



Received on 02 December, 2016; received in revised form, 06 February, 2017; accepted, 02 May, 2017; published 01 June, 2017

## ANXIOLYTIC EFFECT OF ETHANOLIC EXTRACT OF FLOWERS OF *CHRYSANTHEMUM INDICUM* IN ALBINO MICE

D. Sheela <sup>\*1</sup>, V. Srinivasan <sup>1</sup> and E. Sukumar <sup>2</sup>

Department of Pharmacology <sup>1</sup>, Department of Research and Development <sup>2</sup>, Saveetha Medical College and Hospital, Saveetha Nagar, Thandalam, Chennai - 602105, Tamilnadu, India.

### Keywords:

*Chrysanthemum indicum*,  
Anti-anxiolytic effects, Elevated plus  
maze, Open field, Ethanolic extract

### Correspondence to Author:

**D Sheela**

Tutor,  
Department of Pharmacology,  
Saveetha Medical College &  
Hospital, Saveetha Nagar,  
Thandalam, Chennai - 602105,  
Tamilnadu, India.


**E-mail:** sheelapharm15@yahoo.in

**ABSTRACT:** Mental disorders are commonly occurring and often seriously impairing in many countries throughout the world. The total number of people suffering from anxiety or depression amount to 615 million, Close to 10% of the world's population is affected. *Chrysanthemum indicum* is an ancient herbal medicine used as an anti-arthritis, anti-inflammatory and anti-anxiety in many Asian countries. The elevated plus-maze (EPM) and open field test has been most familiar tool in the investigation of the neurochemical transmission basis of anxiety, for testing anxiolytic drugs. The anxiolytic activities of Ethanolic and Chloroform extracts of *Chrysanthemum indicum* Linn flowers were evaluated using the elevated plus-maze (EPM) and Open field. The ethanolic extract results are suggestive of anxiolytic activity in dosages around 250 and 500 mg/kg, as expressed by elevation of the time spent on the open arms in the plus-maze; a decrease of freezing and increase of deambulation in the open field test.

**INTRODUCTION:** Anxiety generally is defined as a state of diffuse, vague, very unpleasant feeling of fear or apprehension <sup>1</sup>. Anxiety is one of the common psychiatric disorders affecting all the age group people around the world. In most of the countries, the life time prevalence of panic disorder is around 7-9% and in India it is been reported to be about 1%. According to WHO, the prevalence of generalised anxiety disorder in common population is about 8.5% and in worldwide population, it is about 16.6% <sup>2</sup>. Compounds varying in selectivity as 5-HT<sub>1A</sub> receptor antagonists having benzo diazepine-like activity are recent targets to assess antianxiety effects in mice <sup>3</sup>.

A common factor responsible for affecting both physiological and psychological state in human is stress. Anxiety and other mental health disorder require a life time treatment <sup>4</sup>. An Indian study has reported the incidence of childhood psychiatric disorder was about 18 per 1000 per year <sup>5</sup>. Anxiety, if it is been untreated it may lead to depression and also increases the risk of cardiovascular morbidity and mortality <sup>6</sup>. The various other mental disorders like generalised anxiety, social phobias, agoraphobia, claustrophobia, panic disorder, obsessive compulsive disorder also require a chronic treatment. Anxiety is been treated by using some of the common drugs like selective serotonin reuptake inhibitor and benzodiazepines which are known for its side effect such as suicidal intention, decreased alertness, sexual dysfunction and dependency <sup>7</sup>.

With the above known side effects, the researcher aims in treating the generalised anxiety with the

<b>QUICK RESPONSE CODE</b> 	<b>DOI:</b> 10.13040/IJPSR.0975-8232.8(6).2573-77
	Article can be accessed online on: <a href="http://www.ijpsr.com">www.ijpsr.com</a>
<b>DOI link:</b> <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.8(6).2573-77">http://dx.doi.org/10.13040/IJPSR.0975-8232.8(6).2573-77</a>	

safe and effective drug by using the alternate traditional treatment. Herbs have been used for centuries by the folk in the traditional treatment of anxiety. Some of the herbs and other nutrients used by the folk as traditional medicine in calming the mind and elevating the mood are Passion flower, lavender, lemon balm, black cohosh, Ginkgo biloba, Kava, St. John wort (SJW), Valerian root and others like amino acid lysine, S-adenosyl – Methionine, B-Vitamin, inositol, choline, omega -3 fatty acid, gamma amino butyric acid (GABA), tryptophan, 5-hydroxyl tryptophan and other cation magnesium<sup>8, 9</sup>. The beneficiary use of the herbs and other alternative nutrients in treating anxiety and depression was reviewed and published over few decades<sup>10</sup>.

*Chrysanthemum indicum*, an ancient herb used in the traditional medicine shows various biological effect which has been documented in a number of studies<sup>11</sup>. They are the traditional Chinese medicine reported to possess anti-arthritis, anti-inflammatory, immunomodulatory property. The plant extract was used as analgesic, antipyretic and also in reducing the blood pressure, respiratory disorders, deterioration of bone and muscles<sup>12</sup>. The aerial parts of the plant used are Stem, flower and leaves 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<sup>13</sup>.

There are studies which show the aqueous extract of the *chrysanthemum indicum* to be used in relieving anxiety<sup>14</sup>. The present study aims in the ethanolic extract of *Chrysanthemum indicum* and also to find the effective dose of the extract in anxiety.

## MATERIALS AND METHOD:

**Authentication of plant material:** Flowers were procured from Tirunelveli district, Tamil Nadu. The flower was identified and authenticated by a renowned Botanist.

**Drugs and chemicals:** Diazepam was obtained from GlaxoSmithKline Pvt. Ltd. and petroleum ether, methanol (LR grade) were procured from H. Chandanmal & Co, Chennai.

## Preparation of extract:

**Alcoholic extract of *Chrysanthemum indicum*:** The shade dried flowers of the *Chrysanthemum indicum* weighing about 10 gm were subjected to the 90 % ethanolic extraction using the Soxhlet apparatus and then filtered. The filtrate was concentrated using a rotary vacuum evaporator. The alcoholic extract of the *chrysanthemum indicum* was about approximately 2 % in the end.

**Chloroform extract of *Chrysanthemum indicum*:** About 10 gm shade dried flowers were extracted three times using lab grade chloroform using the Soxhlet apparatus and were filtered. The filtrate was concentrated using a rotary vacuum evaporator. The chloroform extract yielded about 1% only.

**Animals:** Female Swiss albino mice (weighing 30-40 gm) bred and maintained in the animal house facility of Saveetha Medical College were procured for the study. Animals were randomly allotted to five groups with six animals in each group. Animals were housed in polypropylene cage with three animals per cage and the dry husk was used as the bedding material. Animals were fed with water and food *ad libitum* and maintained at controlled room temperature of about (18 ± 2 °C) with a 12:12h light and dark cycle. The animals selected for the study was brought to the department of pharmacology for acclimation the environment. The guidelines of the “committee for the purpose of control and supervision of experiments on animals (CPCSEA, India) was strictly followed for the maintenance of animals in this study. The study was carried out after the prior approval from the animal ethics committee [SU/CLAR/RD/009/2016].

**Oral administration of extract:** The alcoholic extract and the chloroform extract of the *Chrysanthemum indicum* was freshly prepared using hydroxyl methyl cellulose as the vehicle. About 250 mg/kg and 500 mg/kg of both the extract were given orally by gavage and the diazepam was maintained as a standard drug which was injected intraperitoneally. The extract was given at a volume of 1ml/kg of the body weight of the animal and the test was carried out only after 30 minutes.

**Different treatments:**

The following treatments were employed in the study:

**Group 1-** vehicle (1% HMC in distilled water)

**Group 2-** Standard drug (Diazepam 2.5 mg/kg p.o. suspended in HMC vehicle)

**Group 3-5-** Test groups (EE and CE extracts of *Chrysanthemum indicum* at two different doses- 250, 500 mg/kg p.o. suspended in HMC vehicle)

The effects of the drugs were estimated 30 min after the administration of the dose. In each experiment, apparatus was cleaned using 5% ethanol before introducing the next animal to preclude the possible cueing effects of odours left by previous subjects.

**Animal Model for Anxiety:**

**Elevated plus maze:** Anxiolytic effect of the extract was evaluated using elevated plus maze. The standard elevated plus maze consists of two open arms (25x5x0.5 cm) and two closed arms (25x5x16 cm) with the wall connected to the central zone (5x5x0.5cm) at a height of about 50 cm from the floor. The open arms wall is too small to decrease the number of falls, whereas the closed arms had a high (16 cm) wall to enclose the arm. Each animal was placed in the central zone facing the open arm<sup>15</sup>. During the 5 min experiment, behaviour of the mice was noted as a) number of entries into the open arm & closed arm b) average time spent by the animal in open arm. The

antianxiety activity was recorded as average time spent by the animals in the open arms of the EPM.

**Open field apparatus:** The open field apparatus was used to conduct the open field test in open aired Plexiglass cube with the dimension of 40 cmx40cmx30cm. The each animal was individually placed in the same left corner of the arena and was allowed to explore for 5 mins. The floor of the field was subdivided into 16 squares of equal size (4 center, 12 peripheral). A camera was mounted on the apparatus and was used to record the movement of the animal which would be used to evaluate the anxiety behaviour<sup>16</sup>. The open field apparatus should be cleaned before introducing the other animal with 70 % ethyl alcohol and allow it to dry in order to avoid the bias during the experiment.

The parameters used to evaluate the exploratory behaviour and anxiety in this instrument is

- Number of squares crossing with all four paws
- Number of center square entries
- Duration of stay in the central square
- Rearing frequency
- Grooming frequency

**Statistical analysis:** All the result values were expressed in Mean  $\pm$  SEM calculated using SPSS statistical software package. One Way Analysis of Variance (ANOVA) was performed, followed by Post hoc test to compare the inter-group difference. A P value of <0.05 was considered to be statistically significant.

**RESULTS:**

**TABLE 1: ANXIOLYTIC EFFECT OF ETHANOL AND CHLOROFORM EXTRACT OF FLOWERS OF *CHRYSANTHEMUM INDICUM* AND DIAZEPAM ON ELEVATED PLUS MAZE. DATA ARE EXPRESSED AS MEAN  $\pm$  SEM**

Group	Dose (mg/kg)	Open arm		Closed arm	
		Number of entries	Time spent(s)	Number of entries	Time spent(s)
Control	-	4.83 $\pm$ 0.30	103.33 $\pm$ 1.3	4.00 $\pm$ 0.25	91.17 $\pm$ 0.54
Diazepam	2.5 mg/Kg	7.00 $\pm$ 0.36*	156.50 $\pm$ 1.3	3.50 $\pm$ 0.22	61.33 $\pm$ 0.66
EE	250 mg/Kg	5.50 $\pm$ 0.22	114.00 $\pm$ 1.7	4.67 $\pm$ 0.21	130.17 $\pm$ 0.54
EE	500 mg/Kg	6.33 $\pm$ 0.21*	122.33 $\pm$ 0.8	4.33 $\pm$ 0.33	85.33 $\pm$ 0.33
CE	250 mg/Kg	4.17 $\pm$ 0.30	83.67 $\pm$ 1.4	4.33 $\pm$ 0.21	85.67 $\pm$ 0.95
CE	500 mg/Kg	4.50 $\pm$ 0.22*	92.67 $\pm$ 0.98	4.33 $\pm$ 0.42	83.67 $\pm$ 0.66

\*P < 0.05 (ANOVA, Post hoc) compared with multiple groups

Control= 1% HMC, STD=Diazepam (2.5 mg/kg p.o.). EE= Ethanolic extract (250, 500 mg/Kg p.o.), CE= Chloroform extract (250, 500 mg/Kg p.o), Results are expressed as mean $\pm$ SEM (n=5); P<0.05 was considered statistically significant.

**TABLE 2: ANXIOLYTIC EFFECT OF ETHANOL AND CHLOROFORM EXTRACT OF FLOWERS OF *CHRYSATHAMUM INDICUM* AND DIAZEPAM ON OPEN FIELD. DATA ARE EXPRESSED AS MEAN ± SEM**

Group	Dose (mg/kg)	Deambulation		Behavior		
		centre	periphery	Freezing time(s)	Grooming time(s)	Number of fecal bolus
Control	-	12.17 ± 0.6	37.33 ± 0.61	13.11 ± 0.30	30.67 ± 0.8	3.67 ± 0.21
Diazepam	2.5 mg/Kg	22.50 ± 0.61*	44.67 ± 0.21	11.38 ± 0.13	28.50 ± 0.56	4.50 ± 0.22
EE	250 mg/Kg	13.83 ± 0.91	53.17 ± 0.60	7.43 ± 0.16	30.83 ± 0.40	3.33 ± 0.21
EE	500 mg/Kg	21.83 ± 0.74*	57.50 ± 0.34	6.96 ± 0.05	32.67 ± 0.42	3.33 ± 0.21
CE	250 mg/Kg	10.00 ± 0.57	51.33 ± 0.42	7.73 ± 0.14	28.33 ± 0.33	4.33 ± 0.21
CE	500 mg/Kg	15.00 ± 0.81*	54.83 ± 0.30	8.33 ± 0.13	33.17 ± 0.74	3.50 ± 0.34

\*P < 0.05 (ANOVA, Post hoc) compared with multiple groups

Control= 1% HMC, STD=Diazepam (2.5 mg/kg p.o.). EE= Ethanolic extract (250, 500 mg/Kg p.o.), CE= Chloroform extract (250, 500 mg/Kg p.o.), Results are expressed as mean±SEM (n=5); P<0.05 was considered statistically significant.

**DISCUSSION:** Psychiatric disorders are now considered as a universal problem where there is a need for safety treatment. Benzodiazepines have been used for more than 40 years for the treatment of several types of anxiety<sup>17</sup>. The alternation of neurotransmitters, GABA, serotonin, adrenaline and dopamine play the important role in the development of psychiatric problems<sup>18</sup>. The current drugs which are used for the treatment of anxiety have failed to reduce the untoward effects produced by them. Anxiety disorders require a chronic treatment and the above mentioned reason have forced the researchers for the alternate traditional method to overcome the current scenario. The Indian history shows the custom of using the medicinal herbs for various neurological conditions<sup>19</sup> and even the present drug is being used in using in china for treating Parkinson's disease (PD)<sup>20</sup>. However, to date there have been fewer scientific observations of this flower extract which has led to the current study to demonstrate the anxiolytic effect of this flower extract using elevated plus maze and open field apparatus.

When the lab animals are removed from the home cage and produced to the new environment, the alteration in the behaviour of the animals is termed as anxiety. The most common lab method used in the animals to evaluate the anxiolytic effect was elevated plus maze<sup>21, 22</sup>. The normal behaviour of aversion towards the open area and the greater preference towards the protective area was used to demonstrate the effectiveness of the anxiolytic effect. The ethanolic and chloroformic extract of the flowers of the *Chrysanthemum indicum* increased the time spent and the number of entries in the open arm. There was a significant increase in

the entries and the time spent in the open arm of ethanolic extract than the chloroformic extract of the flowers (**Table 1**). Open field test used the emotional state of the animal to evaluate the anxiolytic effect of the animal<sup>23, 24</sup>. The oral administration of the ethanolic extract of *Chrysthamum indicum* has produced a marked increase in the time spent in the centre and significant reduction in the grooming time. The extract produced anxiolytic effect in a dose dependant manner (**Table 2**).

**CONCLUSION:** The present study has revealed the anxiolytic activity of the ethanolic extract and chloroformic extract of *Chrysthamum indicum* at 250, 500 mg/kg dose. The ethanolic extract has marked anxiolytic effect than the chloroformic extract. The presence of the phytochemicals like flavinoids, phenols, and terpinoids may influence the anxiolytic effect of the herbal plants. Further research may be needed to isolate and quantify the phytochemicals which may be responsible for the anxiolytic effect<sup>25</sup>.

**ACKNOWLEDGMENT:** Authors are thankful to Dr. C.B. Tharani, Prof & HOD, Dept of Pharmacology, Dr. R. Vijayaraghavan, Director of Research and Dr. S Porchelvan, Prof of Statistics, Dept of Social & Preventive Medicine to help conduct.

**DECLARATION OF INTEREST:** The authors declare that there is no conflict of interest.

#### REFERENCES:

- Gautam S, Jain A, Gautam M, Vahia VN, Gautam A. Clinical Practice Guidelines for the Management of



- Generalised Anxiety Disorder (GAD) and Panic Disorder (PD). Indian Journal of Psychiatry. 2017; 59(5):67.
2. Steel Z, Marnane C, Iranpour C, Chey T, Jackson JW, Patel V, Silove D. The global prevalence of common mental disorders: a systematic review and meta-analysis 1980–2013. International journal of epidemiology. 2014; 19:dyu038.
  3. Pytka K, Partyka A, Jastrzębska-Więsek M, Siwek A, Gluch-Lutwin M, Mordyl B, Kazek G, Rapacz A, Olczyk A, Gałuszka A, Błachuta M. Antidepressant-and Anxiolytic-Like Effects of New Dual 5-HT 1A and 5-HT 7 Antagonists in Animal Models. PloS one. 2015; 10(11):e0142499.
  4. Yang YC, Boen C, Gerken K, Li T, Schorpp K, Harris KM. Social relationships and physiological determinants of longevity across the human life span. Proceedings of the National Academy of Sciences. 2016; 113(3): 578-83.
  5. Khairkar P, Pathak C, Lakhkar B, Sarode R, Vagha J, Jagzape T, Damke S, Saoji N. A 5-year hospital prevalence of child and adolescent psychiatric disorders from Central India. The Indian Journal of Pediatrics. 2013; 80(10): 826-31.
  6. Ouakinin SR. Anxiety as a risk factor for cardiovascular diseases. Frontiers in psychiatry. 2016; 7.
  7. Offidani E, Guidi J, Tomba E, Fava GA. Efficacy and tolerability of benzodiazepines versus antidepressants in anxiety disorders: a systematic review and meta-analysis. Psychotherapy and psychosomatics. 2013; 82(6):355-62.
  8. Alramadhan E, Hanna MS, Hanna MS, Goldstein TG, Avila SM, Weeks BS. Dietary and botanical anxiolytics. Medical Science Monitor. 2012; 18(4): RA40-8.
  9. Qureshi NA, Al-Bedah AM. Mood disorders and complementary and alternative medicine: a literature review. Neuropsychiatr Dis Treat. 2013; 9(639):58.
  10. Lakhan SE, Vieira KF. Nutritional and herbal supplements for anxiety and anxiety-related disorders: systematic review. Nutrition Journal. 2010; 9(1):1.
  11. Chen YF, Zhao MH, Yan M, Shi GB, Hou GX, Huang Y, Wang X, Zhao QC. Analgesic activity of the aqueous fraction from the ethanolic extract of *Chrysanthemum indicum* in mice. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2011; 66(7): 538-42.
  12. Wu LY, Gao HZ, Wang XL, Ye JH, Lu JL, Liang YR. Analysis of chemical composition of *Chrysanthemum indicum* flowers by GC/MS and HPLC. Journal of Medicinal Plants Research. 2010; 4(5): 421-6.
  13. Hwang ES, Kim GH. Safety Evaluation of *Chrysanthemum indicum* L. Flower Oil by Assessing Acute Oral Toxicity, Micronucleus Abnormalities, and Mutagenicity. Preventive nutrition and food science. 2013; 18(2):111.
  14. Hong SI, Kwon SH, Kim MJ, Ma SX, Kwon JW, Choi SM, Choi SI, Kim SY, Lee SY, Jang CG. Anxiolytic-like effects of *Chrysanthemum indicum* aqueous extract in mice: possible involvement of GABAA receptors and 5-HT1A receptors. Biomolecules & therapeutics. 2012; 20(4): 413.
  15. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of neuroscience methods. 1985; 14(3):149-67.
  16. Gould TD, Dao DT, Kovacsics CE. The open field test. Mood and anxiety related phenotypes in mice: Characterization using behavioral tests. 2009; 1-20.
  17. Dell'Osso B, Lader M. Do benzodiazepines still deserve a major role in the treatment of psychiatric disorders? A critical reappraisal. European Psychiatry. 2013; 28(1):7-20.
  18. Bystritsky A, Khalsa SS, Cameron ME, Schiffman J. Current diagnosis and treatment of anxiety disorders. PT. 2013; 38(1):30-57.
  19. Kaur N, Sarkar B, Gill I, Kaur S, Mittal S, Dhiman M, Padala PR, Perez-Polo R, Mantha AK. Indian Herbs and their Therapeutic Potential against Alzheimer's disease and Other Neurological Disorders. Neuroprotective Effects of Phytochemicals in Neurological Disorders. 2017; 9:79.
  20. Sarrafchi A, Bahmani M, Shirzad H, Rafieian-Kopaei M. Oxidative stress and Parkinson's disease: New hopes in treatment with herbal antioxidants. Current pharmaceutical design. 2016; 22(2):238-46.
  21. Janus C, Hernandez C, deLelys V, Roder H, Welzl H. Better Utilization of Mouse Models of Neurodegenerative Diseases in Preclinical Studies: From the Bench to the Clinic. Mouse Models for Drug Discovery: Methods and Protocols. 2016; 311-47.
  22. Kelly JR, Borre Y, O'Brien C, Patterson E, El Aidy S, Deane J, Kennedy PJ, Beers S, Scott K, Moloney G, Hoban AE. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. Journal of Psychiatric Research. 2016; 82:109-18.
  23. Díaz-Morán S, Estanislau C, Ca T, Blázquez G, Ráez A, Tobe A, Fernández-Teruel A. Relationships of open-field behaviour with anxiety in the elevated zero-maze test: focus on freezing and grooming. World Journal of Neuroscience. 2014;
  24. Landgraf D, Long JE, Proulx CD, Barandas R, Malinow R, Welsh DK. Genetic Disruption of Circadian Rhythms in the Suprachiasmatic Nucleus Causes Helplessness, Behavioral Despair, and Anxiety-like Behavior in Mice. Biological psychiatry. 2016; 10.
  25. Nugroho A, Lim SC, Choi J, Park HJ. Identification and quantification of the sedative and anticonvulsant flavone glycoside from *Chrysanthemum boreale*. Archives of pharmacological research. 2013; 36(1):51-60.

**How to cite this article:**

Sheela D, Srinivasan V and Sukumar E: Anxiolytic effect of ethanolic extract of flowers of *Chrysanthemum indicum* in albino mice. Int J Pharm Sci Res 2017; 8(6): 2573-77.doi: 10.13040/IJPSR.0975-8232.8(6).2573-77.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)