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Evaluation Of Anti Anxiety Activity of Methanol Extract of Aegle Marmelos (Bael Fruit Tree) Leaves in Rats

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Abstract: *Anxiety is one of the most common mental disorders, characterised by changes in mood, behaviour, somatic function, and cognition. Benzodiazepines and SSRIs are most commonly employed drugs for the treatment of anxiety. Synthetic drugs available for treatment of anxiety have various adverse effects. Drugs obtained from natural sources are known to cause fewer side effects compared to synthetic drugs despite of same ability to cure disease.*

Methanol extract of Aegle marmelos (AM, 70mg/kg, 140mg/kg and 210mg/kg), 2% of gum acacia, diazepam 2mg/kg are administered orally to randomly divided albino rats of either sex. Anxiolytic activity is assessed by Elevated plus maze (EPM) and Actophotometer (locomotor activity) models.

In EPM and Actophotometer models unpaired t test was used to assess anxiolytic activity between groups. In EPM total number of entries and time spent on open arm are increased in AM and standard compared to control. AM (210mg/kg) has shown significant increase in open arm entries ($p<0.03$) and time spent in open arm compared to standard. AM (210 mg/kg) has shown statistically significant decrease in locomotor activity ($p<0.01$) compared to standard in actophotometer model. Other doses of AM (70mg/kg, 140mg/kg) have shown significant difference compared to control but not statistically significant with standard in both models.

Conclusion: *Methanolic extract of Aegle marmelos showed significant anxiolytic activity at higher doses (210mg/kg).*

Key words: *Aegle marmelos, Anxiolytic activity, Elevated plus maze, Actophotometer,*

I. Introduction:

Anxiety is one of the most common mental disorders, which affects more than 10-15% of the population [1]. Anxiety is an adaptive response which prepares person to face challenges in life [2]. Anxiety disorders are characterised by changes in mood, behaviour, somatic function, and cognition Symptoms of anxiety are commonly associated with depression, panic disorder, agoraphobia and other specific phobias, obsessive-compulsive disorder, eating disorders, and some personality disorder(1). Currently benzodiazepines and SSRIs are most commonly employed drugs for the treatment of anxiety [1].

Aegle marmelos (AM) is an ayurvedic medicinal tree commonly known as the bael fruit tree, bilva patra in local language (Kannada) is found all over India [3]. This is also known as golden apple tree, stone apple tree. The tree has various medicinal properties. Several studies on different parts of AM showed that the plant has antidiarrheal , anti diabetic, anticancer, radio protective, antifungal, antimicrobial, anti micro filarial, anti-inflammatory, antipyretic and analgesic activities[3,4].

Synthetic drugs available for treatment of anxiety have various adverse effects like drowsiness, fatigue, tolerance, hypersensitivity reactions (2). Drugs obtained from natural sources are known to cause fewer side effects compared to synthetic drugs despite of same ability to cure disease. Therefore this study is undertaken to evaluate the antianxiety property of methanol extract of Aegle marmelos leaves in rats.

II. Objectives:

- To evaluate the antianxiety activity of the methanol extract of Aegle marmelos leaves in albino rats.

III. Materials And Methods:

Plant materials:

The leaves of Aegle marmelos were collected from their natural habitat in davangere city Karnataka state, India.

Preparation of extract:

The shade dried leaves of AM were powdered using a mechanical grinder. Coarse powder of 300 g was successively extracted with 1.5 L of petroleum ether, chloroform and methanol, in a soxhlet apparatus at 60–70° C temperature each for 10–12 h [3, 7]. Petroleum ether and chloroform are used for defatting following which

methanol is used for extraction. Methanol extracted fluid is kept in hot water bath till methanol is completely gets evaporated and semisolid mass is obtained. The yield of methanol extract is weighed and stored in desiccator till it is used for experiment.

Standard drug used: Diazepam

Selection of animals:
Inclusion criteria:

- Healthy albino rats weighing 200-250 g of either sex with normal behaviour and activity[1].
- Animals from institutional animal house (JJM Medical College, Davangere Karnataka.) were used for the study.

Exclusion criteria:

- Pregnant and diseased animals are not included in the study.
- Animals used for other experiments within 4 weeks

Duration of study: 2 months
Instruments required:

- Elevated plus maze
- Actophotometer

IV. Procedure:

Elevated plus maze (EPM) test:
Principle: This model is based on natural behaviour of rodents for open spaces and fear of height. Rodents always tend to avoid the open areas and stay in darker areas, more enclosed spaces. When animal is placed on EPM anxious animals spend more time in enclosed arms and non-anxious animals explore and spend more time on open arms.

- The plus-maze consists of two open arms, 43 × 15 cm (L ×W), and two enclosed arms, 43 × 15×23cm (L ×W×H), opened to the top, arranged in such way that the two open arms are faced opposite to each other. The maze is elevated to a height of 70 cm.
- The rats weighing 200–250gms body weight are randomly selected irrespective of sex and grouped into 6 groups so that each group consisting of 6 rats .(LD50 of AM is >2000mg/kg in rats)[6].

    1. Normal saline/ gum acacia(2%) 10ml/kg (control)
    2. Diazepam 2mg/kg (standard)
    3. AM 70mg/kg (t1)
    4. AM 140mg/kg (t2)
    5. AM 210mg/kg (t3)

- After one hour of oral administration of the test drug or the standard, the rat is placed at the centre of the maze, facing towards one of the enclosed arms[5].
- After 5min of observation the following parameters are noted:
    1. The number of entries into open arm and closed arms
    2. Time spent in the open and enclosed arms

Digital actophotometer:
Principle: A continuous beam of light from six lights was made to fall on corresponding photoelectric cells, the photoelectric cells will get activated when an animal crossed the beam of light and thereby cuts off (crossing) the rays of light falling on it. These crossings are counted automatically for a period of 10 min by the machine. The number of cuts off was taken as a parameter of the locomotor activity of the rats.

- Rats (200-250 g) are randomly selected into 6 groups to receive study drugs (6 rats in each group).
    1. Normal saline/ GA(2%). 10ml/kg (control)
    2. Diazepam 2mg/kg (standard)
    3. AM 70mg/kg (t1)
    4. AM 140mg/kg (t2)
    5. AM 210/kg (t3)

- Rats are placed in the digital actophotometer 1 hr after drug administration and number of crossings are counted for a period of 10 min and noted.

Digital Actophotometer

Statistical analysis

In EPM and Actophotometer models unpaired t test was used to assess anxiolytic activity between groups. Results are represented as mean & SD. P values less than 0.05 were considered as significant.

V. Results:

Actophotometer model

Methanol extract of AM at the doses of 70mg/kg and 140mg/kg did not show any statistically significant changes ($p>0.05$) compared to standard (Diazepam), though there is significant decrease in the locomotor activity (Fig. 1) compared to control group ($p<0.001$) and AM at the dose of 210 mg/kg has shown statistically significant change with ($p<0.01$) compared to standard and control group suggesting significant anxiolytic activity (table1&2).

Elevated plus Maze model:

In this model different doses (70 mg/kg, 140 mg/kg, and 210 mg/kg) of methanol extract of AM have shown significant, dose dependent increased open arm activity by increase in time spent (Fig. 2) and number of entries into open arms compared to control (table 1&2). AM at 210 mg/kg has shown statistically significant increase in the number of entries into open arm ($p<0.03$) compared to standard and significant increase in the time spent in open arm which was statistically not significant compared to standard.

VI. Discussion:

Present study showed that AM attenuated anxiety parameters in both elevated plus maze test and actophotometer model. Different doses (70 mg/kg, 140 mg/kg, 210 mg/kg) of AM has shown significant, dose dependent increased open arm activity by increasing time spent in and number of entries into open arms in elevated plus maze test and significant decrease in the locomotor activity in actophotometer. Anxiolytic activities of AM at the dose of 210 mg/kg were comparable with that of standard. Anxiolytic activity of diazepam is due to its GABA facilitatory action through GABA-A receptors[1, 2], as pharmacological profile of methanol extract of AM was similar to that of benzodiazepines in our study, it is possible that methanol extract of AM might possess similar mechanism of action.

Various studies have shown presence of flavonoids in phytochemical screening of Aegle marmelos, are responsible for anxiolytic effect of AM through benzodiazepine receptors[3, 4]. Therefore, flavonoids present in the Aegle marmelos may be responsible for the anti-anxiety activity in present study. Further studies are required to know the exact mechanism responsible for antianxiety activity.

AM treated group showed significant anxiolytic activity as shown by standard anxiolytic drug diazepam. Various studies on AM have shown presence of phyto constituents other than flavonoids like tannic acid, phenols, marmesinin, ascorbic acid, eugenol, skimmianine and saponin etc which may possesses anxiolytic properties[2, 3]. Aegle marmelos can be a safe and effective drug for the treatment of number of anxiety disorders, more extensive study is necessary to determine the exact mechanism of action of the extract and its active ingredients.

VII. Figures and Tables:

Figure.1

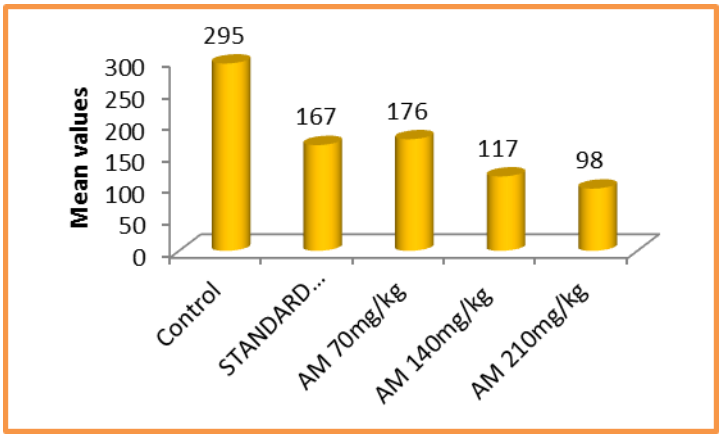

Fig. 1: Effects of GA (control), Diazepam (Standard), AM (70mg/kg, 140 mg/kg, 210 mg/kg) on locomotor activity (mean values) in actophotometer. P (<0.01) is significant in AM (210 mg/kg) treated group. n=6.

Figure. 2

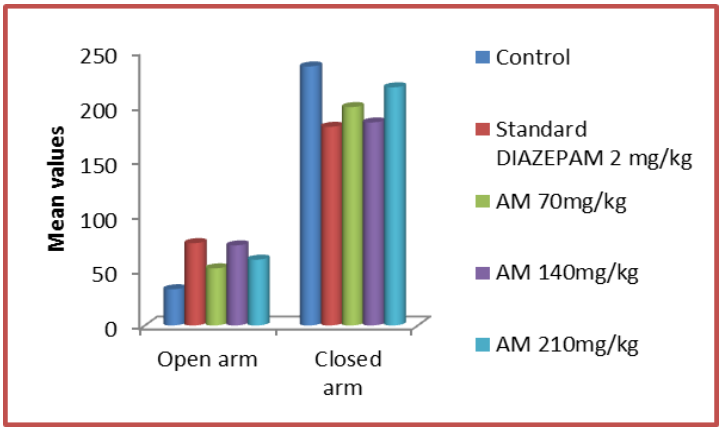

Fig. 2: Effects of control (2%GA), Standard (Diazepam 2mg/kg) and AM (70 mg/kg, 140 mg/kg, 210 mg/kg) treated groups on mean duration of time spent in seconds, in EPM model

TABLE.1: Effects of Diazepam, AM (70 mg/kg, 140mg/kg, 210mg/kg) in actophotometer and EPM. Results are expressed as Mean value± SD (standard deviation). n=6 (no. of animals in each group), GA- Gum acacia, DZ- Diazepam, AM- Aegle Marmelos

		ACTOPHOTO METER	**ELEVATED PLUS MAZE**			
Groups		Locomotor activity	Number of entries in		Time spent in	
			Open arm	Closed arm	Open arm	Closed arm
Group-A	Control (2%GA)	295.17 ± 32.59	2.33±1.21	2.50±1.87	32.67±24.92	236.17±28.34
Group-B	Standard (DZ)	166.67 ± 53.30	4.50±1.87	4.17±2.14	74.83±42.62	180.5 ± 16.68
Group-C	AM 70mg/kg	175.83 ± 35.49	3.83±0.98	2.83±1.83	52.33±19.92	198.83±42.76
Group-D	AM 140mg/kg	117.17 ± 38.76	3.83±1.47	3.00±1.41	72.67±29.32	184.50±26.26
Group-E	AM 210mg/kg	98.00 ± 8.39	2.33±1.03	2.00±0.89	59.50±14.73	217.17±15.75

TABLE.2: Comparison of results between the groups (control, standard and test groups) using unpaired t test and significance.

Group comparison	**Unpaired t test & Significance**				
	Locomotor activity	Mean Number of entries in		Time spent in	
		Open arm	Closed arm	Open arm	Closed arm
A Vs B	5.03, P<0.001	2.38, P<0.03	1.44, NS	2.09,P<0.06	4.14,P<0.002
A Vs C	6.07, P<0.000	2.36, P<0.04	0.31, NS	1.5, NS	1.78, NS
A Vs D	8.61, P<0.000	1.92, NS	0.52, NS	2.55,P<0.02	3.26,P<0.008
A Vs E	14.35, P<0.000	00, NS	0.59, NS	2.27,P<0.04	1.43, NS
B Vs C	0.35, NS	0.77, NS	1.16, NS	1.17, NS	0.98, NS
B Vs D	1.84, NS	0.68, NS	1.11, NS	0.11, NS	0.31, NS
B Vs E	3.11, P<0.01	2.48, P<0.03	2.29, P<0.04	0.83, NS	3.91,P<0.003

NS- Not significant (p>0.05) compared to other group, P value less than 0.05 is significant.

VIII. Conclusion:

The present study was designed to evaluate the anxiolytic effect of methanol extract of Aegle marmelos in rats using actophotometer and elevated plus maze models and results are compared with standard drug (Diazepam).

Methanol extract of Aegle marmelos has shown significant anxiolytic activity in both EPM and actophotometer models. AM administration at all the three doses (70mg/Kg, 140mg/Kg, 210 mg/kg p.o) have shown dose dependent significantly increased open arm activity in EPM by increasing time spent and number of entries into open arms and decreased the number of entries into closed arm as compared to those of control. In actophotometer model, results have shown significant dose dependent decrease in locomotor activity compared to control and standard. AM treated groups showed anxiolytic activity as shown by standard drug Diazepam and results are comparable in both EPM and Actophotometer models.

In conclusion methanol extract of Aegle marmelos showed significant anxiolytic activity probably due to GABA facilitatory action of phytoconstituents such as flavonoids, tannic acid marmesinin, phenols, saponin etc. Hence Aegle marmelos may become potential resource for natural psychotherapeutic agent against various anxiety related disorders with fewer side effects compared to current therapy.

Further, extensive studies are required to determine the exact mechanism and its active ingredient responsible for antianxiety activity.

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