Journal of Complementary and Alternative Medical Research

10(2): 8-16, 2020; Article no.JOCAMR.60337
ISSN: 2456-6276

Evaluation of Antioxidant Potentials of *Ocimum gratissimum* and *Xylopia aethiopica* in Alcohol-Induced Hepatotoxic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ESB designed the study, performed the statistical analysis. Author NCCO managed the analyses of the study and wrote the draft of the manuscript. Author EON managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2020/v10i230158
Editor(s):
(1) Dr. Francisco Cruz-Sosa, Metropolitan Autonomous University Iztapalapa Campus, México.
Reviewers:
(1) Someshwar Dattatraya Mankar, Pravara Rural College of Pharmacy, India.
(2) Erisnaldo Francisco Reis, Centro Universitário Faveni, O. Centro Educacional Dom Alberto, Brazil.
(3) Shalini Purwar, Banda University of Agriculture and Technology, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60337

Original Research Article

Received 08 June 2020
Accepted 13 August 2020
Published 27 August 2020

ABSTRACT

Aims: This study evaluated the antioxidant potentials of *Ocimum gratissimum* and *Xylopia aethiopica* on Alcohol-induced Hepatotoxicity in Albino rats.
Study Design: The study is an experimental case-controlled study.
Place and Duration of Study: This study was conducted at the Department of Physiology, University of Port-Harcourt, Nigeria.
Methodology: Fifty-five (55) healthy adult male albino rats with an average weight of about 150-200 g were used for the study. They were divided into 11 groups of 5 Rats each and subjected to different treatments of the aforementioned herbs. All the animals received humane treatments according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health. Fresh leaves of *Ocimum gratissimum* and *Xylopia aethiopica* were bought from the Mile 3 market in Port-Harcourt. The herbs were separately extracted using maceration method. Absolute ethanol at a volume of 1 ml/kg (0.79 g/kg) was used to induce

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hepatotoxicity in rats. At the end of the treatment period, rats in all the groups were anaesthetized with chloroform and blood samples collected by jugular puncture. Total antioxidant capacity was analyzed using colorimetric method, whilst, malondialdehyde levels were determined using ELISA method. SPSS version 22.0 was used to analyse data generated and p values less than 0.05 were considered significant.

Results: Results show that intraperitoneal injection of 1 ml/kg ethanol significantly raised plasma MDA levels and significantly decreased TAOC in induced rats. Treatment with 200, 400 and 600 mg/kg b.wt. of aqueous extract of *Ocimum gratissimum* and *Xylopia aethiopica* however effectively reduced ethanol induced raised activity of the MDA levels and increased the activity of TAOC in the rats, but the effects were not dose dependent and a combination of both herbs did not produce better therapeutic effect.

Conclusion: Based on our findings, we conclude that *Ocimum gratissimum* and *Xylopia aethiopica* used separately could reduce oxidative stress in alcohol induced hepatotoxic rats.

Keywords: Ocimum gratissimu; Xylopia aethiopica; alcohol-induced; hepatotoxicity; rats.

1. INTRODUCTION

Over the past several years and in recent times, there has been an upsurge in alcohol ingestion amongst the young and elderly, and males and females, which could be due to social, environmental, biological and/or psychological factors. This upsurge in alcohol ingestion is however said to be linked to alcohol-related disorders, which increasingly causes morbidity and mortality all over the world [1].

Alcohol induces hepatotoxicity through the production of excess amount of reactive oxygen species (ROS) and free radicals, and impairs antioxidant defence mechanisms, resulting in oxidative stress (Wu and [2]. During the metabolism of ethanol, free radicals such as ethyl and 1- hydroxyethyl radicals are generated [3] by microsomal monooxygenase system, and also by the type of cytochrome P-450 system which can be induced by ethanol known as CYP2E1 [4]. The generated free radicals then initiate lipid peroxidation, which in turn, destroys cellular membrane, causing lysomal enzyme to leak out of the hepatocytes, and thus leading to autolysis [5].

Ingestion of large amounts of ethanol for a long period of time produces fatty liver, hepatomegaly, alcoholic hepatitis, fibrosis, and cirrhosis [6]. Approximately eighty percent of heavy drinkers develop steatosis, ten to thirty-five percent develop alcoholic hepatitis, and ten percent develop liver cirrhosis [7]. In animals treated with alcohol, histological examination of hepatocytes revealed large number of cytoplasmic vacuoles, nuclei showing pyknosis, and infiltration by lymphocytes [8].

Ocimum gratissimum and *Xylopia Aethiopica* are two known traditionally used herbal plants for the treatment of various diseases and health conditions. *Ocimum gratissimum* is a perennial herb which belongs to the Lamiaceae family, and widely distributed in tropical areas especially India and also in West Africa [9]. It is widely distributed across Nigeria and some other parts of the world [10]. Several native names have been given to it in India such as Vriddhutulsi, Ram tulsi and Nimma tulasi [11]. In Nigeria, the Edo people named it ebamwonkho, the Igbo people named it nchu-anwu, and the Yoruba people named it effinrin-nla, while the Hausas named it Dai doya togida. Due to the aromatic flavour of this vegetable, it is commonly referred to as scent-leaf, and is served as a source of condiment and stimulation in soup by the eastern Nigerians (Igbos) [12]. It is also widely used in Ayurvedic preparations. [13] stated that the major constituents of *Ocimum gratissimum* are aromatic and volatile oil, linolenic acid, oleic acid, alkaloid, flavonoid and saponin, while [14,15,16] stated that through the phytochemical screening of the leaf extract of *Ocimum gratissimum*, the plant was found to contain alkaloids, saponins, tannins, anthraquinone, flavonoids, steroids, terpenoids and cardiac glycosides.

Ocimum gratissimum is used in folk medicine for the treatment of several health conditions such as diabetes, bacterial infections, tumor, cancer, plasmodiasis, helminthiasis, fever, epilepsy, pneumonia, bronchitis, infection of the upper respiratory tract, diarrhoea, conjunctivitis, headache and dermatitis [17] and methotrexate-associated nephrotoxicity [18]. *Ocimum gratissimum* is also used in the amelioration of alcohol-induced hepatotoxicity due to its rich content of polyphenolic compounds with

antioxidant properties, and thus, has the ability to scavenge the free radicals and reactive oxygen species (ROS) which have been generated from alcohol toxicity.

Xylopia *aethiopica* also called Ethiopian pepper, negro pepper, African pepper, Guinea pepper and spice tree, is an angiosperm which belongs to the family Annonaceae and widely distributed in the evergreen rain forests of tropical and subtropical Africa especially in Ghana, Zambia, Mozambique and Angola [19]. The generic name Xylopia originated from the Greek words “xylon pikron” , meaning "bitter wood", while the specie name aethiopica, refers to its origin, Ethiopia, but presently grows most commonly as a crop in Ghana. It has several native names such as, “Uda” in the Eastern part of Nigeria (Igbos), “sesedu” in the Western part of Nigeria (Yorubas), and “kimba” in the Nothern part of Nigeria (Hausas). It matures as a slim, tall tree of approximately 60 cm in diameter and up to 30 m high with a straight stem having a slightly stripped or smooth bark [20], and bears characteristic aromatic fruits, which are slightly curved, slender encapsulated seeds with about fifteen carpels which are arranged in capitula to form bouquets of twelve to twenty bacciferous-like capsules. Its strongly peppery seeds and carpels are used as spices or condiments [21].

Xylopia aethiopica contains pytochemicals, some of which include resins, annonacin, reberoside, avicien, rebersole, alkaloids, tannin, oxalate [22] however stated that past reports on the phytochemical composition of the fruit of *Xylopia aethiopica* revealed the presence of alkaloids, polyphenols, terpenoids and kauranes (a class of diterpenes, namely kaurenoic and xylopic acid).

In folk medicine, *Xylopia aethiopica* is used for the treatment of several health conditions; almost all parts of the plant (bark, fruits, leaves, etc) possess great medicinal value. It is used to treat sores, boils, cough, wounds and cuts, among others. The fruit extract possesses antimicrobial, antifungal, anagelsic, hypoglycemic, anthelmintic, haematopoietic, immune boosting activities, and increases lutenizing hormone and testosterone levels in rats [23]. That fruits of the plant are also used as an abortefacient. It is also added as a stimulant to several other herbal preparations in traditional medicine for the treatment of stomach ache, bronchitis, dysentery, neuralgia and biliousness. Xylopia *aethiopica* can also be used to treat alcohol-induced hepatotoxicity due to its antioxidant property. Its extract has been reported to possess great antioxidant activity [24], thus has the ability to ameliorate the effects of alcohol-induced hepatotoxicity by scavenging the free radicals and reactive oxygen species (ROS) generated from the alcohol toxicity.

Because the liver plays a central role in metabolizing the alcohol, it stands the risk of being the primary target organ for alcohol toxicity; it does this by generating free radicals and reactive oxygen species, and depletes the antioxidant system invivo [25], leading to oxidative stress and thus lipid peroxidation in the liver with the resultant effect of either fatty liver, hepatomegaly, alcoholic hepatitis, fibrosis, or cirrhosis. Therefore, the aim of this study was to evaluate the antioxidant potentials of *Ocimum gratissimum* and *Xylopia aethiopica* on Alcohol-induced Hepatotoxicity in Albino rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Fifty-five (55) healthy adult male albino rats with an average weight of about 150-200 g were purchased from the animal house of the Department of Physiology, University of Port-Harcourt, Nigeria, and used for the study. The rats were divided into 11 groups of 5 rats each; and each group housed in different metallic cages, and fed with standard laboratory chow and water *ad libitum*. They were kept and maintained in a well-ventilated room at a temperature of 25±2°C with 12-hour daylight and 12-hour night light cycles and allowed to acclimatize for two weeks.

2.2 Collection and Identification of Plant Materials

Fresh leaves of *Ocimum gratissimum* and *Xylopia aethiopica* were bought from the Mile 3 market in Port-Harcourt, Rivers State, Nigeria. The plants were identified and authenticated in the Department of Agricultural Science, University of Port-Harcourt, Nigeria.

2.2.1 Aqueous preparation of the plants extracts of *Ocimum gratissimum* and *Xylopia aethiopica*

The leaves were washed in running tap water, air-dried for 14 days, and pulverized using a laboratory mechanical grinder. 50 g powdered material of each plant was macerated in 500 mls distilled water for 48 hours with occasional

shaking to facilitate extraction. The extracts were filtered using Whatman (No. 1) paper, and were evaporated to dryness using rotary evaporator. The extract for the rat treatment is weighed and prepared on weekly bases according to the weight of the rats. The powdered stock was stored at 4°C and used when required.

2.3 Experimental Design

The rats were divided into 11 groups of 5 rats each and given the stated treatments:

- Group 1: (negative control group): rats were not induced.
- Group 2: (Eth Control): injected intraperitoneally with 1ml/kg (0.79 g/kg) absolute ethanol only once every week.
- Group 3: induced and treated orally with 200 mg/kg *Ocimum gratissimum* extract daily daily for six weeks.
- Group 4: induced and treated orally with 400 mg/kg *Ocimum gratissimum* extract daily, for six weeks.
- Group 5: induced and treated orally with 600 mg/kg *Ocimum gratissimum* extract daily for six weeks.
- Group 6: induced and treated orally with and treated orally with 200 mg/kg *Xylopia aethiopica* extract daily for six weeks.
- Group 7: induced and treated orally with 400 mg/kg *Xylopia aethiopica* extract daily for six weeks.
- Group 8: induced and treated orally with 600 mg/kg *Xylopia aethiopica* for six weeks.
- Group 9: induced and treated orally with combination therapy of 200 mg/kg *Ocimum gratissimum* and Xylopia *aethiopica* extracts daily for six weeks.
- Group 10: induced and treated orally with treated orally with combination therapy of 400 mg/kg *Ocimum gratissimum* and Xylopia *aethiopica* extracts daily for six weeks.
- Group 11: induced and treated orally with treated orally with combination therapy of 600 mg/kg Ocimum *gratissimum* and Xylopia *aethiopica* extracts daily for six weeks.

2.4 Blood Sample Collection

At the end of the treatment period, rats in all the groups were anaesthetized with chloroform and dissected, and blood samples collected by jugular puncture into plain bottles, spun using a centrifuge and serum transferred into other plain bottles and stored in the laboratory freezer of a until time for analysis.

2.4.1 Sample analysis

2.4.1.1 Determination of serum malondialdehyde (MDA) level

Method of Assay: Enzyme-linked Immunosorbent Assay (ELISA).

Assay Principle: This ELISA kit makes use of the principle of Competitive-ELISA. The provided microtitre plate has been precoated with MDA. In the process of the reaction, MDA in the sample or standard competes with a fixed quantity of MDA on the solid phase supporter for sites on the Biotinylated Detection antibody specific to MDA. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. A stop solution is then added to terminate the enzyme-substrate reaction, and the change in colour is measured with a spectrophotometer at a wavelength of 450 nm ± 2 nm. The amount of MDA in the samples is then determined by comparing the absorbance of the samples to the standard curve.

2.4.1.2 Determination of the total antioxidant capacity (TAOC)

Method of assay: Colorimetric method.

Assay Principle: In an acid condition, antioxidants are capable of reducing Fe^{3+}-TPTZ to produce blue Fe^{2+}-TPTZ. The antioxidant capacity of the sample can then be calculated through the detection of the absorbance at a wavelength of 593 nm.

Calculation: A graph of the absorbance (x-axis) values of the standard against corresponding concentrations (y-axis) was plot. The concentration of the sample was then calculated based on the absorbance of the sample, using the formula below:

Concentration (y) = 3.2902x + 0.0007, where x = absorbance

2.5 Statistical Analysis

Statistical analysis was done with Statistical Package for Social Sciences (SPSS) of Windows Stat Pack (version 22). Data generated were

recorded as mean and standard deviations (Mean ± S. D), ANOVA (including Turkey's Multiple Comparative Test). P values less than 0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

This study showed that injection of 2 ml/kg (1 ml/kg) ethanol increased oxidative stress in the rats. This is evident in the significant increase (p=0.000) in plasma MDA levels and a marked decrease in total antioxidant capacity of the rats, (Table 1).

This is suggestive of alcohol induced oxidative stress in the liver which led to the high levels of oxidative stress marker in the plasma. This may have occurred due to the accumulation of free radicals generated as pharmacokinetic products of the liver's interaction with ethanol. However, impairment in the functions of endogenous hepatic antioxidant systems, probably induced by ethanol, are also known to contribute to the elevation of free radicals and reactive oxygen species leading to oxidative stress.

The liver's antioxidant system is presumed to have been overwhelmed by the free radicals generated by products of its metabolism of ethanol. This happens when the production of free radicals exceeds the level which the liver's natural antioxidant defense mechanisms can cope with; consequently, creating a cellular oxidative environment which triggers the oxidation of essential biomolecules like DNA, protein and lipids, leading to liver damage.

The generation of hepatic liver peroxidation by free radicals has been proposed as a mechanism for ethanol induced hepatotoxicity. Moreover, it can also be inferred that alcohol induced liver injury depleted the antioxidant system of the treated rats. Reactive oxygen species (ROS) are highly reactive molecules that are naturally generated in small amounts during the body's metabolic reactions and can react with and damage complex cellular molecules such as lipids, proteins, or DNA. It has been reported that in some studies conducted in the rat, extremely high doses of ethanol are required to produce hepatotoxicity with the characteristics of free radical induced lipid peroxidation, when given acutely, though the changes may be potentiated by chronic dosing, [26].

Acute and chronic ethanol treatments is known to increase the production of ROS, lower cellular antioxidant levels, and enhance oxidative stress in many tissues, especially the liver. Ethanol-induced oxidative stress plays a major role in the mechanisms by which ethanol produces liver injury. Many pathways play a key role in how ethanol induces oxidative stress, [27]. Due to their special chemical characteristics, ROS/RNS can initiate lipid peroxidation, cause DNA strand breaks, and indiscriminately oxidize virtually all molecules in biological membranes and tissues, resulting in injury. Ali and colleagues in 2009 reported that among the various methods involved in the hepatotoxic effect of alcohol, one is the oxidative damage through free radical generations, (Ali et al., 2009; Dhanasekaran and [28,29].

However, on treatment with the three doses of aqueous *Ocimum gratissimum,* significant decreases (p=<0.001) in MDA and a corresponding increase in TAOC were observed. This is suggestive of strong antioxidant properties of aqueous *Ocimum gratissimum* extract. Specific biologically important compounds have been identified in extracts from the plant by previous workers [30,31]. The present work also reveals that the extract from the leaves of *Ocimum. gratissimum* possesses good antioxidant potential presumably because of its phytochemical constituents (Thabrew *et al.,* 1998; Halliwell and [32,33].

Table 1. MDA and TAOC levels for induced rats treated with aqueous extract of *Ocimum gratissimum*

Groups	MDA(ng/ml)	TAOC(ng/ml)
Grp 1 (NC)	86.20 ± 17.92	1.90 ± 5.80
Grp 2 (Eth Control)	679.60 ± 157.39[a]	0.10 ± 0.60[a]
Grp 3 (200 mgOcim)	81.40 ± 17.78[b]	2.00 ± 2.50[b]
Grp 4 (400 mgOcim)	101.60 ± 11.88[b]	2.00 ± 2.50[b]
Grp 5 (600 mgOcim)	93.20 ± 25.04[b]	1.90 ± 2.50[b]
F value	66.317	9.720
P value	<0.001	<0.001

Superscripts depict significant p values (Post hoc test), a- compared with NC, b – compared with Eth control

Table 2. MDA and TAOC for induced rats treated with aqueous extract *Xylopia aetiopica*

Groups	MDA(ng/ml)	TAOC(ng/ml)
Grp 1 (NC)	86.20 ± 17.92	2.98 ± 1.59
Grp 2 (EthControl)	679.60 ± 157.39[a]	0.22 ± 0.21[a]
Grp 6 (200 mgXylop)	108.20 ± 11.16[b]	1.96 ± 0.23[b]
Grp 7 (400 mgXylop)	93.00 ± 30.36[b]	2.12 ± 0.17[b]
Grp 8 (600 mgXylop)	165.00 ± 66.14[b]	2.06 ± 0.28[b]
F value	53.372	9.164
P value	<0.001	<0.001
Remark	S	S

Superscripts depict significant p values (Post hoc test), a- compared with NC, b – compared with Eth control

Table 3. MDA and TAOC for induced rats treated with conbination of aqueous extracts of *Xylopia aetiopica* and *Ocimum gratissimum*

	MDA(ng/ml)	TAOC(ng/ml)
Grp 1 (NC)	86.20 ± 17.92	2.98 ± 1.59
Grp 2 (EthControl)	679.60 ± 157.39[a]	0.22 ± 0.21[a]
Grp 9 (200 mgOcimXylop)	152.00 ± 56.00[b]	1.56 ± 0.37
Grp 10 (400 mgOcimXylop)	155.80 ± 49.76[b]	1.26 ± 0.64[a]
Grp 11(600 mgOcimXylop)	99.00 ± 29.76[b]	1.46 ± 0.39
F value	49.748	7.347
P value	<0.001	0.001
Remark	S	S

Superscripts depict significant p values (Post hoc test), a- compared with NC, b – compared with Eth control

Antioxidant property is claimed to be one of the mechanisms of hepato-protective effect of indigenous drugs [34]. Some investigators have also made similar reports on medicinal plants, such as leaves of *M*elia *azedarach* and seeds of *Piper longum* that are rich in similar phytochemicls, [35]. However, the extent to which the phytochemicals can act as anti-oxidants *In vivo* is still poorly understood.

This study shows that the antioxidant property of aqueous extract of *Ocimum* is not dose dependent. Post hoc comparison of the antioxidant properties of the three doses showed that there was a significant increase (p=0.000) in MDA levels when the dose was increased to 400 mg/kg b.wt., while there was no change in the levels of TAOC, indicating that an increase in dose did not enhance the antioxidant system of the rats, rather showed a reduced ability of the extract to ameliorate alcohol induced lipid peroxidation. However, the highest dose of the extract produced a reduced MDA levels, but this reduction was not significant when compared with the mean value for rats that were treated with 400 mg/kg b.wt.

Significant decreases (p=0.000) in MDA levels were seen in the groups of rats treated with all dose concentrations of aqueous *Xylopia aethiopica* when compared with the ethanol induced group, (Table 2). This also suggests that the aqueous extracts of *Xylopia aethiopica* could contain substances that mop up free radicals in the system. This finding is in line with a study by [36] observed that 200 mg/kg of *xylopia aethiopica* (although ethanolic extract), significantly reduced MDA levels and improved the activities of the antioxidant enzymes, Gpx and SOD in ethanol induced albino rats. From a standpoint of a clear understanding of the mechanistic basis of alcohol induced liver toxicity plants such as *Xylopia aethiopica* possessing significant antioxidant or free radical scavenging activities are considered plausible candidates for the prevention of cellular injury occasioned by alcohol.

In this study also, there were significant decreases (p=0.000) in MDA levels in rats that were treated with the three dose concentrations of the combined herbs when compared with values for the induced group. It is presumed that a combination of the aqueous extracts of both plant materials ameliorated free radical induced lipid peroxidation basically due to their phytochemical contents. It has been reported that due to alcohol induced hepatotoxicity, the hepatic cellular signalling network often goes awry due to excessive ROS; moreover, the

protective effects that most plant phytochemicals exert are likely to be the sum of several distinct mechanisms from individual plants, [37]. Several studies have been dedicated to understanding and formulating mechanistic pathways by which these naturally-derived substances could alter the fate of the cell. Particularly those antioxidant properties of phytochemicals that have been implicated as stress-alleviation agents. Some studies have demonstrated a strong relationship between polyphenols and lipid per oxidation, [38].

4. CONCLUSION

Based on our findings, we conclude that *Ocimum gratissimum* and *Xylopia aethiopica* used separately could reduce oxidative stress in alcohol induced hepatotoxic rats.

CONSENT

All authors declare that 'written informed consent was obtained where necessary.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the authors.

ACKNOWLEDGEMENTS

Authors would like to acknowledge the following people for their roles in making the manuscript a success: Chukwuemeka Oguine for funding the study, Pamel Laboratories for experimental analysis and U.A. Obisike for statistical analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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