 determinations. Vitamin C levels were expressed in mg/dl. Means of the same row with different superscript letter differs significantly (P<0.05). ND=normal diet; LPD=low protein diet; ND+J=normal diet with the leaves supplement; LPD+J=low protein diet with the leaf supplement; ND+F=normal diet with iron supplement; LPD+F=low protein diet with iron supplement.

TABLE 3: Effect of *Jatropha tanjorensis* leaves supplement on vitamin E level in rats

Tissues	ND	ND+J	ND+F	LPD	LPD+J	LPD+F
Liver	56.7±3.8 ^a	61.7±5.3 ^b	58.9±4.7 ^a	33.8±2.1 ^c	46.8±3.2 ^d	32.7±3.9 ^d
Plasma	63.3±5.2 ^a	74.7±3.7 ^b	60.0±3.2 ^a	21.6±2.4 ^c	43.6±4.3 ^d	34±3.7 ^c

Values are expressed as mean±SEM. n=6 determinations. Vitamin E levels were expressed in mg/dl. Means of the same row with different superscript letter differs significantly (P<0.05). ND=normal diet; LPD=low protein diet; ND+J=normal diet with the leaves supplement; LPD+J=low protein diet with the leaf supplement; ND+F=normal diet with iron supplement; LPD+F=low protein diet with iron supplement

TABLE 4: Effect of *Jatropha tanjorensis* leaves supplement on superoxide dismutase (SOD) activity in the rats

Tissues	ND	ND+J	ND+F	LPD	LPD+J	LPD+F
Liver	20.1±0.14 ^a	24.0±0.18 ^b	18.7±0.32 ^a	9.8±0.26 ^c	13.6±0.21 ^d	10.2±0.34 ^d
Kidney	18.3±0.13 ^a	17.0±0.11 ^a	18.6±0.12 ^a	11.4±0.13 ^b	14.2±0.16 ^c	9.8±0.20 ^d

Values are expressed as mean±SEM. n=6 determinations. Superoxide dismutase (SOD) activity is expressed as Units g⁻¹ tissue. Means of the same row with different superscript letter differs significantly (P<0.05). ND=normal diet; LPD=low protein diet; ND+J=normal diet with the leaves supplement; LPD+J=low protein diet with the leaf supplement; ND+F=normal diet with iron supplement; LPD+F=low protein diet with iron supplement

whose concentration (expressed in Units/g/ tissue weight) is determined spectrophotometrically at 420nm. Catalase activity in the tissues was estimated as residual H₂O₂ after incubation with the enzyme^[15]. Estimation of lipid peroxidation in the tissues involved the determination of thiobarbituric acid reactive substances (TBARS), which are indicators of membrane lipid peroxidation. Values for TBARS are reported as malondialdehyde (MDA) and quantified using a Molar extinction coefficient of 1.5×10⁵ M cm⁻¹ and expressed as μmole MDA g⁻¹ tissue weight^[14]. The vitamin C con-

tent of the tissues was determined according to the method of Roe and Kuether, (1943). Estimation of vitamin E was based on the method of Desai^[14].

Statistical analysis

The results were expressed as means±SEM. Analysis of variance was used to test for differences in the groups. Duncan's multiple range test was employed to test for significance differences between the means^[31].

RESULTS

The means of weight gain, food intake and dry faecal output are represented in TABLE 1. The rats fed the low protein diet (LPD) consumed significantly (p<0.05) less food with a corresponding reduced weight gain and faecal output than the controls (ND). On the other hand, the rats fed the leaf supplemented diets (LPD+J and ND+J) consumed more food than the LPD group. Hence their gain in weight and faecal output were higher than their LPD counterpart. Supplementation of the protein deficient diet with fesoate tablets (LPD+F) resulted in significantly reduced (p<0.05) food intake, faecal output and a major weight deficit but to a lesser extent when compared with the LPD group. While the normal rats placed on fesoate tablets supplements (ND+F) had significantly high (p<0.05) food intake, gained more weight with a corresponding high faecal output when compared with the control.

TABLES 2 and 3 summarize the effect of *J. tanjorensis* leaves on vitamin C and vitamin E levels. The rats exposed to the LPD+J diet had significantly increased (p<0.05) vitamin C and E levels in both the liver and plasma tissues when compared to the LPD diet-fed rats. While the rats placed on the leaves (ND+J) and the fesoate tablet (ND+F) diet supplements showed normal levels of vitamin C and vitamin E comparable with the control values. The rats fed the LPD+F diet showed no statistical difference (p>0.05) in both vita-

TABLE 5: Effect of *Jatropha tanjorensis* leaves supplement on catalase (CAT) activity in rats

Tissues	ND	ND+J	ND+F	LPD	LPD+J	LPD+F
Liver	20.3±0.41 ^a	21.6±0.32 ^a	20.8±0.50 ^a	16.4±0.21 ^b	18.7±0.33 ^c	16.1±0.43 ^b
Kidney	8.44±0.84 ^a	9.79±0.30 ^b	8.24±0.23 ^a	4.39±0.41 ^c	6.75±0.41 ^d	5.09±0.01 ^c

Values are expressed as mean±SEM. n=6 determinations. Catalase activity is expressed as moles/mg tissue. Means of the same row with different superscript letter differs significantly (P<0.05). ND=normal diet; LPD=low protein diet; ND+J=normal diet with the leaves supplement; LPD+J=low protein diet with the leaf supplement; ND+F=normal diet with iron supplement; LPD+F=low protein diet with iron supplement

min levels relative to the LPD fed rats.

TABLES 4 and 5 depict superoxide dismutase (SOD) and catalase (CAT) activities in the liver and kidney tissues. Statistical analysis revealed significant induction (p<0.05) of SOD and CAT activities in the liver and kidney tissues of the LPD+J fed rats when compared with the malnourished counterpart (LPD). The SOD and CAT activities were normal in the normal rats that were fed on the leaves supplemented diet compared with controls. Supplementation of the low protein diet with fesoate tablet resulted in no significant change (p>0.05) in the SOD and CAT levels when compared with the LPD fed rats which showed marked reduction (p<0.05) in these enzymes levels in contrast to control.

TABLE 6 shows the effect of *Jatropha tanjorensis* leaf on lipid peroxidation (malondialdehyde) level. The rats fed the LPD and LPD+F diets showed significantly increased (p<0.05) level of malondialdehyde (MDA) relative to controls. The MDA level in the LPD+J fed-rats was considerably reduced (p<0.05) compared with the LPD fed rats but was still more than control values. The ND+J and ND+F diet-fed rats however, showed normal levels of MDA when compared with controls.

DISCUSSION

Nutritional oxidative stress in PEM describes an imbalance between the pro-oxidant load and the antioxidant defense as a consequence of inadequate supply of the animals with the necessary nutrients for growth and maintenance of life^[28] The present study evaluated the effect of *Jatropha tanjorensis* leaves on some stress enzymes and antioxidant vitamins in rats exposed to nutritional oxidative stress using a low protein diet.

Results from this study showed evidence of early

TABLE 6: Effect of *Jatropha tanjorensis* leaves supplement on lipid peroxidation (malondialdehyde) level in rats

Groups	ND	ND + J	ND + F	LPD	LPD + J	LPD + F
Liver	54±3.4 ^a	57±4.4 ^b	52±2.4 ^a	32±2.2 ^b	41±1.3 ^c	34±1.5 ^b
Plasma	92.6±2.4 ^a	96.3±4.4 ^a	89.6±3.2 ^a	39.2±1.2 ^b	54.4±3.1 ^c	42.1±1.6 ^b

Values are expressed as mean±SEM. n=6 determinations. Lipid peroxidation level is expressed as $\mu\text{mole MDA g}^{-1}$ tissue. Means of the same row with different superscript letter differs significantly (P<0.05). ND=normal diet; LPD=low protein diet; ND+J=normal diet with the leaves supplement; LPD+J=low protein diet with the leaf supplement; ND+F=normal diet with iron supplement; LPD+F=low protein diet with iron supplement

physical signs of malnutrition particularly, in the rats fed with the PDD diet, from the third week of feeding when compared with the control rats. These symptoms were more pronounced in the rats fed the LPD diet than those fed the leaves supplemented diet. Nevertheless, the malnourished state of these animals further confirms previous studies on induction of PEM in laboratory rats^[1,8,24].

Likewise, the reduction in the body weights of the rats fed the LPD diet also agrees with our previous studies^[8,24] and may be attributed to the anorexic state of these animals. In this state, the body lacks the ability to absorb and use nutrients as well as compensate for nutrient loss. The slight reduction in weight as observed in the LPD+J group even with the leaves supplementation is consistent with earlier reports in which the treatment of PEM with plant protein showed increased growth but with the expected weight for height not attained^[30]. The high body weight gain in the normal rats fed the leaves supplement suggests that the leaves may adequately support growth. The animals in this group also consumed more food and excreted more faeces than their LPD counterpart.

Generally, cells possess a number of mechanisms to protect themselves against attack by reactive oxygen species (ROS). For instance, superoxide dismutase (SOD) removes superoxide (O_2^-) radical by converting it to H_2O_2 which is readily converted to water by catalase and glutathione reductase (GPX)^[9,23]. Nutritional oxidative stress may result in toxicity when the rate at which the free radicals generated exceeds the cell's capacity for their removal.

Likewise, in the malnourished state the concentration of antioxidant enzymes, vitamins, essential polyunsaturated fatty acids (PUFAs) and mineral elements have been reported to be compromised. Thus the body's

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own antioxidant defense system may not be strong enough to protect the cells against damage by ROS. This may account for the reduced level of the antioxidant vitamins (Vitamin C and E) and enzymes (superoxide dismutase and catalase) in the rats fed with the LPD diet relative to control.

Reactive oxygen species can react with a large variety of easily oxidizable cellular components including NADH, NADPH, ascorbic acid, proteins and nucleic acids. The most important effect is that on the membrane PUFAs with the resultant chain reaction of lipid peroxidation with the resultant tissue damage associated with most degenerative diseases^[6,23]. Malondialdehyde (MDA) has been suggested as one of the end products of lipid peroxidative process^[19,23]. In this study, nutritional oxidative stress as a result of low protein diet was confirmed by the increase in lipid peroxidation product (MDA) in the LPD group as compared with the control. Moreover, several papers have reported that diets that are low in protein but high in carbohydrates may induce protein malnutrition and cause oxidative stress thereby enhancing lipid peroxidation^[4,33].

On the other hand, the relatively high levels of the antioxidant vitamins, enzymes and the low level of MDA in the rats fed the leaves supplemented diets (LPD+J and ND+J) suggests that the plant leaves might have provided some protective effect against damage by ROS. These may be attributed to the presence of bioactive compounds in the plant leaves. Accordingly, many plants bioactive compounds have been associated with antioxidant activities in biological systems by acting as scavengers of singlet oxygen and free radicals produced by ROS, thus, reducing oxidative stress. Similarly, our previous study on *Jatropha tanjorensis* leaves showed that it contained some phytochemical compounds such as flavonoids, tannins, saponins that may be responsible for its antioxidative property^[8,24].

For instance, polyphenols are highly reactive compounds due to the fact that they are polydentate ligands with a multiplicity of potential binding sites, hence their capacity to act as antioxidant depend largely upon their molecular structure^[21,32]. Nevertheless, some natural products (e.g. carotenoids, vitamin C) that are biologic antioxidants have their antioxidative properties dependent on oxygen tension and concentration. Moreso, the

antioxidant paradox have proposed that in biological conditions, there is always the tendency for an antioxidant to become a pro-oxidant if a suitable receptor molecule is present to accept the electron and promote autooxidation^[5,14].

The supplementation of the LPD diet with iron (fesolate) tablets still showed all the symptoms of malnutrition and a compromised antioxidant system. This may be explained by the fact that iron supplements alone could not alleviate malnutrition in these animals.

Instead, the presence of iron may promote bacterial growth as well as the production of ROS^[10,34]. In this regard, several cases of increased mortality and morbidity have been linked with oral iron supplementation especially during the early phases of treatment of PEM^[8-10].

Thus, enhancing the antioxidant defense system as well as nutritional support for growth and development during the early stages of rehabilitation may be crucial to the survival of wasted PEM patients^[16]. Therefore, *Jatropha tanjorensis* leaves may provide antioxidative protection against nutritional oxidative stress produced in PEM. But there is need for thorough understanding of the efficacy and the long-term safety of the plant leaves supplement for human consumption.

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