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Research Article
Antioxidant Activities of African Basil (*Ocimum gratissimum*) and African Nutmeg (*Monodora myristica*) in Wistar Rat

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Abstract

Background and Objective: Globally, there is growing interest in plant based antioxidants since some synthetic products are reported as carcinogenic. This research was, therefore, designed to investigate *in vivo* antioxidant activities of African basil and African nutmeg aqueous extracts in Wistar rat. **Materials and Methods:** Fifty female Wistar rats were divided randomly into 10 groups (I-X) of five each. Rats in group I received only water, group II received 10 mg kg^{-1} b.wt., of carbon tetrachloride (CCl_4) and groups III and IV received 200 and 300 mg kg^{-1} b.wt., of *O. gratissimum*, respectively. Groups V and VI received CCl_4 plus 200 mg kg^{-1} b.wt., and CCl_4 plus 300 mg kg^{-1} b.wt., of *O. gratissimum*, respectively. Groups VII and VIII received 200 and 300 mg kg^{-1} b.wt., of *M. myristica*, respectively whereas, groups IX and X received CCl_4 plus 200 and CCl_4 plus 300 mg kg^{-1} b.wt., of *M. myristica*, respectively. Treatments were administered via oral gavage for 60 days. Animals were sacrificed and blood samples were collected for biochemical analysis (alanine transaminase, aspartate transaminase, alkaline phosphatase, albumin, total bilirubin and total protein). **Results:** Rats administered CCl_4 exhibited significant ($p<0.05$) elevation in all the analyzed parameters (except total protein and albumin) compared to the control. Aqueous extracts of *O. gratissimum* and *M. myristica* maintained liver enzymes within the control values, whereas each extract plus CCl_4 reduced liver enzymes compared to CCl_4 treated group. **Conclusion:** The findings of this study suggest that both extracts could offer protection against CCl_4-induced liver damage (oxidative stress) due to their antioxidant activities.

Key words: African basil, African nutmeg, carbon tetrachloride, antioxidants, biochemical analysis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules. They protect cells from impairments caused by unstable molecules known as free radicals[1]. Antioxidants terminate these chain reactions by removing intermediates of free radicals and inhibiting other oxidation reactions by being oxidized themselves[2]. Free radicals are fundamental to any biochemical process and represent an essential part of metabolism. Majority of the degenerative diseases are linked with oxidative stress[3]. However, there is growing interest in plant based antioxidants since some of the synthetic products are reported to be carcinogenic. Some medicinal plants are reliable sources of natural antioxidants which can protect against oxidative stress and related degenerative diseases[4].

Ocimum gratissimum and *Monodora myristica* are medicinal plants widely consumed in Nigeria. *Ocimum gratissimum* is a perennial herb commonly called African basil or 'scent leaf' which belongs to the family of plants known as Lamiaceae[5]. Phytochemical screening of leaves of African basil revealed the presence of alkaloids, saponins, tannins, alkaloids, anthraquinone, flavonoids, steroids, terpenoids and glycosides[6-8]. In traditional medicine, this herb is effective in the management of upper respiratory tract infection, diarrhoea, headache, skin disease, pneumonia, fever and conjunctivitis[9]. On the other hand, *M. myristica* commonly known as African nutmeg is an edible perennial plant which belongs to the Annonaceae family and thrives in evergreen forests of Africa. This herb has a characteristic pleasant fragrance, slightly warm taste and often used to flavor meals. Traditionally, it is used to treat fever, headache, arthritis, eye infection, diabetes mellitus, stomach ache, rheumatism and vomiting[10,11].

Research efforts are being tailored towards the discovery of plants with potent antioxidant activities which could be used as food or introduced into foods as remedy for degenerative diseases[12]. This study was therefore, designed to investigate *in vivo* antioxidant activities of African basil and African nutmeg.

MATERIALS AND METHODS

Time duration of experiment: This study was carried out between August-November, 2019.

Experimental location: This study was conducted at the Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.

Collection and preparation of plant materials: Fresh leaves of *O. gratissimum* and seeds of *M. myristica* were purchased from Marian market, Calabar. They were identified and authenticated at the Department of Botany, University of Calabar, Nigeria. Both plant materials were washed, air-dried and then pulverized with an electric blender (Model 4250 Braun, Germany). About 250 g of each pulverized plant material was soaked in 1 L of distilled water. The mixture was shaken and allowed to stand for 24 h before filtering with Whatman filter paper. The filtrate was evaporated at 60°C using an oven. Appropriate weights of the obtained residue were prepared in distilled water for various concentrations which were stored at 4 until required for use. Each herb was prepared separately following this procedure, except that there was no combination of both herbs.

Experimental animals: Fifty healthy mature female Wistar rats with body weight between 180 and 200 g were purchased from the Department of Biochemistry, University of Calabar, Calabar, Nigeria. The animals were housed in aluminum cages covered with wire mesh and maintained under standard laboratory conditions. They were fed with growers mash daily and allowed unrestricted access to clean water. Animals were handled in accordance with the Helsinki declaration and guiding principles for animal care and use. Department of Research Ethics Committee approved all procedures and protocols (Reference number SC/012/2018-19/005).

Experimental procedure: After a week of acclimatization, the animals were divided into 10 groups (I-X) of five each in a completely randomized design. Rats in group I were given water only and group II received 10 mg kg^{-1} b.wt., of carbon tetrachloride (CCl_4), groups III and IV received 200 and 300 mg kg^{-1} b.wt., of *O. gratissimum,* respectively, groups V and VI received CCl_4 plus 200 mg kg^{-1} b.wt., and CCl_4 plus 300 mg kg^{-1} b.wt., of *O. gratissimum,* respectively, groups VII and VIII received 200 and 300 mg kg^{-1} b.wt., of *M. myristica,* respectively, whereas groups IX and X received CCl_4 plus 200 and CCl_4 plus 300 mg kg^{-1} b.wt., of *M. myristica*, respectively. Treatments were administered via oral gavage for 60 days and the animals were sacrificed under chloroform anesthesia 24 h after the last dose.

Biochemical analysis: Blood samples were collected from the animals by cardiac puncture into test tubes without anticoagulant and centrifuged at 3000 rpm for 10 min. Serum was then collected into plain bottles for biochemical

analysis. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed using the method of Reitman and Frankel[13]. Alkaline Phosphatase (ALP) activity was determined by Rec. GSCC method[14]. Total Protein (TP) was determined by using the Biuret method as reported by Haussament[15]. Albumin was determined according to the method of Peter *et al.*[16]. Total Bilirubin (TB) was evaluated according to the method of Doumas *et al.*[17].

Statistical analysis: The data obtained were subjected to one-way Analysis of Variance (ANOVA) using Predictive Analysis Software (PASW) version 18.0. While, Least Significant Difference (LSD) was used to separate means significant at $p<0.05$.

RESULTS

Results for serum biochemical analysis of rats administered aqueous extracts of *O. gratissimum* and *M. myristica* are presented in Table 1. The results revealed that carbon tetrachloride (CCl_4) significantly ($p<0.05$) increased alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and total bilirubin, while total protein and albumin reduced significantly ($p<0.05$) compared to the control group. Furthermore, there was no significant ($p>0.05$) difference between groups administered aqueous extracts of *O. gratissimum* and *M. myristica* and the control for the analyzed biochemical parameters, except for aspartate aminotransferase (47.47 U L^{-1}) which was significantly ($p<0.05$) lower in the group that received 300 mg kg^{-1} b.wt., of *M. myristica*. It was also observed that administration of each aqueous plant extract plus CCl_4 lowered alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and total bilirubin and increased total protein and albumin significantly ($p<0.05$) compared to the group that received only CCl_4.

DISCUSSION

Serum biochemical analysis provides important information on organ damage in animals, particularly the liver and kidneys[18-20]. The common enzymes employed as indicators of hepatocellular damage are alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Research efforts are presently being tailored towards the discovery of plants with potent antioxidant potentials which could be used as remedy for degenerative diseases. This study was, therefore, designed to investigate *in vivo* antioxidant activities of African basil and African nutmeg.

The findings of this study showed that serum activities of ALP, ALT and AST increased significantly ($p<0.05$) in CCl_4 treated group compared to the control group. This implicated that CCl_4 as a hepatotoxic substance that is capable of being deleterious or lethal to hepatocytes. The AST and ALT are known to be involved in amino acids catabolism and biosynthesis, while ALP catalyzes the hydrolysis of a wide variety of phosphoric acid esters in alkaline medium[21]. So, increase in the activities of these enzymes is proportional to the extent of hepatic damage[22]. Interestingly, aqueous extracts of *O. gratissimum* and *M. myristica* significantly ($p<0.05$) decreased ALP, ALT and AST activities when administered simultaneously with CCl_4. The decrease in serum ALP, ALT and AST suggested that both extracts confer protection against liver/kidney tissues injury, damage or disease, which is often the direct cause of elevation of these enzymes in the blood stream[23]. These findings are consistent with the reports of Hristev *et al.*[24], Obianime *et al.*[25] and Nivetha and Prasanna[26].

The findings of this study also showed that Total Bilirubin (TB) increased significantly ($p<0.05$) in the group administered CCl_4 compared to the control group. The

Table 1: Effects of CCl_4, *O. gratissimum* and *M. myristica* on biochemical indices of Wistar rats

	Groups									
Parameters	I	II	III	IV	V	VI	VII	VIII	IX	X
ALP (U L^{-1})	123.30±1.1^{c}	202.7±1.0^{a}	128.5±1.3^{c}	116.7±1.1^{c}	175.9±0.8^{b}	174.8±0.3^{b}	118.7±0.9^{c}	126.9±0.6^{c}	168.1±1.4^{b}	163.7±1.1^{b}
AST (U L^{-1})	48.20±0.3^{c}	57.33±0.7^{a}	46.38±0.4^{c}	47.93±0.5^{c}	50.38±0.8^{b}	48.13±5.2^{c}	48.10±0.5^{c}	47.47±0.6^{d}	52.85±0.5^{b}	49.82±0.9^{b}
ALT (U L^{-1})	38.87±0.4^{c}	44.84±0.6^{a}	39.12±0.6^{c}	38.94±0.7^{c}	41.51±1.2^{b}	40.13±0.8^{b}	39.26±0.6^{c}	39.08±0.8^{c}	42.15±0.9^{b}	40.38±0.9^{b}
TB (mg dL^{-1})	11.67±0.8^{c}	19.08±0.7^{a}	10.80±0.9^{c}	10.20±1.5^{c}	14.61±1.5^{b}	13.93±0.5^{b}	9.93±0.6^{c}	10.75±1.1^{c}	13.26±0.9^{b}	15.27±0.7^{b}
TP (g L^{-1})	75.25±0.8^{a}	54.23±0.6^{d}	75.68±0.9^{a}	76.93±1.3^{a}	65.55±0.9^{b}	67.13±0.9^{b}	75.52±0.4^{a}	73.63±1.1^{a}	62.23±0.8^{c}	62.33±1.4^{c}
Albumin (g L^{-1})	37.75±0.7^{a}	28.68±0.9^{c}	36.90±0.8^{a}	37.55±0.9^{a}	31.90±0.7^{b}	31.18±0.7^{b}	36.40±0.7^{a}	36.73±1.2^{a}	31.95±1.2^{b}	33.05±1.1^{b}

Values are presented as Mean±SEM, Means followed by the same case letter along the horizontal array indicate no significant difference ($p>0.05$), Group I served as control and received only water, Group II received 1 mL kg^{-1} b.wt., of carbon tetrachloride (CCl_4), Group III received 200 mg kg^{-1} b.wt., of *O. gratissimum*, Group IV received 300 mg kg^{-1} b.wt., of *O. gratissimum,* Group V received CCl_4 plus 200 mg kg^{-1} b.wt., *O. gratissimum*, Group VI received CCl_4 plus 300 mg kg^{-1} b.wt., of *O. gratissimum*, Group VII received 200 mg kg^{-1} b.wt., *M. myristica*, Group VIII received 300 mg kg^{-1} b.wt., of *M. myristica*, Group IX received CCl_4 plus 200 mg kg^{-1} b.wt., *M. myristica*, Group X received CCl_4 plus 300 mg kg^{-1} b.wt., of *M. myristica*, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, TP: Total protein, TB: Total bilirubin

concentration of albumin and bilirubin in serum indicated the secretory and synthetic roles of the liver and could be used to ascertain the occurrence of liver damage[27]. Bilirubin is the main product that results from the destruction of old red blood cells. It is removed from the blood by the liver and thus, is a good indicator of the functional state of this organ. Bilirubin concentration is elevated in the blood either by increased production of bilirubin or decreased liver uptake as a result of liver disease. Egesie *et al.*[28] reported that an increase in the concentration of serum bilirubin suggested the occurrence of liver damage since, it serves as an excretory unit rather than a distributing unit. However, administration of CCl_4 plus *O. gratissimum* or *M. myristica* extract lowered TB significantly ($p<0.05$) compared to the group that received CCl_4 alone, thus demonstrated the ameliorating role of the plant extracts.

Furthermore, this study revealed the significant reduction of albumin in CCl_4 treated group compared to the control. Administration of CCl_4 plus each plant extract elevated albumin level significantly compared to the group that received only CCl_4. Albumin level represents a reliable test to assess the degree of liver damage in animals[29]. Similarly, there was a significant ($p<0.05$) decrease in total protein level in the group treated with CCl_4 compared to the control. This decline or reduction may be due to CCl_4-induced glomerular damage leading to proteinuria[30]. However, CCl_4 plus each plant extract increased total protein significantly ($p<0.05$) compared to the group treated with only CCl_4. This elevation could be due to the protective ability of both herbs against oxidative damage. This study, therefore, demonstrates that CCl_4 induced oxidative stress in Wistar rat and administration of *O. gratissimum* and *M. myristica* extracts offered protection against or ameliorated CCl_4-induced liver damage and oxidative stress.

CONCLUSION

This study demonstrated that administration of CCl_4 can alter the activity of some biochemical indices in Wistar rats. This is evident upon the significant increase in total bilirubin, ALP, ALT and AST concentrations, decrease in albumin and total protein levels. The findings of this research also suggest that *O. gratissimum* and *M. myristica* could protect against or ameliorate CCl_4-induced oxidative damage due to their antioxidant activities. Further research should be carried out to investigate the effects of these herbs on other physio-pathological conditions.

SIGNIFICANCE STATEMENT

This study unveiled the *in vivo* antioxidant activities of *O. gratissimum* and *M. myristica*, suggesting that the herbs could protect against or ameliorate CCl_4-induced oxidative damage. This study will assist researchers to uncover the critical areas of antioxidant activity assessment of herbs that others studies were not able to explore.

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