Full Length Research Paper

Protective effects of *Pleurotus ostreatus* in ameliorating carbon tetrachloride (ccl₄) induced liver injury in Wistar rats

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Since oxidative stress is implicated in the pathogenesis of liver diseases, this study was carried out to evaluate the hepatoprotective, hematoprotective and hypolipidemic effects of *Pleurotus ostreatus* (Oyster mushroom) in Carbon Tetrachloride (CCl₄) induced liver injury in wistar rats. Thirty rats were used for this study. The rats were divided into five groups of six rats per cage. Group I that served as the normal control received distilled water only. Groups II to V served as test groups. Group II received CCl₄ at a dose of 1 ml/kg body weight on the 14th day and 28th day only. Groups III, IV and V received CCl₄ at a dose of 1 ml/kg body weight on the 14th day and 28th day, then, silymarin (100 mg/kg), 25% w/w and 50% w/w of powdered Oyster mushroom respectively. The results revealed that CCl₄ caused a significant (p<0.05) increase in lipid peroxidation judging from the significant (p<0.05) elevated level of malondialdehyde MDA in the hepatic tissues whereas the level or activities of reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) in the liver tissue was significantly (p<0.05) reduced. Liver peroxidation was followed by disruption of proper functioning of the hepatocytes as indicated by the significant increase of liver biomarker enzymes (AST, ALT, and ALP) and decreased serum levels of albumin and total protein. Dyslipidemia and hematotoxicity were also manifested via a significant (p<0.05) increase in the serum levels of triglyceride TG, total cholesterol TC, very low-density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol LDL-C and white blood cells count. These were accompanied by significant (p<0.05) reduction in the serum level of high density lipoprotein cholesterol HDL-C and RBC count and its differentials. Supplementation of powdered Oyster mushroom daily for a period of 28 days to rats led to reversal of these signs of toxicities. The ability of the powdered Oyster mushroom to mitigate against CCl₄-induced hepatotoxicity is probably due to its antioxidant and enzyme modulatory effects.

**Key words**: Hepatoprotective, hematoprotective, hypolipidemic, *Pleurotus ostreatus*, carbon tetrachloride, peroxidation.

INTRODUCTION

Toxic effects of chemicals on internal organs and cellular components such as liver, kidneys, brain, hematopoiesis, lipids, Proteins and nucleic acids have been a matter of public health concern (Rizwan et al., 2014). Carbon tetrachloride (CCl₄) is a common solvent used in various industrial processes and has been reported to be hepatotoxic. It is a potent inducer of oxidative stress and has been associated with the development of liver cirrhosis and hepatocellular carcinoma. The use of natural products as potential remedies for liver injury is gaining interest due to their potential to mitigate the adverse effects of CCl₄. *Pleurotus ostreatus* (Oyster mushroom) is a widely used medicinal mushroom known for its immune-stimulating and antioxidant properties. This study was conducted to assess the hepatoprotective, hematoprotective and hypolipidemic effects of *Pleurotus ostreatus* in CCl₄ induced liver injury in Wistar rats.
tetrachloride is used as an organic solvent in many industries. It is also used as a hepatotoxic agent for the purpose of studying pathogenesis of liver injury (Boll et al., 2001a). Its mechanism of toxicity is in connection with its ability to interact with membrane lipids thus causing peroxidation (Boll et al., 2001b). This process, however, involves series of biotransformation of CCl₄ by hepatic microsomal P₄₅₀ to produce the trichloromethyl radical (CCl₃⁻) which, in the presence of oxygen, is further converted to a peroxy radical (CCl₃OO⁻) involved in peroxidation of internal organs and cellular components (Sharifudin et al., 2013).

The end product of peroxidation that is malondialdehyde (MDA) or 4-hydroxynonenal (HNE), which is highly reactive aldehydes forms adducts with protein and DNA (Kadiiska et al., 2005). When excess production of this Reactive Oxygen Species (ROS) overwhelsms the endogenous antioxidant defense system a condition called oxidative stress occurs (Renugadevi and Prabu, 2010). This has been implicated in the pathogenesis of many diseases. The major antioxidant defenses systems are composed of antioxidant enzymes that include superoxide dismutate SOD, catalase CAT, glutathione peroxidase GPx and non-antioxidant enzymes (GSH) (Klivenyi et al., 2000). SOD defends against oxidative stress by catalyzing the dismutation of superoxide radicals (O₂⁻) into molecular oxygen (O₂) and hydrogen peroxide (H₂O₂) (Mates, 2000). H₂O₂ is neutralized by the combined action of CAT and GPx in all vertebrates (Mourelle et al., 1988).

Reports have shown that carbon tetrachloride causes increased lipid synthesis and a concomitant decrease in the transport of lipids out of the hepatocyte (Okolo et al., 2017; Jayakumar et al., 2008). This imbalance between the synthesis and degradation of lipids may be the direct cause of steatosis or fatty liver associated with CCl₄-induced hepatotoxicity (Kiezcka and Kappus, 1980). Silymarin and colchicine are natural products having antioxidant effects that were found to protect the liver from CCl₄-induced damage (Letteron et al., 1990) by inhibiting cytochrome P450 enzymes. Another natural substance is the hepatic stimulator substance (HSS), also an antioxidant; it protects the liver from failure induced by CCl₄ due to its ability to reduce peroxidation (Mao-Hua et al., 1993). Despite the acclaimed therapeutic uses of these natural products; there relatively scarcity limits their use for the treatment of liver and liver-related diseases. This implies there is a compelling need for a continued search for an efficient and readily available hepatoprotective agent from natural source that could either be used directly or serve as lead compounds for the treatment of liver disease.

Pleurotus ostreatus (Oyster mushroom) is a wood-rotting fungus produced on ligno-cellulose substrates that grow in the tropical and subtropical part of the world. It belongs to the class of basidiomycetes and Agaricaeae family. A hypolipidemic agent called Lovastatin has been isolated as the active chemical principle of P. ostreatus (Liu et al., 1997). Also, its high dietary fiber content, protein, microelements coupled with the presence of plant sterols make it a valuable therapeutic agent (Wasser and Weis, 1999). Vitamin B₁ and B₂ had been reported as constituents of the fungus. Oyster mushrooms contain polyphenols which are scavengers of free radicals (Antonia et al., 2002). Other therapeutic uses of P. ostreatus include inhibition of platelets aggregation and reduction of blood cholesterol (Borchers et al., 1999).

The presence of valuable therapeutic agents in the Oyster mushroom has captured our interest to investigate this fungus as a prospective candidate for the remedy of liver necrosis, hematoxotoxicity and dyslipedemia.

**MATERIALS AND METHODS**

**Collection and Identification of oyster mushrooms**

Oyster mushrooms were collected from decaying dead trees in Edo State, Nigeria. The plant materials were identified and authenticated by a mycologist in the Department of Botany, University of Benin, Edo State. The fresh mushroom samples were crushed to powder by using electronic blender. The powdered oyster mushrooms were then stored in an air-tight container and kept in the refrigerator at 4°C until use.

**Animals**

Thirty (30) adult Wistar rats of both sexes weighing 170-240 g were purchased from National Veterinary Research Institute (NVRI), Vom, Plateau State. Food and water were provided ad libitum. Animals were exposed to controlled environmental temperature (28 ± 2°C), relative humidity (50 ± 5%) and 12-h light or darkness. After obtaining ethical approval (ABUCAUC/2018/028), this study was conducted in accordance with the principle governing the handling procedures of experimental animals as laid down by the Ahmadu Bello University Committee on Animal Use and Care. All the animal care and treatment procedures were respected following the guidelines established by the University's committee on animal use and care. Efforts were made to minimize the number of animals used and their sufferings by strictly following the ethical guidelines for investigations of experimental pain in conscious animals as described.

**Acute toxicity study**

Acute oral toxicity (AOT) of oyster mushrooms was investigated using Wister rats in a method described by Lorke (1983). The animals were fasted for 12 h (overnight) prior to the experiment.

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The animals were divided into five groups of six animals each and were administered with single dose of oyster mushroom powder orally at doses of 1, 2, 4, 6 and 8 g/kg body respectively. The animals were observed for mortality up to 48 h (acute) and for another 14 days for sub chronic toxicity. Calculated LD₅₀ was done after oral administration of variable doses.

**Preparation of oyster mushroom**

The grower mash fed to the animals consisted of 25 and 50% w/w of oyster mushroom.

**Grouping of the experimental animals**

The CCl₄ was prepared as described previously (Okolo et al., 2017): rats were divided into five groups, each group consisting of six animals thus: Group I: (Control) received 0.5 ml of distilled water daily for a period of 28 days; Group II: (CCl₄−induced) animals received 0.5 ml of distilled water daily for 28 days and CCl₄ at a dose of 1 ml /kg body weight on the 14th day and 28th day; Group III: animals (Standard control) received aqueous form of silymarin at a dose of 100 mg/kg body weight daily for 28 days and CCl₄ at a dose of 1 ml /kg body weight on the 14th day and 28th day; Group IV: animals received grower mash supplemented with 25% w/w of powdered Oyster mushroom daily for 28 days and CCl₄ at a dose of 1 ml /kg body weight on the 14th day and 28th day; Group V: animals received grower mash supplemented with 50% w/w of powdered Oyster mushroom daily for 28 days and CCl₄ at a dose of 1 ml /kg body weight on the 14th day and 28th day. After 24 h of the last treatment, all the animals were fasted overnight and were anaesthetized with chloroform. They were then sacrificed by cervical decapitation and the blood was collected into heparinized and non-heparinized tubes. Serum was collected from the non-heparinised tubes by centrifugation at 4000 rpm for 15 min and was stored at 20°C till analysis. The liver tissue was collected and perfused with normal saline to remove blood and used for the preparation of tissue homogenate.

**Assay for liver function biomarkers**

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured spectrophotometrically by the methods described by Reitman and Frankel (1957), and Belfield and Goldberg (1971) respectively. Total protein was determined by the method of Henry (1964).

**Lipid profiles assay**

The serum levels of triglyceride and total cholesterol were determined according to the principle described by Tietz (1990) and Siedel (1983), respectively. The HDL-cholesterol levels were determined by the method of Assmann (1983) and LDL-cholesterol levels were calculated using Friedwald’s formula (1972).

**Antioxidant assay**

**Lipid peroxidation (LPO)**

This was estimated by measuring MDA in the liver homogenate using the method described by Ohkawa et al (1979). The principle involves measuring the absorbance of pink colour complex formed from the reaction of MDA with thiobarbituric acid in acidic medium at 534 nm. Absorbance was expressed in nmol/g protein.

**Determination of enzymatic antioxidants in liver homogenate**

**Catalase activity (CAT)**

The activity of CAT was measured as described by Aebi (1984). The principle involves the reaction of CAT with excess H₂O₂. Exactly after a minute, the remaining H₂O₂ reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore. The colour intensity was inversely proportional to the absorbance measured at 240 nm. The enzymatic activity of CAT was expressed in units/mg cellular protein.

**Superoxide dismutase activity (SOD)**

The activity of SOD was assayed according to the method of Nishikimi et al. (1979). The principle of this method involves the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye (NBT). The absorbance was read at 505 nm using a spectrophotometer and the enzyme activity of SOD was expressed in units/mg cellular protein. All CCl₄ administrations were made intraperitoneally.

**Glutathione peroxidase activity (GPx)**

The activity of GPx was determined spectrophotometrically (Paglia and Valentine, 1967). GPx catalyzes the oxidation of glutathione. Where glutathione reductase and NADPH are present, the oxidized glutathione is immediately converted to the reduced form with a simultaneous oxidation of NADPH to NADP⁺. GSH-Px activity was measured at 340 nm by the decrease of NADPH absorbance using extinction coefficient of 6.22 mM expressed in unit/mg-protein.

**Assay of reduced glutathione (GSH) level**

The level of reduced glutathione was assayed using the colorimetric method described by Beutler et al. (1963).

**Determination of glutathione transferase (GST) level**

The activity of GST was assayed by monitoring the reaction between 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione. The reaction was accompanied by an increase in absorbance which was measured at 340 nm. The rate of increase is directly proportional to the GST activity in the sample (Habig et al., 1974).

**Hematological assay**

White blood cells (WBCs), Red blood cells (RBCs) counts, hemoglobin concentration (Hb), hematocrit percent (Hct %), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were determined using a Neubauer counting chamber Japan, (Dacie and Lewis, 1991).

**Statistical analysis**

The data were expressed as mean± SD and the difference among treatment groups was analyzed using one-way analysis of variance.
RESULTS

The results obtained from this study revealed that CCl₄ causes a significant (p<0.05) increase in lipid peroxidation; judging from the significant (p<0.05) elevated level of malondialdehyde MDA in the hepatic tissues whereas the levels or activities of reduced glutathione, catalase, superoxide dismutase, glutathione peroxidase and glutathione S-transferase in the liver tissues were significantly reduced (p<0.05). This was also followed by a significant increase in liver biomarker enzymes such as AST, ALT, and ALP with a corresponding decrease in the serum levels of albumin and total protein. Dyslipidemia and hepatotoxicity were also manifested via a significant (p<0.05) increase in the serum levels of TG, TC, VLDL-C, LDL-C and WBC count. These were accompanied by a significant (p<0.05) reduction in the serum levels of HDL-C and RBC count and its differentials. Supplementation of powdered oyster mushroom daily for a period of 28 days to the sample animals studied led to reversal of these signs of toxicities. There was no death recorded following the acute toxicity studies, suggesting that Pleurotus ostreatus has no associated toxicity (Tables 1 to 4; Figure 1).

DISCUSSION

The liver is susceptible to toxic substances because of its key role in neutralizing and excreting xenobiotics. In fact,
it has been reported that over 700 drugs are involved in the pathogenesis of liver injury (Friedman et al., 2003). The mechanism of CCl₄-induced hepatic injury involves rapid biotransformation of CCl₄ to trichloromethyl radical (CCl₃), and trichloromethyl peroxyx which are very reactive radicals that causes lipid peroxidation and decreased activities of antioxidant defense system in the liver tissues (Muriel, 1997). The alarming increase of drug-induced liver injury indicated that there is need for continuous search for hepatoprotective agents from medicinal plants.

In this present study, intraperitoneal injection of CCl₄ resulted in a significant (p<0.05) increase of malondialdehyde MDA level in the liver accompanied by a significant (p<0.05) decrease in the activities or level of GSH, CAT, SOD, GPx and GST in the hepatic tissues. This observation could be attributed to free radical generation from the peroxidation of polyunsaturated fatty acids measured as MDA and lipid hydroperoxides LPO. These findings conform with previous studies as reported by Poli (1993). The increase in MDA level in this study confirmed the pro-oxidant and hepatotoxic effect of CCl₄, manifested likely due to the failure of antioxidant defense mechanisms in the hepatocytes to prevent the formation of excessive free radicals (Al-Dosari, 2010).

The observed decreased in the activities of the enzymatic and the non-enzymatic antioxidant system in the hepatic tissue of the sample animals injected with CCl₄ may perhaps be an indication of overwhelming of the system by the free radicals generated. This does not however mean reduction in the amount of the enzymes but an indication of saturation as a result of increased...
utilization of non-enzymatic and enzymatic antioxidant system. Supplementation of powdered Oyster mushroom daily for a period of 28 days to the sample animals investigated in this study led to increased level and activities of these non-enzymatic and enzymatic antioxidant systems respectively. This strongly suggests the antioxidant potential of Oyster mushroom.

Consequent to the liver membrane damage by CCl₄OO- following the intraperitoneal injection of CCl₄, activities of liver biomarker enzymes such as AST, ALT and ALP, assayed in this study commonly refer to as liver function indices or markers increased tremendously in the plasma. The observed increase in the activities of these liver enzymes was likely as a result of leakage of these enzymes from liver tissue into the plasma. These leakages could be due to LPO of cell membranes which had been reported to be the cause of loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability. Interestingly, from this study, Supplementation of powdered Oyster mushroom daily for a period of 28 days reversed these hyper activities of these enzymes to nearly normal activities as compared to normal rats (group I). This above observation strongly suggests the hepato-protective effect of Oyster mushroom and may be related to its ability to mitigate against LPO which in turn stabilizes the integrity of the hepatic tissue membranes thus preventing the leakage of these liver enzymes. Albumin and other proteins are synthesized in the liver and an impairment of the liver affects its ability to synthesize these biomolecules. Low serum levels of these proteins are considered a sign of hepatotoxicity.

In this study, intraperitoneal injection of CCl₄ resulted in a significant (p<0.05) decrease in the serum levels of albumin and total protein as compared to the normal rats (group I). This may suggest the inhibition of these proteins synthesis by CCl₄-generated peroxy radical (CCl₃OO-). Our findings is inconsistent with previous studies as reported by Wessam (2013).The results from this study also show that supplementation of grower mash with 50% powdered Oyster mushroom daily for 28 days caused an increase (p<0.05) in serum level of albumin and total protein as compared to the CCl₄ injected rats (group II).This may indicate the ability of the Oyster mushroom to mitigate against oxidative liver damage thus restoring liver synthetic function. Liver plays a key role in the metabolism and transport of lipids. The results of this findings reveal a significant (p<0.05) increase in the serum levels of triglyceride TG, total cholesterol TC, very low density lipoprotein cholesterol VLDL-C and low density lipoprotein cholesterol LDL-C accompanied by a significant (p<0.05) reduction in the serum level of high density lipoprotein cholesterol HDL-C upon intraperitoneal injection of CCl₄ as compared to normal rats (control I). These results are in tandem with the findings of El-Habibi et al. (2009). Hyperlipidemic effect of CCl₄ as evident in this study may be related to its positive effect on acetate transport to the liver, esterification of fatty acids and disruption of membrane phospholipid. Others may include inhibition of synthesis of the bile acids and fatty acid β oxidation (Elshater et al., 2013).

Supplementation of grower mash with 50% powdered Oyster mushroom daily for 28 days reversed these elevated lipid profiles with concomitant increase in serum level of high density lipoprotein cholesterol HDL-C. Although an Oyster mushroom hypolipidemic effect is poorly understood, it may likely be related to inhibition of oxidative stress and hydroxymethylglutary-CoA (enzyme that catalyses the rate of limiting step of cholesterol biosynthesis) (Maduka et al., 2014). Intraperitoneal injection of CCl₄ to rats in this study also revealed alteration in hematological parameters of the rats.

The results showed that there was significant increase (P < 0.05) in WBC count with a corresponding decrease in RBC count and its differentials as compared to normal control. Our findings are consistent with the findings of Saba et al. (2010). The decreased RBCs count and its indices might be attributed to the oxidative stress imposed by CCl₄ injection (Sule et al., 2012). Destruction of hematopoiesis with consequent reduction in the rate of formation of RBC and its indices may be another reason for this reduction (Essawy et al., 2010).Whereas the increase in the WBCs count might be due to the defensive mechanism of immune system (Oluyemi et al., 2007).

Supplementation of powdered Oyster mushroom daily for 28 days to sample rats investigated led to a reversal of the altered hematological parameters to near normal level, suggestive of heptato-protective potentials. In this study, supplementation of powdered Oyster mushroom at the dose of 50% of the feed given to the rats appears to be more effective as compared to group iii which received silymarin.

Conclusion

In conclusion, daily supplementation of powdered Oyster mushroom subsided greatly dyslipidemia, hepatotoxicity and hematotoxicity associated with injection of CCl₄. Although its mechanism of action is unknown, it is believed to be associated with its ability to mitigate against LPO which in turn stabilizes the integrity of the hepatocyte membrane. Others may include enhancing the activities of enzymatic and non-enzymatic antioxidants in the liver. Research is in progress to isolate the bioactive compound responsible for these observed positive effects.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.
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