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ANTI-ANXIETY ACTIVITY OF AEGLE MARMELOS FRUIT EXTRACTS IN MICE

Maninder Kaur*, Abhishek, Ajeet Pal Singh and Amar Pal Singh

Department of Pharmacology, St. Soldier Institute of Pharmacy, Lidhran Campus, Behind NIT (R.E.C), Jalandhar-Amritsar by Pass, NH-1, Jalandhar-144011, Punjab, India.

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***Corresponding Author**
Maninder Kaur
Department of Pharmacology, St. Soldier institute of Pharmacy, Lidhran Campus, Behind NIT (R.E.C), Jalandhar-Amritsar by Pass, NH-1, Jalandhar-144011, Punjab, India.

ABSTRACT

Anxiety is one of the most common mental disorders, characterized by changes in mood, behavior, somatic function, and cognition. Benzodiazepines and SSRIs are most commonly employed drugs for the treatment of anxiety and provided various side effects. Drugs obtained from natural sources are known to cause fewer side effects compared to synthetic drugs despite of same ability to cure disease. Aegle marmelos is an ayurvedic medicinal tree belongs to family Rutaceae. It is commonly known as apple tree, stone apple tree found all over India. Aqueous and Ethanolic fruit extracts of Aegle marmelos used to evaluate Anti-Anxiety activity. These extracts administered orally in two different doses 100 mg/kg and 200 mg/kg as compare to dose of Standard drug (diazepam) 1 mg/kg. Plasma Corticosterone Level was also estimated by collected blood from tail vein after behavioral parameters. The results of our study demonstrated that the ethanolic and aqueous extracts of Aegle marmelos fruit has significant anxiolytic activity in LDM and HBT models of anxiety and also shown significant results in biochemical estimation.

KEYWORDS: Aegle marmelos, Anti-Anxiety, Corticosterone levels, Light dark model (LDM) and Hole board test (HBT).

INTRODUCTION

Worldwide 500 million people suffer from mental and behavioral disorders.[1] Some of leading causes of disability and premature death worldwide are psychiatric conditions. Anxiety is a CNS disorder.[2,3] and common emotional phenomenon in humans.[4] It can be

measured as “intact” condition, which approximately totally disturbs the routine life of the person.[5] It is negative emotional state characterized by feelings of worry and nervousness, accompanied by specific somatic, cognitive, and behavioral manifestations involving multiple brain regions, including amygdala, locus ceruleus, frontal cortex.[6,7] and show imbalance in several neurotransmitters including norepnephrine (NE), GABA, dopamine and serotonin.[8] Anxious state present itself in type of phobia, panic attacks, post-traumatic stress disorder, social anxiety disorder or generalized anxiety disorder.

A number of synthetic drugs were used in the first half of the 20th century including the barbiturates and the carbamates (meprobamate) and some prolong to be used today.[9] Benzodiazepines are prescribed more frequently than barbiturates because they have a higher margin of safety in overdose. On the other hand, benzodiazepines are associated with abuse, dependence, and withdrawal symptoms. Phenobarbital and other long-acting barbiturates were generally used to treat anxiety.[10] To reduce the side effects of synthetic drugs and provided an effective treatment for various health conditions, herbs are great alternatives to pharmaceutical drugs.[11] Indian medicinal plants are provided huge resource of several pharmacologically active constituents, which are commonly used in home remedies against multiple ailments.[12]

Aegle marmelos (Common name Bael), a plant of Indian origin having remarkable therapeutic potential, it is belong to family Rutaceae. It is known by the several other names in the different country and also outside of the country.[13] Bael is a much known plant for the people of any part of India, as it is related to the Hindu Religious. It is believed that the bael fruit is the symbol of lord Shiva and its leaf is top of the demand in the season of ‘Sawan’.[14] Aegle marmelos has several medicinal properties which are used in traditional medicinal system to cure a variety of diseases like: - Asthma, Gastrointestinal problem, Cardiac and circulatory stimulatory activities, Epilepsy, Hypoglycaemic, Rheumatism and useful in inflammation of rectum.[15]

It contains various chemical compounds such as: Limonene, aegelin, lupeol, rutin, marmeline, marmesinin, α-Phellandrene.[16] skimmianine, scoparone, scopoletin, umbellliferone, marmesin and skimming,[17] Fagarine, Marmin,[18] D-limonene, Cineol, Citronellal, Citral, P-cyrnene.[19]

The present study was planned to evaluate the anti anxiety activity of *Aegle marmelos* fruit extracts in mice.

MATERIAL AND METHODS

Source of data:- All experiments were planned to generate data from the laboratory studies i.e.; experiments were performed as described in reference, experimental studies in journals and in textbooks available with college, SSIP library, Jalandhar, and various institutions.

Methods for collections

Drugs and Chemicals: - Diazepam was obtained from Sigma chemical, USA and S. D. Fine Chem. Ltd., India.

Plant material: - The whole part of plant Aegle marmelos was collected from the campus, St. Soldier Institute of Pharmacy, Jalandhar. This plant was identified and Morphological authenticated by Dr. M.S. Bhatti, Professor & Head, Botanical and Environmental Sciences, GNDU, Amritsar, India wide letter 1378/B& ES dated 13-11-2018.

Preparation of plant extracts: - An extract was mixture of phytochemicals from any plant which was obtained by extraction of specific parts of the plant. The crude extracts of different parts of *Aegle marmelos* were prepared using different solvents such as ethanol and distilled water. The different methods used for extraction were as follows:

Ethanolic extraction: Plant powder (10 g) was successively extracted with 250 ml of ethanol, in a Soxhlet apparatus at 75°C and 63°C respectively each for 10 to 12 h. Extracts were filtered through Whatman no.1 filter paper. The extracts were concentrated by keeping in water bath at 40°C till all the solvent had completely evaporated from mixtures.

Aqueous extraction: The powdered plant material was mixed with distilled water and magnetically stirred in a separate container for overnight at room temperature. The residue was removed by filtration through Whatman no.1 filter paper and the aqueous extracts were lyophilized and stored in airtight containers. All extracts were stored in sterilized containers at -20°C until used for testing. At the time of testing, the extracts were reconstituted in Dimethyl Sulphoxide (DMSO).[20]

Animals:-Healthy, adult swiss albino mice of either sex weighing (25-40 g), maintained under standard laboratory conditions, at temperature 25 ± 2°C and a 12 hr light-12 hr dark period was employed for the experimentation. Food and water will be provided ad libitum.

Acute oral toxicity study: - Acute toxicity study for the ethanolic and aqueous extract of "Aegle marmelos" fruit was done according to the OECD guidelines No: 423 and low, medium and high dose was selected for treatment.

Method

The overnight fasted mice were divided into 06 groups, each group consisting of 6 or 7 animals. The EAM & AAM were given in various doses (50, 100, 200,500 and 1000 mg/kg) by oral route with a gawage. After administration of the extract, the animal was observed continuously for the first 2 hours and at 24 hrs to detect changes in behavioral responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep, and coma also monitored up to 14 days for the toxic symptoms and mortality. The same was repeated for AAM in another 04 groups. After 14 days of acute oral toxicity the survival mice was rehabilitated and reused for experimentation.[21]

Methods

The methods for extract of aqueous and ethanolic *Aegle Marmelos* fruit could be used by the dose dependent study and time dependent study and then Fix the dose of the extracts of aqueous and ethanolic Aegle Marmelos fruit on the basis of the literature review and dosage regimen can be scheduled**.**

Parameters for anxiety

The behavioral effects of an acute or sub acute (10-day course) was orally administered. "*Aegle Marmelos*" (100 or 200 mg/kg) ethanolic and aqueous extracts were evaluated in mice by Light Dark Model (LDM) and Hole Board Test (HBT). The effect of diazepam (DZP; 1 mg/kg) was also assess.

Light-dark model: - The testing apparatus consists of a light and a dark chamber divided by photocell equipped zone. A polypropylene animal cage, 44x 21x21 cm. is darkened with black spray over one-third of its surface. A partition containing a 13 cm long x 5 cm high opening separates the dark one third from bright two thirds of the cage. An electronic system used four sets of photocells across the partition automatically counts numbers of crossings and the time spent in the light and dark compartments. Naïve male mice and rats are placed into cage. The animals will be treated 30 min before the experiment with the test drugs or vehicle and then observed for 10 min.[22]

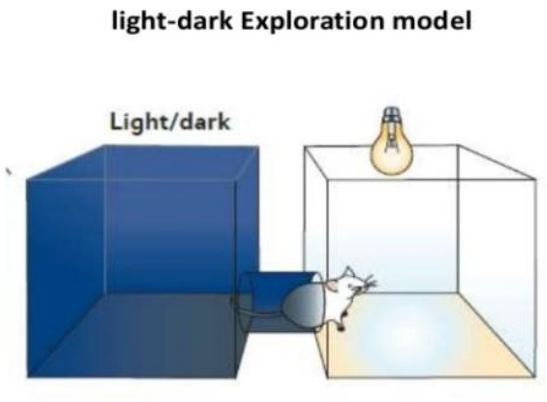


Fig. 1: Light dark model.

Hole board test:- The Hole board consists of wooden chamber (40 × 40 × 25 cm) with 16 holes (diameter of 3 cm) on the floor, elevated from the ground so that the mice could peep through the holes. Each mouse was placed individually in the apparatus for recording the parameters like latency to the first head dips, no. of head dips in the holes, total time spent with the head dips, no. of rearing, no. of defecation units.[23]

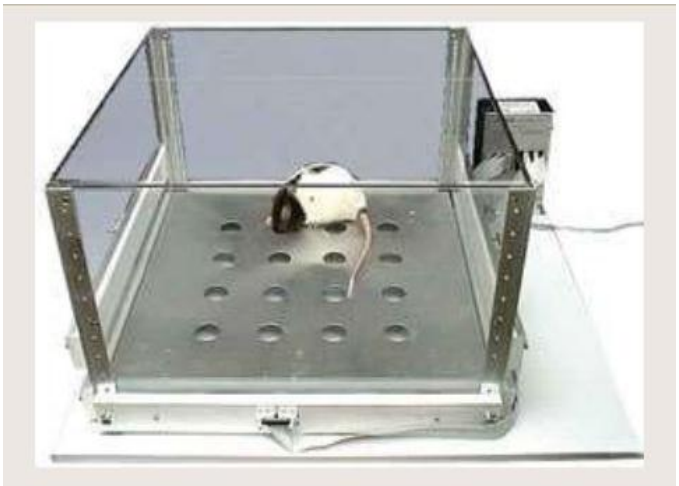


Fig. 2: Hole board test.

Biochemical estimation

Collection of blood samples

On 15th day, blood (0.3 ml) was withdrawn from tail vein from all groups of mice. Blood samples were centrifuged at 2500 rpm for 10 min using refrigerated centrifuge (Paramount scientific works, Ambala cantt, India) to separate the plasma, which was used for estimation of corticosterone levels.

Estimation of plasma corticosterone levels

The quantitative estimation of corticosterone levels in the blood plasma was performed by the method of Bartos and Pesez, 1979. To 1.0 ml of sample in ethanol, 0.50 ml of 0.10 % solution of p-nitroso-N, N-dimethylaniline in ethanol was added and the tubes were immersed in ice water for 5 min, and then 0.50 ml of 0.10 N sodium hydroxide was added. The tubes were plugged with cotton-wool, and were let to stand at 0°C for 5 h, protected against light. To the above solution, 2.0 ml of buffer for pH 9.8, 5.0 ml of 0.10 % solution of phenol in ethanol and 0.50 ml of 1.0 % aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at 20±2°C for 10 min. The solution was read at 650 nm using UV-visible spectrophotometer (UV 3200 UV-VIS Spectrophotometer, Somajiguda, Hyderabad).[24]

Statistical analysis

All the results were expressed as standard error of mean (S.E.M.). Data was analyzed using one-way ANOVA (Graph pad prism version 5.00 software) followed by suitable post test Dunnett's t-test. $p<0.05$ was considered as statistically significant.

RESULTS

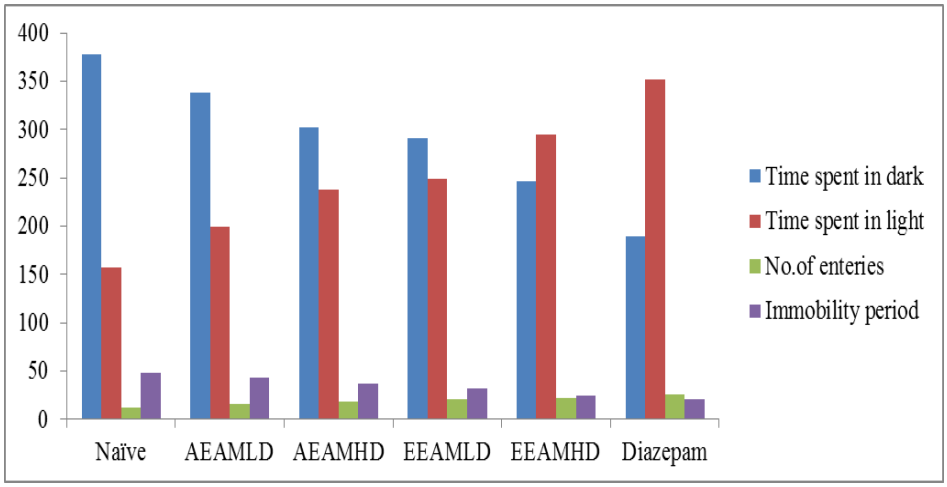
Light-dark model

Observation table: - Table 1: Showing effects of different doses of *Aegle marmelos* in anxiolytic activity as compared to standard (diazepam).

S. No.	Groups for treatment	Dose (mg/kg)	Time spent		No. of entries	Immobility period (sec.)
			In Light (sec.)	In Dark (sec.)		
1	Naïve	Normal saline	157±1.00	377.6±0.20	12±0.12	48.6±1.60
2	AEAM	100	199.3±0.34	338.1±1.02*	16±1.20*	42.8±1.95
3	AEAM	200	237.3±0.51*	302.6±1.52*	18±2.00*	37.1±0.65*
4	EEAM	100	248.8±2.54*	291±0.52*	21±1.96*	32.5±0.24*
5	EEAM	200	294.3±1.04**	246.1±2.00**	22±1.02**	24.6±0.09**
6	Diazepam	1	351.3±0.24***	189.3±0.23***	26±0.24***	21.3±0.16***

When compared with the control group. All values represent = Mean ± SEM, n = 6/7 in each group.

*P<0.05, **P<0.01, ***P<0.001



Graph 1: Showing effects of different doses of Aegle marmelos in anxiolytic activity as compared to standard (Diazepam).

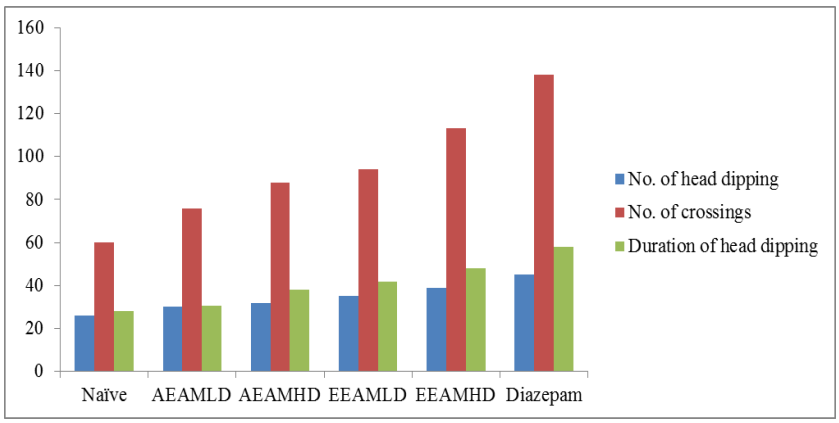
Hole board test

Observation table: - Table 2: Showing effects of different doses of *Aegle marmelos* in anxiolytic activity as compared to standard (diazepam).

S. no.	Groups for treatment	Dose (mg/kg)	No. of head dipping	No. of crossings	Duration of head dipping (in sec.)
1	Naïve	Normal saline	26±2.42	60±3.50	28±2.80
2	AEAM	100	30±2.50	76±3.57*	30.5±2.92
3	AEAM	200	32±2.85	88±3.62*	38.1±3.50*
4	EEAM	100	35±3.25*	94±3.76*	41.6±3.65*
5	EEAM	200	39±3.75**	113±3.80**	48±3.82**
6	Diazepam	1	45±3.80***	138±3.92***	58.1±4.52***

When compared with the control group. All values represent = Mean ± SEM, n = 6/7 in each group.

*P<0.05, **P<0.01, ***P<0.001



Graph 2: Showing effects of different doses of Aegle marmelos in anxiolytic activity as compared to standard (Diazepam).

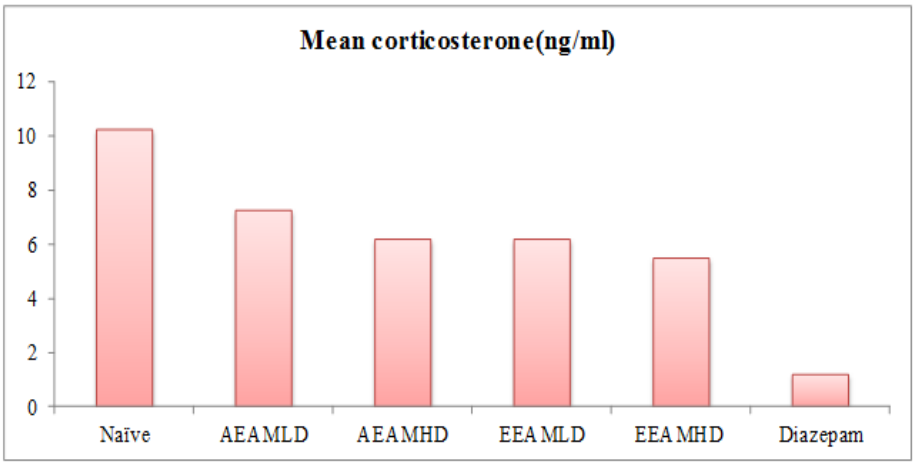
Estimation of plasma corticosterone levels

Observation table: - Table 3: Showing effect of different doses of *Aegle marmelos* on Plasma corticosterone level as compared to standard (diazepam).

S. No.	Groups for treatment	Dose (mg/kg)	Mean corticosterone (ng/ml)±SE
1	Naïve	Normal saline	10.26± 0.51
2	AEAM	100	7.25± 1.45
3	AEAM	200	6.21±2.26*
4	EEAM	100	6.20±1.29*
5	EEAM	200	5.50±0.30**
6	Diazepam	1	1.22±0.34***

When compared with the control group. All values represent = Mean ± SEM, n = 6/7 in each group.

*P<0.05, *P<0.01, **P<0.001



Graph 3: showing effects of different doses of Aegle marmelos on Plasma corticosterone level as compared to standard (Diazepam).

DISCUSSION

The neurobiology of anxiety disorder is not fully known. Anxiety disorders are due to involvement of GABAergic, serotonergic, involvement. The adrenergic and dopamanergic system have also been shown to play a role in anxiety. Dysregulation of the GABAergic, serotoninergic, dopaminergic and adrenergic neurosystems have all been implicated in the pathophysiology of anxiety.

In addition to GABA, 5-HT plays an important role in the development and the persistence of anxiety disorders. Many studies have shown that patient with anxiety disorder have genetic

polymorphism in the 5-HT transporter. Anxiety disorder can also be due to free radical induced damage to GABAnergic and serotoninergic systems.

The wide spread traditional use of Aegle marmelos for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrates that the Aegle marmelos extracts had anti-anxiety activity in mice by Light- Dark model and Hole board model. The choice of test methods not only determines effectiveness but in some instances also gives an indication of the mechanism(s) of the test substance.

The light/dark box is also widely used for screening anxiolytic or anxiogenic drugs in mice. Anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar and can be evaluated according to the number of transitions in to and the time spent in the light chamber, whereas increase in these parameters is considered to reflect anxiolytic-like properties. Reduction in the number of entries, time spent and rearing behavior in the light chamber is regarded as markers of anxiety. Our extracts of AM (100 and 200 mg/kg) showed significantly increased time spent in light box with significant reduction in immobility period.

The hole-board test provides a simple method indicates that head-dipping behavior is sensitive to changes in the emotional state of the animal and suggests that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behavior. In this study, extracts of AM significantly increased the number and duration of head dips with peak effects produced at doses of 100 and 200 mg/kg respectively, suggesting anti-anxiety effect.

The Plasma corticosterone level in mice is increased when they got anxiety. So when the plasma corticosterone level was checked after parameters of anxiety it turn out to be increased in control group and decreased in Diazepam.

The observed anxiolytic effect of the title plant may be due to the agonistic effect on GABA/benzodiazepine receptor complex or 5-HT1A receptor or antagonize the 5-HT1B receptor.

The present study showed that the Ethanolic extract of Aegle marmelos is more effective as compare to aqueous extracts, whereas less effective than standard drug (Diazepam) in both parameters of anxiety and corticosterone.

CONCLUSION

The present study was designed to evaluate the anti-anxiety activity of Aegle marmelos fruit extracts in mice using light-dark model and hole board test and results are compared with standard drug (Diazepam). Most of the drug as well as Diazepam acts through BZD receptors as an anxiolytic. It may be the probable mechanism action of our tested herbal compound. In LDM extracts of AM had showed decrease the time spent in the dark box and induced a significant increase in the time spent in the light box as well as the number of crossings between the light and dark boxes. In HBT extracts of AM had showed significantly increased number of head dipping, no. of crossings and increased duration of head dipping. Result indicates that Bael (*Aegle marmelos*) contains number of phytoconstituents in extracts like: - flavonoids, tannic acid, phenols, ascorbic acid, eugenol, alkaloids, coumarins (marmelosin, marmesin, marmin, imperatorin and scopoletin), skimmianine and saponin etc. Flavonoids present in the Aegle marmelos may be responsible for the anti-anxiety activity in present study.

In conclusion Aegle marmelos fruit extracts showed significant anti-anxiety activity probably due to GABA facilitatory action of phytoconstituents such as flavonoids, tannic acid, marmesinin, phenols, saponin etc. The particular compound had not been isolated whose responsible for Anxiolytic activity. Further work would be required to establish the exact mode of action and its active ingredient responsible for anti anxiety activity.

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REFERENCES

1. Barbotte, E., Guillemin, F., Chan, N., & Group, L. Prevalence of impairments, disabilities, handicaps and quality of life in the general population: a review of recent literature. The International Journal of Public Health (Bulletin of World Health Organization), 2001; *79*: 1047-1055.
2. Kjernised KD, Bleau P. Long-term goals in the management of acute and chronic anxiety disorders. Can J Psychiatry, 2004; 49(1): 515-655.
3. Weinberger DR. Anxiety at the Frontier of molecular medicine. N Engl J Med. 2001; 344(16): 1247-9.

4. Cle´ment Y, Calatayud F, Belzung C. Genetic basis of anxiety-like behaviour: A critical review. Brain Res Bull, 2002; 57: 57-71.
5. Neeraj G and Dinesh D. Neurochemical modulation of anxiety disorders. International Journal of Pharmacy and Pharmaceutical Sciences, 2010; 2 (1).
6. Philippe N. Anxiety disorders and GABA neurotransmission: A Disturbance of modulation. Neuropsychiatric Disease and Treatment.
7. Gani R and Bhat Z. A. Anxiety Disorders and Herbal Medicines. International Journal of Pharmaceutical Sciences and Research, 2018; 9(3): 844-853.
8. Arzoo and Parina K. Natural gifts for treating anxiety. International Journal of Institutional Pharmacy and Life Sciences, 2017; 7(6).
9. Almokhtar A. Adwas, J.M. Jbireal and Azab Elsayed Azab. Anxiety: Insights into Signs, Symptoms, Etiology, Pathophysiology, and Treatment. East African Scholars J Med Sci, 2019; 2(10): 580-591.
10. Cloos JM, Ferreira V, Curr Opin Psychiatry, 2009; 22: 90–95.
11. Mrs. Rajwinder K, Dr. Neha T, Ms. Seju P, Mrs. Vanita M. A review article for herbal drugs as anxiolytic activity. International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR), 2017; 5(2).
12. Sharma Ganesh N, Dubey Susheel K et al. International Journal of Current Pharmaceutical Review and Research, 2011; 1(3): 12-22.
13. Sharma Prabodh C, Bhatia V et al, A Review on Bael Tree. Natural Product Radiance, 2007; 6(2): 171-178.
14. Maity P, Hansda D et al, Indian Journal of Experimental Biology, 2009; 47: 849-861.
15. Sanyogita S, Ajeet S, Navneet. Ethnomedicinal, Pharmacological and Phytochemistry of Aegle marmelos (linn.) Corr: A Review. Int. J. Pharm. Sci. Rev. Res., 2019; 55(1): 85-90.
16. Abhijit D, Neeta L, Musarrat N, Abhijeet G and Rupa V. Ethnological and Ethno-medicinal Importance of Aegle marmelos (L.) Corr (Bael) Among Indigenous People of India, 2014; 1.
17. Mathew G, Lincy J and Sreelakshmi R. Phytochemical and pharmacological screening of in vivo anti-inflammatory activity of Aegle marmelos (L.) Corr. Serr, 2016.
18. Sunita Verma, R. Pharmacological potential study on bael (Aegle marmelos): Rutaceae, 2017.
19. Vijayalakshmi S. And Venkatalakshmi P. An Updated Review on the Phytopharmacological Significance of Aegle Marmelos (L.). World Journal of Pharmaceutical Research, 2017; 6(16).

20. Jayesh M, Smita N, Shraddha K. Formulation And Evaluation Of Antibacterial Activity of an Herbal Ointment Prepared From Crude Extracts Of Aegle Marmelos, (Bael). Int J Pharm Pharm Sci, 2014; 6(2).
21. The Organization of Economic Co-Operation and Development (OECD). The OECD Guideline for Testing of Chemicals: 423 Acute Oral Toxicity, OECD, Paris, 2001; 1-14.
22. Springer, H. Gerhard Vogel (Ed.), drug discovery and evaluation (pharmacological assay), 2nd edition: Page no, 430.
23. Kavaya Sree K, Vijusha M, Rajani A and Hemamalini K. Screening of Behavioural, Muscle Co-ordination and Anxiolytic activities of Methanolic extract of Holoptelea Integrifolia (Roxb), November, 2013.
24. A. Mukherjee and S. Banerjee; Effect of Metabolic Syndrome on Anxiety in Mice. Indian Journal of Pharmaceutical Sciences, 2018; 80(3): 453-459.