INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

STUDY AND EVALUATION OF ANTIDEPRESSANT LIKE PROPERTY OF ETHANOLIC SEED EXTRACT OF *ELAEOCARPUS GANITRUS* IN ANIMAL MODEL OF DEPRESSION

Swati Hardainiyan *[1], Bankim Chandra Nandy [2], Krishan Kumar [1]
[1]Department of Food and Biotechnology, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India
[2]Department of Pharmaceutical Science, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India
*Corresponding Author Email: swati.pandithar@gmail.com

Article Received on: 06/02/17 Approved for publication: 08/03/17

DOI: 10.7897/2230-8407.080445

ABSTRACT

Depression is a widespread psychiatric disorder affecting around 5% population of the world. It is fourth leading cause of disease trouble universal by ranked and it is expected to turn into the second most immobilizing disorder. Moreover, it is not easy to expect which patient will retort to whichever given treatment. At present obtainable antidepressant drugs are effective and harmless, but limitations range from a delayed start of action to a considerable rate of non-responders. In the systems of traditional medicine, numerous plants and formulations have been used to take care of depression for thousands of years. We have reported antidepressant activity of EG. Therefore, the present study was start to evaluate the antidepressant potential of fruit extract of *Elaeocarpus ganitrus* and fraction of *Elaeocarpus ganitrus* in forced swim test (FST) and tail suspension test (TST). The mice were divided into six groups, each group containing five animals. Test drug *Elaeocarpus ganitrus* (EG) were suspended in distilled water. The vehicle (10ml/kg, p.o), Imipramine HCl (10mg/kg, p.o), EGE and EGF (50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg, p.o. respectively) were administered 1 hour prior to study. Duration of immobility was noted in both the models. In our study, Imipramine HCl, EGE and EGF significantly reduced the duration of immobility in both experimental models as compared to the animals in the control group. The antidepressant activity of EGE and EGF were comparable to that of standard drug Imipramine HCl. The results of the present study indicate the potential for use of EG as an adjuvant in the treatment of depression.

Keywords: Forced swim test, Tail suspension test, *Elaeocarpus ganitrus*, Depression, Imipramine HCl.

INTRODUCTION

Depression is a very serious disabling and life-threatening disorder [1, 2]. Depression symptoms includes depressed mood, lack of energy, appetite, loss of pleasure, inability to concentrate, feelings of guilt, and thoughts of suicide[3,4]. Depression is characterized by annoyances in sleep and hunger as well as shortages in cognition and energy. Worthlessness, thoughts of guilt and suicide are common in depression[5]. The occurrence of depression in general population is estimated to be around 5%. At the present time 121 million people are approximation to be ill with from depression. An approximated 9.5% of women and 5.8% of men experience a depressive incident in their lifetime with suicide as one of the most common results of depression[6, 7]. It is precious to appear for antidepressants from plants with confirmed advantage and favourable benefit-to-risk ratio [8]. Presently available treatment of depression is regularly associated with some unwanted side effects and it is effective only in an induced portion of the patients[9]. A search for new pharmacotherapy from medicinal plants for psychiatric diseases has progressed significantly in the past decade. A huge number of herbal preparations for antidepressant activity have been assessed in a diversity of animal models[10].

Elaeocarpus ganitrus belongs to family Elaeocarpaceae. Genus *Elaeocarpus* contains approximately 350 species and distributed in India [11, 12]. *Elaeocarpus ganitrus* is commonly known as Rudraksha and grows in India, South-East Asia, Indonesia, New Guinea, Australia, Guam, and Hawaii[13]. Traditional medicinal plants are a lot cheaper, nearby available and easily unpreserved, rare or like simple medicinal preparations [14]. Ayurvedic physician, claim that used decoctions made from this fruit successfully in the treatment of mental disease reported by Chopra[15] *et al* (1956) and Nadkarni[16] (1954). Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders[17, 18].

Rudraksha is used in Ayurveda for the treatment of mental diseases, epilepsy, asthma, hypertension, liver diseases and arthritis. According to Ayurveda, fruits are appetizer, bitter, sedative and helpful in treatment of nerve pain, epilepsy, cough, migraine and bronchitis[19]. In folk medicine, the flesh of the fruit is prescribed in epileptic fits. The powder of beads is used for expelling thick and sticky phlegm due to its emetic property. For acquiring tranquility and relaxed mental state, the beads are worn over the body. Internally the powder of the beads is used in neurological disorders, pschycological instability, cardiac depression, restlessness and insomnia. Ethanol extract of the fruit reportedly known to exhibit sedation, hypnosis, tranquillizing, anticonvulsive, anti-epileptic and antihypertensive properties[20]. Quercetin, Gallic and ellagic acids are important constituents of seeds of E. *sphaericus*[21]. In the present study, plant E. *ganitrus* was evaluated for antidepressant activity. Literature shows that by tradition this plant is being use in the treatment of depression but no scientific and research data is presented / reported to treat depression using this plant. Our effort is to establish the scientific data of this plant as cheap,

common and affordable, effective, safe, readily available substitute antidepressant agent.

MATERIALS AND METHOD

Sample Collection

Plant material (fruit) was collected from the herbal garden of Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India in the month of April-May 2013. *Elaeocarpus ganitrus* (Family: Elaeocarpaceae) were identified by Department of Botany, University of Rajasthan, Jaipur. The specimen conserved in the Herbarium (Voucher specimen: RUBL- 211325) for the reference. Ripened fruits of plant were shade dried, powdered and extracted with 90% ethanol for 48 h by soxhlet extraction method. Then, ethanol was separated under reduced pressure to get solid mass. The hydro-alcoholic extract was dried and stored in refrigerator until further use. Imipramine hydrochloride was received a gift sample from Harika Drugs Pvt. Ltd. Hyderabad, India

Treatments

Distilled water used as untreated control and Imipramine hydrochloride was used as reference standard drug. The drug was dissolved in distilled water and administered to animals through oral route at doses of 10 mg/kg body weight[22, 23]. *Elaeocarpus ganitrus* extract used as test drug. Animals were intended into six groups containing five animals each (n=5). All the solutions were prepared freshly on the same day and administration and administrated orally in a volume of 300µl per 30g of the body weight of mice. Drugs and vehicle were administered orally 60 minutes prior to the experiment[24]. Group I and II, served as control and standard respectively. The animals of group III, IV, V and VI were treated with EGE of 50, 100, 150 and 200 mg/ kg body weight, respectively (table-1). The animals were grouped and administered drugs showed in Table 1. The recommended dose of extract was used to calculate the dose for experimental animal. The selection dose of Imipramine HCl hydrochloride used was based on previous study[28].

Animal's model system maintenance

Swiss albino mice (20-25 g) were purchased from the experimental animal facility of Indian Veterinary Research Institute (IVRI), Bareilly, Utter Pradesh, India and maintained in a clean rodent room. Animals had free access to standard food diet and tap water ad *libitum*. After randomization into various groups and before the study, the animals were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and housed in polypropylene cages layered with husk and kept in a semi-natural light/dark condition (12 hours light/12 hours' dark). Animal study was conducted according to the IAEC Guidelines and all the animals were used and care as per the norms stated in IAEC guidelines, in Jayoti Vidyapeeth Women's University, Jaipur, with due permission from Institutional Animal Ethics Committee (R. No. 1402/a/10/CPCSEA).

Acute Toxicity Assay

The Swiss albino mice (20–25 g) were divided into six groups separately and were treated orally with extracts of E. *ganitrus* at 50, 100, 200, 400, 600, 800 and 1000 mg/kg, body weight doses for safe dose analysis. Animals treated with extracts of E. *ganitrus* did not show any behavioral changes (from 50 to 1000mg/kg body weight), toxic reaction or mortality[25, 26, 27]. An acute oral toxicity study of E. *ganitrus* extracts for the determination of lethal dose was carried out in mice by administering different doses according to the method described by Hule AK[26] *et al*., 2011. It was observed that the extract was nontoxic up to the dose of 5.0g/kg body weight and was used in different doses for further studies.

ANIMAL MODEL FOR ANTIDEPRESSANT ACTIVITY

FST (Forces Swim Test) 'or' PST (Porsolt Swim Test)

For the determination of antidepressant activity, FST protocol was employed[29]. During the test, mice were individually placed in a glass chamber containing water from which they cannot escape (20 cm in height, 14 cm in diameter) filled 10 cm high at 25 ± 2°C. The forced swim test was carried out on mice separately forced to swim in an open cylindrical container. All animals were forced to swim for duration of immobility during the 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test. Immobility period was considered as the time spent by the mice to float in water with no struggle and making only those movements necessary to keep its head above the water[30]. In order to check the fitness level of every test animal, a pre-test was carried out 24 h before the FST by focusing each test animal to a session of 15 min swimming. The duration of immobility was recorded. Decrease in the period of immobility during the FST was taken as evaluate of antidepressant activity[29, 30, 31, 32].

TST (Tail Suspension Test)

The overall duration of immobility induced through tail suspension was determined according to the method explained previously[33, 34] as a means of evaluating potential antidepressants. In the Tail Suspension Test, a mouse is suspended by the tail, so that its body hangs in the air and rodent facing downward. Mice both acoustically and visually isolated were suspended 50 cm higher than the bottom by adhesive tape placed approximately 1 cm from the tip of the tail. At first the animals tried to run away by making energetic movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of body and hanged passively. The total duration of immobility was noted during last 4 minutes of 6 minute period[31]. All animal was used only once. The procedures were accomplished after 1 hour of administrating the drug orally to animals. When the animal stops struggling, and hang up itself immobile, it is considered to have "given up". Longer time of immobility is attributing of a depressive-like condition[31, 35].

Statically analysis

The immobility time in forced swimming test and tail suspension test were analyzed by means of analysis of variance (ANOVA). Whenever ANOVA was significant, further comparisons between control and drug-treatment groups were performed. The data were expressed as mean (±) standard error of mean (S.E.M.) and the Statistical comparisons of data were performed using One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. The values obtained were compared with the vehicle control group and were considered statistically significant when $P<0.05$. Statistical tests were applied by using computerized GraphPad Prism software (V.5.0).

Table 1: Effect of E. *ganitrus* extract on immobility time in the Forced Swim Test (FST) using mice

Groups	Treatment	Mean duration of immobility (sec) ± S.E.M.
Group-1	Control (D.W)	155.8±1.15
Group-2	Standard Imipramine HCl (10mg/kg)	90.2±1.77
Group-3	♦EGE-1 (50mg/kg)	147.6±2.87
Group-4	♦EGE-2 (100mg/kg)	124±2.30
Group-5	♦EGE-3 (150mg/kg)	95.6±2.71
Group-6	♦EGE-4 (200mg/kg)	140.2±1.98

Test solutions were administered orally 60 min prior to the test. Values represented mean ± S.E.M. (n=5), P<0.05, vs. control (group 1).
♦*Elaeocarpus* ganitrus extract.

Table 2: Effect of E. *ganitrus* extract on immobility time in the Tail Suspension Test using mice

Groups	Treatment	Mean duration of immobility (sec) ± S.E.M.
Group-1	Control (D.W)	162.2±1.28
Group-2	Standard Imipramine HCl (10mg/kg)	94.4±1.66
Group-3	♦EGE-1 (50mg/kg)	156±2.19
Group-4	♦EGE-2 (100mg/kg)	130.8±1.77
Group-5	♦EGE-3 (150mg/kg)	99±3.52
Group-6	♦EGE-4 (200mg/kg)	140.8±1.82

Test solutions were administered orally 60 min prior to the test. Values represented mean ± S.E.M. (n=6), *P<0.05, vs. control (group 1).
♦*Elaeocarpus ganitrus* extract.

Table 3: Effect of E. *ganitrus* fraction on immobility time in the Forced Swim Test (FST) using mice

Groups	Treatments	Mean duration of Immobility(sec) ± S.E.M.
Group-1	Control (D.W)	122 ± 1.41
Group-2	Std. Imipramine HCl HCl (10mg/kg)	84.4 ± 1.56
Group-3	*EGF-1 (50mg/kg)	85.6 ± 0.92
Group-4	*EGF-2 (100mg/kg)	116.6 ± 1.63
Group-5	*EGF-3 (150mg/kg)	119.8 ± 1.15
Group-6	*EGF-4 (200mg/kg)	121.8 ± 0.86

Test solutions were administered orally 60 min prior to the test. Values represented mean ± S.E.M. (n=6), P<0.05, vs. control (group 1).
**Elaeocarpus* ganitrus Fraction.

Table 4: Effect of *E. ganitrus* Fraction and Imipramine HCl on immobility time in the Tail Suspension Test using mice

Groups	Treatments	Mean duration of Immobility(sec) ± S.E.M.
Group-1	Control (D.W)	132.8 ± 1.06
Group-2	Std. Imipramine HCl (10mg/kg)	91.4 ± 0.92
Group-3	*EGF 50mg/kg	94.6 ± 1.80
Group-4	*EGF 100mg/kg	120.4 ± 1.07
Group-5	*EGF 150mg/kg	122.2 ± 1.39
Group-6	*EGF 200mg/kg	129.4 ± 0.92

Test solutions were administered orally 60 min prior to the test. Values represented mean ± S.E.M. (n=6), *P<0.05, vs. control (group 1).
**Elaeocarpus* ganitrus Fraction

RESULT AND DISCUSSION

In the past study of E. *ganitrus* revealed that, the fruit extract of E. *ganitrus* was good source of phenolic constituents. The plant of E. *ganitrus* is reservoir of probably valuable bioactive constituents which offer the same as drugs; there is no doubt, make available newer guides and evidences for current drug design by synthesis[36]. Here is huge possibility for further research on E. *ganitrus* and more pharmacological and clinical can be accomplished to examine the unexploited possible of this plant.

In this aspect, the study was carried out to evaluate the antidepressant activity of fruit extract of E. *ganitrus*. The study of Pemminati S [24] *et al.,* (2014) revealed that gallic acid has the potential to be exploited as an adjuvant in depression treatment and other mood disorders.

In the present study, ethanolic extract (50, 100, 150 and 200 mg/kg, p.o) administered for 10 successive days to mice, produced significant antidepressant like effect in mice in both TST and FST and their effectiveness were found to be similar to Imipramine HCl (10 mg/kg, po). TST and FST are two of the most generally used behavioral tests in rodents for evaluating drugs having antidepressant-like activity[23]. These tests are quite sensitive and relatively definite to all major classes of antidepressants.

Forced Swim Test (FST)

The possible antidepressant effect of EGE and EGF after oral administration was studied in the forced swimming test. The result effect of ethanol seed extract and fraction of *Elaeocarpus ganitrus* are shown in Table No 2 & 4. Duration of immobility is a measure of antidepressant activity was recorded in the last 4 minutes of 6 minutes' test session. Statistically significant reduction in duration of immobility was observed in *Elaeocarpus ganitrus* extract (EGE-1, EGE-2, EGE-3 and EGE-4) and fraction of *Elaeocarpus ganitrus* (EGF-1, EGF-2, EGF-3 and EGF-4) treated animals. In this test, animals treated with four doses of EGE (50, 100, 150 and 200 mg/kg) and four doses of EGF (50, 100, 150 and 200 mg/kg) showed decreases in their immobility times, which were significant (147.6±2.87, 124±2.30, 95.6±2.71, and 140.2±1.98 respectively; p<0.001 for

extract) and (116.6 ± 1.63, 85.6 ± 0.92, 119.8 ± 1.15 and 121.8 ± 0.86 respectively; p<0.001 for fraction) when compared with control (155.8±1.15 for extract and 122 ± 1.41 for fraction). Similarly, animals treated with Imipramine HCl (10 mg/kg), as expected, showed a significant decrease in the immobility time (90.2±1.77 and 84.4 ± 1.56 p<0.001) for extract and fraction respectively. The effect of 100 and 150 mg/kg dose of extract has almost equivalent to Imipramine HCl treated animals. But 150m/kg dose of extract gives the best result virtually equal to Imipramine HCl treated animals (P<0.001) and the effect of 50 mg/kg dose of the fraction gives the best result almost equal to the standard (Imipramine HCl) treated animals (P<0.001). The extract and fraction shortened remarkably the immobility period during the forced swimming test in to the comparison with control and exhibited a dose dependent antidepressant activity. ANOVA analysis shows that all test groups were significantly different form control group (P<0.001).

Tail Suspension Test (TST)

The result effects of ethanol seed extract and fraction of *Elaeocarpus ganitrus* are shown in Table 3 & 5. Duration of immobility is a measure of antidepressant activity was recorded in the last 4 minutes of 6 minutes' test session statistically. Significant reduction in duration of immobility was observed in ethanolic extract (EGE-1, EGE-2, EGE-3 and EGE-4) and fraction (EGF-1, EGF-2, EGF-3 and EGF-4) of *Elaeocarpus ganitrus* treated animals. In this test, animals treated with four doses of extract (EGE 50, 100, 150 and 200 mg/kg) and four dose of fraction (EGF 50, 100, 150 and 200 mg/kg) showed decreases in their immobility times, which was significant for extract (147.6±2.87, 124±2.30, 95.6±2.71 and 140.2±1.98 respectively; p<0.001) and for fraction (94.6 ± 1.80, 120.4 ± 1.07, 122.2 ± 1.39 and 129.4 ± 0.92 respectively; p<0.001) when compared with control of extract and fraction (155.8±1.15, 132.8±1.06; respectively). Similarly, animals treated with Imipramine HCl (10 mg/kg), as expected, showed a significant decrease in the immobility time of extract and fraction (90.2±1.77 and 91.4 ± 0.92; p<0.001 respectively). The effect of 150 mg/kg dose of extract and 50mg/kg dose of fraction has nearly equivalent to Imipramine HCl treated animals (P<0.001).

DISCUSSION

In previous study, researcher said that Gallic acid have the antidepressant like activity. Gallic acid showed antidepressant-like activity in stressed and unstressed mice probably appropriate to its antioxidant activity and throughout inhibition of MAO-A activity and reduce in plasma nitrite levels. As well, gallic acid also showed antidepressant-like activity in stressed mice most likely during decrease in plasma corticosterone levels[37]. The fruits of E. *sphaericus* are used in Ayurveda for treatment of mental diseases, asthma, hypertension, epilepsy, liver diseases and arthritis. Singh[38] *et al.*, 2000 showed that in order benzene (BE), chloroform (CE), acetone (AE), petroleum ether (PE), and ethanol (EE) extracts (50-200 or 200 mg/kg, i.p, or 200 mg/kg, p.o) of dried E. *sphaericus* fruits, showed significant anti-inflammatory action against both acute and sub-acute models, barbiturate-hypnosis potentiation, analgesic and antiulcerogenic activities in rats. All the extracts, except PE and EE decreased swim stress immobility in mice indicating some degree of antidepressant activity. All the extracts defended guinea-pigs against bronchospasm induced by histamine and acetylcholine aerosols. Chemically, the extracts showed the presence of glycosides, steroids, alkaloids and flavonoids. Ayurveda reveals an amount of single and compound drug formulations of plant derivation that are used in the treatment of psychiatric disorders[18] and are claimed to have an improved side-effect profile than conventional drugs. Most of the drugs that are currently being used in the treatment of depression have poor effects that affect the quality of life of the patient. This guides to patient's refusal to medication, which more complicates the problem[17].

In the present study we have evaluated the antidepressant activity of EG in TST and FST. The development of immobility when rodents are suspended by their tail during TST and when they are placed in an inescapable cylinder of water during FST reflects the termination of their determined escape-directed behavior. Conventional drugs reliably decrease the duration of immobility in animals during these tests. This decrease in duration of immobility is considered to have a good predictive value in the evaluation of potential antidepressant agents[29]. In the present study, EG in the highest dose tested (200mg/kg) was not superior to Imipramine HCl in both the experimental models. But lower dose (150mg/kg) of E. *ganitrus* was not superior to Imipramine HCl but showed equivalent to standard drug.

Tannic acid has been exposed to be a non selective inhibitor of monoamine oxidase, thus increasing the levels of monoaminergic neurotransmitters in the brain[39]. Chronic use of gallic acid has been shown to have a neurotropic exploit on the hypothalamus[40]. A further probable mechanism of action is the decrease of oxidative stress make during depression, through the polyphenols and tannic acid present in EO[40]. Several polyherbal formulations prescribed by medical practitioners are: Chaihu-Shugan-San, Catuama, Banxia-houpu, EuMil, Mentat, Siotone, Kami-shoyo-san and Sho-ju-sen[41]. It has been examined that the main components of these preparations include: *Ocimum sanctum, Asparagus racemosus* Willd, *Withania Somnifera, Emblica officinalis* Gaertn., *Centella asiatica*, *Nardostachys jatamansi* DC, *Evolvulus alsinoides* Linn., *Panax ginseng, Bacopa moninieri, Acorus calamus* Linn., *Valeriana Jatamansi* Jones, *Tinospora cordifolia (*willd*.)* Miers. Ex Hook. f. & Thoms, *Terminalia chebula* Retz, *Terminalia bellirica* Roxb, *Sasa kurinensis* Makino et Sinata, *Celastrus paniculatus* Willd, *Saussurea* lappa C.B. Clarke, *Pinus densiflora* Sieb. Et Zucc. *and Tribulus terrestris* Linn[42-47].

CONCLUSION

The finding of the present investigation suggests the antidepressant activity of E. *ganitrus* in FST and TST models of depression. E. *ganitrus* significantly reduced the immobility period in both FST and TST. The extract also had high level of phenol and flavonoids and was so safe at least up to 5000 mg/kg. However, further studies are necessary for complete understanding the antidepressant activity of E. *ganitrus* Such identified potential and natural constituents could be exploited as cost effective food additives for human and animal health. Thus, it may be concluded that *Elaeocarpus ganitrus* produced antidepressant-like effect in mice in both FST and TST. The efficacy of the *Elaeocarpus ganitrus* was comparable to that of Imipramine HCl. Further work was necessary to elucidate the mechanism of action involved in the antidepressant activity of *Elaeocarpus ganitrus* with special references to phytochemicals. As mentioned above, there are a number of medicinal plants and formulation that possess antidepressant activity comparable to clinically effective synthetic antidepressants. Thus, plants based formulations can be effectively used for the treatment of mild to moderate cases of depression, fewer side effects than the older synthetic agents.

ACKNOWLEDGEMENTS

The authors are thankful to Harika Drugs Pvt. Ltd. Hyderabad, India, for providing the free gift sample of Imipramine hydrochloride. The author also wishes to thank the Jayoti Vidyapeeth Women's University, Jaipur, Rajastshan for providing facilities and laboratory for the study.

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Cite this article as:

Swati Hardainiyan *et al*. Study and evaluation of antidepressant like property of ethanolic seed extract of *Elaeocarpus ganitrus* in animal model of depression. Int. Res. J. Pharm. 2017;8(4):35-40 http://dx.doi.org/10.7897/2230-8407.080445

Source of support: Nil, Conflict of interest: None Declared