Journal of Applied Pharmaceutical Research
Volume 11 Issue 3, Year of Publication 2023, Page 41 – 47
DOI: 10.18231/j.joapr.2023.11.3.41.47

Check for updates

Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | *JOAPR*
www.japtronline.com *ISSN: 2348 – 0335*

EXPERIMENTAL EVALUATION OF ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITIES OF METHANOLIC EXTRACT OF TRITICUM AESTIVUM (WHEATGRASS) IN ALBINO MICE

Saroj Kothari*, Ajay Gupta

Article Information
Received: 14th October 2022
Revised: 23rd March 2023
Accepted: 4th May 2023
Published: 31st August 2023

Keywords
Antianxiety, antidepressant, Triticum aestivum, elevated plus maze, light and dark box

ABSTRACT

Background: Anxiety and depression are common psychiatric conditions. The present study was carried out to find antianxiety and antidepressant activity of methanolic extract of *Triticum aestivum* (wheat grass) in mice. **Materials and methods:** The methanolic extract of *Triticum aestivum* (META) was screened for antianxiety activity by elevated plus maze (EPM) and light and dark box (LDB) and for antidepressant activity by forced swim test (FST) and tail suspension test (TST) in mice. Animals were divided into four groups having six animals in each group. Group I served as control and received gum acacia aqueous suspension 10 ml/kg, Groups II and III served as test groups and received META 200 and 400 mg/kg, respectively, Group IV served as standard group and received diazepam 1 mg/kg for antianxiety activity and fluoxetine (20 mg/kg) for antidepressant activity once daily for thirty days. **Result**: META 200 and 400 mg/kg showed significant ($P < 0.01$) dose-dependent increase in entries and stay in the open arms in EPM and entries and stay in the light compartment in LDB as compared to control. Antianxiety effect of META at dose of 400 mg/kg was comparable ($P > 0.05$) with diazepam 1mg/kg. META 200 and 400 mg/kg also produced dose-dependent significant ($P < 0.01$) antidepressant effect, indicated by reduction in the immobility time as compared to control in both FST and TST. The antidepressant activity of META at dose of 400 mg/kg was comparable ($P > 0.05$) with fluoxetine 20 mg/kg. **Conclusion:** Results of our study suggested that META possess dose-dependent significant antianxiety and antidepressant activities.

INTRODUCTION

Modern pattern of lifestyle is associated with increased incidence of anxiety and depression. Anxiety is less severe disorder of adaptive response, characterized by tension, fear, apprehension, lack of concentration and is associated with sympathetic and somatic symptoms like tachycardia, tremors, sweating, fatigue, sleep disturbance, and GIT distress [1]. A depressive episode in a sufferer is characterized by feelings of sadness, irritability, and empty feeling, and loss of interest in pleasure and activities. Some patients may also have poor

*Department of Pharmacology, Gajra Raja Medical College, Gwalior, Madhya Pradesh, India

***For Correspondence:** **saroj.kothari@rediffmail.com**

concentration, feelings of excessive guilt and worthlessness, hopelessness, and thoughts of dying or suicide. Sometimes bodily symptoms like pain, fatigue and weakness may also present [2]. Depression is not only the most common psychiatric disease but also an important cause of morbidity worldwide [3]. A wide range of medicine is available for the treatment of disorders of anxiety, like benzodiazepines (e.g.diazepam, alprazolam, and clonazepam), which have adverse effects like lightheadedness, confusion, weight-gain and altered sexual function, and newer drugs like azapirones (e.g. buspirone and gepirone) having adverse effects like dizziness, tinnitus, headache, and nervousness [4,5]. Frequently prescribed standard drugs for depression include selective serotonin reuptake inhibitors (SSRI), e.g., fluoxetine, escitalopram, sertraline, etc. although safe and effective, but associated with various adverse effects like, dry mouth, altered taste, urinary retention, mental confusion, sedation, postural hypotension, and cardiac arrhythmia [6]. Thus, synthetic drugs available for anxiety and depression have other limitations besides adverse effects including the slow onset of action, poor response, and remission.

Herbal medicines may be a successful option in the treatment of anxiety and depression since a large number of herbal preparations have demonstrated psychotherapeutic activities and are widely accepted as safe and effective treatment for many illnesses [7,8]. Wheatgrass is the young grass of the plant *Triticum aestivum* Linn belonging to the family Poaceae. *T. aestivum* has anti-proliferative, hypolipidemic and anti-Alzheimer properties [9–11] Wheatgrass is rich in vitamins A, B complex, C, and E, and minerals like calcium, iron, magnesium and potassium and is also mentioned as an antioxidant [12].

Fresh wheat grass juice consumption is conventionally very common as a dietary supplement in old age group individuals and is also used as a part of various green tea preparations. A literature survey revealed that the antianxiety, and antidepressant properties of the methanolic extract of *T. aestivum* have not been studied therefore, the present study is planned to evaluate the antianxiety, antidepressant properties of methanolic extract of wheatgrass in albino mice.

MATERIALS AND METHODS

Plant material

The grass of *T. aestivum* used in this study was grown indoors at home. The earthen pot was filled with 2.5 inches of growing medium composed of three parts of soil and one part of compost. Overnight soaked *T. aestivum* seeds were then evenly spread over it and further covered with 0.5 inches of soil. Small quantities of water were sprinkled evenly over the soil and 3-7 hours of indirect sunlight was allowed daily for the growth of the grass. On the tenth day, when grass is about 6 inches tall, it is cut 0.5 inches above the surface of the soil [13]. *T. aestivum* (Wheat) grass is identified and authenticated by Dr. Arti Garg scientist E and head of the office, Government of India, Ministry of Environment, Forest and Climate Change, Botanical Survey of India, Central Regional Centre, 10 Chatham Lines, Allahabad–211002, Authentication certificate dated 05.08.2022

Preparation of extract

The collected wheat grass was washed thoroughly with tap water and dried at room temperature in the absence of sunlight. One kg of dry grass was obtained from 6.25 kg of wet wheatgrass. The dried grass was powdered using the grinder and extracted with 2.5 L of 99.9% pure methyl alcohol (V/V) using a soxhlet apparatus for 16 h giving 8.5% (W/W) of extract of *T. aestivum.* META was then stored in amber colored bottle for further use [14].

Phytochemical Screening

META was subjected to phytochemical tests for the presence of bioactive compounds by standard methods as described by Harbourne [15].

Animals

Albino male mice weighing 25-30 g raised in the animal house of the Department of Pharmacology, Gajra Raja Medical College, Gwalior, were used for the study. These were maintained at 24±2°C, humidity 50±5% with12 h light and dark cycle and kept on standard pellet diet (Pranav Agro Industries, Delhi, India) and water *ad libitum*. The care and maintenance of animals were as per the approved guidelines of the Committee for Control and Supervision of Experiments on Animals in India. The Institutional Animal Ethics Committee approved the protocol. (Registration number 846/GO/Ere/S/04/CPCSEA).

Drugs and Chemicals

Gum acacia 2% suspension, Methanol 99.9% (V/V), Diazepam (Tab Valium 2 mg- Abbott Healthcare Pvt. Ltd.), Fluoxetine (Cap Flunil 20 mg- Intas Pharmaceuticals Ltd.), Drugs used in the study were purchased from local medical stores.

Study of antianxiety activity

The test compound was screened for antianxiety activity by elevated plus maze, EPM & light dark box, LDB in albino mice.

Elevated Plus Maze

The EPM consisting of two open arms (16 × 5 cm) and two enclosed arms (16×5× 12 cm) was used. The maze was elevated to the height of 25 cm above the floor. On the 30th day, animals were placed individually at the center of the EPM with their head facing towards the open arm. Total time of stay and number of entries in different arms were recorded during 5 min period. An arm entry is defined as the presence of all four paws in the arm [16]. Several rearing and head dipping were also counted. EPM was cleaned with 70% ethanol after each reading to eliminate any possible bias due to odor.

Light and Dark Box

The light and dark box apparatus consisted of two compartments (20×20×20 cm), one painted white and illuminated and the other painted black which is kept dark. These two compartments connected by a door (5×5 cm). The light source is kept at 50 cm above the compartment's roof. On the 30th day, animals were placed individually at the center of the illuminated white chamber (light compartment) with their head facing the side wall. Total time of stay and number of entries in light and dark compartments were recorded during 5 min period. An entry is defined as the presence of all four paws in the respective compartment [17]. LDB was carefully wiped with 70% ethanol after each trial, to eliminate the possible bias due to the odor of the previous animal.

Study of Antidepressant Activity

The antidepressant activity was studied using the forced swim test (FST) and tail suspension test (TST) in albino mice.

Forced Swim Test

Mice were individually placed to swim in an open cylindrical container (diameter 13 cm, height 24 cm) containing water (22±2°C) to a depth of 10 cm (Tail does not touch the bottom of cylinder). When mice ceased to struggle and swim and remained floating in water, only moving to keep their head above water, it was considered immobile. On the 30th day in a single session, mice were placed to swim in a narrow cylinder from which they could not escape. The immobility period was recorded during the last 4 min of the 6 min test session by summing the total time spent immobile, which were the short periods of slight activity where the mice made those movements necessary to maintain their head above water. During the first 2 min of the test session, mice show a high frequency of exploratory and escape-directed behavior, the last 4 min is the time during which the mice show the most immobility [18]. Following every session, the animals were removed from the cylinder, dried with towels and placed in the heated cage for 15 min before returning to their home cage.

Tail Suspension Test

This method is based on the assumption that when a mouse is suspended by tail, it shows alternating agitation and immobility; the immobility is indicative of a state of depression. Mice were suspended on the edge of a table, 50 cm above the ground, with the help of tape placed approximately 1 cm from the tip of the tail on the 30th day. During a 6 min test session, the immobility period was recorded. When mice did not show any movement in body and hung passively, it was considered to be immobile [19].

Study Design

Mice were divided into four groups having six animals in each group as follows. Group1: Control received 2% gum acacia at the dose of 10 ml/kg. Groups 2 and 3: Test drug-treated groups META200 and META400 received META200 mg/kg and META 400 mg/kg, respectively. Group 4: Standard drug-treated group DZP1 received diazepam 1mg/kg and group FXT20 received fluoxetine 20 mg/kg for antianxiety and antidepressant activities respectively. All the animals received respective treatments as 2% gum acacia suspension by gavage once daily at 10 AM for 30 days. Doses of META for antianxiety and antidepressant activity were selected based on the previous studies using *T. aestivum* [20].

Statistical Analysis

The data collected after experiments were represented as mean ± SEM and were analyzed using SPSS software version 20. A One-way ANOVA test was applied, followed by Tukey's multiple comparison tests. $p<0.05$ was considered as statistically significant.

RESULTS

Phytochemical analysis

Presence of alkaloids, flavonoids, tannins, amino acids, protein, carbohydrate, cardiac glycoside, saponins, coumarins, and terpenoids were revealed on phytochemical analysis of META.

Assessment of Antianxiety Activity

META200 showed an increase of 6% and 31% in entries and stay respectively in the open arms in EPM and an increase of 2% and 15% in entries and stay respectively in the light area in LDB as compared to GA10 and were significant (p<0.01). META400 showed an increase of 9% and 47% in entries and stay respectively in the open arms in EPM and increase of 5% and 25% in entries and stay respectively in the light area of LDB as compared to GA10 and were significant ($P < 0.01$). Results of META400 were also significant as compared to META 200 ($p<0.01$). The standard drug DZP1showed increases of 9% and 48% in entries and stay respectively in the open arms in EPM and increases of 5% and 26% in entries and stay respectively in the light area in LDB as compared to GA10 and were significant ($p<0.01$). Results of the DZP1 were also significant as compared to META 200 ($p<0.01$). META 400 was comparable (p>0.05) with the DZP1 group (Table 1, Figure 1).

META 200 and META 400 showed dose-dependent increases in rearing and head dipping respectively in EPM as compared to GA10 and the effect was significant (p<0.01) The standard drug Diazepam treated group DZ1 showed significant increase in rearing and head dipping in EPM as compared to GA10 and META200 (p<0.01). All the parameters for the antianxiety activity of META 400 were comparable ($p>0.05$) with the DZP1 treated group (Table 1, Figure 1).

Table 1: Effect of methanolic extract of *Triticum aestivum* on the number of entries, time of stay, number of rearing and head dipping using EPM in mice

Groups	Number of Entries		Time spent(sec)		Rearing	Head dipping
	Open arm	Closed arm	Open arm	Closed arm	Both arms	Both arms
GA10	2.33±0.21	4.00±0.36	22.33±0.84	277.66±0.84	3.00±0.25	4.16±0.16
META200	5.50±0.22[a]	7.33±0.21[a]	114.33±3.52[a]	185.66±3.5[a]	6.80±0.30[a]	9.33±0.21[a]
META400	10.66±0.33[ab]	12.66±0.21[ab]	161.50±0.80[ab]	138.50±0.08[ab]	10.50±0.22[ab]	13.33±0.33[ab]
DZP1	11.50±0.22[ab]	13.66±0.21[ab]	166.16±1.10[ab]	133.83±1.10[ab]	11.00±0.25[ab]	14.67±0.70[ab]

GA10=Gumacacia10ml/kg, META200 and META400=META200mg/kg and 400mg/kg respectively, DZP1=Diazepam 1 mg/kg. Each group consists of 6 animals (n=6). Values are expressed as mean ±SEM, One-way ANOVA followed by Tukey's multiple comparison tests. [a]$P<0.01$as compared to GA10, [b]$P<0.01$as compared to META200mg/kg

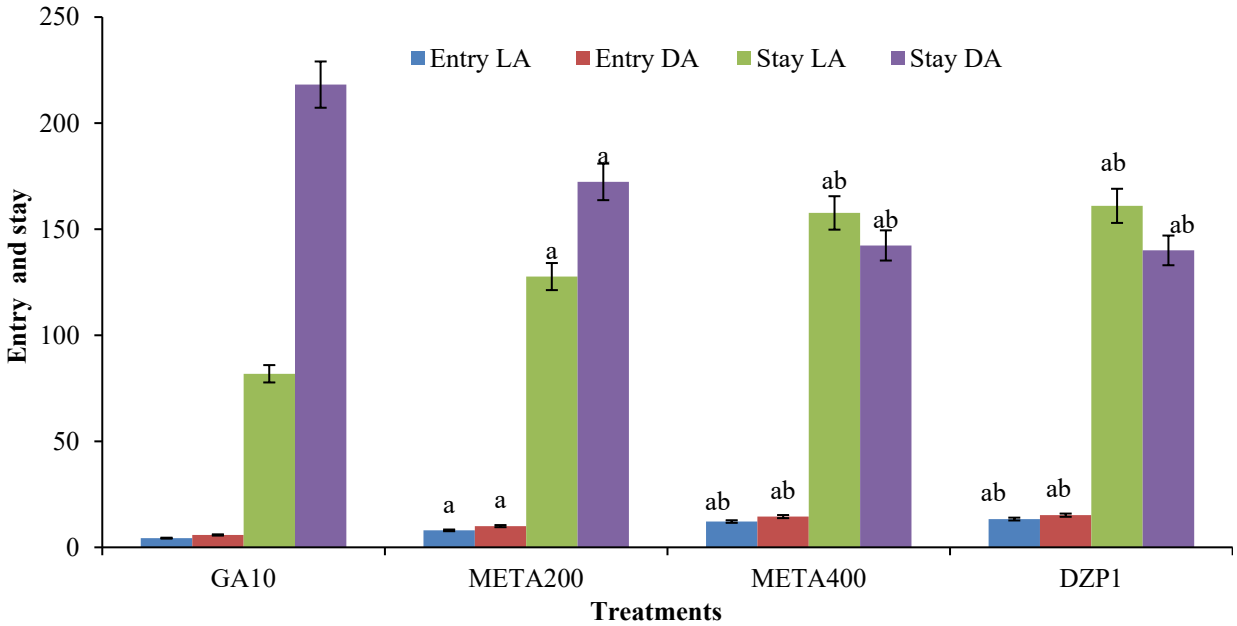

Figure 1: Effect of methanolic extract of *Triticum aestivum* on entries and stay in light and dark area using LDB in mice

GA10=Gumacacia10ml/kg, META200 and META400=META200mg/kg and 400mg/kg respectively, DZP1=Diazepam 1 mg/kg. Each group consists of 6 animals (n=6). Values are expressed as mean ±SEM, One-way ANOVA followed by Tukey's multiple comparison tests. [a]$P<0.01$as compared to GA10, [b]$P<0.01$as compared to META200mg/kg, LA = Light area of light and dark box, DA= Dark area of light and dark box

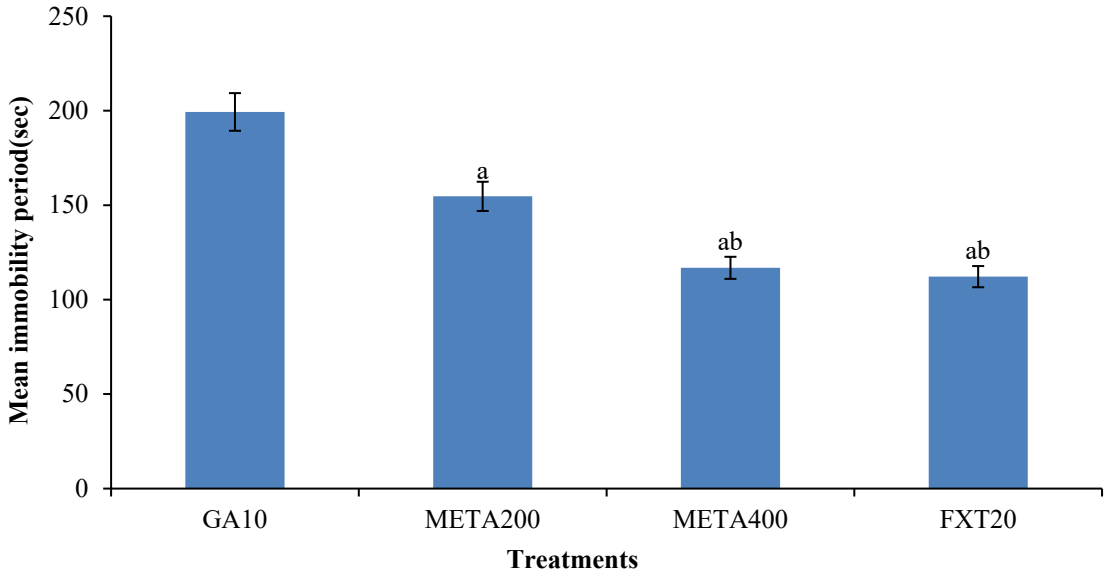

Figure 2: Effect of methanolic extract of *Triticum aestivum* on Immobility period in Tail suspension test in mice

GA10= Gum acacia 10ml/kg, META200 and META400= META200mg/kg and 400mg/kg respectively, FXT20= Fluoxetine20 mg/kg. Each group consists of 6 animals (n=6). Values are mean ±SEM, one way ANOVA followed by Tukey's multiple comparison tests. [a]*P*<0.01as compared to GA10, [b]*P*<0.01as compared to META200mg/kg

Table 2: Effect of methanolic extract of *Triticum aestivum* on the Immobility period in forced swim test in mice

Treatments	Mean Immobility Period (sec)
GA10	117.33±1.80
META200	95.66±1.89[a]
META400	82.00±1.23[ab]
FXT20	80.00±0.93[ab]

GA10= Gumacacia 10ml/kg, META200 & META400 is META200mg/kg & 400mg/kg respectively, FXT20= Fluoxetine 20 mg/kg. Each group consists of 6 animals (n=6). Values are mean ±SEM, One-way ANOVA followed by Tukey's multiple comparison tests. [a]*P*<0.01as compared to GA10, [b]*P*<0.01as compared to META200mg/kg

Assessment of Antidepressant Activity

META200 lowered the mean immobility time by 18% and 22% as compared to GA10 in FST and TST respectively and was significant ($p<0.01$). META400 lowered the mean immobility time by 30% and 43% as compared to GA10 in FST and TST respectively and the effects were significant ($p<0.01$). Effects of META 400 were also statistically significant as compared to META 200 in FST and TST models ($p<0.01$). Standard drug FXT20 lowered the mean immobility time by 31% and 44% respectively as compared to GA10 in FST and TST respectively and was significant ($p<0.01$). The results of FXT20 were also significant as compared to META 200 ($p<0.01$). Anti-depressant effects of META 400 were comparable with FXT20 ($p>0.05$) in both FST and TST models (Table 2, Figure 2).

DISCUSSION

T. aestivum grass is highly valued for its nutritional and medicinal properties. It has a protective effect against oxidative stress- induced psychotic illness as reported by Kulkarni et al. [12]. Fresh wheatgrass has attracted researchers all over the world to explore its pharmacological properties. Elevated plus-maze is the simplest, valuable and commonly used apparatus to explore anxiolytic responses produced by the test drugs. It is used to test almost all types of anti-anxiety agents. Animal's movement in open arms is more fear provoking than the closed arms. With the use of anxiolytic, the number of entries and time spent in the open arms is found to be increased as mentioned by Latha et al. [21].

The light-dark box test is based on the inherent nature of mice to avoid brightly illuminated places. Manu et al reported that with the use of anxiolytic the time spent in the light compartment found to be increased [22]. In the present study META200 and 400mg/kg showed significant dose-dependent increase in entries and stay in the open arms in EPM and entries and stay in the light compartment in LDB as compared to control. This is in

accordance with earlier study by Shrivastava et al. [20]. Plant extracts which contain flavonoids have shown antianxiety activity in study by Gupta et al. [23]. Flavonoids present in wheatgrass as reported by Zendehbad et al. [24] may act by modulating the gamma amino butyric acid (GABA) receptors thereby increasing the frequency of chloride channel opening and resulting in the neuronal hyperpolarization similar to that of standard drugs benzodiazepines seen by Khan et al. [25].

The brain derived neurotrophic factor (BDNF) maintains neuronal preservation and the brain plasticity and its decreased levels are associated with anxiety like behavior reported in study by Sechi et al. [26]. *T.aestivum* reported to increase BDNF in hippocampus might also contribute in anxiolytic activity of *T. aestivum* as per the study of Khalil et al. [27]. Thus, antianxiety activity of META in our study might be due to flavonoids, increased GABA and BDNF levels in the brain. In the present study, the antidepressant activity of META in albino mice was evaluated by using FST and TST methods. Stress induced in animals initiates an important reaction leading to depression. FST and TST animal models are well known tests to create physical stress and there by development of depression. These models of depression are reliable and quick behavioral screening tests for antidepressants. When the animal is immobile it reflects to a state of behavioral despair and is unable to adapt to the stress. Both these models are widely used to screen new antidepressant drugs.

In our study, administration of META200 and 400 mg/kg showed significant dose-dependent antidepressant activity in both FST and TST models. Results of our study on depression in mice are in accordance with the earlier studies done with alcoholic and aqueous extracts of *T.* aestivum by Charan et al. and Shrivastava et al. respectively [28,20] where protection against depression was reported in mice. Antidepressant activity of *T. aestivum* in our study can be due to activation of the brain monoaminergic system in the brain due to flavonoids present in *T. aestivum*. A wide range of plant-derived flavonoids are found to cross the blood-brain barrier and can influence brain functions by different ways to protect against depression reported by Pannu et al. [29]. Wheatgrass is rich source of flavonoids and are involved in increase in neurogenesis and neurotrophic factors as is in study by Zendehbad et al. [24]. Thus, antidepressant activity of META in our study might be due to its antioxidant potential and neuroprotective flavonoids.

However, the study has its share of limitations. There is a need to conduct further *in vivo* studies with more animals and more behavioral animal models and *in vitro* experiments such as GABA receptor binding assay, benzodiazepine receptor binding assay, serotonin receptor binding assay for antianxiety activity; and MAO inhibition assay, measurement of monoamines, and their metabolites levels measurement of BDNF level to know the exact mechanism of antidepressant activity of META.

CONCLUSION

META showed significant anxiolytic and antidepressant activity. Hence, it may be served as a potential resource for natural psychotherapeutic agents against stress-related disorders such as anxiety and depression.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Saroj Kothari contributed in planning study, designing the manuscript, analyzing the data, proof reading and reviewing the final manuscript. Ajay Gupta contributed in planning the study, conducting literature survey, collecting the data, proof reading

REFERENCES

[1] Hosseini SE, Hosseini SA. The therapeutic effects of medicinal plants on depression and anxiety disorders. *Report Health Care*, **4,** 67-80 (2018)

[2] Kessler R.C. The costs of depression. *Psychiatr Clin North Am*, **35**,1–14 (2014)

[3] Kenda M, Glavac NK, Nagy M, Dolenc MS. Medicinal Plants Used for Anxiety, Depression, or Stress Treatment: An Update. *Molecules* **27(18)**, 6021 (2022)

[4] Bandelow B, Michaelis S, Wedekind D. Treatment of anxiety disorders. *Dialogues Clin Neurosci*, **19**, 93–107 (2017).

[5] James MO, Robert RB, Richard C, Shelton P. Drug therapy of depression and anxiety disorders. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 13th ed. New York: McGraw-Hill; 2018. p. 267-78.

[6] Arroll B, Chin WY, Martis W, Goodyear-Smith F, Mount V, Kingsford D, Humm S, Blashki G, Macgillivray S. Antidepressants for treatment of depression in primary care:

A systematic review and meta-analysis. *J Prim Health Car*e, **8**,325–334(2016)

[7] Yeung KS, Hernandez M, Mao JJ, Haviland I, Gubili J. Herbal medicine for depression and anxiety: A systematic review with assessment of potential psycho-oncologic relevance. *Phytother Res,* **32**,865–891(2018).

[8] El-Akhal J, Humulescu I, Ionita R, Postu PA, Ungureanu E, Hancianu M, Bencheikh R, Robu S, Cioanca O, Hritcu L. Anxiolytic and Antidepressant-Like Effects of Conyza canadensis Aqueous Extract in the Scopolamine Rat Model. *Plants*, **10**, 645 (2021).

[9] Rajoria A, Mehta A, Mehta P, Ahirwal L, Shukla S, Bajpai VK. Evaluation of antiproliferative and hepatoprotective effects of wheat grass (*Triticum aestivum)*. *Acta Biol Hung,* **68**,150–161 (2017).

[10] Kothari S, Jain AK, Mehta SC, Tonpay SD. Hypolipidemic effect of fresh *Triticum aestivum* (wheat) grass juice in hypercholesterolemic rats. *Acta Pol Pharm*, **68(2),** 291-4 (2011).

[11] Bitra VR, Rapaka D, Mathala N, Akula A. Effect of wheat grass powder on aluminum induced Alzheimer's disease in wistar rats. *Asian Pacific J Trop Med,* **7,** S278–S281 (2014).

[12] Kulkarni SD, Tilak JC, Acharya R, Rajurkar NS, Devasagayam TPA, Reddy AVR. Evaluation of the antioxidant activity of wheatgrass *(Triticum aestivum* L.) as a Function of Growth under Different Conditions. *Phytother Res*, **20**, 218–227(2006).

[13] Kothari S, Jain AK, Mehta SC, Tonpay SD. Effect of fresh *Triticum aestivum* grass juice on lipid profile of normal rats. *Ind J Pharmacol*, **40**,235-36 (2008).

[14] Kothari S, Gupta A, Chaturvedi V. Effect of methanolic extract of Triticum aestivum (Wheat grass) on learning and memory in mice. *Int J Green Pharm*, **17 (2)**, 171-176(2023).

[15] Harborne JB. Phytochemical methods: A guide to Modern Techniques of Plant Analysis. 2nd ed. London: Chapmann and Hall; 1983.

[16] Bora KS, Pant A. Evaluation of anxiolytic activity of *W. chinensis* Merrill leaves. *J Phytopharmacol*, **7(1**), 19-24 (2018).

[17] Bourin M, Hascoet M. The mice light/dark box test. *Eur J Pharmacol*, **463**, 55-65 (2003).

[18] Castagne V, Moser P, Roux S, Porsolt RD. Rodent models of depression: Forced swim and tail suspension behavioral despair tests in rats and mice. *Curr Protoc Neurosci,* **55**,8-10 (2011).

[19] Pamulaparthi AR, Prathap VR, Banala MA, Nanna RS. Experimental evaluation of antidepressant and antianxiety activities of aqueous leaf extracts of *Senna alata* (L.) roxb. using *in vitro* animal models. *Int J Curr Pharm Res,* **8**, 60-3 (2016).

[20] Shrivastava AK, Magar PT, Shrestha L. Effect of aqueous extract of barley and wheat grass in stress induced depression in Swiss mice. *J Ayur Integr Med*, **13**, 100630 (2022).

[21] Latha K, Rammohan B, Sunanda BP, Maheswari MU, Mohan SK. Evaluation of anxiolytic activity of aqueous extract of *Coriandrum sativum* Linn. in mice: A preliminary experimental study. *Pharmacogn Res* **7(1),** S47-51 (2015)

[22] Manu G, Padmanabha ST, Chandrakantha T, Ravishankar M. Evaluation of antianxiety activity of ethanolic extract of leaves of *Ocimum sanctum* (Tulsi) in albino mice. *Natl J Physiol Pharm Pharmacol*, **7**, 827-30 (2017).

[23] Gupta V, Sharma R, Bansal P, Kaur G. Bioactivity guided isolation of potent anxiolytic compounds from leaves of *Citrus paradisi*. *AYU*, **39**, 21-8 (2018).

[24] Zendehbad SH, Mehran MJ, Malla S. Flavonoids and phenolic content in wheat grass plant (*Triticum aestivum*) *Asian J Pharm Clin Res*,**7(4)** 4184-187(2014).

[25] Khan IN, Sarker MMI, Ajrin M. Sedative and anxiolytic effects of ethanolic extract of Calotropis gigantea (Asclepiadaceae) leaves. *Asian Pac J Trop Biomed,* **4(Suppl 1**): S400-S404 (2014).

[26] Sechi S, Chiavolelli F, Spissu N, Di Cerbo A, Canello S, Guidetti G, Fiore F, Cocco R. An antioxidant dietary supplement improves brain-derived neurotrophic factor levels in serum of aged dogs: preliminary results. *J Vet Med*, Article ID 412501, 1–9 (2015).

[27] Khalil MG, Ali AA, Hassanin SO, Al-Najjar AH, Ghosh S, Mahmoud MO. Comparative study on the effect of EGCG and wheat grass together with mental and physical activities against induction of Alzheimer's disease in both isolated and socialized rats. *Phytomed Plus* **2**, 100146 (2022).

[28] Charan K. Evaluation of Anti-Depressant Activity of Hydro Alcoholic Extract of Triticum Aestivum. *Pharma Tutor*, **2(8),** 224-225 (2014).

[29] Pannu A, Sharma PC, Thakur VK, Goyal RK. Emerging Role of Flavonoids as the Treatment of Depression. *Biomolecules,* **11(12),** 1825-1916 (2021)