EFFECT OF ETHANOLIC EXTRACT OF OCIMUM GRATISSIMUM ON SODIUM NITRITE-INDUCED CEREBRAL CORTEX TOXICITY IN ADULT WISTAR RATS

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ABSTRACT

Aim: The effect of ethanolic leaf extract of Ocimum gratissimum on sodium nitrite-induced cerebral oxidative stress in adult Wistar rats was evaluated.

Methods: Twenty four adult Wistar rats of weight between 150g to 250g were divided into six groups of four rats each. Group I was the Control and received 2ml/kg bwt of distilled water, Group II received 54mg/kg bwt of $NaNO_2$, Group III received 750mg/kg bwt of the extract and 54mg/kg bwt of $NaNO_2$, Group IV received 375mg/kg bwt of the extract and 54mg/kg bwt of $NaNO_2$, Group V received 54mg/kg bwt of $NaNO_2$ and 2ml/kg bwt of Olive oil while Group VI received 2ml/kg bwt of Olive oil. The administration was oral for 21 days after which the animals were sacrificed and blood collected for hematological and biochemical analyses and the tissues processed for histological studies.

Results: The result showed a dose dependent decrease in the mean body weight of the animals in Groups III and IV and a significant increase in serum levels of MDA and decrease in SOD, GPX and CAT in Group II ($P < 0.05$). Histological studies showed degenerative changes in Group II with less degeneration in Group IV.

Conclusion: The result showed that O. gratissimum in a dose-dependent manner may be used in the management of neurodegenerative conditions that involve free radical production.

Key words: Sodium nitrite, Cerebral cortex, Ocimum gratissimum

INTRODUCTION

Hemoglobin is the primary oxygen-transport protein in vertebrates and can be converted into methemoglobin by multiple agents, including nitrites, with consequent loss of its oxygen-carrying capacity which may predispose to hypoxia (Baky et al., 2010). Several free radical species are generated during the course of nitrite-induced oxidation of hemoglobin (Fan and Steinberge, 1996; Greene et al., 2003). Although the brain represents only 2% of the body weight, it receives 15% of the cardiac output and consumes 20% of the total body oxygen (Magistretti and Pellerin, 1996). There is a critical dependence of brain function on continuous and efficient usage of oxygen. The organ's heightened vulnerability and sensitivity to alterations in oxygen supply may result inthe most common disorders of the brain, such as Alzheimer's disease, Parkinson's disease, Huntington's disease which have been found to be associated with alterations in cerebral oxygen metabolism (Lowry et al., 1964; Ishii et al., 1996). The brain is particularly vulnerable to the effects of reactive oxygen species due to its high demand for oxygen (Halliwell and Gutteridge, 2007). Reactive Oxygen Species (ROS) are highly reactive and short-lived and are known to cause damage to cellular components including lipid, DNA, protein, carbohydrate, and other biological molecules, which may consequently lead to many pathological processes such as cancer, cardiovascular diseases, diabetes, inflammation and neurodegenerative diseases (Marouf, et al., 2010; Abramov, 2003). Sodium nitrite is a chemical with a variety of

applications and can be toxic to humans. Sodium Nitrite poses health risk on exposure and is an irritant to the eye, lungs and skin and toxic when consumed (Gladwin et al., 2004). It oxidizes hemoglobin to methemoglobin which contains ferric iron rather than ferrous iron present in hemoglobin. Acute intoxication is manifested primarily by methemoglobin formation and resultant hypoxia (Patel and Chu, 2011). Nitrites and Nitrates have been shown to react with various amines and amides to form carcinogenic nitroso compounds (Stoewsand, et al., 1972; Zheng and Wang, 2001).Herbs have been used safely and effectively for many centuries (Murray, 2004). Ocimum gratissimum belongs to the family Lamiaceae and found mostly in the tropical countries including: Nigeria, India, North and South America, Mexico and Brazil (Njoku et al., 1997; Akimoladun, et al., 2007). It is traditionally used to relief pains, treatment of rheumatism, diarrhea, high fever, convulsions, diabetes, eczema, piles and as a repellant (Chitwood, 2003; Hotlets, et al., 2003, Pessoa et al., 2003; Abdullahi et al., 2012). The present work is aimed at evaluating the effect of ethanolic leaf extract of Ocimum gratissimum against sodium nitrite-induced oxidative stress in cerebrum of adult Wistar rats.

MATERIALS AND METHODS

Experimental Protocol
Twenty-four apparently healthy adult Wistar rats of both sexes weighing between 150g to 250g were purchase from the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria Kaduna State. The animals were acclimatized for three weeks in the Department Animal house. The animals were fed with standard pellet and water ad libitum throughout the experimental period. 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 was done in the herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria with voucher number 285.The leaves were washed with distilled water and air dried for the period of one week and was extracted in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. The dried fresh leaves were grounded into coarse powder of 500g. The powder was subjected to absolute ethanol extraction using Soxhlet apparatus for 10 hours.

The extract was concentrated by using evaporating dish to dryness in a Water bath regulated at 60^0C and 10%w/w dark green extract was obtained.

Chemicals and Reagents
12g of sodium Nitrite manufactured by May and Baker limited Dagenhan 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 LD_{50} of 2500mg/kg body weight (Rabeloet al., 2003). The stock solution was prepared by dissolving 12g of the extract in 160 ml of Olive oil to form the stock solution, 30% (750mg/kg body weight) and 15% (375mg/kg body weight) 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 1 received 2ml/kg bwt of distilled water, Group 2 received 54mg/kg bwt of $NaNO_2$, Group 3 received 750mg/kg bwt of the extract+54mg/kg bwt of $NaNO_2$, Group 4 received 375mg/kg bwt of the extract+54mg/kg bwt of $NaNO_2$, Group 5 received 54mg/kg bwt of $NaNO_2$+2ml/kg bwt of Olive oil, Group 6 received 2ml/kg bwt of Olive oil.

Animal Sacrifice
After the last day of administration, the animals were left for 48 hours and were fasted overnight before sacrificed. The animals were humanely sacrificed by cervical dislocation and the blood collected through cardiac puncture for hematological and biochemical analyses. Incisions were made through the mid sagital suture and the brains were removed and fixed in Bouin`s fluid. The tissues were processed, sectioned and stained with hematoxylin and eosin and Cresyl fast violet methods.

ESTIMATION OF OXIDATIVE PARAMETERS
Determination of catalase activity
Catalase 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 catalase activity

was obtained from the graph of the standard curve.

Determination of superoxide dismutase (SOD) activity
Superoxide Dismutase (SOD) activity was determined by a method described by Fridovich, (1989). Absorbance was measured every 30 seconds up for a total of 150 seconds 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 535nm.

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 was reported as Mean ± Standard Error of Mean (SEM). One way Analysis of Variance (ANOVA) was used to compare the means with values of $p<0.05$ was considered to be statistically significant. Sigmastat 2.0 (SystatInc, Point Richmond, CA) was used for the statistical analysis.

RESULTS

Physical Observation
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 was observed to be normal.

Weight Changes
The mean body weight 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. Though the mean change in the weight across the Groups did not show statistical significance, the animals in Group III showed a significance decrease in the mean final body weight when compared with the initial body weight as shown in Table 1.

Table 1: Show Morphometric Parameters Cerebella, Initial and Final Body Weight (g).

GRP	ADMINS	IN.B.W	F.B.W	C.BRUM
1	Control	193.75 ±31.52	220.75±27.77	1.19 ±0.03
II	NaNO$_2$ only	203.00±31.72	216.75±30.19	1.12±0.06
III	NaNO$_2$+H.D extract	218.75±36.50	213.00±30.89*	1.08±0.04
IV	NaNO$_2$ +LD extract	210.50±9.04	207.25±27.09*	1.05±0.42
V	NaNO$_2$ + Olive oil	193.75±25.03	216.00±28.44	1.04±0.02
VI	Olive oil only	186.25±22.65	225.75±24.50*	1.08±0.03

GRP =group. ADMINS=administration. IN.B.W=initial body weight, g= grams F.B.W=final body weight. C.BRUM=cerebellum.

Oxidative Enzymes
The effect on the level of oxidative enzymes such as Superoxide dismutase (SOD), Malondialdyde (MDA), Glutathione peroxidase (GPX) and Catalase (CAT) showed no statistical significance difference in the parameters. The mean SOD of group II showed 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.

Table 2: Showing oxidative parameters

GROUP	ADMINSTRATION	SOD(U/mg)	MDA(nmol/mg)	$CAT(\mu\frac{mol}{mg})$	GPX(U//mg)
I	Control	2.35±0.25	1.70±0.20	47.5±3.50	52.50±3.50
II	NaNO2 Only	2.25±0.15	1.80±0.05	41.5±1.50	46.50±4.50
III	NaNO2+High dose of extract	2.40±0.00	1.55±0.10	47.5±2.50	46.00±2.00
IV	NaNO2+Low dose of extract	2.40±0.20	1.40±0.10	44.50±5.50	49.00±3.00
V	NaNO2+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= Malondialdihyde, GPx= Gluthione peroxidase, CAT= Catalase.

Haematological Parameters

The mean value of pack cell volume (PCV), red blood cell count (RBC), haemoglobin count (Hb), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of the experimental animals were studied. The result showed no statistical significance differences in the mean PCV across the Groups. The mean WBC of the animals in Groups II, V and VI showed significance increase when compared with the Control ($P \leq 0.05$). The mean Hb of the treated animals did not show any statistical significant difference when compared with the Control. The result of the mean RBC of the treated goops showed statistical significance difference ($P \leq 0.05$.) compared with the Control (Tables 3 and 4).

Table 3: Showing haematological parameters

GRP	ADMINS	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$_2$+H.D extract	40.50±2.53	5.35 ± 0.49*	13.50±0.86	17.65 ± 1.45
IV	NaNO$_2$+L.D 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

H.D=high dose, L.D=low dose, GRP=groups, ADMINS=administration, PCV= packed cell volume, RBC= red blood cell count, Hb = haemoglobin count, WBC = white blood cell count,* = p≤ 0.05

Table 4: Showing haematological parameters

GROUP	ADMINS	MCV(cu μ)	MCHC(%)	MCH(pg)
I	Control	6.04±0.54	0.33±0.0005	20.12±1.79
II	NaNO$_2$ only	6.59±0.27	0.34±0.0055	22.052±0.67
III	NaNO$_2$+H.D extract	7.84±1.02	0.33±0.0004	26.14±3.41
IV	NaNO$_2$+L.D extract	6.31±0.43	0.33±0.0004	20.93±1.42
V	NaNO$_2$+Olive oil	4.22±0.36	0.33±0.0004	13.83±1.20
VI	Olive oil only	3.81±0.34	0.33±0.0022	13.036±0.99

GRP = groups, ADMINS = administrations, H.D=high dose, L.D = low dose, GRP=groups, ADMINS= administration, MCV= mean corpuscular volume, MCH=mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration.

Differential White Blood Cell Count

The result of differential white blood cell count showed a decrease in the mean MON in Groups II, IV, V and VI when compared with the Control. There was a significance increase in the mean NEU in Groups III and V when compared with the Control. The mean LYM in the treated Group was observed to decrease when compared with the Control. However, there was a significance increase in Group II when compared with Groups III and IV. The mean EOS was increased in Groups II and V when compared with the Control as shown in Table 5.

Table 5: Showing the differential white blood count (%)

GRP	ADMINS	MON	LYM	NEU	EOS
I	Control	2.00 ± 0.00	10.50 ± 1.3	85.75 ± 1.3	2.75 ± 0.48
II	$NaNO_2$ only	1.25 ± 0.25	16.25 ± 2.4	80.50 ± 23	3.25 ± 0.48
III	$NaNO_2$+H.D extract	2.00 ± 0.00	20.50 ± 3.6	77.50 ± 1.5	2.25 ± 0.25
IV	$NaNO_2$+L.D extract	1.50 ± 0.29	16.25 ± 2.0	79.50 ± 1.7	2.00 ± 0.41
V	$NaNO_2$+Olive oil	1.50 ± 0.50	18.75 ± 1.5	78.50 ± 2.7	3.25 ± 0.75
VI	Olive oil only	1.50 ± 0.29	17.25 ± 1.8	76.25 ± 2.7	3.25 ± 0.48

H.D=high dose, L.D=low dose, GRP=groups, ADMINS=administrations, MON=Monocytes, LYM=Lymphocytes, NEU=Neutrophyls, EOS = Eosynophyls

Histological Observation

The result of the histological observation of the section of cerebral cortex of the experimental animals showed some histological changes in the rats administered with $NaNO_2$ with or without extract and Olive oil as shown in the Plates. The animals in the Control showed normal architecture of Pyramidal cell layer, Pyramidal cells, Stellate cells of cerebral cortex as shown in Plate 1. Group II animals showed area of cortical degeneration with some degenerating cells, fibres and some normal pyramidal cells and Stellate cells in the cerebral cortex as shown in Plate 2. Group III showed degenerating cell with neuronal clumping in the cerebral cortex as shown in Plate 3. Group IV animals showed mild degeneration of cells, cellular aggregation and clumping of cells in the cerebral cortex as shown in Plate 4. Group V showed areas of cortical degeneration, nuclei aggregation and necrotic pyramidal cell and stellate cells as shown in Plate 5.While the animals in Group VI showed normal architecture of Pyramidal cell layer, Pyramidal cell, Stellate cells of cerebral cortex as shown in Plate 6.

DISCUSION

The result of the present study showed decrease in physical activities in Group II which could be due to reduced energy generation as a result of hypoxia as a result of methemoglobin formation (Zeidi, 2010). This may also be due to increased level of sodium nitrite 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 and Porter, et al., (1993), had stated that $NaNO_2$ increases the rate of catabolic reaction. The apparent decrease in the mean body weight observed in Groups III and IV could be due to the hypoglycaemic and diuretic effect of the extract. The result of the present study was in agreement with Effriam et al., (2003) and Akinmoladum et al., (2007), who showed that aqueous and ethanol leaf extracts of Ocimum gratissimum possess hypoglycaemic effects on normoglycaemic and neonatal streptozocin-induced diabetes. In general the reduction in body weight may be attributed to the decrease in food intake or direct cytotoxic effects of sodium nitrite treatment (Njoku et al., 1997). The chemical reactivity of $NaNO_2$ with hemoglobin may enhance iron-mediated toxicities and nitrite is known to cause free radical generation (Volkoet al., 2006; Cristiana et al., 2006), as it can stimulate oxidation of ferrous ions in oxy-hemoglobin to form methemoglobin as well as various ROS (Gladwin, 2004; Baky, 2010). The result of the present study showed that sodium nitrite significantly impaired the oxidative status in the animals. This effect was presented by the significant elevation in brain malondialdehyde (MDA), an index of lipid peroxidation and a significant reduction in the levels of GPX and in addition to depletion of SOD and CAT activities. The present result is in agreement with Hotlets et al., (2003), who showed asignificant increase in the mean SOD, GPX, CAT and a decrease in the mean MDA which could be as a result of ameliorative effect of O. gratissimum extract (Abdullahi et al., 2012). The present result agrees with the findings of Fagbohun, et al., (2012); Akinmoladun et al., (2007).
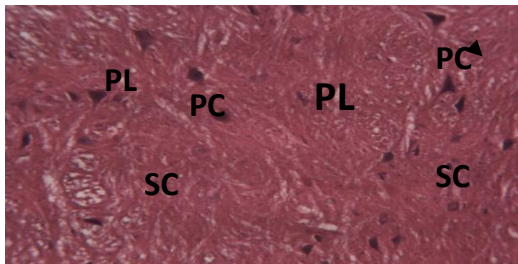
Plate 1: A section of the cerebral cortex of the Control Group (G1) showing normal architecture of Pyramidal cell layer (PL),Pyramidal cell (PC), Stellate cells (SC) (H and E X250).
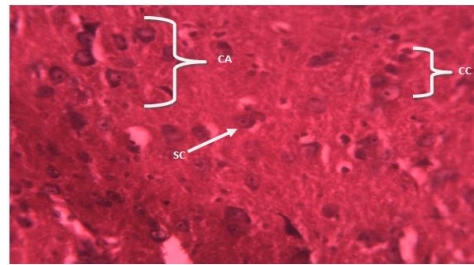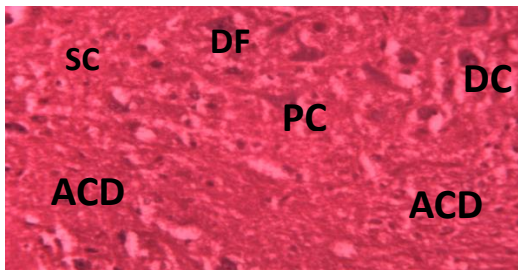

Plate 2: A section of the cerebral cortex of animals in Group II showing some degenerating cell (DC) and fibre (DF), area of cortical degeneration (ACD) with some normal pyramidal cell (PC) and Stellate cells (SC) (H&E x 250).

Plate 3: A section of the cerebral cortex of the animals in Group III showing Stellate cell (SC), with degenerating cell (DC) and neuronal clumping (NC) (H&E ×250)

Plate4: A section of the cerebral cortex of the animals in Group IV showing Stellate cell (SC) cellular aggregation (CA) and clumping of cells (CC) (H&E×250)
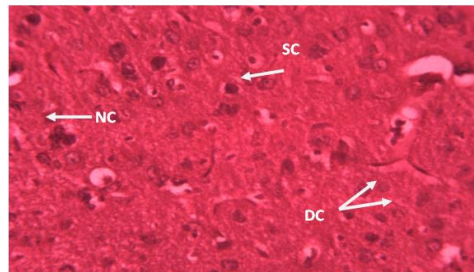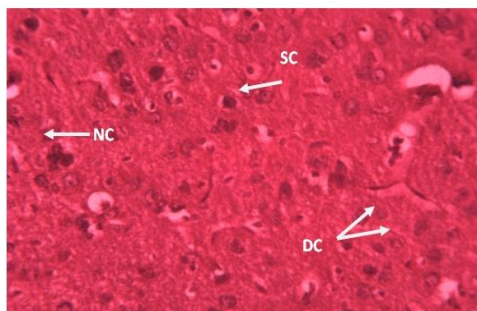

Plate 5: A section of the cerebrum of the animals in Group V showing areas of cortical degeneration (ACD) nuclei aggregation (NA) and necrotic pyramidal cell (NPC) and a stellate cells (H&E Stain, ×250)
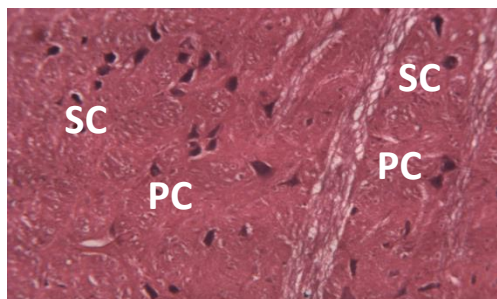

Plate 6: A section of the cerebral cortex of the animals in Group VI showing stellate cell (SC) and pyramidal cells (PC) that appeared normal (H&E ×250)

The extract from the leaves of O. gratissimum possesses good antioxidant potential presumably because of its phytochemical constituents (Rabelo et al., 2003; Chitwood et al., 2003). The study of haematological indices is one of the important measures for diagnosis of diseases. Alteration in blood parameters may be due to changes in cellular integrity, membrane permeability and metabolism, or due to exposure to toxic chemicals (Rahman et al., 2009). The result of the present study showed a decrease in PCV, RBC and Hb concentration in the experimental Groups treated with $NaNO_2$ which may be due to anemia as a consequence of the toxic effect of sodium nitrite on bone marrow, spleen and liver (Greene et al., 2003;

Hajieva and Behl, 2006). Imaizumi et al. (1980) and Ishii et al. (1996), had reported that this reduction might be due to sodium nitrite administration, which was accompanied by a remarkable increase of methemoglobin level. The nitrite ion, its metabolites and lipid peroxidation products which react with sulfo-hydryl groups of the lipid bilayer and protein components of erythrocyte membrane and alter their structure (Calabrese et al., 1983; Rahman et al., 2009). The histological study of the cerebrum showed some degenerating cells, fibers and area of cortical degeneration with neuronal clumping in the cerebral cortex. This could be due to methemoglobin formation as a result of nitrite ingestion and the increase in the

concentration of nitrite which is toxic. The present result is in agreement with Porter, et al. (1993), who showed that nitrite convert haemoglobin to methemoglobin and Orafidayo, et al. (200l), who reported that O. gratissimum has toxic potentials that should not be over looked. The present result is in agreement with Akinmoladun, et al., (2007), who reported that O. gratissimum possesses good antioxidant properties due to its phytochemical constituent.

CONCLUSION

Administration of O. gratissimum extract protects the brain against oxidative stress-induced tissue damage and ameliorates the energy failure in damaged brain tissues induced by sodium nitrite. The present study recommends the beneficial use of O. gratissimum in a controlled manner in the management of neurodegenerative conditions that involve free radical production in the brain.

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