Neuropharmacological Activities of *Evolvulus alsinoides* Plant Extract in Swiss Albino Rats

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KEYWORDS
Evolulus alsinoides, antidepression, behavioral tests, oxidative stress parameters, neurophamacological activities.

ABSTRACT
The aim of the present study was to investigate the neuropharmacological (antidepressant) activities of *Evolvulus alsinoides* whole plant extract in swiss albino rats. Anti-depressant activities of the methanolic extract of *Evolvulus alsinoides* were assessed by behavioral tests like Forced Swimming Test (FST), Tail Suspension Test (TST), and Locomotor Activity Test in male Swiss albino rats. By measuring the levels of oxidative stress markers in the rat brain homogenates, including plasma nitrites, plasma corticosterone and catalase (CAT), reduced glutathione, malondialdehyde (MDA), and superoxide dismutase, we were also able to investigate the neuropharmacological activities. Methanolic extract of the *Evolvulus alsinoides* plant showed significant neuropharmacological activity in Swiss albino rats. In both TST and FST, they significantly reduced the immobility duration and demonstrated dose-dependent activity ($p<0.001$). It demonstrated much more activity than imipramine in FST at 400mg kg-1 ($p<0.001$). Compared to the control group, they demonstrated a statistically significant increase in swimming time ($p < 0.001$). According to our research, *Evolvulus alsinoides* exhibited significant antidepressant properties. It appears that the primary mechanism underlying this action is catecholamine reuptake inhibition.
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INTRODUCTION

Depression, also referred to as major depressive illness, is typified by a chronically down mood and diminished interest in or enjoyment of activities. This condition can impact cognition, behavior, emotions, and overall well-being (Murillo-Zamora et al, 2016). According to reports, the incidence of major depressive disorder (MDD) has risen significantly over the past ten years by nearly 20%. Depressive disorders are now recognized as severe and incapacitating illnesses that impact over 300 million individuals globally, and there has also been a corresponding rise in the number of patients who are violent and aggressive. (Ormel et al, 2019) Despite ongoing research, the underlying mechanisms of major depressive disorder (MDD) remain incompletely understood, and many patients do not respond adequately to current treatments.

Major depressive disorder has been treated with a variety of approaches, including pharmaceuticals like lithium and selective serotonin reuptake inhibitors (SSRIs), medical devices like deep brain stimulation, bright light therapy, and electroconvulsive therapy (Rosenthal et al, 2016), and adjunctive therapies like physical activity and music therapy. While antidepressant medications and psychotherapy are accessible, many individuals do not achieve full remission, and recurrence or regression of the symptoms is common among those who do recover. Currently, there is no clear-cut treatment that can effectively lessen the long-term effects of depression.

Evolvulus alsinoides (L), a member of the Convolvulaceae family, is a perennial herb characterized by its small, hairy, procumbent nature and compact, branched rootstock with woody attributes (Austin, 2008). The leaves of *Evolvulus alsinoides* are recommended for the treatment of mental health issues and asthma, while the roots and stem extract are used in Sri Lanka to treat depression and dysentery (Rajaqkaruna et al, 2002). It is also employed in managing conditions such as insanity, epilepsy, and nervous debility. As a brain tonic, E. alsinoides is used in Ayurveda to treat amnesia, asthma, and neurodegenerative illnesses (Goyal and Singh, 2005). Scientific investigations have demonstrated the diverse potential of E. alsinoides, including its effects on the central nervous system, such as depression, anxiety reduction, tranquillization, antidepressant properties, stress reduction, neurodegenerative disease management, anti-amnesic effects, antioxidant activity, lipid-lowering properties, immunomodulatory effects, pain relief, antifungal and antibacterial properties, anti-diabetic activity, anti-ulcer properties, anti-cataleptic effects, and cardiovascular activity (Sethiya et al, 2009).

Thus, the goal of this investigation is to evaluate the neuropsychiatric impacts of methanolic extract from *Evolvulus alsinoides* in Swiss albino rats. The study aims to offer insights into the possible therapeutic utility of the *Evolvulus alsinoides* methanolic extract compound as a depression treatment option by assessing both behavioral and biochemical attributes.

MATERIALS AND METHODS

Collection of Sample and Other Ingredients

The fresh *Evolvulus alsinoides* plants were collected from the Thenkasi district in the month of July 2022 and authenticated (Reg No: XCH-40454) by Dr.S.Mutheeswaran, Scientist, Xavier Research Foundation, St.Xavier's College, Playamkottai, Tamil Nadu, India.

Extraction of *Evolvulus alsinoides*

Following collection, the *Evolvulus alsinoides* plants were thoroughly rinsed using distilled water. Subsequently, the entire plants were subjected to drying in a cabinet drier. Once dried, the plants were finely ground and sieved, resulting in a well-prepared powder of *Evolvulus alsinoides*. This powder was then carefully stored in an airtight container for future applications. 10g of the dried powdered material was macerated for 24 hours at room temperature with 50 ml of methanol to perform the extraction process. The extracts underwent centrifugation (3000×g) three times, and the clear supernatants were combined for each respective solvent. After filtering the mixed supernatants through the Whatman No. 1 filter paper, the residue was extracted once more using the same solvent. Finally, all the extracts were pooled together and allowed to undergo a drying process. The resulting crude extracts were then employed for further analysis.

Antidepression Activity

Experimental animals: Swiss male albino rats 6 per group with average weight 120 – 150g were acclimatized to the experimental room under conditions of 23±2°C temperature as well as a 12-hour light-dark cycle. Female rats had not been included in the study due to reports indicating that estrogen, a female sex hormone, may have antidepressant effects. Water and food were available to the animals at all times. Before and after the drugs were administered, they fasted for two hours. The Institutional Animals Ethics Committee (IAEC) met on March 23, 2023, and approved the experimental procedure. Guidelines for animal care procedures were provided by the Government of India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Ref: SBCP/2022-23/ CCSEA/IAEC/I(2)/F16/368).

Acute Toxicity Studies

The methanol extract of *Evolvulus alsinoides* underwent acute oral toxicity testing following the revised OECD (2002) guidelines No. 423. Animals were monitored for behavioral changes hourly for four hours and daily for fourteen days. Rats receiving oral dosages of the extract up

to 2000mg/kg displayed no toxicity signs. For the purpose of the following research, the dosages of the extract were chosen to range from 100mg/kg to 400mg/kg.

Experimental Design

Rats were put into five groups (n = 5). The animals had been administered medication 60 minutes before the research "began.

Group I: Normal control- rats received normal saline 2ml/kg orally
Group II: Positive control- rats received imipramine 30mg/kg orally
Group III: rats received MEEA 100mg/kg orally
Group IV: rats received MEEA 200mg/kg orally
Group V: rats received MEEA 400mg/kg orally

Procedure for ARS

To induce acute restraint stress (ARS), rats were individually placed in plastic rodent restraint devices for 12 hours. This method effectively restricted their physical movements without causing pain. Animals were denied water and food during this time of stress exposure. After 12 hours of restraint, the rats were released from the devices, and 40 minutes after release, they underwent behavioral tests followed by biochemical measurements. In contrast, rats in the normal control group were housed in standard animal cages within the experimental room.

Behavioral tests

Depression-like behaviors in rats were evaluated using tests like the Locomotor Activity Test and TST, conducted 40 minutes after subjecting them to restraint stress. Each rat also underwent a 10-minute pretest for the forced swim test (FST) simultaneously. Relevant samples were given out the next day, 23.5 hours later, and the primary test was carried out 30 minutes later. Rats placed under restraint were compared to a control group, and following the FST on the 90th day, oxidative stress markers levels like catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), and lipid peroxidation (LPO) had been assessed.

Tail Suspension Test

After subjecting the animals to restraint stress, each rat was individually suspended by the end of its tail utilizing micropore adhesive tape, positioned approximately 1cm from the end, in a suspension box with the head 50 cm above the floor. This occurred 40 minutes after the restraint stress procedure. For six minutes in total, each rat was suspended, and the last four minutes of that time were used to measure the immobility period. While hanging passively and without movement, the rat was said to be immobile. Antidepressant medication usually shortens the duration of immobility during this test.

Forced Swimming Test

On the fourteenth day of the experiment, each rat was given ten minutes to swim on their own to get used to the environment. The relevant samples were then given out 23.5 hours later, and on the fifteenth day, the primary test was run 30 minutes later. Rats were used in the experiment, and they were kept in a cylindrical tank 40 cm in diameter and 60 cm in height with 30 cm of fresh water kept at 25°C ± 1°C. To maintain consistency and avoid any impact from previously used water on the following behaviors, the water was replaced after every rat. Each rat exhibited vigorous movement in the initial 2-minute period of the test. An observer manually recorded the length of immobility for the next 4 minutes of the 6-minute testing period. Rats had been considered motionless when they floated upright as well as moved very slightly to keep their heads above water. Rats were dried with a cotton towel after their swim and put back in their own cages. When compared to a control group, an increase in immobility time suggests depressive-like effects, while a decrease in immobility duration supports antidepressant-like benefits.

Locomotor Activity Test

To evaluate the locomotor activity of albino rats, thirty rats in all were split up into five groups of six rats each (n = 6). Methanol extract was given to the experimental groups at doses of 25mg/kg, 50mg/kg, and 100mg/kg respectively, while imipramine hydrochloride served as the standard treatment. Control rats were administered physiological saline. Thirty minutes before the experimental trials began, the treatments for the control and experimental animals were administered.

The albino rats were placed in an open field apparatus consisting of a 45 cm diameter arena divided into 16 equal areas. This occurred 15 hours after the final treatment. The rats were positioned in the arena's center, and their behavioral responses, including rearing frequencies, locomotion, and defecations, were observed for a period of 5 minutes.

Biochemical estimations: Collection of blood samples

On the 90th day, both unstressed and stressed rats underwent testing for antidepressant activity. On the 91st day, one hour after administering the drug, blood samples (1.0–1.5mL) had been collected from the rat's retro-orbital plexus. Following the separation of the plasma, the levels of corticosterone and nitrite were assessed using a refrigerated centrifuge (Remi, Mumbai, India) set at 350 g for ten minutes.

Estimation of plasma nitrite levels

The Green et al. (1982) approach was utilized to determine the levels of plasma nitrite. One part of "a 5% w/v aqueous solution of m-phosphoric acid was mixed with one part of 0.1% w/v N-(1-Naphthyl) ethylene diamine dihydrochloride, and the combination was refrigerated at 0°C for 60 minutes. Following a 0.5 mL/0.5 mL mixture of this solution and plasma, the mixture was left to stand at room temperature in the dark for 10 minutes. The absorbance at 546 nm was then measured using a UV-visible spectrophotometer.

Estimation of plasma corticosterone levels

The method outlined by Bartos and Pesez in 1979 had been used to assess plasma corticosterone levels. Initially, 1.0 mL of the sample was mixed" with 0.50 mL of a 0.10% w/v solution of p-nitroso-N, N-dimethylaniline in ethanol. The liquid was mixed with 0.50mL of 0.10M sodium hydroxide after it had cooled for five minutes in ice water. Cotton wool was used to seal the tubes, which were then left in the dark at 0°C for five hours.

Then, 2.0mL of sodium carbonate/bicarbonate buffer (pH-9.8), 5.0mL of phenol in ethanol (0.10% solution), and 0.50mL "of potassium ferricyanide (1.0 w/v aqueous solution) had been added to the mixture. Subsequently, the tubes were incubated in a water bath at 20 ± 2°C for 10 minutes. Lastly, the absorbance of the solution at 650 nm was measured using a UV–visible spectrophotometer.

Biochemical estimations in brain homogenate

On the ninetieth day, after blood samples had been taken, the mice were beheaded and their brains removed. After the isolated brain samples were rinsed in a cold buffer (pH 7.4) containing 0.02 M ethylenediamine tetraacetic acid (EDTA), 0.1 M Tris", and 0.25 M sucrose, they were weighed. Using a Teflon glass homogenizer, the brain samples had been homogenized in nine "liters of cold 0.25M sucrose–0.1 M Tris–0.02 M EDTA buffer (pH-7.4). Next, the homogenate was placed in a refrigerated centrifuge and centrifuged twice at 350g for ten minutes at 4°C. The obtained pellet was disposed of and the supernatant was collected.

The recovered supernatant was then centrifuged" for 20 minutes at 4°C at 8064 g. Assays for superoxide dismutase, reduced glutathione, malondialdehyde, as well as catalase activity were conducted using the supernatant that was produced from this step.

Determination of Malondialdehyde Level

The spectrophotometric assay method using TBA (thiobarbituric acid) was utilized to detect the level of malondialdehyde (MDA), which is utilized as an indication of lipid peroxidation.

Ohkawa et al. first developed this approach in 1979. In this experiment, TBA reacts with specific lipid peroxidation products at a high temperature and acidic environment to form a compound that is pink in color.

250µl of liver homogenate, "500µl of distilled water, 500µl of 15% trichloroacetic acid (TCA), and 500µl of 0.37% TBA were employed in the experiment. The TCA and TBA solutions were prepared in 0.25 M HCl. For ten minutes, the mixture was boiled in a bath of boiling water. The samples undergo a 10-minute, 12,000×g centrifugation after cooling. The absorbance of the resulting supernatant was determined with a spectrophotometer set at 535 nm.

Determination of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity had been measured employing a colorimetric technique" that was reported by Misra and Fridovich in 1972. When oxygen is present and epinephrine is oxidized at an alkaline pH, superoxide radical anions (O2·) are produced indirectly. Then, spectrophotometric measurements were made at 485 nm to determine the pink-colored oxidation product of epinephrine, known as adrenochrome. (Hara et al, 1972)

Estimation of reduced glutathione

The technique reported by Jollow et al. in 1974 was used to measure reduced glutathione levels. First, 1.0 mL of 10% v/v diluted post-mitochondrial supernatant was combined with a corresponding volume of 4% w/v sulfosalicylic "acid, and the mixture was allowed to incubate for at least one hour at 4°C. The proteins were then precipitated by centrifuging the mixture at 81g for 15 minutes at 4°C.

The test was carried out by adding 0.2mL of 5,5-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, 0.1mM, pH-8.0) and 0.1mL of the supernatant to a total volume of 3.0mL. The phosphate buffer was added at a concentration of 0.1M", pH 7.4. Immediately after, a spectrophotometer was used to measure the yellow color that had emerged at "412nm. Using a molar extinction value of 1.36 × 10^4 M^-1 cm^-1, glutathione (GSH) concentrations had been calculated and expressed in micromoles per milligram of protein.

Estimation of catalase activity

Catalase activity had" been determined following the approach outlined by Saint-Denis et al, 1988. The reaction mixture for this experiment "included 1.0mL of hydrogen peroxide (0.019M), 3.0 mL of total volume, 1.95mL of phosphate buffer (0.05M, pH-7.0), and 0.05mL of post-mitochondrial supernatant (diluted 10% v/v). Using a spectrophotometer set at 240 nm, changes in absorbance were observed.

The extinction coefficient of hydrogen peroxide (43.6 M^-1 cm^-1) had" been employed to evaluate the catalase activity. The results were revealed as micromoles of hydrogen peroxide broken down in a minute per milligram of protein.

Statistical "analysis

The Mean ± S.E.M. is used to present all results. A one-way analysis of variance (ANOVA) was performed on the data using SPSS software 2.0, with a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Acute Toxicity Studies

At a dose of 2000mg/kg, a methanolic extract of *Evolvulus alsinoides* in Swiss albino rats did not trigger any physiological alterations or mortality.

Antidepression Activities

Under stressful conditions, the hypothalamic-pituitary-adrenal (HPA) axis increases blood levels of glucocorticoids, such as cortisol in primates and corticosterone in rodents. Depression has been connected to increased blood glucocorticoid levels resulting from over-activation of the HPA axis (Pan et al, 2006). Neurogenesis, excitability, neuronal survival, and memory formation are all impacted by cortisol. By affecting these brain activities, high cortisol levels may exacerbate depressive symptoms (Sousa et al, 2008).

Behavioural Tests

Table 1: Effect of Methanolic Extract of *Evolvulus alsinoides* on mice behaviors in TST

Group No.	Drug and dose	Tail Suspension test Duration of Immobility (Sec)		
		30 days	60 days	90 days
1	Control	185 ± 6.12	174 ± 5.8	182 ± 5.16
2	Imipramine 30mg/kg	93.4 ± 3.71***	63.9 ± 3.65***	46 ± 2.98***
3	Evolvuls alsinoides 100 mg/kg p.o	151.6 ± 3.24*	128.5 ± 4.7*	106.3 ± 3.2**
4	Evolvuls alsinoides 200 mg/kg p.o	135.2 ± 2.43*	98.2 ± 3.5***	89.8 ± 3.59***
5	Evolvuls alsinoides 400 mg/kg p.o	94.6 ± 3.5**	67.9 ± 2.48***	47.6 ± 1.3***

Each group has n=4, with the mean ± SEM. Group I and Group II were contrasted. Group II was compared with the remaining groups. There were significant changes to the mean values *** = P< 0.001 highly significant; ** = P< 0.01 moderately significant; * = P< 0.05 significant

Table 2: Effect of Methanolic Extract of *Evolvulus alsinoides* on mice behaviors in Forced Swim Test

Group No.	Drug and dose	Duration of Immobility (Sec)		
		30 days	60 days	90 days
1	Control	171.20 ± 3.21	173.41 ± 3.53	172.87 ± 2.91
2	Imipramine 30mg/kg	32.10 ± 0.75***	28.45 ± 0.72***	27.52 ± 0.65***
3	Evolvuls alsinoides 100 mg/kg p.o	165.20 ± 3.01	120 ± 1.25	75.8 ± 3.5*
4	Evolvuls alsinoides 200 mg/kg p.o	132.40 ± 1.72	86.3 ± 2.30*	57.2 ± 3.2***
5	Evolvuls alsinoides 400 mg/kg p.o	94.3 ± 2.30*	47.20 ± 1.31**	32.8 ± 4.1***

The values in each group (n = 4) are mean ± SEM; Group I was compared to Group II. The remaining groups were compared using Group II. There were significant "changes to the mean values *** = P< 0.001 highly significant; ** = P< 0.01 moderately significant; * = P< 0.05 significant

Table 3: Effect of Methanolic Extract of *Evolvulus alsinoides* on mice behaviors in Locomotor Activity

Group No.	Drug and dose	Locomotor Activity in Minutes			
		Before Treatment	30 days	60 days	90 days
1	Control	51 ± 0.12	51 ± 0.12	53 ± 0.23	53 ± 0.35
2	Imipramine 30mg/kg	54 ± 0.20	98 ± 0.39***	114 ± 0.45***	121 ± 0.28***
3	Evolvuls alsinoides 100 mg/kg p.o	52 ± 0.22	60 ± 0.21	84 ± 0.29	115 ± 0.30**
4	Evolvuls alsinoides 200 mg/kg p.o	51 ± 0.17	72 ± 0.34	103 ± 0.28**	126 ± 0.18***

5	**Evolvuls alsinoides 400 mg/kg p.o**	53 ± 0.28	96 ± 0.17*	116 ± 0.37**	132 ± 0.27***

Each group has n=4, with the mean ± SEM. Every group was contrasted with group I. There were significant "changes to the mean values *** = P< 0.001 highly significant; ** = P< 0.01 moderately significant; * = P< 0.05" significant

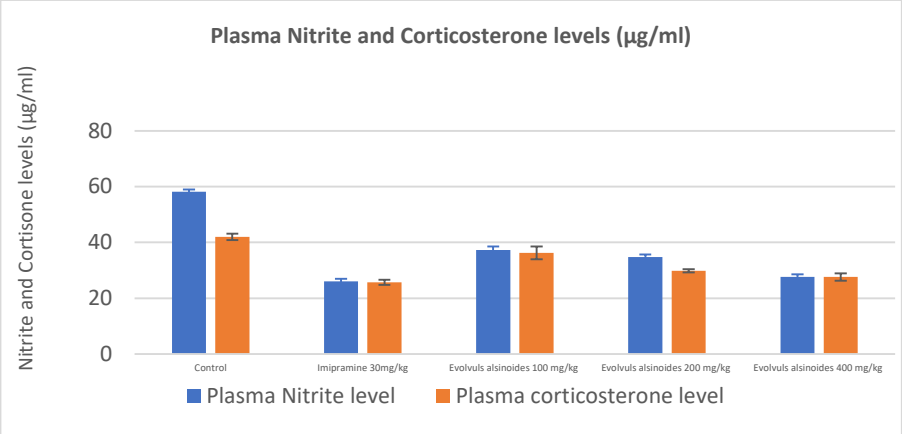

Fig 1: Effect on the levels of Plasma Nitritre and Cortisterone in mice by oral administration of *Evolvulus alsinoides* methanol extract

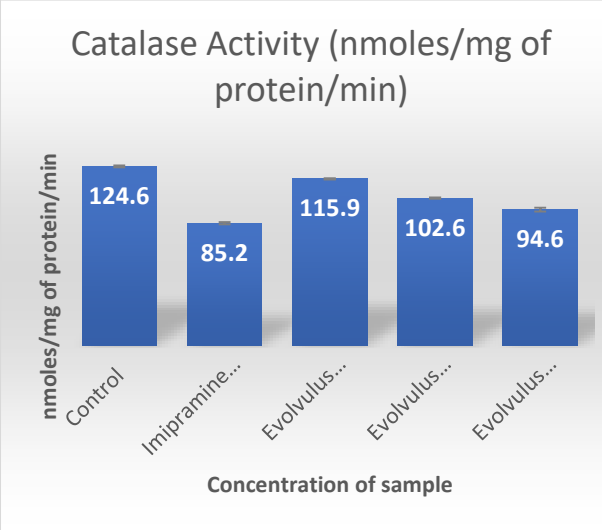

Fig 2: Effect on the MDA acitvity in mice by oral administration of *Evolvulus alsinoides* methanol extract

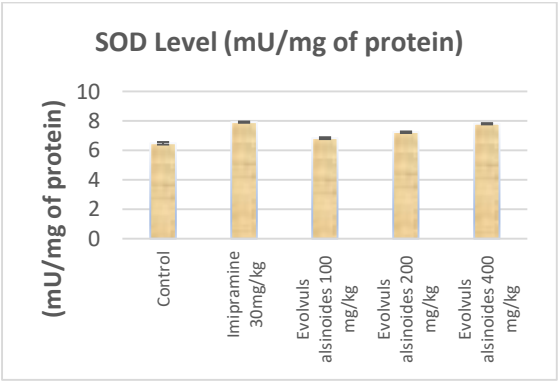

Fig 3: Effect on the levels of SOD in mice by oral administration of *Evolvulus alsinoides* methanol extract

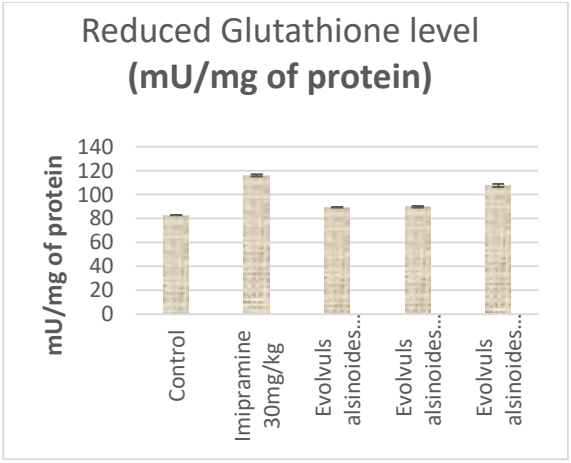

Fig 4: Effect on the levels of reduced glutathione in mice by oral administration of *Evolvulus alsinoides* methanol extract

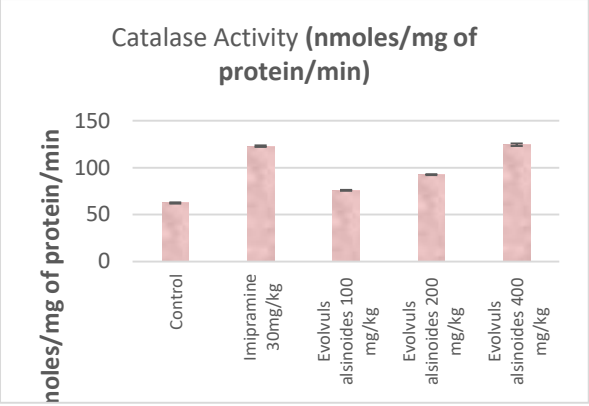

Fig 5: Effect on catalase activity in mice by oral administration of *Evolvulus alsinoides* methanol extract

In this study, the methanol extract of the *Evolvulus alsinoides* plant was administered daily for 90 days, revealing substantial antidepressant-like effects in Swiss albino rats. Acute Restraint Stress (ARS) is widely accepted as the most reliable animal model for studying depressive behaviors akin to those observed in humans (Willner, 2005). Tests like the FST, TST, as well as locomotor activity, were used to see whether the *Evolvulus alsinoides* extract altered the rats' depressive-like behavior.

When compared to control rats, ARS enhanced the total number of immobility phases in both TST and FST, revealing that depression-like behavior was effectively induced. Stressed rats' immobility times were considerably lowered in both TST and FST when they received oral treatments with imipramine (30 mg/kg) and various concentrations of methanol extract (100, 200, and 400 mg/kg). These treatments also showed considerable antidepressant-like effects. Rats' locomotor activity was also significantly boosted by imipramine and the methanol extract, indicating that the drugs had stronger central nervous system stimulant effects.

In this study, administering the methanol extract of *Evolvulus alsinoides* to rats exposed to various stressors for 90 days led to increased levels of malondialdehyde in the brain and nitrite in the plasma. Simultaneously, there was a decline in brain concentrations of reduced glutathione and catalase activity, along with plasma corticosterone. These outcomes are consistent with prior investigations where acute restraint stress (ARS) had deleterious effects on antioxidant defenses in the brain, leading to a reduction in glutathione levels and catalase activity, as well as a rise in lipid peroxidation and nitrite levels (Dhingra and Bansal, 2015).

CONCLUSION

In summary, the methanol extract of *Evolvulus alsinoides* demonstrated significant antidepressant-like effects in Swiss albino rats, potentially by lowering plasma corticosterone levels, and increasing nitrite levels. It may be able to boost the overall response to antidepressant treatment through the integration of several drugs that have similar but diverse effects. Furthermore, this strategy might enable a more potent antidepressant effect without requiring larger amounts of a specific medication, which could result in more undesirable side effects. (Szopa et al, 2023) These results suggest that further exploration of *Evolvulus alsinoides* extract for treating depression in humans.

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