WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Exploring the Potential of *Eleusine Coracana* Aqueous Extract as a Natural Anti-Depressant in Experimental Mice

Ranjitha R Nayak [1], Satish S [2]

[1]PG Scholar, Department of Pharmacology, Srinivas College of Pharmacy Mangalore (Karnataka) India 574143
[2]Professor, Department of Pharmacology, Srinivas College of Pharmacy Mangalore (Karnataka) India 574143

ABSTRACT:

The study aimed to evaluate the anti-depressant activity of the aqueous extract of Eleusine coracana (Finger Millet) grains in experimental mice using the forced swim test (FST) and tail suspension test (TST), both commonly used models for assessing antidepressant effects. Two doses of the extract, 200 mg/kg and 400 mg/kg (administered orally), were tested for 15 days, with Imipramine (10 mg/kg, oral) used as a standard reference. The primary endpoints were the total duration and frequency of immobility, which are indicators of depressive-like behavior. Results showed that the 400 mg/kg dose of the extract significantly reduced the immobility time in both the FST and TST, compared to control and standard groups, indicating a notable antidepressant effect. The 200 mg/kg dose showed a less pronounced effect but still provided some reduction in immobility. These findings suggest that the aqueous extract of Eleusine coracana exhibits dose-dependent antidepressant activity. The study provides preliminary evidence supporting the potential use of Eleusine coracana as a natural therapeutic agent for depression, with its beneficial effects possibly linked to the modulation of neurotransmitter systems involved in mood regulation. Further studies are needed to identify the active compounds responsible for these effects and explore their clinical potential.

Keywords: Anti-depressant activity; *Eleusine coracana*, Forced swim test; Imipramine; Tail Suspension test.

Introduction:

Everyone experiences brief sadness, but clinical depression (major depressive disorder) is different, with severe symptoms affecting mood, thoughts, and daily activities. Research suggests that biological, psychological, environmental, and genetic factors contribute to depression [1]. These issues can become persistent or recurrent, which significantly impairs a person's capacity to attend to their daily obligations [2].

MDD is widespread, causing significant role impairment and severe symptoms. Despite more treatment options, inadequate treatment remains a concern. Depression often accompanies chronic medical conditions and can sometimes be caused by them, frequently coexisting with other health issues [3]. Reduced serotonergic function contributes to depression, with SSRIs' effects likely due to regulating various serotonin receptor subtypes [4]. Patients with serious depression may have varied outcomes depending on whether they receive a variety of therapy options or only antidepressants [5].

The lifetime risk of depression is 5-12% for men and 10-25% for women. 15% of severely depressed patients may take their own lives. Only about 30% achieve remission with a single medication. Combination therapy targeting serotonin and catecholamines like NE and DA is often necessary for effective treatment [6]. The development of MDD may be influenced by dysfunction of the serotonin 1A receptor (5-HT1A) [7]. Unipolar depressive disorders account for 4.4% of the worldwide disease burden, according to a recent estimate from the worldwide Burden of Disease study [8].

Global Millets Year the health advantages of millets have garnered significant attention worldwide in 2023. A crucial component of grains in Indian cuisine, millets are nutri-cereals. *Eleusine coracana*, also referred to as finger millet, is a valuable millet crop that has medicinal, nutritional, and commercial value [9].

Several plants are being used for the treatment of depression such as *Glycyrrhiza uralensis, Curcuma longa, Lafoensia pacari, Bupleurum falcatum, Siphocampylus verticillatus, Hypericum reflexum L, Allium macrostemon, Piper tuberculatum, Mitragyna speciose, Rosmarinus officinalis L., Asparagus racemosus Linn, Magnolia bark and ginger rhizome, Valeriana officinalis, Ginkgo biloba, Ocimum sanctum, Withania somnifera, Mimosa pudica, Allium sativum, Areca catechu* and more [10]. *"Eleusine coracana*" is one among them and also literature review revealed that *Eleusine coracana* grains possess antidepressant activity [11]. Though there is a paucity of scientific data for its antidepressant activity, hence the present study was selected to investigate the antidepressant activity of *Eleusine coracana* in mice. Based on its use in Ayurveda for the treatment, it is hypothesized that "*Eleusine coracana*" may possess antidepressant activity which needs to be investigated. Hence the *Eleusine coracana* grains are selected for the study.

Methodology:

Collection and authentication of plant material:

The *Eleusine coracana* grains used for the current study were procured from the local shop in Alevoor, Udupi District, Karnataka. The grains were authenticated by Dr. Siddaraju M N Assistant Professor and Research Guide, Department of Botany, University College Mangalore.

Preparation of extract:

The seeds were properly cleaned under running water and then soaked for four hours at room temperature in sterile distilled water. These were then given another three days to dry in the sun, ground into a powder, and extracted using an aqueous solvent through a maceration process. After the solvent was removed, the crude extract that was thus produced was kept in a closed container for additional research [12].

Preliminary qualitative phytochemical analysis:

Table 1: Preliminary Phytochemical analysis

S. No	phytochemicals	Test	Observation	Inference
1.	Alkaloids	Wagner's Test	Reddish brown precipitate	Presence of Alkaloids
2.	Alkaloids	Mayer's test	White or creamy precipitate	Presence of Alkaloids
3.	Flavonoids	Shinoda test	Formation of a pink or crimson-red color.	Presence of Flavonoids
4.	Saponins	Froth's test	Foams formation	Presence of saponin.
5.	Glycosides	Borntrager's Test	Pink or red colour solution	Presence of Glycosides
6.	Tannins	Ferric chloride test	Dark green color or blue-green	Presence of tannins.
7.	Carbohydrates	Fehling's Test	Formation of Brick red ppt	Presence of Carbohydrates.
8.	Proteins	Biuret test	Appearance of violet or pink color	Presence of proteins
9.	Aminoacids	Ninhydrin test	Appearance of blue colour	Presence of aminoacids

Experimental Animals:

Healthy albino mice (20-25g) of either sex used for the experiment were collected from the animal house of Srinivas College of Pharmacy, Mangalore. They are maintained under standard conditions (temperature 22 ± 2°C, relative humidity 60±5%, and 12h light/dark cycle) and have free access to a standard pellet diet and water ad libitum. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding.

Approval of Research Protocol:

The Institutional Animal Ethics Committee reviewed and approved the experimental protocol (Approval no. SCP/IAEC/F150/P216/2023 Dated 04/08/2023). All the procedures were performed following the IAEC constituted as per the direction of the CCSEA. All the animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". The animals were acclimatized for at least one week before use.

Evaluation of Antidepressant Activity:

Preparation of Doses:

The dried extract of *Eleusine coracana* was weighed and dispersed in the water to prepare 200mg/kg and 400mg/kg according to the body weight of the animal and 1ml of extract was administered to each animal orally [13].

Evaluation of Antidepressant Activities by Animal Models

A. Forced Swim Test:

In 1978, Porsolt *et al*. created the forced swim test (FST), commonly referred to as the "behavioral despair" test, as a mouse model for predicting the clinical efficacy of antidepressant medications [14]. A novel behavioral technique for "inducing a depressed state in mice" is FST. The most frequently accepted experimental model for evaluating the antidepressant activity in the experimental animals is the cylinder due to its versatility and simplicity [15].

Treatment Groups:

Swiss Albino Mice of either sex weighing between (20-25g) were divided into following 4 groups of six animals each.

Experimental Design of FST

GROUPS NUMBER	GROUP NAME	TREATMENT	DOSE
Group 1	Vehicle control	Vehicle (Water)	10ml/kg p.o
Group 2	Standard	Imipramine	10ml/kg, p.o
Group 3	AEECG low dose	AEECG	200mg/kg, p.o
Group 4	AEECG high dose	AEECG	400mg/kg, p.o

Study chart:

From day 1-15 animals will be administered with drugs as mentioned above except standard. Standard are administered 1hour prior testing (15^{th} day). One day before the experiment they are brought to laboratory with free access to food and water.

Procedure:

The following protocol was used to describe this novel behavioral paradigm in mice: "Mice were dropped into a cylinder (38 cm in height, 22 cm in diameter, and 15 cm of water at 23–25°C) one hour after a single dose oral administration of standard drug, and they were left for six minutes." Since there wasn't any immobility seen in the first two minutes, only the latter four minutes' worth was recorded. It was calculated to see how long a mice remained still in a minute. When a mouse stopped trying to swim and just stayed floating in the water, it was considered immobile and simply needed to move its head above the water [15].

After 6minutes mice are removed, dried and returned to their home cages. Each mouse will be subjected to this procedure 24hr prior (pretest) and 1hr (test) after the respective drug administration for 6min in a test session where the immobility duration will be recorded for the last 4min on both days. Water in the cylinder was changed after subjecting each animal to test because used water has been shown to alter the behavior [16].

Evaluation

A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water making only movements necessary to keep its head above water. Duration and number of episodes of immobility in last 4 minutes out of 6 min is measured in control animals and animals tested with various doses of a test drug and standard and compared.

B. Tail Suspension Test:

The TST, in contrast to the FST, was developed to evaluate behavioral despair in mice before being expanded to include rats. Mice are hung by their tails using a hook for six minutes during a TST, which is comparable to the six-minute mouse FST methodology. Animal movements and struggle are classified as searching behavior, while times of immobility are classified as waiting behavior. Antidepressants reduce immobility in the TST, just as they do in the FST. This test offers a seemingly less stressful alternative to the FST and abolishes the risk of hypothermia, but it is not suitable for adult rats because of their weight [17].

In this experiment known as the "tail suspension test," an animal's tail is suspended, and it exhibits behavior ranging from trying to flee to giving up and eventually going into sadness. In 1985, Lucien Steru and associates developed and used this test to gauge depressed behavior in mice. The TST operates on a similar basis as the FST, which is the progressive growth of depressive behaviors in animals in the face of an unavoidably stressful environment [18].

Treatment

Swiss Albino Mice of either sex weighing between (20-25g) were divided into following 4 groups of six animals each.

Experimental Design of TST

GROUPS NUMBER	GROUP NAME	TREATMENT	DOSE
Group 1	Vehicle control	Vehicle (Water)	10ml/kg p.o
Group 2	Standard	Imipramine	10ml/kg, p.o
Group 3	AEECG low dose	AEECG	200mg/kg, p.o
Group 4	AEECG high dose	AEECG	400mg/kg, p.o

Procedure

From day 1-15 animals will be administered with drugs as mentioned above except standard. Standard are administered 1hour prior testing (15^{th} day). All group animals will be subjected to tail suspension test. Mice were considered immobile when they hang passively and completely motionless. Each mouse

will be subjected to this procedure 24hr prior (training session) for 15 min and 1hr after the respective drug administration (test session) for 6min in test session where the duration of immobility will be recorded for last 4min on both days [19].

Evaluation:

Mice were considered immobile when they hang passively and completely motionless. Duration and total number of episodes of immobility in last 4 min out of 6 min is measured in controls and animals tested with various doses of a test drug and standard and compared.

Statistical Analysis

The data of pharmacological experiments were expressed as mean ± SEM. Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, CA, USA). Data of 5-HTP induced head-twitch responses in mice was analyzed by two-way analysis of variance (ANOVA) followed by Turkey's (multiple comparison test). Data of biochemical parameters were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. A value of $P<0.05$ was considered to be statistically significant.

Results

Preliminary Phytochemical Screening:

Table 2: Preliminary Phytochemical Screening of Aqueous Extract of *Eleusine coracana* Grains

Name of the chemical test	Results
Alkaloids	+
Flavonoids	+
Carbohydrates	+
Tannins	+
Saponins	+
Phenolic Acids	-
Steroids	+
Alkaloids	+
Glycosides	+
Proteins	+
Volatile oils	-
Starch	+

Pharmacological Assessment of Antidepressant Activity:

Following were the results, showing the antidepressant activity of *Eleusine coracana* grains.

Forced Swim Test:

The groups treated with the AEECG both 200mg/kg and 400mg/kg, showed significant reduction in the duration of immobility in comparison to the standard drug imipramine in the mice during the forced swim test.

Table 3: Effect of Imipramine and AEECG on immobility period of mice in forced swim test:

Group No.	Groups	Treatment	Dose	Duration of Immobility in FST in Seconds (Mean ± SD)
I	Control (Vehicle)	Distilled water	10ml/kg p.o	251.500 ± 0.764
II	Standard group	Imipramine	10mg/kg, p.o.	151.167 ± 0.654***
III	Test group	AEECG	200mg/kg, p.o.	212.333 ± 0.422*
IV	Test group	AEECG	400mg/kg, p.o.	181.000 ± 5.033**

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

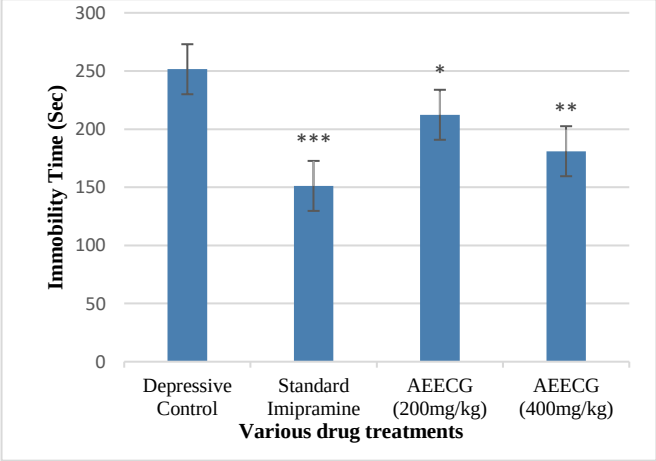

Fig 1: Effect of standard drug imipramine and AEECG on immobility period of mice in FST

Table 4: Effect of Imipramine and AEECG on number of episodes of immobility (frequency) of mice in FST:

Group No.	Groups	Treatment	Dose	Duration of Immobility in FST in Seconds (Mean ± SD)
I	Control (Vehicle)	Distilled water	10ml/kg p.o.	5.167±0.753
II	Standard group	Imipramine	10mg/kg, p.o.	7.833 ± 1.169***
III	Test group	AEECG	200mg/kg, p.o.	6.833 ± 0.753*
IV	Test group	AEECG	400mg/kg, p.o.	7.667 ± 0.816**
All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.				

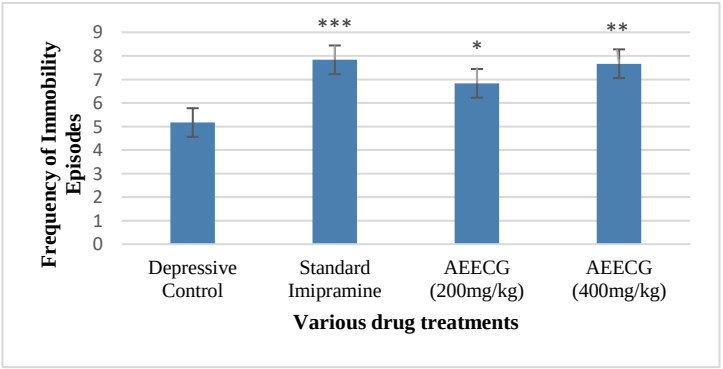

Figure 2: Effect of standard drug imipramine and AEECG on frequency of immobility episodes of mice in FST

Tail Suspension Test:

The groups treated with the AEECG both 200mg/kg and 400mg/kg, showed significant reduction in the duration of immobility in comparison to the standard drug imipramine in the mice during the TST.

Table no. 5: Effect of imipramine and AEECG on immobility period of mice in tail suspension tests.

Group No.	Groups	Treatment	Dose	Duration of Immobility in Seconds in TST (Mean ± SD)

I	Control (Vehicle)	Distilled water	10ml/kg p.o.	130.000 ± 0.577
II	Standard group	Imipramine	10mg/kg, p.o.	80.444 ± 0.601***
III	Test group	AEECG	200mg/kg, p.o.	92.833 ± 0.864**
IV	Test group	AEECG	400mg/kg, p.o.	91.138 ± 0.601**
All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.				

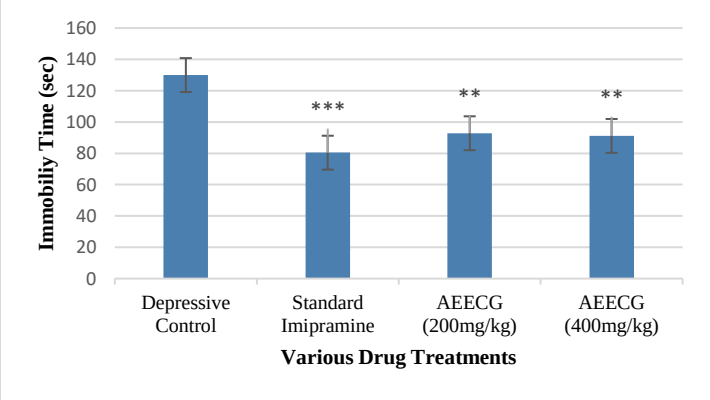

Figure 3: Effect of standard drug imipramine and AEECG on immobility period of mice in TST

Table 6: Effect of imipramine and AEECG on number of episodes of immobility (frequency) of mice in tail suspension tests.

Group No.	**Groups**	**Treatment**	**Dose**	**Duration of Immobility in Seconds in TST (Mean ± SD)**
I	Control (Vehicle)	Distilled water	10ml/kg orally	6.167 ± 1.169
II	Standard group	Imipramine	10mg/kg, p.o.	7.833 ± 0.753***
III	Test group	AEECG	200mg/kg, p.o.	7.333 ± 0.813*
IV	Test group	AEECG	400mg/kg, p.o.	8.400 ± 0.894**
All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.				

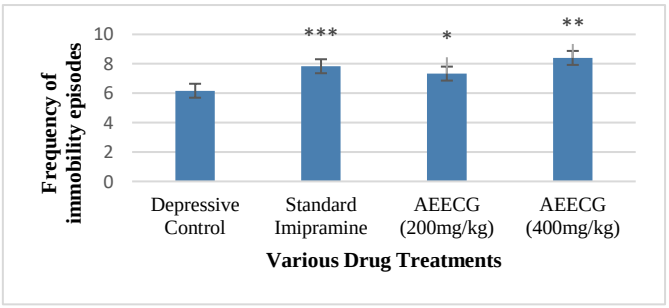

Figure 23: Effect of standard drug imipramine and AEECG on frequency of immobility episodes of mice in TST

Discussion

Anxiety and depression, key psychosomatic disorders causing morbidity, have shown an alarming increase in prevalence. Depression, characterized as a pathological mood state, can vary in severity and is the most common mood disorder, sometimes accompanied by delusions and hallucinations [20]. Despite significant progress in antidepressant therapies, including TCAs, SSRIs, SNRIs, and MAOIs, concerns regarding their safety and efficacy remain

[21]. Depression impacts behavior, mood, and health, contributing to 12.3% of the global disease burden, projected to rise to 15-20%. Its strong association with suicide underscores the importance of effective treatment [22].

In this study, the potential antidepressant activity of AEECG (derived from *Eleusine coracana* grains) was assessed using the Forced Swim Test (FST) and Tail Suspension Test (TST), models widely accepted for evaluating antidepressant efficacy. The Porsolt FST measures immobility as an indicator of behavioral despair [23]. Treatment with AEECG (200 mg/kg and 400 mg/kg) reduced immobility times similar to imipramine (10 mg/kg), demonstrating significant antidepressant activity. Tail suspension test (TST) is one of the simplest and reliable methods available for the study of antidepressant action of any given drug. In this experiment an animal is suspended by its Tail, and it exhibits behavior ranging from trying to flee to giving up and eventually going into sadness [24].

In most research, imipramine hydrochloride has been the usual medication. It works by blocking NE reuptake. Imipramine hydrochloride appears to have a positive effect in the FST model because it increases the availability of NE and 5HT at the postsynaptic location after reuptake inhibition.

Phytochemical analysis showed that AEECG contained flavonoids and amino acids such as tryptophan, both of which contribute to CNS benefits and may enhance antidepressant activity. Furthermore, oxidative stress has been implicated in major depression, correlating with symptom severity. The antioxidant properties of AEECG potentially aid in reducing oxidative stress, complementing its antidepressant effect.

Main classes of antidepressant medications are studied by these tests, which are fairly specific and highly sensitive. Immobility in TST is a reflection of a depressed condition that can be lessened by a number of medications that are useful as treatments for depression in people [25]. The TST is said to be more pharmacologically sensitive and less stressful than the FST [26].

FST and TST have proven to be helpful in basic research related to the neurobiology and genetics of mood disorders. In this present study anti-depressant activity of test drug in experimental animal model, test drug significantly produced the antidepressant activity. Hence the current test drug may be useful in depression. It is worth for further investigations for isolation of more bioactive molecules for the treatment and using more experimental paradigms are required for further confirmation of antidepressant potential of AEECG in the treatment of MDD.

Conclusion

The aqueous extract of Eleusine coracana grains (AEECG) demonstrated significant antidepressant activity in mice, as shown by reduced immobility time in both the Tail Suspension Test (TST) and Forced Swim Test (FST). The effect was comparable to the standard antidepressant drug, imipramine. The observed antidepressant potential of AEECG is likely due to the synergistic action of its phytoconstituents, including alkaloids, flavonoids, amino acids, proteins, and saponins. Oral administration of AEECG at doses of 200 mg/kg and 400 mg/kg showed antidepressant effects similar to imipramine (10 mg/kg) in both the FST and TST, with a more pronounced benefit at higher doses. The findings indicate that AEECG possesses significant antidepressant activity.

These results highlight AEECG's potential as a new candidate for depression treatment, opening the possibility for the development of novel antidepressant therapies. However, further research is necessary to identify and characterize the active compounds and to elucidate the precise mechanisms underlying AEECG's antidepressant effects, as the exact pathways remain unclear.

References:

1. Karp DA. Speaking of Sadness: Depression, Disconnection, and the Meanings of Illness. Oxford University Press. 2006;28-39.
2. Kumar KS, Srivastava S, Paswan S, Dutta AS. Depression symptoms, causes, medications, and therapies. Pharma. Innov. 2012;1-37.
3. Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA. 2003;289(23):3095-105.
4. Amidfar M, Kim YK. Recent developments on future antidepressant-related serotonin receptors. Curr. Pharm. Des. 2018;24(22):2541-8.
5. Al-Harbi KS. Treatment-resistant depression: therapeutic trends, challenges, and future directions. Patient Prefer Adherence. 2012;369-88.
6. Sanmukhani J, Anovadiya A, Tripathi CB. Evaluation of the antidepressant-like activity of curcumin and its combination with fluoxetine and imipramine: an acute and chronic study. Acta. Pol. Pharm. 2011;68(5):769-75.
7. Savitz J, Lucki I, Drevets WC. 5-HT1A receptor function in major depressive disorder. Prog Neurobiol. 2009;88(1):17-31.
8. Glue P, Donovan MR, Kolluri S, Emir B. Meta-analysis of relapse prevention antidepressant trials in depressive disorders. Aust. N Z J. Psychiatry. 2010;44(8):697-705.
9. Kumar A, Nadarajan N, Rajesh R. Nutraceutical value of finger millet [Eleusine coracana (L.) Gaertn.] and their improvement using omics approaches. Front Sustain Food Syst. 2021;5:684447.
10. Jawaid T, Gupta R, Siddiqui ZA. A review on herbal plants showing antidepressant activity. Int. J. Pharm. Sci. Res. 2011;2(12):3051-7.

11. Jagati P, Mahapatra I, Dash D. Finger millet (Ragi) as an essential dietary supplement with key health benefits: A review. Int. J. Home Sci. 2021;7(2):94-100.
12. Nikam P, Kangralkar VA, Godabole S, Goundar D, Kelkar P. Evaluation of antidepressant activity of seed extract of Eleusine coracana L. in experimental rats. Pharmacologyonline. 2022;12(1):1314-20.
13. Kareem AM, Bello SO, Etuk E, Arisegi SA, Umar MT. Evaluation of the hypoglycemic and hypolipidemic effects of aqueous Eleusine coracana seed extract. Int. Archive. Med. Sci. 2019;1(3):35-41. Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF. Factors influencing behavior in the Forced swim test. Physiol. Behav. 2013;118:227-39.
14. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology (Berl). 2005;177:245-55.
15. Shashikumara S, Prathima C, Sibgatullah M. Evaluation of antidepressant activity of ethanolic extract of Alangium salviifolium (L. f.) Wangerin in Swiss albino mice. Biomed. Pharmacol. J. 2017;10(1):249-58.
16. Shashikumara S, Prathima C, Sibgatullah M. Evaluation of antidepressant activity of ethanolic extract of Alangium salviifolium (L. f.) Wangerin in Swiss albino mice. Biomed. Pharmacol. J. 2017;10(1):249-58.
17. Gencturk S, Unal G. Rodent tests of depression and anxiety: construct validity and translational relevance. Cogn. Affect Behav. Neurosci. 2024;24(2):191-224.
18. Jun G. Tail suspension test. In: Sachdeva M, editor. The ECPH Encyclopedia of Psychology. Springer. 2024;1-2.
19. Das MC, Rao S, Sri K. Antidepressant activity of Brahmi in albino mice. J. Clin. Diagn. Res. 2014;8(3):35–7.
20. Fekadu N, Shibeshi W, Engidawork E. Major depressive disorder: pathophysiology and clinical management. J Depress Anxiety. 2017;6(1):255-7.
21. Chen KX. Quantitative structure–activity relationship analysis of aryl alkanol piperazine derivatives with antidepressant activities. Eur. J. Med. Chem. 2009;44:4367–75.
22. Dhingra D, Bhankher A. Behavioural and biochemical evidences for antidepressant-like activity of palmatine in mice subjected to chronic unpredictable mild stress. Pharmacol. Rep. 2014;66:1-9.
23. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioral despair in rats: A new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 2018;47:379-91.
24. Jun G. Tail suspension test. In: Sachdeva M, editor. The ECPH Encyclopedia of Psychology. Springer. 2024;1-2.
25. Paget GE, Barnes JM. Evaluation of drug activities: pharmacometrics. In: Laurence DR, Bacharach AL, editors. Toxicity Tests. New York: Academic Press. 2022;135–42.
26. Porsolt RD, Bertin A, Jalfre M. Behavioral assessment of antidepressant activity in rodents. Arch. Int. Pharmacodyn. Ther. 2007;229:327-36.