Original Article

Effect of ethanolic extract of *Ocimum gratissimum* on sodium nitrite-induced cerebellar cortex toxicity in adult Wistar rats

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Abstract

Introduction: Intoxication of nitrites mainly from food and water constitute a potential hazard with a resultant hypoxia. **Aim:** The aim was to study the effects of ethanolic leaves extract of *Ocimum gratissimum* on sodium nitrite ($NaNO_2$)-induced cerebellar toxicity in adult Wistar rats. **Materials and Methods:** Twenty-four adult Wistar rats weighing 150–250g were divided into six groups of four rats each. Group I was the control and received distilled water, Group II received 54 mg/kg body weight (bwt) of $NaNO_2$, Group III received 750 mg/kg bwt of the extract and 54 mg/kg bwt of $NaNO_2$, Group IV received 375 mg/kg bwt of the extract and 54 g/kg bwt of $NaNO_2$, Group V received 54 mg/kg bwt of $NaNO_2$ and 2 ml/kg bwt of olive oil, and Group VI received 2 ml/kg bwt of olive oil. The administration was by oral route and lasted for 21 days, after which the animals were sacrificed and blood collected for analyses, and the tissues were processed for histological studies. **Results:** The result showed a decrease in the mean bwt of the animals in Groups III and IV, a significant increase in serum levels of malondialdehyde and a decrease in superoxide dismutase, glutathione peroxidase, and catalase in Group II. The result of the hematological analysis showed a significant increase in red blood cells, white blood cells, mean corpuscular volume, and mean corpuscular hemoglobin ($P < 0.05$). The result of histological studies showed degenerative changes in Group II with less degeneration in Group IV. **Conclusion:** The result showed that *O. gratissimum* in a controlled manner may be useful in the management of neurodegenerative conditions that involve free radical generation and reduction in brain energy production.

Key words: Cerebellum free radical, enzymes, hypoxia, oxidative stress, *Ocimum gratissimum*

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INTRODUCTION

Reactive oxygen species (ROS) including free radicals such as superoxide anion (O^-), hydroxyl (HO), hydrogen

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peroxide (H_2O_2), and peroxyl radicals ($RCOO^-$) are active oxygen components that are by-product of normal body metabolism (Halliwell and Gutteridge, 2007). ROS are highly reactive and short-lived and are known to cause damage to cellular components including lipid, deoxyribonucleic acid, protein, carbohydrate, and other biological molecules. They consequently lead to many pathological processes such as cancer, cardiovascular diseases, diabetes, inflammation, and neurodegenerative diseases (Marouf, *et al.*, 2010).

Several free radical species are generated during the course of nitrite-induced oxidation of hemoglobin (Hb) (Fan and Steinberg, 1996). Under normal physiologic conditions, aerobic metabolism of glucose is the primary metabolic fuel for energy production in the brain (Lowry, *et al*., 1964; Greene, *et al*., 2003). Although the brain represents only 2% of the body weight (bwt), it receives 15% of the cardiac output and consumes 20% of the total body oxygen (Magistretti and Pellerin, 1996). Many of the most common disorders of the brain, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple system disorder, progressive supranuclear palsy, and mitochondrial encephalomyopathy, have been found to be associated with alterations in cerebral oxygen metabolism (Ishii, *et al*., 1996). The brain is particularly vulnerable to the effects of ROS due to its high demand for oxygen and its abundance of highly peroxidisable substrates.

Sodium nitrite ($NaNO_2$) is a chemical with a variety of applications. On its own, it can be toxic to humans, but when integrated into specific processes such as the preservation and curing of food, it can be a safeguard from much more dangerous bacteria that can grow in the food. $NaNO_2$ are in many types of fertilizers, pesticides, herbicides, fabrics and have some applications in medicine. $NaNO_2$ poses health risk on exposure. It is irritant to the eye, lungs, and skin and is toxic when consumed. It is not often handled outside professional settings because of its toxicity in high concentrations (Abramov, *et al*., 2003; Abdullahi, *et al*., 2012). $NaNO_2$ oxidizes Hb to methemoglobins which contain ferric iron (Fe^{3+}) rather than ferrous iron (Fe^{2+}) present in Hb. Ferric ions have slightly greater affinity for O^2 which shift the oxygen dissociation curve to the left resulting in decrease release of O_2 to the tissue (Patel and Chu, 2011). Acute intoxication is manifested primarily by methemoglobin formation and resultant hypoxia (Calabrese, *et al*., 1983; Hajieva and Behl, 2006). Nitrites, ingested from food mainly from cured meats and water supply, constitute a potential toxicity. Nitrites and nitrates have also been shown to react with various amines and amides to form carcinogenic nitroso compounds (Stoewsand, *et al.*, 1973). Much attention has been focused on the use of antioxidants, especially natural antioxidants, for the improvement of human health (Zheng and Wang, 2001; Cristiana, *et al*., 2006).

Herbs have been used safely and effectively for many centuries and are free of most of the side effects associated with synthetic drugs (Njoku, *et al*., 1997; Murray, 2004). *Ocimum gratissimum* belongs to the family *Lamiaceae* and found mostly in the tropical countries. It is traditionally used to relief pains and also used in the treatment of rheumatism, diarrhea, high fever, convulsions, diabetes, eczema, and piles and as a repellant (Chitwood, 2003; Hotlets, *et al.*, 2003; Pessoa, *et al.*, 2002). The present work was aimed at studying the effects of ethanolic leaves extract of *O. gratissimum* against $NaNO_2$ induced toxicity in adult Wistar rats.

MATERIALS AND METHODS

Experimental Protocol

Twenty-four apparently healthy adult Wistar rats of both sexes weighing between 150 and 250 g were purchased from the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Kaduna State. The animals were acclimatized for 3 weeks in the Department Animal House. The animals were fed with standard pellet and water *ad libitum* throughout the experimental period under controlled environment of 12 h cycle of light and dark cycle at room temperature. The rats were divided into six groups of four rats each. Fresh leaves of *O. gratissimum* were purchased from Sabon Gari Market Zaria, Kaduna State-Nigeria. Identification and authentication were carried out at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, with voucher number 285.

The leaves were washed, rinsed with distilled water and, air-dried for the period of 1 week was extracted in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The dried fresh leaves of *O. gratissimum* were grounded into coarse powder of 500 g. The powder was subjected to absolute ethanol extraction using soxhlet apparatus for 10 h. The extract was concentrated by using an evaporating dish and was slowly evaporated to dryness in a Water bath regulated at 6°C, and 10% w/w dark green color of the extract was obtained.

Chemicals and Reagents

Twelve grams of $NaNO_2$ manufactured by May and Bakers Limited, Dagenham, England, was purchased from Steve Moore Chemicals Limited, Samaru, Zaria-Nigeria. Goya olive oil was purchased from Beautiful Gate Pharmaceutical Limited, Samaru, Zaria-Kaduna State, Nigeria. Growers feed from Vital feed was obtained from Samaru Market Zaria, Kaduna, Nigeria, and was used to feed the animals throughout the experimental period.

Experimental Procedure

The dose of the extract was determined using the LD_{50} of 2500 mg/kg bwt (Rabelo, *et al*., 2003). The stock solution was prepared by dissolving 12 g of the extract in 160 ml of olive oil to form the stock solution; 30% (750 mg/kg bwt) and 15% (375 mg/kg bwt) of the LD_{50} were used in this study for the high and low dose, respectively. The animals were randomly divided into six groups of four animals per group. Group I received 2 ml/kg bwt of distilled water, Group II received 54 mg/kg bwt of $NaNO_2$, Group III received 750 mg/kg bwt of the extract + 54 mg/kg bwt of $NaNO_2$, Group IV received 375 mg/kg bwt of the extract + 54 g/kg bwt of $NaNO_2$, Group V received 54 mg/kg bwt of $NaNO_2$ + 2 ml/kg bwt of olive oil, and Group VI received 2 ml/kg bwt of olive oil. The experimental procedure was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Animal Sacrifice

After the last day of administration, the animals were left for 48 h and were fasted overnight before sacrificed. The animals were humanely sacrificed by cervical dislocation and the blood collected through cardiac puncture for hematological analysis, and some blood were centrifuged at 2500 rpm and serum collected for biochemical analysis.

Incision was made through the midsagittal suture, and the brains were removed and fixed in Bouin's fluid. The tissues were processed, sectioned, and stained with hematoxylin and eosin method and Cresyl fast violet method.

Estimation of Oxidative Parameters

Determination of catalase activity

Catalase (CAT) activity was determined using the method described by Sinha (1972), and the absorbance was read at 570 nm. Standard cure was made by plotting the absorbance obtained at various levels of the assay. The CAT activity was obtained from the graph of the standard curve.

Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined by a method described by Fridovich (1989). Absorbance was measured every 30 s up for a total of 150 s at 480 nm from where the SOD activity was calculated.

Assessment of Lipid Peroxidation

Lipid peroxidation as evidenced by the formation of TBARS was measured by the method of Niehaus and Samuelson (1968). The absorbance of the pink supernatant was measured against a reference blank using a spectrophotometer at 535 nm.

Assay of Reduced Glutathione Concentration

Reduced glutathione (GSH) concentration measurements were done according to the method of Ellman (1959) as described by Rajagopalan, *et al.* (2004), and the absorbance was read at 412 nm.

Statistical Analysis

Data were reported as mean ± standard error of mean. One-way analysis of variance and Duncan's Multiple range test were used to compare the means with values of $P < 0.05$ was considered to be statistically significant. Sigmastat version 2.0 (Systat Inc., Point Richmond, CA) was used for the statistical analysis.

RESULT

Physical Observations

Some physical observations were made during the period of administration. The animals in Group II were observed to be weak and breathe faster when compared to the control and Group VI. The animals in Groups III and IV were observed to feed well and drank a lot of water when compared to the animals in the control. The animals in the control and in Group VI showed increased activities in their locomotion and feeding habit to be normal.

Weight Changes

The mean bwt of animals in the control and Groups II, V, and VI were observed to increase during the period of administration while the animals in Group VI showed a more rapid increase in weight when compared with others. The mean change in weight in all the groups did not show statistical significance. Meanwhile, the animals in Group III showed a significance decrease in the mean final bwt when compared with the initial bwt as shown in Table 1.

Oxidative Enzymes

The effect on the level of oxidative enzymes such as SOD, malondialdehyde (MDA), glutathione peroxidase (GPX), and CAT showed no statistically significant difference in the parameters. The mean SOD of Group II showed

Table 1: Morphometric parameters cerebella, initial and final body weight (g)

GRP	ADMINS	IN.B.W	F.B.W	C.BLLUM
I	Control	193.75±31.52	220.75±27.77	0.46±0.03
II	$NaNO_2$ only	203.00±31.72	216.75±30.19	0.49±0.02
III	$NaNO_2$ + H.D extract	218.75±36.50	213.00±30.89*	0.49±0.02
IV	$NaNO_2$ + L.D extract	210.50±9.04	207.25±27.09*	0.46±0.02
V	$NaNO_2$ + olive oil	193.75±25.03	216.00±28.44	0.45±0.03
VI	Olive oil only	186.25±22.65	225.75±24.50*	0.41±0.03

GRP - group, ADMINS - administration, IN.B.W - Initial body weight, F.B.W - Final body weight, C.BLLUM - Cerebellum. *$P \leq 0.05$

significant decrease when compared to the control. There was significance increase in the mean MDA in Group II when compared to the control. The mean GPX in Groups II, III, V, and VI showed significant decrease when compared to the control. The mean CAT in Groups II, IV, V, and VI were observed decrease when compared to the control as shown in Table 2.

Hematological Parameters

The mean value of pack cell volume (PCV), red blood cell count (RBC), Hb count, white blood cell count (WBC), mean corpuscular volume, mean corpuscular Hb (MCH), and MCH concentration of the experimental animals were studied. The result showed no statistically significant differences in the mean PCV across the groups. The mean WBC of the treated animals were statistically significanct ($P \leq 0.05$) when compared to the control. The mean WBC of the animals in Groups II, V, and VI showed significant increase when compared with the Control. The mean Hb of the treated animals did not show any statistically significant difference when compared with the control. The result of the mean RBC of the treated groups showed statistically significant difference ($P \leq 0.05$) when compared with the control [Table 3].

Histological Observation

The result of histological observation of the sections of the cerebellar cortex of the animals showed some histological changes in the rats administered with $NaNO_2$ with or without the extract and olive oil. The control showed normal architecture and orientation of the molecular layer, granular layer, and Purkinje layer with normal Purkinje cells of the cerebellar cortex as shown Plate 1. Group II showed some degeneration of the Purkinje cell layer and Purkinje cells of the cerebellar cortex as shown in Plate 2. Group III showed degeneration of the Purkinje cells in the cerebellar cortex as shown in Plate 3. Group IV animals showed mild degeneration Purkinje cell along with the Purkinje cell layer of the cerebellar cortex as shown in Plate 4. Group V showed the molecular layer and granular layer, Purkinje cell along with the Purkinje cell layer as shown in Plate 5. Whereas animals in Group VI showed normal orientation of the molecular layer, granular layer, and Purkinje layer with normal Purkinje cells of the cerebellar cortex as shown in Plate 6.

Table 2: Oxidative parameters

Group	Administration	SOD (U/mg)	MDA (nmol/mg)	CAT (μ × mol/mg)	GPX (U/mg)
I	Control	2.35±0.25	1.70±0.20	47.5±3.50	52.50±3.50
II	$NaNO_2$ only	2.25±0.15	1.80±0.05	41.5±1.50	46.50±4.50
III	$NaNO_2$ + high dose of extract	2.40±0.00	1.55±0.10	47.5±2.50	46.00±2.00
IV	$NaNO_2$ + low dose of extract	2.40±0.20	1.40±0.10	44.50±5.50	49.00±3.00
V	$NaNO_2$ + olive oil	2.35±0.05	1.70±0.20	40.50±3.00	44.50±3.50
VI	Olive oil only	2.45±0.10	1.70±0.10	42.50±4.00	45.5±3.50

SOD - Superoxide dismutase, MDA - Malondialdehyde, GPx - Glutathione peroxidase, CAT - Catalase

Table 3: Hematological parameters

Group	Administration	PCV (%)	RBC (cell/mm³)	Hb (g/dl)	WBC (cell/mm³)
I	Control	41.75±3.43	7.03±0.75*	13.90±1.12	16.58±0.86
II	$NaNO_2$ only	40.50±4.94	6.10±0.35*	13.52±1.51	30.18±2.17
III	$NaNO_2$ + high dose extract	40.50±2.53	5.35±0.49*	13.50±0.86	17.65±1.45
IV	$NaNO_2$ + low dose extract	45.25±2.78	7.28±0.68*	15.05±0.94	18.63±0.89
V	$NaNO_2$ + olive oil	42.25±2.06	10.27±0.47	14.05±0.69	36.00±0.67
VI	Olive oil only	45.00±4.02	11.48±0.91	14.89±1.27	33.00±1.63

*$P \leq 0.05$. PCV - Packed cell volume, RBC - Red blood cell count, Hb - Hemoglobin count, WBC - White blood cell count

DISCUSSION

The decrease in physical activities observed in experimental animals could be a result of reduced energy generation due to hypoxia resulting from methemoglobin formation. This may also be due to the increased level of $NaNO_2$ in the body leading to increased catabolic process in the body. The results of this study are in agreement with Grant and Butler (1989) who reported that $NaNO_2$ reduce energy generation due to hypoxia-induced methemoglobin formation. Porter, *et al.* (1993) had shown that $NaNO_2$ increases the rate of catabolic reaction thus leading to the decrease in the mean bwt observed in Groups III and IV could be due to the hypoglycemic and diuretic effect of the extract. The present result is in agreement with the findings of Effraim, *et al.* (2003), which showed that the aqueous and ethanol extracts of *O. gratissimum* leaves possessed hypoglycemic effects on normoglycemic and neonatal streptozocin-induced diabetes model. The rats treated with $NaNO_2$ showed significant decrease in mean bwt when compared the control throughout the experiment periods, and this reduction in weight may be due to the reduction in food consumption (Grant and Butler, 1989; Patel and Chu, 2011).

The chemical reactivity of $NaNO_2$ with Hb may enhance iron-mediated toxicities. Nitrite is known to cause free radical generation (Rahman, *et al.* 2009; Zaidi, 2010), as it can stimulate oxidation of ferrous ions in oxy-Hb to form methemoglobin (Gladwin, 2004; Baky, 2010). The result of the present study showed that $NaNO_2$ significantly impaired the oxidative status in the animals. This effect was shown by the significant increase in brain malondialdehyde, an index of lipid peroxidation in Group II animals and a significant reduction in the levels of GPX, in addition to decrease in SOD and CAT activities when compared with the control. The present result is in agreement with Hayashi, *et al*. (2004) and Valko, *et al*. (2006), which showed that there was a significant

increase in the mean SOD, GPX, CAT and a decrease in the mean MDA. This could be as a result of ameliorative effect of *O. gratissimum* extract. The present result agrees with the findings of Fagbohun, *et al.* (2012) and Akinmoladun, *et al.* (2007) which showed that the leaf extract of *O. gratissimum* possesses antioxidant potential presumably because of the phytochemical constituents.

The cerebellum of animals in Groups II and III showed some degenerating cells with degeneration of the Purkinje cell layer and Purkinje cells of the cerebellar cortex. This could be due to methemoglobin formation due to nitrite ingestion and increase in concentration of nitrite and extract which could be toxic. The present result was in agreement with Imaizumi, *et al*. (1980) and Porter, *et al.* (1993) who showed that nitrite convert Hb to methemoglobin and Orafidiya, *et al.* (200l) had reported that *O. gratissimum* leaves have toxic potentials that should not be overlooked. Group IV treated with low dose of extract showed mild degeneration of cells which could be as a result of the antioxidant effect of the extract. This result is in agreement with Akinmoladun, *et al.* (2007) who reported that *O. gratissimum* possess good antioxidant properties due to its phytochemical constituents. Meanwhile, Group V showed more cytoarchitectural damage when compared with Group IV while the animals in the control and Group VI showed normal cytoarchitecture of the cerebral and cerebellar cortex.

CONCLUSION

Administration of *O. gratissimum* leaves extract have shown to significantly protect the brain against oxidative stress-induced tissue damage and ameliorates the energy failure in damaged brain tissues induced by $NaNO_2$. The current study greatly recommends the beneficial use of *O. gratissimum* in a dose-controlled manner in the management of neurodegenerative conditions that involve free radical generation and reduction in brain energy production, particularly in hypoxic states.

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Conflicts of Interest
There are no conflicts of interest.

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