Potential protective effects of barley and wheat grains on cardiovascular damage induced by tramadol

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Abstract

The present study was conducted to evaluate the potential protective effects of barley and wheat grasses against tramadol induced cardiovascular damage, biochemical changes and oxidative stress in adult male albino rats. Thirty six male albino rats (150±10 g) were divided into two main groups, the first main group was fed on basal diet and kept as normal control. The second main group was fed on basal diet and administrated tramadol (30mg/kg/day) orally for 30 days to induced cardiovascular damage and oxidative stress in male rats. The rats in the second main group (30 rats) were divided into five subgroups (each group consisted of 6 rats). Subgroup1; received tramadol and kept as tramadol group, subgroup2; received tramadol + barley grass (250mg/kg/day), subgroup3; received tramadol + barley grass (500mg/kg/day), subgroup4; received tramadol + wheat grass (250mg/kg/day) and subgroup5; received tramadol + wheat grass (500mg/kg/day) orally for 30 days. Biological evaluation was carried. Relative liver, kidney and heart weight were calculated. Serum was separated from the blood and assessed lipid profile and serum antioxidant markers at the end of the experiment. Results: indicated that lipid profile and serum antioxidant markers were restored significantly in barley and wheat groups at high doses (500mg/kg/day) as compared to the positive control group., thus might due to chemical composition of barley and wheat grasses can be considered as a good source for dietary fiber, polyphenols and antioxidant compounds. In conclusion, this study reveals the health benefits of barley and wheat grasses in functional foods for cardiovascular damage, and patients with heart disease.

Key words: Tramadol, antioxidant & oxidant parameters and lipid profile.
Introduction

Barley (*Hordeum vulgare L.*) is the fourth cereal crop in the world, has high dietary fiber content; its malt for functional food is not only the world’s largest material for beer, but also often used as one of 300 species being used in Chinese herbal medicine. Regular consumption of barley grass reduces the risk of chronic diseases (diabetes, cancer, obesity, cardiovascular disease, etc.), based on phytochemicals including β-glucan, phenolic acids, flavonoids, lignans, tocols, phytosterols, and folate (*Idehen et al., 2017* and *Minaiyan et al., 2014*). It is preventive inflammatory and cardiovascular diseases has exhibited activities against all human platelet agonists inhibited both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism, which elevated the SOD and GSH-Px activities (*Gul et al., 2014*).

Barley grass has young green leaves and stem of vegetative growth stage from seedling at 10 days after sprouting (barley sprout) to elongation stage (barley green) for nutritional peak before the start of reproductive cycle of barley (*Park et al., 2015*); anti-arthritis, reducing cholesterol, and antioxidant (*Lahouar et al., 2014*). So has played an important role in human health, coevolution and functional ingredients as well as major mechanism in therapeutic role (*Gao et al., 2015*).

Wheatgrass (*Triticum Aestivum L.*) is one of the cereal grasses mostly used as a functional drink, has quickly become “the new age espresso”, and mostly used in the form of smoothies, juices, salads, tablets, and powders (*Ben, 2002*). Wheatgrass juice contains minerals and trace elements including calcium, iodine, magnesium, selenium, zinc, chromium, antioxidants like vitamin C, vitamin E, β-carotene, vitamin B1, antianemic factors like vitamin B12, iron, folic acid, pyridoxine, abscisic acid, ferulic acid, and vanillic acid – the concentrations of which increase with the
germination period. These are promoted as a source of antioxidant, the most important being O-glycosyl isovitexin, superoxide dismutase, catalase, carotenoids, other bioactive compounds, polyphenols play role in the prevention of cardiovascular diseases (Ashok, 2011).

Wheatgrass (Triticum Aestivum L.) contain nutrition support may require being adapted over time to sustain metabolic strength and endorse recover. Chlorophyll’s astringent properties make it great for heavy periods and bleeding gums. Amazingly, the chlorophyll molecule found in plants is remarkably similar to the hemoglobin molecule found in humans. That makes the chlorophyll found in wheat grass very good for stimulating hemoglobin and red cell production and makes it a great food for anemia. Fat soluble chlorophyll is a rich source of beta-carotene and best form of vitamin K. It has significant antioxidant and anticancer effects (Shyam et al., 2007)

Tramadol was discovered and manufactured in 1962 for the first time by German company (Grunenthal GmbH) for the cure of pain while being announced in the market by the name ‘Tramadol’ in 1977 (Patterson, 2018). Tramadol a substitute artificial opioid anesthetic, which is used to treat modest or severe pain (Pinho et al. 2013). Tramadol after being absorbed, it reached to the liver, after circulation in the blood throw heart, it is extracted by the kidneys. Therefore, these organs are reflecting tramadol toxicity (Barbosa et al. 2017). Unfortunatry, it was abused and accompanied by worrying phenomenon among Egyptian public (Ahmed et al., 2018). Tramadol induced oxidative stress and cause cardio vascular damage (Haytham et al., 2020).

So, this study was aimed to find the potential effects of barley and wheat grasses due to their antioxidant
abilities against tramadol induced cardio vascular damage in male rats.

**Materials and Methods**

**Materials**

Barley grass and wheat grass have been obtained from Agriculture seeds, Herbs and Medicinal Plants Company, Cairo, Egypt. Casein, minerals, vitamins, and cellulose were obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments, Tanta, Egypt. Thirty male albino rats (Sprague Dawely) Strain were purchased from the Vaccine and Immunity Organization, Helwan Farm, Cairo, Egypt. Tramadol tablets, each contains 225 mg tramadol hydrochloride obtained from October Pharma Company (Giza, Egypt).

**Methods**

**Preparation of Wheat and Barely grass powder**

**Soaking:** Wheat and barley grains were first cleaned and eliminated from dust, broken particles, and other foreign materials and then soaked for 12 hr in tap water at room temperature, with a ratio of 1:5 (grain : water (w/v)). Then non-imbibed water was disposed. Grains were soaked for removing an anti-nutrient because of its strong binding affinity to minerals (Brady et al., 2007).

**Germination:** The previously soaked seeds were individually spread on wet jute bags, covered by muslin cloth and one more wet jute bag. Then, the seeds were sprinkled by water every 12 hr. till finishing the germination period (72 hr.). The germinated seeds were picked gently with the sprouts, washed, carefully drained, dried in the oven at 50°C for 24 hr., crushed by blender and stored in labeled polyethylene bags until uses and analysis (Mansour and El-Adawy, 1994)
Nutritional Characteristics

Chemical Composition: Barley grass and wheat grass were chemically analyzed for moisture, fat, protein, fiber, and ash, according to methods of (A.O.A.C., 2000). Total carbohydrates were estimated by difference. Whereas, the energy calculated by the factor of 4, 9 and 4 for proteins, fats, and carbohydrates, respectively (Chaney, 2006).

Total Minerals of Fe and Zn were extracted by wet acid-digestion method, using mixture of nitric acid and perchloric acid (HNO₃: HClO₄, 5: 1 w: v). Total Fe and Zn in the digested solution were measured by atomic absorption spectrophotometry (Thermo Elmental, 300VA, UK) (Lindsey and Norwell, 1969).

Vitamin C and E Analysis: Spectrophotometer (Model No 6300, Designed and made in UK by I en way LTD) was used to estimate vitamin C levels. While, retention time of the vitamin E acetate peak is about 10 minutes. The column efficiency determined by the number of theoretical plates is not less than 3000 and the tailing factor is not more than 1.5 for the vitamin E acetate peak as described by (Anonymous, 1966).

Chlorophyll Analysis: In this research, Chlorophyll were analyzed by HPLC (reversed-phase) using water 600 system which provided with auto samples injector, degasser, pump in addition to water 996 UV (visible photodiode array detector) operating at 450nm. Then, the data were saved and processed by Millennium 4.00 software (production of Waters, Stockholm, Sweden). The absorption spectra were recorded between 250 - 500 nm (Rodriguez-Amaya, 1997).

Experimental Design: Thirty six albino male rats (Sprague Dawely). After seven days the adaptation period, rats were
randomly divided into two main groups; the first group control (-) had 6 rats that were fed on basal diet which composed of 12 g of casein (85 % protein); corn oil (10 % fat); minerals mixture (4 % minerals); vitamins mixture (1% vitamins); cellulose (4% fiber); and corn starch (71 % starch), according to (Jerome et al., 2002); The used vitamins and salts mixture component were that recommended by Campbell, (1963) and Hegsted et al., (1941), respectively. Water supply was given ad-libitum and checked daily. The second group (n= 30 rats), divided into five subgroups each group had 6 rats, one kept as a control (+) and fed on basal diet, the other four groups were fed on basal diet supplemented with Barley grass at 250 mg kg\(^{-1}\), 500 mg kg\(^{-1}\) and wheat grass at 250 mg kg\(^{-1}\), 500 mg kg\(^{-1}\) respectively. Rats received tramadol at dose (30 mg/kg/day), orally for 30 days. This dose is 1/10 of LD50. LD 50 is 286–300 mg/kg (Elkhateeb et al., 2015). Rats received barley and wheat grasses at doses (250, 500 mg/kg/day). This doses were freshly prepared, suspended in distilled water and taken orally for 30 days (Abed et al., 2017).

**Biological Evaluation:** During the experimental period, feed intake, body weight gain % (BWG %), and feed efficiency ratio (FER) was recorded every week. After sacrificed organ weight relative to body weight % were calculated as described by Chapman et al., (1959). Liver, kidney, heart and tests were removed from each rat, carefully washed with saline solution, dried with filter paper and weighted based on Drury and Wallington (1980).

**Biochemical analysis**
Serum total cholesterol (T.C), triglycerides (T.G) and HDL-c were determined according to methods of Allain et
Serum VLDL-c and LDL-c were being calculated using equation described by Friedwald et al., (1972), Atherogenic Index (AI) according to the formula of Kikuchi et al., (1998) respectively.

Antioxidant and oxidant parameters in serum: Malondialdehyde (MDA), Nitric oxide (No), Catalase (CAT), and Glutathione peroxidase (GPx) were determined in serum according to the methods of (Ruiz-Larrea et al., 1994; Moshage et al., 1995; Goth et al., 1984 and Ahmadvand et al., 2014) respectively.

Antioxidant and oxidant parameters in heart tissues: Malondialdehyde (MDA), Nitric oxide (No), Catalase (CAT), and reduced glutathione (GSH) were determined in kidneys tissues according to the methods of (Placer et al., 1966; Green et al., 1982; Aebi, 1974 and Maral et al., 1977) respectively.

Statistical Analysis: Data were represented as means ± standard deviation (SD). Differences were statistically analyzed by one-way analysis of variance (ANOVA test) using SPSS 16 software package and considered significant at P values < 0.05 Armitage and Berry (1987).

Results and discussions
Chemical composition:
The chemical composition (on dry weight basis per 100 g) of wheat and barley grass are presented in Table (1). It is noticed from the data that fat, protein, carbohydrate, fiber, and moisture for wheat grass representing 1.56, 21.76, 43.02, 12.68, and 14.48 % respectively, giving energy representing 323.88 kcal. Comparing with chemical
composition of barley grass, as fat, protein, carbohydrate, fiber, and moisture showed 2.37, 19.31, 40.4, 18.5 and 11.92% respectively, giving energy representing 334.17 kcal.

Table (1): Chemical composition of dried wheat and barley grass (per 100 g)

<table>
<thead>
<tr>
<th>Nutritional component</th>
<th>Protein %</th>
<th>Fat %</th>
<th>Moisture %</th>
<th>Fiber %</th>
<th>Ash %</th>
<th>Carbohydrate %</th>
<th>k.cal</th>
<th>Chlorophyll (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat grass</td>
<td>21.76</td>
<td>1.56</td>
<td>14.48</td>
<td>12.68</td>
<td>6.5</td>
<td>43.02</td>
<td>323.88</td>
<td>499.5</td>
</tr>
<tr>
<td>Barley grass</td>
<td>19.31</td>
<td>2.37</td>
<td>11.92</td>
<td>18.5</td>
<td>7.5</td>
<td>40.4</td>
<td>334.17</td>
<td>539.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minerals and vitamins</th>
<th>Iron mg</th>
<th>Zinc mg</th>
<th>Vit. C mg</th>
<th>Vit. E mg</th>
<th>Total polyphenol (%)</th>
<th>Total flavonoids (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat grass</td>
<td>5.27</td>
<td>3.90</td>
<td>14.4</td>
<td>12.8</td>
<td>1.05</td>
<td>162.12</td>
</tr>
<tr>
<td>Barley grass</td>
<td>14.7</td>
<td>4.93</td>
<td>26.1</td>
<td>14.9</td>
<td>1.15</td>
<td>148.33</td>
</tr>
</tbody>
</table>

Also Table (1) showed that represents vit. C, vit. E, Chlorophyll, Iron, zinc, Total polyphenol and Total flavonoids for wheat grass representing (14.4, 12.8, 499.5, 5.27, 3.90) mg, 1.05% and 162.12 μmol/l respectively. Comparing with vit. C, vit. E, Chlorophyll, Iron, zinc, Total polyphenol and Total flavonoids for barley grass (26.1, 14.9, 539.9, 14.7, 4.93) mg, 1.15% and 148.33 μmol/l respectively.

Wheatgrass contains minerals and trace elements including calcium, iodine, magnesium, selenium, zinc, chromium, antioxidants like vitamin C, vitamin E, β-carotene, vitamin B1, anti-anemic factors like vitamin B12, iron, folic acid, pyridoxine, abscisic acid, ferulic acid, and vanillic acid – the concentrations of which increase with the
germination period. Similarly, barley grass is much more important and the early production of cereal crop (A.O.A.C., 2010).

Rana et al., (2011) mentioned that barley grass is nutritionally very similar to wheatgrass. There are no main differences between the two foods though, with barley grass containing little more calcium, potassium, chlorophyll, and enzymes than wheatgrass. Barley grass has detoxifying and antioxidant effects. It is rich in antioxidant compounds, such as flavonoids, polyphenols, superoxide dismutase (SOD), saponarin, lutonarin, Se, tryptophan, chlorophyll, vitamins (pro vitamin A, C, and E), dietary fiber (Zeng et al., 2018). Barley plays a main role in health. Zeng et al., (2020) reported that barley grass has antioxidant activity as flavonoids, SOD, vitamins, and dietary fiber as β-glucans.

2. Biological evaluation of investigated rats:

Feed intake (FI), body weight gain % (BWG%) and feed efficiency ratio (FER) of treated rats by tramadol are shown in Table (2). The data revealed that FI, BWG% and FER of positive rats (17.13 ± 0.56 g ,22.00±3.68 % and 0.05±0.02 ), respectively were declined significantly decrease than these of negative control rats (18.90 ± 0.23 g , 30.71±4.02 % and 0.16± 0.02 ), in contrast The obtained results recorded significant increase in all treated groups when compared with the positive control group. The data in FI showed non-significant differences between all treated groups, on the other hand all treated groups with barley grass or wheat grass recorded non-significant changes in this parameter, as compared to the negative control group. The best value for BWG was observed in tramadol group which treated with wheat grass (250 mg/kg), followed by barley grass (250 mg/kg), respectively. (28.21± 5. 98 & 25.43± 2.20 %). The best values for FER were observed in
the group treated with wheat grass (250 mg/Kg) and barley grass (250mg/kg) (0.12±0.02 & 0.08± 0.01)

**Table (2): Effects of barley grass and wheat grass powder on feed intake, body weight gain and feed efficiency ratio of rats treated with tramadol (mean±SD, n=6)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>FI (g)/day</th>
<th>BWG (g)</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>18.90 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.71±4.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive control</td>
<td>17.13 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.00±3.68&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.05±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Barley grass (250mg/kg)</td>
<td>18.90 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.43±2.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Barley grass (500mg/kg)</td>
<td>19.02±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.57±1.23&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wheat grass (250mg/Kg)</td>
<td>19.26±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.21±5.98&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.12±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wheat grass (500mg/kg)</td>
<td>19.22±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.38±4.53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.09±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Means with different superscript letters in the same column were different significantly at p≤0.05

According to studies of **Oka et al., (2015)** who reported that administration of tramadol has inhibitory effects on the appetite centers of the hypothalamus which may explain the low food intake in these groups. Also these results were in harmony with **Elbadrawy and Elkewawy (2019)** mentioned that there were significant decreases in weight gain, feed intake and feed efficiency ratio in tramadol group. They explained that tramadol led to some intestinal disturbances such vomiting, nausea and constipation with changing in appetite. **Abd El-Mottaleb et al., (2019)** cleared that there was decrease in body weight of rats given tramadol 30 mg/kg/day for three months. In contrast **Ali et al., (2020)** showed that receiving tramadol at 3 doses 25 mg/kg, 50 mg/kg and 75 mg/kg of body weight did not alter feed intake but reduced body weight in rats.

**Ikeguchi et al., (2014)** revealed that adding barley leaf powder in the diet increased the fecal weight as it
contain water-soluble dietary fiber and stimulating gastrointestinal tract by pH lowering. Jorige and Akula (2015) reported that regular consumption of the wheatgrass can develop the gastrointestinal system. Wheat grass juice reduce body weight as it contains selenium which improved function of the thyroid gland and contain potassium which aspects in coming off of water weight. So that, managing body weight. Also, wheatgrass blocks the stomach, thus suppressing the appetite (Husain et al., 2017).

Daotong et al., (2018) investigated and revealed the ability of barley leaf contains abundant plant fibers, which are important substrates for the metabolism. Ghoniem et al., (2018) cleared that administration fresh wheat grass Juice to rats received high fat diet reduced body weight gain. Barley grass is a health drink in many parts of India. It is suppressing obesity and cholesterol levels (Thatiparthi et al., 2019). Oral administration of polysaccharides isolated from barley leaf improved the decrease of body weight (Han et al., 2020).

Relative organs weight
Table (3) shown the results of relative weight of liver, spleen, lungs, and heart to body weight in rats' treated with Tramadol after feed on barley grass and wheat grass. Liver weight was significantly increased at (p<0.05) in positive group when compared to normal rats’ group 3.17±0.17 and 2.06±0.16 %, respectively). However, there is insignificant difference between all treat groups. On contrast, the mean value of kidney weight was decreased significantly at (p <0.05) in the control positive group, as compared to normal rats’ group. The best results recorded in barley grass (500mg/kg) at 0.51±0.05 %. In heart, weight was significantly increased at (p<0.05) in positive group when compared to normal rats’ group. Generally, all treated group had significant decrement of heart, organ weights compared to the positive group.
Table (3): Effects of barley grass and wheat grass powder on relative organs weight of rats treated with tramadol (mean±SD, n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver %</th>
<th>Kidney %</th>
<th>Heart %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>2.06±0.16b</td>
<td>0.50±0.07a</td>
<td>0.22±0.04c</td>
</tr>
<tr>
<td>Positive control</td>
<td>3.17±0.17a</td>
<td>0.37±0.12c</td>
<td>0.40±0.05a</td>
</tr>
<tr>
<td>Barley grass (250mg/kg)</td>
<td>2.08±0.40b</td>
<td>0.54±0.19a</td>
<td>0.32±0.06b</td>
</tr>
<tr>
<td>Barley grass (500mg/kg)</td>
<td>2.37±0.19b</td>
<td>0.51±0.05a</td>
<td>0.27±0.03c</td>
</tr>
<tr>
<td>Wheat grass (250mg/Kg)</td>
<td>2.06±0.20b</td>
<td>0.43±0.09b</td>
<td>0.22±0.05c</td>
</tr>
<tr>
<td>Wheat grass (500mg/kg)</td>
<td>2.16±0.10b</td>
<td>0.47±0.04b</td>
<td>0.23±0.05c</td>
</tr>
</tbody>
</table>

- Means with different superscript letters in the same column were different significantly at p≤0.05

Relative organ weight estimations are an important part of the toxicological valuation of chemical ingredients. **Foda, (2010)** reported that adding young green barley leaves powder might improve kidney, liver and heart weight. **Lakshmi et al., (2015)** who studied the consumption wheat grass extract orally (200 and 400mg/kg) by rats for 20 following days before oral receiving of sodium arsenite significantly preserved kidney weights of experimental rats towards normal after arsenic exposure.

**Adikwu and Nelson, (2018)** observed that absolute and relative organ weights were not significantly altered in the tramadol treated rats in comparison to control. Also, the finding is in harmony with **Obembe and Olatoke, (2019)** recorded that admiration tramadol (50mg/kg) of male wistar rats had no significant effect on relative organs weight. Three compounds (Choline, magnesium and Potassium), found abundantly in wheatgrass, help the liver to stay vital and healthy. Moreover choline works to prevent the deposition of fat. Magnesium helps to draw out excess fat in the same way. Magnesium sulfate (Epsom
salties) draws pus from an infection, and potassium acts as an invigorator and stimulant. Wheatgrass extract contain apigenin, Flavonoids like apigenin are known to improve hepatic functions in high-fat diet-induced diabetic rats (Zhang et al., 2018). Hence the hepatoprotective effect of wheatgrass diet could be due to the presence of flavonoids.

Biochemical analysis:

Lipids profile:

Results recorded in Table (4) showed the effects of barley grass and wheat grass powder on total cholesterol and triglycerides of rats treated with tramadol. The data revealed that, total cholesterol and triglyceride record the highest significant increase for tramadol group as compared to normal group. Moreover, barley grass (500 mg/kg) followed by wheat grass (500 mg/kg) recorded the highest significant decrease in total cholesterol and triglyceride as compared with other treated groups.

Table (4): Effects of Barley grass and Wheat grass powder on total cholesterol and triglycerides of rats treated with tramadol (mean±SD, n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg dl⁻¹)</th>
<th>Triglycerides (mg dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>110.0±2.03e</td>
<td>67.00 ± 1.00d</td>
</tr>
<tr>
<td>Positive control</td>
<td>174.0±2.02a</td>
<td>117.07±2.52a</td>
</tr>
<tr>
<td>Barley grass (250mg/kg)</td>
<td>140.7±2.51b</td>
<td>81.33±4.51b</td>
</tr>
<tr>
<td>Barley grass (500mg/kg)</td>
<td>122.3±2.52d</td>
<td>75.00±2.00c</td>
</tr>
<tr>
<td>Wheat grass (250mg/Kg)</td>
<td>132.0±1.02c</td>
<td>87.47±3.21b</td>
</tr>
<tr>
<td>Wheat grass (500mg/kg)</td>
<td>127.0±2.07d</td>
<td>79.76±5.51bc</td>
</tr>
</tbody>
</table>

- Means with different superscript letters in the same column were different significantly at p≤0.05

Cholesterol-lowering effects have been attributed to the hexacosyl alcohol and beta-sitosterol fractions of barley leaf extract. Beta-sitosterol is thought to act by inhibiting
the intestinal absorption of cholesterol and accelerating its catabolism to bile acid (Yu et al., 2004). Treatment with barley grass juice at (200 and 400 mg/kg) restored the lipid profile to normal compared to high fat diet obese group showed a significant decrease in serum TC, TG levels (Jhansyrani et al., 2019)

Furthermore, the ethanolic extract of wheatgrass was found to improve blood lipid profiles (TC, TG) and reduced the inflammation of non-alcoholic fatty liver and obesity in mice fed with high-fat and choline-deficient diet. It was demonstrated that the wheatgrass extract exerted the hepatoprotective mechanisms by improving insulin resistance and lipid metabolism (Oh et al., 2019)

**Lipoprotein fractions:**

Results in Table (5) explained plasma level of (VLDL, HDL, LDL and atherogenic index). Plasma lipoprotein fraction proved significantly decrease in HDL level. On contrast it showed significantly increase in VLDL, LDL levels and (AI) for tramadol group as compared to normal group. Barley grass (500 mg/kg) followed by wheat grass (500 mg/kg) recorded the best result for an HDL level as compared to tramadol group. Moreover, Barley grass (500 mg/kg) followed by wheat grass (500 mg/kg) revealed the highest result of for LDL level compared to positive control group (57.00±1.00 mg dl⁻¹). The best VLDL result showed in Barley grass (500 mg/kg) (15.00±2.00 mg dl⁻¹). In addition, the highest result of atherogenic index record in Barley grass (500 mg/kg) as closet to normal group (1.91±0.09) as compared with all grasses groups.
Table (5): Effects of barley grass and wheat grass powder on antioxidant parameters in serum of rats treated with tramadol (mean±SD, n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>HDL (mg dl⁻¹)</th>
<th>LDL (mg dl⁻¹)</th>
<th>VLDL (mg dl⁻¹)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>56.03±3.61</td>
<td>40.6±2.00</td>
<td>13.4±2.00</td>
<td>0.96±0.07d</td>
</tr>
<tr>
<td>Positive control</td>
<td>29.04±1.08</td>
<td>91.00±1.02</td>
<td>23.07±2.02</td>
<td>2.3±0.04a</td>
</tr>
<tr>
<td>Barley grass (250mg/kg)</td>
<td>47.00±2.00</td>
<td>76.8±2.01</td>
<td>16.2±1.01</td>
<td>1.97±0.09b</td>
</tr>
<tr>
<td>Barley grass (500mg/kg)</td>
<td>50.33±5.03</td>
<td>57.00±1.01</td>
<td>15.00±2.01</td>
<td>1.44±0.08c</td>
</tr>
<tr>
<td>Wheat grass (250mg/Kg)</td>
<td>45.33±1.5</td>
<td>69.17±2.03</td>
<td>17.49±2.02</td>
<td>1.91±0.09b</td>
</tr>
<tr>
<td>Wheat grass (500mg/kg)</td>
<td>49.67±4.04</td>
<td>61.37±1.03</td>
<td>15.95±1.03</td>
<td>1.55±0.07c</td>
</tr>
</tbody>
</table>

- Means with different superscript letters in the same column were different significantly at p≤0.05

Jhansyrani et al., (2019) reported that treatment with barley grass juice (200 and 400 mg/kg, p.o), restored the lipid profile to normal compared to high fat diet obese group showed a significant decrease in serum LDL and VLDL phospholipids and increase HDL levels.

Furthermore, antioxidants have a major share in the management of obesity by reducing the levels of glucose, triglycerides and LDL cholesterol in blood, increasing energy expenditure and fat oxidation, as well as lowering body weight and adiposity (Garcia-Lafuente et al., 2009 and erra et al., 2009). Previous studies have indicated the beneficial effects of barley grass in the treatment of chronic diseases like atherosclerosis, CVD, cancer, metabolic syndrome etc., but scientific evidence for their effect against obesity is lacking which prompted us to plan the current work.

Chauhan, (2014) investigated the antioxidant activity of wheatgrass at various levels of protection from radical scavenging and inhibition of free radical induced
membrane damage, reducing the levels of cholesterol, triglycerides and LDL cholesterol, increasing HDL cholesterol in blood.

**Antioxidant and oxidant parameters in serum:**

Results registered in Table (6) showed serum nitric oxides (NO), malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GPX). The data revealed that, there were significant increase for NO and MDA and significant decrease for CAT and GPX for tramadol group as compared to normal group. Barley grass (500 mg/kg) followed by wheat grass (500 mg/kg) recorded significant decrease in serum NO and MDA. In contrast barley grass (500 mg/kg) and wheat grass (500 mg/kg) revealed highly significant increase in serum CAT, GPX as compared to tramadol group and all grasses groups.

**Table (6): Effects of Barley grass and Wheat grass powder on antioxidant parameters in serum of rats treated with tramadol (mean±SD, n=6)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO (nmol ml⁻¹)</th>
<th>MDA (nmol ml⁻¹)</th>
<th>CAT (ng ml⁻¹)</th>
<th>GPX (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>45.87 ±1.23ᵃ</td>
<td>42.00 ±3.79ᵃ</td>
<td>56.37 ±2.29ᵃ</td>
<td>39.25 ±3.18ᵃ</td>
</tr>
<tr>
<td>Positive control</td>
<td>97.50 ±3.53ᵃ</td>
<td>131.50 ±1.09ᵃ</td>
<td>28.75 ±4.01ᵈ</td>
<td>12.50 ±2.12ᵉ</td>
</tr>
<tr>
<td>Barley grass (250mg/kg)</td>
<td>73.75 ±3.88ᵇ</td>
<td>90.00 ±5.65ᵇ</td>
<td>43.75 ±5.30ᵇ</td>
<td>34.75 ±1.01ᵇ</td>
</tr>
<tr>
<td>Barley grass (500mg/kg)</td>
<td>51.25 ±1.76ᶜ</td>
<td>54.50 ±4.94ᶜ</td>
<td>30.00 ±2.82ᶜ</td>
<td>15.50 ±1.41ᵈ</td>
</tr>
<tr>
<td>Wheat grass (250mg/Kg)</td>
<td>79.50 ±2.19ᵇ</td>
<td>88.00 ±5.6ᵇ</td>
<td>47.00 ±3.89ᵇ</td>
<td>30.75 ±4.59ᵇ</td>
</tr>
<tr>
<td>Wheat grass (500mg/kg)</td>
<td>53.25 ±1.06ᶜ</td>
<td>59.75 ±1.06ᶜ</td>
<td>33.37 ±3.18ᶜ</td>
<td>22.50 ±2.12ᶜ</td>
</tr>
</tbody>
</table>

- Means with different superscript letters in the same column were different significantly at p≤0.05.

The present work detected a highly significant increase in serum of NO, MDA levels and significant reduction in serum of CAT and GPX levels in tramadol group as compared to normal control. The current results confirmed the results of an earlier study directed by
Ahmed and Kurkar, (2014) who suggested that tramadol causes lipid peroxidation and increase in MDA levels. The major reduction in GSH activity can be described by its consumption through the purification of reactive oxygen metabolites (Hamza and Al-Harbi, 2014). Hindawy et al., (2019); Nazifi et al., (2019); and Omar et al., (2019) confirmed the present results about administration tramadol increased serum MDA and NO due to oxidative stress, increasing the lipid peroxidation and increasing the generation of free radicals.

Our results indicated that barley grass and wheat grass (500 mg/kg) recorded significant decrease in serum of NO, MDA. Also they revealed significant increase in serum CAT, GPX as compared to tramadol group and all grasses groups. Barley grass is rich in antioxidants which overturn lipid peroxidation, such as polyphenols, saponarin, lutonarin, superoxide dismutase (SOD), Se, chlorophyll, vitamins (pro vitamin A, C and E) and flavonoids (Zeng et al., 2018). Thatiparthi et al., (2019) showed that barley grass juice (Hordeum vulgare L.) has potent antioxidant action. Barley sprouts juice inhibited the increase of malondialdehyde level and the reduction of catalase activities (Mohamed et al., 2019). Barley grass possesses antioxidant properties (Deng et al., 2020). Wheatgrass has many antioxidant compounds as Selenium, pro vitamin A, C, E, Carotene, transhydrogenase and superoxide dismutase (SOD) cytochrome oxidase (Padalia et al., 2010). Wheatgrass contains great amount of antioxidants, thus it can be used as an antioxidant phytomedicine against oxidative stress produced by chemotherapeutic drugs (Sachin et al., 2013). Durairaj et al., (2014) cleared that administration of wheatgrass to male albino rats returned levels of antioxidants to normal as superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, vitamin E, and vitamin C, which had decreased resulting from alcohol which induced oxidative stress.
Antioxidant and oxidant parameters in heart tissue:

Results registered in Table (7) showed antioxidant enzymes heart tissue; nitric oxides (NO), malondialdehyde (MDA), catalase (CAT) and reduced glutathione (GSH). The data revealed that, there were significant increase for NO and MDA and significant decrease for CAT and GPX for tramadol group as compared to normal group. Barley grass (500 mg/kg) followed by wheat grass (500 mg/kg) recorded significant decrease in serum NO and MDA. In contrast barley grass (500 mg/kg) and wheat grass (500 mg/kg) revealed highly significant increase in serum CAT, GSH as compared to tramadol group and all grasses groups.

Table (7): Effects of Barley grass and Wheat grass powder on antioxidant parameters in heart tissue of rats treated with tramadol (mean±SD, n=6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO (nmol ml⁻¹)</th>
<th>MDA (nmol ml⁻¹)</th>
<th>CAT (ng ml⁻¹)</th>
<th>GSH (ng mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>32.25 ±1.06⁶</td>
<td>49.12 ±4.06⁶</td>
<td>52.05 ±1.70⁴</td>
<td>7.70 ±0.14⁴</td>
</tr>
<tr>
<td>Positive control</td>
<td>94.50 ±0.70⁴</td>
<td>142.50 ±6.6⁴</td>
<td>22.57 ±2.01⁴</td>
<td>1.80 ±0.07⁴</td>
</tr>
<tr>
<td>Barley grass (250mg/kg)</td>
<td>72.00 ±2.82⁷</td>
<td>75.25 ±3.98⁷</td>
<td>49.75 ±2.35⁷</td>
<td>4.05 ±0.42⁷</td>
</tr>
<tr>
<td>Barley grass (500mg/kg)</td>
<td>45.50 ±5.07⁶</td>
<td>65.50 ±2.82⁶</td>
<td>34.60 ±2.27⁶</td>
<td>2.65 ±0.21⁶</td>
</tr>
<tr>
<td>Wheat grass (250mg/Kg)</td>
<td>73.87 ±2.65⁷</td>
<td>81.50 ±2.12⁷</td>
<td>48.37 ±3.35⁷</td>
<td>4.67 ±0.17⁷</td>
</tr>
<tr>
<td>Wheat grass (500mg/Kg)</td>
<td>51.25 ±5.13⁶</td>
<td>67.50 ±0.70⁷</td>
<td>38.82 ±1.87⁶</td>
<td>2.72 ±0.38⁶</td>
</tr>
</tbody>
</table>

- Means with different superscript letters in the same column were different significantly at p≤0.05

Zeng et al., (2018) revealed that barley grass is rich in antioxidants which overturn lipid peroxidation, such as polyphenols, saponarin, lutonarin, superoxide dismutase (SOD), Se, chlorophyll, vitamins (pro vitamin A, C and E) and flavonoids. Thatiparthi et al., (2019) showed that barley grass juice has potent antioxidant action. Barley sprouts juice inhibited the increase of malondialdehyde level and the reduction of catalase activities (Mohamed et
Moreover the antioxidant activity of wheatgrass was observed at various levels of protection from radical scavenging and inhibition of free radical induced membrane damage (Chauhan, 2014). Reduction of MDA and NO levels, increased activity of antioxidant enzymes (CAT, and GSH), and improvement of hematological parameters were observed in rats exposed to toxic metals that were treated with wheatgrass. This indicated that a diet rich in phenolic contents can prevent oxidative stress by boosting antioxidant enzymes and reverse tissue damage (Ajiboye et al., 2020).

Conclusion and recommendation
The results from this study focus on the effect of barley and wheat grains on cardiovascular damage induced by tramadol. As tramadol induced oxidative stress and cause cardiovascular damage. Thus, the study reveals the health benefits of barley and wheat grasses due to chemical composition that can be considered a good source for dietary fiber, polyphenols and other antioxidant compounds which raised the nutritional value in functional foods for cardiovascular damage and patients with heart disease.
References


Foda, M. (2010). Biochemical studies on antioxidants extracted from young green barley leaves. Master of Science in Agriculture science (Biochemistry). Department of Biochemistry Faculty of Agriculture Benha Universty, Benha, Egypt.


The potential protective effects of barley and wheat sprouts against cerebral infarction caused by tramadol and its biochemical changes in adult rats

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The study was conducted to evaluate the potential protective effects of barley and wheat sprouts on cerebral infarction caused by tramadol and its biochemical changes in adult rats. Sixty and thirty adult albino rats (150 ± 10 g) were divided into two main groups, the first group was fed the normal diet and given water by mouth for 30 days, while the second group was fed the normal diet and given tramadol (30 mg/kg/day) by mouth for 30 days to induce cerebral infarction. Then, the rats in the second main group were divided into five subgroups (each subgroup consisted of 6 rats): subgroup 1; tramadol; subgroup 2; tramadol + barley sprout (250 mg/kg/day); subgroup 3; tramadol + barley sprout (500 mg/kg/day); subgroup 4; tramadol + wheat sprout (250 mg/kg/day), and subgroup 5; tramadol + wheat sprout (500 mg/kg/day) through oral tube for 30 days. The biological evaluation included the diet consumed and the body weight gained, as well as the absorption efficiency of the diet.
ووزن الأعضاء كذلك تحليل دهون الدم ومضادات الأكسدة في السيرم وفي نسيج القلب.

النتائج: تشير إلى التحسن المعنوي في تحليل دهون الدم ومضادات الأكسدة للمجموعات التي تناولت حشائش الشعير والقمح بجرعات عالية (500 ملجم/ كجم يوم) عند مقارنتها بالمجموعة الضابطة الموجبة. وهذا يرجع إلى التركيب الكيميائي لحشيشة الشعير والقمح التي تعتبر مصدر جيد للالياف الغذائية والمواد الفينولية ومضادات الأكسدة. وخلصت الدراسة إلى الفوائد الصحية لحشيشة الشعير والقمح لمرضى القلب والأوعية الدموية.

الكلمات المفتاحية: الترامادول، مضادات الأكسدة ومؤشرات المؤكسدات، دهون الدم.