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AN EXPERIMENTAL STUDY TO EVALUATE THE EFFECTS OF *AEGLE MARMELOS* AND IT'S COMPARISON WITH PIRACETAM ON LEARNING AND MEMORY IN WISTAR RATS

Himani [1], Shankar Pratap [1], R. C. Verma*[2], Sharad Leve [1], Amod Sachan [1] and R.K. Dixit [1]

Department of Pharmacology, K.G.M.U. [1], Lucknow, Uttar Pradesh, India
Department of Pharmacology, R.I.M.S. [2], Safai, Etawah, Uttar Pradesh, India

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Correspondence to Author:

Dr. R. C. Verma

Lecturer, Department of Pharmacology, R.I.M.S, Safai, Etawah, Uttar Pradesh, India

E-mail: rakeshverma.kgmu@gmail.com

ABSTRACT: Present study was done to evaluate the memory enhancing effect of *Aegle marmelos* and its comparison with the present standard treatment Piracetam in Wistar rats. The learning and memory was assessed using the elevated plus maze. The findings of the present study indicate that, *Aegle marmelos* extract treated animals at a dose of 100 mg/kg p.o. as well as 200 mg/kg p.o. showed significant decrease in transfer latency from 6th day to 7th day ($p<0.05$), but the percentage reduction was more with higher dose. When compared to the control group, this reduction was statistically significant ($p<0.05$). These findings demonstrate the facilitatory effect of *Aegle marmelos* on learning and memory (nootropic activity). This may be attributed to the involvement of neurotransmitters like acetylcholine, serotonin, noradrenaline and dopamine, which play a vital role in the cognitive functions.

INTRODUCTION: Cognition is the physiological process of knowing, including awareness, perception, reasoning, and judgment. Cognitive functions mainly categorized into memory, attention, creativity and intelligence. It is subjective in nature and can be affected by number of factors including ageing, stress, hypertension, various pathological conditions such as dementia, Parkinson's disease (PD), Alzheimer's disease (AD), schizophrenia, cancer and Human immunodeficiency virus (HIV).

Cognitive enhancement may be defined as the amplification or extension of core capacities of the mind through improvement or augmentation of internal or external information processing systems [1].

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Many different strategies are proposed to enhance cognition. Most interventions target either disease pathologies or the processes underlying normal cognition, particularly synaptic plasticity. Strategies and treatments for cognition enhancement are given as follows:

- Environmental enrichment and exercise
- Nutrients
- Herbal medicines
- Pharmaceutical drugs

There are various agents, which can help to improve or prevent memory loss known as nootropic drugs. Nootropic agents have selective facilitatory effect on integrative functions of the central nervous system particularly on intellectual performance, learning capacity and memory. Nootropic drugs such as piracetam, aniracetam and donezipil are being used for improving memory, mood and behaviour but the resulting side effects associated with them have limited their use in patients.

Memory can be divided into mainly three types:

- Short-term memory (lasts for seconds or at the most minutes),
- Intermediate long-term memory(lasts for days to weeks)
- Long-term memory (once stored, can be recalled up to years or even a lifetime later).

Now a days scientific interest in medicinal plants has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of allopathic medicines. *Aegle marmelos*, commonly known as a bael, is one of the gifts of nature to mankind. Numerous pharmacological studies have been conducted on different parts of *Aegle marmelos.* This plant is having great potential to cure the diseases like diabetes, hyperlipidemia, peptic ulcer, diarrhoea, dysentery, cancers etc. It has also shown its effect as cardio protective, anti bacterial, anti fungal, radio protective, anti pyretic, analgesic, antioxidant, hepatoprotective, and many more.

However, there are only few studies of *Aegle marmelos* pertaining to central nervous system activities. Hence in the present study, Neuropsychopharmacological effects of *Aegle marmelos (*Bael) (including antidepressant, nootropic, anxiolytic, analgesic activities and effect on motor function) were studied in wistar rats.

MATERIALS AND METHODS: The study was conducted in the Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow (Erstwhile Chhatrapati Shahuji Maharaj Medical University). Ethical clearance was obtained from the Institutional Animal Ethics Committee before conducting the study.

Experimental Animals & Rearing Conditions: Adult healthy Male Wistar rats weighing 160-200 gm had been used in study. Animals had been obtained from CPCSEA-certified animal house [Indian Institute of Toxicology Research, Lucknow (IITR)]. They were allowed to access normal rat pellet diet and water *ad libitum* and were kept in Institutional animal house under temperature controlled environment [25 ± 2°C] with 12 hours' light and dark cycle. The animals were housed for two weeks prior to the experiments to acclimatize to new environment.

The maintenance of the animals and the experimental procedures were in accordance with the guiding principles of Institutional Animal Ethics committee and the 'Guide for the Care and Use of Laboratory Animals', National Research Council, 1996 (Latest revision in 2011).

The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India were followed.

Dosage Forms, Doses and Sources of the Drugs: Following drugs were used in this study.

(1) Test Drugs - Bael (*Aegle marmelos*)- Drug (extract form) was dissolved in normal saline and administered orally with the help of feeding cannula in a doses of 100 mg/ kgbw and 200 mg/ kgbw [2]. It was purchased from market (Aegle marmelos extract, Himalaya Drug Company).

(2) Standard drugs - Piracetam : Dose 200 mg/kgbw i.p. [3].

Tablets were purchased from government authorized medical store.

Experimental Protocol: The present study had been designed to evaluate Neuropsychopharmacological effects of *Aegle marmelos* (Bael) that includes antidepressant, nootropic anxiolytic, analgesic activities and effect on motor function in male wistar rats.

Animal Groups: A total number of 24 Male Wistar rats were included in the study. They were kept in Institutional Animal House under standard conditions.

All the animals received normal rat pellet diet and water *ad libitum*. All the animals were allowed to get acclimatised to the new environment for period of 2 weeks.

Rats were randomly divided into 4 groups, each group containing 6 rats: All Groups were used to evaluate the effect of *Aegle marmelos* on learning and memory and its comparison with that of piracetam. Three weeks after the evaluation of effect on learning and memory, all groups were used to evaluate the effects of *Aegle marmelos* on anxiety. The effects were compared with those of diazepam.

Neuropsychopharmacological Evaluation: Adult male Albino Wistar rats weighing between 160- 200 gms were used. All the animals allowed to get to acclimatize to the new environment for period of 2 weeks. They were provided with normal rat pellet diet & water *ad libitum*. Following validated behavioural models of rodents were used to assess the neuropsychopharmacological effects of *Aegle marmelous* extract.

Assessment of learning and memory facilitating activity in rats using Elevated plus maze (EPM) model:

Grouping: Albino Wistar rats weighing between 160- 200 gms were randomly divided into 4 groups, each group containing 6 rats.

Group 1: Rats were administered normal saline p.o (1 ml)

Group 2: Rats were administered aegle marmelous extract (100 mg/kg) p.o.

Group 3: Rats were administered aegle marmelous extract (200 mg/kg) p.o.

Group 4: Rats were administered Standard drug (piracetam 200 mg/kgbw i.p)

Procedure: This test was used to assess the retention of learning and memory [4]. Transfer latency was assessed using the elevated plus maze (EPM). The EPM is made up of wood with two open arms (40 x 10 cm) and two closed arms with a wall (40 x 10 x 41 cm) and a central platform (10 cm×10 cm. The maze was kept in a dimly lit room elevated 50 cm above floor level. All arms were arranged in such a way that the two arms of each type were opposite to each other to give the apparatus a plus sign appearance.

For the assessment of transfer latency (TL) each animal was placed at the end of an open arm facing away from the central platform. Transfer latency was defined as the time taken by the animal to move into any one of the closed arms with all its four paws. If the animal did not enter into the one of the arm within 90secs, it was gently pushed into one of the covered arms and the TL was assigned as 90secs. Transfer latency was recorded with the help of a stop watch.

Rats were treated with respective doses (vehicle/ standard drug/ extract) for consecutive 6 days. On the first day (6^{th} day of drug treatment), each rats was placed at the end of an open arm facing away from the central platform and TL was recorded (in seconds) on that training day. The animals were allowed to explore to the maze for 10 secs and then returned to its home cage. Retention of this learned-task (memory) was examined 24hrs after the first day trial (i.e. on 7th day, 24 hr after last dose). Significant reduction in transfer latency value indicates improvement in memory [5].

During the entire experiment room level lighting was kept consistent. The procedure was conducted in a sound attenuated room. Every time before placing each animal the apparatus was thoroughly cleaned.

Assessment: Transfer latency was examined on 6th day and after 24hrs i.e. on 7th day. The mean for each group was determined and comparison was done between control and drug treated groups.

Oral drug administration: The desired doses of drugs were calculated on the basis of body weight and dissolved in normal saline just prior to administration. It was then loaded in a syringe fitted with a 16 G ball tipped feeding needle. The rat was restrained using a towel in the left hand. The head of the rat were restrained using the thumb and index finger whereas the tail of the rat was restrained between the ring and little finger. While the rat was lying on the left palm, the ball-tipped feeding needle was introduced from the side of mouth in to the pharynx and then let in to the esophagus when the animal was in the act of swallowing. The drug was then injected in to the esophagus of routs.

Statistical analysis: A one-sample Kolmogorov-Smirnov test was used to investigate whether the variables were normally distributed. The one way analysis of variance (ANOVA) was used to assess the comparability of the groups assigned to the treatment groups. Independent *t* test/Tuke's pairwise comparison was used to compare the different parameters like immobility time, transfer latency, open arm activity, number of visits, reaction time and duration of stay on rotarod between respective treatment groups. Differences in treatment effects within groups and between the treatment and control groups were tested by a multivariate analysis of variance repeated-measures design with treatment

type as a between-subject factor (2 groups) and treatment effect (baseline compared with follow-ups) as a within-subject factor. A significant *P* value for the treatment effect indicated a change over time in the combined values of the groups and was further investigated by using a paired *t* test for each individual group. Between group differences in treatment effect were indicated by significant interactions between treatment effect and treatment type. The percent change from baseline to follow-ups was also calculated for each group. Statistical significance was based on a two-tailed *P* value < 0.05.

OBSERVATIONS AND RESULTS:

Assessment of Learning and Memory: The learning and memory effect on different treatment group (Group 1: Control, Group 2: *Aegle marmelous* extract 100 mg/kg, Group 3: *Aegle marmelous* extract 200 mg/kg, Group 4: Piracetam 200 mg/kgbw) have been summarized in **Table 1**. The learning and memory was insignificantly different between Day 6 (30.50±2.42) and day 7 (31.00±3.52) in Group 1 (p>0.05). However, the learning and memory was significantly different between Day 6 (24.03±1.78) and day 7 (22.78±2.49) in group 2 (p<0.05). Similar observation was found for Group 3 and Group 4. There was 21.2% reduction from Group 1 to Group 2, 30.1% in group 3 and 57.1% in group 4 at day 6. Similar trend was found at day 7.

TABLE 1: EFFECT OF *AEGLE MARMELOUS* EXTRACT ON LEARNING AND MEMORY USING ELEVATED PLUS MAZE

Groups	DAY 6 Transfer latency (sec) (Mean±SD)	DAY 7 Transfer latency (sec) (Mean±SD)	p-value (Day 6 to Day 7)	Mean percentage change with respect to group 1	Mean percentage change with respect to group 2
Group 1	30.50±2.42	31.00±3.52	p>0.05	--	--
Group 2	24.03±1.78	22.78±2.49	<0.05*	21.2	26.5
Group 3	21.33±2.25	19.15±1.13	<0.05*	30.1	38.2
Group 4	13.08±1.74	11.17±1.47	<0.05*	57.1	64.0

* Statistically significant

The learning and memory was significantly different between Group 1 and Group 2 (p<0.05), Group 3 (p<0.05) and Group 4 (p<0.01) at 6 day. The learning and memory was significantly different between Group 2 and 3 (p<0.01) at day 6. The learning and memory was also significantly different between Group 3 and 4 (p<0.01) at day 6. Almost similar observation was found at day7 (**Table 2**).

TABLE 2: COMPARISON OF LEARNING AND MEMORY (ELEVATED PLUS MAZE) BETWEEN GROUPS

Groups		6 day		7 day	P-value
		Mean difference	p-value	Mean difference	
Group 1 vs	Group 2	6.46	<0.05*	9.67	<0.05*
	Group 3	9.16	<0.05*	11.85	<0.01*
	Group 4	17.41	<0.01*	19.83	<0.01*
Group 2 vs	Group 3	2.70	>0.05	2.17	>0.05
	Group 4	10.95	<0.01*	10.16	<0.01*
Group 3 vs	Group 4	8.25	<0.01*	7.98	<0.05*

* Statistically significant

DISCUSSION: Any mental illness significantly affects feeling, thinking and behaviour of person. Stresses of life can precipitate number of mental illnesses. Modern day life style leads to numerous stressful conditions. Moreover their prevalence is increasing day by day. Stress might influence learning-and-memory processes by suppression of adult neurogenesis or by affecting neurochemical systems (for example, catecholamines, opiates, glucocorticoids). Due to the various side effects of allopathic drugs used for treatment of these diseases, there is continuous search for alternative treatment. So it is prudent to look for options which are efficacious & safer. Indigenous system of medicine including natural herbs are time tested way of treatment. Herbal medicines emphasize the prevention of diseases, rejuvenation of our body systems, maintain balance and harmony and extend the life span [6]. Medicinal herbs are indispensible part of traditional medicine practiced all over the world due to easy access, low cost and ancestral experience. Number of plants have been being used for management of mental illness. Some of them are as follows:

- For treatment of anxiety- *Bacopamonniera* [7], *Citrus paradise* [8], *Azadirachta indica, Centella asiatica*;
- For treatment of depression- *Allium cepa* [9], *Bacopamonniera* [10], *Centella asiatica, Curcuma longa*
- For improving learning and memory- *Ginkgo biloba, Glycyrrhiza glabra, Piper longum, Bramhi, Shatavari, Shankhapushpi*.

Several activeconstituents, which can be of immense importance as drugs, are the precursors for synthesis of many drugs [11]. Their effectiveness, low cost and comparative freedom from serious toxic effects make these medicines not only popular but also an acceptable mode of treating diseases even in modern times. Due to the various unavoidable adverse effects of available allopathic medicines, management of various diseases without any untoward side effects is still a challenge for modern medical science. So several herbal plants having various bioactive phytochemicals, possessing several activities and no or very less adverse effects, have been explored.

Number of studies have shown beneficial effects of *Aeglemarmelos as* antiviral, antibacterial, antifungal, anticancer, antihyperlipidemic, antidiabetic and antioxidant agents. However, there are only few studies pertaining to neuropsychopharmacological actions of *Aegle marmelos*. Many phytoconstituents like flavonoids, saponins, quercetin, phenols, skimmianine and ascorbic acid have shown very important role in management of psychiatric illnesses.

The herbal plants which are used for treatment of various psychiatric illnesses in traditional medicines contains these phytoconstituents. Phytochemical screening of *Aegle marmelos* have shown the presence of many phytoconstituents including flavonoids, saponins, quercetin, phenols, skimmianine and ascorbic acid [12]. Hence, we hypothesised that, due to the presence of these important phytoconstituents similar to the other herbal plants being used for many psychiatric illnesses, *Aegle marmelos* could have the potential place in treatment of such type of illnesses.

The present study was undertaken to explore the Neuropsychopharmacological effects of *Aegle marmelos* (Bael) in wistar rats.

The dose of *Aeglemarmelos* was based on previous studies [2]. Extract form needs less amount to be administered, previous trials and experimental studies have been mostly performed using extract (ethanolic or aqueous) forms and also they are soluble in normal saline. Therefore we have chosen the extract-form in our study.

We have chosen the oral route for administering the herbs as a drug, as this route is natural & usual route of taking herbal drugs if prescribed by a physician. This route doesn't need assistance of others and is quite easy in terms of intake. A total number of 24 male Wistar rats were included in the study. Rats were randomly divided in to 4 groups, each group containing 6 rats. All the animals were allowed to get acclimatised to the new environment for period of 2 weeks. Group 1 to 4 were used to evaluate the effect of *Aeglemarmelos*on learning, memory and anxiety.

Learning is defined as the acquisition of information and skills. Subsequent retention of that information is called memory. In the present study elevated plus-maze model was used to assess the learning and memory in animals. Transfer latency (TL) was defined as the time taken by the animal to move from end of open arm into any one of the closed arms with all its four paws. TL of first day (at day 6) reflected learning behaviour of animals, whereas TL of second day (at day 7) reflected the retention of information or memory. Piracetam, a nootropic drug was used as a standard drug. Nootropics are a class of psychotropic agents with selective facilitatory effect on integrative function of the central nervous system, particularly on intellectual performance, learning capability and memory [13].

The findings of the present study indicate that, *Aeglemarmelos*extract treated animals at a dose of 100 mg/kg p.o. as well as 200 mg/kg p.o. showed significant decrease in transfer latency from 6th day to 7th day ($p<0.05$), but the percentage reduction was more with higher dose (Table 1). Also when compared to the control group, this reduction was statistically significant ($p<0.05$) (Table 2). These findings demonstrate the facilitatory effect of *Aeglemarmelos* on learning and memory (nootropic activity). This may be attributed to the involvement of neurotransmitters like acetylcholine, serotonin, noradrenaline and dopamine, which play a vital role in the cognitive functions) [14].

Piracetam treated animals also showed the significant decrease in transfer latency from 6th day to 7th day ($p<0.05$); the percentage decrease was more than both doses of *Aeglemarmelos*and control (table 1, 2) indicating improvement in learning and memory as consistent with the previous studies [3]. Piracetam is used as protective agent because of its antioxidant properties [15, 16].

The oxidative stresses, generation of free radicals, harmful by-products of oxidative metabolism are known to cause organic damage to the living system. It is hypothesized that increasing antioxidant levels in the organism might retard or reverse the damaging effects of free radicals on neurons.

*A. marmelos*has free radical scavenging activity. Its phytoconstituents Eugenol [17] and Marmesinin [18] , have potent antioxidant property. Also the antioxidant phytochemicals such as flavonoids, alkaloids, sterols, tannins, phlobotannins and flavonoid glycosides possess free radical scavenging activity [19]. In addition, these phytochemical (alkaloids, flavonoids and saponins) have been shown to possess nootropic activity and thereby support the aforementioned findings [20].

Piracetam activates brain adenylate kinase and produces a significant increase in the cerebral glucose utilisation in the whole brain of rats [21] Also in man, an increase in glucose utilisation was observed [22]. Piracetam has also been observed to increase the blood flow in some situations, probably because of decreased platelet aggregation, enhancement of red blood cell deformability and reduction in adherence of damaged erythrocytes to endothelial cells [23]. It is proposed that the beneficial effect of nootropics may be the result of improvement in cerebral circulation and brain metabolism [24]. Results like these have often led to the classification of the piracetam-1ike nootropics as cerebral metabolic enhancers.

Flavonoids and flavonoid-rich fruits are well reported to modulate neuronal signalling pathways crucial in inducing synaptic plasticity [25]. It has been postulated that effects of flavonoids in the brain are mediated by an ability to protect neurons against injury induced by neurotoxins and neuroinflammation. They also enhance existing neuronal function, stimulate brain blood flow and induce neurogenesis [26, 27].

Although the exact mechanism of action is not elucidated. But the reported pharmacological activities of *A. marmelos* extract could also be attributed to the presence of flavonoids.Hence, due to the presence of a number of phytoconstituents including flavonoids, quercetin, tannic acid, phenols, eugenol, marmesinin, ascorbic acid, skimmianine and saponinetc or any other mechanisms, *Aeglemarmelos* possessnootropic properties. *Aeglemarmelos*can be a safe and effective indigenous drug for the treatment of number of psychiatric disorders including anxiety and depression. However, a more extensive study is necessary to determine the exact mechanism of action of the extracts and its active compound(s).

CONCLUSION: The present study was designed to evaluate the neuropsychopharmacological effects of *Aegle marmelos* extract. From the results of present study following conclusions may be drawn -

- Findings of present study indicat the facilitatory effect of *Aeglemarmelos* on learning and memory (nootropic activity). *A. marmelos*extract treated animals at both the doses (100 mg/Kg, 200 mg/Kg, p.o) showed significant decrease in transfer latency on 6th and 7th day when compared to the control group, thus demonstrating the nootropic activity.

Higher dose was more effective than lower dose.

- *Aeglemarmelos* extract showed anxiolytic activity in elevated plus-maze and Y-maze models. *A. marmelos*extract administration at both the doses (100 mg/Kg, 200 mg/Kg, p.o) significantly increased open arm activity in EPM by increasing time spent and number of entries into open arms and decreased the number of visits in Y-maze, as compared to those of control. Effect of higher dose was more than lower dose.

Present study [an experimental study to evaluate the neuropsychopharmacological effects (nootropic) of *Aeglemarmelos* extract] shows that *Aeglemarmelos* possessesnootropicproperties. All these effects could be attributed a number of phytoconstituents including flavonoids, quercetin, tannic acid, phenols, eugenol, marmesinin, ascorbic acid, skimmianine and saponin. These phytoconstituents are also present in other herbal extracts which are in use since ages.

These phytoconstituents are supposed to be safe without any major adverse effects. These findings are in favour of using *A. marmelos* as memory enhancing drug. However the results from present study have limitations in the form of short duration of study, only one or two selected models and less number of animals. The other limitation of this study is lack of measurement of various biochemical parameters at various time intervals. Large scale animal study with inclusion of more animal models and biochemical parameters will strengthen the findings of present study. If *A. marmelos* passes through the positive results in animal study, clinical studies may be planned in future.

No wonder that *A. marmelos* will become a safe and effective indigenous drug for the treatment of number of psychiatric disorders including anxiety and depression.

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