ORIGINAL RESEARCH PAPER
Volume-8 | Issue-9 | September - 2019 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EVALUATION OF ANTIDEPRESSANT EFFECT OF AQUEOUS EXTRACT OF AEGLE MARMELOS LEAVES IN ADULT MALE ALBINO MICE

Pharmacology

Dr. S. Prasannakumari* M.D Pharmacology Assistant Professor, Department of Pharmacology, GMC- ESI Hospital, Coimbatore. *Corresponding Author

Dr. M. Shanthi M.D Professor, Institute of Pharmacology, Madurai medical college, Madurai.

Dr. K. Latha M.D Associate Professor, Department of Pharmacology, GMC- ESI Hospital, Coimbatore.

ABSTRACT

AIM: To evaluate the antidepressant effect of aqueous extract of Aegle marmelos leaves in Swiss male albino mice by tail suspension method.
MATERIALS AND METHOD: 30 inbred adult male albino mice (20 – 25 grams) will be procured from central animal house,Madurai Medical college, Madurai. Animals will be preconditioned in the lab for one week before the experiment to get accustomed to the lab condition. They will be divided into five groups of six animals in each group. Group I will served as control received normal feed and water, group II as standard received tablet Fluoxetine (20mg/kg) orally, group III, IV,V as test groups received Aegle marmelos extract (75,150 & 300 mg/kg) orally. The antidepressant effect of Aegle marmelos was evaluated using tail suspension test (TST) and the decrease in duration of immobility was compared with the standard drug Fluoxetine.
RESULTS: The values obtained were expressed as mean ± SD. Statistical analysis of differences between groups was carried out using one-way analysis of variance (ANOVA). Probability (P) value of <0.05 was taken as the level of statistical significance. It is concluded that aqueous extract of Aegle marmelos possess potential antidepressant effect when comparable to that of tablet Fluoxetine.

KEYWORDS

Aegle marmelos, Antidepressant, Fluoxetine, Tail suspension test

1.INTRODUCTION:

Depression is a chronic debilitating disease. It's a major psychotic illness characterized by intense despair, sadness, loss of concentration, mental slowing, pessimistic worry and lack of pleasure. It is associated with physical symptoms like insomnia or hypersomnia, altered eating pattern with anorexia, weight loss and over eating.[1] According to World Health Organization (WHO) over 4.4 % of global population suffer from depression.[2] it is distributed over all age groups. The burden is fifty percent more in females and it will be the second important cause of global disease burden by the year 2020. The worst part is the relevance of the data may be tip of the ice berg.Pharmacotherapy is the common and most productive intervention in the treatment of MDD (Major Mood Depression), which helps to attain remission and also prevents reoccurrence. Among the drugs available Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin Noradrenalin Reuptake Inhibitors (SNRIs) are the most commonly used drugs. They belong to second generation antidepressant drugs[3].Tricyclic Antidepressants (TCAs) and Monoamine Oxidase Inhibitors (MAOIs) are referred to as first generation drugs but currently not preferred because of lack of selective targeting and an array of adverse effects like cardiac conduction abnormalities. Among the many newer antidepressants available, SSRIs are claimed to be safe with better tolerability profile but they too have adverse effects[4].Another major problem with these drugs is lack of patient compliance which could be due to social stigma and also due to factors like cost, fear of discontinuation, drug dependence, and lack of motivation and follow up, almost fifty percent of the patients don't adhere to therapy[5]. Over the past decades alternative medicine has increased and variety of studies has been conducted and their use is greater in persons with symptoms or diagnosed as depression[6]. India is well known for natural vegetation and herbal medicines. About 80% of the world's population depends wholly or partially on traditional medicine for its primary health care needs[7,8] India's most fabulous ancient medicine is Aegle marmelos, commonly known as Bael belonging to the family Rutaceae[9]. In Hindu culture, the leaves are indispensable offering to the 'Lord Shiva'. The leaves has been widely used, due to its various medical properties has been proved potential drug for the treatment of depression. Drugs obtained from natural sources are known to cause fewer side effects with some ability to cure disorders in much same way as their synthetic counterpart. Therefore this study was undertaken to evaluate the antidepressant property of aqueous extract of Aegle marmelos leaves in mice.

2.MATERIALS AND METHODS:

In the present study, the antidepressant activity of Aegle marmelos was evaluated in swiss albino mice by tail suspension test. Approval was obtained from Institutional Animal Ethical Committee of Madurai Medical College, Madurai, before commencing the experiment.

PLANT MATERIAL : The leaves of Aegle marmelos were collected within Madurai city, Tamil Nadu, India. The species was identified and authenticated by Dr. Stephen, taxonomist , of American college, Madurai and the further processing was done by Pharmacology department of Madurai Medical College, Madurai. This study was done for a period of 14 days in March 2017.

PREPARATION OF AQUEOUS EXTRACT: The leaves were dried at room temperature and made into powder using mortar and pestle. Powered 100g leaves was extracted with distilled water for 24 hours and filtered with sterile Whatman's number 1 filter paper. The filtrate obtained was concentrated under reduced pressure using rotavapour (Buchi model). The green colour residue obtained was stored in refrigerator at 4°C until required. The extract was weighed and reconstituted daily, with distilled water according to the dosage needed (75mg/kg ,150 mg/kg & 300mg/kg) and administered orally for a period of 14 days.

FLUOXETINE: Fluoxetine tablets was used as a standard drug in the dose of 20 mg/kg, dissolved with distilled water and administered by oral route.

EXPERIMENTAL ANIMALS:30 adult male albino mice weighing 20-25 g were procured from central animal house, Madurai Medical college, Madurai were used for the experiment. The animals were housed in polypropylene cage at room temperature with a12hours:12hours light/dark cycle. They had free access to food and water ad libitum. They were acclimatized to laboratory conditions for at least 1 week before starting the study.The study followed the principles of CPCSEA and utmost care was taken while handling the animals and adequate care was provided to them during and after experimentation.

PROCEDURE: The animals were divided into 5 groups of 6 animals in each group. Group I served as control, group II as standard, group III, IV and V served as test groups respectively. Drugs were administered orally, using oral feeding tube fit on a 1 ml syringe, once daily in the morning for a period of 14 days, according to the groups as per the table below.

TABLE NO 1: STUDY GROUPS

I	CONTROL	Normal feed and Water
II	STANDARD	Normal feed and Water + T.Fluoxetine(20 mg/kg) Orally
III	TEST1	Normal feed and Water + Aqueous extract of Aegle marmelos (75 mg/kg) OrallyIVTEST2Normal feed and Water + Aqueous extract of Aegle marmelos (150 mg/kg)VTEST 3Normal feed and Water + Aqueous extract of Aegle marmelos (300 mg/kg)

TAIL SUSPENSION TEST:

On the day 0, tail suspension was carried out for the control one by one in group wise. On day 7 and on day14, after one hour of drug administration the tail suspension test was carried out for all the 6 animals in standard, test I, test II and test III group at a time.The tail suspension box is a rectangular chamber made of plywood painted in brown color to provide the contrast. The dimensions of the box were height 55 cm width 90cm and depth 11.5 cm. It was divided in to six chambers by placing dividers, so that each chamber had a width of 15cm. There was a provision in the roof of each compartment to hang the mice. The space in each compartment was adequate to prevent the mouse from getting into contact with the wall. The distance between the floor and the tip of nose of the suspended mouse was around 20-25cms. A detachable tray was placed in the bottom of each compartment to collect the excreta.

FIGURE 1: TAIL SUSPENSION TEST.

Clear hollow cylindrical plastic tubes of dimension 4 cms x 1.5cms were introduced into the tail and used as climb stoppers. An adhesive tape of length 17cm were cut for each mouse and at 2 cm from one end a mark was made. The 2cm marked portion of the tape was applied to the tip of the tail leaving 2-3 mm free and it was adhered securely. It was done for all the six animals in a group consecutively .Care was taken so that the tape is strong enough to hold the mouse's weight, and not too sticky while removing. The camera with a timer set up was positioned in a way so that the view of the tail suspension box was not obscured and the recording would not be interrupted. All the six mice in the group were suspended back to back by attaching the free end of the adhesive tape that measures 15 cm to the hook at the roof of all six compartments. Without any interruption for the next six minutes the immobility time was recorded using the camera that was already positioned. The recordings were saved at the end of six minutes. The animals were taken off the chamber; the tapes were removed with proper care. They were placed back into their cage and observed for a week.

TAIL SUSPENSION TEST –BEHAVIORAL

ANALYSIS[10]Assessment of the behavior of all animals was done from the video recordings. Total observation period was six minutes. Immobility time was assessed. Important facet of this assessment was to differentiate between mobile and immobile state of the animal. Behaviors that were associated with escapism like all four limb movements, attempt to touch the side walls, shaking of the body, running like movements were considered as mobility. While small movements involving forelimb without any hind limb involvement, movements due to oscillatory movement of the tape because of momentum gained by previous motion of the animal were considered as immobility. We have used the whole period of six minutes for assessment; because mostly in tail suspension test the mice tend to be immobile more during the early period of the test. The immobility period was recorded by this method in all the groups after the corresponding treatment, and the scores were analyzed statistically.

3.RESULTS:

Tail suspension test was conducted one hour after drug administration on day 0, day 7 and day 14 for all animals in the group. The duration of immobility was recorded. The mean immobility period of all the six animals in the group was calculated. The control group I received distilled water in addition to normal feed and water. It is expressed as Mean± S.D. The mean immobility period in seconds on day 7 and day 14 were 198.83 ± 14.95 and 194.33 ± 11.48The standard group II received fluoxetin 20mg/kg in addition to normal feed and water. It is expressed as Mean ± S.D. The mean immobility period in seconds on day 7 was 120.16 ± 19.06 and on day 14 it was 126.83 ± 13.4The mice in the test group- III received aqueous extract of Aegle marmelos (75 mg/kg) orally in addition to normal feed and water. The mean immobility period in seconds on day 7 was 131.83 ±25.76 and on day 14 it was 128.83 ± 12.57The mice in the test group- IV received aqueous extract of Aegle marmelos (150 mg/kg) orally in addition to normal feed and water. The mean immobility period in seconds on day 7 was 141.5±15.69 and on day 14 it was 145.5±11.11The mice in the test group- V received Aqueous extract of Aegle marmelos (300 mg/kg) orally in addition to normal feed and water. The mean immobility period in seconds on day 7 was 138.33±16.71 and on day 14 it was 132.5±15.1Results were analyzed using the following statistical test.IBM SPSS (statistical package for social sciences) software version 20 was used for statistical analysis. One way ANOVA and Bonferroni post hoc test was applied.In this study five independent groups were compared, so One-way ANOVA was used for analysis. There is a difference in the mean immobility time between groups. F value is significant (<0.05) for the degree of freedom.

FIGURE 2: THE MEAN IMMOBILITY PERIOD ON DAY 7 IN ALL GROUPS

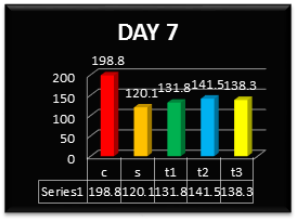
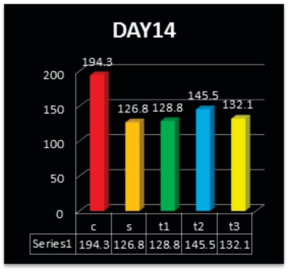


FIGURE 3: THE MEAN IMMOBILITY PERIOD ON DAY 14 IN ALL GROUPS



Both on day 7 and day 14, there is a significant difference in the immobility period when we compare control with all the other groups. The difference is not significant when we compare the corresponding groups on day 7 and 14.Once it was resolved that that there was difference exists between the means among the five groups, to determine where the difference exist Bonferroni post hoc test was applied. It showed there is a significant difference in the immobility period of standard and all three test groups on day 7 when compared with control group.

4.DISCUSSION:

Depression is a highly prevalent psychiatric illness globally, based on the recent studies prevalence is expected to increase in future. It affects all age groups like teenagers, adults and old age of both sexes. The incidence of suicidal attempt is around 50% and 15% of those cohort die due to suicide[11]. Thereby the disease has to be corrected effectively and promptly.Even though various groups of medicine are currently available, no drug is free from adverse effects. Tolerability and safety at over doses is also a problem with many antidepressants. Non compliance is approximately 40%. In a good compliant patient who is adequately managed with drugs alone, remission is 60-70%**.** Hence an ideal drug exhibiting better safety and tolerability, which cures depression most efficiently is sough for.

There are certain pitfalls in the pharmacotherapy of depression like lack of efficacy, late onset of therapeutic effectiveness, adverse

reaction and non compliance. The majority of alternative medicines in use are now turning back towards the herbal medicines, as these medicines are more safe and effective.[12] On a recent survey conducted by WHO globally, around 20,000 medicinal plants are being profusely used either in pharmaceutical industry or in folk medicine system[13]. In our tradition, various herbs are used since ancient times as a remedy for depression. They were found to have less adverse effects with good efficacy , St.John's wort , Kava, Saffron and Turmeric are proved to be effective antidepressant acting through various mechanisms[14]. Till now, an ideal antidepressant is a hot topic of research. Extensive experimental and clinical studies prove that Aegle marmelos possess antidepressant property[15].They form the easily available sources for healthcare purpose in rural and tribal areas.In this study, the antidepressant property of Aegle marmelos was evaluated using tail suspension test by observing the changes in the immobility period. Among the behavioral models available, Forced Swim Test (FST) and Tail Suspension Test (TST) are widely adopted animal models for evaluating the antidepressant effect. Tail suspension test is based on the innate behavior, with a greater sensitivity and high predictability. TST is easy to perform and well suited for high throughput screening of new compounds.The variation in the immobility period was compared between groups. In tail suspension model Aegle marmelos has shown a dose dependent significant decrease in the duration of immobility. The variation in the immobility period was compared between groups on day 0, day 7 and day 14. On day 7 and day 14, in the tail suspension test there was a significant decrease in the duration of immobility in the standard group when compared with the control group and the P value was < 0.001 level. The changes in the immobility period among the three test groups at different doses were also significant at 0.000 levels when compared with the control. There was no significant difference among the three test groups. The study has established the antidepressant property of Aegle marmelos in animal model, at different doses when comparable with that of fluoxetine . Depression is a result of multiple etiological factors which include free radical injury of neurons also. Various studies on Aegle marmelos have shown the presence of various phytoconstiuents like flavonoids, tannic acid, phenols ,ascorbic acid, eugenol, skimmianine and saponin etc could be the cause for its free radical scavenging effect[16,17]. The reducing capacity of a compound may serve as significant indicator of its potential antioxidant property[18,19]Antioxidant property of Aegle marmelos is already proved by various studies[20]. So by preventing the free radical injury it could have helped in depression. In the study conducted by Deepa Halemani and Kothari et a[21] reported that methanolic leaf extract of Aegle marmelos showed significant antidepressant activity probably by modifying monoamine levels at post synaptic sites.Hence from these study, it proves Aegle marmelos has antidepressant property due to the presence of flavanoids and phenols major phytoconstituents , that act through various mechanism like monoamine transmission enhancement and also by its antioxidant effect.

5.CONCLUSION:

Depression is a major public health problem. It contributes to 4th leading cause of global disease burden ultimately resulting in a great economic loss to the society due to loss of productivity and utility of medical resources. Pharmacotherapy is the corner stone in the management of major depressive disorder. By combining non-pharmacological therapy like Cognitive Behavioral Therapy (CBT) and Interpersonal therapy, depression is almost curable[22].It was observed that aqueous extract of Aegle marmelos at low dose (75 mg/kg) and at high dose (300 mg/kg) showed significant antidepressant effect on 14th day, when comparable to that of tablet Fluoxetine (20 mg/kg).The study objective has been achieved. Further studies need to be done with more number of animals and different experimental model , to know the exact molecular and biological mechanism behind Aegle marmelos as an antidepressant . Identification and separation of the active principle that is responsible for the antidepressant effect has to be further evaluated. That will help the society in the near future.

CONFLICT OF INTERESTS: None.

6.REFERENCES :

1. S. K. Srivastava. A Complete Textbook of Medical Pharmacology. vol 1. New Delhi: Avichal Publishing Company; 2014. 664p.1v
2. World Health Organisation. Depression and Other Common Mental Disorders Global Health Estimates. Geneva: World Health Organization ; 2017.11p. 3v.
3. Irving Kirsch, Brett J. Deacon, Tania B. Huedo-Medina, Alan Scoboria, Thomas J. Moore, Blair T. Johnson. Initial Severity and Antidepressant Benefits: A Meta-Analysis of Data Submitted to the Food and Drug Administration. PLoS Medicine. 2008; 5(2): 260-67.
4. James M. O' Donnel and Richard C. Shelton. Drug Therapy of Depression and Anxiety Disorder. In: Laurence L. Brunton, Bruce A. Chabner, Bjorn C. Knollmann (eds.) Goodman & Gilman's The pharmacological basis of therapeutics, 12th edition. New York: McGraw Hill; 2011: p 404-405.
5. Randy A. Sansone, Lori A. Sansone. Antidepressant Adherence: Are Patients Taking Their Medications?. Innov Clin Neurosci. 2012; 9(4–5):41–46.
6. C.Rajeshkannan, S.Murugesan, G.Lakshmanan .Anxiolytic and antidepressant properties of Aegle marmelos: An overview. Joural of Pharmacognosy and Phytochemistry. 2014; 3 (1):118-122.
7. Gangadhar M,Shraddha K,Ganesh M. Antimicrobial screening of garlic (Allium sativum) extract and their effect on glucoamylase activity in vitro. J ApplPharm Sci. 2012;2(01):106-108.
8. Rahman MS; SalehinMF, Jamal M, Parvin A, Alam MK. Antibacterial activity of Argemone Mexicana. Res J Mad Plant. 2011;5(5):621-626.
9. Das SK,Roy C. The protective role of Aegle marmelos on aspirin induced gastro-duodenal ulceration in albino rat model: a possible involvement of antioxidants. Saudi J Gastroenterol. 2012 ;18(3):188-194.
10. Adem Can, David T. Dao, Chantelle E. Terrillion, Sean C. Piantadosi, Shambhu Bhat,Todd D. Gould. The Tail Suspension Test. J Vis Exp. 2012; (59): 376-9.
11. Ammerican Association Of Suicidology. Depression and Suicide Risk. USA: Ammerican Association Of Suicidology. 2014.
12. Saharah Vikas Anand, Principles of Pharmacognosy, 1st edition, Agrobios publication, Jodhpur, 2008, 11-12.
13. Kar ASshutosh, Pharacognosy and Pharmaco biotechnology, 2nd edition, New Age International Publishers, New Delhi,2007, 5-7.
14. Jerome Sarris; Herbal Medicines in the Treatment of Psychiatric Disorders: A Systematic Review. Phytotherapy Research. Aug 2007; Volume 21, Issue 8: 703-716.
15. N.P.Atul, V,D,Nilesh, A.R.Akkatal, S.K.Kamlakar. A review on Aegle marmelos: a potential medicinal tree. International Research Journal of Pharmacy 3(8):86-91.August 2012 .
16. Pushpendra K. Patel, Jyoti Sahu, Lokesh Sahu, Narendra K. Prajapati, B.K.Dubey. Aegle marmelos: A Review on its Medicinal Properties. International Journal of Pharmaceutical and Phytopharmacological Research 2012, 1(5): 332-341.
17. Bramhachari PV, Reddy YK. Phytochemical examination , Antioxidant and radical scavenging activity of Aegle marmelos (L) Correa extracts .J Pharm Res, 2010;3(12):3023-3025
18. Chandra Dinesh: Analgesic Effect of Aqueous and Alcohol Extract of Madhuca Indica Longifolia. Indian Journal of Pharmacology.2001,33:108-111.
19. Pawar Rahul S, Bhutani KK: Protobasic Acid Glycosides from Madhuca Indica with Inhibitory Activity on Free Radical Release from Phagocytes.Journal Natural product 2004,67:668-671.
20. Upadhya S,Shanbhag KK, Suneetha G, Balachandra Naidu M,Upadhya S et al . A study of hypoglycemic and antioxidant activity of Aegle marmelos in alloxan induced diabetic rats. Indian J Pharmacol 2004; 48(4):476- 480.
21. Saroj Kothari, Manish Minda, S.D. Tonpay et al .Anxiolytic and Atidepressant activities of methanol extract of Aegle marmelos leaves in mice. Indian journal of Physiology and Pharmacology 54(4):18-28.
22. Benjamin J. Sadock, Virginia A. Sadock, Mood disorders. In: Pocket Hand book of Clinical Psychiatry. 5th Edition. Philadelphia; Lippincott Williams & Wilkins;2010. p.175- 200.