Original Research Article

Wheat Grass Mediated Modulation of Histoarchitecture and Antioxidant Status Offers Protection against Carbon Tetrachloride Induced Hepatotoxicity

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ABSTRACT

Introduction: Wheatgrass (Triticum aestivum) is believed to prevent occurrence of many diseases. However, studies have not been conducted to ascertain its role in prevention of hepatotoxicity.

Aim: Study was planned to elucidate role of wheatgrass if any on liver function test (LFT), antioxidants enzymes and histoarchitecture in hepatotoxicity induced by Carbon tetrachloride (CCl₄).

Material & Methods: 42 female Wistar rats were divided into 7 groups. Group 1: Rats were given normal saline SC. Group 2: CCl₄ was administered SC at dose of 2ml/kg b.wt twice/week for 4 weeks. Group 3-6: - Rats received wheatgrass orally in water at different doses of 20mg, 40mg, 60mg and 80mg/100g b.wt and CCl₄ as was given to group 2. Group 7- Rats received wheatgrass alone at a highest dose of 80mg/100g b.wt. The effect of different treatments was studied on LFT at end of 2 weeks and 4 weeks. Also, glutathione (GSH), lipid peroxidation (LPO) were analyzed and histological studies were conducted at 4 weeks.

Results: Activity of alkaline phosphatase (ALP), Aspartate aminotransferase(AST) and Alanine aminotransferase (ALT) increased significantly in CCl₄ group at 2 and 4 weeks. Interestingly, supplementation of wheatgrass at all doses brought down increased activity of ALT, AST and ALT but there was more pronounced decrease with 80 mg dose of wheatgrass at both the time durations of 2 and 4 weeks. It was also found that GSH level decreased and LPO increased significantly in CCl₄ treated rats whereas in wheatgrass treated groups, GSH levels increased while LPO decreased as compared to CCl₄ group. Histologically, there was necrosis, portal triaditis & lobular inflammation in CCl₄ group.

Conclusion: Therefore, protection was observed with wheatgrass which may not be significant at 2 weeks but values were significant at 4 weeks. Conclusion: Wheatgrass supplementation at a dose of 80mg/100g is more effective in controlling hepatotoxicity induced by CCl₄.

Keywords: Antioxidant, Carbon tetrachloride, Hepatotoxicity, Histoarchitecture, LFT, Wheatgrass

INTRODUCTION

Human population is constantly exposed to toxic chemicals in the environment. Further, excessive use of drugs in functional disorders of various organs is a matter of serious concern. The liver, because of its strategic anatomical location and its large capacity for metabolic conversions, is
continuously exposed to different kind of xenobiotics and therapeutic agents. The response of liver to noxious agents having varied steatognoic effects is currently of great interest.

There is a need to develop useful hepato protective drugs and in that attempt various experimental models have been designed, where liver toxicity is induced by different innocuous agents. A large number of such agents having diverse effects on the liver have been investigated and among them most often used and best studied are: ethano \[1,2\] and carbon tetrachloride (CCl4).\[3\] CCl4 is the most investigated of all the hepatotoxins. Its toxic effects include the damage to the mucosal membrane and also the necrosis of hepatocytes. Both in vivo and in vitro studies suggest that CCl4 may injure liver cells by accelerating the phospholipase C mediated degradation of membrane phospholipids.\[4\] Earlier studies from our laboratory have also shown the adverse effects of CCl4 on hepatic histoarchitecture and liver marker enzymes.\[5\] In the present study, we have selected carbon tetrachloride as a hepatotoxin of choice due to its few inherent properties and most of its effects are best studied which show highly reproducible results with specific mode of action, thus making it a useful experimental toxin.\[6,7\]

In the last decade, a great deal of research has been devoted to the study of natural products with antioxidant activity. The beneficial effects of fruits and vegetables are very likely due to many of their components such as dietary fibre, micronutrients, and antioxidants.\[8\] The present study was mainly carried out to evaluate the efficacy of hepatoprotective agents against carbon tetrachloride toxin. The cereal grasses - wheatgrass, barley grass, Alfa-Alfa have been known to boost health and vitality both in humans and animals.

Wheatgrass (Triticum aestivum) has been known to treat a number of conditions in human including the common cold, cough, bronchitis, fever and infections and inflamed mouth and throat, skin disorders like haemorrhoids, psoriasis, ivy, eczema and burns\[9,10\] Other benefits of wheatgrass are: removal of toxins from the body, improving blood sugar balance, prevention of tooth decay, improving haemoglobin production, keeping hair healthy, aiding digestion and reducing high blood pressure levels.\[11\]

Wheat grass juice helps to improve the health status and lifespan in terminally ill cancer patients. The extract of wheat grass when applied to known chemical mutagens, decreased their cancer causing ability by up to 99 percent\[12-14\] which suggests that wheat grass may also have cancer preventing property.

However, studies have not been conducted to elucidate the hepatic-protective potential of wheatgrass. So, the present study was designed to examine the protective potential of wheat grass, if any, on the liver of rats intoxicated with CCl4.

**MATERIALS AND METHODS**

**Chemicals:** Carbon tetrachloride was purchased from Merck (India), Bombay. Corn oil was commercially available, wheatgrass tablets were a generous gift from Sarvaayush Ayurved & Herbals, Pune, India. All other reagents used were of analytical grade.

**Animals:** The animals used in this study were female Wistar rats in the weight range of 150-200g and were acclimatized for one week prior to subjecting them to different treatments. They were maintained in polypropylene cages under standard conditions. Animals were maintained as per the principles and guidelines of ethical committee of animal care of Panjab University, Chandigarh, in accordance with Indian national law on animal care and use.
**Experimental design:** 42 animals were segregated in following seven groups.

**Group 1:** Rats in this group were fed standard laboratory feed and water ad libitum throughout the period of experimentation and they served as normal controls.

**Group 2:** Rats in this group were given subcutaneous injection of CCl₄ mixed with corn oil in 1:1 ratio at dose of 2ml/kg body weight (b.wt.) twice a week for two time durations of 2 and 4 weeks. [15]

**Group 3-6:** Animals in these groups were given wheatgrass orally at a dose of 20mg, 40 mg, 60mg, 80mg/100g b.wt, respectively every day. Wheatgrass tablet weighing 500 mg/tablet was dissolved in water. Animals belonging to groups 3-6 also received injections of CCl₄ mentioned twice a week for two time durations of 2 and 4 weeks. Wheatgrass treatment to animals belonging to group 3 to 6 started 2 weeks prior to CCl₄ treatment and wheatgrass treatment continued for 4 weeks.

**Group 7:** Animals in this group were given wheatgrass treatment alone at a highest dose i.e. 80mg/100g b.wt everyday.

Food consumption, body weight and physical activity were recorded every week. Blood samples were drawn by ocular vein puncture using a fine sterilized capillary at the second week and end of the experiment. Serum was separated for various estimations.

Liver of each rat was excised and opened longitudinally and analysed histologically.

**Body weights:**

A careful record of body weight of animals belonging to all groups was kept throughout the study. The animals were weighed weekly and finally before sacrificing them at the end of 4 weeks. A daily record of food as well as water intake was also maintained throughout the study.

**Estimations of liver marker enzymes in serum:**

The Alkaline phosphatase (ALP) enzyme activity was measured by using the method of Wooton [16] and the enzyme activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated according to Reitman & Frankel [17] method. The above estimations were done in serum at different time intervals of 2 and 4 weeks.

**Reduced Glutathione and Lipid peroxidation:**

The rats were sacrificed by giving adequate ether anaesthesia and livers were removed immediately and washed with ice chilled saline. Tissues were weighed and homogenates were prepared in 0.1 mM tris HCl buffer (pH 7.4) by using Teflon Fitted Potter-Elvejhem type homogeniser. The homogenates were centrifuged at 10,000g for 10 min at 4 °C and pellets were discarded. The supernatants were used for various biochemical estimations.

Lipid peroxidation (LPO) and reduced glutathione (GSH) levels were analysed using established biochemical procedures as follows:

LPO was estimated by method of Okhawa, [18]

Estimation of GSH was performed by following the method of Ellman's reagent [19] and protein by Lowry’s method [20]

**Histopathological studies**

For the histopathological observation at light microscope level, fresh tissue pieces of liver were immersed fixed in buffered formalin. Following an overnight fixation, the specimen were dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax. Blocks were made and 5-7 micro metre thick sections were double stained with hematoxylin and eosin and observed under microscope.

**Statistical Analysis:**

The statistical significance of the data was determined using one way analysis of variance (ANOVA) followed by a multiple post hoc test (student Newman Kewls) with
RESULTS

The data obtained from various experiments conducted in this study are depicted in tables (1-5). The data from various treatment groups have been compared with the normal control animals. However, results obtained from wheatgrass+CCl4 treated group were additionally compared with that of CCl4 treated group.

Mortality:
During this study, we did not observe any evidence for mortality as all the animals survived during the entire course of the experiment.

Body weight changes:
The variations in the body weight of the animals subjected to different treatments are shown in table

<table>
<thead>
<tr>
<th>Group</th>
<th>1 week</th>
<th>2 week</th>
<th>3 week</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>150.34±16.21</td>
<td>160.18±21.22</td>
<td>180.45±26.04</td>
<td>195.43±25.51</td>
</tr>
<tr>
<td>CCl4 treated</td>
<td>150.12±18.56</td>
<td>127.13±16.82</td>
<td>112.9±18.07</td>
<td>91.56±16.41</td>
</tr>
<tr>
<td>CCl4 + 20mg WG</td>
<td>165.21±22.24</td>
<td>132.78±22.61</td>
<td>113.45±20.13</td>
<td>100.67±16.62</td>
</tr>
<tr>
<td>CCl4 + 40mg WG</td>
<td>162.89±25.16</td>
<td>145.21±20.31</td>
<td>140.34±24.05</td>
<td>125.42±18.47</td>
</tr>
<tr>
<td>CCl4 + 60mg WG</td>
<td>154.43±21.75</td>
<td>150.15±18.79</td>
<td>148.81±17.24</td>
<td>135.56±12.16</td>
</tr>
<tr>
<td>CCl4 + 80mg WG</td>
<td>150.18±23.94</td>
<td>165.73±16.18</td>
<td>173.23±23.69</td>
<td>193.33±24.16</td>
</tr>
<tr>
<td>80mg WG alone</td>
<td>155.45±26.18</td>
<td>163.67±21.05</td>
<td>171.32±21.75</td>
<td>186.94±25.33</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.D., n = 6

All values are expressed in g

1P<0.05, 2P<0.01, 3P<0.001 by one way ANOVA when values are compared to controls
4P<0.05, 5P<0.01, 6P<0.001 by one way ANOVA when values are compared to CCl4 treated animals

Aminotransferases

Activities of serum enzymes ALT, AST and ALP are shown in the table 2, 3, & 4 respectively. A significant increase in the enzyme activities of serum ALT, AST and ALP was observed following treatment with CCl4 at both the time durations of 14 days and 28 days when compared with activities of normal control rats. However, wheatgrass extract treatment with 40 to 80mg dose for a period of 28 days resulted in significant decrease in the activities of ALT, AST and ALP. Treatment with 80mg dose to CCl4 treated rats showed an appreciable decrease at both the intervals of 14 days and 28 days and the activities were brought back almost within normal limits. Wheatgrass treatment alone at 80mg dose did not reveal any significant change in the activities of all the enzymes when compared to normal control rats and activities were within normal limits.
The light microscopic examination of liver of normal control rats showed normal histology. The liver lobules, hepatocytes were normal and the mitochondria were prominent. Further, Kupffer cells and portal tracts including bile ducts revealed normal morphology. Similar observations were made for the control animal given only 80mg dose of wheatgrass (figure 1a).

On the other hand, CCl4 treatment caused drastic alterations in hepatic architecture after 4 weeks of treatment. The liver lobules were differentiated into nodules and outlined after 4 weeks of treatment. The liver lobules of normal control rats showed normal histoarchitecture findings:

Table 2: Effect of wheatgrass on ALT activity (U/L) of normal and CCl4 treated animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>36.96±6.5</td>
<td>33.31±3.05</td>
</tr>
<tr>
<td>CCl4 treated</td>
<td>152.95±12.21</td>
<td>189.98±21.81</td>
</tr>
<tr>
<td>CCl4+20mg WG</td>
<td>129.45±14.04</td>
<td>142.08±24.02</td>
</tr>
<tr>
<td>CCl4+40mg WG</td>
<td>116.67±14.60</td>
<td>96.27±23.54</td>
</tr>
<tr>
<td>CCl4+60mg WG</td>
<td>102.09±18.07</td>
<td>72.90±13.39</td>
</tr>
<tr>
<td>CCl4+80mg WG</td>
<td>57.08±11.76</td>
<td>64.89±16.78</td>
</tr>
<tr>
<td>80mg WG alone</td>
<td>34.05±3.16</td>
<td>37.24±4.12</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.D., n = 6

All values are expressed in U/L

<table>
<thead>
<tr>
<th>Groups</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.60±5.30</td>
<td>25.60±5.31</td>
</tr>
<tr>
<td>CCl4</td>
<td>94.85±12.81</td>
<td>17.94±16.33</td>
</tr>
<tr>
<td>CCl4+20mg WG</td>
<td>89.37±16.18</td>
<td>80.98±38.56</td>
</tr>
<tr>
<td>CCl4+40mg WG</td>
<td>62.24±10.61</td>
<td>61.9±10.98</td>
</tr>
<tr>
<td>CCl4+60mg WG</td>
<td>42.56±5.61</td>
<td>53.67±15.2</td>
</tr>
<tr>
<td>CCl4+80mg WG</td>
<td>34.34±18.89</td>
<td>31.22±5.61</td>
</tr>
<tr>
<td>80mg WG alone</td>
<td>24.08±5.61</td>
<td>23.35±4.09</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.D., n = 6

All values are expressed in U/L

Atiopxidants:

The levels of GSH were significantly decreased (p<0.001) following CCl4 treatment where as LPO levels showed a significant increase in hepatic tissue (table 5).

Interestingly, wheatgrass treatment at the doses of 40-80 mg/100g b.wt. resulted in a significant increase in the levels of GSH and decreased LPO level which confirmed the preventive action. However, treatment of wheatgrass at 80mg alone to normal rats did not show any significant changes in any of the biochemical indices.

Table 3: Effect of wheatgrass on AST activity (U/L) of normal and CCl4 treated animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>28.53±7.46</td>
<td>27.69±14.18</td>
</tr>
<tr>
<td>CCl4 treated</td>
<td>96.48±15.23</td>
<td>115.09±17.42</td>
</tr>
<tr>
<td>CCl4+20mg WG</td>
<td>91.23±18.47</td>
<td>82.09±16.14</td>
</tr>
<tr>
<td>CCl4+40mg WG</td>
<td>63.66±16.14</td>
<td>59.64±15.42</td>
</tr>
<tr>
<td>CCl4+60mg WG</td>
<td>58.63±12.34</td>
<td>52.59±22.18</td>
</tr>
<tr>
<td>CCl4+80mg WG</td>
<td>40.67±10.11</td>
<td>38.37±14.61</td>
</tr>
<tr>
<td>80mg WG alone</td>
<td>29.13±9.64</td>
<td>26.94±15.24</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.D., n = 6

All values are expressed in U/L

<table>
<thead>
<tr>
<th>Groups</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.08±11.76</td>
<td>64.89±16.78</td>
</tr>
<tr>
<td>CCl4</td>
<td>37.24±4.12</td>
<td>37.24±4.12</td>
</tr>
<tr>
<td>CCl4+20mg WG</td>
<td>34.05±3.16</td>
<td>37.24±4.12</td>
</tr>
<tr>
<td>CCl4+40mg WG</td>
<td>30.57±4.23</td>
<td>30.57±4.23</td>
</tr>
<tr>
<td>CCl4+60mg WG</td>
<td>26.94±15.24</td>
<td>26.94±15.24</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.D., n = 6

All values are expressed in U/L

Table 4: Effect of wheatgrass on ALP activity (U/L) of normal and CCl4 treated animals

Antioxidants:

<table>
<thead>
<tr>
<th>Groups</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.34±0.02</td>
<td>0.23±0.013</td>
</tr>
<tr>
<td>CCl4</td>
<td>1.63±0.04</td>
<td>0.71±0.025</td>
</tr>
<tr>
<td>CCl4+20mg WG</td>
<td>1.56±0.04</td>
<td>0.50±0.014</td>
</tr>
<tr>
<td>CCl4+40mg WG</td>
<td>1.72±0.06</td>
<td>0.47±0.019</td>
</tr>
<tr>
<td>CCl4+60mg WG</td>
<td>1.89±0.09</td>
<td>0.44±0.021</td>
</tr>
<tr>
<td>CCl4+80mg WG</td>
<td>2.06±0.01</td>
<td>0.33±0.024</td>
</tr>
<tr>
<td>80mg WG alone</td>
<td>2.22±0.07</td>
<td>0.23±0.036</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.D.

All values are expressed in µ moles of MDA formed/mg protein and in µ moles of non-protein-SH/g tissue as for GSH, n = 6

Table 5: Effect of wheatgrass on levels of GSH and LPO of normal and CCl4 treated animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH(µ moles GSH/gm tissue)</th>
<th>LPO(µmoles of MDA formed/mg protein)</th>
<th>Proteins(mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.71±0.025</td>
<td>0.23±0.013</td>
<td>127.03±9.6</td>
</tr>
<tr>
<td>CCl4</td>
<td>0.50±0.014</td>
<td>0.47±0.019</td>
<td>111.45±6.75</td>
</tr>
<tr>
<td>CCl4+20mg WG</td>
<td>0.47±0.019</td>
<td>0.44±0.021</td>
<td>116.01±7.56</td>
</tr>
<tr>
<td>CCl4+40mg WG</td>
<td>0.33±0.024</td>
<td>0.23±0.036</td>
<td>122.78±5.22</td>
</tr>
<tr>
<td>CCl4+60mg WG</td>
<td>0.23±0.036</td>
<td>0.15±0.006</td>
<td>125.6±7.88</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.D.

All values are expressed in n moles of MDA formed/mg protein for LPO, µ moles of non-protein-SH/g tissue as for GSH, n = 6

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The portal tracts were expanded and showed mild lymphomononuclear infiltrates. The hepatocytes showed variation in size and there were giant cells with more than one nucleus. Fatty changes were widespread and there was steatonecrosis but no inflammation. Liver cells contained abundant eosinophilic conditions (Fig 1b).

Administration of wheatgrass to CCl₄ treated rats resulted in marked improvement in overall histoarchitecture especially with 60mg and 80mg dose. The liver showed minor fatty changes with 80 mg dose (Fig. 1c). Other doses also showed eosinophilic hyaline or fluffy inclusion with no major distortion of hepatic histoarchitecture.

**DISCUSSION**

CCl₄ is an established potent toxin that is metabolized by a microsomal drug-oxidizing system to a more toxic metabolite, the CCl₃ radical, which initiates peroxidative changes in polyunsaturated fatty acid constituents of various bio membranes. [21]

The present investigations revealed that the rats treated with CCl₄ did not gain body weights throughout the study, while control animals and those given wheatgrass with CCl₄ treatment put on weight steadily and showed appreciable net gain in body weights at the end of study. It is emphasized that the decrease in body weight can be a result of physiological response of the rats to diminished feed intake due to CCl₄ toxicity, rather than as a result of primary toxic effects of CCl₄ itself. Maximum weight gain was found with 80mg dose of wheatgrass which is due to the increase in protein content as indicated in table 1. These findings are supported by studies conducted on old mice treated with wheat sprouts extracts which showed a recovery of hepatocyte DNA levels when compared with the old untreated ones. [22] After the treatment, the increase in DNA and protein contents observed in aged animals was
comparable to that present in young mice hepatocytes.

Alkaline phosphatase is a lysosomal enzyme, which is loosely bound and is released into the blood stream when lysosomal membranes are disintegrated. \[23\] The activity of alkaline phosphatase was found to be significantly enhanced (p<0.05 to p<0.001) by CCl\textsubscript{4} treatment for 4 weeks. These results are in conformity with earlier findings from our laboratory \[24\] and could be well attributed to the toxic effects of CCl\textsubscript{4} on membranous lipids, CCl\textsubscript{4} is known to cause peroxidation of polyunsaturated lipids of biomembranes, resulting in the disruption of the membranous integrity and ultimately releasing the enzyme from the cell. \[25\] These observations are consistent with the observed increase in the extent of NADPH-dependent lipid peroxidation due to CCl\textsubscript{4} toxicity.

Wheatgrass supplementation to CCl\textsubscript{4} intoxicated rats resulted in almost normal levels of alkaline phosphatase in serum at the end of the study. The protective effects in CCl\textsubscript{4} treated rats suggests that wheatgrass contributes in the breakdown of the different membranous structures to varying extents, thus preventing the leakage of the enzymes into the blood stream. The transaminases are amongst the important, specific liver enzymes that interconnect the metabolism of proteins and carbohydrates. Similar results were observed regarding the activities of both the transaminases in serum, which were found to be increased significantly (p<0.05 to p<0.001) as a result of CCl\textsubscript{4} toxicity. Both of these transaminases are liver-specific enzymes and are considered to be very sensitive and reliable indices in serum for measuring hepatoxic as well as the hepatoprotective or curative effects of various compounds. \[5\] Wheatgrass treatment of CCl\textsubscript{4} intoxicated rats furnished very interesting results, since it restored the activities of both hepatic AST and ALT almost to within the normal limits. The activities of serum AST and ALT were significantly reduced as compared to the levels in the CCl\textsubscript{4}-treated group and maximum reduction was found in 80 mg dose. Three compounds: Choline, magnesium and potassium, present abundantly in wheatgrass, help the liver to stay vital and healthy. Choline works to prevent the deposition of fat. Magnesium helps to draw out excess fat in the same way and potassium acts as an invigorator and stimulant. \[26,27\] Though the mechanism of protection of wheatgrass is still obscure, the present results suggest, that it probably helps in regulating protein metabolism, which in turn controls AST and ALT activities.

In the present study, we have also observed significantly reduced GSH content. GSH, acting through enzymes as substrates or a co factor, is known to be an important cellular antioxidant. Burk et al concluded that GSH protect cells against CCl\textsubscript{4} induced lipid peroxidation. \[28\] So, when these rats were supplemented with wheatgrass, GSH levels increased and came closer to normal values at a dose of 80 mg/100g b.wt dose. Recent studies have also shown protective effect of wheatgrass on alcohol induced oxidative stress in rats where the levels of TBARS and LH were significantly (p ≤ 0.05) increased in alcohol + ΔPUFA group, which were found to be reduced on treatment with wheatgrass. The levels of both enzymatic and nonenzymatic antioxidants were reportedly decreased (p ≤ .05) in alcohol + ΔPUFA group, which were found to be restored on treatment with wheatgrass. \[29\] Another study also reported that Wheat grass supplementation with a high-fat diet resulted in improved lipid levels (decreased total cholesterol and increased HDL-C) together with significantly reduced MDA levels and
increased GSH and vitamin C levels. These results indicate the beneficial role of wheat grass in ameliorating hyperlipidemia and the associated oxidative stress. [20]

These observations are also consistent with the results of histological findings, which show that wheatgrass is remarkably efficient in preserving the structural integrity of the liver.

CCl₄ reaches to its maximum concentration in liver parenchyma within two hours of administration. [25] This hepatotoxin acts directly on parenchymal cells of liver and lipid containing structural components of the cell are affected by the presence of lipid soluble, non polar lipid solvent. Thus, compositional, functional and morphological changes in all cytoplasmic membrane systems of the liver cells would be expected to occur during the times of maximum concentration of carbon tetrachloride in the liver. [21] The results obtained during the current investigations revealed that CCl₄ treatment for 4 weeks caused centrolobular necrosis of the parenchymal cells of liver. However, CCl₄ treated animals which were supplemented with wheatgrass exhibited minor structural changes and fatty changes were negligible with 80mg of wheatgrass. The major benefit of wheat grass in diseased conditions appears to be linked to the presence of biologically active compounds and minerals as well as antioxidant potential of bioflavonoids such as apigenin, quercitin, luteoline. Furthermore, indole compounds namely choline and laetrile present in it may also be responsible for its therapeutic potential. [31-34] The presence of 70% chlorophyll, which is almost chemically identical to hemoglobin, in wheat grass shall make it more useful in various clinical conditions involving hemoglobin deficiency and other chronic disorders.

**CONCLUSION**

Hence, the present study concludes that wheatgrass is effective in providing protection to liver during CCl₄ toxic conditions and may prove to be beneficial in protecting the damage to liver during other diseased conditions. Therefore, it will be interesting to understand the physiological and molecular mechanisms underlying the action of wheatgrass in future studies.
Conflict of interest: None

Funding Agency: Indian Council of Medical Research, Delhi, India

Authors’ Contribution:
1. JK Kamboj was responsible for all the experimental and analytical part of the paper.
2. Prof. SV Rana is corresponding author who mainly looked into the oxidative stress part of the experiment and provided all the required chemicals and necessary guidance.
3. Prof Kim Vaiphei supervised the histological part of the experiment. She analyzed and confirmed the histological findings of the work.
4. Prof. DK Dhawan played a major role in paper editing and also helped in liver enzymes analysis and also provided all the necessary chemicals and glassware.

Key Message
Human population is constantly exposed to toxic chemicals in the environment. The liver, because of its strategic anatomical location and its large capacity for metabolic conversions, is continuously exposed to different kind of xenobiotics and therapeutic agents. There is a need to develop useful hepatoprotective drugs without any side effects. In the last decade, a great deal of research has been devoted in India and worldwide to study the effect of natural products with antioxidant activity. The present study was mainly carried out to evaluate the efficacy of hepatoprotective agents against carbon tetrachloride toxin. The cereal grasses - wheatgrass, barley grass, Alfa-Alfa have been known to boost health and vitality both in humans and animals. Therefore, it will be interesting to understand the physiological and molecular mechanisms underlying the action of wheatgrass which can prove to be really beneficial for people who opt for alternative source of medicine, especially in India.

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