Assessment of Ameliorative Properties of Methanol Extract of Pleurotus ostreatus Cultivated with Extract of Allium cepa on Oxidative Stress Markers of CCl₄ Induced Hepatotoxicity in Wistar Rats

Ogbeifun, H. E., Anacletus, F. C. and Ighorodje, C.C.

Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.

Introductio

The large size of the liver is well positioned and properly organized to carry out a major role in protein, fat and carbohydrate metabolism. It is the place where metabolites and waste products like ammonia are converted to harmless substances. It also serves a role of producing plasma proteins and maintaining stable blood glucose concentration. It participates in the release of glucose from its storage form (glycogen) decomposing it to glucose when required (glycogenolysis) and producing glucose from non-carbohydrate like ammonia (gluconeogenesis) (Ward and Daly, 1999; Kniec, 2001; Pocock and Gillian, 2006; and Krishnendu, 2012).

Diseases associate with liver have become a major problem all over the world and are connected with high rate of diseases that result to death (Baranisrinivasan et al; 2009). In developed countries, the major cause of liver disease is excessive alcohol consumption and viral induced chronic liver diseases while in developing countries hepatitis B and C viruses, parasitic diseases, hepatotoxic drugs, high doses of paracetamol and environmental toxins are the common frequent causes.

Herbal medicine and plant-based preparations are used to alleviate diseases. Over the past thirty years, the use of herbal drugs have increased as it is known that if used properly, herbs can help treat various conditions and have fewer side effects compared to conventional medicine (Kala et al., 2006). Plants can synthesize different chemical compounds and some of these compounds exhibit pharmacological activity, this is the basis of herbal drug. These plants contain bioactive compounds that can affect one or more identified biological process such as improving homeostasis, free radical scavenging ability, cholesterol lowering capability, anti-inflammatory, antimicrobial, antiviral, anticancer and antiparasitic activity (Manjulika et al; 2004).

Materials and Methods

Experimental Plants

Red Allium cepa (Onion) bulbs and Pleurotus ostreatus fruiting bodies were bought from Choba Market, Choba, Port Harcourt, Rivers State and identified at Department of Plant Science, University of Port Harcourt, Rivers State Nigeria.

Experimental Animals

Seventy Wistar rats weighing between 100-200g of three months old breed, purchased from Department of Biochemistry, University of Port Harcourt Animal House were used for this research. The animals were Randomly Selected,
weighed and distributed into seven groups. These animals were put in plastic cages and left under suitable laboratory conditions for two weeks to acclimatize to the new environment. The cages were cleaned daily. The animals were fed with commercial growers mash product of Top feeds Ltd., Sapele, Nigeria and water served ad libitum. The animals' body weights were recorded before commencement of treatment.

Methods

Preparation of Allium cepa extract by soxhlet method.

Fresh, healthy Red bulbs of Allium cepa were washed, sliced into small pieces and blended in a warring blender. Four hundred grams of the sample was placed in a Soxhlet extractor that was inserted on a filter paper. The extractor was connected to a pre-weighed dried distillation flask and acetone was poured into the distillation flask through the condenser, joined to the Soxhlet extractor. This set up was clamped on a retort stand. Cold water from the jet was permitted to move continuously into the condenser, and the heated solvent refluxed as a result. Onion sample in the solvent that was poured into the distillation flask was extracted in the process of refluxing continuously. When onion extract was observably extracted completely from the sample under test, the condenser and the extractor were disconnected, and the solvent was heated to concentrate the onion extract. Air oven was used to dry the flask to constant weight and re-weighed to get the crude weight of onion extract (Sheema et al., 2015).

Method of cultivation of Pleurotus ostreatus fruiting body with extract of Allium cepa bulb.

This involves 5 stages:

(i) Preparation of tissue culture of Pleurotus ostreatus using Potato Dextrose Agar (PDA) medium.

Potato Dextrose Agar (PDA) medium was prepared by pealing 200g Irish potatoes and boiled in water for some minutes. This was filtered and the filtrate was made up to 1000ml. Twenty grams of glucose and 20 grams of powdered Agar were added to the filtrate and this was stirred properly and shared into two conical flasks of 500ml which were covered with cotton wool and foil paper held with rubber band. The two conical flasks were placed inside the pressure pot and sterilized for 15 minutes. This was filtered and the filterate was made up using Potato Dextrose Agar (PDA) medium. This involves 5 stages:

(ii) Mushroom substrate preparation

The substrate used were 93kg of saw dust, 7kg of wheat brown, 400g of calcium carbonate and 60 to 65% of water. These were mixed properly after which they were bagged and sterilized for 4 hours before the spawns were transferred into them and were kept in the incubation room for one month for ramification to occur.

(v) Development of the full grown fruiting bodies of Pleurotus ostreatus

After ramification had taken place, the bags were cut open and were watered for duration of two weeks before the fruiting bodies started emerging and were harvested after maturation (Vasil and Thorpe, 1998).

Methanol extraction of Pleurotus ostreatus cultivated with Allium cepa extract

Dried Pleurotus ostreatus were blended in a warring blender, 300 grams of Pleurotus ostreatus sample was macerated in 300ml of methanol for three days in a macerating jar. Then the sample was filtered using a Whatman No.1 filter paper. The filtrate was concentrated with a rotary evaporator at 65°C and was finally dried in a thermostat water bath at 60°C to become an extract (Chaturvedi, 2011).

Table 1.1. Result of the Effect of Methanol Extract of Pleurotus ostreatus on Superoxide Dismutase (SOD) activities (unit/mg).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Day10</th>
<th>Day20</th>
<th>Day30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control (NC).</td>
<td>2.40±0.05abc</td>
<td>2.60±0.07abc</td>
<td>2.58±0.18abc</td>
</tr>
<tr>
<td>2</td>
<td>CCl₄ treated only.</td>
<td>0.29±0.00ab</td>
<td>0.69±0.00bc</td>
<td>0.79±0.00ab</td>
</tr>
<tr>
<td>3</td>
<td>CCl₄ + 100mg/kg extract.</td>
<td>0.72±0.04ab</td>
<td>1.78±0.33bc</td>
<td>1.11±0.00ac</td>
</tr>
<tr>
<td>4</td>
<td>CCl₄ + 200mg/kg extract.</td>
<td>0.68±0.13b</td>
<td>2.13±0.02b</td>
<td>1.47±0.25</td>
</tr>
<tr>
<td>5</td>
<td>CCl₄ + 300mg/kg extract.</td>
<td>0.94±0.03ab</td>
<td>2.00±0.00b</td>
<td>1.89±0.33</td>
</tr>
<tr>
<td>6</td>
<td>CCl₄ + 5.2mg/kg Livolin.</td>
<td>0.50±0.24ac</td>
<td>1.95±0.00ce</td>
<td>1.32±0.32ac</td>
</tr>
<tr>
<td>7</td>
<td>CCl₄ + 200mg/kg extract+5.2mg/kg Livolin+50mg/kg Vitamin C.</td>
<td>1.07±0.04abc</td>
<td>2.33±0.33bc</td>
<td>2.33±0.33bc</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± Standard error of mean; n =3 per group.

Values in the same column with common superscript letter (a, b, c) are significantly different at P < 0.05.

Superscript A (a) represents significant difference when group 1 is compared to other groups at P < 0.05.

Superscript B (b) represents significant difference when group 2 is compared to other groups at P < 0.05.

Superscript C (c) represents significant difference when group 6 is compared to other groups at P < 0.05.

Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at P < 0.05.
(vi) Catalase activity determination

The activity of Catalase was determined as described by Brisswanger, (2004). Catalase acts to prevent accumulation of H₂O₂, by converting it to O₂ and H₂O. This composition of H₂O₂ was monitored spectrophotometrically at 480nm.

(vii) Determination of Superoxide Dismutase (S.O.D) Activity

The activity of SOD was determined as described by Fridovich (1997). Adrenaline auto-oxidizes rapidly in aqueous solution to adreno-chrome, whose concentration can be determined at 420nm using spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme SOD inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anions; the degree of inhibition is a reflection of the activity of SOD and is determined at one unit of the enzyme activity.

(viii) Thiobarbituric Acid Reactive Substances Assay (TBARS)

TBARS assay is an establishment for quantifying lipid peroxidation by measuring the formation of TBARS according to the method of Tripathi et al., (2001). This assay is based on the reaction of a chromogenic reagent, 2 thiobarbituric acid with malondialdehyde (MDA) at 25°C to give a red species absorbing at 535nm.

Statistical Analysis

### Table 1.2. Result of the Effect of Methanol Extract of *Pleurotus ostreatus* on Catalase (CAT) activities (unit/mg).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control (NC).</td>
<td>1.39±0.00ₐ</td>
<td>1.31±0.01ₐ</td>
<td>1.47±0.03ₐ</td>
</tr>
<tr>
<td>2</td>
<td>CCl₄ treated only.</td>
<td>0.38±0.00ₐ</td>
<td>0.20±0.00ₐ</td>
<td>0.23±0.00ₐ</td>
</tr>
<tr>
<td>3</td>
<td>CCl₄ + 100mg/kg extract.</td>
<td>1.07±0.30</td>
<td>0.98±0.06</td>
<td>0.75±0.17</td>
</tr>
<tr>
<td>4</td>
<td>CCl₄ + 200mg/kg extract.</td>
<td>1.48±0.33</td>
<td>1.16±0.15</td>
<td>0.88±0.20</td>
</tr>
<tr>
<td>5</td>
<td>CCl₄ + 300mg/kg extract.</td>
<td>1.59±0.10ₐ</td>
<td>1.23±0.12ₐ</td>
<td>0.99±0.34</td>
</tr>
<tr>
<td>6</td>
<td>CCl₄ + 5.2mg/kg Livolin.</td>
<td>0.96±0.35ₐ</td>
<td>1.05±0.01ₐ</td>
<td>0.75±0.07ₐ</td>
</tr>
<tr>
<td>7</td>
<td>CCl₄ + 200mg/kg extract+5.2mg/kg Livolin+ 50mg/kg Vitamin C.</td>
<td>1.55±0.16ₐ</td>
<td>1.28±0.07ₐ</td>
<td>1.19±0.16ₐ</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± Standard error of mean; n =3 per group.

Values in the same column with common superscript letter (a, b, c) are significantly different at P < 0.05.

Superscript A (⁺) represents significant difference when group 1 is compared to other groups at P < 0.05.

Superscript B (⁺⁺) represents significant difference when group 2 is compared to other groups at P < 0.05.

Superscript C (⁺⁺⁺) represents significant difference when group 6 is compared to other groups at P < 0.05.

Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at P < 0.05.

### Table 1.3. Result of the Effect of Methanol Extract of *Pleurotus ostreatus* on TBARS Levels (umol/mg).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control (NC).</td>
<td>1.12±0.00ₐ</td>
<td>1.20±0.00ₐ</td>
<td>1.18±0.00ₐ</td>
</tr>
<tr>
<td>2</td>
<td>CCl₄ treated only.</td>
<td>1.31±0.01ₐ</td>
<td>1.30±0.01ₐ</td>
<td>1.36±0.10ₐ</td>
</tr>
<tr>
<td>3</td>
<td>CCl₄ + 100mg/kg extract.</td>
<td>1.12±0.01ₐ</td>
<td>1.09±0.04</td>
<td>1.06±0.07ₐ</td>
</tr>
<tr>
<td>4</td>
<td>CCl₄ + 200mg/kg extract.</td>
<td>1.00±0.00ₐ</td>
<td>0.68±0.33</td>
<td>0.99±0.01ₐ</td>
</tr>
<tr>
<td>5</td>
<td>CCl₄ + 300mg/kg extract.</td>
<td>0.99±0.02ₐ</td>
<td>0.07±0.03ₐ</td>
<td>0.89±0.03ₐ</td>
</tr>
<tr>
<td>6</td>
<td>CCl₄ + 5.2mg/kg Livolin.</td>
<td>1.11±0.00ₐ</td>
<td>1.01±0.00ₐ</td>
<td>0.87±0.29ₐ</td>
</tr>
<tr>
<td>7</td>
<td>CCl₄ + 200mg/kg extract + 5.2mg/kg Livolin + 50mg/kg Vitamin C.</td>
<td>0.98±0.07ₐ</td>
<td>0.09±0.00ₐ</td>
<td>0.83±0.01ₐ</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± Standard error of mean; n =3 per group.

Values in the same column with common superscript letter (a, b, c) are significantly different at P < 0.05.

Superscript A (⁺) represents significant difference when group 1 is compared to other groups at P < 0.05.

Superscript B (⁺⁺) represents significant difference when group 2 is compared to other groups at P < 0.05.

Superscript C (⁺⁺⁺) represents significant difference when group 6 is compared to other groups at P < 0.05.

Values without superscript shown no significant difference when group 1, 2 and 6 are compared to other groups at P < 0.05.

All data were presented as Means ± SD, and were analyzed using the One Way Analysis Of Variance (ANOVA). The results were considered significant when p values are less than 0.05 (p<0.05) and non-significant when p values are greater than 0.05 (p>0.05).

**Discussion**

The effect of methanol fruiting body extract of *Pleurotus ostreatus* cultivated with crude extract of red bulb Allium cepa on oxidative stress markers of carbon tetrachloride induced hepatotoxicity in wistar rats were investigated.

Onions are common kitchen spices which posses so many health benefits; this vegetable increases the health values of many foods when added to them (Khiari et al., 2009).

The cultivation of *Pleurotus ostreatus* with *Allium cepa* extract shows a fast growth of the mycelium when compared to the growth of the mycelium without *Allium cepa* extract.

Oxidative stress has been shown to be involved as primary factor in progression of many degenerative ailments like cancer, diabetes type 2, atherosclerosis, cataracts, liver disease, neurodegenerative disorders, etc. (Jayakumar et al.,2006) and it occurs when there is an over production of reactive oxygen species.
These reactive species if not deactivated, their chemical reaction can cause injury to macromolecules in cells like proteins, carbohydrates, lipids, and nucleic acids. High level of ROS are dangerous and can cause oxidation of biomolecular substances that can result to cell damage and eventual death which results to various ailments and disorders (Halliwell and Gutteride, 2000).

Carbon Tetrachloride has been established to cause hepatotoxicity in both man and experimental subjects (Adewale et al., 2013) and has since been used in suitable modern form for the screening of hepatoprotective activities of different sources of natural products. Administration of CCl_{4} to experimental rats induced chronic liver injury that results to fibrosis, scar production and damage of normal tissue architecture (Chaudhary et al., 2010).

Medicinal plants owe their therapeutic features to the presence of various phytochemicals in their leaves, stems, barks, roots, and fruits (Sofowora, 2008). Fruiting body extract of Pleurotus ostreatus has been recognized to be possible source of antioxidants and have the ability to highly stop lipid peroxidation (Chaudhary et al., 2010). The occurrence of compounds like phenolic compounds, and flavonoid are responsible for the fruiting bodies of Pleurotus ostreatus protection due to their antioxidant features (Liu, 2004; Gupta et al., 2011) that scavenge reactive oxygen species.

The result of Table 1.1 and 1.2 on day 10 shows a significant decrease at P<0.05 in the activities of SOD and CAT in group 2 when compared to group 1. Groups treated with the dosage of methanol extract of Pleurotus ostreatus showed a significant increase at P<0.05 in the activities of SOD and CAT when compared to group 2. This explanation was also applicable to the activities of SOD and CAT on day 20 and 30.

This improvement to near normal level was an indication of stabilization of plasma membrane as well as repair of hepatic parenchyma.

The result of Table 1.3 on day 10 shows a significant increase at P<0.05 in the levels of TBARS in group 2 when compared to group 1. Groups treated with the dosage of methanol extract of Pleurotus ostreatus showed a significant decrease at P<0.05 in the level TBARS when compared to group 2. This explanation is also applicable to TBARS on day 20 and 30.

**Conclusion**

From this research, it has been established that CCl_{4} induced a significant damage to the liver tissues causing high level of lipid peroxidation, but methanol fruiting body extract of Pleurotus ostreatus cultivated with red bulb Allium cepa effectively ameliorated this effect and as such can be used in the treatment of Liver diseases.

**References**


used in traditional medicine. *Journal of pharmacy and pharmaceutical sciences, 6*: 539-542.


