Research Article

EVALUATION OF NOOTROPIC ACTIVITY OF AEGLE MARMELOS EXTRACT USING DIFFERENT EXPERIMENTAL MODELS IN RATS

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ABSTRACT
In the present study *A. marmelos* selected for evaluation of its anti-amnesic activity and also to study its influence on cholinergic system of the brain of rats, because there were no reporters on its anti-amnesic activity being evaluated pharmacologically. The electroshock (MES) induced amnesia model and scopolamine a well known anticholinergic drug was another model also used to produce loss of memory. Chronic exposure to MES for 7 days produced a significant decrease in latency to expose to electroshock grid in step down latency and increased the time of transfer latency in elevated plus maze. The same effect was also seen in scopolamine exposed animals, we found that there was a significant increase in acetylcholinesterase enzyme activity in MES exposed and also in Scopolamine exposed rats. Whereas, administration of ethanol extracts of leaf of *A. marmelos* simultaneously with MES and scopolamine exposure for 7 days prevented the impairment of memory consolidation and also reduced the Acetyl cholinesterase enzyme activity in all parts of the brain. Daily administration of extracts significantly attenuated the amnesic effect of both MES and scopolamine, which was also observed in performance of learned tasks in elevated plus maze and step-down apparatus.

Keywords: Acetyl cholinesterase, Anti-amnesic activity, Scopolamine, Transfer latency.

INTRODUCTION
It is believed that herbal drugs are relatively safe and exhibit a remarkable efficacy in the treatment of chronic ailments. According to an estimate, for nearly quarter are being used for medicinal purpose[1]. About 80% of people in developing countries depend on traditional systems of medicine for primary health care[2]. One fourth of the prescriptions filed by pharmacies each year are of substances derived from plants, and when the drugs obtained from microorganisms and animals are added in, the total rises to 40%. Some 120 chemicals extracted in pure form from about 90 species of higher plants are used in medicine throughout the world; a wide range of plant species being used locally for medicinal purposes[3].

Despite the vast availability of medicinal plants, *Aegle marmelos* plant selected in our study and the case for the collection of material and its unique importance in this area. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them.

Aegle marmelos belongs to the family Rutaceae. Its common name is Bilwa, the tree grows wild in dry forests on hills and plains of central and southern India and Burma, Pakistan and Bangladesh, also in mixed deciduous and dry dipterocarp forests of former French Indochina. It is a

medium sized tree (average height is 8.5 metres tall), with spines on the branches and very aromatic. It matures in about 10 years. Trunk is about 50 cms across. Bark grey outside, rough, peeling off irregularly into small flakes. Leaves are pale green, trifoliate. It is used in several indigenous drug preparations for general health and other disease conditions, has been shown to posses antimicrobial, antidiabetic, antipyretic, antidiarrhoeal, anti-inflammatory properties. More than 30 identified compounds from the leaves of *Aegle marmelos* have been reported[4-6].

The present study looking into scientific exploration of ethanol extract of *Aegle marmelos* as prospective anti-amnesic activity and also to study its influence on cholinergic system of the brain of rats.

MATERIALS AND METHODS
Plant material and preparation of the extract
The leaves of *Aegle marmelos* were collected in and around the Kuvempu University campus. The plant was authenticated by comparing with the herbarium voucher specimen deposited at Kuvempu University herbaria. The leaves were shade dried, powdered mechanically (sieve No. 10/44), 200g of powdered material was soaked in 100ml of ethanol for 48 h. It was filtered by using Whatman no.1 filter paper. The solvent was distilled out completely from the filtrate under the reduction pressure in Rota vapour

Animal collection
Adult male Albino Wistar rats weighing 150 – 200g were acclimatized in a well ventilated animal house condition and were fed with commercial feed. There were no significant differences in the body weights of the treated rats when compared with control, either at the beginning or at the end of the study period. Institutional animal ethics committee (IAEC) approved the experimental protocol and care of the animals was taken as per guidelines of CPCSEA Department of animal welfare, Govt. of India. All the animals were dosed once in a day with respective drugs for seven days. All drugs were given through

per oral route (p. o.) to all group animals as showed in Table 1.

The animals were trained on the 0 (zero) day and the acquisition of memory was tested on the day 1, later the animals were subjected to induction followed by drug treatment, that was continued for up to day 7. Then, the animals were subjected for the retention test on the day 7. Soon after the passive avoidance task (step–down latency) and elevated plus maze (transfer latency) the animals were sacrificed for Acetylcholinesterase enzyme estimation.

Screening tests for memory
a. Transfer latency
The maze was elevated to a height of 50cm, the animals were individually placed at the end of either of the open arms and the time taken for the animal to move from open to closed arm (Transfer latency, TL) was taken as the criterion of task. The animals were allowed to explore the apparatus for 30 seconds.

After 24 hours of the first exposure, TL was again noted on the day 1 of the study for determining the acquisition. Five minutes later the animals of Group 3, 5 and 6 received electroshock of 150mA for 0.2 seconds through a pair of ear electrode from an Electroconvulsiometer and then the animals were dosed with respective drug and kept in their home cage. Similarly, animals of Group 4, 7, and 8 received scopolamine (0.3mg/kg body weight) and then were dosed with respective drug and returned to their home cage. The electroshock/scopolamine and dosing with drug continued for up to 7 days and on 7th day the animals were subjected to the retention test 25min. after the last dose, for evaluating the transfer latency keeping the time period of 60 seconds as cut off criterion.
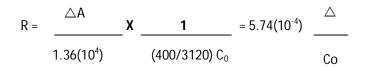
b. Step-down latency
Pole climbing apparatus chamber is used for passive avoidance response where pole is replaced by a wooden platform fixed on electrified grid floor. When rats stepped off the platform, they receive a continuous foot shock from grid floor. The normal reaction of rat was to jump back to the wooden platform. After about 4-5 trials the animals

acquired the passive avoidance response and they refrained from stepping down. The criterion was reached when the animal remained on the platform for at least 60 seconds.

The animals were then subjected for the passive avoidance test on the day 1, and the time was noted down for step down latency taking 60 seconds as cut-off period. Five minutes later again the process is repeat as shown in Transfer latency method.

c. Acetylcholinesterase enzyme activity

Exactly 60 minutes after the electroshock and scopolamine treatment the rats were decapitated by gillette, and the whole brain were taken out quickly. The cerebral cortex, cerebellum, medulla oblongata and midbrain were dissected out then suspended in phosphate buffer and weighed accurately. The different regions of the brain viz. cortex, cerebellum, medulla oblongata and midbrain were homogenized in a tissue homogenizer. To this, 100µl of Ellman's reagent was added and then taken into the photocell. The absorbance was set to zero at 412nm. 20µl of the substrate (Acetyl thicholine iodide) was added. A change in the absorbance per minute was noted. The rate of moles of substrate hydrolyzed per minute per gram of tissue was later calculated as per the following equation.

$$R = \frac{\triangle A}{1.36(10^4)} \times \frac{1}{(400/3120)\, C_0} = 5.74(10^{-4}) \frac{\triangle}{C_0}$$

Where

$\triangle A$ = Change in absorbance per minute (mean change in absorbance from the 1st to 7th minute was taken).
C_0 = Original concentration of the tissue.
R = Rate in moles substrate hydrolysed per minute per gram of tissue.

Statistical Analysis

The step-down latency and transfer latency were analysed using the Student's paired't' test (two tailed). A probability level of $P<0.01$ was considered as significant.The rat brain acetyl cholinesterase activity of different groups were analysed using ANOVA, followed by Dunnett's 't' test for individual comparision of groups, viz.

1. Normal control group vs. Negative control group (MES induced and Scopolamine induced).
2. Normal control group vs. Positive control group.
3. Negative control – MES induced group vs. MES induced- Treatment group.
4. Negative control – Scopolamine induced group V/S scopolamine induced - Treatment group.

Probability level of $P<0.0001$ for One way ANOVA was considered as significant, and for post test (Dunnett's 't'test), a probability level of $P<0.01$ was considered as significant.

RESULTS
Nootropic activity of *Aegle marmelos*

Transfer Latency: The animals were subjected to transfer latency to evaluate the retrieval of memory in behavioral paradigm after a period of 7 days of acquisition trial, to know the effect of extracts on the long term memory.

The normal control animals produced highly significant ($P<0.0001$) retrieval of memory in behavioral paradigm. In the positive control group, the animals treated with Standard drug (Mentat) reduced the time taken in transfer latency, did not produced significant activity. In the

negative control group, the animals exposed to MES and Scopolamine produced significant (P<0.01) loss of memory in behavioral paradigm. In the treatment group, the animals exposed to MES and treated with *A. marmelos* leaf extract, MES and treated with Standard drug (Mentat) produced significant (P<0.01) activity. In the treatment group, the animals exposed to scopolamine and treated with *A. marmelos* leaf extract and scopolamine exposed, treated with Standard drug (Mentat) reduced the time taken to perform the task in elevated plus maze it did not produce moderately significant retrieval of memory in behavioral paradigm (Table 2).

Step-down Latency
The animals were subjected to step-down latency for evaluation of retrieval of memory in Passive Avoidance Task (PAT) after a period of 7 days of acquisition trial, to know the effect of extracts on the long term memory. The normal control animals produced highly significant (P<0.0001) retrieval of memory in passive avoidance task (PAT). In the positive control group, the animals treated with Standard drug (Mentat) showed highly significant (P<0.0001) retrieval of memory. In the negative control group, both MES and scopolamine exposed animals produced significant (P<0.0001), loss of memory in PAT. In the treatment group, the animals exposed to MES and treated with leaf extract of *A. marmelos* produced significant (P<0.01) retrieval of memory, where as Standard drug (Mentat) increased the step-down latency period but of no significance retrieval was recorded. In the treatment group, the animals exposed to scopolamine and treated with leaf extract of *A. marmelos* showed significant (P<0.01) activity, whereas Standard drug (Mentat) treated animals produced highly significant (P<0.0001) retrieval of memory in PAT (Table 2)

Acetylcholinesterase enzyme activity
The animals were sacrificed at the end of the study period of 7 days after last dose by evaluating Step-down latency and Transfer latency, to dissect and isolate the brain. Then different parts of brain were dissected

and subjected for the estimation of Acetyl cholinesterase enzyme activity and the results were expressed as Mean±SEM (moles X10^{-6}/minute/gram of tissue)

The animals of positive control group, negative control (MES exposed) and negative control (scopolamine exposed) groups were compared with normal control group, whereas the MES induced – treatment group was compared to negative control scopolamine induced group (Table 3).

The animals of positive control group treated with Standard drug (Mentat) (3.110±0.08327, 3.200±0.1400, 3.437±1.032 and 2.400±0.1531) produced significant (P<0.01) reduction of Acetylcholinesterase enzyme activity in comparison with normal control (7.387±0.3078, 9.223±0.2955, 9.783±0.5349 and 3.930±0.4251) in different parts of brain viz. cortex, medulla, midbrain and cerebellum respectively (Table 3).The animals of negative control group exposed to MES (9.307±0.2143, 11.31±0.2857, 12.53±0.1904 and 7.420±0.3205) and animals exposed to scopolamine (8.927±0.2392, 11.20±0.1444, 13.58±0.1910 and 6.367±0.2834) produced significant increase in Acetylcholinesterase enzyme activity as compared to the normal control group which produced significant (P<0.01) loss of memory (Table 3).

In the treatment group, the animals exposed to MES and treated with leaf extract of *A. marmelos* (5.563±0.3993, 3.287±1.303, 5.583±1.197 and 2.663±0.2437) and MES induced Standard drug (Mentat) (4.887±1.178, 4.213±0.6444, 4.130±0.6245 and 1.680±0.2117) significantly (P<0.01) decreased the Acetylcholinesterase enzyme activity in comparison with negative control MES exposed group in different parts of brain viz. cortex, medulla, midbrain and cerebellum respectively (Table 3).

In the treatment group, the animals exposed to scopolamine and treated with Standard drug (Mentat) (4.717±0.3060, 4.743±0.4879, 4.537±0.3606 and 2.530±0.06557). Leaf extract of *A. marmelos* (3.713±0.3068, 5.283±0.3132, 5.250±0.4838 and 2.163±0.2369) animals produced significant (P<0.01) reduction in Acetylcholinesterase enzyme activity as

compared to negative control scopolamine exposed group (Table 3).

DISCUSSION

Traditionally *A. marmelos* is known to be very effective in enhancing the memory, promoting intellect and is used as brain tonic. Its influence on cholinergic activity of the rat brain was studied because there are many reports suggesting loss of memory, which is associated with decreased cholinergic activity. In the present study, the anti-amnesic activity of ethanol extract of *A. marmelos* is evaluated and compared with the established memory enhancing poly herbal preparation "Mentat", that is available in the market as tablet manufactured by Himalya Herbal Healthcare. The electroshock induced amnesia model was used in the present study, which produces retrograde amnesia interfering with memory consolidation, with concomitant reduction in brain acetylcholine levels. Chronic exposure to MES for 7 days produced a significant decrease in latency to expose to electroshock grid in step down latency and increased the time of transfer latency in elevated plus maze. The same effect was also seen in scopolamine exposed animals. This suggested that application of MES and scopolamine disrupts the acquisition, retention and consolidation of a learned task[7]. In our present study, we found that there was a significant increase in acetylcholinesterase enzyme activity in MES exposed and also in Scopolamine exposed rats. Whereas, administration of extracts of leaf of *A. marmelos* simultaneously with MES and scopolamine exposure for 7 days prevented the impairment of memory consolidation and also reduced the Acetyl cholinesterase enzyme activity in all parts of the brain.

A deficient cholinergic system has been implicated for the progressive decline of learning and memory in various neuropsychiatric disorders[8]. From the study, we observe that, there is a good correlation of loss of memory and loss of cholinergic activity which include both acetylcholine content and acetylcholinesterase enzyme activity. We also observe that retention of memory was associated with decreased Acetyl cholinesterase enzyme activity. Madepalli *et al.,* (1994)[9] reported that Acetylcholine content and acetylcholinersterase enzyme activity have an inverse relationship on memory retention. In the present study, we measured the acetylcholinesterase enzyme activity of different part of rat brain and that supports the findings of Madepalli *et al.*, (1994)[9] because acetyl cholinesterase enzyme activity increased after MES and scopolamine treatment, which decreased by treatment with ethanol extract of *A. marmelos.* The results from the present study suggest that ethanol extracts of *A. marmelos* possess antiamnesic activity as it reversed the memory impairment produced by MES and scopolamine. The results suggest that these extracts improve cognition by decreasing the level of acetylcholinesterase enzyme activity.

Table 1: Grouping of animals

Group(n)	Sub Group
Normal Control	**Group 1:** Normal Control (0.5% Tween 60),
Positive Control	**Group 2:** Standard (Mentat)
Negative Control	**Group 3**: MES induced (150mA for 0.2 sec),
	Group 4: Scopolamine induced (0.3mg/kg b.w.),
Treatment group MES + Drug treated	**Group 5**: Ethanol extract of *A.marmelos* (200mg/kg b.w.),
	Group 6: Standard (Mentat)
Treatment group Scopolamine (0.3mg/kg b.w) + Drug treated	**Group 7:** Ethanol extract of *A.marmelos* (200mg/kg b.w.),
	Group 8: Standard (Mentat)

Table 2: Nootropic activity of ethanol extract of *A. marmelos* leaf - Summary of Transfer latency and Step-down latency

Group(n)	Sub Group	Transfer Latency (In Seconds) in Elevated Plus Maze		Step-down Latency (In Seconds) in Step-down apparatus	
		Before	After	Before	After
		Day 1	Day 7	Day 1	Day 7
Normal Control	Normal Control	22.36±1.634	6.492±0.5852***	17.41±3.014	25.57±2.700***
Positive Control	Standard (Mentat)	28.89±6.007	19.04±4.447*	16.76±1.799	60.00±0.000***
Negative Control	MES induced	6.074±1.094	22.18±1.277**	19.19±1.443	10.31±0.8922***
	Scopolamine induced	24.71±4.325	47.12±5.485**	27.43±3.445	11.73±2.402***
Treatment group MES + Drug treated	*A. marmelos*	20.91±2.889	17.49±2.492**	26.14±3.868	34.32±4.896**
	Standard (Mentat)	26.96±3.601	13.03±1.872**	9.668±1.300	15.24±2.522*
Treatment group Scopolamine + Drug treated	*A. marmelos*	20.02±1.948	11.49±0.5782*	8.482±0.5590	16.34±1.031**
	Standard (Mentat)	21.30±9.572	15.48±7.693*	32.04±2.235	60.00±0.000***

n=5 number of animals in each group. Values are expressed as Mean±SEM.
Students 't' Test – Paired, two   tailed
*** $P<0.0001$ ** $P<0.01$ * $P<0.05$

Table 3: Nootropic activity of ethanol extract of *A. marmelos* leaf - Summary of Acetyl cholinesterase enzyme activity in different parts of brain

Group(n)	Sub Group	Acetylcholinesterase Enzyme activity (Mean±SEM) (In moles X10^{-6}/min/g of tissue)			
		Cortex	Medulla	Midbrain	Cerebellum
Normal Control	Normal Control	7.387±0.3078	9.223±0.2955	9.783±0.5349	3.930±0.4251
Positive Control	Standard (Mentat)	3.110±0.08327**	3.200±0.1400**	3.437±1.032**	2.400±0.1531**
Negative Control	MES induced	9.307±0.2143**	11.31±0.2857**	12.53±0.1904**	7.420±0.3205**
	Scopolamine induced	8.927±0.2392**	11.20±0.1444**	13.58±0.1910**	6.367±0.2834**
Treatment group MES + Drug treated	*A. marmelos*	5.563±0.3993#	3.287±1.303#	5.583±1.197#	2.663±0.2437#
	Standard (Mentat)	4.887±1.178#	4.213±0.6444#	4.130±0.6245#	1.680±0.2117#
Treatment group Scopolamine + Drug treated	*A. marmelos*	3.713±0.3068††	5.283±0.3132††	5.250±0.4838††	2.163±0.2369††
	Standard (Mentat)	4.717±0.3060††	4.743±0.4879††	4.537±0.3606††	2.530±0.06557††

n =3 number of animals in each group.
Values are expressed as Mean±SEM
One Way Analysis of Variance (ANOVA) followed by Dunnett's 't' test
** $P<0.01$ – Compared to Normal Control.
#$P<0.01$ – Compared to Negative Control – MES induced.
†† $P<0.01$ – Compared to Negative Control – Scopolamine induced.

CONCLUSION

In the present study scientific evaluation was carried out by using ethanol extract of leaf of *A. marmelos* to prove nootropic potential. In conclusion data produced from the study shows significant neuro-protection and memory enhancement by extracts of A. *marmelos* at a dose of 200mg/kg body weight, which might also be useful as supportive adjuvant in treatment of elderly memory loss, hence *A. marmelos* can be used for the management of Alzheimer's disease and other neurodegenerative disorders.

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