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Research Article

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# Study on Nootropic activity of *Aegle marmelos L* fruits

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**Abstract**

The present study carried out to evaluate the nootroptic protective effect of *Aegle marmelos*. Fresh *A. marmelos* fruits were used for methanolic extract preparation and used for the nootroptic protective activity on scopalamine induced amnesia in rats . The spatial memory was assessed by Morris water maze, and biomarker enzyme levels were estimated (acetylcholinesterase, malondialdehyde, Superoxide dismutase) to determine the nootropic activity. The outcome of current research suggest that methanolic extract of *A. marmelos* fruits significantly reversed the scopalamine induced spatial memory deficits and also inhibits the changed levels of biomarker enzymes due to scopalamine. In conclusion, the separation of the bioactive compounds from *A. marmelos* fruits can be used as an adjuvant medicine along with allopathic medicine in the treatment of cognitive disorders.

**Key words:** *Aegle marmelos*, Fruits, Amnesia, Biomarker enzymes.

## 1. Introduction

The cognitive disorders and their impairments are commonly associated with definable neuropathological, metabolic or toxic changes in brain and are characterized by confusion, disorientation (Fibiger, 1990). Neurons basic structural and functional units of the brain and are generally sensitive and they are not regenerated when the it damaged. The damage of the brain caused by occur due to different factors like exocytotoxicity, oxidative stress, $Ca^{2+}$ over load, increased rate of cellular oxidative reactions due to increment of metal ions etc and neurodegenerative disorders like Alzheimer's disease, Parkinsonism, and Huntington's chorea etc (Kopelman, 2002; Dukin, 2009). According to the reports on dementia and impaired cognition are the clinical features for major neurodegenerative disorders. The clinical features are dementia, progressive deterioration of thought, judgment, language skills, visual-spatial perception and mood (Loy *et al.,* 2014). In traditional medicine various medicinal plants have been using for neuroprotection (Sharma *et al.,* 2005) and in recent studies different medicinal plants have been reporting scientifically their memory enhancement (Russo and Borrelli, 2005; Visweswari *et al.,* 2010). In this regard, the present work carried out to evaluate the nootroptic protective effect of *Aegle marmelos* fruit extract.

*Aegle marmelos* Linn, is an traditional medicinal plant belong to the family Rutacae present around the subtropical region (Sharma *et al.,* 2007).The traditional healers use dry powder of fruit with mustard oil for the treatment of burn cases. (Pamar CMK Kaushal, 1982). Fruits are also used in diarrhea, gastric troubles, constipation, laxative, tonic, digestive, stomachic, dysentery, brain and heart tonic, ulcer, antiviral, intestinalparasites, gonorrhoea, epilepsy, toys, edible, jam, preservative (Kaushik P Dhiman, 1999; Vijay *et al.,* 2010). Therefore, the present study is aimed to evaluate the Nootropic effect of methanolic extract of *Aegle marmelos L* fruits.

## 2. Materials and Methods

### *2.1 Plant Collection and Extraction*

The *A. marmelos* fruits were procured from the Laila Impex, Vijayawada, Andhra Pradesh. The fruits were extracted with methanol by using soxhlet apparatus. After evaporation of the solvent under

reduced pressure crude extract was obtained and it is used for further studies.

*2.2 Chemical*

The chemical and reagents used in present study were analytical grade.

*2.3 Experimental animals*

Albino Wister Strain rats weighing 180-250gms of either sex were used which were obtained from S.N.Ghosh Enterprises, Kolkata. Animals were divided into groups (N=6) in cages at an ambient temperature and 45-55% Relative Humidity with 12 hours light/dark cycle. They had free access to pellet chow diet (Rayans Biotechnologies Pvt. Ltd, Hyderabad) and water *ad libitum*. The experimental protocols were approved by the institutional animal ethics committee of Andhra University (Approval no Reg.no.516/01/A/CPCSEA) under the regulation of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

*2.4 Acute toxicity studies*

The acute toxicity of methanolic extract of *A. marmelos* fruits was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). The albino rats of single sex, were selected in to two groups of consisting of 6 animals. They were maintained for one week before the experiment, under room temperature and allowed free access to water and diet. The animals were subjected for acute toxicity study using each extract at a dose of 2000 mg/kg orally in 2 groups at regular intervals of time, *i.e.*, 1, 2, 4, 8, 12 and 24 h. During this time, the animals were under observation for their mortality

*2.5 Experimental design for Nootropic activity*

Animals were divided into three groups, each consisting of six Wistar rats. Group I received 1% CMC (7 days), Group II received scopalamine (1mg/kg, i.p.) 30 min before acquisition trial (7 days), Group III received methanolic extract of *A. marmelos* (400mg/kg) dissolved in 1%CMC 30 min before the administration of scopalamine (1mg/kg) before the acquisition trial (7 days). The nootropic protection was measured by using Morris water maze task on spatial memory described by Morris (1984) and brain biomarker enzymes (Neurochemical study) acetyl cholinesterase (AChE), malondialdehyde (MDA) and superoxide dismutase (SOD) levels using diagnostic kits (Ohkawa *et al.*, 1979; Ellman *et al.*, 1961; Kakkar *et al*., 1984).

*2.5.1 Morris water maze task*

In the present study, we have used the Morris water maze to know the effect of methanolic extract of *Aegle marmelos.* on spatial memory. The water maze task was run during seven consecutive days after the 1$^{st}$ day treatment. It was done during the light period approximately between 08:00 to15:00h. A circular tank was used as described by Morris (1984). The pool was 180cm in diameter and 60cm high, filled with 23 ± 1$^0$C water, and placed in a room that was rich in permanently located spatial cues including shelves, posters and illumination lights. The pool was divided into four quadrants designated northeast (NE), northwest (NW), southeast (SE) and southwest (SW). Position of the escape platform (9cm diameter) was changed daily in the pseudo-random order (in the center of the NW,SW,NE or SE quadrants,1.5cm below the water surface, equidistant from the sidewall and the center of the pool**.** The platform was provided for escape from water. Four different start points were equally spaced around perimeter of the pool at points NE, SE, SW and NW. On each training day, the three start points were used once only a pseudo-random sequence (i.e. each trial was started from the different point). First, the rat was placed on the platform for15s for orientation. Then the rat was put in the water, facing wall of the pool, in one of the three quadrants that did not contain the platform, in a random sequence. The time of finding the escape platform was measured. The rats were placed on platform for 15s, if they did not find it within 60s. The rat that failed to reach the platform was given a latency score 60s.The inter-trial interval was10min. At the end of session, all animals were towel and fan dried and returned to their home cages. The animals were trained during four consecutive days, each given one session of three trials daily. During each trial session, the time taken to find the hidden platform (latency) was recorded. Spatial working memory was assessed after the last training trial sessions, the platform was removed from the pool and rats were allowed to swim for 60s

to search for it. A record was kept of the number of crossing over the platform position in the pool quadrant where the platform had been previously placed.

*2.5.2 Estimation of Biochemical parameters*

The brains from each rat were removed after the study, washed with normal saline and kept on ice. Then, their weighs were recorded and they were homogenized in cold phosphate buffer ().1M, pH 7.4) using homogenizer as standard procedures. The homogenates was centrifuged at 1000rpm at $4^{o}$c for 3mins and the supernatant divided into three portions, one of which was used for measurement of malondialdehyde (MDA), The other was centrifuged at 3000rm at $4^{o}$C for 15min was used to estimate acetylcholinesterase levels; and the remaining supernatant was again centrifuged at 12000 rpm at $4^{o}$C for 15mins and used for the measurement of Superoxide dismutase (SOD).

*2.5.2.1 Estimation malondialdehyde* (MDA)

MDA levels were estimated as per Ohkawa *et al* 1979. The method is, to a sample of 0.2mL tissue homogenate 0.2mL of 8.1% sodium dodecyl sulphate, 1.5mL of 20% acetic acid solution and 1.5mL of 0.8% aqueous solution of TBA were added, then solution was make up to 5mL with distilled water. The solution was heated for 60min at 95°C in oil and cooled under tap water. Then 5mL of n-butanol and pyridine (15:1 v/v) was added and shaken vigorously. Then solution was centrifuged for 10min at 4000rpm, the supernatant was separated and measured its absorbance at 532nm.

*2.5.2.2 Estimation of superoxide dismutase* (SOD)

SOD levels were estimated as per method described by Kakkar *et al.*, 1984. The brin homogenize in ice cold phosphate buffer (0.1M) was centrifuged at 12000rpm for 15min at 4°C. Then, to 0.1 mL supernatant, 1.2ml of 0.052M sodiumpyrophosphate buffer (pH 8.3) 0.1ml of 186 µM phenazinemethosulphate, 0.3ml of 300 µM nitrobluetetrazolium, 0.2ml of 780 µM NADH. Then, solution was incubated for 30sec at 30°C, then reaction will stop by adding 0.1mL glacial acetic acid. Then solution was shaken by adding 4mL n-butonol and then centrifuged for 10min at 4000rpm. The supernatant was separated and measured its absorbance at 560nm against control which was prepared using 0.1mL of distilled water devoid of 0.1mL of homogenate and SOD levels were expressed as units/mg proteins.

*2.5.2.3 Estimation of acetyl cholonesterase* (AChE)

AChE levels were measured as per procedure Ellaman *et al.*, 1961 using spectrophotometer on basis of color formation (yellow) when thiocholine reacts with dithiobis nitro benzoate ions forming cholonesterase. The sample was first treated with 5, 5'-dithionitrobenzoic acid (DTNB) and the optical density (OD) of the yellow colour compound formed during the reaction at 412 nm every minute for a period of three minutes was measured.

*2.5.3 Statistical analysis*

The results were expressed as (MEAN±SD). Differences in AChE, MDA, SOD were determined by factorial One way ANOVA. Individual groups were compared using turkey's test. Differences with $P<0.05$ were considered statistically significant. Statistical analysis was performed using Prism Software.

**3. Results and Discussion**

Acute toxicity of the selected plant extracts were tested as per OECD guidelines. There was no behavior signs such as alertness, motor activity, breathlessness, restlessness, diarrhea, tremor, convulsion and coma were observed at the administered doses. The rats were physically active and no death was recorded even at the dose of up to 2000 mg/kg body weight in the tested groups of animals.

Scopalamine treated rats showed more latency period and less number of crossings over the platform when compared to normal control. Methanolic extract of *A. marmelos L.* administered along with scopalamine significantly improved spatial memory by decreasing the latency period and increase in the number of crossings over the platform. This indicates that the selected plant extract have the memory enhancement activity (Fig. 1).

Results of the biomarker enzyme assays also indicating the same, i.e. the MDA and AChE levels

increased and SOD levels were decreased due to the induction on scopalamine in group II. But, these enzyme levels are normal in Group I and the altered enzymes levels due to scopalamine were restored by the selected plant extract treatment in Group III (Fig. 2, Fig. 3 and Fig. 4).

Many medicinal plants are used in treatment of cognitive decline. Earlier studies have reported that *A. marmelos* have different biological activities (Narayan P Yadav and Chanotia, 2009).The different extracts of the *A. marmelos* reported about the Antioxidant, Antidiabetic etc. The chronic diseases, neurotoxins and cognitive disorders damage the brain and its function and finally cause the death due to different diseases. In the present study, the scopalamine cause the brain damage, so the latency periods for the animals were increased and also biomarker enzymes levels also varied. But, in the selected extract treatment group the latency period and enzyme levels became normal. This may be due to the brain tissue restoration capacity of the *A. marmelos.*

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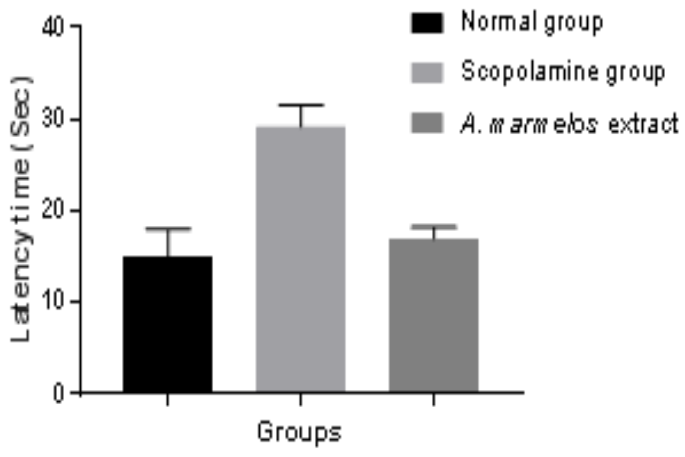


**Fig 1.** Effect of methanolic extract of *Aegle marmelos L.* on Morris water maze

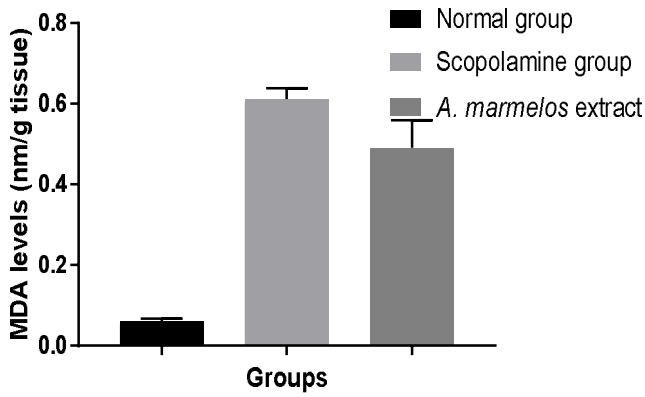


**Fig 2.** Effect of methanolic extract of *Aegle marmelos L.* on MDA levels in brain tissue

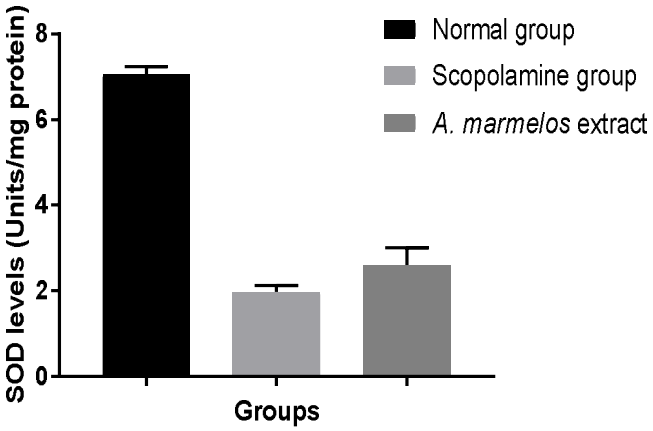


**Fig 3.** Effect of methanolic extract of *Aegle marmelos L.* on SOD levels in brain tissue

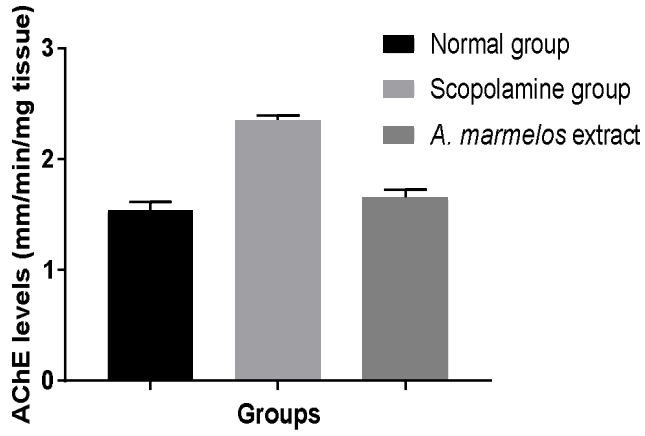


**Fig 4.** Effect of methanolic extract of *Aegle marmelos L.* on AchE in the brain

**Conflicts of interest**

Author has none to declare.

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