REPORT

Protective effect of *Cucurbita pepo* fruit peel against CCl₄ induced neurotoxicity in rat

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Abstract: *Cucurbita pepo* is a common vegetable used all over the world. In folk medicine it is used in gastroenteritis, hepatorenal and in brain anomalies. In the present study, protective effect of *Cucurbita pepo* fruit peel against CCl₄-induced neurotoxicity in rats was investigated. In this study, 36 Sprague-Dawley female rats (190±15 g) were randomly divided into 6 groups of 6 rats each. Group I was given 1 ml/kg bw (body weight) of corn oil intraperitoneally (i.p); Group II, III and IV were treated with 20% CCl₄ in corn oil (1ml/kg bw i.p). However, animals of Group III and IV were also treated with CPME (methanol extract of *C. pepo* fruit peel) at 200 and 400mg/kg bw respectively. Animals of Group V and VI were administered only with CPME at 200 and 400mg/kg bw respectively. These treatments were administered 3 days a week for two weeks. Administration of CCl₄ cause acute neurotoxicity as depicted by significant depletion (P<0.05) in the activities of antioxidant enzymes; catalase, superoxide dismutase, peroxidase, glutathione reductase, glutathione-S-transferase, glutathione peroxidase, quinone reductase, while enhanced the γ-glutamyl transferase level in brain samples. CCl₄ intoxication decreased the reduced glutathione (GSH) level whereas markedly (P<0.05) enhanced lipid peroxidation in brain samples. Co-treatment of CPME significantly (P<0.05) protected the brain tissues against CCl₄ constituted injuries by restoring activities of antioxidant enzymes and ameliorated lipid peroxidation in a dose dependent fashion. These neuroprotective effects might be due to the presence of antioxidant constituents.

Keywords: *Cucurbita pepo*, CCl₄, oxidative stress, catalase, superoxide dismutase, reduced glutathione, lipid peroxidation.

INTRODUCTION

Carbon tetrachloride (CCl₄) intoxication as experimental model resembles oxidative stress caused by different pathophysiological conditions. Many studies have revealed that CCl₄ induced oxidative stress is significantly contributed in degeneration of tissues (Recknagel *et al*., 1989; Sahreen *et al*., 2014a). Tissue CCl₄-induced injuries have been manifested in different organs including brain, lungs and kidneys, however the major effect of CCl₄ intoxication was reported in liver (Khan *et al*., 2012; Khan *et al*., 2013; Sahreen *et al*., 2014a). Still limited studies are available for brain tissue, however experimental studies have shown that like the hepatic tissue, brain tissue is also sensitive to CCl₄ (Jung and Henke, 1996).

Carbon tetrachloride metabolism by cytochrome p450 produces free reactive radicals more important are the trichloromethyl (•CCl₃) and trichloromethyl peroxo (•OOCCl₃) radicals. Trichloromethyl peroxo radicals remove hydrogen (H) atoms from unsaturated fatty acids producing lipid free radicals. Hydroperoxides of lipids have marked tendency to react with oxygen to generate more reactive radicals, which consequently initiated the process of lipid peroxidation. If radical scavengers do not neutralize these peroxyl radicals, then these radicals continue to remove H atoms from lipid molecules and propagate the chain reaction of lipid peroxidation (Recknagel *et al*., 1991) resulting in alteration of permeability of the membranous system with consequent loss of cellular Ca²⁺ homeostasis and sequestration. This process enhances the level of degenerative enzymes and subsequently results in cellular damages (Halliwell and Gutteridge, 1999). As compared to trichloromethyl peroxyl radical, trichloromethyl (•CCl₃) radical is more involved in covalent binding reactions (Slater, 1982). Trichloromethyl (•CCl₃) radical react covalently with sulfhydryl (SH) groups, such as protein thiols and GSH (reduced glutathione) which result in abnormal protein function. Reactive aldehydes, especially 4-hydroxynonenal are the fatty acids degradation products, which bind to proteins functional groups and hinder the important enzymes functions. CCl₄ have genotoxic potential due to ability of •CCl₃ to produce reactive species that covalently bind to DNA and induces chromosomal rearrangements (Diaz and Castro, 1981).

Since herbs have wide contribution in many areas of human life such as nutrition, flavoring, cosmetics, beverages and medicine. During recent decades medicinal plants were studied extensively for their antioxidant...
potential (Bokhari et al., 2013; Sahreen et al., 2014b). The intake of fresh vegetables, fruit and other dietary natural products having antioxidants possess marked role to mitigate cancer and cardiovascular anomalies (Wilcox et al., 2004). The risk of mortality from these diseases lowers down by the higher intake of plant foods. About 60% anti-infective and antitumoral agents have natural origins, which are available commercially (Cragg et al., 1997).

Cucurbitacins are highly diverse oxygenated triterpenoid molecules possessed by different genera of Cucurbitaceae family including Cucurbita pepo (Tannin-Spitz, 2007; Haritunians et al., 2008; Wakimoto et al., 2008). It is reported that cucurbitacins have excellent antioxidant potential and this potential is due to their free radical scavenging ability like singlet oxygen, hydroxyl radical and superoxide anions. Moreover various others reports proved that cucurbitacins have excellent potent antioxidant, anti-inflammatory and anti-proliferative activities. The most important thing is that these compounds were found safe for body cells without having any toxic effect.

Cucurbita pepo is a gourd like squash of Cucurbitaceae family. Many active components are present in it. The pharmacological investigations of Cucurbita pepo indicated the presence of active compounds such as hexane cucurbitane glycoside, syringic acid, cucurbitane type triterpenes, alkaloids, tannin and vitamin A, E, and C (Wang, 2007). Sugars, fats and fibers are also present. Minerals such as iron, boron, cobalt, zinc, magnesium, potassium and calcium are present. Both D-chiro-inositol (a molecule having insulin like activity), and antioxidants are thought to be responsible for protective effects of pumpkin (Oloyede et al., 2014). Anti mutagenic and anti cancer effects of C. pepo have also been established (Parasad et al., 2011). Ingestion of dried fruit of C. pepo lowered the cholesterol level and reduced the hepatic damage (Silveria et al., 1996) and ulcer (Sarkar and Guha, 2008). Polysaturated fatty acids are the most prominent and integral constituents of brain tissues. It has been investigated that CCl4 induces injuries in various tissues through lipid peroxidation (Khan et al., 2013). Cucurbita pepo is used as brain tonic and other disorders in local system of medicine (Gossell-Williams et al., 2011; Adnaik, 2012). Plant based products are mostly endowed with antioxidants to prevent the oxidative stress caused by ROS. This relationship has led to considerable interest in determining the free radical scavenging capacity of extracts. In this study protective effects of Cucurbita pepo fruit peel were investigated against neuronal oxidative toxicity induced with CCl4 in rats.

MATERIALS AND METHODS

Extract preparation
At maturity fruits of Cucurbita pepo were collected in August 2011 from the local market of Rawalpindi, Pakistan and were peeled off. Peels were then shade dried for four weeks. 2kg of dried peels were ground and extracted with 4 liters of 95% methanol for 3 days and filtered (Khan and Zehra, 2013). Filtrate was evaporated on rotary evaporator to obtain methanol extract (CPME) and kept at 4°C for additional in vivo investigations.

Animals and experimental design
Healthy Sprague-Dawley female rats (36 rats) of weight 190±15g were maintained at room temperature (25±3°C) in ordinary cages. Rats were divided into 6 groups (06). Animals of Group I were administered with 1ml/kg bw (body weight) of corn oil intraperitoneally 3 days a week for two weeks. Group II, III and IV were given 20% CCl4 in corn oil (1ml/kg bw) intraperitoneally 3 days a week for two weeks. Rats of Group III and IV were also co-treated with C. pepo peel extract (CPME) at 200 and 400 mg/kg bw intraperitoneally 3 days a week for two weeks. Rats of Group V and VI were given CPME only at 200 and 400 mg/kg bw intraperitoneally 3 days a week for two weeks. Authentication of the protocol for the present study was granted by Ethical Committee of Quaid-i-Azam University Islamabad for laboratory animal feed and care. All experiments were conducted at the primate facility at Quaid-i-Azam University, Islamabad.

Body weights of rats in each group were recorded before and at the end of experiment. After sacrifice under anesthesia brains were taken and washed with saline solution, weighted and processed for various biochemical studies.

Biochemical studies of brain samples
Whole brain was homogenized with 10 times (w/v) ice cold phosphate buffer (100 mM, pH 7.4). Supernatant was taken after centrifugation at 12000×g for 30 min at 4°C for various biochemical studies.

Assay for total protein
Quantity of protein in brain sample was estimated with standard biuret method. For this assay standard AMP diagnostic kit (Krenngasse 12, 8010 Graz, Austria) was used.

Assay for antioxidant parameters
Activity of catalase (CAT) and peroxidase (POD) was determined by using hydrogen peroxide and guaiacol as the substrates respectively (Chance and Maehly, 1955). Superoxide dismutase activity (SOD) was assessed by using phenazine methosulphate (Kakkar et al., 1984). Glutathione-S-transferase activity (GST) was estimated according to Habig et al. (1974) whereas the method reported by Carlberg and Mannervik (1975) was used to estimate the glutathione reductase activity (GSR). To estimate the glutathione peroxidase assay (GSH-Px) in brain samples method of Mohanddas et al. (1984) was followed. Activity of quinone reductase (QR) was determined according to Benson et al. (1980). Reduced
glutathione contents (GSH) in the brain homogenate were determined by following the method of Jollow et al. (1974). However, estimation of lipid peroxidation (TBARS) was carried out according to the method of Wright et al. (1981).

**STATISTICAL ANALYSIS**

Means ± standard deviation (SD) were determined for each group. Treatment effects for different groups were assessed by computer software SPSS 13.0 through one way analysis of variance. Significance level among treatments was determined by post hoc LSD at 0.05% and 0.01% probability level.

**RESULTS**

**Effect of CPME on body and brain weight of rat**

The protective potential of CPME against the general toxicity of CCl₄ in rat is given in table 1. In this experiment body weight of the rats was significantly (P<0.01) decreased whereas absolute brain and relative brain weight was increased with CCl₄ treatment against the untreated rats. The protective capacity of CPME on the CCl₄ induced toxicity was evident as it increased (P<0.05) the body weight, while decreased the absolute brain and relative brain weight in a dose dependent manner. Co-administration of CPME at 400mg/kg bw showed the highest protective potential for the body weight, absolute brain and relative brain weight in relation to CCl₄ treated animals. In comparison to untreated animals administration of CPME alone at both 200 and 400mg/kg bw did not alter (P>0.05) the above parameters.

**Effect of CPME on CAT, POD and SOD in brain**

The antioxidant potential of CPME was determined by estimating different radical scavenging assays such as the activity level of CAT, POD and SOD in brain of rat (table 2). The level of antioxidant enzymes; CAT, POD and SOD and total protein content was decreased by the toxicity of CCl₄ in rat’s brain with respect to untreated animals. The functional integrity of these enzymes mutually protect the body by scavenging of free radical damages and constitute the first line defense to maintain a balance in cellular activities. The extract used in our study (CPME) showed the potential to counteract the formation of free radicals due to CCl₄ toxicity in brain thereby elevated the activity level of CAT, POD and SOD and restore the total protein concentration. The results obtained from this study show that administration of CPME at 200 and 400mg/kg bw did not influence the activity level of CAT, POD and SOD and the content of total protein (P>0.05) in brain samples with respect to the control group.

**Effect of CPME on GSR, GST, GSH-Px and QR in brain**

Antioxidants present in the extract (CPME) under study

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<table>
<thead>
<tr>
<th>Table 1: Effect of CPME on % increase in body weight, absolute brain weight and relative brain weight of rat</th>
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<tbody>
<tr>
<td><strong>Treatments</strong></td>
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</tr>
<tr>
<td>Control</td>
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<tr>
<td>CCl₄ (1 ml/kg bw)</td>
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<tr>
<td>CPME (200 mg/kg bw) + CCl₄</td>
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<td>CPME (400 mg/kg bw) + CCl₄</td>
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<tr>
<td>CPME (200 mg/kg bw)</td>
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<tr>
<td>CPME (400 mg/kg bw)</td>
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</table>

Mean ± SD (n=6 number)

*,**, indicate significance from the CCl₄ group at P<0.05 and P<0.01 probability level
+, ++ indicate significance from the control group at P<0.05 and P<0.01 probability level

<table>
<thead>
<tr>
<th>Table 2: Effect of methanol extract of Cucurbita pepo peels on CAT, POD and SOD of rat’s brain</th>
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<tr>
<td><strong>Treatment</strong></td>
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Mean ± SD (n=6 number)

*,**, indicate significance from the CCl₄ group at P<0.05 and P<0.01 probability level
+, ++ indicate significance from the control group at P<0.05 and P<0.01 probability level
have the potential to stop the chain reactions and to alleviate the burden of oxidative stress induced with CCl₄ in rat’s brain. Activity level of phase II antioxidant enzymes such as GSR, GST, GSH-Px and QR was increased whereas activity of γ-GT was decreased (P<0.01) in brain samples by CPME co-administration, which were adversely affected by the CCl₄ intoxication to rats (table 3). The effect of CPME was dose-dependent with peak effect produced at 400mg/kg bw to scavenge the CCl₄ induced oxidative stress. In this study, the effect of CPME at different doses (200 and 400mg/kg bw) alone was not significantly different (P>0.05) from the control group.

**Effect of CPME on TBARS and GSH in brain**
Intraperitoneal injection of CCl₄ to rats caused the induction of oxidative injuries in brain with an increase in lipid peroxidation (concentration of TBARS) while a decrease in the concentration of reduced glutathione (GSH). Co-administration of CPME produced significant reduction in lipid peroxidation while enhance GSH level in rat’s brain (P<0.01). This effect was dose-dependent with peak effect produced at the highest dose of 400mg/kg bw (table 4). However, CPME at doses of 200, 400mg/kg bw alone did not affect the lipid peroxidation and concentration of GSH as comparable with control rats.

**DISCUSSION**
Enhanced lipid peroxidation and alteration in the antioxidant status are significantly related with the oxidative stress induced disorders. Free radicals most likely are involved in lipid peroxidation of the membranous system. Brain tissues are considered to be more vulnerable to lipid peroxidation to reactive oxygen species of CCl₄ on account of its aerobic metabolic activity and greater accumulation of polyunsaturated fatty acids in the neuronal membranes (Del Maestro and Mc Donald, 1987; Adams et al., 1991). CCl₄ after administration is concentrated in different organs. Liver and brain tissues rapidly intake and metabolize the CCl₄ in to highly injurious reactive oxygen species such as trichloromethyl (•CCl₃) and trichloromethyl peroxy (•OOCCl₃) radical (Sanzgiri et al., 1997; Weber et al., 2003). Human body is protected from free radical damages by a cooperative scavenging mechanism of antioxidant enzymes and dietary nutrients. Enzymic antioxidants include; SOD, POD, CAT and GSH-Px. Their coordinated system is essential for free radical detoxification. SOD converts superoxide radical which is highly reactive to H₂O₂ while GSH-Px and CAT decompose H₂O₂ and protect the body while minimizing the potential damages of hydroxyl (OH) radicals. According to Szymonik-lesiuk et al. (2003) CCl₄ intoxication results in changes in expression level and consequently decreases the levels of CAT and SOD in liver tissue. Our present investigation corroborates with the above findings and reflected that CCl₄ intoxication lead to significant reduction in phase I enzymes such as SOD, POD and CAT in brain tissue. Two-week administration of CPME significantly restored the levels of SOD, POD and CAT towards normal group.

### Table 3: Effect of methanol extract of *Cucurbita pepo* peels on brain GSR, GST, GSH-Px, QR and γ-GT of rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSR (nM/min/mg protein)</th>
<th>GST (nM/min/mg protein)</th>
<th>GSH-Px (nM/min/mg protein)</th>
<th>QR (nM/min/mg protein)</th>
<th>γ-GT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.11±7.57**</td>
<td>206.9±13.1**</td>
<td>52.94±6.54**</td>
<td>131.2±9.70**</td>
<td>5.0±0.63**</td>
</tr>
<tr>
<td>CCl₄ (1 ml/kg bw)</td>
<td>33.84±3.05</td>
<td>120.7±6.89</td>
<td>23.12±5.33</td>
<td>68.29±4.88</td>
<td>10.4±1.06</td>
</tr>
<tr>
<td>CPME (200 mg/kg bw) + CCl₄</td>
<td>67.02±2.30*</td>
<td>166.6±28.4*</td>
<td>33.77±9.94*</td>
<td>98.7±11.5*</td>
<td>7.78±1.74*</td>
</tr>
<tr>
<td>CPME (400 mg/kg bw) + CCl₄</td>
<td>72.7±10.1**</td>
<td>189.2±13.1**</td>
<td>45.34±7.25**</td>
<td>111.4±8.36**</td>
<td>6.11±0.94*</td>
</tr>
<tr>
<td>CPME (200 mg/kg bw)</td>
<td>83.2±9.22*</td>
<td>205.2±23.2**</td>
<td>51.57±4.32**</td>
<td>128.2±16.8**</td>
<td>4.92±0.62*</td>
</tr>
<tr>
<td>CPME (400 mg/kg bw)</td>
<td>80.8±11.2*</td>
<td>208.4±29.4**</td>
<td>54.25±4.78**</td>
<td>132.0±17.6**</td>
<td>4.81±0.74*</td>
</tr>
</tbody>
</table>

Mean ± SD (n=6 number)
*”, ** indicate significance from the CCl₄ group at P<0.05 and P<0.01 probability level
*, + + indicate significance from the control group at P<0.05 and P<0.01 probability level

### Table 4: Effect of CPME on brain GSH and TBARS of rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (µM/g tissue)</th>
<th>TBARS (nM/min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.03±0.41**</td>
<td>4.13±0.80**</td>
</tr>
<tr>
<td>CCl₄ (1 ml/kg bw)</td>
<td>2.05±0.34**</td>
<td>9.14±1.16**</td>
</tr>
<tr>
<td>CPME (200 mg/kg bw) + CCl₄</td>
<td>4.05±0.69*</td>
<td>5.99±0.46**</td>
</tr>
<tr>
<td>CPME (400 mg/kg bw) + CCl₄</td>
<td>4.26±0.64**</td>
<td>4.86±1.11**</td>
</tr>
<tr>
<td>CPME (200 mg/kg bw)</td>
<td>5.26±0.42**</td>
<td>4.28±0.35**</td>
</tr>
<tr>
<td>CPME (400 mg/kg bw)</td>
<td>4.96±0.74**</td>
<td>4.13±0.67**</td>
</tr>
</tbody>
</table>

Mean ± SD (n=6 number)
*, ** indicate significance from the CCl₄ group at P<0.05 and P<0.01 probability level
*, + + indicate significance from the control group at P<0.05 and P<0.01 probability level
Glutathione system includes different phase two metabolizing enzymes e.g. GSH-Px, GST and GSR. These enzymes metabolize different toxins and also regulate genomic and biochemical changes (Mc Gregor and Lang, 1996). Reduced glutathione is an important thiol protein, which participates in different defense processes against oxidative stress. It eliminates superoxide anion and \( \text{H}_2\text{O}_2 \) from the body. Low level of GSH in living organisms is responsible for tissue injury and various anomalies related to oxidative stress (Limon-Pacheco et al., 2007). Reduced glutathione scavenge diverse free radicals effectively. In systems of oxidative stress GSH is oxidized to GSSG by GSH-Px activity while GSR activity converted back GSSG to GSH (Meister and Anderson, 1983). Glutathione peroxidase is found in the cell membrane. It is thought that GSH is usually dependent on GSH-Px for its protective system against oxidative breakdown of lipids, which occur by GSH-Px mediated reduction of endogenous \( \text{H}_2\text{O}_2 \) (Sing and Pathak, 1990; Khan and Siddique, 2012). According to Adams et al. (1991) GSH-Px more efficiently detoxify \( \text{H}_2\text{O}_2 \) than CAT and SOD in brain tissues. Moreover, GSH-Px in combination with GSH terminates lipid peroxidation chain reaction by amelioration of \( \text{H}_2\text{O}_2 \) and many other hydroperoxides (Sun et al., 1988; Mak et al., 1996). Results reported in this study suggested that free radicals generated by metabolism of \( \text{CCl}_4 \) cause significant reduction in protein concentration as well as the catalytic potential of phase II enzymes e.g. GSH-Px, GSR, GST in brain samples. Protective potential of the CPME is evident in this study as its administration along with \( \text{CCl}_4 \) ameliorated the oxidative stress by increasing the activities of GSR, GSH-Px, GST and QR and protein content whereas reduces the activity of \( \gamma\)-GT against the \( \text{CCl}_4 \) treated group similar to other findings (Farombi et al., 2002; Khan and Zehra, 2013).

Reactive oxygen species formed by \( \text{CCl}_4 \) intoxication can covalently bind to macromolecules and in cellular membranes can promote lipid peroxidation. Peroxidation of polyunsaturated fatty acids generates malonaldehydes (Khan and Younus, 2011; Saeed et al., 2012). Superoxide dismutase eliminates peroxide anion radicals and inhibits the propagation of fatty degeneration (Escobar et al., 1996). In this study \( \text{CCl}_4 \) administration significantly increased the TBARS while decreased the GSH level in brain tissues. Increase in TBARS levels indicated \( \text{CCl}_4 \) induced oxidative damages in brain tissues, similar to other reports (Clemedson et al., 1990; Krasteva et al., 2007; Park et al., 2008). In present investigation, the TBARS level in the brain tissues moved towards normal group after treatment with CPME. The elimination of \( \text{CCl}_4 \) induced neuronal toxicity with the extract may involve restoration of antioxidant enzymes (Srivastava and Shivanandappa, 2010; Sahreen et al., 2013).

Our study also revealed the marked changes on body and organ weight induced by the administration of \( \text{CCl}_4 \) in rat. Intraperitoneal administration of \( \text{CCl}_4 \) significantly decreased the body weight of rat, which was suggested due to degeneration and necrosis of body tissues (Noyan et al., 2006). Findings of this study are similar to the above reports where \( \text{CCl}_4 \) intoxication reduced the body weight conversely to the increase in absolute brain and relative brain weight. Decrease in body weight while increase in absolute and relative brain weight was significantly restored to normal control group by treatment with CPME. According to Khan and Ahmed, (2009) and Lin et al. (2008) significant loss in body and gain in brain weight due to \( \text{CCl}_4 \) was restored significantly by \textit{Digera muricata} and \textit{Solanum nigrum}, respectively.

**CONCLUSION**

Methanol extract of \textit{Cucurbita pepo} fruit peel showed significant neuroprotective activity against \( \text{CCl}_4 \) induced alterations, which might be due the presence of its bioactive compounds. Further research work for isolation and purification of bioactive constituents is needed.

**REFERENCES**


Sanja Zaib and Muhammad Rashid Khan


