Ameliorative Efficacy of Methanolic Extract of *Corchorus olitorius* Leaves against Acute Ethanol-Induced Oxidative Stress in Wistar Rats

ABSTRACT

Aim: This study is aimed at investigating the ameliorative efficacy of methanolic extract of *C. olitorius* leaves against acute ethanol-induced oxidative stress in ~~wistar~~ Wistar rats.

Methods: Fresh plants of *C. olitorius* were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 14 days and milled into powder using an electronic blender. The extraction was done using soxhlet apparatus and methanol as the solvent. The methanol was evaporated in a rotary evaporate at 35 ^{o}C with a yield of 2.17 g which represents a percentage yield of 8.68%. The extract was preserved in the refrigerator until when needed. Twenty adult male ~~wistar~~ Wistar rats with body weight between 120 and 150 g were used for this study. They were acclimatized for seven days during which they were fed *ad libitum* with standard feed and drinking water. They were randomly divided into four groups of five rats each. Animals in groups 1 and 2 were administered saline solution while those in groups 3 and 4 were administered *C. olitorius* 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, SOD and GPX but decrease GSH. These effects were regulated by *C. olitorius* administration.

Conclusion: *C. olitorius* was able to remedy the effect of ethanol by regulating the oxidative stress biomarkers, thus possesses ameliorative efficacy against ethanol-induced oxidative stress and can protect the body against free radicals arising from oxidative stress.

Keywords: ameliorative efficacy, *C. olitorius* leaves, ethanol, oxidative stress

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1. INTRODUCTION

Corchorus olitorius (~~malvaceae~~Malvaceae) is a plant native to both tropical and subtropical regions throughout the world with mallow leaves commonly consumed as a leafy vegetable. The leaves have been reportedly used in ethnomedical practices to treat ache and pain, dysentery, malaria, enteritis, fever, gonorrhea, pectoral pains and tumors [1]. *C. olitorius* is a green leafy vegetable popularly consumed among the Yorubas of southwestern Nigeria where it is commonly called Ewedu. Among the Igbos of southeastern, Nigeria, it is called Ahihara, while in English, the plant is known as jute mallow or bush okra. *C. olitorius* plant is not found in Nigeria only but also in other countries such as Egypt, Sudan, Malaysia, South America, and the Caribbean [2,3,4]. Nutritional substances; including calcium, potassium,

phosphate, iron, ascorbic acid, carotene and large amount of mucilaginous polysaccharides have all been identified in the plant [5]. ~~The phytochemical composition and its toxicity have also been investigated.~~ Medicinally, *C. olitorius* are used as a demulcent, diuretic, purgative, bitter tonic, laxative, refrigerant, carminative and lactagogue [6]. The leaves extract has given positive results in the management of chronic cystitis and dysuria. Its reported high antibacterial activity gives credence to its use traditionally for the treatment of dysentery, fever and gonorrhea [5,6]. Its hypoglycemic and hypolipidaemic effect has also been reported [7].

Excessive acute or chronic alcohol consumption poses a serious health hazard and can result into several metabolic disorders in hepatic and extra-hepatic diseases [8]. 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 [9,10]. Alcohol overuse is also characterized by central nervous system (CNS) intoxication symptoms, impaired brain activity, poor motor coordination, and behavioral changes [11]. Excessive alcohol consumption commonly causes hepatic, gastrointestinal, nervous and cardiovascular injuries leading to physiological dysfunctions [12]. 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 [13,14]. 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 [15]. 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 [16].

There is an increasing global interest concerning the use of medicinal plants in the prevention and treatment of different pathologies [17, 18]. The beneficial effects of plants are attributed to the presence of secondary metabolites such as polyphenols, tannins, terpenoids, alkaloids, flavonoids [19]. 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 [20]. Many plants have been shown to possess antioxidant potentials [21,22]. 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 [23,24]. 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 [25,26]. These constituents selectively target toxins and pathogens without significant detrimental effect on the human host. This study therefore sought to investigate the ameliorative efficacy of *C. olitorius* leaves against acute ethanol-induced oxidative stress in ~~wistar~~ Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Material

Fresh plants of *C. olitorius* were harvested from the Institute of Agricultural Research and

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Training, Moor Plantation, Ibadan and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 14 days and milled into powder using an electronic blender (Moulinex). The extraction was done using soxhlet apparatus and methanol as the solvent. About 25 g of the powder was packed into the *thimble* of the soxhlet extractor~~.~~ ~~and~~ 250 ml of methanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle. The solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the methanol was evaporated in a rotary evaporate at 35 ^{o}C with a yield of 2.17 g which represents a percentage yield of 8.68%. The extract was preserved in the refrigerator until when needed.

2.2. Animal Treatment

Twenty adult male ~~wistar~~ 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 *C. olitorius* 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 [27]. 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~~for further analysis.

2.3 Biochemical Analyses

2.3.1 Determination of Hepatic Marker Enzymes Activities

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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 [28].

2.3.2 Determination of Oxidative Stress Biomarkers

Determination of Lipid Peroxidation (LPO), Reduced Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione peroxidase (GPX) were carried out according to the methods previously described by Airaodion et al. [29].

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~~ analysis of ~~Variance~~ 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

One major finding of this study was that *C. olitorius* leaves indeed unhinged and perturbed

the activities of hepatic marker enzymes and oxidative stress biomarkers in the animals used. These perturbations were reflected as up/down regulation of the activities of these enzymes as shown in **Tables 1** and **2**.

Table 1: Effect of *C. olitorius* on Hepatic hepatic Marker marker Enzymes enzymes of Experimental experimental Ratsrats.

Hepatic Marker Enzymes	Control	70% Ethanol only	*C. olitorius* Extract only	*C. olitorius* Extract + 70% Ethanol
AST (IU/L)	110.27 ± 3.22^{ac}	132.84 ± 5.78^{b}	101.22 ± 3.53^{a}	109.06 ± 3.83^{c}
ALT (IU/L)	46.85 ± 2.17^{a}	56.49 ± 3.64^{b}	44.45 ± 1.32^{a}	48.13 ± 2.92^{c}
ALP (IU/L)	15.60 ± 2.57^{ac}	22.79 ± 1.82^{b}	12.84 ± 1.23^{a}	17.41 ± 2.62^{c}
LDH (IU/L)	184.40 ± 9.13^{ac}	206.55 ± 10.10^{b}	197.18 ± 11.29^{a}	191.83 ± 14.05^{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 *C. olitorius* on Oxidative oxidative Stress stress Biomarkers biomarkers of Experimental experimental Ratsrats.

Oxidative Stress Biomarkers	Control	70% Ethanol only	*C. olitorius* Extract only	*C. olitorius* Extract + 70% Ethanol
LPO (nmol MDA/mg protein)	11.76 ± 1.08^{a}	18.91 ± 2.27^{b}	10.02 ± 1.38^{a}	12.53 ± 1.27^{a}
GSH (mg/ml)	4.68 ± 0.10^{a}	3.06 ± 0.78^{b}	5.06 ± 0.57^{a}	4.28 ± 0.82^{a}
CAT (Mm H_2O_2/mg protein)	13.04 ± 1.04^{a}	22.00 ± 2.29^{b}	11.56 ± 2.43^{a}	17.34 ± 1.85^{c}
SOD (U/mg protein)	8.62 ± 1.21^{a}	13.93 ± 2.23^{b}	8.91 ± 1.28^{a}	9.63 ± 1.22^{a}
GPX (U/mg protein)	6.08 ± 0.16^{a}	8.73 ± 1.17^{b}	5.24 ± 0.84^{a}	6.11 ± 1.23^{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

4. DISCUSSION

Green leafy vegetables and fruits have been reported to have some health benefits [21,22]. Consequently, *C. olitorius* leaf is a green leafy vegetable popularly used as food and in traditional medicine for the management of some diseases [30]. However, there is dearth of information on the possible mechanisms of action by which these vegetables exert their health benefits. 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 [31,32]. The protective effect of antioxidants against alcohol-induced liver injury in many studies further supports the involvement of oxidative stress [30,33].

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 [34]. High levels of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) are an indicator of hepatotoxicity or liver diseases [35]. 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 [36]. Exposure to toxic chemicals causes alterations in some tissue enzyme activities [37,38]. 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 [39].

In this study, the activities of AST, ALT, ALP and LDH were not significantly different ~~when animals treated~~ upon treatment with *C. olitorius* extract only ~~were~~ when 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 pretreatment with *C. olitorius* extract were compared with those of the control and *C. olitorius* extract only groups at $p<0.05$ (table 1).

This might be an indication that ethanol causes liver damage to the animals. However, when animals pretreated with *C. olitorius* 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, ALP and LDH. This could be that pretreatment with *C. olitorius* extract resulted in increased transcription of some genes involved in glucose uptake, glycolysis and lipogenesis [40]. Glucose represses the induction of inducible operons by inhibiting the synthesis of cyclic ~~Adenosine~~ 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~~ adenosine ~~Triphosphate~~ triphosphate (ATP) [41,42]. 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 *C. olitorius* 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 [43]. The significant decrease observed in the activities of ALT and AST in *C. olitorius*-treated animals when compared to those induced without pretreatment showed that *C. olitorius* 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 [44]. It is often employed to assess the integrity of the plasma membrane, since it is localized predominantly in the microvilli in the bile

canaliculi, located in the plasma membrane. Since ALP ~~hydrolyses~~ hydrolyzes 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 as it may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver [45]. 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 *C. olitorius* extract.

The elevation in the activities of markers such as ALT, AST and LDH in the liver tissue of animals without pretreatment with *C. olitorius* 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 [46]. According to Kottaimuthu [47], 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 increase, antioxidant defense system decrease equivalently resulting in oxidative stress [48]. This suggests that the administration of ethanol might have weakened the liver membrane of the rats with subsequent penetration and elevation of the hepatic biomarker enzymes.

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 [49,50]. Lipid peroxidation, a primary mechanism of cell membrane destruction and cell damage is a common feature of both acute and chronic alcohol consumption [51,52]. 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. *C. olitorius* 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. [46] who reported the hepato- and neuro-protective effects of watermelon juice on acute ethanol-induced oxidative stress in rats. It is also in agreement with the report of Airaodion et al. [29] who study the hepatoprotective effect of *parkia biglobosa* on acute ethanol-induced oxidative stress in wistar 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 [52]. 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. [31] 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 *C. olitorius*-treated rats prior to ethanol-administration may be due to the direct ROS—scavenging effect of *C. olitorius* or an increase in GSH synthesis. This is consistent with the report of Airaodion et al. [29] who study the hepatoprotective effect of *parkia biglobosa* on acute ethanol-induced oxidative stress in ~~wistar~~ Wistar rats.

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 [53]. In this study, a significant increase was observed in the activity of catalase in control animals and those treated with *C. olitorius* extract only when compared with ethanol-induced animals with *C. olitorius* extract pretreatment. This contradicts the findings of Airaodion et al. [29] who reported a non-significant difference when animals were treated with *parkia biglobosa*. The activity of catalase in animals pretreated with *C. olitorius* prior to ethanol induction was significantly reduced when compared with those without *C. olitorius* pretreatment. This might be that ethanol-induced oxidative stress generated elevated ROS in the liver which CAT tend to combat, thereby increasing its activity. *C. olitorius* 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 Orieke et al [54]. Increased CAT activity in this study following acute ethanol exposure suggests elevated ethanol oxidation and formation of ~~oxidising~~ oxidizing product-acetaldehyde. This is in agreement with the study of Airaodion et al. [29] and Oyenihi et al. [46] 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 [55]. These radicals have been reported to be deleterious to polyunsaturated fatty acids and proteins [56]. In this study, no significant difference was observed in the activity of SOD in control animals and those treated with *C. olitorius* extract only when compared with ethanol-induced animals with *C. olitorius* extract pretreatment. The activity of SOD in animals pretreated with *C. olitorius* prior to ethanol induction was significantly reduced when compared with those without *C. olitorius* pretreatment. This might be that ethanol-induced oxidative stress generated elevated ROS in the liver which SOD tend to combat thereby increasing its activity. *C. olitorius* 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 Orieke et al [54]. The increased activity of SOD observed in ethanol induced animals contradicts the study of Halliwell and Gutterberidge [57] who reported that SOD activity was considerably reduced during ethanol intoxication.

Glutathione ~~preroxidase~~ peroxidase (GPX) is another enzymic antioxidant that acts as a defense against oxidative stress. In this study, no significant difference was observed in the activity of GPX in control animals and those treated with *C. olitorius* extract only when compared with ethanol-induced animals with *C. olitorius* extract pretreatment. The activity of GPX in animals pretreated with *C. olitorius* prior to ethanol induction was significantly reduced when compared with those without *C. olitorius* pretreatment. This might be that ethanol-induced oxidative stress generated elevated ROS in the liver which GPX tend to combat thereby increasing its activity. *C. olitorius* was able to reduce the ROS generation with subsequent decrease in GPX activity due to its high phytochemical content and antioxidant potential as reported by Orieke et al [54]. The increased activity of GPX observed in ethanol induced animals contradicts the studies of Airaodion et al. [29] who observed no significant difference in

the activity of GPX in the study of hepatoprotective effect of *parkia biglobosa* on acute ethanol-induced oxidative stress in wistar rats and that of Yang et al. [58] who also observed no significant difference in GPX activities in rats hepatocyte exposed to varying concentrations of ethanol at an incubation time of 12 hours. 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 [53,59]. Catalase and glutathione peroxidase further detoxify H_2O_2 into H_2O and O_2 [56]. Thus, SOD, catalase and GPX function mutually as enzymatic antioxidative defense mechanism to counter the deleterious effect of ROS.

45. CONCLUSIONS

The results of this study indicated ~~that indeed~~ ethanol induced oxidative stress as shown in the perturbation of the biomarkers. *C. olitorius* leaves was able to remedy this effect by regulating the oxidative stress biomarkers, thus possesses ameliorative efficacy against ethanol-induced oxidative stress and can protect the body against free radicals arising from oxidative stress. Regular consumption of this vegetable is hereby recommended.

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Comment [SaA5]: Title?????????????