IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

ANTIDEPRESSANT ACTIVITY OF FLOWER EXTRACT OF *CHRYSANTHEMUM INDICUM* L IN EXPERIMENTAL ANIMAL.

Avani K. Shewale*, N. S. Naikwade, P. L. Ladda, Sudhir Patil

Appasaheb Birnale College of Pharmacy, Sangli. Dis- Sangli, Maharashtra, India 416 416

ABSTRACT

Depression is a common symptom in today's scenario. The Ethanolic extract of *Chrysanthemum indicum* Linn (EECI) contains flavanoids compound which has shown therapeutic potential in neurological diseases. The study was undertaken to evaluate the antidepressant activity of EECI using the Despair Swim Test (DST) & Tail Suspension Test (TST). The Swiss Albino mice weighing about 20-25 gm were used. The animals were divided into 4 groups, each group comprising of 6 animals,(n=6). Group I was controlling received Distilled Water (10ml/kg per oral), Group II Standard Imipramine HCl (10gm/kg per oral) and Group III & IV Test group, receives EECI (250& 500 mg/kg per oral respectively). All drugs were administered for 10 days. The results were analyzed using one way ANOVA followed by Dunnett test, $p<0.05$ was considered as significant. The effect of EECI on immobility periods of mice was assessed in DST & TST. The effect of EECI was compared with that of control. The effect of 500mg/kg showed a significant reduction in immobility time of mice in both DST & TST. The present study suggests that possible antidepressant activity of EECI in mice on the 500mg/kg drug administration is more than 250mg/kg.

Keywords*:* - *Chrysanthemum indicum*Linn, Imipramine HCl, Despair Swim Test, Tail Suspension Test.

INTRODUCTION

Depression is a common, chronic, recurring disorder with some property like low cognitive & emotional reaction that imposes high expanses to patients & remedial systems [1]. Depression is a common disorder with a prevalence of about 15% during lifecycle and today it is considered as the main reason for disability around the world & is in 4th rank among 10 main reasons for world load disease [1]. Depression affects not only patients but also their friends & families. Social withdrawal, lack of motivation, sexual dysfunction, sleep disorder (in 75% of the patient), depressed mood are the main symptoms of depression. [2]

Depression is a significant contributor to the global burden of disease and affects people in all communities across the world. There are several types of depression. A person with major depression experiences symptoms of depression that last for more than two weeks. A person with dysthymia experiences episodes of depression that alternate with periods of feeling normal. A person with bipolar disorder, manic-depressive illness, experiences recurrent episodes of depression and extreme elation. A person with Seasonal Affective Disorder (SAD) experiences depression during the winter months when day length is short. Although the exact cause of depression is unknown, research suggests that depression is linked to an imbalance of the neurotransmitters serotonin, norepinephrine, and dopamine in the brain. [3] Factors that may contribute to depression include heredity, stress, chronic illnesses, certain personality traits (such as low self-esteem), and hormonal changes.[4]

Today depression is estimated to affect 350 million people. The World Mental Health Survey was conducted in 17 countries found that on average about 1 in 20 people reported having episodes of depression in the previous year. Depressive disorders often start at a young age; they reduce people's functioning and often are recurring. For these reasons, depression is considered the leading cause of disability worldwide in terms of total years lost due to disability. The demand for curbing depression and other mental health conditions is on the rise globally. A recent World Health Assembly called on the World Health Organization and its member states to take action in this direction. [5]

Recently, internal stressors such as variation in serum levels of cholesterol, triglycerides, sugar, coagulation factors have been reported to be involved in the development of depression [2]. Currently, certain drugs including Tricyclic antidepressant, serotonin and noradrenaline reuptake inhibitor, & *Monoamine oxidase* inhibitors used to treat depression [2].

Depression symptoms & signs are fully treated in only 1/3rd of people & recurrence risk persists. Besides that, they can cause several adverse reactions like sleepiness, dry mouth, constipation & impotence. [2]

Many plants have been a traditional claim in the treatment of several harmful diseases still they are the second leading cause of premature death or disability all over the world by the year 2020. Medicinal herbs are becoming popular day by day. Both developed and developing countries have a great demand for medicinal plants due to increased identification of natural products and it is sometimes the primary source of health care available to the poor. Since old time herbal sources are useful in the treatment of many diseases.[6]

As per the literature review, the most medicinal plants exerted antidepressant effect through the synaptic regulation of serotonin, noradrenaline, and dopamine, regulating the activity of the hypothalamic-pituitary-adrenal axis, reinforcing antioxidant defense system. Since the medicinal plants and their active compounds can relieve depression through different pathways and hence are considered a new source to produce antidepressant. [2]

Some of the herbs used by the folk as a traditional medicine in calming mind & elevating mood are Passionflower, Lavender, Ginkgo biloba and phytoconstituents like amino acid lysine, s-adenosyl methionine, B-vitamin, choline, gamma-aminobutyric acid (GABA), tryptophan and other action magnesium. [7]

Chrysanthemum Indicum L. is an aromatic, perennial plant producing a clump of stem 25100 cm tall from rhizome [8]. *Chrysanthemum indicum* L. belongs to family Asteraceae [9]. The plant contains lipids, phenols, terpenoids, and flavonoids as a phytoconstituents. [10]

Chrysanthemum indicum L.an ancient herb used in the traditional medicines showed various biological effects which are documented in various studies. They are reported to have anti-arthritic, anti-inflammatory activity. The plant extract was also used as an analgesic, antipyretic, respiratory disorder, deterioration of bone and muscles [7]. It was also proved to be effective to inhibit the agglutination of blood platelet and promote the myocardial blood circulation and white cell phagocytosis, and therefore it was used to treat many diseases such as furuncle[11]. Furthermore, tea of *Chrysanthemum indicum* Linn has been used to treat anxiety by facilitating relaxation and curing insomnia. Recently, some studies have suggested that *Chrysanthemum Indicum* has anti-apoptotic effects in vitro and in vivo.

Review of the plant –

Plant Profile:

***Chrysanthemum indicum* L: [10,11]**

Figure No. 1- *Chrysanthemum Indicum*. flower

Chrysanthemum is a cosmopolitan genus, comprising about 300 species of herbs and undershrub, among which a few yields the commercial insecticide known as Pyrethrum. Several species of Chrysanthemum are ornamental and grown in gardens for their large, showy, multicolored flowers. In India, it is cultivated on a large scale only in Kashmir, though successful trials of cultivation have been reported at Kullu, Palampur, Mayurbhanj, Kumaun, Assam, Karnataka, Kerala, and Kodaikanal1. Its flowers yield an important insecticide, i.e. the pyrethrins. The aerial parts of the plant used are Stem, flower and leave specifically the tea prepared from the flowers of *Chrysanthemum indicum* is been used in Korea to relieve anxiety and to enhance the mood, antioxidant and DNA damage preventive activity was found in the flower extract.

Chrysanthemum indicum Linn is considered to be a native of China and Japan and is extensively cultivated in the Indian gardens for its ornamental multi-colored flowers. The flower has a sharp bitter taste and is said to be stomachic and aperient. In Indo-China, the leaves are used as depur ant and prescribed in *1 migraine. A survey of the literature on Chrysanthemum species shows that these plants are the valuable sources of various polyacetylp α k -lenic compounds, a sesquiterpene lactone, sesquiterpene ketone, 7 g Q monoterpenes, camphene alcohol, and flavonoid compounds. Chronicle investigation on the flowers of this plant *Chrysanthemum indicum* by the earlier workers revealed that this is a rich source of carotenoids.

Taxonomy of plant:

Table No. 1: -Taxonomy of flower *Chrysanthemum indicum* L

Kingdom	Planta
Clade	Angiosperms
Order	Asteroids
Family	Asteraceae
Genus	Chrysanthemum
Species	*C.indicum*
Binomial Name	*Chrysanthemum indicum* L.

Chemistry Flower: Glycoside, chrysanthemin, on hydrolysis glucose & cyanidin; stachydrine, oil, and vitamin A.

Edible Uses: The flower heads are pickled in vinegar. Young leaves - cooked. An aromatic tea is made from the leaves, Seed.

Medicinal Uses: The whole plant is antiphlogistic, blood tonic, depurative, febrifuge, and vulnerary. The plant is used in China to treat eye ailments. In conjunction with black pepper, it is used in the treatment of gonorrhea. The leaves are depurative. They are used in China in the treatment of migraine. The flowers are aperient, bitter, hypotensive, stomachic and vasodilator. They contain the glycoside chrysanthemin that yields glucose and cyanidin on hydrolysis, together with stachydrine and essential oil. They have an antibacterial action, inhibiting the growth of *Staphylococcus, E. coli, streptococcus, C. diphtheriae, Bacillus dysenteriae*.

The flowers are used in the treatment of furuncle, scrofula, deep-rooted boils, inflammation of the throat, eyes, and cervix, eczema, itchiness of the skin and hypertension. They have a rejuvenating effect when used over a long period. An essential oil obtained from the plant contains chrysanthenone; this is active on the brain center affected by Parkinson's disease.

The plant is used in China to treat eye ailments. In conjunction with black pepper, it is used in the treatment of gonorrhea. The leaves are depurative. They are used in China in the treatment of migraine. The flowers are used in the treatment of furuncle, scrofula, deep-

rooted boils, inflammation of the throat, eyes, and cervix, eczema, itchiness of the skin and hypertension. They have a rejuvenating effect when used over a long period.

MATERIALS AND METHODS

I. Procurement, Authentication, Drying and Extraction of plant:

Plant (*Chrysanthemum indicum* L.) was collected in August 2018 from the Satara region (Dist - Satara) Maharashtra (India). The Plant (*Chrysanthemum indicum* L.) material was authenticated by Prof.M.D.Wadmare Sir Department Of Botany, Smt. Kasturbai Walchand College Sangli.

II. Preparation of plant extract:

The powdered flower of the plant (*Chrysanthemum indicum* L.) was exhaustively extracted with 90 to 95% ethanol in a Soxhlet's apparatus. • The ethanolic extract was obtained, collected and concentrated at room temperature. Then the extracts were stored until use for further study. • Average % yield of the ethanolic extract of *Chrysanthemum indicum* L. was found to be 32.1%.

III. Experimental animals:

All experiments were carried out using male and female, Swiss Albino mice. Young (6-8 weeks) mice weighing around 18-24 gm used in the present study. The animal had free access to food and water and they were housed in a natural light-dark cycle. Animals were acclimatized for at least 5 days to the laboratory condition before the behavioral experiment. Experiments were carried out between 900h and 1600h. The experimental protocol was approved by the Institutional Animal Ethics Committee (AIEC) IAEC/ABCP/02/2018-19 and care of laboratory animals were taken as per guidelines of CPCSEA, Ministry of Forest and Environment of India.

Experimental design:

The animals were divided into 04 groups and each group contains 06 mice. Treatments were given for 10 days by the oral route. The immobility period was noted on the 1st and 10th day.

Groups for Forced Swim Test:

Group Treatment Dose, Route of administration

Group, I Distilled Water 10 ml/kg p.o. 2

Group II Imipramine 10 mg/kg p.o. 3

Group III- Ethanolic Extract of *Chrysanthemum indicum* 250mg/kg p.o.

Group IV- Ethanolic Extract of *Chrysanthemum indicum* 500mg/kg p.o.

Evaluation of Antidepressant Activity:

Despair Swim Test [31, 32]

Despair Swim Test was proposed as a model to test antidepressant activity by Porsolt in 1977. The mouse was individually forced to swim in a plastic cylinder having dimensions (height: 40 cm; diameter: 18 cm) containing freshwater of 15 cm height and maintained at 250c (± 30c). The mouse was placed in the cylinder for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min. the activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. The immobility period was recorded for 05 min. After 05 min. the mouse was removed and allowed to dry before being returned to their home cages.

Tail Suspension Test [31, 33]

The "Tail Suspension Test" has been described by Steru et al. (1985) as a facile means of evaluating potential antidepressants. The mouse was suspended on the edge of a shelf 58 cm above a tabletop by adhesive tape placed approximately 01 cm from the tip of the tail. The duration of immobility was recorded for 05 min. Mice will be considered immobile when they hung passively and completely motionless.

Estimation of Serotonin [39]

Preparation of Serotonin Sample -

On the last day of the experiment, mice were sacrificed, the Whole-brain was dissected out and the subcortical region (including the stratum) was separated. Weight tissue was weight and homogenized in 5 ml HCL – Butanol solution for about 01 min. The sample was then centrifuged for 10 min. at 2000 rpm. An aliquot supernatant phase (01ml) was removed and added to a centrifuge tube containing 2.5 ml heptane and 0.31 ml HCL of 0.1M. After 10 min. the content in the centrifuge tube was centrifuged under the same condition as above to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2ml) was then taken for the estimation of Serotonin.

Standard Serotonin preparation –

Serotonin standards were prepared in distilled water and HCl Butanol (1:2). Stock solution of serotonin (500µg/ml) solution was prepared by dissolving 5mg of serotonin in 10ml of Distilled water and HCl Butanol (1:2). From this stock solution, a 1ml standard sample was diluted to 1000 ml of distilled water & HCl Butanol (0.5 µg/ml) was prepared for further process. The working standard of concentration ranging from 0.05-0.5 µg/ml was prepared by transferring appropriate volume to 10 ml volumetric flask and volume was made with DW & HCl Butanol. The absorbance is read against blank at 360-470 nm in Photoflurimeter.

Estimation of Serotonin –

To 0.2 ml aqueous extract 0.25 ml of O-Pthaldehyde reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at for serotonin 360-470 nm in the spectrofluorimeter.

Statistical Analysis and documentation of Results:-

The values are expressed as Mean ± SEM for six mice in each group. The Statistical Analysis was performed using one way ANOVA followed by Dunnett's test. (Graph pad prism version 7.04).p-value < 0.05 was taken as statistically significant.

RESULT

Sr No	Test	Observation	Inference
1	**Test for Carbohydrates** – a.Benedict's test	 +	 Present
2	**Test for Alkaloids** – a. Dragendroff's test b. Hager's test c. Wagner's test	 + + +	 Present Present Present
3	**Test for Protein –** a.Millon's test	 +	 Present
4	**Test for Flavonoids** a. Shinoda test b. Sulfuric acid test c. Lead acetate test	 + + +	 Present Present Present
5	**Test for Glycosides –** a.Killer killani test b. Legal's test c. Baljet test and. d. Modified borntrager's Test	 + + + +	 Present Present Present Present
6	**Test for Tannins –** a. Lead acetate test b. Bromine water test c. Pot. Dichromate test d.FeCl3 test e. Acetic Acid solution	 + + + + +	 Present Present Present Present Present

Evaluation of Despair Swim Test

Table No. 2 -Effect of Ethanolic Extract of *Chrysanthemum indicum* L. flower on DST:

Sr. No.	Drug Treatment	Immobility time (sec.)	
		1st Day	10th Day
1	Control - Distilled water (10ml/kg, p.o.)	230.8±6.635	226.0±6.197 (2.079%)
2	Standard – Imipramine (10mg/kg, p.o.)	122.5±3.819****	112.3±2.028**** (8.326%)
3	Test I - EECI (250mg/kg, p.o.)	196.7±6.606***	137.8±3.260**** (29.944%)
4	Test II - EECI (500mg/kg, p.o.)	185.5±3.222****	119.3±3.809**** (35.687%)

ANOVA followed by Dunnett's test (n = 6). ***p<0.001, ****p < 0.0001 as compared to control Values were expressed in a Mean ± SEM. The results were analyzed statistically by the one - group. The values in the bracket indicate that the % reduction in Immobility time.

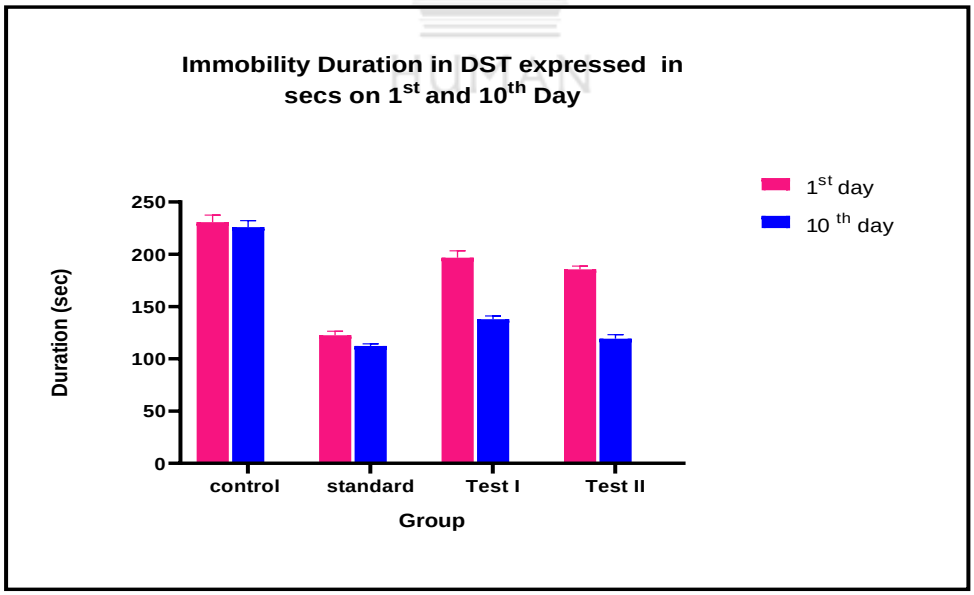

Figure No. 2- Effect of *Chrysanthemum indicum* flowers extract on the immobility period in Despair swim Test.

In this test animal treated with two doses of 250mg/kg and 500mg/kg showed significant **** p<0.0001 decrease in immobility time (196.7±6.606, 185.5±3.222) on 1st day and the

10th day also (137.8±3.260, 119.3±3.809) respectively when compared with control group (230.8±6.635) on 1st day and 10th day (226.0±6.197) similarly animal treated with Imipramine (10mg/kg) showed significant decrease in immobility time (122.5±3.819, 112.3±2.028).

Estimation of Serotonin in the DST model:

Table No. 3 - Level of Serotonin in Brain tissue homogenate in the DST model:

Sr. No.	Treatment	The concentration of serotonin in µg/gm of wt tissue
1	Control - Distilled water(10ml/kg, p.o)	87±0.3651
2	Standard- Imipramine(10mg/kg, P.o)	95.33±0.4216****
3	The test I - EECI (250mg/kg, p.o.)	92.50±0.5627****
4	Test II -EECI (500mg/kg, p.o.)	93.50±0.4282****

Values are expressed in a Mean ± SEM. Statistical analysis of data was carried out by one way ANOVA followed by Dunnett's test. ****p<0.0001as compared to control group.

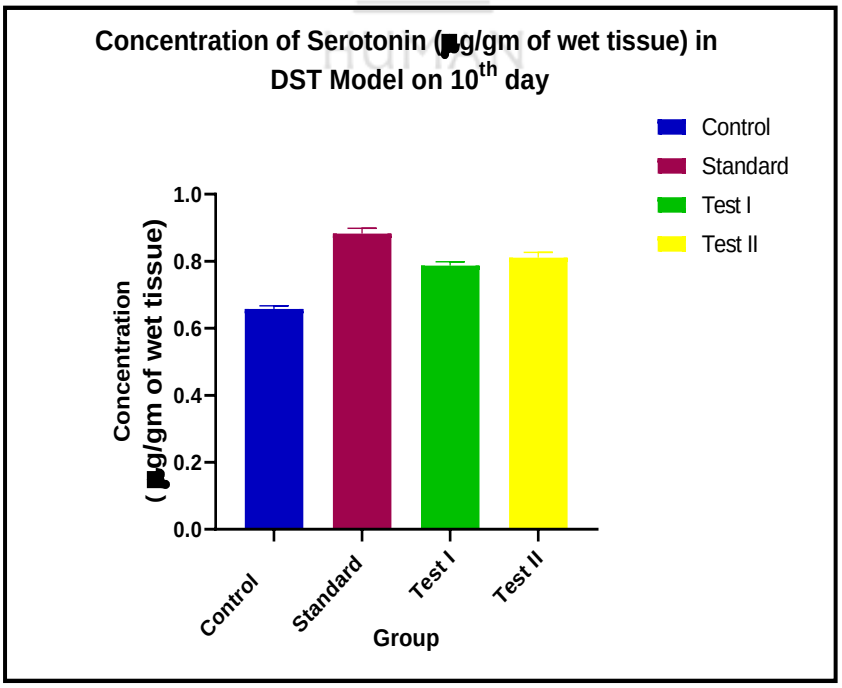

Figure No. 3 -Level of Serotonin from brain tissue homogenate in the DST model.

The neurotransmitter levels from brain tissue homogenates were also estimated. In this estimation animal treated with 2 doses of *Chrysanthemum indicum* 250 mg/kg and 500 mg/kg showed significant increase in the level of Serotonin ****p<0.0001 respectively when compared with the control group (87±0.3651) similarly animal treated with Imipramine (10mg/kg) showed a significant increase in level of Serotonin.

Evaluation of Tail Suspension Test

Table No. 4 -Effect of Ethanolic Extract of *Chrysanthemum indicum* L. flower on Tail Suspension Test:

Sr No	Drug Treatment	Immobility time (sec)	
		1st day	10th Day
1	Control - Distilled water (10ml/kg, p.o)	242±4.953	196.7±3.556 (18.71%)
2	Standard- Imipramine(10mg/kg, P.o)	124.8±3.525****	114.5±1.688**** (8.25%)
3	Test I - EECI (250mg/kg, p.o.)	216.5±2.705***	161.7±3.159**** (25.311%)
4	Test II - EECI (500mg/kg, p.o.)	209.3±3.964****	136.3±2.140**** (35.021%)

Values were expressed in a Mean ± SEM. The results were analyzed statistically by the one - way ANOVA followed by Dunnett's test (n = 6). ***p<0.001,****p< 0.0001 as compared to control group. The values in the bracket indicate that the % reduction in Immobility time.

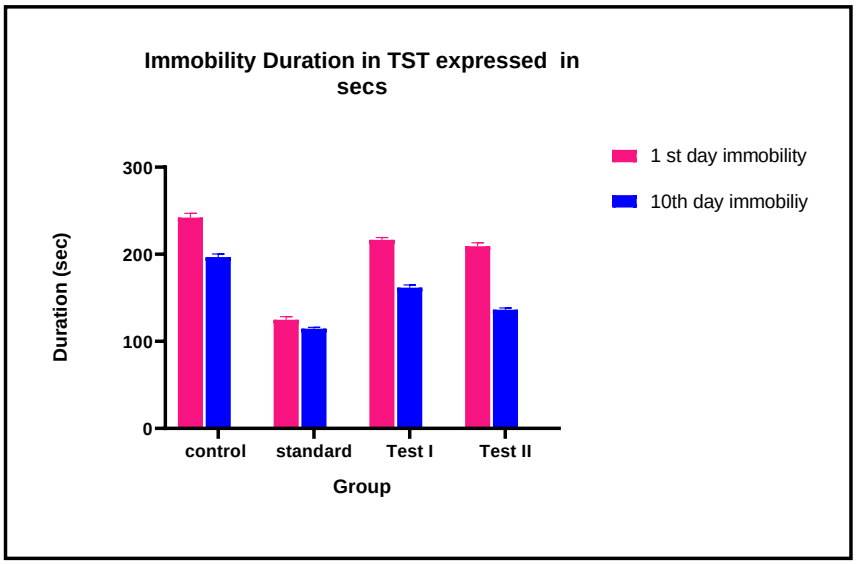

Figure No. 4 - Effect of *Chrysanthemum indicum* flowers extract on the immobility period in Tail Suspension Test.

In this test animal treated with two doses of 250mg/kg and 500mg/kg showed significant **** p<0.0001 decrease in immobility time (216.5±2.705, 209.3±3.964) on 1st day and the 10th day also (161.7±3.159, 136.3±2.140) respectively when compared with control group (242±4.953) on 1st day and 10th day (196.7±3.556) similarly animal treated with Imipramine (10mg/kg) showed significant decrease in immobility time (124.8±3.525, 114.5±1.688).

Estimation of Serotonin in TST model:

Table No. 5 - Level of Serotonin in Brain tissue homogenate in TST model:

Sr No	Drug Treatment	The concentration of serotonin in µg/gm of wt tissue
1	Control - Distilled water(10ml/kg, p.o)	88±0.3651
2	Standard- Imipramine(10mg/kg, P.o)	95.33±0.4216****
3	Test I - EECI (250mg/kg, p.o.)	92.50±0.4282****
4	Test I - EECI (500mg/kg, p.o.)	93.33±0.4944****

Values are expressed in a Mean ± SEM. Statistical analysis of data was carried out by one way ANOVA followed by Dunnett's test. ****p <0.0001 as compared to control group.

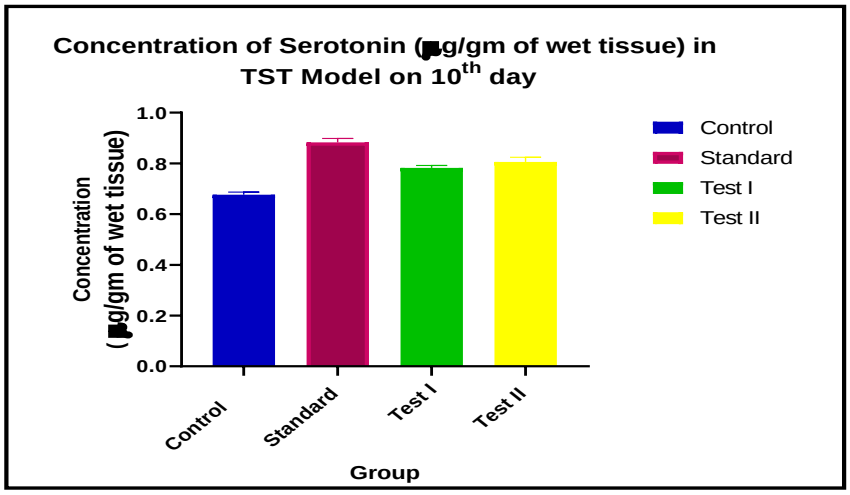

Figure No. 5 - Level of Serotonin from brain tissue homogenate in the TST model.

The neurotransmitter levels from brain tissue homogenates were also estimated. In this estimation animal treated with 2 doses of *Chrysanthemum indicum* 250 mg/kg and 500 mg/kg showed significant increase in the level of Serotonin ****p<0.0001 respectively when compared with the control group (88±0.3651) similarly animal treated with Imipramine (10mg/kg) showed a significant increase in level of Serotonin.

DISCUSSION

Depression and mental health problems in general and sensible neurological disorders, in particular, are widely prevalent in modern fast-paced life with a multitude of a stressful life. As many as10-15% of individuals with this disorder and up to 25% of those with bipolar disorder, display suicidal behavior during the lifetime. It is currently leading one of the causes of morbidity and mortality. Despite advances in treatment and frequently used antidepressants are associated with the increasing incidence of relapse adverse effects and drug interaction.

A variety of chemical and synthetic drugs are available to treat depression, but most patients fail to tolerate adverse effects due to drugs. Moreover, only 50% of patients experience a complete recovery.

Currently, studies are being increasingly conducted to detect new and economical drugs to treat depression with no adverse effect. Depression is a heterogeneous mood disorder

characterized by regular negative moods, feeling helplessness and is caused by decreased brain level of monoamines such as noradrenaline, dopamine, serotonin.

Since the initial hypothesis of depression has been formulated long ago and proposing that symptoms of depression due to functional deficiency of cerebral monoaminergic transmitters such as Noradrenaline, serotonin, and dopamine located at synapses. Imipramine hydrochloride acts by inhibiting Noradrenaline and Serotonin reuptake and has been used as a standard drug in majority studies. The beneficial effect of Imipramine hydrochloride in the forced swimming test model seems to be due to increased availability of Noradrenaline and serotonin at the postsynaptic site following reuptake inhibition.

Phytochemical components especially alkaloids, saponins, flavonoids, phenols, carbohydrates have been reported to have antidepressant activity. Despair Swim Test (DST) and Tail Suspension Test (TST) are the most commonly used models for the screening of the new antidepressant activity. Both models predispose rodents to the state of behavioral despair which is comparable to human depression. Both paradigms are widely accepted behavioral models for assessing pharmacological and antidepressant activity. Immobile behavior shows a lowered mood. The agents that decrease this behavior are presumed to have antidepressant effects. The same pattern of immobility was observed in the present study which can be correlated with findings of Porsolt et al. in both model DST and TST.

In DST test when animal forced to swim in a restricted area, initially has vigorous activity and then showed immobile posture & its movement were restricted to those movements that keep its head above water (Porsolt et al., 1977). The reduction in the immobility time was observed in the both group 250mg/kg (137.8±3.260) & 500mg/kg (119.3±3.809) but 500mg/kg group showed maximum decrease in immobility time (****$p<0.0001$).

The percentage decrease in the immobility of Test group II 500mg/kg (35.68%) was comparable with Test group I 250mg/kg (29.94%) in the DST model.

Based on the hypothesis of depression monoamine such as Serotonin, Noradrenaline and Dopamine play an important role in the development of depression. In this investigation, the level of biogenic amine are also estimated and result indicated that the level of Serotonin was increased significantly, in 500 mg/kg ethanolic flowers extract of *Chrysanthemum indicum* treated group (test group II) since extract contain chemical constituent pyrethroids,

sesquiterpenoids, flavonoids, coumarins, triterpenoids, steroids, phenolics, purines, lipids, aliphatic compounds, and monoterpenoids acts as precursor of the synthesis of the biogenic amine & might be responsible for antidepressant activity. In test II (500mg/kg) group (93.50±0.4282) showed a significant increase when compared to the whole control group. Standard drug Imipramine (95.33±0.4216) also showed an increase in the level of Serotonin.

In TST, immobility reflects a state of despair that can be reduced by several agents that are therapeutically effective in human depression. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression.

In TST result showed that the administration of *Chrysanthemum Indicum* produces a diminution of immobility time of mice exposed to TST. In the present study extracts 250mg/kg (161.7±3.159) and 500mg/kg (136.3±2.140) produce a significant antidepressant effect and their efficacies were found to be comparable to standard drug Imipramine (114.5±1.688).

The percentage decrease in immobility was observed in the TST model, the Test group II 500mg/kg showed significant results (35.02%) as compared with Test group I 250mg/kg (25.31%).

Among the treated group for the Tail Suspension test model *Chrysanthemum indicum*, 500mg/kg group (35.02%) showed a maximum decrease in immobility time (****$p<0.0001$) as similar to DST model (35.68%). Brains serotonin level was also increased in the treated and standard group. In the test group increase in serotonin levels in the brain as compared to the control group.

The preliminary phytochemical screening indicated that the presence of Flavonoids, Alkaloids, and Glycoside in flower of *Chrysanthemum indicum* L, have been shown to possess anti-depressant effect. Plants with an antidepressant activity that contains flavonoids, polysaccharide, alkaloids, saponins, and polyphenols include *Justicia gendarussa Burm*, *Morusme syzygial*, *Momordica malaria*, *Passiflora foetida*, and *Eclipta alba*. Therefore, the observed antidepressant effect observed with EECI could be due to the presence of one or more of these secondary metabolites.

CONCLUSION

The finding of the present investigation suggests the antidepressant activity of *Chrysanthemum indicum* in DST and TST models of depression. *Chrysanthemum Indicum* L significantly reduced the immobility period in both DST and TST.

Further, it is found that the group treated with 500mg/kg dose shows a more significant restoration in the immobility time and increased level of brain serotonin.

In conclusion, we can say that the Ethanolic extract of flowers of *Chrysanthemum indicum* L is having the potential to be considered as an antidepressant but at the same time more extensive is required to support antidepressant action.

However, the precise mechanism by which extract produced antidepressant-like effect is not completely understood. Further studies would be necessary to evaluate the contribution of the chemical constituents. These chemical constituents are active and they have observed antidepressant activity as it remains to be determined which components are responsible for these effects.

REFERENCES

1. Kopaie M R, M. Bahmani, K.Saki. The Effect of the most important medicinal plant of two important psychiatric disorders (anxiety &depression). Asian Pacific Journal of Tropical medicine. 2014; 7(1):S34-S42.
2. Rabiei Z, Rabiei S. A review on the antidepressant effect of medicinal plants. Bangladesh Journal of Pharmacology. 2017 Mar 1;12(1):1-1.
3. World Health Organization. The World Health Report 2001: Mental health: new understanding, new hope. World Health Organization; 2001. https://www.who.int/whr/2001/en/ Accessed on – 10-2-2019.
4. Ashwani A, Preeti V. A review on pathophysiology, classification and long term course of depression. IRJP. 2012;3:90-6.
5. World Health Organization's 2008, the Global Burden of Disease 2004update. http://www.who.int/healthinfo/global_burden_disease/GBD_ report_2004.accessed on 22-12-2019.
6. Sutar RC, Kasture SB, Kalaichelvan VK. Evaluation of the antidepressant activity of leaf extracts of Holopteleaintegri Folia (roxb) planch in experimental animals. Int J Pharm Pharm Sci. 2014; 6(6):250-3.
7. Sheela D. Anxiolytic effect of ethanolic extract of flowers of *Chrysanthemum indicum* in Albino mice, International Journal of Pharmaceutical Science & Research.2017; 8(6):2573-2577.
8. Kirtikar and Basu. Indian Medicinal Plant, Periodical Expert Book. 2nd Ed. Mumbai Publication.1935; 2:1380-81.
9. *Chrysanthemum indicum* L[internet] Available from https://en.m.wikipedia.org/wiki/chrysanthemumindicum. Accessed on 5-7-2018.
10. Kumar A, Singh SP, Bhakuni RS. Secondary metabolites of Chrysanthemum genus and their biological activities. Current science. 2005 Nov 10:1489-501.
11. Chae SC. An Up-To-Date Review of Phytochemicals and Biological Activities in Chrysanthemum Spp. Biosciences Biotechnology Research Asia. 2016 Jun 25; 13(2):615-23.

12. Kumar BA, Lakshman K, Velmurugan C, Sridhar SM, Gopisetty S. Antidepressant activity of methanolic extract of Amaranthusspinosus. Basic and clinical neuroscience. 2014; 5(1):11.
13. htpp://WWW.Theinformationalstandered.org>home>epilepsyadvice&information>helthmatters (accessed 17 Aug 2018)
14. Antidepressant MedicationWhat You Need to Know About Depression Medication. http://www.helpguide.org/mental/medications_depression.htm Accessed 05.3.2014
15. http://www.psnpaloalto.com/wp/wp-content/uploads/2010/12/Depression-Diagnostic Criteria-and-Severity-Rating.pdf) (cited at 5/10/2014 01.04 pm)
16. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Harrison's™ principles of internal medicine, The Mac Graw-Hill Companies.
17. J.T. Dipiro et al. *Pharmacotherapy A Pathophysiologic Approach*, 2008, 7th Ed. 1123-1134.
18. Buzsáki G. The hippocampo-neocortical dialogue. Cerebral cortex. 1996 Mar 1; 6(2):81-92.
19. Rosene DL, Van Hoesen GW. The hippocampal formation of the primate brain. Cerebral cortex 1987 (pp. 345-456). Springer, Boston.
20. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues in clinical neuroscience. 2006 Dec; 8(4):383.
21. Kaplan RM, Ries AL, Prewitt LM, Eakin E. Self-efficacy expectations predict survival for patients with chronic obstructive pulmonary disease. Health Psychology. 1994 Jul; 13(4):366.
22. Köhler C, Chan-Palay V, Steinbusch H. The distribution and orientation of serotonin fibers in the entorhinal and other retro hippocampal areas. Anatomy and Embryology. 1981 Jan 1; 161(3):237-64.
23. Hensler JG. Serotonergic modulation of the limbic system. Neuroscience &Biobehavioral Reviews. 2006 Jan 1; 30(2):203-14.
24. Tuchtenhagen F, Daumann J, Norra C, Gobbelé R, Becker S, Pelz S, Sass H, Buchner H, Gouzoulis-Mayfrank E. High-intensity dependence of auditory evoked dipole source activity indicates decreased serotonergic activity in abstinent ecstasy (MDMA) users. Neuropsychopharmacology. 2000 Jun 1; 22(6):608-17.
25. Stahl SM, Stahl SM. Stahl's essential psychopharmacology: neuroscientific basis and practical applications. Cambridge university press; 2008.Apr 1132.
26. Bortolato M, Chen K, Shih JC. Monoamine oxidase inactivation: from pathophysiology to therapeutics. Advanced Drug Delivery Reviews. 2008 Oct 1; 60(13-14):1527-33.
27. Youdim MB. Why do we need multifunctional neuroprotective and neurorestorative drugs for Parkinson's and Alzheimer's diseases as disease-modifying agents? Experimental neurobiology. 2010 Jun 1; 19(1):1-4.
28. Pizzinat N, Marti L, Remaury A, Leger F, Langin D, Lafontan M, Carpéné C, Parini A. High expression of monoamine oxidases in human white adipose tissue: evidence for their involvement in noradrenaline clearance. Biochemical Pharmacology. 1999 Dec 1; 58(11):1735-42.
29. Fagervall I, Ross SB. Inhibition of monoamine oxidase in monoaminergic neurons in the rat brain by irreversible inhibitors. Biochemical pharmacology. 1986 Apr 15; 35(8):1381-7.
30. Horstink M, Tolosa E, Bonuccelli U, Deuschl G, Friedman A, Kanovsky P, Larsen JP, Lees A, Oertel W, Poewe W, Rascol O. Review of the therapeutic management of Parkinson's disease. Report of a joint task force of the European Federation of Neurological Societies (EFNS) and the Movement Disorder Society-European Section (MDS-ES). Part II: late (complicated) Parkinson's disease. European Journal of Neurology. 2006 Nov; 13(11):1186-202.
31. Gitlin LN, Hauck WW, Dennis MP, Schulz R. Depressive symptoms in older African-American and white adults with functional difficulties: the role of control strategies. Journal of the American Geriatrics Society. 2007 Jul; 55(7):1023-30.
32. Ashwani A, Preeti V. A review on pathophysiology, classification and long term course of depression. IRJP. 2012; 3:90-6.
33. Singh JN, Sunil K, Rana AC. Antidepressant activity of methanolic extract of Foeniculum vulgare (fennel) fruits in experimental animal models. Journal of Applied Pharmaceutical Science. 2013 Sep 1; 3(9):65-70.
34. H.Garhard Vogel (Ed). In: Chapter E, W.H.Vogel, B.A.Scholkens, J.Muller, W.F.Vogel (eds.) Drug discovery & evaluation.2nd Ed. New York, Sringer - Verlag; 2002: 545 – 575.

35. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Archives Internationals De Pharmacodynamie et de Therapie. 1977 Oct; 229(2):327-36.
36. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology. 1985 Mar 1; 85(3):367-70.
37. Tripathi KD. Essentials of medical pharmacology 5 th Ed. Panama 2008; 6:445-7.
38. Kumar KS, Srivastava S, Paswan S, Dutta AS. Depression-symptoms, causes, medications, and therapies. The Pharma Innovation. 2012 May 1; 1(3, Part A):37.
39. Habibur R., M.C Eswaraiah. Simple spectroscopic Methods for estimating Brain Neurotransmitter, Antioxidant Enzyme of laboratory animal-like Mice: A review: Pharmatuor, Art – 1244.
40. Khandelwal K R. Practical Pharmacognosy techniques and experiments, 21st Ed, Nirali Prakashan Pune 2011, 5:25-29.
41. Arokiyaraj S, Arasu MV, Vincent S, Prakash NU, Choi SH, Oh YK, Choi KC, Kim KH. Rapid green synthesis of silver nanoparticles from *Chrysanthemum indicum* L and its antibacterial and cytotoxic effects: an in vitro study. International Journal of Nanomedicine. 2014; 9:379.
42. Hussaini B, Tula MY, Onyeje GA, Memi GG, Nne UI. Effect of *Chrysanthemum indicum* Aqueous Extract on Some Biochemical and Haematological Parameters in Albino Rats. International Journal of Biochemistry Research & Review. 2018 Jul 20:1-8.
43. Rajeshkumar S, et al. Hepatoprotective activity of aqueous extract of *Chrysanthemum indicum* L flower on paracetamol-induced liver injury in Albino mice. Asian Journal of Pharmaceutical and Clinical Research. 2016; 9(3): 246-249.
44. Rajendran P, Parasuraman G, Jain T, Dutta R. A Cross-Sectional Study to Assess the Quality of Sleep and Level of Stress among First-Year Medical Students in a Private Medical College in Chennai, Tamil Nadu. Indian Journal of Public Health Research & Development. 2018 Aug 1;9(8).
45. Wu XL, Li CW, Chen HM, Su ZQ, Zhao XN, Chen JN, Lai XP, Zhang XJ, Su ZR. Anti-inflammatory effect of supercritical-carbon dioxide fluid extracts from flowers and buds of *Chrysanthemum indicum* L. Evidence-Based Complementary and Alternative Medicine. 2013;2013.1-13
46. Depression [INTERNET] https://www.who.int/news-room/fact-sheets/detail/depression accessed on 12-3-2019.
47. Mannan A, Abir AB, Rahman R. Antidepressant-like effects of methanolic extract of Bacopamonniera in mice. BMC complementary and Alternative Medicine. 2015 Dec;15(1):337.