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ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITIES OF METHANOL EXTRACT OF *AEGLE MARMELOS* LEAVES IN MICE

SAROJ KOTHARI*, MANISH MINDA AND S. D. TONPAY

Department of Pharmacology,
Gajara Raja Medical College,
Gwalior – 474 009

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Abstract : The objective of the present study was to evaluate the anxiolytic and antidepressant activities of methanol extract of *Aegle marmelos* (AM) leaves as well as its interaction with conventional anxiolytic and antidepressant drugs using elevated plus maze and tail suspension test in mice. Albino mice were treated with AM (75, 150 and 300 mg/kg, po), imipramine (20 mg/kg, po), fluoxetine (20 mg/kg, po), and combination of sub-effective dose of AM with imipramine or fluoxetine. Effects were observed on (a) time spent on (b) number of entries into (c) number of stretch attend postures (d) number of head dips in arms of elevated plus maze and on duration of immobility in tail suspension test. Effects of pre-treatment with prazosin (0.062 mg/kg, po), haloperidol (0.1 mg/kg, po) and baclofen (10 mg/kg, po) were also studied on AM induced decrease in duration of immobility. Effects of AM (75, 150 and 300 mg/kg po) were observed on locomotor activity using photoactometer. Results showed that AM significantly ($P<0.05$) and dose dependently increased proportionate time spent on and number of entries into open arms while decreased number of stretch attend postures and head dips in closed arms. Dose dependent and significant ($P<0.05$) anti-immobility effect was found in mice treated with AM. Combination of AM (75 mg/kg, po) with imipramine (5 mg/kg, po) or fluoxetine (5 mg/kg, po) also produced significant ($P<0.05$) anxiolytic and antidepressant activity. Antidepressant activity of AM (150 mg/kg, po) was significantly ($P<0.05$) decreased by prazosin, haloperidol and baclofen. Methanol extract showed insignificant ($P>0.05$) effect on locomotor activity of mice. It is concluded that AM possess potential anxiolytic and antidepressant activities and it enhances the anxiolytic and antidepressant activities of imipramine and fluoxetine.

Key words : *Aegle marmelos* anxiolytic antidepressant elevated plus maze tail suspension test

INTRODUCTION

Anxiety and depression are the most common stress related mood disorders, causing disability and premature death (1). Despite shortcomings, the monoamine hypothesis of anxiety and depression-like states remains largely accepted (2). Most of

*Corresponding Author : Dr. (Mrs.) Saroj Kothari, 2-A J.A. Hospital Campus, Near Medical College Square, Lashkar, Gwalior – 474 009; Email : saroj.kothari@rediff.com; Ph.: 91-751-4082868; Mob.: 09827322002

the antidepressant drugs are found effective against anxiety disorder in addition to depression (3). Advances made in neuroscience suggest that dysfunction of the GABAergic system in addition to monoamine deficit contributes to the pathophysiology of anxiety and depression (4). $GABA_B$ receptor antagonists have antidepressant-like potential and have also been shown to increase 5-hydroxytryptamine and dopamine neurotransmission (5). Synthetic drugs available for treatment of anxiety and depression have various adverse effects including drowsiness, ataxia with benzodiazepines and insomnia, libido with selective serotonin reuptake inhibitors (6). Drugs obtained from natural sources are perceived to have fewer side effects while having same ability to cure disorders in much the same way as their synthetic counterparts. Recently the search for novel pharmacotherapy from medicinal plants for psychiatric illness has progressed significantly and thus revealed pharmacological effectiveness of different plant species in a variety of animal models (7).

Aegle marmelos (AM) a highly reputed ayurvedic medicinal tree commonly known as the bael fruit tree is found all over India. The tree is endowed with various medicinal properties. Several studies on different parts of AM showed that the plant possess antidiarrhoel (8), antidiabetic (9), anticancer (10), radioprotective (11), antifungal (12), antimicrobial (13), antimicrofilarial (14), antiinflammatory, antipyretic and analgesic activities (15). AM has been used in nervous disorder and as tonic for brain (16, 17). Phytochemical analysis of AM leaves have shown to contain several bioactive compounds including essential oils (e.g. marmenol, β-caryophyllene, α-humulene), triterpenoids (e.g. lupeol, β and γ-sitosterol), flavonoid (e.g. rutin), alkaloids (e.g. rutacin, aegeline, aegelinine), coumarins (e.g. marmesinin, umbeliferone), condensed tannins, anthocyanins and flavonoid glycosides (15, 18–21).

Despite the widespread uses of the plant, no scientific work is reported in literature regarding the effect of AM leaves against anxiety-depression like states therefore, present study was undertaken to evaluate anxiolytic and antidepressant effects of methanol extract of *Aegle marmelos* (AM) leaves using elevated plus maze test and tail suspension test in mice. Studies were also conducted to find its interaction with conventional anxiolytic and antidepressant drugs in order to elucidate its role in modulation of central monoamines.

MATERIALS AND METHODS

Plant materials

The leaves of *Aegle marmelos* were collected from their natural habitat from Gwalior city, Madhya Pradesh, India in the month of April 2008. The plant leaves were identified by Dr. K. K. Koul, Professor & Head, Department of Botany, Jiwaji University, Gwalior, India and a voucher specimen (skam/08) has been retained in our laboratory for further reference.

Preparation of extract

The shade dried leaves were powdered using a mechanical grinder and passed through 40 mesh sieve. Powder (300 g) was successively extracted with 1.5 L of

petroleum ether, chloroform and methanol, in a soxhlet apparatus at 60–70°C each for 10–12 h consecutively. Solvents used were of analytical grade. Methanol was removed from the extract under vacuum and a semisolid mass was obtained. The yield of methanol extract was 11.50% (w/w). It was stored in sterile amber coloured storage vials in refrigerator until used for experiment.

Selection of animals

Albino mice weighing 20-25 g of either sex raised in institutional animal house (G.R. Medical College, Gwalior, M.P.) were used for the study. They were maintained under standard laboratory condition on 12 h day/night cycle and with free access to food (Pranav Agro Industries, Delhi, India) and water. The animals were acclimatised to the laboratory conditions prior to experimentation. All the experiments were carried out between 10.00 h to 16.00 h at ambient temperature. The animals were drawn at random for test and control groups. Institutional animal ethics committee constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiment on Animals approved the protocol. (Registration number 846/ac/04/CPCSEA)

Elevated plus maze test (EPM)

The elevated plus maze test is a rapid and selective technique (22) for detecting anxiolytic drug effects under identical conditions. For more sensitive measures of effects of new anxiolytic compounds, risk assessment behaviors (behavior related to anxiety/fear) such as stretch attend postures and head dips were also measured in addition to measure of time spent on and number of entries into arms in the EPM (23). The plus maze was in the shape of a cross or plus with two closed arms each with roof open measuring 30×5×20 cm, extending from a central region (5×5 cm) running along a north-south axis and two open arms each measuring 30×5 cm running east-west. The wooden apparatus was elevated to a height of 50 cm from the floor in a dimly illuminated room. Mice were placed individually in the central area of the maze with open access to any arm. The amount of time spent on and number of entries in both open and closed arms whereas numbers of stretch attend postures and head dips in closed arms were measured manually during the 5 min test period. An arm entry was defined as all four feet in the arm. Stretch attend posture was defined as mice stretching forward and then retracting to original position from closed (protected) or open (unprotected) arms. Head dipping is defined as mice protruding the head over the edge of closed or open arms down towards floor. The apparatus was cleaned after each mouse was tested to remove any residue or odour. For the purpose of analysis, open arm stay was quantified as the amount of time that the mouse spent in open arm relative to the total amount of time spent in open and closed arm (open/total×100) and open arm entries were quantified as the number of entries in open arm relative to the total number of entries in open and closed arm (open/total×100).

Tail suspension test (TST)

Tail suspension test is behaviour despair model of depression, employed in rodents to predict antidepressant potential by decreasing immobility period produced by several different classes of antidepressant

drugs (24). It has been reported that tail suspension test is less stressful and has higher pharmacological sensitivity than forced swim test, the other commonly employed model to study antidepressant activity (25). Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min period. The animal was considered immobile when it did not show any movement of the body except for those required for respiration and hanged passively.

Digital photoactometer

Mice were placed in the digital photoactometer (MAC, New Delhi, India) 1 h after drug administration. A continuous beam of light from six lights was made to fall on corresponding photoelectric cells, the photoelectric cell got activated when an animal crossed the beam of light and thereby cuts off the rays of light falling on it. These cut-offs were counted automatically for a period of 10 min and the figure was taken as a measure of the locomotor activity of the animal (26).

Drugs

Imipramine, fluoxetine, prazosin, baclofen (Sun Pharmaceutical Industries Baroda), haloperidol (RPG Life Sciences Ltd., Mumbai), diazepam (Ranbaxy) were purchased and used in the study.

Vehicle

All the drugs and extract used in the study were suspended in 2% gum acacia and were administered orally to the respective animals 1 h prior to start of experiment. The volume for oral administration was 10 ml/kg.

Experimental design

Different groups of mice were used to explore antianxiety, antidepressant and locomotor activity. To ensure consistency of experience prior to the test session, animals were brought to the testing room 1 h prior to the start of behaviour testing. Test room lighting, temperature and noise level were kept constant for all mice used in the study. Mice were divided into various groups according to the treatments they received. Each group consisted of 6 animals. Individual mice were subjected to test 1 h after drug administration. Mice were tested in EPM and TST only once in order to avoid influence of repeated experience on anxiolytic and antidepressant activities of drugs.

Drug treatments

Mice were treated with methanol extract of AM at the dose of 75, 150, 300 mg/kg for dose dependent effect against EPM induced anxiety-like behaviour, TST induced depression-like behaviour and on locomotor activity. Doses of AM were selected on the basis of earlier studies conducted using methanol extract of AM for evaluation of its analgesic activity (27). To compare the effects of test drug with standard anxiolytic drugs mice were treated with diazepam (2 mg/kg), imipramine (20 mg/kg) and fluoxetine (20 mg/kg). To compare effects of test drug with standard antidepressant drugs mice were treated with imipramine (20 mg/kg) and

fluoxetine (20 mg/kg). To assess influence of AM on anxiolytic activity of standard drugs, mice were treated with diazepam (0.5 mg/kg), imipramine (5 mg/kg), fluoxetine (5 mg/kg) alone and in combination with AM (75 mg/kg). Similarly to assess influence of AM extract on antidepressant activity of standard drugs mice were treated with imipramine (5 mg/kg), fluoxetine (5 mg/kg) alone and in combination with AM (75 mg/kg). To assess interaction with alpha$_1$, dopaminergic and GABA$_B$ receptors, AM (150 mg/kg) were administered after ½ h to each group of the animals treated with prazosin (0.062 mg/kg), haloperidol (0.1 mg/kg), baclofen (10 mg/kg) and effects were observed on duration of immobility in TST. Mice treated with vehicle (2% gum acacia) at the dose of 10 ml/kg, served as control group.

Statistical analysis

Results are represented as Mean±SEM. All the data were analyzed using one-way ANOVA followed by Tukey multiple comparison tests. P values <0.05 were considered as statistically significant.

RESULTS

Effect of AM against EPM induced anxiety-like behaviour in mice

Results of the present study showed that methanol extract of AM at the dose of 75 mg/kg did not show significant change (P>0.05) in mean time spent on and in mean number of entries into open arms as well as in mean number of protected stretch attend postures and head dips as compared to control (Table I). This suggests that AM at the dose of 75 mg/kg is sub effective. AM at the dose of 150 and 300 mg/kg showed proportionate increase by 20 and 26 percent respectively in mean time spent on and by 15 and 27 percent respectively in mean number of entries into open arms while decrease by 29 and 55 percent respectively in mean number of protected stretch attend postures and by 37 and 68 percent respectively in mean number of protected head dips as compared to control mice. Anxiolytic activities of AM (150 and 300 mg/kg) were dose dependent and significant (P<0.05) as compared to control and AM (75 mg/kg) (Table I).

TABLE I: Effects of AM, diazepam, imipramine and fluoxetine in the elevated plus maze.

Treatment (mg/kg)	*Time spent (seconds)*		*Number of entries*		*Number of SAP*	*Number of HD*
	Open arms	*Closed arms*	*Open arms*	*Closed arms*	*Open arms*	*Closed arms*
GA10 ml/kg	21.66±3.63	220.50±2.99	0.16±0.16	4.83±0.47	12.16±0.60	8.00±0.57
AM 75	24.00±3.07	211.66±3.02	0.33±0.21	7.00±0.36	11.83±0.60	7.33±0.44
AM 150	46.66±4.66*[a]	185.00±2.90*[a]	2.50±0.22*[a]	13.66±0.42*[a]	8.66±0.42*[a]	5.00±0.54*[a]
AM 300	61.33±2.45*[ab]	166.66±2.47*[ab]	4.33±0.42*[ab]	11.50±0.36*[ab]	5.50±0.42*[ab]	2.58±0.41*[ab]
DZ 2	68.66±2.89*[ab]	132.50±4.61*[ab]	4.50±0.42*[ab]	10.83±0.30*[ab]	5.33±0.42*[ab]	2.08±0.58*[ab]
IMI 20	61.66±2.80*[ab]	146.33±2.62*[ab]	3.33±0.21*[ab]	8.16±0.30*[ab]	5.16±0.47*[ab]	2.25±0.44*[ab]
FLU 20	67.83±2.81*[ab]	130.00±5.08*[ab]	4.80±0.47*[ab]	10.66±0.42*[ab]	4.33±0.4*[ab]	2.00±0.57*[ab]

Values are expressed as Mean±SEM, n=6, *P<0.05 as compared to GA treated group. [a]P significant as compared to AM (75 mg/kg) treated group [b]P<0.05 as compared to AM (150 mg/kg) treated group using ANOVA and Tukey multiple comparisons test SAP = stretch attend postures HD = head dips GA = gum acacia AM = *Aegle marmelos* DZ = diazepam, IMI = imipramine, FLU = fluoxetine

Administration of diazepam (2 mg/kg), imipramine (20 mg/kg) and fluoxetine (20 mg/kg) caused proportionate increase by 34, 30 and 34 percent in mean time spent on and by 27, 29 and 31 percent in mean number of entries into open arms respectively. Same treatments showed decrease by 57, 58 and 64 percent in mean numbers of protected stretch attend postures and by 74, 71 and 75 percent in mean number of protected head dips respectively as compared to control (Table I). The anxiolytic activity of standard drugs was significant (P<0.05) as compared to control and AM (75 and 150 mg/kg).

Effect of combination of sub-effective dose of AM with that of standard drugs against EPM induced anxiety-like behaviour in mice

Administration of diazepam (0.5 mg/kg), imipramine (5 mg/kg) and fluoxetine (5 mg/kg) did not show significant change in mean time spent on and mean number of entries into open arms as well as in mean number of stretch attend postures and mean number of head dips in closed arms as compared to control (P>0.05) suggesting these doses of standard drugs to be sub-effective. Similar non significant (P>0.05) changes on all the parameters in EPM were produced by combination of sub-effective dose of AM (75 mg/kg) with that of diazepam (0.5 mg/kg) as compared to control, AM (75 mg/kg) and diazepam (0.5 mg/kg) alone suggesting AM is not potentiating diazepam in EPM test (Table II). Sub-effective dose of AM (75 mg/kg) in combination with that of imipramine (5 mg/kg) showed proportionate increase by 27 percent in mean time spent on, and by 26 percent in mean number of open arm entries while decrease by 61 percent in mean number of stretch attend postures and by 72 percent in mean number of head dips as compared with control (Table II). Antianxiety activity of AM with imipramine was significant (P<0.05) as compared to control, AM (75 mg/kg) and imipramine (5 mg/kg) alone. Sub effective dose of AM (75 mg/kg) with that of fluoxetine (5 mg/kg) also

TABLE II: Effects of combination of sub-effective dose of AM with that of diazepam, imipramine and fluoxetine in the elevated plus maze.

Treatment (mg/kg)	*Time spent (seconds)*		*Number of entries*		*Number of SAP*	*Number of HD*
	Open arms	*Closed arms*	*Open arms*	*Closed arms*	*Open arms*	*Closed arms*
GA10 ml/kg	21.66±3.63	220.50±2.99	0.16±0.16	4.83±0.47	12.16±0.60	8.00±0.57
AM 75	24.00±3.07	211.66±3.02	0.33±0.21	7.00±0.36	11.83±0.60	7.33±0.44
DZ 0.5	23.00±1.89	218.83±3.71	0.33±0.21	7.16±0.87	11.33±0.55	7.16±0.57
IMI 5	22.50±0.76	218.00±2.86	0.40±0.32	8.16±0.94	11.50±0.42	7.10±0.30
FLU 5	23.16±2.05	223.66±3.06	0.50±0.22	7.35±0.95	11.08±0.66	6.60±0.66
AM 75+DZ 0.5	22.66±1.85	219.83±3.20	0.41±0.20	6.50±0.76	11.25±0.40	6.25±0.44
AM 75+IMI 5	62.50±1.38*[ab]	168.83±2.37*[ab]	3.66±0.36*[ab]	10.16±0.33*[ab]	4.80±0.49*[ab]	2.54±0.27*[ab]
AM 75+FLU 5	65.16±2.25*[ac]	166.83±2.71*[ac]	4.00±0.36*[ac]	9.33±1.50*[ac]	4.00±0.53*[ac]	2.29±0.45*[ac]

Values are expressed as Mean±SEM, n=6
*P<0.05 as compared to GA treated group
[a]P<0.05 as compared to AM (5 mg/kg) treated group
[b]P<0.05 as compared to IMI (5 mg/kg) treated group
[c]P<0.05 as compared to FLU (5 mg/kg) treated group
using ANOVA and Tukey multiple comparisons test
SAP = stretch attend postures HD = head dips GA = gum acacia
AM = *Aegle marmelos* DZ = diazepam, IMI = imipramine, FLU = fluoxetine

showed proportionate increase by 27 and 26 percent in mean time spent on and mean number of entries into open arms respectively whereas decrease by 67 and 75 percent in mean number of stretch attend postures and in mean number of head dips respectively in closed arms as compared to control. Antianxiety activity of AM in combination with fluoxetine was significant (P<0.05) as compared to control, AM (75 mg/kg) and fluoxetine (5 mg/kg) alone. These results suggest that AM is potentiating effects of imipramine and fluoxetine in EPM (Table II).

Effect of AM against TST induced depression-like behaviour in mice

Mice treated with AM (75 mg/kg) did not show significant decrease in duration of immobility in TST (P>0.05). Animals received AM (150 and 300 mg/kg) showed decrease by 23 and 30 per cent respectively in mean duration of immobility as compared to control. Anti-immobility activity due to AM (150 and 300 mg/kg) were dose dependent and significant (P<0.05). Mice treated with imipramine (20 mg/kg) and fluoxetine (20 mg/kg) showed decrease by 32 and 34 per cent respectively in mean duration of immobility as compared to control (Fig. 1). The antidepressant effect of standard drugs were significant (P<0.05) as compared to control, AM (75 and 150 mg/kg).

Effect of combination of sub-effective dose of AM with that of standard drugs against TST induced depression-like behaviour in mice

Administration of imipramine (5 mg/kg) and fluoxetine (5 mg/kg) did not show significant change in duration of immobility as compared to control (P>0.05) suggesting

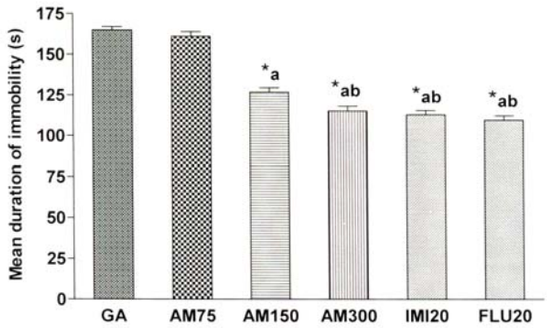

Fig. 1: Effects of *Aegle marmelos* (75, 150, 300 mg/kg), imipramine (20 mg/kg) and fluoxetine (20 mg/kg) on mean duration of immobility in seconds in tail suspension test. Values are expressed as Mean± SEM, n=6 *P<0.05 as compared to GA (10 ml/kg) treated group [a]P<0.05 as compared to *Aegle marmelos* (75 mg/kg) treated group [b]P<0.05 as compared to *Aegle marmelos* (150 mg/kg) treated group using ANOVA and Tukey multiple comparisons test.
GA = gum acacia, AM = *Aegle marmelos*, IMI = imipramine, FLU = fluoxetine

these doses to be sub effective. Combination of sub-effective dose of AM with that of imipramine showed decrease by 30 per cent in mean duration of immobility as compared to control. Antidepressant activity of AM with imipramine was significant (P<0.05) as compared to control, AM (75 mg/kg) and imipramine (5 mg/kg) alone. AM at the dose of (75 mg/kg) in combination with fluoxetine (5 mg/kg) produced decrease by 34 percent in mean duration of immobility as compared to control, Antidepressant activity of AM with fluoxetine was significant (P<0.05) as compared to control, AM (73 mg/kg) and fluoxetine (5 mg/kg) alone. These results show that AM is potentiating effects of imipramine and fluoxetine in TST (Fig. 2).

Effect of prazosin, haloperidol and baclofen pre-treatment on immobility period due to AM in TST

Treatment with single dose of prazosin (0.062 mg/kg) showed significant (P<0.05) 32

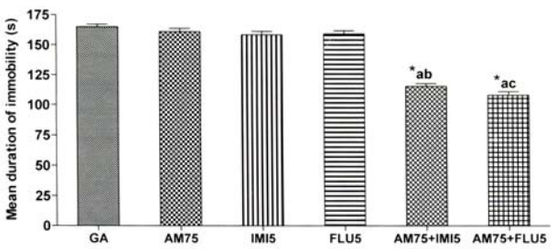

Fig. 2 : Effects of combination of sub-effective dose of *Aegle marmelos* (75 mg/kg) with that of either imipramine or fluoxetine (5 mg/kg) on mean duration of immobility in seconds in tail suspension test. Values are expressed as Mean±SEM, n=6. *P<0.05 as compared to GA (10 ml/kg) treated group [a]P<0.05 as compared to *Aegle marmelos* (75 mg/kg) treated group; [b]P<0.05 as compared to imipramine (5 mg/kg) treated group; [c]P<0.05 as compared to fluoxetine (5 mg/kg) treated group; using ANOVA and Tukey multiple comparisons test GA = gum acacia, AM = *Aegle marmelos*, IMI = imipramine, FLU = fluoxetine

percent increase in mean duration of immobility as compared to control. Pre-treatment with prazosin (0.062 mg/kg) showed significant (P<0.05) increase in mean duration of immobility by 47 percent as compared to AM (150 mg/kg) alone (Table III). Haloperidol (0.1 mg/kg) showed significant increase in mean duration of immobility by 37 percent as compared to control (P<0.05). Pre-treatment with haloperidol (0.1 mg/kg) caused significant 57 percent increase in mean duration of immobility as compared to AM (150 mg/kg) alone (P<0.05). Mice treated with baclofen showed significant increase by 11 percent in mean duration of immobility as compared to control (P<0.05). Pre-treatment with baclofen (10 mg/kg) caused significant (P<0.05) increase by 27 percent in mean duration of immobility as compared to AM (150 mg/kg) alone. Result suggesting that pre-treatments with prazosin, haloperidol and baclofen significantly attenuated effect of AM in TST (Table III).

TABLE III : Effects of prazosin, haloperidol and baclofen pre-treatment on AM induced decrease in duration of immobility in mice.

Treatment	*(mg/kg, po)*	*Duration of immobility(s)*
Vehicle (GA)	10 ml/kg	164.83±2.22
AM	150	126.83±2.72*
Prazosin	0.062	217.00±3.47*
Prazosin+AM	0.062+150	178.5±3.19[a]
Haloperidol	0.1	225.66±3.25*
Haloperidol+AM	0.1+150	198.66±1.99[a]
Baclofen	10	183.50±2.27*
Baclofen+AM	10+150	161.66±2.84[a]

Values are expressed as means±SEM, n=6
* P<0.05 as compared to GA (10 mg/kg) treated group
[a] P<0.05 as compared to AM (150 mg/kg) treated group
GA = gum acacia; AM = *Aegle marmelos*

Effect of AM leaf extract on locomotor activity in mice

AM extract at the dose of (75, 150 and 300 mg/kg) did not show any significant change (P>0.05) in the locomotor function of mice, as compared to the control (Table IV).

TABLE IV : Effects of AM on locomotor activity in mice.

Treatment (mg/kg, po)	*Number of counts*
GA 10 ml/kg	628±4.58
AM 75	632±7.84 NS
AM 150	634±5.65 NS
AM 300	647±8.06 NS

Values are expressed as Mean±SEM, n=6
NS = not significant (P<0.05) as compared to GA treated group
GA = gum acacia AM = *Aegle marmelos*

DISCUSSION

Elevated plus maze is well established paradigm has a long and successful history in assessing .anxiety-like behaviour in rodents. The model is based on natural aversion of rodents for open spaces (afraid possibly of falling off). Rodents tend to avoid the open areas, especially when they are brightly lit, favouring darker, and more enclosed spaces. Inconsistent results with anxiolytic compounds and a desire for more targeted therapeutic treatments suggest that

scoring additional, ethologically relevant behavioural indicators (e.g. stretch attend postures and head dips) may provide more sensitive measures of the effects of new anxiolytic compounds. This ethological approach overcome locomotor confounds and has increased the value of plus maze as important tool to study anxiolytic activity (28). Present study showed that AM attenuated anxiety parameters in the elevated plus maze test. AM administration at the dose (150 and 300 mg/kg) significantly and dose dependency increased open arm activity by increasing time spent on and number of entries into open arms while decreased risk assessment behaviour by decreasing number of stretch attend posture and head dips. Anxiolytic activities of AM (300 mg/kg) were comparable to standard drugs diazepam, imipramine and fluoxetine.

Tail suspension test is widely employed animal model for testing antidepressant activity. It is based on the principle that suspending mice upside down leads to a characteristic behaviour of immobility after initial momentary struggle. This behaviour reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression (24). In the present study, methanol extract of AM at the dose of 150 and 300 mg/kg administered to mice 1 h before they were subjected to tail suspension test showed dose dependent significant anti-immobility activity. The antidepressant activity of AM at the dose of 300 mg/kg were comparable to imipramine and fluoxetine.

Combination of sub-effective dose of AM (75 mg/kg) with that of diazepam (5 mg/kg) did not show significant anxiolytic activity ($P>0.05$) suggesting that methanol extract of leaves of AM did not potentiate effect of diazepam in EPM. It has been well investigated and established that anxiolytic activity of diazepam is due to its GABA facilitatory effect on $GABA_A$ receptors (29) and result of present study show non involvement of $GABA_A$ receptors in anxiolytic activity of AM. Combination of sub-effective dose of AM (75 mg/kg) with either imipramine (5 mg/kg) or fluoxetine (5 mg/kg) produced dose dependent increase in open arm activity and decrease in risk assessment behaviour in EPM and decrease in duration of immobility in mice subjected to TST after single dose. These results suggest that AM enhances anxiolytic as well as antidepressant effects of imipramine and fluoxetine.

Imipramine prevents reuptake of nor adrenaline and serotonin resulting in their increased availability in the synapse and therefore an increase in adrenergic and serotonergic neurotransmission (30). Fluoxetine is selective serotonin reuptake inhibitor facilitates serotonergic neurotransmission (31). Since catecholamine and 5-hydroxytryptamine is implicated in etiology of anxiety and depression, the positive effect of these drugs in EPM and TST seems to be due to increased availability of these neurotransmitters at the post synaptic receptor sites. Anxiolytic and antidepressant activity of AM achieved at sub-effective level in combination with imipramine or fluoxetine suggest involvement of AM extract in increasing monoamines level at post synaptic sites.

AM extract did not show significant changes in the locomotor activity of mice as compared to the control value ($P>0.05$) so the extract did not produce any motor effects. This indicates that increased motor activity was not involved in the action seen in EPM and TST. Further, it also confirms the assumption that

anxiolytic and antidepressant effects of AM are specific.

Earlier studies have reported involvement of alpha$_1$ receptors in mediating antidepressant activity of agents which facilitate noradrenergic neurotransmission and it is attenuated by pre-treatment with alpha$_1$ receptor blocker prazosin (32). In accordance to this present study showed that antidepressant activity of AM was significantly reversed by pre-treatment of animals with prazosin suggesting interaction of AM extract with alpha$_1$ receptors. Decreased dopamine neurotransmission is also linked with depression and studies demonstrated that blockade of dopamine receptor by haloperidol increased duration of immobility (33). In this study, pre-treatment with haloperidol also significantly reversed antidepressant action of AM this may be attributed to the interaction of AM extract with dopamine receptors. Moreover, recent increase in understanding of the molecular mechanism of depression and anxiety has provided alternative molecular targets for these disorders. In particular receptors, within GABA systems provide a diversity of drug targets and molecular, biological and behavioural studies of these receptors have revealed the important role they play in anxiety and depression (34). In support of this it is reported that GABA$_B$ receptor antagonist increased swimming behaviour in forced swim test suggesting that blockade of GABA$_B$ receptors may serve as novel strategy for the treatment of depression (35). In contrast to this GABA$_B$ receptor agonist baclofen is reported to exacerbate depression-like behaviour in animal model of depression (36). Our results showed that pre-treatment with baclofen significantly attenuated antidepressant activity of AM, it is tempting to suggest interaction of AM with GABA$_B$ receptors.

In conclusion, methanol leaf extract of *Aegle marmelos* showed significant anxiolytic and antidepressant activities possibly by increasing monoamines level at post synaptic sites. Hence *Aegle marmelos* may be served as a potential resource for natural psychotherapeutic agent against stress related disorders such as anxiety and depression.

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