Allium cepa extract attenuates CCl4-induced oxidative Stress in rat brain

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Ameliorative effects of Allium cepa extract on carbon tetrachloride neurotoxicity in rat

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Introduction: Carbon tetrachloride (CCl4) has been used extensively to evaluate the probable mechanisms of toxicity in experimental animals. The CCl4-induced oxidative stress in rat brain has been established. This study aims to investigate the neuro-protective effect of Allium cepa extract administration to rats given a single dosage of CCl4 (1 ml/kg bw).

Materials and Methods: Thirty rats were divided into five groups (I–V) containing six rats each. The plant (aqueous extract of A. cepa) was given at oral doses of 100 and 200 mg/kg day for 20 days after which CCl4 in olive oil vehicle (1 ml/kg bw) was administered to rats in Groups II-V. Thereafter, the brains were harvested for biochemical assays: Lipid peroxidation (LPO), glutathione (GSH) (reduced), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) activities.

Results: Elevated levels of LPO and reduced GSH activity were noted in the brain (forebrain, midbrain, and hindbrain) of rats (negative control group of rats) when compared to the positive control group. Equally, a severe decrease in the activities of GR, GST, CAT, GPx, and SOD in the brain of rats induced with CCL4 but not treated with the extract was noted. However, treatment with different doses of the extract significantly (P < 0.05) reversed the trends as was noticed in the extract-treated groups of rats when compared with the negative control group.

Conclusion: This study indicates that extract of A. cepa possesses anti-neurotoxic effect in rats.

Keywords: Allium cepa, aqueous extract, carbon tetrachloride, neuroprotective

INTRODUCTION

Living organisms continuously make reactive oxygen species (ROS) for the preservation of normal physiology and are kept in a balanced state through the antioxidant defense system. When ROS is overproduced above the range of antioxidant defense system, oxidative stress results in the cell which causes damages to macromolecules such as protein, DNA, and lipids.[1] Free radicals refer to molecules which have unpaired electron in their outer orbit.[2] They cause oxidative stress leading to injury in cellular membrane with subsequent change in metabolic processes. ROS is involved in the etiology of several disease conditions such as neurodegenerative disorders, cardiovascular diseases, cancer, and aging.[3-5]

Carbon tetrachloride (CCl4) has been commonly used to induce hepatotoxicity, especially in experimental animals. To develop models for liver and kidney injury, CCl4 is the chemical substance mostly used.[6,7] It has been demonstrated from recent studies that a 1 ml/kg bw of CCl4 given as a single hepatotoxic dose is likewise neurotoxic to rats.[8] The metabolism of CCl4 starts with proxy chloromethyl and trichloromethyl free radicals formation, through the activity of cytochrome P450 oxygenase system in the endoplasmic reticulum. The trichloromethyl radical then reacts with several essential biological substances such as proteins, fatty acids, lipids, amino acids, and nucleic acids.[9,10]

When compared to other organs of the body, the brain is the most vulnerable to oxidative stress probably due to its remarkably high level of oxygen consumption and due to its low level of antioxidant enzymes, and it is rich in polyunsaturated fatty acids (PUFA) together with a high quantity of non-heme iron.[11,12] Compounds with neurotoxic properties induce
oxidative stress by causing lipid peroxidation (LPO) besides altering the antioxidant defenses of the brain.[13-15]

Onion is of the genus *Allium*, Family *Amaryllidaceae*. Onion species are cultivated at diverse altitudes in North America, Europe, Asia, and Africa.[16,17] The color of red onion (*Allium cepa*) is attributed to their anthocyanins contents, while flavonoids is responsible for the brown and yellow color of onion skin.[18] *A. cepa* is one of the richest dietary sources of flavonoids.[19] The major flavonoids found in onions consist of kaempferol, quercetin, and myricetin.[20] Varieties of onion are available commercially, namely, white, red, and yellow with a varied amount of flavonoids and phenolic compounds in them.[21-23] *A. cepa* is used for the prevention and treatment of several diseases including cancer,[24] diabetes,[25,26] cataract,[27] coronary heart disease,[28] microbial infections,[29] hypercholesterolemia, and obesity.[30,31] These activities are mostly related to the thiosulfinates, flavonoids, and sterols of the plant. *A. cepa* displays antioxidant activity which is attributed to the flavonoids-queretin, myricetin, kaempferol, and catechin.[32]

Although the hepatotoxic effects of CCl₄ are well understood and widely reported, there is little information about the neurotoxic effects of CCl₄. Equally, the effect of *A. cepa* has not been explored on neuropathic pain.[33] Besides, there is a paucity of information on the neuroprotective effects of *A. cepa*. Therefore, in this study, we evaluated the neurocurative potential of *A. cepa* against CCl₄-induced toxicity.

**MATERIALS AND METHODS**

**Chemicals**

1-chloro-2,4-dinitrobenzene, thiobarbituric acid (TBA), glutathione (GSH) reduced, oxidized glutathione (GSSG), and 2,4-dinitrophenylhydrazine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

**Collection of Plant Materials**

*Allium cepa* (red onion bulbs) were bought from Uselu Market, Benin City, and identified in the Department of Plant Science, University of Benin, Edo State, Nigeria.

**Onion Seed Extract**

The onion bulb was washed with distilled water, and each layer was peeled off manually and was all rewashed. The layers were air-dried for 1 week. They were thereafter homogenized using mortar and pestle. A part of 50.0 g of the dried sample was soaked in 50 ml of distilled water. The mixture was allowed to stand for 24 h with intermittent shaking. The mixture was filtered using muslin cloth and then Whitman no. 1 filter paper. The filtrate was concentrated at 40°C to dryness under reduced pressure using rotary evaporator, and the dried extract was kept in sterile bottle and stored at 4°C until use. The dried extract was later reconstituted in freshly prepared sterile distilled water, and concentrations of 100 and 200 mg/ml were prepared before administration.

**Animals**

Adult male albino rats of Wistar strain weighing 200–250 g were purchased from the animal house of the Department of Anatomy, Delta State University, Abraka, Nigeria. The animals were fed a pellet diet (Top Feed, Ltd., Sapele, Delta State, Nigeria) and water *ad libitum*. The animals were kept in a controlled environment in standard conditions of temperature and humidity with an alternating 12 h light and dark cycle. The animals used in this study were maintained in accordance with the guidelines on the care and well-being of research animals.[34]

**Dosage and Treatment**

Rats were divided into five groups containing six rats each. The plant extract was employed at oral doses of 100 and 200 mg/kg day and was fed to rats by gavage:

- Group I - Served as control and received distilled water only for 21 days.
- Group II - Served as negative control and received CCl₄ in olive oil vehicle only.
- Group III - Animals received 100 mg/kg-day extract orally for 21 days.
- Group IV - Animals received 200 mg/kg-day extract orally for 21 days.
- Group V - Animals received 100 mg/kg bw of silymarin for 21 days.

On the 29th day, animals from Groups II-V were injected intraperitoneally with CCl₄ in olive oil vehicle at a dosage of (1 ml/kg bw), the dose at which hepatotoxicity occurs,[35] and 48 h after CCl₄ administration, the rats were sacrificed and the brain dissected on ice to get the different regions, namely, forebrain, midbrain, and hindbrain, which were processed immediately for biochemical assays. Thereafter, the processed tissues were used for the following assays/methods: LPO,[36] GSH,[37] glutathione reductase (GR),[38] glutathione peroxidase (GPx),[39] glutathione-S-transferase (GST),[39] catalase (CAT),[40] and superoxide dismutase (SOD).[41] and protein concentration was estimated by the method of Lowry et al.[42]

**Statistical Analysis**

The values are expressed as mean ± standard deviation (SD). The results were evaluated using the GraphPad Prism 6 software and evaluated by one-way ANOVA followed Tukey’s *post hoc* *P* < 0.05 was considered to be statistically significant.

**RESULTS**

**LPO**

Effects of onion extracts on LPO were measured by the formation of free MDA in the different regions of the brain following exposure to CCL₄, as shown in Table 1. On CCL₄ administration, MDA levels increased significantly from 1.18 ± 0.06 to 5.80 ± 0.70 nmol/mg proteins in the forebrain region. However, treatment with the high dose of the onion aqueous extract and the standard drug significantly inhibited the formation of MDA in the forebrain region.
Similarly, treatment with the high dose of onion extract caused a decrease in the MDA levels from 4.32 ± 0.20 to 1.45 ± 0.30 and 4.17 ± 0.20 to 1.29 ± 0.10 in the midbrain and hindbrain regions, respectively. Furthermore, a decrease in the MDA levels was observed in the silymarin-treated groups from 4.32 ± 0.20 to 1.37 ± 0.17 and 4.17 ± 0.20 to 1.26 ± 0.17 in the midbrain and hindbrain regions of the rats, respectively.

### Reduced GSH Levels

Table 1 shows the concentrations of GSH in the different regions of the brain homogenates. A significant ($P < 0.05$) decrease in GSH concentration was observed in CCl4-treated rats, 9.16 ± 0.96, 8.39 ± 0.84, and 7.61 ± 0.69 μg/mg protein for forebrain, midbrain, and hindbrain, respectively, compared with the normal control group (15.40 ± 1.62, 16.71 ± 0.92, and 20.22 ± 1.54 μg/mg protein for the forebrain, midbrain, and hindbrain, respectively). Administration of the aqueous extract of onion for 21 consecutive days afforded a dose-dependent protection against GSH depletion in all brain regions examined.

### GPx Activity

The activity of GPx in the brain samples is shown in Table 2. A significant decrease ($P < 0.05$) was observed for the CCL4-treated rats in the three regions of the brain evaluated from 22.08 ± 1.37 to 8.26 ± 1.02 (forebrain) and 11.52 ± 0.62 to 6.42 ± 0.46 (midbrain), while treatment with the onion extract caused a significant increase ($P < 0.05$) when compared with the CCL4-treated group in the forebrain and midbrain regions of the rats. Similarly, a significant increase ($P < 0.05$) was also observed in the silymarin-treated groups compared with the CCL4-treated group.

### GST and GR Activities

GST activities (11.29 ± 1.0, 9.84 ± 0.65, and 7.51 ± 0.59 nmol/min/mg protein for forebrain, midbrain, and hindbrain, respectively) were significantly decreased ($P < 0.05$) in brain tissue homogenates of CCl4-treated rats, as compared to control rats (44.44 ± 1.89, 42.07 ± 1.04, and 27.91 ± 1.48 nmol/min/mg protein for the forebrain, midbrain, and hindbrain, respectively); however, the extract could dose-dependently restore the activity toward normal [Table 2]. Furthermore, the silymarin-treated rats exhibited a pattern similar to the extract-treated groups. In the same vein, a similar trend was noted in the GR parameter. A significant decrease ($P < 0.05$) in this enzyme activity was observed in CCl4-treated rats when compared with those in control animals. Treatment with either high or low dose of onion extract or silymarin significantly restored the activities of the enzyme in all regions of the brain [Table 2].

### SOD and CAT Activities

The activity of SOD in brain tissue homogenate was significantly decreased in CCl4-treated animals, compared with control. However, pre-treatment with onion extract and the standard drug showed a significant increase in SOD activity in all regions of the brain examined [Table 3]. Similarly, the CAT activity in CCl4-treated rats was significantly decreased
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Effect of onion extract on some antioxidant enzymes activities in different groups in the brain

Table 2: Effect of onion extract on some antioxidant enzymes activities in different groups in the brain

<table>
<thead>
<tr>
<th>Group</th>
<th>GPx (forebrain)</th>
<th>GPx (midbrain)</th>
<th>GPx (hindbrain)</th>
<th>GST (hindbrain)</th>
<th>GST (midbrain)</th>
<th>GST (forebrain)</th>
<th>CAT (forebrain)</th>
<th>CAT (midbrain)</th>
<th>CAT (hindbrain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.37 ± 0.69</td>
<td>1.53 ± 0.18</td>
<td>1.73 ± 0.17</td>
<td>3.05 ± 0.14</td>
<td>3.05 ± 0.14</td>
<td>3.05 ± 0.14</td>
<td>4.53 ± 0.14</td>
<td>4.53 ± 0.14</td>
<td>4.53 ± 0.14</td>
</tr>
<tr>
<td>II</td>
<td>1.11 ± 0.49</td>
<td>2.61 ± 0.24</td>
<td>2.61 ± 0.24</td>
<td>1.95 ± 0.12</td>
<td>1.95 ± 0.12</td>
<td>1.95 ± 0.12</td>
<td>3.12 ± 0.12</td>
<td>3.12 ± 0.12</td>
<td>3.12 ± 0.12</td>
</tr>
<tr>
<td>III</td>
<td>3.57 ± 0.49</td>
<td>5.61 ± 0.24</td>
<td>5.61 ± 0.24</td>
<td>3.95 ± 0.12</td>
<td>3.95 ± 0.12</td>
<td>3.95 ± 0.12</td>
<td>6.12 ± 0.12</td>
<td>6.12 ± 0.12</td>
<td>6.12 ± 0.12</td>
</tr>
<tr>
<td>IV</td>
<td>10.24 ± 0.22</td>
<td>22.95 ± 0.81</td>
<td>22.95 ± 0.81</td>
<td>17.95 ± 0.67</td>
<td>17.95 ± 0.67</td>
<td>17.95 ± 0.67</td>
<td>25.12 ± 0.59</td>
<td>25.12 ± 0.59</td>
<td>25.12 ± 0.59</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six animals per group. Group I: Positive control (animal+distilled water only), Group II: Negative control (animal+CCl4), Group III: Animals+100 mg/kg-day of extract), Group IV: Animals+200 mg/kg-day of extract), Group V: Animals+100 mg/kg bw of silymarin). *Values with different superscripts along a column are statistically different (P<0.05)

DISCUSSION

A. cepa has been widely used for cooking purposes and in traditional medical systems since ancient times. A number of A. cepa varieties are commercially available with differences in biological activities due to varied phytoconstituents. However, their neuroprotective effects and neuropathic potentials are yet to be fully explored.[33] CCl4 crosses the cell membranes easily and is distributed in tissues due to its lipophilic nature. Although CCl4 is speedily taken up both by the liver and brain, relative to the liver, its neurotoxic effects are poorly understood.[43] It has been reported that a single dose of CCl4 (1 ml/kg bw) induces oxidative stress in the brain.[6,35] Oxidative stress is usually associated with numerous neurological diseases. It has been reported that there are increases in free radical during seizures,[46] signifying the importance of OS in the pathogenesis of neurological disorders such as epileptic seizures.[45] Hence, the present study was designed to study the neuroprotective effect of A. cepa aqueous extract against CCl4-induced neurotoxicity in rats.

To sustain adequate GSH level, it is necessary to prevent CCl4-induced damages in the cell.[46] GSH plays the role of coordinator in the body's antioxidant defense system. It acts as a scavenger of free radicals and supports the preservation of protein sulfhydryl groups. GSH is an essential source of reducing power in oxidative stress induced by ROS.[47] In this study, CCl4 administration drastically reduced the brain concentration of GSH, in all regions of the brain. In vivo studies by other workers also indicate that CCl4 decreases the GSH level in brain tissues.[8,48] However, cotreatment with A. cepa extract to CCl4-treated rats restored the GSH levels in the brain to near normal and could have resulted to a rise in the antioxidants store of cells. The levels of GSH (reduced form) are critical for the maintenance of structural and functional integrity of several organs.[49]

One measure of membrane damage and alteration in the function and structure of cellular membranes is LPO, measured by the formation of free MDA.[50] CCl4 as well as its metabolites is able to initiate a chain of reactions (LPO) by abstracting hydrogen from PUFA. In this study, an increase in MDA levels in groups of animals with CCl4-induced damage was noted. It has been well demonstrated that production of ROS, together with hydrogen peroxide and superoxide anions, increases in the brains of rats exposed to seizures.[51,52] Nevertheless, A. cepa coadministration led significantly to lowering of LPO and restoration of the antioxidant defense system. The results showed that the aqueous extract of A. cepa possesses a protective action against the ROS-induced damage resulting from CCl4. Thus, CCl4 treatment amplifies LPO in cellular membrane, whereas coadministration of A. cepa extracts diminished it. This observation is in concord with

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the reports of Rahul et al.[53] that A. cepa produced a major decrease in the global I/R-induced increase in the level of TBARS when compared to control.

Results of this study revealed a significant reduction in the activities of GPx and GR in rats treated with CCl4. This may be due to an increase in induced LPO and free radical damages.[54] Nonetheless, coadministration with A. cepa extract showed improvement in the antioxidant system and resulted in a significant increase in GPx and GR activities. GPx enzyme is known to catalyze the reaction of hydroperoxides and reduced GSH to yield hydroperoxides and GSH disulfide (GSSG). To convert GSSG to the reduced form GSH, GR is important in supporting the reverse reaction which leads to reduced GSH and NADPH.[55] Thus, A. cepa extract coadministration sustained GR and GPx activities within normal ranges in this study.

GST plays a critical role in shielding against ROS-mediated cells injury by detoxification of lipid hydroperoxides produced as a result of oxidative damage.[56] A decrease in the activity of GST in all the brain regions by CCl4 was observed in our results, and this is capable of compromising the brain’s biochemical antioxidant defenses which were simultaneous with a significant increase in the oxidative parameter MDA. However, cotreatment with either the extract or standard drug (silymarin) was able to restore the activity of this enzyme in a dose-dependent manner.

GR, GPx, GST, CAT, and SOD constitute a common supportive group of anti-ROS defense system.[57] The coordinate activity of antioxidant system has been known to be very essential for free radical detoxification. SOD decreases the concentration of extremely reactive superoxide radical, and this is by changing it to $H_2O_2$ but GPx and CAT decompose $H_2O_2$ and safeguard tissues from the very reactive hydroxyl radicals. Furthermore, SOD is the most predisposed enzyme against tissue damage. It scavenges superoxide anion ($O_2^-$), thereby converting it to hydrogen peroxide to reduce the free radical-induced toxic effects.[58]

In the present study, CCl4 was observed to cause a significant reduction in SOD and CAT. The decreased activity of SOD in CCl4 toxicity may result in the accumulation of $H_2O_2$, $O_2^-$, or the products formed through their degradation.[55] The reduced activity of SOD in the brain tissue seen in the CCl4-treated rats may be as a result of antioxidative enzymes inactivation or increased in LPO level. However, pre-treatment with A. cepa or silymarin was able to significantly restore the activities of SOD as compared to the CCl4 treated group. Thus, in this investigation, A. cepa and silymarin showed a significant increase in SOD activities of enzymes, suggesting that A. cepa has antioxidative activity as reported by Lee and Jung.[59]

CAT is an enzyme commonly found in virtually all living organisms that are exposed to oxygen. It is a hemoprotein which defends cells against the accumulation of $H_2O_2$ by disintegrating it into $H_2O$ and $O_2$ or by consuming it as an oxidant where it acts as a peroxidase. We observed from this study that treatment with CCl4 significantly decreased the level of CAT in the brain tissues. This CCl4-mediated neurotoxicity in rats may well be as a result of the generation of superoxide radicals or reduction of NADPH, or elevation in the level of LPO, or combination of all.[60] However, coadministration with A. cepa extract considerably increased the levels of CAT. Thus, treatment with A. cepa extract in all doses attenuated the obviously higher levels of the enzymes and produced a later recovery toward the normal range.

In the present investigation, administration of CCl4 to rats was shown to cause oxidative stress in their brain and the damage was coupled with significantly reduced activities of antioxidant enzymes such as CAT, SOD, GPx, GR, and GST. A significant rise in lipid peroxides was also seen in the animals treated with CCl4, while a significant reduction in GSH in the brain tissues, thus signifying the severity of CCl4-induced OS in rats. However, cotreatment with A. cepa, on the other hand, restored the activities of GSH and antioxidant enzymes toward the control level of animals, thereby precluding the toxic effects of CCl4. The results of this study agree to an extent with previous reports, which have shown that A. cepa mitigated CCl4-induced neuropathic pain in rats and was ascribed to the quercetin and high total flavonoid content.[33] Furthermore, A. cepa has been demonstrated to cause a significant reduction in cerebral damage and oxidative stress. The bioactive fraction said to be responsible for this activity was found to contain a high amount of total phenolic and total flavonoid content.[53] Thus, the results of this study agree largely with previous neurotoxicity studies involving CCl4 and A. cepa.[33,59,61-64]

**CONCLUSION**

The findings of this investigation indicated that aqueous extract of A. cepa demonstrated a significant decrease in oxidative stress and cerebral damage and also ameliorated the damage to the brain. The probable mechanism of neuroprotection could be attributed to the existence of phytoconstituents in the extract. Thus, the aqueous extract of A. cepa has neurocurative benefits.

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**Table 3**: Effect of onion extract on SOD and CAT activities in different groups in the brain

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (forebrain) (U/mg protein)</th>
<th>SOD (midbrain) (U/mg protein)</th>
<th>SOD (hindbrain) (U/mg protein)</th>
<th>CAT (forebrain) (U/mg protein)</th>
<th>CAT (midbrain) (U/mg protein)</th>
<th>CAT (hindbrain) (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.33±0.28ab</td>
<td>5.55±0.40ab</td>
<td>5.31±0.20ab</td>
<td>5.09±0.39ab</td>
<td>4.76±0.17de</td>
<td>4.51±0.29ab</td>
</tr>
<tr>
<td>II</td>
<td>1.21±0.06e</td>
<td>1.33±0.19e</td>
<td>1.29±0.16e</td>
<td>1.44±0.26e</td>
<td>0.96±0.09e</td>
<td>0.98±0.08e</td>
</tr>
<tr>
<td>III</td>
<td>3.13±0.19de</td>
<td>3.23±0.10bc</td>
<td>3.01±0.37bc</td>
<td>3.16±0.11de</td>
<td>3.13±0.09de</td>
<td>2.76±0.08e</td>
</tr>
<tr>
<td>IV</td>
<td>4.86±0.48bc</td>
<td>4.79±0.42bc</td>
<td>4.65±0.40bc</td>
<td>5.01±0.28bc</td>
<td>4.64±0.17cd</td>
<td>4.01±0.45bc</td>
</tr>
<tr>
<td>V</td>
<td>5.16±0.32d</td>
<td>4.86±0.27c</td>
<td>5.21±0.19e</td>
<td>5.00±0.17bc</td>
<td>4.68±0.16cd</td>
<td>4.35±0.17c</td>
</tr>
</tbody>
</table>

Values are mean±SD of six animals per group. Group I=Positive control (animal+distilled water only), Group II=Negative control (animal+CCl4), Group III=Animals+100 mg/kg-day of extract, Group IV=Animals+200 mg/kg-day of extract, Group V=Animals+100 mg/kg bw of silymarin. * Values with different superscripts along a column are statistically different (P<0.05). SOD: Superoxide dismutase, CAT: Catalase.
potentials on the brain and may, therefore, be a probable candidate for the management of cerebral damage.

REFERENCES


