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Antioxidant Enzyme Levels in Rats Fed Wholly Compounded Diet Supplemented With Orange-Fleshed Sweet Potato Leaves *(Ipomoea batatas)*

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ABSTRACT: Orange-fleshed sweet potato (*Ipomoea batatas*) is a nutrient rich plant known for its high content of beta carotene, vitamins, minerals, and antioxidants. The leaves are rich in polyphenols, flavonoids, and other bioactive compounds that contribute to their potent antioxidant properties. This study investigates the effect of orange fleshed sweet potato supplemented diet on antioxidant enzyme levels in rats. Thirty (30) Wistar rats were used for the experiment. The rats were categorized into five (5) groups; group 1, the normal control (0% orange-fleshed sweet potato leaves), Group 2, 3 and 4 received 25%, 50% and 100% of orange-fleshed sweet potato leaves supplemented diet, respectively and group 5 received 100 % pumpkin leaves supplemented diet (Positive control). After feeding for six (6) weeks, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx) levels were evaluated. The results show a significant increase ($p < 0.05$) in all antioxidant enzymes assayed in rats fed 50% and 100% OFSP when compared to control group. Supplemented orange fleshed sweet potato leaves *in vivo* study has been shown to boost the levels of antioxidant enzymes and ameliorate the effect of oxidative stress, which is the basis of most diseases.

Keywords: Orange fleshed sweet potato, *Ipomoea batatas*, Antioxidant enzymes, Oxidative stress.

Introduction

Oxidative stress is a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the resulting damage (Pizzino *et al.,* 2017). Oxidative stress leads to many problems in humans and they are connected with pathophysiology of many diseases (Blokhina *et al.,* 2003; Lipinski, 2001), which includes inflammation (Halliwell, 2006), cancer (Reuter *et al.,* 2010), atherosclerosis and aging (Finkel and Holbrook, 2000).

Orange-fleshed sweet potatoes, a variant distinguished by its vibrant orange color, are valued for their high beta-carotene content, a precursor of vitamin A, which is essential for maintaining healthy vision, immune function and skin integrity (Neela and Fanta, 2019). In addition to beta carotene, the tubers and leaves of this plant are rich in vitamins C, E, iron, potassium and dietary fiber, making them a highly nutritious food source (Alam, 2021; Endrias *et al.,* 2016). The leaves have special attributes such as adaptability in wider topography, good productivity in short durations and balanced nutritional composition (Trancoso-Reyes *et al.,* 2016). They contain significant levels of polyphenols, including chlorogenic acid and caffeoylquinic acid, which contribute to their antioxidant activity (Pazos *et al.,* 2022). These compounds have been associated with various health benefits, including anti-inflammatory, anti-carcinogenic, cardio-protective and antioxidant effects (Steed and Truong, 2008; Neela and Fanta, 2019). While previous studies have primarily focused on the tubers of the orange-fleshed sweet potato, the leaves, which are often considered a byproduct, are an underutilized source of nutrients and antioxidants. The potential health benefits of incorporating orange fleshed sweet potato leaves (OFSP leaves) into the diet, particularly their role in modulating antioxidant enzyme activity, remain largely unexplored.

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are critical components of the body's defense system against oxidative stress. Enhancing the levels of these enzymes through dietary interventions could be a viable strategy to improve overall health and reduce the risk of chronic diseases related to oxidative stress, such as cardiovascular diseases, diabetes, and neurodegenerative disorders (Valko *et al.*, 2007).
This study aims to fill this gap by investigating the effects of orange fleshed sweet potato leaves supplemented diet on the antioxidant enzyme levels in rats compared with rats fed with pumpkin leaves.

Materials and methods

Plant collection and experimental design: Orange fleshed sweet potato leaves were obtained from Nigeria's National Root Crops Research Institute (NRCRI), Umudike, Abia State. The plant was identified and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. The herbarium specimen was deposited with voucher number: UBH-1493. After harvesting, the leaves were separated from the vine, washed under running water and dried at room temperature. They were then grinded using electric grinder into fine powder (Ayeleso *et al.*, 2018).
Thirty (30) albino rats were purchased from the Department of Anatomy (Animal House) University of Benin, Benin City. The rats were randomly divided into five (5) test groups of 6 rats each and transferred to standard steel. The rats were acclimatized for two weeks. Group 1, serving as the control group, was fed a formulated diet consisting of maize, corn flour, fish meal, groundnut meal, bone meal, and a vitamin premix. Group 2 received the same formulated diet with the addition of 25 % orange-fleshed sweet potato (OFSP) leaves. Group 3 diet was enhanced with 50 % OFSP leaves, while group 4 was provided with a diet containing 100 % OFSP leaves. Lastly, Group 5, the positive control group, was fed a diet containing 100 % Ugwu leaves (*Telfairia occidentalis*) (Aluyor *et al.,* 2024; Oboh *et al*., 2024). Blood samples were collected by cardiac puncture into plain bottles.
Telfairia occidentalis leave was used as positive control due to its high antioxidant properties revealed from scientific researches (Kulczyński *et al.*, 2020).
The table below shows the rats feed composition for the different groups

Table 1: Rats feed composition for the different groups

Constituents (g)	Group 1	Group 2	Group 3	Group 4	Group 5
Maize	22	22	22	22	22
Wheatbran	38	38	38	38	38
Soybeanmeal	5	5	5	5	5
Palm Kernelcake	20	20	20	20	20
Brewersdriedgrain	10	10	10	10	10
Bonemeal	2	2	2	2	2
Limestone	1	1	1	1	1
Salt	0.3	0.3	0.3	0.3	0.3
Vit-minpremix	1	0.75	0.5	0	0
OFSP	0	0.25	0.5	1	0
Pumpkin	0	0	0	0	1
Lysine	0.2	0.2	0.2	0.2	0.2
Meth+cysteine	0.5	0.5	0.5	0.5	0.5
Total (g)	100	100	100	100	100

Assay of antioxidant enzymes (AOE): Antioxidant enzymes assayed in this study included Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Glutathione Reductase (GR).
Assay of catalase enzyme: The activity of serum catalase was determined using the kinetic procedure originally described by Cohen et al. (1970). The method involved monitoring the decomposition of hydrogen peroxide spectrophotometrically, with the rate of decrease in absorbance directly reflecting catalase activity.
Assay of glutathione peroxidase (GPx): GPx activity was assessed following the kinetic method reported by Flohe and Gunzler (1984). The procedure measured the rate of oxidation of reduced glutathione in the presence of hydrogen peroxide, and changes in absorbance were recorded to estimate enzyme activity.
Assay of glutathione reductase (GR): The activity of glutathione reductase was determined according to the method described by Ellman (1959). The procedure monitored the reduction of oxidized glutathione to its reduced form, with absorbance changes measured to calculate enzyme activity.

Assay of superoxide dismutase (SOD): SOD activity was determined using the kinetic method of Misra and Fridovich (1972). The method involved measuring the inhibition of adrenaline auto-oxidation by superoxide dismutase, and enzyme activity was expressed based on the degree of inhibition observed spectrophotometrically.

Data analysis: Data was subjected to statistical analysis using the IBM SPSS statistics software version 25 and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean ± SEM. In addition, LSD *post-hoc* test was used. Values of $p < 0.05$ were considered significant.

Results

Antioxidant enzyme (SOD, CAT, GPx, and GR) activities are presented in Table 2. The antioxidant activities showed significant variations across the dietary groups ($p < 0.05$).

Table 2: Antioxidant enzyme activities in experimental diet of rats supplemented with varying amount of OFSP.

Antioxidant Enzyme	Group 1	Group 2	Group 3	Group 4	Group 5	F value	*P* value
SOD (U/mg protein)	2.28 ± 0.20[a]	1.78 ± 0.30[b]	3.89 ± 0.60[c]	4.10 ± 0.30[d]	3.49 ± 0.03[e]	9.45	0.000
CAT (U/mg protein)	100.03 ± 2.00[a]	100.37 ± 8.00[b]	121.25 ± 1.00[c]	113.52 ± 2.00[d]	111.33 ± 0.20[e]	619.00	0.001
GPx (U/mg protein)	2.03 ± 0.03[a]	1.97 ± 0.07[b]	2.30 ± 0.05[c]	2.34 ± 0.02[d]	2.27 ± 0.08[e]	15.60	0.000
GR (U/mg protein)	2.09 ± 0.02[a]	2.18 ± 0.01[b]	2.14 ± 0.01[c]	2.12 ± 0.01[d]	2.11 ± 0.01[e]	7.18	0.001

Generally, supplementation with OFSP increased enzyme activity compared to the control, with the highest levels observed in Groups 3 and 4. This indicates a dose-dependent enhancement of antioxidant defense, suggesting that OFSP supplementation improves oxidative stress regulation in rats.

Superoxide Dismutase (SOD) activity significantly varied among the groups ($p = 0.000$). Group 1 (Control) showed a baseline SOD activity of 2.28 ± 0.20 U/mg protein. The inclusion of 25% OFSP leaves in group 2 resulted in a decrease in SOD activity (1.783 ± 0.30 U/mg protein).

A statistically significant elevation in SOD activity was recorded in groups 3 (3.887 ± 0.60 U/mg protein) and 4 (4.103 ± 0.30 U/mg protein), relative to the control and suggesting higher concentrations of OFSP leaves can enhance SOD activity. The 100 % Ugwu leaves group (Group 5) showed slightly lower SOD activity (3.488 ± 0.03 U/mg protein) compared to the 100% OFSP leaves group, indicating that while ugwu leaves also have a positive effect, OFSP leaves may be more effective in increasing SOD activity.

There was a significant difference in catalase (CAT) activity among the groups ($p < 0.05$). The control group (Group 1) exhibited a CAT activity of 100.03 ± 2.0 U/mg protein. Similar to SOD, group 2 (25% OFSP leaves) showed no significant increase (100.37 ± 8.00 U/mg proteins). However, a marked increase in CAT activity was observed in group 3 (50% OFSP leaves) at 121.25 ± 1.00 U/mg protein, which was the highest among all groups. Group 4 (100% OFSP leaves) and group 5 (100% ugwu leaves) demonstrated slightly lower CAT activities (113.52 ± 2.00 and 111.33 ± 0.20 U/mg protein, respectively) compared to group 3, suggesting an optimal concentration for OFSP leaves in enhancing CAT activity.

Glutathione Peroxidase (GPx) activity showed significant differences among the groups ($p < 0.05$). The control group (Group 1) had a GPx activity of 2.03 ± 0.03 U/mg protein. Groups 2 (25% OFSP leaves) and 5 (100% ugwu leaves) showed slightly reduced GPx activities (1.97 ± 0.07 U/mg protein and 2.27 ± 0.08 U/mg protein, respectively). However, the highest GPx activities were observed in groups 3 (50% OFSP leaves) and groups 4 (100% OFSP leaves) with values of 2.30 ± 0.05 and 2.34 ± 0.02 U/mg protein, respectively, indicating that higher percentages of OFSP leaves can enhance GPx activity.

Glutathione Reductase (GRx) activity also showed a significant difference ($p < 0.05$). The control group (Group 1) had a GRx activity of 2.088 ± 0.02 U/mg protein. Group 2 (25% OFSP leaves) showed a slight increase in GRx activity (2.182 ± 0.01 U/mg protein), whereas Groups 3 (50% OFSP leaves), 4 (100% OFSP leaves), and 5 (100% ugwu leaves) had lower GRx activities (2.138 ± 0.007, 2.117 ± 0.005, and 2.112 ± 0.007 U/mg protein, respectively). This indicates that a moderate inclusion of OFSP leaves may enhance GRx activity, but higher concentrations do not further increase this effect.

Discussion

An observed increase in enzyme activities following OFSP supplementation suggests improved antioxidant capacity, which may contribute to the protection of tissues from oxidative damage. These results are consistent with previous studies indicating that OFSP leaves, being rich in β-carotene, polyphenols, and flavonoids, can enhance the activity of endogenous antioxidant enzymes (Adebayo *et al.,* 2019; Oboh *et al.,* 2010). Similarly, *Telfairia occidentalis* has been reported to enhance antioxidant defense, although in this study its effect appeared slightly less pronounced than OFSP at equivalent concentrations, which agrees with findings from Okoli et al. (2018) showing differential potency among green leafy vegetables. The apparent peak activity at 50% inclusion for CAT and GPx may reflect a threshold effect where moderate supplementation optimally stimulates enzyme activity, while higher concentrations do not produce proportional increases.

The data suggest that OFSP leaves may be more effective than *T. occidentalis* in modulating antioxidant enzyme activities, supporting their potential role in dietary management of oxidative stress related conditions. These findings highlight the value of promoting OFSP as part of dietary interventions against oxidative stress. However, further studies using longer feeding trials and molecular markers are needed to confirm the mechanistic pathways.

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