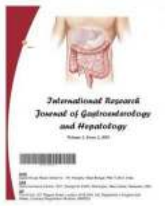

International Research Journal of Gastroenterology and Hepatology

2(1): 1-11, 2019; Article no.IRJGH.49538

Hepatoprotective Effect of *Parkia biglobosa* on Acute Ethanol-induced Oxidative Stress in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIA conceptualized and designed the study and also wrote the draft of the manuscript. Author EOO managed the analyses of the study. Authors UO and APA managed the literature searches. Author ARA wrote the protocol while author EOA performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Hui Jiang, Department of Hepatobiliary Pancreatic Surgery, The Second People's Hospital of Neijiang, China.
Reviewers:
(1) Mustafa Sevindik, Akdeniz University, Turkey.
(2) Raju Senthil Kumar, Swamy Vivekanandha College of Pharmacy, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/49538

Original Research Article

Received 28 March 2019
Accepted 10 June 2019
Published 17 June 2019

ABSTRACT

Aim: This study is aimed at investigating the hepatoprotective effect of *Parkia biglobosa* on acute ethanol-induced oxidative stress in wistar rats.
Methods: *P. biglobosa* was purchased from a local market at Orita-Challenge area of Ibadan, Nigeria. They were sun dried and milled into powder using an electronic blender (Moulinex). The powder was extracted using n-hexane. Twenty adult male wistar rats with body weight between 120 and 150 g were purchased from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were housed in Imrat animal house, Ibadan. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water. They

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were randomly divided into four groups of five rats each. Animals in groups 1 and 2 were administered normal saline solution while those in groups 3 and 4 were administered *P. biglobosa* extract for twenty-one days. The animals were administered the extract and saline solution at a dose of 4 ml per 100 g body weight 12 hourly via oral route of administration. At the end of the treatment, they were fasted overnight and animals in groups 2 and 4 were exposed to a single dose of 70% ethanol at 12 ml/kg body weight to induce oxidative stress. After 12 hours of ethanol administration, the animals were anaesthetized using diethyl ether and were sacrificed. Liver was excised, weighed and homogenized in 50 mmol/L Tris–HCl buffer (pH 7.4) and then centrifuged at 5000 × g for 15 minutes for biochemical analysis. Supernatants were immediately kept frozen until when needed.

Results: Ethanol-induced oxidative stress significantly increased the activities of AST, ALT, LDH, LPO, CAT and SOD but decrease GSH. However, it has no effect on GPX. These effects were regulated by *P. biglobosa* administration.

Conclusion: *P. biglobosa* was able to remedy the effect of ethanol by regulating the oxidative stress biomarkers, thus possesses prophylactic efficacy against ethanol-induced oxidative stress and can protect the liver against free radicals arising from oxidative stress.

Keywords: Parkia biglobosa; ethanol; oxidative stress; hepatoprotective effect.

1. INTRODUCTION

Excessive acute or chronic alcohol consumption poses a serious health hazard and can result into several metabolic disorders in hepatic and extra-hepatic diseases [1]. Alcohol is a commonly used hepatotoxin in experimental hepatopathy. Although the pathogenesis of alcohol-induced liver disease is not clearly defined, there is evidence that ethanol-induced liver injury is due to oxidative stress that leads to fibrosis and impaired liver functions [2,3]. Alcohol overuse is also characterized by central nervous system (CNS) intoxication symptoms, impaired brain activity, poor motor coordination, and behavioral changes [4]. Excessive alcohol consumption commonly causes hepatic, gastrointestinal [5], nervous and cardiovascular injuries leading to physiological dysfunctions [6]. Cellular disturbances resulting from excessive alcohol consumption results in increased formation of oxidative stress biomarkers such as malondialdehyde (MDA); reduction in the level of reduced glutathione level and a decrease in the activities of antioxidant enzymes [7,8]. Free radicals and reactive oxygen species (ROS) have been implicated in the oxidative damage of biomolecules and various organs of the body. Studies have shown the crucial role free radicals play in the pathogenesis of many human diseases namely, cardiovascular and pulmonary diseases, some types of cancer, immune/ autoimmune diseases, inflammation, diabetes, cataracts and brain dysfunction such as Parkinson and Alzheimer [9]. However, the deleterious effect of free radicals can be corrected by antioxidants – both enzymatic and nonenzymatic. Oxidative stress is known to arise when there is an imbalance between free radical production (especially reactive oxygen species; ROS) and endogenous antioxidant defense system. This shift in balance is associated with oxidative damage to a wide range of biomolecules including lipids, proteins, and nucleic acids, which may eventually impair normal functions of various tissues and organs [10].

There is an increasing global interest concerning the use of medicinal plants in the prevention and treatment of different pathologies [11,12]. The beneficial effects of plants are attributed to the presence of secondary metabolites such as polyphenols, tannins, terpenoids, alkaloids, flavonoids [13]. Considering the central role played by free radicals in the initiation and progression of many diseases, the use of natural products with antioxidant constituents has been proposed as an effective therapeutic and/or preventive strategy against diseases and the search for potent and cost-effective antioxidants of plant origin has since increased [14]. Many plants have been shown to possess antioxidant potentials [15,16]. This has thus raised interest in the investigation of commonly consumed plants for their phytochemicals with nutritional and chemotherapeutic potentials. Therefore, the need to argument synthetic chemotherapeutic compounds with natural products is the drive for the exploitation of natural products from plants; as they may have little or no side effects yet meeting the nutritional, chemotherapeutic and economic needs [17,18]. Moreover, despite the efforts of pharmaceutical companies in the

production of synthetic antibiotics, there yet exists a marked increase in pathogen population exacerbated by multi drug resistant microorganisms. Consequently, there is increased research into phytochemicals for the effective therapeutics combat of this menace. The therapeutic effects of plant-based drugs have been documented to be due to the phytochemicals that constitute the plants [19,20]. These constituents selectively target toxins and pathogens without significant detrimental effect on the human host.

P. biglobosa is a perennial tropical plant legume predominantly distributed within the savannah region of West Africa [21]. *P. biglobosa* is also known as African locust bean while in Nigeria its local names include; Origili in Igbo, Dorowa in Hausa and Iru in Yoruba [22]. The African locust bean has gained its popularity from the consumption and economic value of its bean seeds. In West Africa especially Nigeria, the beans are usually fermented to yield a product popularly called "Dawadawa". Dawadawa is a black, tasty seasoning, rich in protein which is commonly used as condiment in local soups and as a dietary protein source [23,24,25]. Furthermore, other parts of the plant such as the fruit and stem have also been exploited. The stem bark was reported to have anti-snake venom activities [26], the fresh fruit pulp can be used as mineral supplement [27]. Besides the culinary use of the African locust bean, its chemotherapeutic attributes have been explored [22,28,29]. In traditional medicine practices, a decoction of the stem bark has been used as a hot mouthwash to relieve toothaches [30]. A mixture of the root and leaves has been reported to be an efficacious remedy for the treatment of sore eyes and dental caries [31]. This study is therefore aimed at investigating the hepatoprotective effect of *P. biglobosa* on acute ethanol-induced oxidative stress in wistar rats.

2. MATERIALS AND METHODS

2.1 Sample Preparation

P. biglobosa (African locust bean) was purchased from a local market at Orita-Challenge area of Ibadan, Nigeria. They were sun dried and milled into powder using an electronic blender (Moulinex). The powder was extracted according to the method described by Airaodion et al. [22]. 2.5 kg powder of ALB was extracted with n-hexane (boiling point 40–60°C) in a soxlet extractor (Sri Rudram Instrument, Chennai, India) for 18 hours. The defatted, dried sample was repacked and then extracted with methanol. Briefly, the dried marc was extracted with methanol in the soxlet apparatus for 10 hours. The methanol solution was subsequently concentrated in a rotatory evaporator at 40°C.

2.2 Animal Treatment

Twenty adult male wistar rats with body weight between 120 and 150 g were purchased from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were housed in Imrat animal house, Ibadan. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into four groups of five rats each. Animals in groups 1 and 2 were administered normal saline solution while those in groups 3 and 4 were administered *P. biglobosa* extract for twenty-one days. The animals were administered the extract and saline solution at a dose of 4 mL per 100 g body weight 12 hourly via oral route of administration. At the end of the treatment, they were fasted overnight and animals in groups 2 and 4 were exposed to a single dose of 70% ethanol at 12 mL/kg body weight to induce oxidative stress. The dosage of ethanol used in this study has been documented to induce tissue toxicity and oxidative damage in rats [32]. After 12 hours of ethanol administration, the animals were anaesthetized using diethyl ether and were sacrificed. Liver was excised, weighed and homogenized in 50 mmol/L Tris–HCl buffer (pH 7.4) and then centrifuged at 5000 × g for 15 minutes for biochemical analysis. Supernatants were immediately kept frozen until when needed.

2.3 Biochemical Analyses

2.3.1 Determination of ALP, AST, ALT and LDH activities

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities were determined using Randox commercial Enzyme kits according to the method of Reitman and Frankel [33].

2.3.2 Assessment of lipid peroxidation (LPO)

Lipid peroxidation was determined according to the method of Varshney and Kale [34] based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA): an end product of lipid peroxidation. Briefly, 0.4 ml of the sample was mixed with 1.6 ml of Tris–KCl buffer and 0.5 ml of trichloroacetic acid (TCA, 30%). This was followed by the addition of 0.5 ml of TBA (0.75%). The reaction mixture was heated in a water bath for 45 minutes at 80 °C, cooled in ice and centrifuged at 3000 × g for 5 minutes. Absorbance of the resulting supernatant was determined at 532 nm against a reference blank of distilled water. Lipid peroxidation in units/mg protein was computed with a molar extinction coefficient of 1.56×10^5 $m^{-1}cm^{-1}$.

2.3.3 Reduced glutathione (GSH) assay

The method of Jollow et al. [35] was used in estimating the concentration of reduced glutathione (GSH). Liver homogenates were deproteinized by the addition of 0.15 M sulphosalicyclic acid (1:1, v/v). The protein precipitate was centrifuged at 4000 × g for 5 min. Thereafter, 0.5 mL of the supernatant was added to 4.5 mL of 0.001 M Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB). At 412 nm, absorbance of the mixture was read against a blank consisting of 0.5 mL of de-proteinizing agent diluted with water (1:1) and 4.5 mL of 0.001 M DTNB. The concentration of reduced glutathione is proportional to the absorbance. GSH concentration was extrapolated from calibration curve prepared with GSH standards.

2.3.4 Estimation of catalase (CAT) activity

Catalase (CAT) activity was determined by the method of Sinha [36] based on the reduction of dichromate (in acetic acid) to chromic acetate in the presence of H_2O_2. Briefly, the assay mixture contained 4 mL of H_2O_2 solution (800 mol) and 5 ml of phosphate buffer (0.01 M, pH 7.0). 1 ml of diluted sample (1:10) was rapidly mixed with the reaction mixture at room temperature. 1 ml portion of the reaction mixture was withdrawn and blown into 2 mL dichromate/acetic acid reagent (1:3 by volume) at 60 s intervals. The chromic acetate then produced is measured calorimetrically at 570 nm for 3 minutes at 60 s intervals after heating the reaction mixture in a boiling water bath for 10 minutes. Catalase activity expressed as mol H_2O_2 consumed/min/ mg protein.

2.3.5 Estimation of superoxide dismutase (SOD) activity

The activity of SOD was determined by using Oxiselect Superoxide Dismutase Activity Assay described by Mohammad et al. [37]. Tissues were homogenized on ice using mortar and pestle in 7 mL of cold 1X Lysis Buffer per gram tissue followed by centrifugation at 12000 ×g for 10 minutes. The supernatant of tissue lysate was then collected and kept at -80°C until further analysis. Superoxide anions generated by Xanthine/Xanthine Oxidase system were detected with a Chromagen Solution by measuring the absorbance reading at 490 nm using POLARstar Omega Reader. The activity of SOD was determined as the inhibition percentage of chromagen reduction.

2.3.6 Estimation of glutathione peroxidase (GPX) activity

Glutathione peroxidase enzyme was determined according to Rotruck et al. [38]. Briefly, 500 μL of tissue homogenates were mixed with 500 μL of assay buffer (potassium phosphate 30 mM, pH 7.0), 100 ml of sodium azide (NaN_3; 10 mM), 200 μL of reduced glutathione (GSH; 4 mM), 100 μL hydrogen peroxide (H_2O_2; 2.5 mM), and 6 ml of distilled water. The whole reaction mixture was incubated at 37°C for 3 min after which 0.5 mL of TCA (10%) was added and thereafter centrifuged at 3000 rpm for 5 minutes. 1 mL of the supernatants was added to 2 mL of K_2HPO_4 (0.3 M) and 1 mL of DTNB and the absorbance was read at 412 nm.

2.4 Statistical Analysis

Results are expressed as mean ± standard error of the mean (S.E.M). The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

Acute and chronic alcohol exposures have been documented to increase the generation of reactive oxygen species (ROS). Many investigations have revealed a decreased level of antioxidants and increased production of free radicals in animals and humans following excessive ethanol exposure [39,40]. The protective effect of antioxidants against alcohol-

induced liver injury in many studies further supports the involvement of oxidative stress [41,42].

Investigating the use of plant materials as a potent remedy for various ailments has not only authenticated their efficacies, but has also identified the pharmacological roles of the individual bioconstituents of these plant materials. Phytochemistry of the pods of *P. biglobosa* has been reported to contain an array of medically important secondary metabolites including saponins, tannins, cardiac glycosisides, flavonoids, carotenoids, steroids, terpenoids and reducing sugars which is a strong indication that the plant has potential medicinal values [43]. In addition, Airaodion et al. [22] reported the effect of *P. biglobosa* on fasting blood sugar and lipid profile of animals on albino rats. However, the action of *P. biglobosa* extract against ethanol-induced oxidative stress has not been reported so far.

Evaluation of liver function is very important when analyzing toxicity of drugs and plant extracts because of its relevance for the survival of the organism [44]. High levels of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) are an indicator of hepatotoxicity or liver diseases [45]. Studies on the alterations of these enzymes might reflect the metabolic abnormalities and cellular injuries in some organs. The liver and kidney have extremely important function in detoxification and excretion of metabolic wastes and xenobiotics [46]. Exposure to toxic chemicals causes alterations in some tissue enzyme activities [47, 48]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are distributed extensively in several different organs and have important roles in carbohydrate and amino acid metabolic pathways and their activities is established to change under several physiological and pathological circumstances [49].

In this study, the activities of AST, ALT, ALP and LDH were not significantly different when animals treated *P. biglobosa* extract only were compared with those of the control group at $p<0.05$. A significant increase was observed when the activities of AST, ALT, ALP and LDH in animals induced with 70% ethanol without treatment with *P. biglobosa* extract were compared with those of the control and *P. biglobosa* extract only groups at $p<0.05$. This might be an indication that ethanol causes liver damage to the animals. However, when animals treated with *P. biglobosa* extract before the induction of oxidative stress by ethanol were compared with those induced without pretreatment, a significant decrease was observed in the activities of AST, ALT and LDH. This could be that pretreatment with *P. biglobosa* extract resulted in increased transcription of some genes involved in glucose uptake, glycolysis and lipogenesis [50]. Glucose represses the induction of inducible operons by inhibiting the synthesis of cyclic Adenosine monophosphate (cAMP) a nucleotide that is required for the initiation of transcription of a large number of inducible enzyme systems including the Lac operon. Cyclic AMP (cAMP) is required to activate an allosteric protein called catabolite activator protein (CAP) which binds to the promoter CAP site and stimulates the binding of ribonucleic acid (RNA) polymerase to the promoter for the initiation of transcription, but cAMP must be available to bind to CAP which binds to deoxyribonucleic acid (DNA) to facilitate transcription. In the presence of glucose, adenylase cyclase (AC) activity is blocked. AC is required to synthesize cAMP from Adenosine Triphosphate (ATP) [51,52]. Therefore if cAMP levels are low, CAP is inactive and transcription does not occur. Thus the effect of glucose in suppressing these inducible enzymes is by lowering cyclic AMP level. The *P. biglobosa* extract might have lowered cAMP in animals thus causing inhibition of these inducible enzymes. ALT is considered most reliable hepatocellular injury because it is solely confined to the liver, unlike AST and LDH which are also abundantly present in other body organs such as the kidneys, brain, and hearts [53]. The significant decrease observed in the activities of ALT and AST in *P. biglobosa*-treated animals when compared to those induced without pretreatment showed that *P. biglobosa* protected the liver from damage by ethanol-induced oxidative stress.

Alkaline phosphatase (ALP) is involved in the hydrolysis of a wide range of phosphomonoester substrates. It is a marker enzyme for the plasma membrane and endoplasmic reticulum of tissues [54]. It is often employed to assess the integrity of the plasma membrane, since it is localized predominantly in the microvilli in the bile canaliculli, located in the plasma membrane. Since ALP hydrolyses phosphate monoesters, its significant increase in ethanol-induced animals without pretreatment could constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital process

Table 1. Effect of *P. biglobosa* on hepatic marker enzymes of experimental rats

Hepatic marker enzymes	Control	70% Ethanol only	*P. biglobosa* extract only	*P. biglobosa* extract + 70% ethanol
AST (IU/L)	110.27 ± 3.22^{a}	132.84 ± 5.78^{b}	108.89 ± 4.82^{a}	114.84 ± 3.34^{a}
ALT (IU/L)	46.85 ± 2.17^{a}	56.49 ± 3.64^{b}	45.28 ± 3.72^{a}	48.49 ± 3.64^{a}
ALP (IU/L)	15.60 ± 2.57^{ac}	22.79 ± 1.82^{b}	13.59 ± 0.70^{a}	19.79 ± 1.02^{bc}
LDH (IU/L)	184.40 ± 9.13^{ac}	206.55 ± 10.10^{b}	183.54 ± 8.06^{a}	190.55 ± 11.10^{c}

Values are presented as Mean±S.E.M, where n = 5. Values with different superscript along the same row are significantly different at p<0.05

Legend: AST = Aspartate Amino Transferase, ALT = Alanine Amino Transferase, ALP = Alkaline Phosphatase, LDH = Lactate Dehydrogenase

Table 2. Effect of *P. biglobosa* on oxidative stress biomarkers of experimental rats

Oxidative stress biomarkers	Control	70% ethanol only	*P. biglobosa* extract only	*P. biglobosa* extract + 70% ethanol
LPO (nmol MDA/mg protein)	11.76 ± 1.08^{a}	18.91 ± 2.27^{b}	10.25 ± 1.41^{a}	10.30 ± 0.92^{a}
GSH (mg/mL)	4.68 ± 0.10^{a}	3.06 ± 0.78^{b}	4.58 ± 0.13^{a}	4.31 ± 0.64^{a}
CAT (Mm H_2O_2/mg protein)	13.04 ± 1.04^{a}	22.00 ± 2.29^{b}	12.82 ± 1.08^{a}	17.34 ± 1.85^{c}
SOD (U/mg protein)	8.62 ± 1.21^{a}	13.93 ± 2.23^{b}	7.35 ± 0.86^{a}	9.05 ± 1.10^{a}
GPX (U/mg protein)	6.08 ± 0.16^{a}	6.52 ± 0.21^{a}	5.88 ± 0.19^{a}	5.80 ± 0.14^{a}

Values are presented as Mean±S.E.M, where n = 5. Values with different superscript along the same row are significantly different at p<0.05.

Legend: LPO = lipid peroxidation, GSH = Glutathione, CAT = Catalase, SOD = Superoxide Dismutase, GPX = Glutathione Peroxidase

as it may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver [55]. Consequently, this may adversely affect the facilitation of the transfer of metabolites across the cell membrane of ethanol-induced animals without pretreatment. This effect was remedied by pretreatment with *P. biglobosa* extract.

The elevation in the activities of markers such as ALT, AST and LDH in the liver tissue of animals without pretreatment with *P. biglobosa* might be due to cellular necrosis of hepatocytes, which causes increase in the permeability of the cell. Lactate dehydrogenase (LDH) is an index of cell damage including hepatotoxicity and the endothelial disruption in blood vessel. The significant increase observed in the activity of LDH might be suggestive of the beginning of cytolysis, which is a possible indication of membrane damage including the endothelial membranes of blood vessels. This disruption of endothelial membrane, directly or indirectly, as reported earlier, includes the generation of reactive oxygen species in endothelial cells [56]. According to Kottaimuthu [57], free radicals attack unsaturated fatty acids in the membranes resulting in membrane lipid peroxidation which decreases membrane fluidity, leakage of enzyme and loss of receptor activity as well as damage membrane proteins leading to cell inactivation. As lipid peroxidation progressively increased, antioxidant defense system decrease equivalently resulting in oxidative stress [58]. This suggests that the administration of ethanol might have weakened the liver membrane of the rats with subsequent penetration and elevation of AST, ALT and LDH in the liver [59].

Alcohol metabolism results in oxidative and nitrosative stress via elevation of $NADH/NAD^{+}$ redox ratios, induction of nitric oxide synthase (NOS) and NADPH/xanthine oxidase [60,61]. Lipid peroxidation, a primary mechanism of cell membrane destruction and cell damage is a

common feature of both acute and chronic alcohol consumption [62,63]. The presence of a high concentration of oxidisable fatty acids and iron in liver significantly contributes to ROS production. A rise in lipid peroxidation level is only identified if there is oxidative damage due to the increase in free radical generation. Generally under normal conditions, the animals tend to maintain a balance between generation and neutralization of ROS in the tissues. But, when the organisms are subjected to xenobiotic stress, the rate of production of ROS including O_2^-, H_2O_2, OH^-, ROO^{--}, exceeds their scavenging capacities. All the organisms have their own cellular antioxidant defense system composed of both enzymatic and non-enzymatic components. Enzymatic antioxidant pathway consists of SOD, CAT and GPX. Superoxide anion O_2^- is dismutated by SOD to H_2O_2, which is reduced to water and molecular oxygen by CAT or is neutralized by GPX, which catalyzes the reduction of H_2O_2 to water and organic peroxide to alcohols using GSH as a source of reducing equivalent. Glutathione reductase (GR) regenerates GSH from oxidized glutathione (GSSG), which is a scavenger of ROS as well as a substrate for other enzymes. Glutathione S-transferase (GST) conjugates xenobiotics with GSH for exclusion.

In this study, acute ethanol exposure significantly elevated the malondialdehyde (MDA) levels in the liver indicating enhanced peroxidation and breakdown of the antioxidant defense mechanisms. Decomposition products of lipid hydroperoxide such as malanoldehyde and 4-hydroxynonenal, can cause chaotic cross-linkage with proteins and nucleic acids, which plays an important role in the process of carcinogenesis. In this investigation, hepatic lipid peroxidation (LPO) activities show significant increase due to ethanol intoxication. Furthermore, extensive damage to tissues in a free radical mediated LPO results in membrane damage and subsequently decreases the membrane fluid content. *P. biglobosa* pretreatment significantly reversed these alterations causing a significant decrease in MDA levels, suggesting its protective effects against ethanolic-induced oxidative damage. This is consistent with the study of Oyenihi et al. [56] who reported the hepato- and neuro-protective effects of watermelon juice on acute ethanol-induced oxidative stress in rats.

Glutathione (GSH) is a tripeptide (L-α-glutamylcysteinol glycine) which is highly abundant in all cell compartments and it is the major soluble antioxidant. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism [63]. Glutathione detoxifies hydrogen peroxide and lipid peroxide by donating electron to hydrogen peroxide to reduce it to water and oxygen protecting macromolecules such as lipids from oxidation. In this study, the decrease in the reduced glutathione level in animals treated with ethanol only is connected with ethanol-induced oxidative stress and direct conjugation of GSH with acetaldehyde and other reactive intermediates of alcohol oxidation. This result is in agreement with the finding of Pinto et al. [39] who reported that acute ethanol treatment caused reduction in the glutathione levels in different tissues. The significant increase ($P < 0.05$) in the glutathione levels in the liver of *P. biglobosa*-treated rats prior to ethanol-administration may be due to the direct ROS—scavenging effect of *P. biglobosa* or an increase in GSH synthesis.

Catalase (CAT) contributes to ethanol oxidation, by oxidizing a small amount of ethanol in the presence of a hydrogen peroxide (H_2O_2) generating system to form acetaldehyde [64]. In this study, no significant difference was observed in the activity of catalase in control animals and those treated with *P. biglobosa* extract only when compared with ethanol-induced animals with *P. biglobosa* extract pretreatment. The activity of catalase in animals pretreated with *P. biglobosa* prior to ethanol induction was significantly reduced when compared with those without *P. biglobosa* pretreatment. This might be that ethanol-induced oxidative stress generated elevated ROS in the liver which CAT tend to combat, thereby increasing its activity. *P. biglobosa* was able to reduce the ROS generation with subsequent decrease in CAT activity due to its high phytochemical content and antioxidant potential as reported by Igbowe et al [43]. Increased CAT activity in this study following acute ethanol exposure suggests elevated ethanol oxidation and formation of oxidising product-acetaldehyde. This is in agreement with the study of Oyenihi et al. [56] and Oh et al. [65] who reported a significantly higher CAT activity after ethanol treatment.

Superoxide dismutase (SOD) plays an important role in reducing the effect of free radicals attack, and SOD is the only enzymatic system quenching O_2- to oxygen and H_2O_2 and plays a significant role against oxidative stress [66]. These radicals have been reported to be

deleterious to polyunsaturated fatty acids and proteins [67]. In this study, no significant difference was observed in the activity of SOD in control animals and those treated with *P. biglobosa* extract only when compared with ethanol-induced animals with *P. biglobosa* extract pretreatment. The activity of SOD in animals pretreated with *P. biglobosa* prior to ethanol induction was significantly reduced when compared with those without *P. biglobosa* pretreatment. This might be that ethanol-induced oxidative stress generated elevated ROS in the liver which SOD tend to combat thereby increasing its activity. *P. biglobosa* was able to reduce the ROS generation with subsequent decrease in SOD activity due to its high phytochemical content and antioxidant potential as reported by Igbowe et al. [43]. The increased activity of SOD observed in ethanol induced animals contradicts the study of Halliwell B, Gutterberidge [68] who reported that SOD activity was considerably reduced during ethanol intoxication.

Glutathione preroxidase (GPX) is another enzymic antioxidant that acts as a defense against oxidative stress. The lack of significant effect in GPX activity observed in this study after ethanol treatment may be due to the duration of exposure to ethanol. This result is in agreement with the study of Yang et al. [69] who observed no difference in GPX activities in rats hepatocyte exposed to varying concentrations of ethanol at an incubation time of 12 h. The toxicity of ethanol is related to the product of its metabolic oxidation. Acetaldehyde and acetate, produced from the oxidative metabolism of alcohol are capable of forming adducts with cellular macromolecules, causing oxidative damage and affecting metabolic processes [64,70]. Catalase and glutathione peroxidase further detoxify H_2O_2 into H_2O and O_2 [67]. Thus, SOD, catalase and GPX function mutually as enzymatic antioxidative defense mechanism to counter the deleterious effect of ROS.

4. CONCLUSION

The results of this study indicated that ethanol induced oxidative stress as seen in the perturbation of the biomarkers. *P. biglobosa* was able to remedy this effect by regulating the oxidative stress biomarkers, thus possesses prophylactic efficacy against ethanol-induced oxidative stress and can protect the liver against free radicals arising from oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lieber CS. Ethnic and gender differences in ethanol metabolism. Alcohol. Clin. Exp. Res. 2000;24 (4):417–418.
2. Wu D, Cederbaum AI. Alcohol, oxidative stress, and free radical damage. Alcohol Res. Health. 2003;27:277–284.
3. Ronis MJ, Hakkak R, Korourian S, Albano E, Yoon S, Ingelman-Sundberg M, Lindros KO, Badger TM. Alcoholic liver disease in rats fed ethanol as part of oral or intragastric low-carbohydrate liquid diets. Exp. Biol. Med. 2004;229 (4):351–360.
4. Gohlke JM, Griffith WC, Faustman EM. Computational models of ethanol-induced neurodevelopmental toxicity across species: Implications for risk assessment, Birth Defect Res. B: Dev. Reprod. Toxicol. 2008;83(1):1–11.
5. Reddy SK, Husain K, Schlorff EC, Scott RB, Somani SM. Dose response of ethanol ingestion on antioxidant defense system in rat brain subcellular fractions, Neurotoxicology. 1999;20(6):977–987.
6. Lieber CS. Hepatic and other medical disorders of alcoholism: From pathogenesis to treatment. J. Stud. Alcohol. 1998;59(1):9–25.
7. Nadro MS, Arungbemi RM, Dahiru D. Evaluation of *Moringa oleifera* leaf extract on alcohol-induced hepatotoxicity. Trop. J. Pharm. Res. 2007;5(1):539–544.
8. Das SK, Vasudevan DM. Alcohol-induced oxidative stress. Life Sci. 2007;81(3):177–187.
9. Rahman T, Hosen I, Islam MT, Shekhar HU. Oxidative stress and human health. Adv Biosci Biotechnol. 2012;3:997–1019.
10. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional

foods: Impact on human health. Pharmacogn Rev. 2010;4:118–126.
11. Gorinstein S, Yamamoto K, Katrich E, Leontowicz H, Lojek A, Leontowicz M, Trakhtenberg S. Antioxidative properties of Jaffa sweeties and grapefruit and their influence on lipid metabolism and plasma antioxidative potential in rats. Biosci. Biotechnol. Biochem. 2003;67(4):907–910.
12. Ramana KV,Singhal SS, Reddy AB. Therapeutic potential of natural pharmacological agents in the treatment of human diseases. BioMed Res. Int. 2014: 1–4.
13. Chikezie PC, Chiedozie O, Ibegbulem FN. Bioactive principles from medicinal plants. Res. J. Phytochem. 2015;9(3):88–115.
14. Morales-González JA. Oxidative stress and chronic degenerative diseases—a role for antioxidants. InTech, Rijeka, Croatia. 2013;500.
15. Airaodion AI, Olatoyinbo PO, Ogbuagu U, Ogbuagu EO, Akinmolayan JD, Adekale OA, Awosanya OO, Agunbiade AP, Oloruntoba AP, Obajimi OO, Adeniji AR, Airaodion EO. Comparative assessment of phytochemical content and antioxidant potential of *Azadirachta indica* and *Parquetina nigrescens* leaves. Asian Plant Research Journal. 2019;2(3):1-14.
16. Airaodion AI, Ibrahim AH, Ogbuagu U, Ogbuagu EO, Awosanya OO. Akinmolayan JD, Njoku OC, Obajimi OO, Adeniji AR, Adekale OA. Evaluation of Phytochemical Content and Antioxidant Potential of *Ocimum gratissimum* and *Telfairia occidentalis* leaves. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2019;7(1):1-11.
17. Airaodion AI, Obajimi OO, Ezebuiro CN, Ogbuagu U, Agunbiade AP, Oloruntoba AP, Akinmolayan JD, Adeniji AR, Airaodion EO. Prophylactic efficacy of aqueous extract of *Curcuma longa* leaf against indomethacin-induced ulcer. International Journal of Research. 2019;6(1):87-91.
18. Airaodion AI, Olayeri IM, Ewa AO, Ogbuagu EO, Ogbuagu U, Akinmolayan JD, Agunbiade AP, Oloruntoba AP, Airaodion EO, Adeniji AR, Obajimi OO, Awosanya OO. Evaluation of *Moringa oleifera* leaf potential in the prevention of peptic ulcer in wistar rats. International Journal of Research. 2019;6(2):579-584.
19. Airaodion AI, Ogbuagu U, Ogbuagu EO, Airaodion EO, Agunbiade AP, Oloruntoba AP, Mokelu IP, Ekeh SC. Investigation of aqueous extract of *Zingiber officinale* root potential in the prevention of peptic ulcer in albino rats. International Journal of Research and Innovation in Applied Science. 2019;4(2):64-67.
20. Airaodion AI, Adekale OA, Airaodion EO, Ogbuagu EO, Ogbuagu U, Osemwowa EU. Efficacy of combined extract of *Curcuma longa* and *Moringa oleifera* in the prevention of peptic ulcer in albino rats. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2019;7(2):1-8.
21. Campbell-Platt G. African Locust Bean (*Parkia* species) and its West African fermented food product, Dawadawa. Ecol. Food Nutr. 1980;9:123-132
22. Airaodion AI, Airaodion EO, Ogbuagu EO, Ogbuagu U, Osemwowa EU. Effect of oral intake of african locust bean on fasting blood sugar and lipid profile of albino rats. Asian Journal of Research in Biochemistry. 2019;4(4):1-9.
23. Wokoma EC, Aziagba GC. Sensory evaluation of dawa dawa produced by the traditional fermentation of African yam bean (*Sphenostylis stenocarpa* Harms) seeds. J. Appl. Sci. Environ. Mgt. 2001;5: 85-91.
24. Achi OK. Traditional fermented protein condiments in Nigeria. Afr. J. Biotechnol. 2005;4:1612-1621.
25. Chukwu O, Orhevba BA, Mahmood BI. Influence of Hydrothermal Treatment on proximate composition of fermented Locust Bean (Dawadawa). J. Food Technol. 2010; 8: 99-101.
26. Asuzu IU, Harvey AL. The antisnake venom activities of *Parkia biglobosa* (Mimosaceae) stem bark extract. Toxicon. 2003;42:763-768.
27. Omojola MO, Afolayan MO, Adebiyi AB, Orijajogun JO, Thomas SA, Ihegwuagu NE. Further physicochemical characterization of *Parkia biglobosa* (Jacq.) Benth fruit pulp as a mineral supplement. Afr. J. Biotechnol. 2011;10: 17258-17264.
28. Asase A, Oteng-Yeboah AA, Odamtten GT, Simmonds MSJ. Ethnobotanical study of some Ghanaian anti-malarial plants. J. Ethnopharmacol. 2005;99:273-279.
29. Builders MI, Tarfa F, Aguiyi JC. The potency of African locust bean tree as antimalarial. J. Pharmacol. Toxicol. 2012; 7:274-287.
30. Ajaiyeoba EO. Phytochemical and antibacterial properties of *Parkia biglobosa*

and *Parkia bicolor* leaf extracts. Afr. J. Biomed. Res. 2002;5:125-129.

31. Millogo-Kone H, Guissou IP, Nacoulma O, Traore A. Comparative study of leaf and stem bark extracts of *Parkia biglobosa* against Enterobacteria. Afr. J. Tradit. Complement Altern. Med. 2008;5:238-243.
32. Han Y, Xu Q, Hu JN, Han XY, Li W, Zhao LC. Maltol, a food flavoring agent, attenuates acute alcohol-induced oxidative damage in mice. Nutrients. 2015;7(1): 682–696.
33. Reitman S, Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. American Journal of Clinical Pathology. 1957;28:56-58.
34. Varshney R, Kale RK. Effects of Calmodulin antagonist on radiation induced lipid peroxidation in microsomes. Int. J. Radiat. Biol. 1990;58:733–743.
35. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3,4bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11:151–169.
36. Sinha AK. Colorimetric assay of catalase. Anal. Biochem. 1972;47(2):389–394.
37. Mohammad MK, Mohamed MI, Zakaria AM, Razak HR, Saad WM. Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) juice modulates oxidative damage induced by low dose X-ray in mice. BioMed Research International. 2014;1-6.
38. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 4073;179(1973):588–590.
39. Pinto C, Cestero JJ, Rodríguez-Galdón B, Macías P. Xanthohumol, a prenylated flavonoid from hops (*Humulus lupulus* L.): protects rat tissues against oxidative damage after acute ethanol administration. Toxicol. Rep. 2014;1:726–733.
40. Han Y, Xu Q, Hu JN, Han XY, Li W, Zhao LC. Maltol, a food flavoring agent, attenuates acute alcohol-induced oxidative damage in mice. Nutrients. 2015;7(1): 682–696.
41. Du J, He D, Sun LN, Han T, Zhang H, Qin LP, Rahman K. Semen Hoveniae extract protects against acute alcohol-induced liver injury in mice. Pharm. Biol. 2010; 48(8):953–958.
42. Seif HSA. Ameliorative effect of pumpkin oil (*Cucurbita pepo* L.) against alcohol-induced hepatotoxicity and oxidative stress in albino rats. Beni Suef Univ. J. Basic Appl. Sci. 2014;3(3):178–185.
43. Igwo-Ezikpe MN, Ogbunugafor HA, Gureje AP, Ezeonwumelu IJ. Phytochemical, antioxidant and antimicrobial properties of *Parkia biglobosa* (African Locust Bean) pods. The Bioscientist. 2013;1(2):182-191.
44. Owoade AO, Adetutu A, Airaodion AI Ogundipe OO. Toxicological assessment of the methanolic leaf extract of *Bridelia ferrugelia*. The Journal of Phytopharmacology. 2008;**7**(5): 419-424.
45. Brautbar N, Williams J II. Industrial solvents and liver toxicity: Risk assessment, risk factors and Mechanisms. International J of Hygiene and Environmental Health. 2002;25(6):497-492.
46. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals, 5th Ed. Academic Press, London. 1999;829-44.
47. Jarrar BM, Mahmoud ZN. Histochemical demonstration of changes in the activity of hepatic phosphatases induced by experimental lead poisoning in male white rats (*Rattus norvegicus*). Toxicol Ind Health. 1999;15:1–9.
48. Gholipour-Kanani H, Shahsavani D, Baghishani H. Effect of exposure to sublethal levels of potassium cyanide on serum and tissue enzymes in roach fish (*Rutilus rutilus*). Online J Vet Res. 2013; 17(5):245-55.
49. Al-Ghanim KA. Effect of cypermethrin toxicity on enzyme activities in the freshwater fish *Cyprinus carpio*. African Journal of Biotechnology. 2014;13(10): 1169–1173.
50. Towle HC, Kaytor EN, Shih HM. Regulation of the expression of lipogenic enzymes by carbohydrates. Annual Review of Nutrition. 1997;17:405-433.
51. Zubay G, Schwartz D, Beckwith J. Mechanism of activation of catabolite-sensitive genes: A positive control system. Proceedings of the National Academy of Sciences. 1970;66(1):104-110.
52. Todar K. Regulation and control of metabolism in Bacteria: In: Todar's Online Textbook of Bacteriology. 2008;1-5.
53. Johnson PJ. The assessment of hepatic function and investigation of jaundice. In: Marshall, WJ, Bangert SK, editors. Clinical Biochemistry – Metabolic and Clinical

Aspects. Churchill Livingstone, New York. 1995;217-36.
54. Wright PJ, Plummer DT. The use of urinary enzyme measurement to detect renal damage caused by Nephrotoxic compounds. Biochem. Pharmacol. 1974; 23:98-112.
55. Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. Toxicology. 1993; 81:173-179.
56. Oyenihi OR, Afolabi BA, Oyenihi AB, Ogunmokun OJ, Oguntibeju OO. Hepato- and neuro-protective effects of watermelon juice on acute ethanol-induced oxidative stress in rats. Toxicology Reports. 2016;3: 288–294.
57. Kottaimuthu R. Ethnobotany of the valaiyans of karandamalai, Dindigul district, Tamil Nadu, India. Ethnobot. Leaflets. 2008;12:195-203.
58. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr. Sci. 2002;83:30-38.
59. Haorah J, Ramirez SH, Floreani N, Gorantla S, Morsey B, Persidsky Y. Mechanism of alcohol-induced oxidative stress and neuronal injury. Free Radic. Biol. Med. 2008;45(11):1542–1550.
60. Manzo-Avalos S, Saavedra-Molina A. Cellular and mitochondrial effects of alcohol consumption. Int. J. Environ. Res. Public Health. 2010;7(12):4281–4304.
61. Sathiavelu J, Senapathy GJ, Devaraj R, Namasivayam N. Hepatoprotective effect of chrysin on prooxidant-antioxidant status during ethanol-induced toxicity in female albino rats. J. Pharm. Pharmacol. 2009; 61(6):809–817.
62. Ramezani A, Goudarzi I, Lashkarboluki T, Ghorbanian MT, Abrari K, Salmani ME. Role of oxidative stress in ethanol-induced neurotoxicity in the developing cerebellum. Iran. J. Basic Med. Sci. 2012;15(4):965.
63. Livingstone C, Davis J. Review: Targeting therapeutics against glutathione depletion in diabetes and its complications. Br. J. Diabetes Vasc. Dis. 2007;7(6):258–265.
64. Agarwal DP. Genetic polymorphisms of alcohol metabolizing enzymes. Pathol. Biol. 2001;49(9):703–709.
65. Oh SI, Kim CI, Chun HJ, Park SC. Chronic ethanol consumption affects glutathione status in rat liver. J. Nutr. 1998;128(4): 758–763.
66. Meydan D, Gursel B, Bilgici B, Can B, Ozbek N. Protective effect of lycopene against radiation-induced hepatic toxicity in rats. Journal of International Medical Research. 2011;39(4):1239–1252.
67. Murray RK, Granner DK, Mayes PO, Rodwell VW. Harper's illustrated biochemistry. 26th Edn., Appleton and Lange Medical Publication/McGraw Hill, USA. 2003;693.
ISBN-13: 9780071389013.
68. Halliwell B, Gutterberidge JMC. Free radicals in biology and medicine. 4th Ed. Oxford, UK: Oxford University; 2006.
69. Yang SS, Huang CC, Chen JR, Chiu CL, Shieh MJ, Lin SJ, Yang SC. Effects of ethanol on antioxidant capacity in isolated rat hepatocytes. World J. Gastroenterol. 2005;11(46):7272.
70. Shirpoor A, Minassian S, Salami S, Khadem-Ansari MH, Yeghiazaryan M. Alpha-lipoic acid decreases DNA damage and oxidative stress induced by alcohol in the developing hippocampus and cerebellum of rat. Cell. Physiol. Biochem. 2008;22(5–6):769–776.

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