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PHARMACOLOGICAL EVALUATION OF ANTI- DEPRESSANT ACTIVITY OF ETHANOLIC EXTRACT (FLOWER) OF CHRYSANTHEMUM INDICUM IN ALBINO MICE

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ABSTRACT:

AIM: The objective of this study is to assess the antidepressant properties of the ethanolic extract of the flower of *Chrysanthemum indicum* in albino mice.

METHODOLOGY: A control group was given distilled water, a standard group was given Imipramine hydrochloride (10 mg/kg) as the reference medication, and two test groups were given two different doses of the ethanolic extract of *Chrysanthemum indicum* (EECI) at 250 and 500 mg/kg over the course of a two-week treatment period. Every medication and test sample was administered orally by gavage. The mice were put through the tail suspension test (TST) and the despair swim test (DST) to assess the antidepressant effects of EECI.

RESULTS: According to the results of this study, EECI has antidepressant properties in both the DST and TST models of depression. Interestingly, EECI significantly decreased the despair swim test duration of immobility ($p < 0.001$). Likewise, in the tail suspension test, the extract dramatically reduced the immobility period ($p < 0.001$).

CONCLUSION: EECI has demonstrated antidepressant activity in the DST and TST. The floral extract of *Chrysanthemum indicum*, which has antidepressant-like properties, is one of the active ingredients in herbal medicine that this study helps identify.

KEYWORDS: *Chrysanthemum Indicum*, Imipramine hydrochloride, Antidepressant effect, Despair swim test, Test of the tail suspension

I. INTRODUCTION

Depression:

Depression is a mood disorder that causes persistent feelings of sadness, emptiness, and loss of joy. It is different from the mood fluctuations that people regularly experience as a part of life. Major life events, such as bereavement or the loss of a job, can trigger depression. But depression is distinct from the negative feelings a person may temporarily have in response to a difficult life event. Depression often persists in spite of a change of circumstances and causes feelings that are intense, chronic, and not proportional to a person's circumstances [1]

Causes of Depression:

There are several possible causes of depression. They can range from biological to circumstantial.

- **Brain chemistry.** There may be a chemical imbalance in parts of the brain that manage mood, thoughts, sleep, appetite, and behavior in people who have depression.[2]
- **Family history.** You're at a higher risk for developing depression if you have a family history of depression or another mood disorder.
- **Early childhood trauma.** Some events affect the way your body reacts to fear and stressful situations.
- **Brain structure.** There's a greater risk for depression if the frontal lobe of your brain is less active. However, scientists don't know if this happens before or after the onset of depressive symptoms.
- **Medical conditions.** Certain conditions may put you at higher risk, such as chronic illness, insomnia, chronic pain, Parkinson's disease, stroke, heart attack, and cancer.[3]

- **Substance use.** A history of substance or alcohol misuse can affect your risk.
- **Pain.** People who feel emotional or chronic physical pain for long periods of time are significantly more likely Trusted Source to develop depression.

Types of Depression:

- **Major Depressive:** An episode significant depressive disorder is a frequent and dangerous condition characterized by recurrent bouts of significant depression. 4.4% of the world's total disease burden is attributed to major depressive disorder. [4]
- **Dysthymia (Minor Depression):** For at least two years, a person with this milder, chronic mood disorder reports feeling down nearly every day. Dysthymia sufferers are susceptible to recurrent periods of major depression, but their symptoms are not as those of major depression. [5]
- **Bipolar Disorder:** Previously referred to as manic-depressive disorder, this condition is characterized by alternating spells of depression and mania or hypomania.
- **Cyclothymia:** Recurrent periods of hypomania and depression are hallmarks of this moderate type of bipolar disorder. [6]
- **Melancholic Depression:** It is typified by a loss of enjoyment in most or all activities, an inability to react to pleasurable stimuli, a depressed mood that is more noticeable than that of loss or grief, early morning awakening, psychomotor retardation, weight loss, or excessive guilt. [7]
- **Atypical Depression:** Increased hunger and weight gain, labile mood, leaden paralysis (a feeling of heaviness in the limbs), hypersomnia, and severe social impairment due to hypersensitivity to perceived rejection from others are all linked to it. [8]
- **Catatonic Depression:** Motor behavior abnormalities are among the signs of this uncommon and severe type of major depression. In this case, the person is silent, nearly unconscious, and either moves aimlessly or in an odd way.

Pathophysiology of depression:

Genetic Vulnerability, Environmental Stress

↓

Changes in Neurobiology

↓

Dysregulation of Neurotransmitters (↓ dopamine, serotonin, and norepinephrine)

↓

HPA Axis Hyperactivity → The cortisol level increased

Neuroinflammation] (↑ Cytokines such as TNF-α and IL-6)

↓

Impairment of Neuroplasticity] (BDNF, hippocampus atrophy) ↓

↓

[Changes in the Functional Brain]

(↓ Prefrontal cortex activity, ↓ amygdala activity)

↓

Depression Clinical Symptoms

(Depression, exhaustion, anhedonia, insomnia, guilt, and thoughts of suicide)

Figure No. Flow chart pathophysiology of depression:

Clinical signs and Symptoms:

- Weight gain or loss,
- physical discomfort,
- substance abuse,
- panic attacks,
- interpersonal issues,
- social isolation,
- suicidal thoughts,

- self-harm

Diagnosis and Test:

A diagnosis of depression may be made by your physician based on:

- **Physical exam:** In addition to performing a physical examination, your doctor could inquire about your health. Depression can occasionally be connected to a physical health issue.
- **Lab tests:** For instance, to ensure that your thyroid is operating correctly, your doctor could do a blood test known as a complete blood count. [9]
- **Psychiatric evaluation:** Your mental health provider will inquire about your symptoms, feelings, thoughts, and patterns of behavior. A questionnaire to assist in answering these questions can be sent to you. .
- **DSM-5:** The DSM-5, which is published by the American Psychiatric Association, has criteria for depression that your mental health provider may utilize. The 21st

II. TREATMENT AND MANAGEMENT**1. Medication:**

- Selective Serotonin Reuptake Inhibitors (SSRIs)- Examples: Fluoxetine (Prozac), Sertraline (Zoloft), Escitalopram (Lexapro)
- Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs)- Examples: Venlafaxine (Effexor), Duloxetine (Cymbalta), Desvenlafaxine (Pristiq)
- Tricyclic Antidepressants (TCAs) - Examples: Amitriptyline (Elavil), Nortriptyline (Pamelor), Fluvoxetine (Tofranil)
- Monoamine Oxidase Inhibitors (MAOIs) - Examples: Phenelzine (Nardil), Tranylcypromine (Parnate), Isocarboxazid (Marplan)

Atypical antidepressant - Examples: Bupropion (Wellbutrin), Mirtazapine (Remeron), Trazodone (Desyrel) [10]

2. Psychotherapy

- **Cognitive behavioral therapy (CBT):** In cognitive behavioral therapy (CBT), a therapist will work with you to uncover unhealthy patterns of thought and identify how they may be causing harmful behaviors, reactions, and beliefs about yourself.[11]
- **Dialectical behavior therapy (DBT):** Dialectical behavior therapy (DBT) is similar to CBT, but puts a specific emphasis on validation, or accepting uncomfortable thoughts, feelings, and behaviors, instead of fighting them.
- **Electroconvulsive therapy (ECT):** Electroconvulsive therapy (ECT) uses electrical currents to induce a seizure, and has been shown to help people with clinical depression. It's used in people with severe depression or depression that is resistant to other treatments or antidepressant medications.[12]

3. Alternative therapies

- **Meditation.** Stress, anxiety, and anger are triggers of depression, but meditation can help change the way your brain responds to these emotions. Studies Trusted Source show that meditation practices can help improve symptoms of depression and lower your chances of a depression relapse.
- **Acupuncture.** Acupuncture is a form of traditional Chinese medicine that may help ease some symptoms of depression. During acupuncture, a practitioner uses needles to stimulate certain areas in the body in order to treat a range of conditions. Research Trusted Source suggests that acupuncture may help clinical treatments work better and may be as effective as counseling. [13]

4. Natural remedies and lifestyle tips

- **Exercise:**
- **Avoid alcohol and substance use**

5. Vitamins

- Vitamins are important to many bodily functions. Research suggests two vitamins are especially useful for easing symptoms of depression:
- **Vitamin B:** B-12 and B-6 are vital to brain health. When your vitamin B levels are low, your risk for developing depression may be Trusted Source higher.

Vitamin D: Sometimes called the sunshine vitamin, vitamin D is important for brain, heart, and bone health.

There may be Trusted Source a link between vitamin D deficiency and depression, but more research is needed.[14]

PLANT DESCRIPTION

Chrysanthemum indicum, a member of the Asteraceae family, is a common plant. Its blooms are tiny and yellow. The health advantages of *Chrysanthemum indicum* are well known around the world. Traditional medicine uses *C. indicum* to treat a variety of ailments, such as stomatitis, colitis, and pneumonia. Additionally, it can help cure symptoms of high blood pressure, whooping cough, vertigo, fever, and sores. *C. indicum* has been used as a culinary ingredient to cover up tastes and in mixed spices. In Korea, it has also been utilized since ancient times to make teas and alcoholic beverages with flowers.[15]



Figure No.1- *Chrysanthemum Indicum*. Flower Synonyms:

- *Matricaria indica*,
- *Dendrathera indicum*.

Taxonomical classification of *chrysanthemum indicum*:

Sr. No.	Scientific classification	
1	Kingdom:	Plantae
2	Clade:	Tracheophytes
3	Clade:	Angiosperms
4	Clade:	Eudicots
5	Clade:	Asterids
6	Order:	Asterales
7	Family:	Asteraceae
8	Subfamily:	Asteroideae
9	Tribe:	Anthemideae
10	Genus:	<i>Chrysanthemum</i> L
11	Species:	<i>Ch. Indicum</i> Linn

Table No.1- Taxonomical classification of *chrysanthemum indicum*:
Pharmacological activity of *Chrysanthemum Indicum*:

- **Anti-tumour Activity:** Sesquiterpenoid exhibits little cytotoxic effects on healthy cells. while acting as an anticancer agent. Sesquiterpenoid has a higher anti-tumor effect in this lactone. [16]
- **Anticancer Activity:** Although chrysanthemum indicum's anticancer 40 efficacy has drawn increased interest, particularly in relation to hepatocellular carcinoma, its exact anticancer mechanism is still unknown and requires further investigation. [17]
- **Anti-inflammatory Activity:** A herbal plant called Chrysanthemum indicum is used to treat a number of immune system-related illnesses. Numerous flavonoids found in Chrysanthemum indicum have antiinflammatory properties. [18]
- **Antiviral Activity:** Chrysanthemum indicum has been shown to have a number of chemicals with antiviral properties. Chrysanthemum in A, Chrysanthemum in B, Chrysanthemum in C, Chrysanthemum in D, and ten more sesquiterpenoids are examples.
- **Neuroprotective Effects:** To assess their neuroprotective effects against H_2O_2 , Chrysanthemum indicum compounds, such as derivatives of caffeoylquinic acid, flavanone glycoside, eriodictyol 7-O- β -D-rutinoside, eriodictyol, eriodictyol 7-O- β -D-glucopyranoside, eriodictyol 7-O- β -D-glucuronide, and hesperetin 7-O- β -D-glucuronide were tested in SH-SY5Y cells. [19]
- **Antidiabetic Effects:** Chrysanthemum indicum has demonstrated promise in blood sugar regulation. It may help control glucose metabolism and enhance insulin sensitivity, according to research, which makes it advantageous for those with diabetes or those at risk of getting the disease. [20]
- **Hepatoprotective Activity:** The concentration of Chrysanthemum indicum is 258.75 mg/ml. According to a different study, chrysanthemum methanolic extract has anti-hepatitis B virus (HBV) properties, which makes it useful for treating a range of liver conditions
- **Analgesic and Antipyretic Effects:** In traditional usage, Chrysanthemum indicum has shown modest analgesic (pain-relieving) and antipyretic (fever-reducing) properties. Its medicinal components, such as flavonoids and essential oil, have been linked to its capacity to lessen fever and discomfort. [21]

III. RESEARCH AND METHODOLOGY:

Collection and Authentication of The Plant:

Chrysanthemum Indicum L. flower plant was collected from R. K. Pharmacy College Hebal Garden Kashipur surai, sathioan, Azamgarh Uttar Pradesh. Yellow-petalled flowers were hand-picked. Following full bloom. The collected plant flower were authenticated from the Department of botany from B.H.U. by Prof. N.K. Dubey the plant scientist.(Voucher specimen no. Astera.2025/01) After the authentication only we made them for ready for extraction process.



Figure No2-Blooming Flower

Extraction:

Continuous hot percolation, or "Soxhlation," was used for extraction. Chrysanthemum Indicum dried and ground flowers were put in a Soxhlet apparatus thimble. Using ethanol (40–60 degrees Celsius) as a non-polar solvent initially, xhlation was carried out at 60 degrees Celsius. Ethanol was used to remove the dried plant material (marc). Soxhlation was carried out for each solvent until the siphon tube showed no more color. Colorless solvent 56 was taken from the siphon tube and evaporated for residue in order to verify that the plant marc had been drained, or that the extraction

process was complete. The absence of residue verified that the extraction process was finished. A rotating vacuum evaporator (Buchi type) was used to evaporate the obtained extracts at 40°C. The following formula was used to get the extractive value:

Experimental animals:

Swiss Albino mice, both male and female, were used in every experiment. The mice utilized in this investigation were young (6–8 weeks) and weighed between 18 and 24 g. The animals were kept in a natural light-dark cycle and given unrestricted access to food and water. Prior to the behavioral trial, the animals were acclimated to the laboratory environment for a minimum of five days. The trials lasted for 21 days. The Institutional Animal Ethics Committee (AIEC) accepted the experimental procedure (1384/PO/Re/S/10/CCSEA), and laboratory animals were cared for in accordance with CPCSEA, Ministry of Forest and Environment of India requirements (IAEC/RK/07/25).

Experimental design:

The animals were split up into four groups, with six mice in each group. Oral treatments were administered for ten days. On the first and tenth days, the immobility period was recorded.

Groups for Despair Swim Test: Group Treatment Dose, Route of administration

1 Group, Ist : Distilled Water 10 ml/kg p.o

2 Group IInd : Imipramine 10 mg/kg p.o.

3 Group IIIrd : Ethanolic Extract of Chrysanthemum indicum 250mg/kg p.o.

4 Group IVth : Ethanolic Extract of Chrysanthemum indicum 500mg/kg

Models for Antidepressant Activity:

Despair Swim Test: Porsolt introduced the Despair Swim investigate as a paradigm to investigate the antidepressant activity. Each mice was made to swim alone in a plastic cylinder that was 40 cm in height and 18 cm in diameter. It contained freshwater that was 15 cm high and kept at 25°C (\pm 30°C). When the mouse was originally put in the cylinder, it was quite active and would swim frantically in circles, try to scale the wall, or dive to the bottom. After two to three minutes, the action starts to slow down and is broken up by longer periods of floating or immobility. Five minutes were recorded during the immobility time. The mice were taken out and given five minutes to dry before being put back in their own cages.[22]

Tail Suspension Test: According to Steru et al. that "Tail Suspension Test" is a simple way to assess possible antidepressants. Adhesive tape was positioned around 01 cm from the tip of the mouse's tail to hang it on the edge of a shelf 58 cm above a tabletop. For five minutes, the immobility period was noted. When mice hang passively and remain entirely still, they are deemed immobile. [23]

IV. RESULTS AND DISCUSSION**Phytochemical Screening**

Phytochemical screening is a series of qualitative tests conducted on plant extracts to identify the presence or absence of various groups of phytochemical compounds. These tests help in the preliminary identification of bioactive compounds in plants and provide insights into their potential pharmacological or medicinal properties. Here are some common phytochemical screening tests performed.

Sr. No.	Phytochemical	Test	Color PPT	Results
1	Alkaloids	Dragendroff's reagent Mayer's reagent Wagner's test	Red White Reddish Brown	Present (+) Present Present
2	Flavonoids	Lead acetate test	Yellow	Present
3	Carbohydrates	molisch's reagent	Brown	Present
4	Tannins	Ferric chloride Test	Blue	Negative
5	Steroids	Liebermann-Burchard	Blue or green	Negative
6	Glycosides	Keller-Killiani Test (Borntrager's test) Legal's test):	Bluish green Red Deep Red	Present Present Present
7	Terpenoids	Salkowski test	Reddish Brown	Present

Table No.3- Chrysanthemum indicum flower extract were undergone for chemical test and results are shown in Antidepressant Activity of EECM by using despair swim test on albino mice:

Despair Swimming Test: Mice were premediated to be stagnant when they were sailing still. The session of traversing water was chronicled for 5-min.

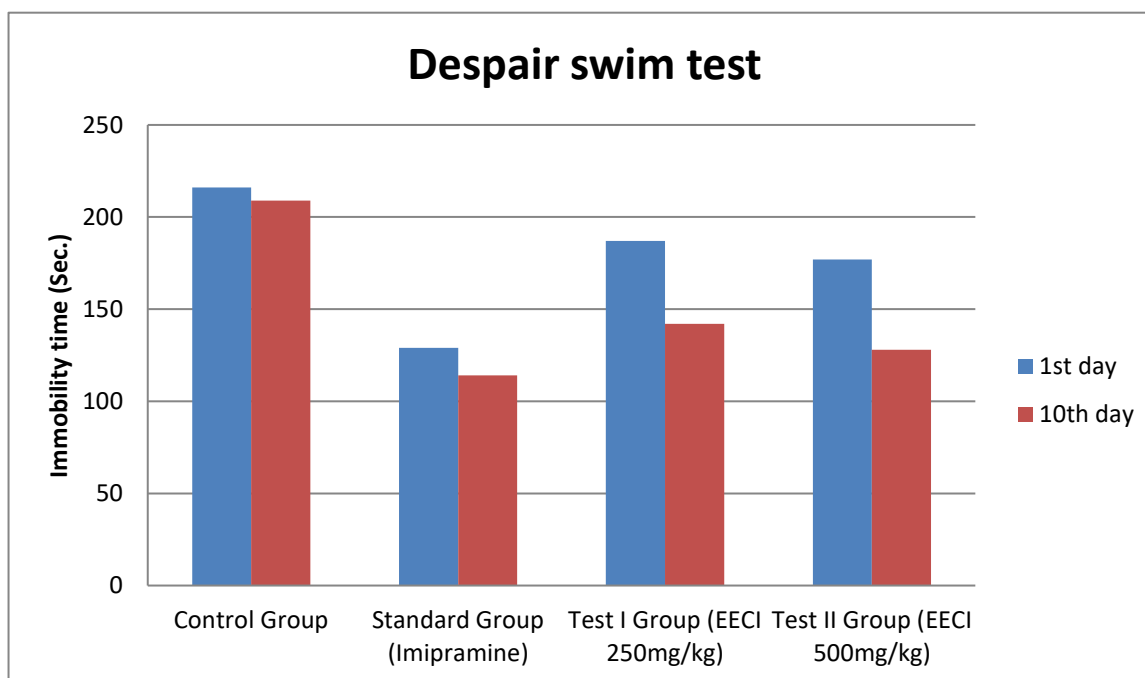
Evaluation of Despair Swim Test

Sr. No.	Drug Treatment	Immobility time (Sec.)	
		1st day	10 th day
1	Control - Distilled water (10ml/kg, p.o.)	216.8±6.635	209.0±6.197 (3.611%)
2	Standard - Imipramine (10mg/kg, p.o.)	129.5±3.819****	114.3±2.028**** (11.737%)
3	Test I - EECI (250mg/kg, p.o.)	187.7±6.606***	142.8±3.260**** (23.921%)
4	Test II - EECI (500mg/kg, p.o.)	177.5±3.222****	126.3±3.809**** (28.845%)

Table No. 4-Effect of Ethanolic Extract of Chrysanthemum indicum flower on DST in Albino Mice:

Dunnett's test (n = 6) is performed after an ANOVA. ****p[^]0.0001 in comparison to control, ***p <0.001 A Mean ± SEM was used to express the values. The one-group statistically examined the outcomes. The percentage decrease in immobility time is shown by the figures in the bracket.

Figure No.3- - Effect of Chrysanthemum indicum flowers extract on the immobility period in Despair swim Test



in swiss albino Mice.

In this experiment, animals given 250 mg/kg and 500 mg/kg respectively demonstrated a significant **** p<0.0001 reduction in immobility time (187.7±6.606, 177.5±3.222) on the first and tenth days as well as (142.8±3.260, 126.3±3.809) in comparison to the control group (216.8±6.635) on the first and tenth days (209.0±6.197). Similarly,

animals given 10 mg/kg of imipramine demonstrated a significant reduction in immobility time (129.5 ± 3.819 , 114.3 ± 2.028).

Antidepressant activity of ECCM by using tail suspension test on albino mice.

Tail Suspension Test: The tail suspension test was conducted in accordance with the previously described procedure.

Evaluation of Tail Suspension Test

Sr. No.	Drug Treatment	Immobility time (sec)	
		1 st Day	10 th Day
1	Control - Distilled water (10ml/kg, p.o)	228 ± 4.953	196.7 ± 3.556 (13.728%)
2	Standard- Imipramine (10mg/kg, P.o)	$114.8 \pm 3.525^{****}$	102.5 ± 1.688 (10.714%)
3	Test I -EECI (250mg/kg, p.o.)	$204.5 \pm 2.705^{***}$	173.7 ± 3.159 (15.061%)
4	Test II-EECI (500mg/kg, p.o.)	$192.3 \pm 3.964^{****}$	$142.3 \pm 2.140^*$ (24.960%)

Table No. 5- Effect of Ethanolic Extract of Chrysanthemum indicum flower on Tail Suspension Test in albino Mice.

A Mean \pm SEM was used to express the values. Dunnett's test ($n = 6$) and a one-way ANOVA were used for statistical analysis of the data. $***p < 0.001$, $****p < 0.0001$ in contrast to the control subject. The percentage decrease in immobility time is shown by the figures in the bracket.

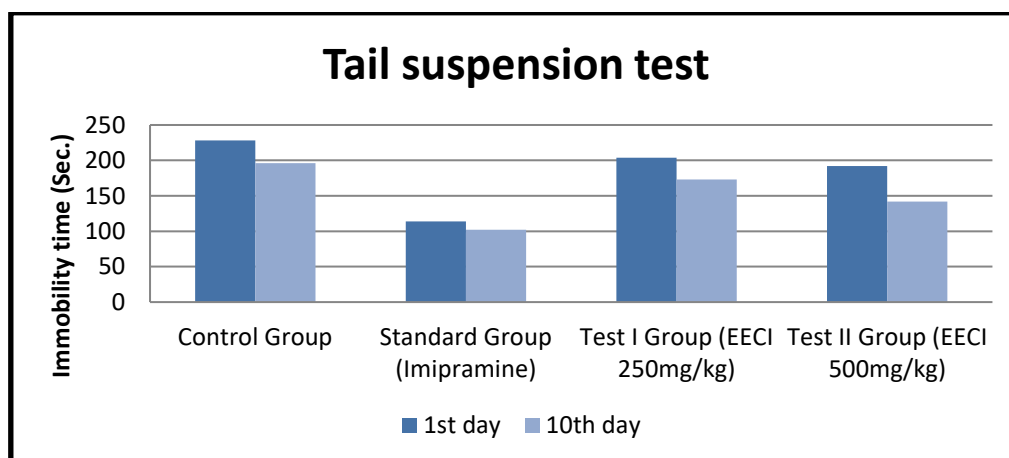


Figure No.4- 10 - Effect of Chrysanthemum indicum flowers extract on the immobility period in Tail Suspension Test in Albino Mice:

When compared to the control group (228 ± 4.953) on the first and tenth days (196.7 ± 3.556), the test animals treated with two doses of 250 mg/kg and 500 mg/kg demonstrated significant **** $p < 0.0001$ decreases in immobility time (204.5 ± 2.705 , 192.3 ± 3.964) on the first day and the tenth day as well as 173.7 ± 3.159 , 142.3 ± 2.140) respectively. Similarly, the animal treated with 10 mg/kg of imipramine showed a significant decrease in immobility time (114.8 ± 3.525 , 102.5 ± 1.688).

V. DISCUSSION

In today's fast-paced, stressful world, depression and mental health issues in general, as well as reasonable neurological illnesses in particular, are quite common. Up to 10–15% of people with this illness and up to 25% of people with bipolar disorder will act suicidally at some point in their lives. It has been observed that some phytochemical components, including alkaloids, saponins, flavonoids, phenols, and carbohydrates, have antidepressant properties. The most popular models for evaluating novel antidepressant effects are the Tail Suspension Test (TST) and the Despair Swim Test (DST). Both methods put rats at risk for behavioral despair, a condition similar to depression in humans. Both paradigms are often used behavioral models to evaluate the antidepressant and pharmacological effects of medications. When an animal is forced to swim in a confined space during the DST test, it first exhibits robust activity before displaying immobility posture and moving only in ways that maintain its head above the water (Porsolt et al., 1977). Both the 250 mg/kg (142.8 ± 3.260) and 500 mg/kg (126.3 ± 3.809) groups showed a decrease in immobility time, although the 500 mg/kg group had the greatest reduction.

In the DST model, the percentage drop in immobility for Test group II 500 mg/kg (28.84%) was similar to that of Test group I 250 mg/kg (23.92%). Monoamines, including serotonin, noradrenaline, and dopamine, are thought to be crucial in the development of depression. Since the 500 mg/kg ethanolic floral extract of *Chrysanthemum indicum* contains the chemical ingredient pyrethroids, the results of this experiment also estimated the level of biogenic amines and showed that the level of serotonin was considerably elevated in the treated group (test group II). In TST, immobility is a sign of hopelessness that can be lessened by a number of medications that are useful in treating depression in people. It is said that this causes animals to experience behavioral despair, which mimics the symptoms of depression in humans. *Chrysanthemum indica* treatment reduces the immobility period of mice subjected to TST, according to TST results. The results of this investigation showed that extracts at 250 mg/kg (173.7 ± 3.159) and 500 mg/kg (142.3 ± 2.140) had a strong antidepressant effect and were as effective as the conventional medication imipramine (102.5 ± 1.688). The TST model indicated a percentage decrease in immobility; Test group II, which received 500 mg/kg, had substantial results (24.96%) in comparison to Test group I, which received 250 mg/kg (15.06%). The 500 mg/kg group (24.96%) of the treatment group for the Tail Suspension test model *Chrysanthemum indicum* had the greatest reduction in immobility time (****p) comparable to the DST model (28.84%). Serotonin levels in the brains of the treatment and control groups were likewise elevated. Serotonin levels in the brain were higher in the test group than in the control group.

According to the initial phytochemical screening, the flower of *Chrysanthemum indicum* L. contains flavonoids, alkaloids, and glycosides, all of which have been demonstrated to have antidepressant properties. Flavonoids, polysaccharides, and alkaloids are found in plants that have antidepressant properties; hence, the antidepressant effect of EECI may be caused by the presence of one or more of these secondary metabolites.

VI. SUMMARY AND CONCLUSION

We summarized these points from study:

- *Chrysanthemum indicum* L. flower extract is a semi-solid extract with a bright yellow color. It has a slightly spicy odor and a sweet & floral taste, providing a unique sensory experience. These physical characteristics make chrysanthemum flower extract visually appealing and offer a pleasant sensory experience when consumed or used in various applications.
- In conclusion, the results of the chemical testing show that the floral extract from *Chrysanthemum indicum* did not include any tannins or steroids. It did, however, test for alkaloids, flavonoids, glycosides, and terpenoids. Furthermore, the Ferric
- mice treated with low and high dosages of *Chrysanthemum Indicum* L. flower extract showed a reduction in immobility time during the Despair Swim Test. This suggests that the extract may have antidepressant-like effects, as

the low dosage reduced immobility time more than the large dose did. The immobility durations were still longer than those of the typical imipramine-treated group, though.

- Mice treated with low and high dosages of *Chrysanthemum Indicum* L. flower extract showed different impacts on struggle time in the Despair Swim Test. The struggle time rose with the low dosage therapy, which would suggest a reduction in depressed behavior. In contrast to the low dosage, the high dose therapy shown a smaller increase in struggle time. Imipramine's effectiveness as an antidepressant was demonstrated in this test by the fact that the standard group receiving treatment showed the longest struggle time.
- The results of this study point to *Chrysanthemum indicum*'s antidepressant properties in both DST and TST models of depression. In both DST and TST, *Chrysanthemum Indicum* L dramatically shortened the immobility duration.
- To sum up, we may state that *Chrysanthemum indicum* L. flower ethanolic extract has the potential to be an antidepressant, but further research is necessary to confirm its antidepressant effects.

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