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Pharmacological Screening of Herbal Extracts of Aegel marmelos and Emblica officinalis for the treatment of Anxiety

Sharma Sheetal *, Vengurlekar Sudha, Jain Sachin Kumar

Oriental College of Pharmacy and Research, Oriental University Indore

Correspondence author:

Sheetal Sharma

PG Research Scholar

Oriental College of Pharmacy and Research

Oriental University Indore

ABSTRACT:

Aim: The Present studies were evaluate antianxiety activity of methanolic composite leaves extract of (Aegel marmelos and Emblica officinalis) on administration in rats.

Method: Male albino Wistar rat were taken and divided into 5 groups and each group contain 4 rats. Diazepam (1mg/kg) and composite extract (50mg/kg, 75mg/kg and 150mg/kg) were suspended in water and administrate orally. In these studies vehicle/ drugs were given 30 min before exposure to experimental model such as actophometer and rotarod. C.E decrease locometry actvity and time taken by rat to falling from the rod in actophometer and rotarod. The activity of rat is compared with standard drugs diazepam (1mg/kg).

Result: The studied shown that at dose of 50mg/kg, 75mg/kg and 150mg/kg composite extract posse's significant anxiolytic activity.

Key words: - Anxiety, Fear, Nervousness, Herbal therapies, Neurological disorder

INTRODUCTION

Anxiety is kind of psychiatrically ill health [1]. Anxiety refers to the experience of worry, restlessness, nervousness, tension, fearfulness, apprehensiveness, panic, and agitation. [2]American Psychiatric Association (APA) says that Anxiety is emotional reaction due to expectation of future risk [3].Nowadays modern life style responsible for increase in case of anxiety and depression [4]. Most common symptoms of anxiety are irritability, uneasiness, rapid or irregular heartbeat, jumpiness, feelings of apprehension, nausea, faintness, and abdomen ache and respiration problems [5].

Glutamatergic hyperactivity in brain injuries, liable for varied events such as mitochondrial pathology, oxidative stress, and cellular communication that responsible for inflammatory response and ultimately cell death. Since glutamatergic overactive is related with characteristic of anxiety, neuroinflammation and oxidative stress. Therefore overactive glutamatergic system deregulationg in balance of inhibitory/excitatory in the brain is liable for anxiety disorder. Presynaptically situated, $mGlu_{2/3}$ receptors are present in some brain areas where glutamate hyperactivity is associated with anxiety, together with the cortex, amygdale, striatum, thalamus, and hippocampus. [6]

There are seven types of anxiety disorder include panic disorder (PD)**,** social phobia (SP)**,** specific phobias (SPP)**,** post-traumatic stress disorder (PTSD)**,** acute stress disorder, obsessive–compulsive disorder (OCD) and anxiety disorders with generalized anxiety, with panic attacks and with obsessive compulsive symptoms.[7]

Treatments of anxiety disorders involve Lifestyle interventions, like physical exercise, psychological interventions, like psychological feature behavioral medical aid, which are difficult to implement and mindfulness-based stress reduction. In synthetic drugs Benzodiazepines class of drugs widely used for treatment of anxiety disorder but it causes various adverse effect such as sedation, confusion, fatigue, depression, anterograde amnesia , disinhibition, irritability, local injection site reaction, tremor, headache, urinary retention etc[8].To overcome from this herbal therapies are considered as alternative or complementary medicines for the treatment of neurological disease.**[9]**

Aegle marmelos origin from eastern ghat or central india. It usually grown in Bihar,Uttar pradesh, Uttaranchal,Madhya Pradesh, Chattisgarh, Jharkhand, Deccan highland and east coast Myanmar and srilanka.[10] It contain alkaloids , terpenoids ,coumerin , phenyl propanoids tannin and flavoinds.[11] Previous studies showed that at dose of 150mg/kg methanolic leaf extract of A*egel marmelos* shown significant anxiolytic activity sing elevated plus maze test [12].

Emblica officinalis, commonly known as Indian gooseberry or amla, is native to the Indian subcontinent. It is found predominantly in India, Pakistan, Sri Lanka, and other Southeast Asian countries. Within India, it grows in

various regions, including the plains, foothills, and up to an altitude of 1,500 meters in the Himalayas. The tree is also cultivated in other parts of the world with suitable climates, such as in tropical and subtropical regions. [13] The phytochemical analysis indicates presence of secondary metabolites like Choline, Betaine, N-methyltyramine and different compounds (phyllantine, phyllantidine). [14] Previous studies shown that 400mg/kg dose of aqueous extract of Emblica officinalis reduced locometry activity so it is useful in treatment of anxiety disorder but it's not shown effect on elevated plus maze model. [15]

MATERIALS AND METHODS

Glass wares: Borosil and ASGI make glass wares were used.

Chemicals: All chemicals used were of analytical grade. Acetic acid S.D. Fines Chemicals; Bombay; Coomassie blue- Sigma Chemical Company, USA, DAB- Sigma Chemical Company, USA, DMSO- Sigma Chemical Company, USA, Dichlomethane Qualigens, Ethanol-Merck/Bengal Chemical; Ethyl acetate-Qualigens; EDTA-Qualigens; Methanol-Merck/Qualigens, Pet. ether, chloroform, methanol, water, molisch reagents, fehling solutions, Dilute hydrochloride, ferric chloride, glacial acetic acid, mercuric chloride, dragendroff reagents, millons reagents, hagers reagents, Ninhydrine reagents, SRBC, Precoated plates, silica gel, Indian ink etc.

Animals: Wistar Albino of either sex (150 to 200 g) was purchased from the CPCSEA approved vendors for antianxiety activity. They were maintained under standard laboratory conditions at 25 ± 2°C and normal 12-hour light-dark cycle were used for the experiment. Commercial pellet diet and water were provided *ad libitum* throughout the course of study. All the experimental trial was carried out in agreement with the CPCSEA guidelines. The study designs were permitted by the Institutional Animal Ethical Committee of Oriental College of Pharmacy and Research, Oriental University, Indore (MP), India.

METHODS

Collection and authentication of plant material

The leaves of *Emblica officinalis, Aegle marmelos,* were collected from outfield medicinal garden near to Ratlam and Indore (M.P.) that show the green color with rough surface. The leaves was washed thoroughly in tap water, dried in shade, finely powdered and used for extraction.

Standard drug: Diazepam tablet I.P Valium®5, were used as a standard drug

Preparation of composite extract: The collected plants leaves were shade dried at room temperature and reduced its size with help of mechanical grinder, and passed through forty meshes sieved. The powdered leaves were extracted with 80% methanol with the Soxhlet at boiling temperature (60^0 C) for 10 h separately. The extract was then evaporated using boiling water bath. final composite extract was dark green in color with dried weight 8.33%.

Phytochemical studies

Stock solution: 1 g of the methanolic plant extracts of *Emblica officinalis and Aegel marmelos* leaves were dissolved in 100 ml of methanol.

- ✓ Test of glycosides

Liebermann's Test: To 2ml of stock solution add 2ml of chloroform and then add 2ml of acetic acid violet to blue to green colaration occur. [16]

- ✓ Test of saponins and tannins

The stock solution was dilute with 20 ml of water and then agitates for 15 min. presence of saponins shown by formation of foam layer. Then 3 ml of these extract add some drops of 1 percent lead acetate. Presence of tannins shown by formation of yellow precipitate. [17]

- ✓ Test for alkaloids

Wager's test: to stock solution add wager's reagent formation of reddish brown precipitate indicate presence of alkaloids. [18]

Preparation of wager reagent

Take 0.25gm of iodine and 1.25gm of potassium iodide and dilute with 250ml water.

- ✓ Test for flavonoids

Alkaline reagent test: to stock solution add some drop of NaOH solution an intense yellow colour produced, which turns to colorless on addition of few drops of dilute acid, shown presence of flavanoids. [19]

Animals and drug treatment: The study included 20 rats. They were divided into 5 groups and each group contains 4 rats. Group-I serve as control and receive only vehicle .Groups II was treated with Diazepam as positive control (1 mg/kg, po).Groups III- V were treated dose of (50, 75,150mg/kg, po). The dose of C.E was prepared at same day of experiment. [20]

Rotarod test: Rotarod is explained by Dunham and Miya in 1957.It is generally used for estimation of neuromuscular management in mice. Rotarod generally consists of rod that is coated with polypropylene foam which provide friction and also to prevent mice from slip of the rod. The space between rod and floor is about 15cm.The rod is drive by motor and the rpm can be maintained at 20 rpm in over study. Animals were trained on rotarod for 2min duration of per trial, with trials 3 per day for two days. On third day, mice were given trial before and after treatment of extract [21]

Actophotometer: actophotometer instrument is employed to watch locomotor activity of mice. Animals were placed in a actophotometer that has constant beams of lights, criss-crossing the chamber and falling on matching photoelectrical cells. When the mouse crosses the light beams every interrupted interruption was recorded for 10min. Total photo beam interruptions represent locomotor activity of mice. [22]

RESULT AND DISCUSSION:

Benzodiazepam class of synthetic drugs are majorly used for treatment of anxiety disorder but its causes many adverse effects such as sedation, confusion, fatigue, depression, anterograde amnesia , disinhibition, irritability, local injection site reaction, tremor etc,. Some adverse effect is common while some are severe such as respiratory depression, suicidality, seizures, bradycardia, cardiovascular collapse, syncope etc which is effect patient's health severely. To overcome from this; natural medicinal plant considered as alternative or complementary medicines for the treatment of anxiety with fewer and no side effects.

Previous studies showed that composite extract (*Aegel marmelos, Emblica officinalis*) have higher antioxidant content instead of individual plants. Many previous studies showed that plants which contain highly antioxidant properties were highly effective in treatment of anxiety disorder due to these in present studies we were take composite extract instead of individual medicinal plants.

Presence of alkaloids, glycoside saponin, tannins, and flavanoids shown by above qualitative chemical test. Presence of flavanoids indicates that composite extract of leaves of medicinal plants have antioxidant properties which is helpful to reduce the anxiety.

The aim of present studies to evaluate anxiolytic properties of composite extract of leaves of medicinal plants using model rotarod and actophometer.

Rotarod:

In above studies it's shown that diazepam and composite extract both are responsible for decreased in time rat falling of the rod when we compare it with control group. When we studied Table 1 we observed that rat falling time from the rod is decreased after administration of composite extract at different doses its showed that all doses (50mg/kg, 75mg/kg and 150mg/kg) posses significant anxiolytic activity. However 150mg/kg doses showed maximum anxiolytic activity.

Actophometer:

In above studies it's shown that diazepam and composite extract both are responsible for decreased in locometry activity of rat when we compare it with control group. When we studied Table 2 we observed that rat showes decreased in locometry activity after administration of composite extract at different doses it's indicate that at all

doses (50mg/kg, 75mg/kg and 150mg/kg) posses significant anxiolytic activity. However 150mg/kg doses showed maximum anxiolytic activity.

S.No.	Treatment	Dose	Time of animals remained without falling from rod(sec.)		
			30 min.	60min.	90min.
1.	Vehicle	Water	172.40 ±3.54	160.51±2.13	155.57±2.45
2.	Diazepam	1mg/kg	165. 51±2.43	139.98±3.38**	107.64±2.25***
3.	C.E(50mg/kg)	(50mg/kg)	170.07±1.44	154.78±2.55	146.12±2.19*
4.	C.E(75mg/kg)	(75mg/kg)	174.41±2.78	152.93±1.99*	139.46±2.04**
5.	C.E(150mg/kg)	(150mg/kg)	165.32±2.42	143.38±2.61**	114.105±3.30***

All values are mean ±SEM (n=4); *p< 0.05,** p<0.01,***p<0.001, when compared to control

Table 1: Table for rotarod

S.No.	Treatment	Dose	Locomotor activity (number of count)		
			Before dose Administration	After 30(min.)	After 60(min.)
1.	Vehicle	Water	253.50±2.10	251.00±2.08	247.50±2.60
2.	Diazepam	1mg/kg	251.00±3.08	224.00±3.16***	189.00±2.27***
3.	C.E(50mg/kg)	(50mg/kg)	253.75±2.39	247.50±2.50	228.75±1.55***
4.	C.E(75mg/kg)	(75mg/kg)	248.75±2.69	232.50±2.10***	218.50±1.94***
5.	C.E(150mg/kg)	(150mg/kg)	253.75±3.04	230.00±2.58***	187.75±2.32***

All values are mean ±SEM (n=4); *p< 0.05, **p<0.01,***p<0.001 when compared to control

Table 2: Table for actophometer

CONCLUSION

Our studied shown that composite methanolic extract of medicinal plant have significant anxiolytic effect on exposure to the rotarod and actophotometer test. C.E at doses (50mg/kg, 75mg/kg and 150mg/kg) posse's an "anxiolytic" activity comparable with the effects of standard drugs diazepam (1mg/kg) while 150mg/kg composite extract showed maximum anxiolytic activity. Methanolic C.E showed significant anxiolytic activity probably due to its antioxidant properties. However further studies are required for better explanations of mechanism of action responsible for anti-anxiety activity of composite extract

REFERENCE

1. Thibaut F (2017). Anxiety disorder a review of current literature. Dialogues in clinical neuroscience, 19(2), 87–88. DOI: 10.31887/DCNS.2017.19.2/fthibaut
2. CE Archana (2013). Antianxiety Effect of Alcoholic Leaf Extract of Plectranthus Amboinicus in Mice. Asian Journal of Biomedical and Pharmaceutical Sciences. 3(18), 49-53.
3. Doukkali Z, Taghzouti K , Bouidida EL H , Nadjmouddine M , Cherrah Y , Alaoui K . (2015) .Evaluation of anxiolytic activity of methanolic extract of Urtica urens in a mice model . . Behavioral and Brain Functions 11(19),1-5 . DOI 10.1186/s12993-015-0063-y.
4. Foyet HS , Tsala DE , Bouba AA , Hritcu L(2012) . Anxiolytic and Antidepressant-Like Effects of the Aqueous Extract of Alafia multiflora Stem Barks in Rodents. Hindawi Publishing Corporation Advances in Pharmacological Sciences,1-8. DOI:10.1155/2012/912041.
5. Solanki G (2013). Anti - Anxiety Drugs – An Overview. International Journal of Biomedical Research, 04 (01),1-4. DOI: 10.7439/ijbr
6. Santos P, Herrmann AP, Elisabetsky E, Piato A(2019). Anxiolytic properties of compounds that counteract oxidative stress, neuroinflammation, and glutamatergic dysfunction: A review. Brazilian Journal of Psychiatry. 41(2),168–178. DOI:10.1590/1516-4446-2018-0005.
7. Bystritsky A , Khalsa S S, Cameron Me , Schiffman J(2013). Current Diagnosis and Treatment of Anxiety Disorders.A peer reviewed journal for managed care and hospital formulary management,38(1): 30-57.
8. Dhaliwal JS, Rosani A, Saadabadi A(2020). Diazepam. NCBI Bookshelf. StatPearls Publishing.
9. Khanum f ,Razack S.(2010).Anxiety-Herbal Treatment: A Review .research and review in biomedicine and biotechnology. 1(2),71-89. DOI:www.rrbb.in
10. Patel P K., Sahu J , Sahu L,prajapati NK,dubey B.K(2012).Aegel marmelos.review : A review on its medicinal properties.international journal of pharmaceutical and phytopharmacological research.1(5),332-341.
11. Patkar AN ,Desai NV, Ranage AA ,Kalekar KS(2012),. A review on aegel marmelos a potencial medicinal tree.international research journal of pharmacy .3 (8).
12. Kothari S, Minda M Tonpay S. D(2010). Anxiolytic and antidepressant activity of methanol extract of aegel marmelos leaves in mice. Indian J Physiol Pharmacol.54(4),318–328.
13. Singh, D., Singh, S., Saluja, S., & Lakhani, P. (2016). Emblica officinalis: A valuable traditional Ayurvedic herb. International Journal of Pharmaceutical Sciences Review and Research, 36(2), 62-67. [DOI: 10.1016/j.jep.2009.07.015].
14. Habib-ur-Rehman., K.A. Yasin, M.A. Choudhary14. Haque, R., B. Bin-Hafeez, I. Ahmad, S. Parvez,N. Khaliq, Atta-ur-Rahman., M.I. Choudhary and S. Malik, 2007. Studies on the chemical constituents of Phyllanthus emblica. Nat Prod Res., 20; 21(9): 775-8
15. El-Desouky, S.K., S.Y. Ryu and Y.K. Kim, 2008. A new cytotoxic acylated apigenin glucoside from Phyllanthus emblica L. Nat Prod Res., 22(1): 91-5

16. Tiwari B.K, Abidi A.B., Rizvi S.I and Pandey K. B(2016) . Phytochemical screening and evaluation of antioxidant potentials of some Indian medicinal plants and their composite extract. Annals of Phytomedicine 5(1): 99-103.
17. Tiwari BK, Kumar D, Abidi A. B, Rizvi SI(2014). Efficacy of Composite Extract from Leaves and Fruits of Medicinal Plants Used in Traditional Diabetic Therapy against Oxidative Stress in Alloxan-Induced Diabetic Rats. Hindawi Publishing Corporation ISRN Pharmacology Volume 2014, 1-7.
18. Gul R, Jan S.U, Faridullah S , Sherani S, Jahan N.Preliminary phytochemical screening quantitative analysis of alkaloidsand antioxidant activityof crude plant extracts from ephedra intermediaindigenous to balochistan. Hindawi Scientific World Journal.1-7, DOI: https://doi.org/10.1155/2017/5873648
19. Neelapu N, Naikwadi DG, Muvvala S , Jadhav K.V(2011). A preliminary pytochemical investigation on the leaves of solanum xanthocarpum.International journal oF research in ayurveda and pharmacy, 2(3),845-850.
20. Shankpal P , Surve S(2020). Evaluation of anti-anxiety effect of nifedipine compared to diazepam in swiss albino mice using behavioural model. International journal of pharmacy and pharmaceutical science. 12 (5), 6-9.
21. Sharma S , Handu S, Dubey A K, Sharma P, Mediratta P, and Ahmed Q M(2017). Anti-anxiety and Anti-depressant Like Effects of Murraya koenigii in Experimental Models of Anxiety and Depression. Anc Sci Life. 36(4): 215–219. DOI: 10.4103/asl.ASL_75_17.
22. Tungmunnithum D, Thongboonyou A,[1] Pholboon A,[1] and Yangsabai A Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines (Basel). 2018 Sep; 5(3): 93. DOI: 10.3390/medicines5030093