



Original Research Article

Effect of aqueous extract of barley and wheat grass in stress induced depression in Swiss mice

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ABSTRACT

Background: The consumption of green juice of Barley and wheat grass is widely increases because of its therapeutic benefits.

Objective: The study aimed to investigate phytochemicals and evaluate the antioxidant and antidepressant activity of aqueous extract of barley and wheat grass.

Material and methods: This study included phytochemical screening, evaluation of antioxidant and antidepressant activities. Four groups consisting of six mice in each group. Negative control with stressed induced mice; imipramine group (100 mg/kg), barley and wheat extract group (400 mg/kg) respectively. Forced swim, tail suspension and elevated plus maize test were carried out to evaluate the antianxiety and antidepressant activity.

Results: Phytochemical screening of barley and wheat grass extract showed secondary metabolites such as alkaloids, Saponin, Tannins, Phenolic, Carbohydrate, Glycosides, Flavonoids, and Proteins. The mean total phenolic content of aqueous extract of barley and wheat grass was 160.996 ± 0.656 , 135.63 ± 1.184 mg equivalent of GAE/g respectively. The total flavonoid content of aqueous extract of barley and wheat grass was 153.42 ± 0.40 , 133.14 ± 0.43 mg equivalent of quercetin/g respectively. The extracts were proved to be an effective radical scavenger in all antioxidant assays. Forced swim and tail suspension test showed a significant ($*p < 0.05$ and $**p < 0.01$) decrease in an immobility time. In elevated plus maize, there was a significant ($**p < 0.01$) increase in average time spent on the open arm of the extract-treated group as compared to the negative control.

Conclusion: It shows that barley and wheat grass extract has antidepressant effect.

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1. Introduction

Depression, a potentially life-threatening psychiatric illness, affects millions of people around the world. Almost one in every five individuals suffers from depression at some stage in their life, with the risk being three times higher (15–18 percent) [1]. The prevalence and association of depression is the major global health concern [2]. The pathophysiology of depression is linked to several neurological theories as well as environmental factors. The stressful lifestyle and life events may trigger major depressive disorder [3,4]. In a study, patients consuming a tryptophan-deficient diet experience a transient drop in

serotonin levels in the brain, which alters the regulation of brain function [5].

The oxidative stress is due to the imbalance in free radical generation which increases reactive oxygen species [6]. It is caused by a disruption or imbalance in the synthesis of reactive oxygen species (ROS) and free radicals [7,8]. The oxidative stress is caused by the accumulation of vast amounts of ROS which participates in degradation of lipids, proteins, and even nucleic acids, resulting in cell damage or death [9].

Several in-vitro antioxidant method has been introduced for the determination of antioxidant activity of different natural sources containing high concentration of phenolic and flavonoid content. The hydrogen atom transfer (HAT) and single electron transfer (SET) are two categories of summarizing all invitro antioxidant activity. The SET method is widely used for the determination of antioxidant activity of different natural sources which included ferric reducing antioxidant

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power (FRAP), 2,2-Diphenyl-1-picrylhydrazyl (DPPH[•]), hydrogen peroxide (H₂O₂), and hydroxyl (OH[•]) radical scavenging activity (HRSA) etc. The present study also used these methods for determination of antioxidant activity of barley and wheat grass extract [10–12].

Antioxidants helps to cure and avoid diseases when consumed in sufficient amounts [13]. Inflammation and neurodegeneration play a vital role in the pathogenesis of depression in the present scenario, but there is no sufficient mechanism that supports the fundamental factor that induces depression. Another hypothesis showed that the generation of free radicals causes mitochondrial dysfunction, which alters fatty acid synthesis, DNA replication and inhibit protein synthesis. Serotonin (5HT), norepinephrine (NE), and dopamine (DA) are quickly oxidized because of production of ROS which may lead to neuropsychiatric illness [14–16]. The overproduction of hydroxyl and superoxide anions lower the monoamine levels in the brain, disrupt the antioxidant processes, and increase oxidative stress in depressed patients [17].

The World Health Organization (WHO) has provided guidelines for managing depression during clinical practice. The antidepressants medications significantly cure the disease [18]. The most popular medications used to treat acute and major depressive disorder are selective serotonin reuptake inhibitors (SSRIs) and selective serotonin-norepinephrine reuptake inhibitors (SNRIs). According to the report, about 63 percent of patients taking SSRIs and SNRIs antidepressant drugs experience more side effects [19]. Because of this, most physicians prefer non-pharmacological therapy also.

Medicinal plants are an important source of natural active compounds that can helps to discover new molecules for the treatment of different types of diseases [20]. Herbal medicines are used to treat mental illnesses, which are becoming more common as natural remedies for the management of depression and anxiety. The World Health Organization and the European Medicine Agency have proved some herbal medicines, such as *Lavandula angustifolia* Mill., *Crocus sativus* L., *Passiflora incarnata* L., and *Valeriana officinalis* L., for the treatment of anxiety and depression because of its lower side effects and withdrawal symptoms [21]. Various pharmacological methods have been developed in recent decades to assess the neuropharmacological activities of medicinal plants [22].

Young barley leaves (*Hordeum vulgare* L.) and wheatgrass (*Triticum aestivum*) belonging to family *Gramineae* is rich in vitamins and minerals. The juice is commonly consumed for its various health benefits which provides nutrition to the cells and detoxifies the human body as it contains bioactive ingredients. Due to the presence of gamma amino butyric acid (GABA), flavonoids, superoxide dismutase (SOD), K-Ca, vitamins (A, C, and E), tryptophan, chlorophylls, bioflavonoids, iron, minerals, and amino acids, it can be used for the treatment of various diseases. Flavones, C-glycosides, Saponin, and Lutonarin are anti-oxidant constituents present in it [23–25]. The green juice of the wheatgrass plant is used for bone marrow and blood-building agents in patients receiving chemotherapy. It is also used for the treatment of thalassemia [24]. Barley and wheatgrass juice, also known as "Jamara ko Juice," is popular among Nepalese people. It is harvested on the seventh day for drinking purposes at a height of 12–14 inches which reveals more benefits [8]. People are generally not aware of the selection and use of these medicinal plants for curing various diseases. Therefore, present study was carried out to elucidate the in-vitro antioxidant property, anti-anxiety and anti-depressant activity through in-vivo study of aqueous extract of barley and wheatgrass in experimental animals.

2. Material and Methods

This study was conducted in the department of pharmacy and pharmacology, Universal College of Medical Sciences (UCMS), Bhairahawa, Rupandehi, Nepal, in December 2019 to May 2020.

2.1. Drugs and chemicals

Imipramine hydrochloride (Himedia), Ferric chloride (SD fine Chem Ltd.), Sulphuric acid (Thermo fischer scientific), Hydrochloric acid (RFCL limited), Gallic acid (SD fine Chem Ltd.), Follin-ciocalteu reagent (SD fine Chem Ltd.), Potassium acetate (SD fine Chem Ltd.), Quercetin (SD fine Chem Ltd.), Ascorbic acid (SD fine Chem Ltd.), Methanol (Merck specialities Pvt. Ltd.), 1,1-diphenyl-2-picrylhydrazyl (SigmaAldrich Pvt. Ltd.), Dimethyl sulfoxide (SD fine Chem Ltd.), Butylated Hydroxy Toluene (Himedia), Butylated Hydroxy Anisole (Himedia), Ammonium acetate (Himedia), Sulphanilamide AR (SD fine Chem Ltd.), Ferrozine (Himedia), Ferrous ammonium sulphate (Himedia).

2.2. Cultivation and collection

For the cultivation of barley and wheat grass, the soil test was done in Agriculture and Forestry University, Faculty of agriculture, Rampur, Chitwan, Nepal. The soil test for cultivation was approved and verified by Mr. Sandesh Bhatta, Assistant Professor, Department of Soil Science and Agri Engineering, Agriculture and Forestry University, on 2076.08.02 BS.

In Bhairahawa, Rupandehi, Nepal, green barley (*H. vulgare*) and wheat grass (*T. aestivum*) were cultivated in indoors facilities. The seeds were first immersed in clean water overnight, and the next day seeds were kept in a moist cotton cloth for sprouting. The soil was prepared with fine particles and combined with equal amounts of organic fertilizers. The barley and wheat sprouts were sown and watered twice a day in a soil bed kept in two separate racks for two different plants, respectively. On the 9th day, the young green grass reached a height of 9–12 cm. The grass was cut with a scissor across the ground surface, and the green grass was collected. After harvesting the wheat and barley grass, they were thoroughly washed under tap water to remove dust and soil. It is stored in a clean tray, and was allowed shade dry and packaged in an airtight container.

2.3. Plant identification and authentication

Plants were cultivated and collected for one month from Bhairahawa, Rupandehi, Nepal. The Barley (*H. vulgare*) and wheat grass (*T. aestivum*) plant herbarium was prepared and submitted to Banaspati Bibhag, Godawari Lalitpur, Kathmandu, Nepal for plant identification and certification. Research officer Ms. Pratiksha Chalise identified the plant, and senior research officer Mr. Subash Khatri gave the certificate, Certificate of Identification No. 160.

2.4. Extraction

The barley and wheat grasses were dried and grounded into coarse particles; the rough and coarse powder was extracted using the soxhlet extraction process. Total 38 and 42 g of coarse powder of *H. vulgare* and *T. aestivum*, respectively. They were accurately weighed and extracted by the Soxhlet extractor, using distilled water at 1:16 ratio solvent for 24 h. The resulting menstruum was heated at 80 °C–90 °C to evaporate the solvent, and the dried extract was collected. The resultant dry extract was weighed, and the percentage yield was measured. The dry extract was placed in a clean airtight container and stored at 4 °C in the refrigerator for further use [26].

$$\text{Yield \%} = \frac{(\text{weight of extract}) \times 100}{(\text{weight of powder})}$$

$$\% \text{ Yield of Barley grass} = \frac{4.86}{38} \times 100$$

$$= 12.79\%$$

$$\% \text{ Yield of Wheat grass} = \frac{6.12}{42} \times 100$$

$$= 14.57\%$$

2.5. In-vitro study

2.5.1. Preliminary phytochemical screening

The preliminary phytochemical screening of aqueous extract of barley and wheat grass was carried out for the detection of alkaloids, amino acids, protein, carbohydrate, glycosides, phenol, steroids, terpenoids, tannin, flavonoid, saponin, etc., [27–29].

2.5.2. Determination of total phenolic content

The total phenolic content of aqueous extract from barley and wheatgrass was analyzed with some modification, using Folin Ciocalteu reagent as a method developed by Lee Y H et al., total 4 mL of 10% Folin Ciocalteu reagent with 6 mL of 75% sodium carbonate (Na_2CO_3), and 1 mL of different concentration samples (0.2–1.0 mg/mL) were used. The solution was incubated for 1 h. The UV-spectrophotometer was used to measure the absorbance against the standard at 765 nm after incubation. Gallic acid (gallic acid/g) was dissolved in 50% ethanol and was used as a standard. The total phenolic content of GAE extract was recorded [8].

$$\text{TPC} = (\text{C} \times \text{V})/\text{M}$$

where,

TPC = total phenolic content of compound (mg of GAE/g sample)
 C = concentration of Gallic acid established from calibration curve (mg/ml)
 V = volume of extract (ml)
 M = weight of methanolic plant extract (g)

2.5.3. Determination of total flavonoid content

The total flavonoid content was calculated by Zhishen J et al., using some modifications to the process. A 0.5 mL of 5 percent (w/v) sodium nitrite (NaNO_2) and 4 mL of barley and wheatgrass aqueous extract of varying concentrations (0.2–1 mg/mL) were incubated for 5 min. After that, 0.5 mL of 10% (w/v) aluminium chloride (AlCl_3) was added after incubation, and 2.5 mL of 1 mol/L sodium hydroxide (NaOH) was added after 10 min of incubation to make up to volume of 12 mL of distilled water shaken for 5 min. After shaking, the absorbance was measured at 510 nm, in terms of mg of Quercetin equivalents/g. The total flavonoid content of the extract was determined [8].

$$\text{TFC} = (\text{C} \times \text{V})/\text{M}$$

where,

TFC = total flavonoids content (mg of Quercetin/g sample)
 C = concentration of quercetin established from the calibration curve (mg/ml)
 V = volume of extract (mL)
 M = weight of plant extract (g)

2.5.4. In-vitro antioxidant assay

The anti-oxidant assay was done by using the methods explained in previous studies. DPPH[•] scavenging activity [30],

Hydrogen peroxide scavenging activity [31], Hydroxyl radical scavenging activity [32], Metal chelating activity [33], Nitric oxide scavenging activity [34], Ferric reducing power [34], and Total antioxidant activity [26].

2.6. In-vivo animal study

2.6.1. Animal

For this research work, Swiss albino mice of either sex (25–30 gm) were purchased from Banaspati Bibhag, Godawari, Lalitpur, Nepal. The mice was cared in a propylene cage and kept at a steady temperature and humidity ($37 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$). The animals were fed on time, had access to clean drinking water, and were kept in a 12 h light/dark cycle. The mice was divided into four groups randomly, each with six mice ($n = 6$). The mice was cared and treated according to standard animal care protocols. The procedure was approved by the Institutional Review Committee (IRC) of the Universal College of Medical Sciences in Bhairahwa, Rupandehi, Nepal, with the IRC approval number UCMS/IRC/229/19.

2.6.2. Experimental design

The mice $n = 24$ were divided into four groups consisting of six mice in each group as follows: negative control group was subjected to induce stress, imipramine group was treated with imipramine 100 mg/kg, barley extract group was treated with aqueous extract of barley grass (400 mg/kg) and wheat extract group was treated with aqueous extract of wheat grass (400 mg/kg).

2.6.3. Acute toxicity study

There was no mortality, and no signs and symptoms of toxicity among the animals upto the dose of 2000 mg/kg. Therefore, the LD_{50} was more than 2000 mg/kg body weight as reported by a previous study for acute toxicity [24].

2.7. Behavioral studies

2.7.1. Forced swim test

The force swim test was done from 10:00 a.m. to 4:00 p.m. Individual mice was first forced to swim in an open cylindrical container (diameter 20 cm, height 40 cm) filled with water up to 30 cm depth at a temperature of $25 \pm 1 \text{ }^\circ\text{C}$. For 6 min, the immobility time was recorded. The imipramine (100 mg/kg) was administered to the imipramine group, and 400 mg/kg of extract were administered orally to barley and wheat grass extract group respectively. The mice were allowed to swim again after 1 h of oral administration of the imipramine and barley and wheat grass extracts, and their immobility time was recorded. When the mice stopped struggling, they became immobile and float motionless in the water, making only movement necessary to keep their heads above water indicate immobility. A decrease in the duration of immobility showed an antidepressant-like effect. The immobility time was calculated on the 0, 2, 4, and 6th days of experiment [23,35].

2.7.2. Tail suspension method

The mice was hung to a wire connected between two 50-cm stands by adhesive tape positioned 1 cm from the tail's tip. When mice did not move, hung passively, and were motionless called immobile. The immobility time of each group of mice was assessed for 6 min. The imipramine (100 mg/kg) was administered to the imipramine group, and 400 mg/kg of extract were administered orally to barley and wheat grass extract group respectively. All the mice of each group were allowed to suspend after 1 h of drug and extract administration, and immobility time was calculated in 6 min. Reduction in duration of immobility showed the antidepressant-like

effect. The duration of immobility was calculated on 0, 2, and 4th, days of the experiment [35,36].

2.7.3. Elevated Plus Maze (EPM)

The Elevated Plus Maze (EPM) apparatus was prepared from plywood and consisted of two open arms (16*5*12 cm) and two closed arms (16*5*12 cm) that extended from a center of the platform that was raised to a height of 25 cm above the floor. Individual mice was positioned in the center of EPM with their heads facing an open arm. The total time spent in open and closed arms was recorded for 6 min., The average time spent per entry in the open arm of EPM was used to measure anxiety level at 0, 2, 4, and 6th days of experiment [37,38].

2.8. Statistical analysis

The results were expressed as means, and the data in the tables and figures represent the Mean \pm SD for antioxidant activity and total phenolic and flavonoid content. For antidepressant activity, all the data analysis was performed using Microsoft Excel 2010 and one-way analysis of variance (ANOVA), followed by Tukey's test comparing all pairs of columns in Graph Pad Prism software (version 5).

3. Result

3.1. Phytochemical screening

Phytochemical screening of barley and wheat grass extracts showed the presence of secondary metabolites such as alkaloids, saponins, tannins, phenolic, carbohydrate, glycosides, flavonoids, and proteins. Among them, terpenoids and sterols were absent. The data were expressed in Table 1.

3.2. Total phenolic contents (TPC)

The total phenolic content of aqueous extract of barley and wheat grass was calculated by using the gallic acid standard curve as mg/g equivalent. The mean TPC of aqueous extract of wheat grass was 135.63 ± 1.184 mg equivalent of GAE/g, and the mean TPC of aqueous extract of barley grass was 160.996 ± 0.656 mg equivalent of GAE/g (Mean \pm SD). The data were listed in Table 2. It was observed that the total phenolic content of the aqueous extract of barley grass was higher than wheat grass.

3.3. Total flavonoid content (TFC)

The total flavonoid content (TFC) of aqueous extract of wheat grass was 133.14 ± 0.43 mg equivalent of quercetin/g, and the

Table 1
Phytochemical screening of aqueous extract of barley grass and wheat grass.

S-N.	Parameters	Aqueous BGE	Aqueous WGE
1	Alkaloids	+	+
2	Tanins	+	+
3	Glycosides	+	+
4	Flavonoids	+	+
5	Phenol	+	+
6	Carbohydrate	+	+
7	Sterols	-	-
8	Terpenoids	-	-
9	Saponins	+	+
10	Protein	+	+

(+) indicates positive, (-) indicates negative.

aqueous extract of barley grass was 153.42 ± 0.40 mg equivalent of quercetin/g (Mean \pm SD). The data were listed in Table 2. It was observed that the total flavonoid content of the aqueous extract of barley grass was higher than wheatgrass extract.

3.4. Determination of antioxidant activity

3.4.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of phenolic compounds was estimated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging process. This assay uses DPPH, and the rate of reaction was directly proportional to antioxidant activity. The percent DPPH radical scavenging activity of aqueous extracts of barley grass and wheatgrass in this assay was 75.05 ± 0.40 percent and 66.99 ± 0.019 percent, respectively (Fig. 1). When compared to aqueous extract of wheat grass, the barley grass extract had a higher radical scavenging activity.

3.4.2. Hydrogen peroxide (H₂O₂) scavenging activity

The ability of the aqueous extracts of barley and wheat grass to scavenge hydrogen peroxide (H₂O₂) was measured and showed in Table 3. The free radical scavenging properties of both plants extracts increase with their concentration. The hydroxyl radical scavenging activity of aqueous extracts of barley and wheat grass was 40.011 ± 0.078 and 31.213 ± 0.640 percentage, respectively. The data showed that the aqueous extract of barley had higher free radical scavenging activity than wheat grass extract.

3.4.3. Hydroxyl radical scavenging activity (HRSA)

In Table 3 demonstrates the assessment of the hydroxyl radical scavenging activity of aqueous extracts of barley and wheat grass. The free radical scavenging properties of both plant extract increase with concentration. The aqueous extract of barley grass had a 77.005 ± 0.024 , and wheat grass had 70.973 ± 0.207 percent hydroxyl radical scavenging activity, respectively. The aqueous extract of barley grass showed the highest hydroxyl radical scavenging activity compared to wheat grass extract.

3.4.4. Metal chelating activity

Table 3 represents the effects of measuring the metal chelating activity of aqueous extracts of barley and wheatgrass. The chelating action of both plant extracts was concentration-dependent. The percent of metal chelating activity of aqueous extract of barley and wheat grass was 39.099 ± 0.0163 and 33.667 ± 0.014 respectively. The aqueous extract of barley grass had higher metal chelating activity than wheat grass.

3.4.5. Nitric oxide radical scavenging activity

Table 3 depicts the nitric oxide radical scavenging activity of aqueous extracts of barley and wheat grass against nitric oxide produced by sodium nitroprusside. The percentage of nitric oxide radical scavenging activity of aqueous extract of barley and wheat grass was $58.992 \pm 0.0623\%$, and 52.602 ± 0.114 respectively. It was observed that the barley grass extract was more effective at scavenging nitric oxide radicals than the wheatgrass extract.

3.4.6. Ferric reducing power activity

In Table 3, the ferric (Fe²⁺) reducing ability of aqueous extracts of barley and wheat grass was evaluated. The radical scavenging properties of both plants extracts increase with concentration. Compared to wheat grass, the aqueous extract of barley grass had more ferric reducing activity.

Table 2
Calculation of Gallic acid and quercetin equivalent of aqueous extract of wheat and barley grass. Mean TPC content of wheat and barley grass extract was 135.63 ± 1.184 and 160.996 ± 0.656 mg CAE/g equivalent respectively. The mean TFC content of wheat and barley grass extract was 133.14 ± 0.43 and 153.42 ± 0.40 mg quercetin/g equivalent respectively. The value was expressed in Mean \pm SD.

Concentration (mg/ml)	Wheat Grass Extract					Barley Grass Extract						
	Abs. of extract	Conc. of Gallic Acid (g/ml)	TPC (mg GAE/g)	Abs. of extract	Conc. of Quercetin (g/ml)	TPC (mg GAE/g)	Abs. of extract	Conc. of Gallic Acid (g/ml)	TPC (mg GAE/g)	Abs. of extract	Conc. of Quercetin (g/ml)	TPC (mg GAE/g)
0.2	0.081	7.21	72.12	0.127	8.904	89.04762	0.091	8.090	80.909	0.115	8	80
0.4	0.12	10.72	107.27	0.188	13.232	132.381	0.138	12.424	124.242	0.165	11.571	115.714
0.6	0.150	13.51	135.15	0.225	15.880	158.809	0.185	16.666	166.666	0.184	12.952	129.523
0.8	0.165	14.87	148.78	0.254	17.976	179.761	0.213	19.181	191.818	0.231	16.309	163.095
1	0.238	21.48	214.84	0.290	20.523	205.238	0.267	24.121	241.212	0.251	17.738	177.381

3.4.7. Total antioxidant activity

In **Table 3**, the cumulative antioxidant activity of aqueous extracts of barley and wheat grass were measured. The inhibitory activities of the aqueous extracts of barley and wheat grass were demonstrated at all concentrations. The overall antioxidant activity of the aqueous extracts of barley and wheat grass was comparable to that of normal compound, ascorbic acid. It was observed that the barley grass extract had good antioxidant activity compared to wheat grass extract.

3.5. Behavioral activity

3.5.1. Forced swim test

Table 4 shows the results of the force swim test. The immobility time was determined on 0, 2, 4, and 6th days after the oral administration of imipramine (100 mg/kg) to imipramine group, and 400 mg/kg of extract to barley and wheat grass extract group respectively and was compared with the negative control group. The immobility time was found to be increased on the 6th day for the negative control group (272.75 ± 4.71) and decreased in imipramine, barley and wheat grass extract group respectively. The immobility time of the imipramine group, barley and wheat grass extract group on the 6th day was 118.75 ± 3.96 , 125.75 ± 3.63 ($p < 0.05$), 138 ± 2.69 ($p < 0.01$), respectively. It was observed that there was a significant reduction in immobility time in barley grass extract group compared to wheat grass extract and negative control group.

3.5.2. Tail suspension test (TST)

The immobility time was observed on 0, 2 and 4th day after the oral administration of imipramine (100 mg/kg) to the imipramine group, barley and wheat grass extract (400 mg/kg) to barley and wheat grass extract group respectively. The immobility time was compared with the negative control group. There was an increase in immobility time in the negative control group on the 4th day (298 ± 4.74) and a decrease in imipramine group (164 ± 4.47) $p < 0.01$, barley extract group (195 ± 5.14) and wheat extract group (208.75 ± 3.96) $p < 0.05$, respectively. The extract-treated group showed a gradual reduction in immobility time than the negative control group suggesting an antidepressant-like effect (**Table 4**).

3.5.3. Elevated Plus Maze (EPM)

The average time spent in the open arm was observed in the elevated plus-maze on 0, 2, 4, and 6th day of the experiment after the oral administration of imipramine (100 mg/kg) to imipramine group, barley and wheat grass extract (400 mg/kg) to barley and wheat grass extract group respectively. The average time spent on the open arm is compared with the negative control group and was found to be less on the 6th day (71.65 ± 2.26). After the treatment, the average time spent on the open arm in the imipramine, barley and wheat grass extract group was 172.5 ± 3.84 , 166.5 ± 3.64 , 157.08 ± 2.43 ($p < 0.01$), respectively (**Table 4**).

4. Discussion

The preliminary phytochemical screening of barley and wheat grass revealed alkaloids, flavonoids, phenols, proteins, carbohydrates, saponin, tannins, and cardiac glycosides in the present study. The steroids and terpenoids were absent in the barley and wheat grass extract in the present study. As reported by Murali M et al., 2016 these bioactive secondary metabolites are responsible for their various therapeutic effects [27]. The various studies showed bioactive compounds like alkaloids, flavonoids, phenols, proteins, carbohydrates, saponin, tannins, cardiac glycosides in

Table 3
In-vitro antioxidant activity of aqueous extract of barley and wheat grass extract at concentration 0.1–1 mg/mL. All values were expressed as Mean ± SD.

Concentration	Hydrogen peroxide (H ₂ O ₂ ●) scavenging activity	Hydroxyl radical (OH●) scavenging activity	Nitric oxide (NO●) scavenging activity	Metal chelating activity	Ferric reducing scavenging activity Absorbance (nm)	Total antioxidant activity Absorbance (nm)
Barley grass extract						
0.2 mg/mL	26.723 ± 0.053	63.850 ± 0.010	42.714 ± 0.054	20.414 ± 0.014	0.085 ± 0.007	0.204 ± 0.022
0.4 mg/mL	30.441 ± 0.040	72.406 ± 0.015	51.172 ± 0.057	34.409 ± 0.027	0.148 ± 0.018	0.251 ± 0.022
0.6 mg/mL	38.917 ± 0.192	77.682 ± 0.050	60.515 ± 0.048	36.438 ± 0.053	0.233 ± 0.041	0.628 ± 0.022
0.8 mg/mL	45.569 ± 0.020	82.602 ± 0.050	68.512 ± 0.094	47.567 ± 0.018	0.483 ± 0.013	0.393 ± 0.044
1 mg/mL	58.404 ± 0.020	88.484 ± 0.024	72.049 ± 0.062	56.666 ± 0.062	0.388 ± 0.051	0.487 ± 0.088
Wheat grass extract						
0.2 mg/mL	23.646 ± 0.433	57.433 ± 0.087	33.487 ± 0.302	16.501 ± 0.029	0.063 ± 0.000	0.157 ± 0.022
0.4 mg/mL	26.168 ± 1.853	66.131 ± 0.100	47.712 ± 0.054	23.674 ± 0.011	0.116 ± 0.038	0.220 ± 0.022
0.6 mg/mL	31.766 ± 0.157	70.909 ± 0.046	56.362 ± 0.043	34.606 ± 0.037	0.196 ± 0.002	0.566 ± 0.038
0.8 mg/mL	35.626 ± 0.312	75.935 ± 0.037	59.900 ± 0.072	42.380 ± 0.083	0.425 ± 0.014	0.361 ± 0.022
1 mg/mL	38.860 ± 0.179	84.456 ± 0.055	65.551 ± 0.108	51.169 ± 0.025	0.334 ± 0.075	0.455 ± 0.022

barley grass extract. The present study also showed a similar result compared to previous studies [26,29].

The plants are also good sources of chlorophyll, vitamins, minerals, enzymes, natural antioxidants, and possess other bioactive compounds. They are a good source of antioxidants and scavenge free radicals, which cause oxidative stress leading to cell death. These free radicals act as mediators in various diseases like cardiovascular disorders, inflammation, neurodegenerative impairments, etc., [39]. Barley and wheat grass have been used as green drinks to treat various diseases like anti-hypertensive, anticancer, anti-obesity, antidiabetic anti-arthritis, hypolipidemic, antioxidant, antidepressant effects, and treatment of thalassemia [40].

The present study estimated the total phenolic content using the Folin-Ciocalteu method, and gallic acid was used as standard. We observed the mean values of total phenolic content (TPC) of barley and wheat grass extract was 160.996 ± 0.656 and 135.63 ± 1.184 mg equivalent of GAE/g, respectively. Generally, barley grass with high phenolic content showed antioxidant activity. Our study showed a higher concentration of total phenolic content in barley than wheat grass extract, comparable to the study reported by various studies [41–43].

Flavonoids are the naturally occurring compounds of poly-phenolic groups, which have antioxidant activities. The antioxidative

property of flavonoids may be due to different mechanisms like scavenging free radicals and metal ion chelation [44]. In the present study, we observed the mean values of total flavonoid content (TFC) of barley and wheat grass extract was 153.42 ± 0.40 and 133.14 ± 0.43 mg equivalent of quercetin/g, respectively. These results indicate that the aqueous extract of barley grass had a higher amount of flavonoid than wheat grass extract. The TFC of barley grass in the present study was higher than the study done by Panthi M et al., 2020 which contains only 18.94–45.76 mg QE/g dried extracts of barley grass [8]. Similarly, the total flavonoid content of wheat-grass extract was higher than 160.10 µmol of quercetin/g equivalent in the study conducted by Qamar A et al., 2018 compared to the present study [45].

DPPH is the most common method to determine free radical scavenging activity. The reducing capacity of DPPH is determined by the decrease in its absorbance at 517 nm induced by antioxidants [42]. In this assay, the mean value of DPPH radical scavenging activity of aqueous extract of barley and wheat grass is 75.05 ± 0.40% and 66.99 ± 0.019%, respectively. The DPPH scavenging activity of wheat grass extract was higher in the present study compared to the study performed by Qamar A et al., 2018 which was 51.690% [45]. Similarly, the DPPH scavenging activity of barley and wheat-grass extract was higher in the present study compared to the

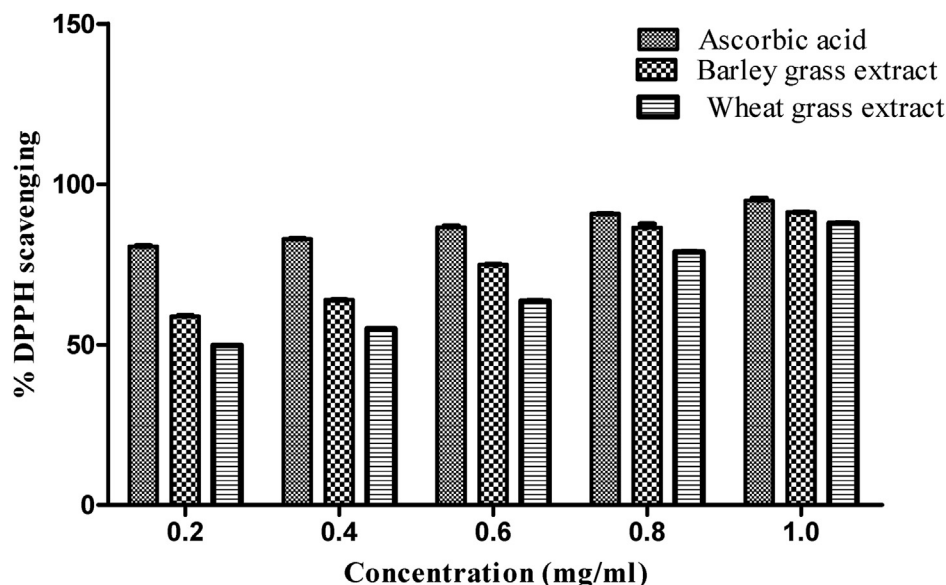


Fig. 1. Comparative DPPH scavenging activity of ascorbic acid and aqueous extract of wheatgrass and barley grass. Each value represents Mean ± SD (n = 3).

Table 4

The result of behavioral study determined the immobility time period in forced swim, tail suspension test and average time spend in open arm in elevated plus maze after the oral administration of imipramine, aqueous extract of *H. vulgare* (barley grass) and *T. aestivum* (wheat grass) in different groups of Albino mice on day 0, 2nd, 4th and 6th respectively. Each treatment group was compared with negative control. All values were expressed as Mean \pm SD * p < 0.05 and ** p < 0.01 in one-way Analysis of Variance (ANOVA) with Tukey's multiple comparison test.

Result of Behavioral studies				
Forced swim test				
Group	0 day	2nd day	4th day	6th day
Negative control	140 \pm 5.09	237.75 \pm 3.03	245.25 \pm 4.81	272.75 \pm 4.71
Imipramine	97.75 \pm 6.45*	148.5 \pm 3.35*	139.25 \pm 2.16*	118.75 \pm 3.96*
Barley grass extract	97.25 \pm 4.96*	155.25 \pm 4.20*	149.5 \pm 3.90*	125.75 \pm 3.63*
Wheat grass extract	94.5 \pm 5.67**	162.25 \pm 3.56**	155 \pm 4.41**	138 \pm 2.69**
Tail suspension test				
Group	0 day	2nd day	4th day	6th day
Negative control	279.25 \pm 3.27	288.5 \pm 4.33	298 \pm 4.74	–
Imipramine	226 \pm 4.74**	178.5 \pm 3.64**	164 \pm 4.47**	–
Barley grass extract	244.75 \pm 5.49*	215 \pm 3.81*	195 \pm 5.14*	–
Wheat grass extract	256 \pm 4.41*	234.5 \pm 4.92*	208.75 \pm 3.96*	–
Elevated Plus Maze Test				
Group	0 day	2nd day	4th day	6th day
Negative Control	90.09 \pm 4.33	86.91 \pm 4.32	80 \pm 2.95	71.65 \pm 2.26
Imipramine	110.67 \pm 3.00**	147.68 \pm 3.00**	157.25 \pm 3.69**	172.5 \pm 3.84**
Barley grass extract	113.08 \pm 3.17**	138.5 \pm 3.28**	148.42 \pm 3.00**	166.5 \pm 3.64**
Wheat grass extract	105.41 \pm 3.54**	127.35 \pm 2.93**	137.83 \pm 2.60**	157.08 \pm 2.43**

previous study (54.8 \pm 0.34%), (38.0 \pm 0.28) done by Hamli S et al., 2017 [46].

Antioxidants activity in various ways, complexation of redox-catalytic metal ions, scavenging free radicals, and decomposition of peroxides [42]. The study showed that aqueous extract of barley grass has higher radical scavenging activity than wheatgrass extract and represents a potent antioxidant activity. Similar action was performed, and the result supports the present study by Durairaj V et al., 2014, [26]. This study determines the different in-vitro antioxidant activity of barley and wheat grass extract. The hydrogen peroxide scavenging activity of wheatgrass extract at 0.8 and 1 mg/mL concentration in the present study was less (35 and 38%) compared to the study conducted by Durairaj V et al., 2014 (57 and 60%) and Al-Awaida WJ et al., 2020 (40%) [26,47]. Similarly, barley grass extract's hydroxyl radical scavenging activity at 0.20 mg/mL concentration in the present study was 63.85%. In contrast, the study done by Shen Y. et al., 2016, showed higher scavenging activity (75%). But barley grass extract at 0.8 and 1 mg/mL concentration showed higher scavenging activity. It may be due to concentration-dependent action of barley grass extract [48]. In another study done by Durairaj et al., 2014, the HRSA of wheat grass extract at 0.8 and 1 mg/mL concentration showed 85 and 97% of hydroxyl scavenging activity which is higher than the present study showing only 75.93 and 84.45%, respectively in the same concentration [26].

The metal chelating activity of wheat grass extract at 0.8 and 1 mg/mL concentration in the present study was less (42.38 and 51.16%) compared to the study done by Durairaj et al., 2014 (50 and 55%) [26]. The percentage of nitric oxide scavenging activity of wheat grass extract at 0.8 and 1 mg/mL concentration in the present study showed a high percentage of scavenging activity (59.90 and 65.55%) compared to the study done by Durairaj V et al., 2014 and Al-Awaida WJ et al., 2020 which was approximately 58 and 60% [26,47].

The anti-oxidant activity of both barley and wheat grass extract was determined by calculating the inhibition capacity of ferric ion to ferrous. In the present study, the result showed that the barley grass extract at 0.8 mg/mL concentration had higher reducing activity than the same extract at 1 mg/mL concentration. A previous

study done by Shen Y et al., 2016 showed the ferric reducing activity of barley grass at 0.25–0.5 mg/mL concentration was constant [48]. This may be because the study used barley grains to determine the ferric reducing power. The research done by Zeng Y. et al., 2020 determined the antioxidant properties of barley extract and found (32.14 \pm 9.35) mean \pm SD. The result indicated that grains had higher functional values [25]. Likewise, the wheat grass extract at 0.8 mg/mL concentration showed higher ferric reducing activity in the present study, comparable with the study done by Kulkarni, S.D. et al., 2006 and Durairaj V et al., 2014, [26,49]. The total antioxidant activity of wheat grass extract at different concentrations (0.2–1 mg/mL) was determined, and higher antioxidant activity was obtained at a 0.6 mg/mL concentration, which is higher than the study done by Durairaj V et al., 2014. In the present study, the extraction of wheat grass was carried out at 72 h in continuous hot Soxhlet apparatus. Still, in a previous study, the extraction procedure is 24 h. The anti-oxidant effect of barley and wheat grass extract was less than the present study which may be due to fewer hours of extraction.

Depression is a complex phenomenon, and its pathophysiology is difficult to understand. The monoamine theory claims that depression is triggered by decreased monoamine neurotransmitters such as serotonin, norepinephrine, or dopamine in the central nervous system (CNS). The most widely researched neurotransmitter in depression is serotonin, and most antidepressants drugs target the serotonin receptor. Since serotonin is made up of tryptophan, tryptophan-deficient amino acids will temporarily lower serotonin levels throughout the brain [5].

In the present study, the anti-depressant effect of aqueous extract of barley and wheat grass was evaluated by various behavioral test methods such as forced swim test (FST), Tail Suspension Test (TST), and Elevated Plus Maze (EPM). A significant decrease in immobility time was observed in FST and TST after the oral administration of aqueous extracts of barley and wheat grass (400 mg/kg), respectively.

After oral administration of barley grass extract at 400 mg/kg, mice were allowed to swim, and the immobility time was determined. It was observed a significant decrease in immobility time (155.25 \pm 4.20) on the 2nd day of the study, compared with the

study done by Yamaura K et al., 2012 and Yamaura K. et al., 2015 [23,37]. There was a significant decrease in immobility time on the 6th day of the experiment, indicating the antidepressant-like effect in barley grass extract.

The Elevated Plus Maze (EPM) was used to assess the anxiety level in mice. Tricyclic anti-depressant (Imipramine 100 mg/kg) was used as a standard drug. After the oral administration of barley and wheat grass extracts in different mice groups, there was a significant decrease in anxiety level in the negative control group. Similarly, the study done by Borah M et al., 2014 and Darwish I E et al., 2013 also showed a significant improvement in major depression and chronic mild stress in rats after using wheat and barley grass extract respectively [24,50].

4.1. Limitation

In the present study, in-vivo antioxidant activity was not performed. The active chemical constituent responsible for antioxidant and antidepressant activity was not elucidated due to a lack of instruments. The fractional extraction method was not performed with different solvents like methanol, ethanol, acetone, and chloroform to characterize active phytoconstituents present in both wheat and barley grass extract.

5. Conclusion

The phytochemical screening of aqueous extract of barley and wheat grass showed bioactive secondary metabolites responsible for its therapeutic effects. Polyphenols and flavonoids are natural antioxidants. The presence of a high amount of total phenolic and flavonoid content showed antioxidant activity. The invitro antioxidant activity also confirms the antioxidant scavenging properties of barley and wheat grass extract.

Similarly, antidepressant and anti-anxiety activity of barley and wheat grass extract showed behavioral improvement in experimental animals on FST, TST, and EPM test models. Thus, the present study suggested the presence of anti-depressant activity in wheat and barley grass extract respectively.

Author contributions

Amit Kumar Shrivastava: conceptualization, study design, validation, investigation, data curation, writing original draft, review and editing, visualization, supervision, project administration.

Pramila Thapa Magar: study design, software, formal analysis, investigation, resources, writing original draft, visualization.

Laxmi Shrestha: Software, validation, formal analysis, writing original draft, review and editing, supervision.

Conflicts of interest

There is no any conflict of interest.

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References

- [1] Bromet E, Andrade LH, Hwang I, Sampson NA, Alonso J, De Girolamo G, et al. Cross-national epidemiology of DSM-IV major depressive episode. *BMC Med* 2011;9(1):1–16.
- [2] Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. *Lancet* 2007;370(9590):851–8.
- [3] Yuluğ B, Ozan E, Gönül AS, Kilic E. Brain-derived neurotrophic factor, stress and depression: a minireview. *Brain Res Bull* 2009;78(6):267–9.
- [4] Kessler RC. The effects of stressful life events on depression. *Annu Rev Psychol* 1997;48(1):191–214.
- [5] Aan het Rot M, Mathew SJ, Charney DS. Neurobiological mechanisms in major depressive disorder. *Canadian Med Association J* 2009;180(3):305–13.
- [6] Maria Michel T, Pulschen D, Thome J. The role of oxidative stress in depressive disorders. *Curr Pharmaceut Des* 2012;18(36):5890–9.
- [7] Grases G, Colom M, Fernandez R, Costa-Bauzá A, Grases F. Evidence of higher oxidative status in depression and anxiety. *Oxid Med Cell Longev* 2014;2014.
- [8] Panthi M, Subba RK, Raut B, Khanal DP, Koirala N. Bioactivity evaluations of leaf extract fractions from young barley grass and correlation with their phytochemical profiles. *BMC Complement Med Ther* 2020;20(1):1–9.
- [9] Pł. Kowalczyński, Radzikowska D, Ivanišová E, Szwęgiel A, Kačániová M, Sawinska Z. Influence of abiotic stress factors on the antioxidant properties and polyphenols profile composition of green barley (*Hordeum vulgare* L.). *Int J Mol Sci* 2020;21(2):397.
- [10] Wang J, Hu S, Nie S, Yu Q, Xie M. Reviews on mechanisms of in vitro antioxidant activity of polysaccharides. *Oxid Med Cell Longev* 2016;2016:1–14.
- [11] Gülcin I. Antioxidant activity of food constituents: an overview. *Arch Toxicol* 2012;86(3):345–91.
- [12] Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala NJP. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants* 2019;8(4):1–12.
- [13] Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M. Free radical scavenging properties of wheat extracts. *J Agric Food Chem* 2002;50(6):1619–24.
- [14] Bajpai A, Verma AK, Srivastava M, Srivastava R. Oxidative stress and major depression. *J Clin Diagn Res* 2014;8(12):4–7.
- [15] Stockmeier CA, Mahajan GJ, Konick LC, Overholser JC, Jurjus GJ, Meltzer HY. Cellular changes in the postmortem hippocampus in major depression. *Biol. psychiatry* 2004;56(9):640–50.
- [16] Wrona MZ, Yang Z, Waskiewicz J, Dryhurst G. Oxygen radical-mediated oxidation of serotonin: potential relationship to neurodegenerative diseases. *Neurodegener DisSpringer* 1996:285–97.
- [17] Khanzode SD, Dakhale GN, Khanzode SS, Saoji A, Palasodkar R. Oxidative damage and major depression: the potential antioxidant action of selective serotonin reuptake inhibitors. *Redox Rep* 2003;8(6):365–70.
- [18] Cuijpers P, Quero S, Dowrick C, Arroll B. Psychological treatment of depression in primary care: recent developments. *Curr Psychiatr Rep* 2019;21(12):1–10.
- [19] Gartlehner G, Wagner G, Matyas N, Titscher V, Greimel J, Lux L. Pharmacological and non-pharmacological treatments for major depressive disorder: review of systematic reviews. *BMJ Open* 2017;7(6):1–13.
- [20] El Gabbas Z, Bezza K, Laadraoui J, Makbal R, Aboufatima R, Chait A. Salvia officinalis induces antidepressant-like effect, anxiolytic activity and learning improvement in hippocampal lesioned and intact adult rats. *Bangladesh J Pharmacol* 2018;13(4):367–78.
- [21] Borgonetti V, Les F, López V, Galeotti N. Attenuation of anxiety-like behavior by *Helichrysum stoechas* (L.) Moench methanolic extract through up-regulation of ERK signaling pathways in noradrenergic neurons. *Pharmaceuticals* 2020;13(12):472.
- [22] Biswas S, Mondol D, Jodder P, Sana S, Saleh MA, Tarafdar AK, et al. Evaluation of neurobehavioral activities of ethanolic extract of *Psidium guajava* Linn leaves in mice model. *Future J Pharm Sci* 2021;7(1):1–12.
- [23] Yamaura K, Nakayama N, Shimada M, Bi Y, Fukata H, Ueno K. Antidepressant-like effects of young green barley leaf (*Hordeum vulgare* L.) in the mouse forced swimming test. *Pharmacogn Res* 2012;4(1):22–6.
- [24] Borah M, Sarma P, Das S. A study of the protective effect of *Triticum aestivum* L. in an experimental animal model of chronic fatigue syndrome. *Pharmacogn Res* 2014;6(4):285–91.
- [25] Zeng Y, Pu X, Du J, Yang X, Li X, Mandal M, et al. Molecular mechanism of functional ingredients in barley to combat human chronic diseases. *Oxid Med Cell Longev* 2020;2020:1–26.
- [26] Durairaj V, Hoda M, Shakya G, Babu SPP, Rajagopalan R. Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass. *Asian Pac J Trop Med* 2014;7:S398–404.
- [27] Murali M, Archa Raj M, Akhil S, Liji R, Kumar S, Nair A. Preliminary phytochemical analysis of wheat grass leaf extracts. *Int J Pharmaceut Sci Rev Res* 2016;40(1):307–12.
- [28] Suriyavathana M, Roopavathi I. Phytochemical characterization of *Triticum Aestivum* (wheat grass). *J Pharmacogn Phytochem* 2016;5(1):283–6.
- [29] Nepal P, Singh M, Baniya A, Singh S, Sainju HK, Shrestha R. Comparative Antioxidant, Antimicrobial and Phytochemical Assesments Of leaves of

- desmostachya bipinnata L. Stapf, Hordeum vulgare L. And drepanostachyum falcatum (nees) keng f. Nepal J Biotechnol 2018;6(1):1–10.
- [30] Shrivastava AK, Thapa S, Shrestha L, Mehta RK, Gupta A, Koirala N. Phytochemical screening and the effect of *Trichosanthes dioica* in high-fat diet induced atherosclerosis in Wistar rats. *Food Frontiers*. 2021;1–10.
- [31] Shalini K, Ilango KJPJ. Preliminary phytochemical studies, GC-MS analysis and in vitro antioxidant activity of selected medicinal plants and its polyherbal formulation. *Phcog Mag* 2021;13(3):648–59.
- [32] Guchu BM, Machocho AK, Mwihiya SK, Ngugi MP. In Vitro Antioxidant Activities of Methanolic Extracts of *Caesalpinia volkensii* Harms, *Vernonia lasiopurpurea* O. Hoffm, and *Acacia hockii* De Wild. *Evid Based Complment Alternat Med* 2020;2020:1–10.
- [33] Kobus-Cisowska J, Szulc P, Szczepaniak O, Dziedzinski M, Szymanowska D, Szymandera-Buszka K, et al. Variability of *Hordeum vulgare* L. cultivars in yield, antioxidant potential, and cholinesterase inhibitory activity. *Sustainability* 2020;12(5):1–14.
- [34] Sasikumar J, Erba O, Egigu MC. In vitro antioxidant activity and polyphenolic content of commonly used spices from Ethiopia. *Heliyon* 2020;6(9):1–7.
- [35] Yousuf S, Haq SM, Rasool A, Zulfajri M, Hanafiah MM, Nafees H. Evaluation of antidepressant activity of methanolic and hydroalcoholic extracts of *Acorus calamus* L. rhizome through tail suspension test and forced swimming test of mice. *J Tradit Chin Med Sci* 2020;7(3):301–7.
- [36] Nandi A, Virmani G, Barve A, Marathe SJE. Dbscorer: an open-source software for automated accurate analysis of rodent behavior in forced swim test and tail suspension test. *Eneuro* 2021;8(6):305–21.
- [37] Yamaura K, Tanaka R, Bi Y, Fukata H, Oishi N, Sato H, et al. Protective effect of young green barley leaf (*Hordeum vulgare* L.) on restraint stress-induced decrease in hippocampal brain-derived neurotrophic factor in mice. *Pharmacogn Magazine* 2015;11(Suppl 1):S86.
- [38] Shrivastava AK, Shrestha L, Gurung S, Joshi B. Anxiolytic and antidepressant-like effect of the ethanolic extract of *Cassia tora* seed in Swiss mice. *J Complement Med Res* 2020;11(1):1–8.
- [39] Kohen R, Nyska A. Invited review: oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 2002;30(6):620–50.
- [40] Zeng Y, Pu X, Yang J, Du J, Yang X, Li X, et al. Preventive and therapeutic role of functional ingredients of barley grass for chronic diseases in human beings. *Oxid Med Cell Longev* 2018;2018:1–15.
- [41] Wangcharoen W, Phimphilai S. Chlorophyll and total phenolic contents, antioxidant activities and consumer acceptance test of processed grass drinks. *J Food Sci Tech* 2016;53(12):4135–40.
- [42] Fogarasi A-L, Kun S, Tankó G, Stefanovits-Bányai É Hegyesné-Vecseri B. A comparative assessment of antioxidant properties, total phenolic content of einkorn, wheat, barley and their malts. *Food Chem* 2015;167:1–6.
- [43] Ragaei S, Abdel-Aal E-SM, Noaman M. Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chem* 2006;98(1):32–8.
- [44] Goveas SW, Abraham A. Evaluation of antimicrobial and antioxidant activity of stem and leaf extracts of *Coscinium fenestratum*. *Asian J Pharmaceut Clin Res* 2013;6(3):218–21.
- [45] Qamar A, Saeed F, Tahir-Nadeem M, Hussain AI, Niaz B, Ullah Khan A, et al. Exploring the phytochemical profile of green grasses with special reference to antioxidant properties. *Int J Food Propert* 2018;21(1):2566–77.
- [46] Hamli S, Kadi K, Addad D, Bouzerzour H. Phytochemical Screening and Radical Scavenging Activity of whole seed of durum wheat (*Triticum durum* desf.) and barley (*Hordeum vulgare* L.) Varieties. *Jordan J Biol Sci* 2017;(4):10.
- [47] Al-Awaida WJ, Sharab AS, Al-Ameer HJ, Ayoub NY. Effect of simulated microgravity on the antidiabetic properties of wheatgrass (*Triticum aestivum*) in streptozotocin-induced diabetic rats. *npj Microgravity*. 2020;6(1):1–10.
- [48] Shen Y, Zhang H, Cheng L, Wang L, Qian H, Qi X. In vitro and in vivo antioxidant activity of polyphenols extracted from black highland barley. *Food Chem* 2016;194:1003–12.
- [49] Kulkarni SD, Tilak JC, Acharya R, Rajurkar NS, Devasagayam T, Reddy A. Evaluation of the antioxidant activity of wheatgrass (*Triticum aestivum* L.) as a function of growth under different conditions. *Phytotherapy Research: Int J Devot Pharmacol Toxicol Eval Nat Prod Derivat* 2006;20(3):218–27.
- [50] Darwish IE, Maklad HM, Diab IH. Behavioral and neuronal biochemical possible effects in experimental induced chronic mild stress in male albino rats under the effect of oral barley administration in comparison to venlafaxine. *Int J Physiol Pathophysiol Pharmacol* 2013;5(2):128.