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Studies on Some Biochemical and Histological Changes Associated with Long Term Consumption of Leaves of *Ocimum gratissimum* L. in Male Rats

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Abstract: This study investigated changes in some biochemical and histological parameters in male rats fed with an *Ocimum gratissimum*-supplemented diet for six months. Biochemical parameters studied include serum protein, cholesterol, lipid peroxidation, glutathione-S-transferase, superoxide dismutase, alanine transaminase, aspartate transaminase, alkaline phosphatase, haemoglobin and white blood cells. The histological characteristics of tissue sections of liver, intestines and testes were also examined. The weight of the animals increased significantly ($p < 0.05$) over the control. There were also significant reductions ($p < 0.05$) in serum protein, cholesterol, lipid peroxidation and haemoglobin in the animals. Superoxide dismutase was also significantly increased ($p < 0.05$) while the changes in glutathione-S-transferase, alanine transaminase, aspartate transaminase and alkaline phosphatase were not significant. White blood cell count was significantly increased ($p < 0.05$). Histological changes in the intestines revealed the presence of increased villi and larger goblet cells. The testes also showed increased number of sperm cells and spermatogonia while there were no visible changes in the histology of the liver.

Key words: *Ocimum gratissimum*, cholesterol, lipid peroxidation, superoxide dismutase, haemoglobin, white blood cells

INTRODUCTION

Plant foods especially vegetables contribute substantially to both local diets and ethno medicine in developing countries especially Nigeria (Okafor, 1980; Gbile and Adesina, 1986). *Ocimum gratissimum* L. called African Basil is one of the most commonly used vegetables in Africa. *Ocimum gratissimum* belongs to the family Lamiaceae and is widely distributed in the tropical and warm regions of the world (Okigbo and Ogbonna, 2006). It is an erect, multi-branched perennial shrub that grows up to a height of two meters with a taproot and many adventitious side rootlets. The leaves are simple, opposite or whorled with several oil glands and possess a peculiar scent smell due to its composition of volatile essential oils. It produces an inflorescence that is capitate and reduces apical dominance while increasing branching. The flowers are zygomorphic, bilobed and bisexual with five petals and sepals and four stamens. The gynecium has two carpels ascending from the ovary. The fruit has a

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group of four nutlets each with a brown seed that has scanty or no endosperm. Leaves of *O. gratissimum* have been found to contain methylchaylcol, linalol, eugenol, thymol and xanthamicrol and the amount produced is dependent on the area that it is cultivated as well as part of the plant (Okujagu *et al.*, 2005; Odebiyi and Sofowora, 1978). Its other constituents include saponins, steroids, camphor, estragol, litral, anethol, hydrocyanamate and terpenes. *Ocimum gratissimum* has both culinary and medicinal uses (Iwu, 1986). It is mainly used as a spice to flavor foods and meats (Okigbo, 1977). Its use as spice is known to reduce microbial load and extend the shelf life of foods. The components of *O. gratissimum* has biological activity such as antidiabetic, antiseptic, antitussive, antihelminthic, antispasmodic and antimicrobial (Gbolade, 2009; Akinyemi *et al.*, 2004; Lopez *et al.*, 2005). The leaves of *O. gratissimum* are used as a laxative and its infusion serves as a relief for respiratory disorders, headaches, fever, diarrhea, dysentery, pile and convulsion (Idu *et al.*, 2005; Danziel, 1980). The whole plant is used as a remedy for gonorrhoea, catarrh conditions, cough, constipation, ringworm, flatulence, hypertension and anemia (Odugbemi and Akinsulire, 2006). Its leaves are used as sponge to remove skin blemishes and have been formulated into skin creams used for treating dermatological disorders as well as toothpastes used in maintaining oral hygiene (Odebiyi and Sofowora, 1978). The mucilaginous nutlets are used for treatment of cough and mixed with a drink used against gonorrhoea and intestinal disturbance. The Igbo name of *O. gratissimum* Nchoanwu literally means mosquito-repellant and thus, its presence around the home is believed to repel mosquitoes as a basis for the prevention of malaria. The extracts of the leaves are applied externally in treatment of conjunctivitis, rheumatic pain, dressing of wounds and lumbago. The hypoglycaemic activity of the plant has also been reported (Aguiyi *et al.*, 2000).

A lot of research has been done on *O. gratissimum* especially on its medicinal properties generating an abundance of information. However, no work has been done on the biochemical and histological changes on tissues such as testes associated with its consumption. This study addresses the effect of consuming *O. gratissimum* leaves for a long term on some biochemical and histological responses in male albino rats.

MATERIALS AND METHODS

This study was conducted from 25th October, 2005 to 4th April, 2007 at the Department of Biochemistry, University of Nigeria, Nsukka, South East, Nigeria.

Preparation of Leaves of *Ocimum gratissimum*

Fresh leaves of *O. gratissimum* were harvested from a family garden farm in Nsukka, South-Eastern Nigeria. The leaves were picked and air-dried at a tropical room temperature (25°C). The dried leaves were then ground to a coarse powdered form using a hammer mill. The proximate analysis of the ground leaves was determined according to the procedure of AOAC (1975).

Formulation of Experimental Diets

Two experimental diets namely Control Diet (CD) and *Ocimum gratissimum*-Supplemented Diet (OGSD) were formulated from commercially available feed grade feedstuffs including maize, corn flour, fish meal, groundnut meal, bone meal and vitamin premix. The Control Diet (CD) was formulated without the inclusion of the ground leaves of *O. gratissimum* while the *O. gratissimum*-Supplemented Diet (OGSD) was formulated with 5% of the ground leaves of *O. gratissimum*. The result of the proximate analysis of the feed

components and the ground leaves of *O. gratissimum* were considered in the formulation of isocaloric and isonitrogenous Control Diet (CD) and *O. gratissimum*-Supplemented Diet (OGSD).

Animal Grouping and Feeding

Twenty male albino rats aged 4 weeks old and with an average weight of 38 ± 3 g were used for the experiment. The animals were randomly distributed into two groups namely Control Group (CG) and *Ocimum gratissimum* Group (OGG) consisting of 10 animals each. The animals in Control Group (CG) were fed Control Diet (CD) while, those in *Ocimum gratissimum* Group (OGG) were fed *Ocimum gratissimum*-Supplemented Diet (OGSD). The two experimental diets and water were given to the animals *ad libitum* for 6 months. The average weights of the animals in the two groups were recorded on a monthly basis.

Phytochemical Analysis

Preliminary phytochemical analysis of the leaves of *Ocimum gratissimum* for the presence of tannins, phytosterols, glycosides, flavonoids, anthocyanidins, alkaloids and saponins was carried out using simple chemical tests as described by Odebiyi and Sofowora (1978).

Collection of Blood and Serum Samples

At the end of the feeding period of 6 months, 6 animals from each group were sacrificed and blood samples were collected in labelled sample bottles with drops of Ethylenediaminetetraacetic acid (EDTA). Blood serum samples were collected in sample bottles without EDTA and allowed to clot before being centrifuged at 5000 rpm for 10 min to obtain the serum.

Collection of Organs and Tissue Samples

Sections of the liver, intestines and testes of the animals in the two experimental groups were promptly excised soon after they were sacrificed and stored in 10% formyl saline.

Determination of Biochemical Parameters and Enzyme Assays

The total serum protein was determined by Lowry *et al.* (1951) method. The concentration of total and free cholesterol were determined by the method described by Searcy and Bergquist (1960). Lipid peroxidation levels were determined by the method described by Wallin *et al.* (1993). Hemoglobin and White Blood Cells (WBC) counts were determined according to methods described by Dacie and Lewis (1990). Glutathione-S-Transferase (GST) was assayed according to the method described by Habig *et al.* (1974). Superoxide Dismutase (SOD) was assayed by the method of Jewett and Rocklin (1993). Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were determined using Randox test kits according to the method described by Reitman and Frankel (1957).

Histological Examination of Organs and Tissues

The histological examination of the organs and tissues were done according to procedures described by Disbrey and Rack (1970) and Drury and Wellington (1967).

Statistical Analysis

The results obtained in this study were expressed as Mean \pm SEM for triplicate determinations. The Analysis of Variance (ANOVA) for a completely randomized design and

Duncan's multiple range tests were used to analyze experimental data. The values were considered significant at $p < 0.05$.

RESULTS

The results of the preliminary phytochemical screening of the leaves of *Ocimum gratissimum* showed that it contained all the phytochemicals that were screened except alkaloids as shown in Table 1.

The result showed that OGSD caused a significant percentage increase ($p < 0.05$) in weight gain when compared to the control. There was a reduction in the level of serum protein which was statistically significant ($p < 0.05$). The lipid peroxidation level was reduced significantly ($p < 0.05$) in animals fed OGSD. Haemoglobin level was also significantly reduced ($p < 0.05$) in the animals fed with OGSD when compared to the control group (Table 2). There was a significant reduction ($p < 0.05$) in total cholesterol in the animals fed OGSD while, WBC count were significantly increased ($p < 0.05$).

Results showed that there was a significant increase ($p < 0.05$) in SOD. The levels of GST and all the liver enzymes including AST, ALT and ALP did not show any significant changes (Table 3).

The histological features of liver, intestine and testes of the animals fed Control Diet (CD) and *Ocimum gratissimum*-Supplemented Diet (OGSD) are presented in Fig. 1a, b to 3a, b, respectively. There were no distinct histological differences between the hepatocytes of the rats fed OGSD and the control group. However, there were histological changes in the intestinal mucosa of rats fed with OGSD which showed increased villi and larger goblet cells. There were increased number of sperm cells and greater number of spermatogonia and spermatocytes in the testes of the animals fed with OGSD when compared to the control group.

Table 1: Qualitative phytochemical constituents of leaves of *Ocimum gratissimum*

Substances	Presence
Phenolic substances	+
Flavonoids	+
Anthocyanidins	+
Phytosterols	+
Tannins	+++
Saponins	++
Alkaloids	ND
Glycosides	++
Cyanogenic glycosides	+
Cardiac glycosides	++

+: Presence, ND: Not detected

Table 2: Effect of *Ocimum gratissimum*-supplemented diet (OGSD) on some biochemical parameters (n = 6)

Experimental groups (diet)	Weight (%)	Protein (mg mL ⁻¹)	Cholesterol (mM L ⁻¹)	Hemoglobin (g dL ⁻¹)	WBC (10 ³ mm ⁻³)	Lipid peroxidation (mg mL ⁻¹)
Control diet (CD)	60.0±7.28	261±16.4	1.43±0.18	9.8±0.50	5.13±0.29	5.03±0.23
<i>Ocimum gratissimum</i> -Supplemented Diet (OGSD)	63.9±3.33*	235±22.1*	0.70±0.08*	9.4±0.70*	9.10±0.54*	3.80±0.20*

* $p < 0.05$

Table 3: Effect of *Ocimum gratissimum*-supplemented diet (OGSD) on some enzyme levels (n = 6)

Experimental groups (diet)	GST (IU L ⁻¹)	SOD (U mg ⁻¹ protein)	AST (IU L ⁻¹)	ALT (IU L ⁻¹)	ALP (IU L ⁻¹)
Control diet (CD)	5.41±0.82	70.7±3.68	88.7±7.41	45.3±5.3	88.6±8.96
<i>Ocimum gratissimum</i> -Supplemented Diet (OGSD)	7.03±1.37	104±11.7*	85.0±16.3	42.7±9.8	93.7±3.86

* $p < 0.05$

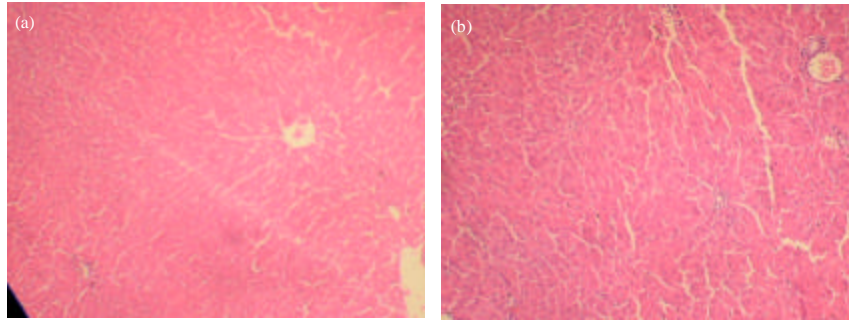


Fig. 1: (a) Photo micrography of a cross section of liver of rats fed with Control Diet (CD). MAG. x 40 and (b) photo micrography of a cross section of liver of rats fed with *Ocimum gratissimum*-Supplemented Diet (OGSD). MAG. x 40

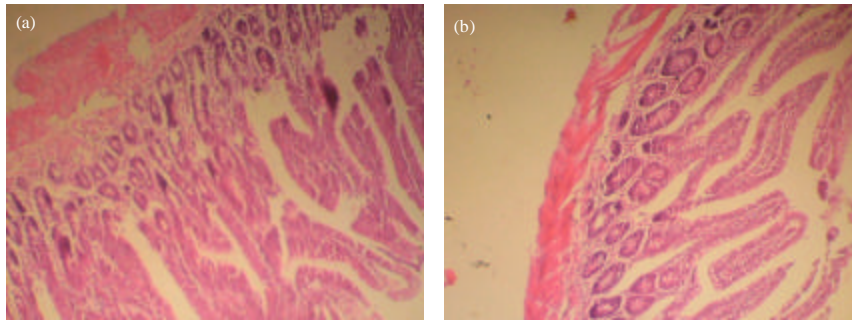


Fig. 2: (a) Photo micrography of a cross section of intestine of rats fed with Control Diet (CD). MAG. x 40 and (b) photo micrography of a cross section of intestine of rats fed with *Ocimum gratissimum*-Supplemented Diet (OGSD). MAG. x 40

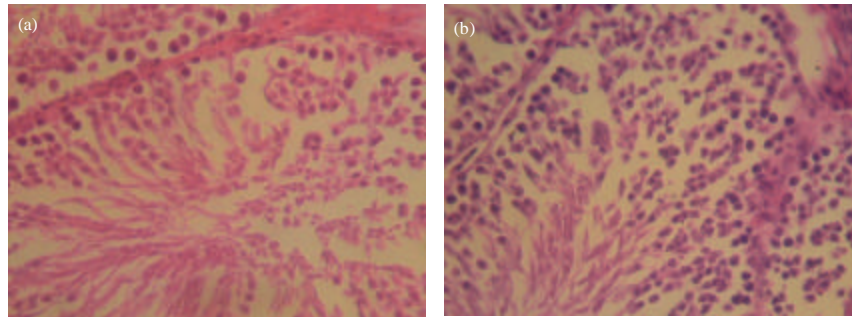


Fig. 3: (a) Photo micrography of a cross section of testes of rats fed with Control Diet (CD). MAG. x 8 and (b) photo micrography of a cross section of testes of rats fed with *Ocimum gratissimum*-Supplemented Diet (OGSD). MAG. x 8

DISCUSSION

The long term consumption of an *Ocimum gratissimum*-Supplemented Diet (OGSD) by male albino rats had some significant effects on the animal's biochemistry and histology as seen in the results obtained from this study. It was observed that OGSD resulted in increased weight gain in the animals which is attributed to the nutrient composition of leaves of *Ocimum gratissimum*. *Ocimum gratissimum* elaborates nutrients that can increase weight such as carbohydrates, lipids, proteins mineral and vitamins (Edeoga *et al.*, 2006). The reduction in lipid peroxidation and increase in superoxide dismutase in animals fed OGSD agrees with the report of Balanehru and Nagarajan (1991), which attributed the effect to oleanolic acid and ursolic acid present in the plant species. Flavonoids and phenolic compounds such as cirsilineol, cirsimaritin, isothymusin, isothymonin, apigenin and rosmarinic acid which are present in the leaves of *O. gratissimum* and are known to exhibit antioxidant activities as well as scavenge superoxide radicals (Kelm *et al.*, 2000). A reduction in lipid peroxidation could be a consequence of the increased SOD activity in the animals fed OGSD which protects cells from the damaging effects of reactive oxygen species (Petkau *et al.*, 1975). This agrees with studies on other commonly consumed tropical plant foods (Iweala and Obidoa, 2009a, b). Lipid peroxidation is implicated in several disease conditions such as cancer. The reduction in lipid peroxidation by consumption of OGSD could provide explanation for the use of *O. gratissimum* in folklore treatment of various diseases. The consumption of *O. gratissimum* significantly reduced the total serum protein. Most plant foods have low amounts of protein which may not even be available during digestion and absorption in the intestines to contribute to serum protein levels (Mgbodile and Campbell, 1972). The reduction in cholesterol by OGSD is in consonance with consumption of plant foods whose non nutrient components have cholesterol-reducing effects (Hassel, 1998; Iweala *et al.*, 2009). Flavonoids which are found in *O. gratissimum* are known to reduce cholesterol (Anderson *et al.*, 1998). This observed effect on cholesterol level tallies with the report by Sarkar *et al.* (1994) and Rai *et al.* (1997) which showed that the administration of *O. gratissimum* reduced serum total cholesterol. Reduction in plasma cholesterol reduces the risk of cardiac-related diseases (Amrani *et al.*, 2006). Hemoglobin levels were significantly reduced in animals fed OGSD. This result concurs with the effect on haemoglobin level obtained with administration of extracts of *O. gratissimum* (Jimoh *et al.*, 2008; Ephraim *et al.*, 2000). The reduction in haemoglobin could be attributed to the absence of haematinic nutrients and also presence of saponins in the leaves of *O. gratissimum* which are toxic to body tissues (Edeoga *et al.*, 2006; Alada, 2000; Watt and Breyer-Brandwijk, 1962). This observation is at variance with the ingestion of a decoction of leaves of *O. gratissimum* as blood tonic in some parts of Nigeria which could be attributed to the inclusion of haematinic nutrients from other sources. The increase in WBC by OGSD is due to its content of anti oxidant phytochemicals such as flavonoids that are known to modulate immune function by protecting lymphocytes (Duthie *et al.*, 1996). The consumption of OGSD did not significantly affect the liver enzymes namely ALT, AST and ALP which are released into the serum during liver damage or toxicity (Edwards *et al.*, 1995). This result shows that *O. gratissimum* may not be toxic to the liver. Some reports have indicated *Ocimum gratissimum* possesses hepatoprotective effects (George and Chaturvedi, 2008; Chaturvedi *et al.*, 2007). These hepatoprotective effects of *O. gratissimum* may be related to the form in which it is administered. Also, GST enzyme was not significantly increased by the consumption of OGSD. However, GST enzyme and other detoxification enzymes have been found to be increased by the alcoholic extracts of leaves of *O. gratissimum* (Banerjee *et al.*,

1996; Aruna and Sivaramakrishnan, 1990). This increased activation of GST is attributed to the presence of bifunctional inducers of phase I and II enzymes possibly extracted by the alcoholic solvent of leaf extract of *O. gratissimum* (Kusamran *et al.*, 1998). The consumption of *Ocimum gratissimum*-Supplemented Diet (OGSD) influenced some changes in the histology of the intestines and testes of the rats. The histological changes observed on the intestines which showed presence of large goblet cells agrees with the observation of Ephraim *et al.* (2003). This observation is related to increased production of lubricating substances such as mucus in the intestines which can promote bowel movement. Bowel movement is important in relieving constipation and even removal of toxins by *O. gratissimum* (Odugbemi and Akinsulire, 2006). In Nigeria, leaves of *O. gratissimum* are commonly used in the preparation of soups consumed to relieve the symptoms of constipation. The absence of any apparent changes in the liver confirmed the earlier observation that leaves of *Ocimum gratissimum* are not toxic to the liver when consumed for a long time. Generally, some tropical vegetables are known to prevent hepatotoxicity in animals (Oboh, 2006). The OGSD consumption impacted a distinct effect on the histology of the testes which showed an increased synthesis of sperm cells and spermatozoa. An increase in spermatogenesis is related to the possibility of increased reproductive capacity in the male rats fed with OGSD. *Ocimum gratissimum* has been shown to affect sexual behavioral score in animals (Kantak and Gogate, 1992).

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