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Research Article

EXTRACTS OF *OCIMUM GRATISSIMUM* LEAVES INHIBITS FE^{2+} AND SODIUM NITROPRUSSIDE INDUCED OXIDATIVE STRESS IN RAT LIVER

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ABSTRACT

This study investigate the antioxidative properties and the ability of aqueous, ethanol and ethyl acetate extracts from *Ocimum gratissimum* (OG) leaves to inhibit some pro-oxidants (Fe^{2+} and sodium nitroprusside) induced lipid peroxidation in rat's liver homogenates *in vitro*. The ability of the extracts to inhibit 25 µM $FeSO_4$ and 7.0 µM sodium nitroprusside induced lipid peroxidation in isolated rat's liver was determined. The results of the study revealed that both pro-oxidants caused a significantly decrease in (p<0.05) accumulation of lipid peroxides. However, aqueous extract of *Ocimum gratissimum* shows a high ability to inhibit lipid production in the liver induced with SNP than Fe^{2+}. Ethanol and ethyl acetate extract of *Ocimum gratissimum* which shows a high ability to inhibit lipid production more when induced with Fe^{2+} than SNP. However, ethyl acetate fraction of *Ocimum gratissimum* shows a higher inhibitory effect on both Fe^{2+} and SNP induced lipid peroxidation in rat's liver. This might be as a result of its significantly higher extractable phytochemicals. Therefore, Fe II and sodium nitroprusside induced oxidative stress could be managed by dietary intake of *Ocimum gratissimum* leaves.

KEYWORDS: *Ocimum gratissimum,* lipid peroxidation, Sodium nitroprusside, Fe^{2+}, antioxidant

INTRODUCTION

Oxidative stress occurs when there is an imbalance between producing reactive oxygen and the biological ability to detoxify the reactive intermediate or easily repair the resulting damage[1]. All forms of life preserve a reducing environment within their cells by enzymes through a constant input of metabolic energy. Disturbances in this normal redox state could lead to producing peroxides and free radicals that damage all compounds of the cell, including proteins, Lipids and DNA[2]. Many *in vitro* and *in vivo* studies reported that polyphenol compounds protect against oxidative stress[3,4]. Some of these medicinal plants used in ethno medicine for the treatment or management of many of these diseases examined for their anti-oxidative properties[5,6]. Many of the metabolites from these medicinal plants especially flavonoids showed potent antioxidant activity *in vitro* and *in vivo*[7,8,9]. Most of the free radical scavenging potential in herbs and spices is because of the redox properties of phenolic compounds which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers[10, 11]. The importance of free radicals and reactive oxygen species (ROS) attracted increasing attention. However, reactive oxygen species and free radical mediated reactions involved in degenerative or pathological processes as aging, cancer, rheumatoid arthritis, coronary heart disease and Alzheimer's disease[12]. Conversely, breakdown products of oxygen such as ROS can be detrimental to cell function and survival[13]. Reactive oxygen species are present as free radicals. Examples of ROS include the hydroxyl superoxide radical, hydrogen peroxide, peroxyl radical, and hypochlorite ion. These are the common forms of ROS that have been considered injurious to sperm survival and function when present in abundance. Although iron is necessary physiologically as components of many enzymes and proteins, free iron in the cytosol and mitochondria could cause considerable oxidative damage by acting catalytically in the production of ROS which have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrate resulting in wide ranging impairment in cellular function and integrity[14]. The mechanism by which iron can cause this deleterious effect is that Fe (II) can react with hydrogen peroxide (H_2O_2) to produce the hydroxyl radical (OH) via the Fenton reaction, whereas superoxide can react with iron (III) to regenerate iron (II) that can participate in the Fenton reaction[15]. The overproduction of ROS can lead to direct attack on the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation.

Sodium nitroprusside (SNP) is an anti-hypertensive drug, which acts by relaxing smooth vascular muscle and dilates peripheral arteries and veins. However, it could cause cytotoxicity through the release of cyanide ion and/or nitric oxide (NO). ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. Malondialdehyde (MDA) is the end product of lipid peroxidation, which is a process where reactive oxygen species degrade polyunsaturated fatty acids. MDA is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism[16].

Many antioxidants compounds, naturally occurring in plant sources identified on free radical or active oxygen scavenger. Many synthetic antioxidant components have shown toxic or mutagenic effects, which shifted the attention onto the naturally occurring antioxidants[17]. Its noted that an extraction solvent selected according to the purpose of extraction such as the nature of interested parts, reagents and equipment's, cost and

safety concerns and so on[18]. *Ocimum gratissimum* Linn (Labiatae) grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linalool extracted from the oil[19]. However, [20] reported that the extracts of *Ocimum gratissimum* has inhibitory effects against iron II and sodium nitroprusside in rat's brain. This study therefore sought to determine the inhibitory effects of extracts of *Ocimum gratissimum* on some hepatotoxin (Fe^{2+} and sodium nitroprusside) induced lipid peroxidation in rat's liver homogenates *in vitro*.

MATERIALS AND METHODS

Collection of plant

Fresh leaves of *O. gratissimum* bought in the market at Ado, Nigeria. The plant identified and authenticated by a plant scientist in the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. A voucher specimen deposited at the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. The yield calculated and the dry extract stored in a refrigerator at -4°C until use for the experiments.

Chemicals and reagents

Chemicals and reagents used such as 1,10-phenanthroline, gallic acid, Folin–Ciocalteau's reagent bought from Sigma–Aldrich, Inc., (St. Louis, MO), trichloroacetic acid (TCA) sourced from Sigma–Aldrich, Chemie GmbH (Steinheim, Germany), dinitrophenylhydrazine (DNPH) from ACROS Organics (New Jersey, USA), hydrogen peroxide, methanol, acetic acid and Fecl3were sourced from BDH Chemicals Ltd., (Poole, England), $CuSO_4 \cdot 5H_2O$, H_2SO_4, sodium carbonate, $AlCl_3$, potassium acetate, Tris–HCl buffer, sodium dodecyl sulphate, FeSO4, potassium ferricyanide and ferric chloride of analytical grade while the water glass distilled.

Preparation of extract

Aqueous extract preparation

The aqueous extract of the powered *Ocimum gratissimum* leaves were prepared using the method of[21]. The percentage yield of extraction calculated as 8 % percent yield.

Percentage yield = Weight of the dry extract / Weight of powdered leaves x 100%

Ethanol extract preparation

The ethanol extract of the powered *Ocimum gratissimum* leaves was prepared using the maceration method described by[22]. The percentage yield of extraction calculated as 12 % yield percent.

Percentage yield = Weight of the dry extract / Weight of powdered leaves x 100%

Ethyl Acetate preparation

The ethyl acetate extract of the powered *Ocimum gratissimum* leaves prepared using the method of[23]. The percentage yield of extraction calculated as 2.08 %.

Percentage yield = Weight of the dry extract / Weight of powdered leaves x 100%

Lipid Peroxidation Test

Preparation of Tissue Homogenates

The rats decapitated under halothane anaesthesia, and the liver rapidly dissected, placed on ice and weighed. This tissue later homogenized in cold saline (1/10 w/v) with about 10 up-and-down strokes about 1,200 rpm in a Teflon glass homogenizer. The homogenate centrifuged for 10 min at 3,000 x g to yield a pellet that discarded and a low-speed supernatant (S1) containing mainly water, proteins, lipids (cholesterol, galactolipid, individual phospholipids, gangliosides), DNA and RNA that kept for lipid peroxidation test[24]. The ethical committee of the Afe Babalola University approved this study. All animals in this study follow the international, national and institutional guidelines for Care and Use of Laboratory Animals as published by the[25].

Lipid Peroxidation and TBA reactions

The lipid peroxidation assay was carried out using the adjusted method of[26].

Statistical analysis

Statistical analysis of difference between groups evaluated by one-way ANOVA followed by student t test. The values $P < 0.05$ regarded as significant.

RESULTS

Lipid peroxidation in rat liver homogenate induced with iron and sodium nitroprusside and the effect of *O. gratissimum* ethanol extracts determined. *Ocimum gratissimum* significantly reduced ($p<0.05$) accumulation of lipid peroxides in a concentration dependent manner for iron and SNP. However, the plant offered greater protection against SNP (62.52%) induced lipid peroxidation compared to iron (54.90%) at the highest tested concentration of the extract (Figure 1). The highest inhibition observed with the 0.33 mg/ml concentration in the liver, with that of the Fe^{2+} being high. Meanwhile, 3.33 mg/ml produced the lowest inhibition in both. The inhibition increased in the 0.33 mg/ml in liver, while the 3.33 mg/ml concentration produced a decreased inhibition in both pro-oxidant used. 3.33 mg/ml has a reduced inhibitory effect against TBARS produced in liver.

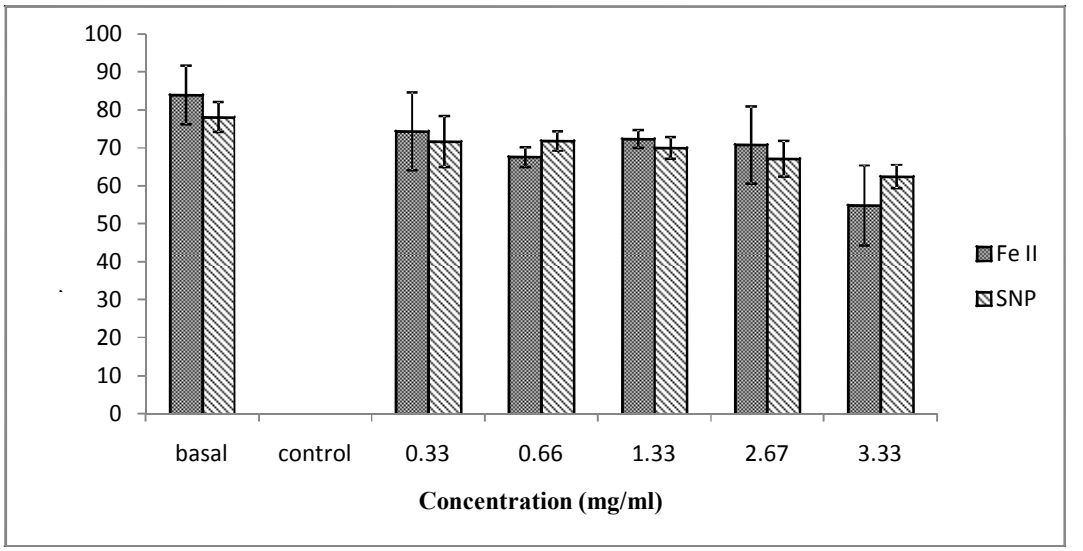

Figure 1: Effect of ethanol extract of *Ocimum gratissimum* on iron sulphate (Fe^{2+}) induced and Sodium nitroprusside (SNP) induced lipid peroxidation in liver homogenate. Each value represent mean ± SEM (n=3). *Basal – rats induced with extract but no pro-oxidant, *Control – rats induced with pro-oxidant but no extract.

Figure 2 shows inhibition of the aqueous extracts with Fe (II) and SNP induced lipid peroxidation in liver of rat. The highest inhibition was observed with the 1.33 mg/ml concentration in the rat liver, thus reflecting the highest inhibitory effect. Meanwhile, 3.33 mg/ml produced the lowest inhibition in both the Fe^{2+} and SNP.

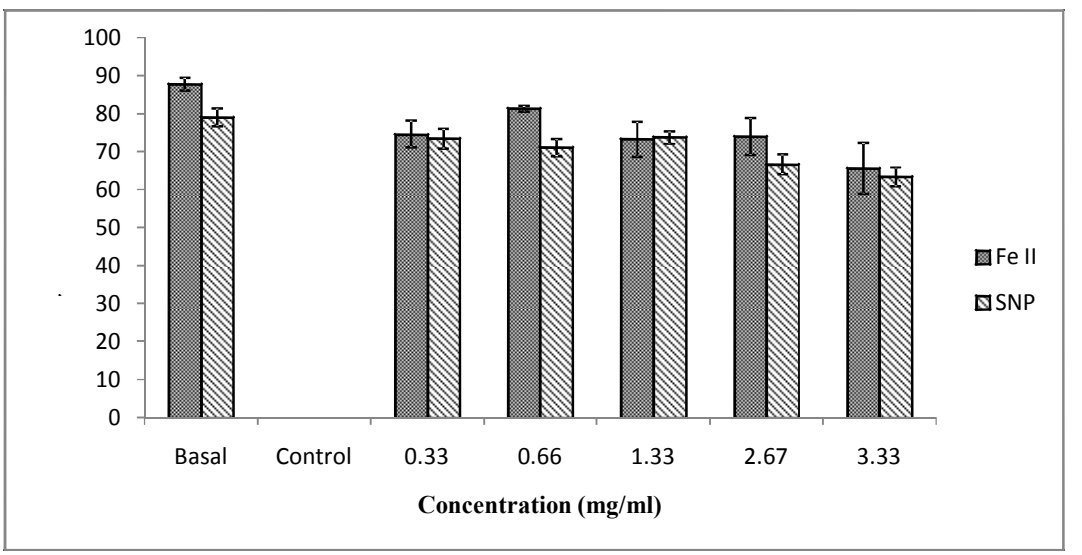

Figure 2: Inhibitory effect of aqueous extract of *Ocimum gratissimum* on iron sulphate (Fe^{2+}) induced and Sodium nitroprusside (SNP) induced lipid peroxidation in liver homogenate. Each value represent mean ± SEM (n=3). *Basal – rats induced with extract but no pro-oxidant, *Control – rats induced with pro-oxidant but no extract.

Figure 3 shows inhibition of the ethyl acetate extracts with Fe (II) and SNP induced lipid peroxidation in liver of rat. *Ocimum gratissimum* caused a significant inhibition ($p<0.05$) in Fe^{2+} induced lipid peroxidation in the liver in a concentration dependent manner (0.33-3.33 mg/ml). The highest inhibition observed to be 1.33 mg/ml concentrations in the rat liver.

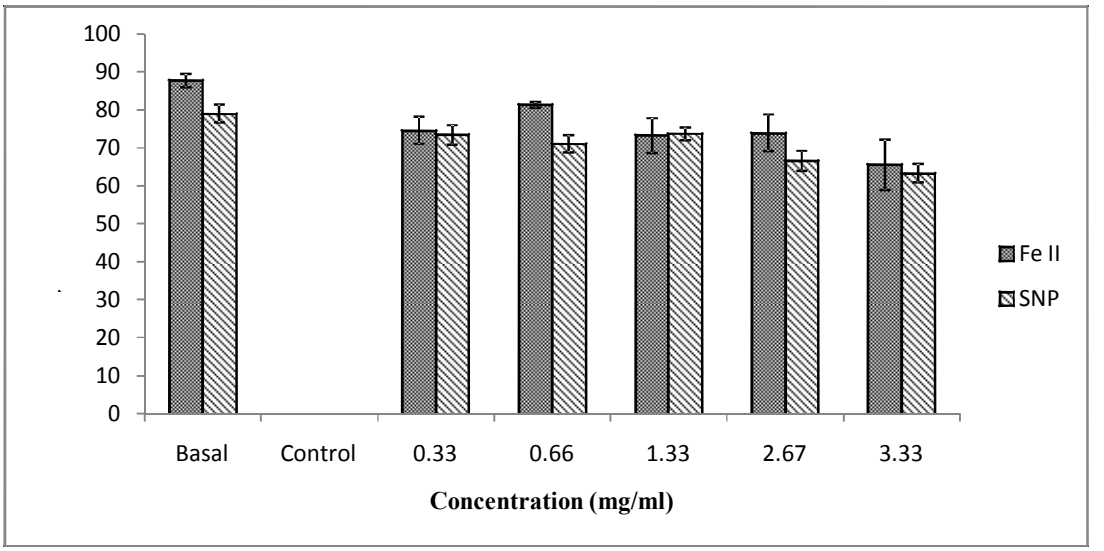

Figure 3: Inhibitory effect of ethyl acetate extract of *Ocimum gratissimum* on iron sulphate (Fe^{2+}) induced and Sodium nitroprusside (SNP) induced lipid peroxidation in liver homogenate. Each value represent mean ± SEM (n=3). *Basal – rats induced with extract but no pro-oxidant, *Control – rats induced with pro-oxidant but no extract.

DISCUSSION

Oxidative stress is associated with several diseases[27]. There also exists a correlation between thiobarbituric acid reactive species (TBARS) as a marker of lipid peroxidation and products that reflect oxidative damage to DNA. Iron is an essential element for normal cellular physiology but excess iron in the body cause cell injury. This is due to the catalytic role it plays in introduction of free radical reactions. However, radicals have been reported to damage cellular lipids, nucleic acid, protein and carbohydrates, resulting in wide-ranging injury to cellular role and integrity. The mechanism involves that Fe (II) can undergo the process of Fenton reaction through reacting with hydrogen peroxide (H_2O_2) to produce (OH)[28]. Iron overload results cellular damage in several tissue including liver and brain which are used in this research work. Storage of iron in the liver leads to liver cirrhosis. The possible mechanism of iron toxicity includes free radical-mediated peroxidation which is readily catalyzed by iron[29]. The protection offered by ethyl acetate extract of *O. gratissimum* suggests that they may be useful in the treatment of liver diseases resulting from this overload. The human body is equipped with antioxidant defense system that deactivates these reactive free radicals. Antioxidant enzymes made in the body and antioxidant nutrient (found in foods) soak up all the energy that these free radicals have, turning them into harmless particles that can be metabolized, so these antioxidants are functional components of that have extra health benefits in the body[30]. Phenols including flavoniods can protect the body cell against the damage caused by reactive oxygen species (ROS). Much of the potentials of *O. gratissimum* is associated with the phenolic content of this medicinal plant[31]. Hepatoprotective potentials of *O. gratissimum* was shown by the ability to chelate iron and protect against the formation of thiobarbituric reactive species (TBARS) in the liver. This tremendous activity may be because of the great phenolic content of this plant as suggested by[20, 31]. This study revealed that ethyl acetate extract of *O. gratissimum* leaf showed a high potency or effectiveness to prevent iron (II) sulfate induced lipid peroxidation in the liver *in vitro* (figure 3) compared to that of ethanol (figure 1) and aqueous extract (figure 2). However, judging by the IC_{50} (extract concentration causing 50% enzyme inhibition) values in figure 3, the plant (IC_{50} = 2.47 mg/ml) had a significantly ($P<0.05$) high inhibitory effect on Fe^{2+} induced lipid peroxidation in the liver homogenate which could be as result of the ability of the extracts to chelate Fe^{2+} and or scavenge radicals in the rat liver. The plant also showed a good iron chelating ability at all the concentration used. Incubation of the isolate liver homogenates in the presence of 7 µM sodium nitroprusside caused a significant decrease in ($p<0.05$) the accumulation of lipid peroxides in a concentration dependent manner for sodium nitroprusside in both the ethanol and ethyl acetate form. However, the aqueous extract of *O. gratissimum* leaf was more effective against sodium nitroprusside induced lipid peroxidation in the liver *in vitro* than Fe^{2+} induced TBARS (figure 2). The plant shows a high ability to inhibit TBARS production more in the liver induced with SNP than Fe^{2+}, as a higher potency ratio of their correlation observed (figure 2). Sodium nitroprusside cause damage to the organ through the release of cyanide which can acts alone or in conjunction with other reactive oxygen species[24, 32, 33]. Decomposition of sodium nitroprusside leads to the production of iron that could sustain lipid peroxidation, through initiating the production of OH radical through Fenton's reaction[30].

CONCLUSION

The aqueous, ethanol and ethyl acetate extracts of *O. gratissimum* were able to protect the liver against Fe (II) and sodium nitroprusside induced lipid peroxidation. However, the ethyl acetate extract of *O. gratissimum* had a higher protective effect. The higher protective effect may be because of their higher antioxidant potentials. These antioxidant properties of *O. gratissimum* may have contributed to its wild use in folk medicine.

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