Evaluation of nootropic activity of leaf extract of *Anacardium occidentale* L.

ABSTRACT

One of the plants that have been used for enthno medicinal purposes in traditional civilization is *Anacardium occidentale* L. of the family Anacardiaceae is native to Brazil also found in tropical countries such as Malaysia and India commonly known as cashew. The purpose of this study is to determine the in-vitro and in-vivo cognitive effects of an ethanolic leaf extract of the herb *Anacardium occidentale* on albino rats. Orally the ethanolic extract was administered in two doses (100 mg/kg and 400 mg/kg). The Elevated Plus maze and Y-maze showed statistically significant improvement in the memory process. The estimation of acetylcholinesterase enzyme in rats brain also shows improvement in the memory process by reducing acetylcholinesterase activity. Disorders related to cognition are one of the major health problems and increasing day by day especially affecting the elder individual. There is no proper medication for the impairment of memory. The study reveals that the ethanolic extract of the leaf of *Anacardium occidentale* has dose-dependent memory-enhancing performance. Synthetic drugs have a lot of side effects, whereas drugs belongs to natural substances have least side effect compared to synthetic one, which has gained a lots of importance. These studies need to be documented effectively. Research findings were contributed to meet the future needs in general healthcare, research, and conservation of endangered species and may give a lead to the discovery of newer drugs.

KEYWORDS: *Anacardium occidentale*, cashew, Nootropic activity, cognitive enhancers, anti-amnestic

INTRODUCTION

Several investigations on ethnomedicinal plants and herbal medicines have been conducted in the past and have been reported. Health effective low-cost traditional are gaining popularity among the people in India to meet the future needs of medicinal plant cultivation and its documentation should be encouraged. India has also treasured this profound knowledge of medicines from the ancient Indian scholars dating back to 5000 years. This knowledge is referred to as “Ayurveda” (In Sanskrit, Ayu=Life, and Veda=Knowledge). (1) The human brain is the centre for controlling physical as well as emotional activities. It is responsible for ‘cognition’ which is one of the salient characteristics of human beings. [2] The

hippocampal, cortical and cerebellar areas of the brain are responsible for the memory processes. The acquired information is encoded as a memory trace in the brain and can be recalled in later stages.[3] Alzheimer's disease is a progressive, age-related neurodegenerative illness marked by severe memory loss, personality changes, abnormal behaviour and decline in cognitive action. [4] Nootropics are otherwise called memory enhancers or savvy drugs. Nootropics work viably because of their activity as an added substance to neurochemicals. These neurochemicals contain hormones, neurotransmitters, and enzymes, where they help in enhancing essential memory capacities.(5) *Anacardium occidentale* leaves are used in medicine due to their therapeutic properties assign to phenolic compounds. *Anacardium occidentale* leaves contain rich secondary metabolites. The secondary metabolites present in the plant shows a great antioxidant property. (6)

However, such data is not available on the plant on its pharmacological action related to the improvement of memory function. Some of the literature mentioned its uses for improvement of memory the leaves of the plant were dried at 40°C, powdered, extracted by infusion with ethanol: water (40:60, v/v) for 7 days(7). These are not satisfied. Hence to access and prove its claim in the treatment of memory disorder the present study is undertaken.

MATERIALS AND METHODS

Collection and Preparation of the extract

The leaves of the proposed plant *Anacardium occidentale* L. were collected from Mangaluru of Dakshina Kannada District, Karnataka, India, during July 2020.

Animals- Albino Wistar rat of both male and female, 4-6 weeks, the weight of 150-200g were obtained from NUCARE animal house, Deralakatte, Mangalore. The animals were housed in cages and kept under standard laboratory conditions (temperature 25 ± 2°) with light and dark cycle (12h/12h). They are let free to access a standard dry pellet diet and water *ad libitum*. The study was approved by institutional ethics committee (IAEC) with Ethical clearance permission number NGSMIPS/IAEC/JUNE-2020/202

Leaf extract- The collected leaves were shade dried under normal room temperature until the leaves were free from moisture or water content. The dried leaves were powdered (coarse powder) by using an electrical grinder. This powder was subjected to the maceration process for the extraction of the phytoconstituents using ethanol as a solvent. The powder was fully

covered with an excess of ethanol in the maceration chamber. The mixture was allowed to macerate for 7 days with occasional stirring. The mixture was then filtered by using a clean muslin cloth. The obtained filtrate was then concentrated by evaporating the ethanol under reduced pressure and controlled temperature. The dried product obtained after evaporating the whole ethanol part was stored in a desiccator for further use.(8)

Phytochemical Screening

Qualitative phytochemical analysis of leaves of *Anacardium occidentale* was carried out.(9)

Drugs and chemicals - Piracetam and Scopolamine were used for the study. The drugs and extract were dissolved in water for injection and were administered intra-peritoneally.

In-vivo Pharmacological evaluation:

Experimental design:

Animals were classified into 06 groups of each six animals.

GROUP I: Animals treated with normal saline served as the control group.

GROUP II: Animals treated with scopolamine (1mg/kg i.p) served as the disease control.

GROUP III: Animals injected with Piracetam (200mg/kg) served as the positive control.

GROUP IV: Animals injected with Scopolamine and treated with Piracetam served as standard drug.

GROUP V: Animals injected with scopolamine and treated with plant extract (100mg/kg p.o.)

GROUP VI: Animals injected with scopolamine and treated with plant extract (400mg/kg p.o.)

In-vivo models

A) Elevated plus-maze

The plus-maze is made up of two opposite open arms (50 × 10 cm) and two closed arms of the same dimensions connected with a central square (10 × 10 cm) to give the apparatus a plus sign appearance. (10)` On the 7^{th} day, after administration of doses, scopolamine was administered to induce amnesia and TL noted after 45min. Retention was recorded after

24hrs.(11) The rat was observed for the locomotion and the time is taken to move from the open arm to any one of the closed arms with its four paws was noted. This obtained time (in seconds) was termed the initial transfer latency (ITL). The rat was pushed gently into the closed arm if it does not move to any of the closed arms within 90 seconds and the ITL was noted or accepted as 90 seconds. The animals were trained before the observation of transfer latency. The retention transfer latency (RTL) was noted after 24 hours of the last dose (ie., on the 8th day) and considered as a measure of retention of the memory (learned-task) (12) (13)

The inflexion ratio can be derived from;

$$IR = L_0 – L_1 / L_1$$

IR= inflexion ratio, Lo= initial transfer latency in seconds, L_1 = Retention transfer latency in seconds.

B) Y maze

For the evaluation of spatial working memory and long-term memory in rodents, a Y maze task was performed. To achieve the goal, food or sweetened water was used as a motive. The primary characteristics recorded for evaluation of drugs effect delivered before or after training for 15 days. The spontaneous alternation task paradigm is the simplest version of the Y-maze task used to measure the spatial working memory in rats. The Y-maze made up of three arms. The arms were randomly designated start arm (S), novel arm (N) and familiar

$$\% \text{ Alterations} = (\text{Number of positive alterations} / \text{Total number of arm entries} – 2) \times 100$$

arm (F). Rats were placed in the start arm in the first trial as a part of training. Rats were allowed to explore the two arms whereas one was kept close. Rats were allowed to explore the three arms in the second trial, and the number and sequence of the arm entry were recorded for a total of 6 minutes. The total number of arm entries indicates the locomotor activity and the spontaneous alternation behaviour was calculated using successive entries into the three arms on overlapping triplet sets (SFN, FNS, and NFS etc). The spontaneous alternation is calculated by the formula: (14)

In-vitro models

Collection of brain samples

The animals were anaesthetized and were sacrificed by cervical dislocation. The skull was cut open and the whole brain was removed carefully which was then weighed, placed on an ice bath, washed and homogenised. For homogenization, about 20 mg of tissue/ml of phosphate buffer of pH 8.0, 0.1M was used, which was then placed in a homogenizer. The homogenate was centrifuged for 10 minutes at 3000 rpm, and the final cloudy supernatant liquid was used to estimate acetylcholine esterase activity. (15)

Estimation of Brain Acetyl Cholinesterase (AChE) Activity by Ellman's method

The Ellman method was used to assess the AChE activity of rat's brain. (16) 0.4ml of rat brain homogenate was added to a cuvette containing 2.6ml phosphate buffer of pH 8.0, 0.1M was taken. 0.1µl of DTNB was added. By bubbling air the content of the cuvette were mixed thoroughly and absorbance was measured at 412 nm. About 20 µl of substrate i.e. acetylthiocholine iodide will be added and a change in absorbance per minute es observed. The formula was used to compute the reaction rate.(17)

$$R = 5.74 \times 10^{-4} \times A/C_0$$

Statistical analysis

The results were expressed as mean ± SEM (standard error of the mean). One way analysis of variance (ANOVA) was used. P-value <0.05 were considered to be statistically significant. For multiple comparisons, Dunnett's test was used. Graph-pad prism version no: 8.0. was used to conduct the statistical analysis.

RESULTS

List 1: Phytochemical analysis

Phytochemical components	***A.occidentale***
Alkaloids	+
Proteins	+
Saponin	+
Phenolic compounds	+
Flavonoids	+

(+ = present ; - absent)

Elevated plus-maze

The effects of scopolamine, piracetam, test extract was evaluated in EPM on 7th and 8th day. Transfer latency on the 7th day of drug treatment reflected the learning behaviour of the animals, whereas transfer latency on the 8th day indicated memory retention. The scopolamine group showed a significant rise in TL when compared to the control group, indicating impairment in learning memory. Test extract showed a decrease in TL in comparison to the scopolamine group. Reduction in Inflexion ratio indicates the induction of amnesia and an increase in plant extract and piracetam treated groups indicates protection from loss of memory and improved cognition. In the presence of amnesia, a higher dose of ethanolic extract of *A.occidentale* (400mg/kg body weight) resulted in a significant reduction in Transfer latency (score: 33.6±0.531) compared to the lower dose group (39.6±0.486) and closely approximated to standard drug Piracetam (32.5±0.565). However, in a dose-dependent manner, both the doses improved spatial learning and memory activity. Furthermore, both the groups showed a drop in RTL on the eighth day compared to the seventh day, elucidating the drugs response to scopolamine-induced learning and memory deficits. In addition, there have been considerable improvements in the inflexion ratio shows its nootropic potential.

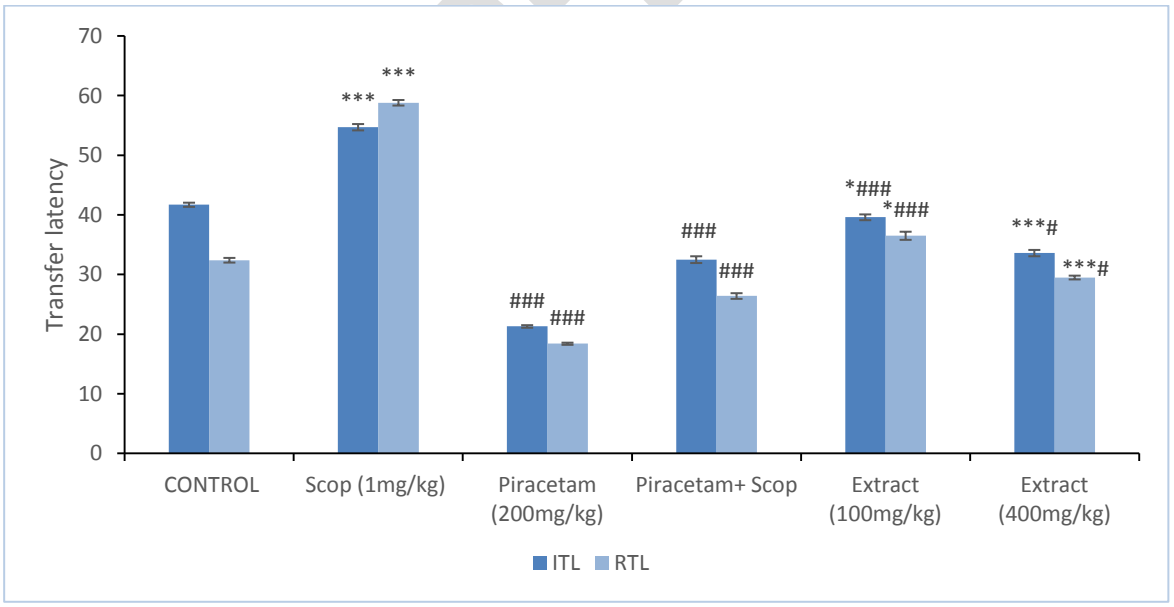

Figure 1: Effect of *Anacardium occidentale* L. leaf extract on Transfer latency in Elevated plus-maze.

The values were taken as mean ± S.E.M of n=(6) values and the statistical analysis was done using one-way ANOVA and compared with control and disease control were ***p<0.001, **p<0.01 and *p<0.05 when compared to the normal control; ### p < 0.001, ## p < 0.01, # p <0.05 when compared to disease control were taken statistically significant.

Y-maze

Percentage alteration was decreased in negative control and increased in plant extract and piracetam groups. Increased percentage alteration indicates that protection from loss of memory and decreased percentage alteration indicates decreased working memory. Using parameters like % alteration and no. of arm entries, the effect on alteration behaviour was investigated.

Effect on % alteration: When rats were treated with scopolamine to induce amnesia, the higher dose of ethanolic extract of *A.occidentale* (400mh/kg) showed a significant alteration response (score: 41.4±2.192) compared to the lower dose (score: 36.5±0.725) and closely approximated to the standard drug (score: 60.1±1.342). both the groups showed a significant increase in% alteration on the 8th day compared to the 7th day.

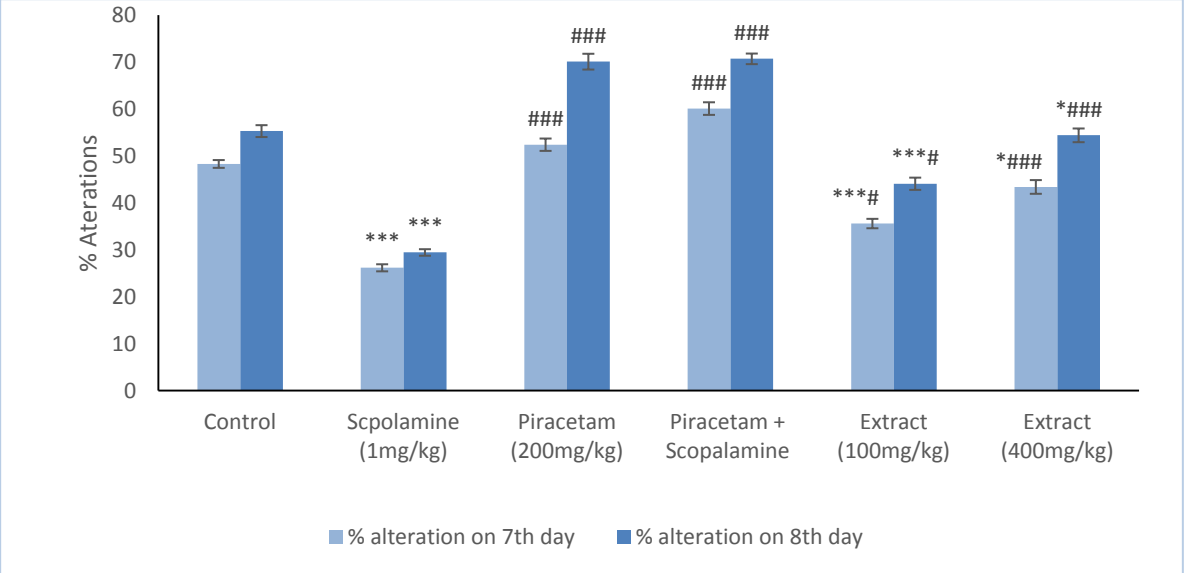

Figure 2: Effect of *Anacardium occidentale* L. leaf extract on % Alteration in the Y maze

The values were taken as mean ± S.E.M of n=(6) values and the statistical analysis was done using one-way ANOVA and compared with control and disease control were ***p<0.001,

**p<0.01 and *p<0.05 when compared to the normal control; ### p < 0.001, ## p < 0.01, # p <0.05 when compared to disease control were taken statistically significant.

Effect on no. of arm entries: The higher dose of the test extract (400mg/kg) resulted in a decrease in the number of arm entries (score: 27.3±0.615 compared to a lower dose (score: 30±0.577) and is close approximately to the standard drug Piracetam (score: 25.8±0.477). As a result, there was a dose-dependent decrease in arm entries on the 8th day compared to the 7th day.

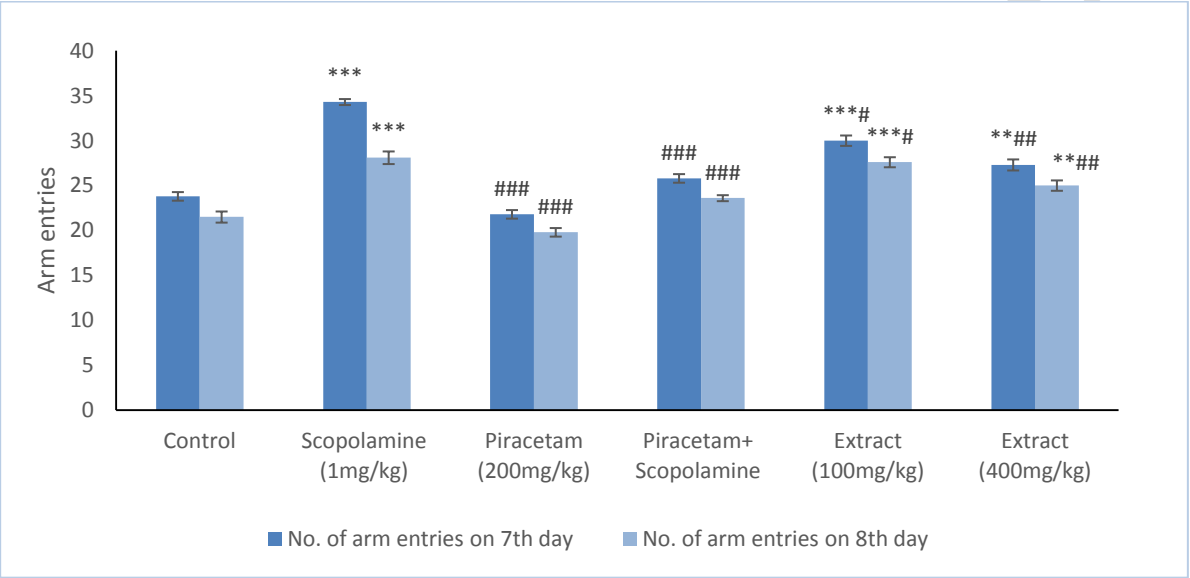

Figure 3: Effect of *Anacardium occidentale* L. leaf extract on No. of arm entries in the Y maze.

The values were taken as mean ± S.E.M of n=(6) values and the statistical analysis was done using one-way ANOVA and compared with control and disease control were ***p<0.001, **p<0.01 and *p<0.05 when compared to the normal control; ### p < 0.001, ## p < 0.01, # p <0.05 when compared to disease control were taken statistically significant.

Estimation of whole-brain acetylcholine esterase (Ach E) activity:

The result of the above study suggested that there was a significant deviation of AchE in the whole brain upon treatment with scopolamine. The amnesia induced group showed a significant dose-dependent decline in AchE on a group of animals pre-treated with *Anacardium occidentale* (100 mg/kg and 400mg/kg) successively for 7 days when compared with scopolamine challenged group.

The extract at a dose showed a comparable decline in AchE levels as the standard Piracetam. The scopolamine treated group shows a significant rise in AchE levels when compared to the control group. The study results reveal the acetylcholine esterase inhibitory activity of the leaf extract of *Anacardium occidentale* at various dose levels. As a result of suppressing the acetylcholinesterase enzyme and boosting acetylcholine level in the rat brain, these drugs improved rat memory.

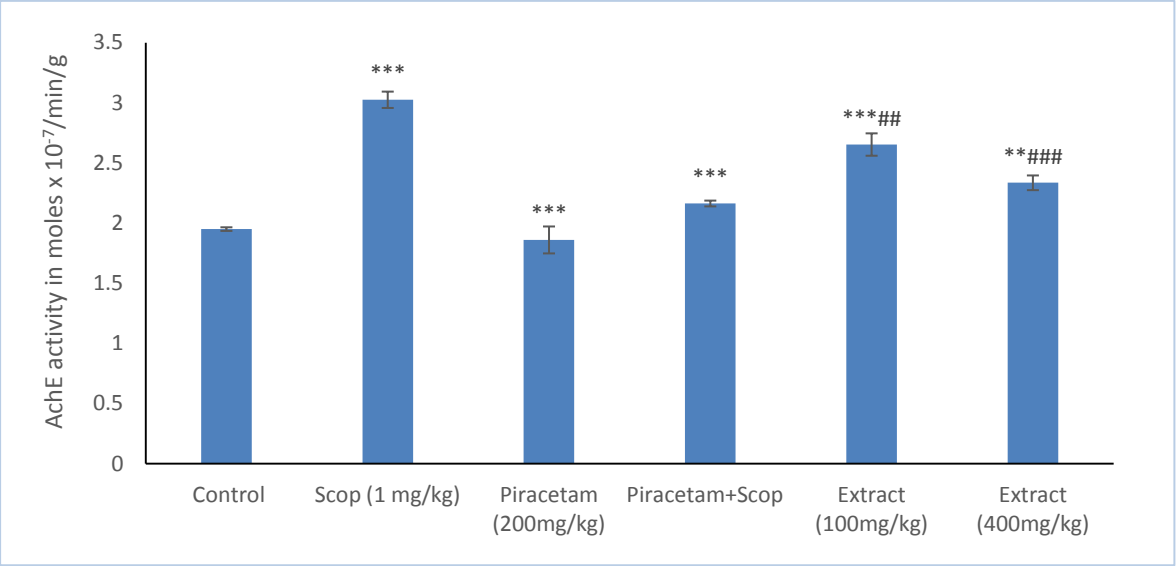

Figure 4 Effect of *Anacardium occidentale* L. leaf extract on Acetylcholinesterase activity

The values were taken as mean ± S.E.M of n=(6) values and the statistical analysis was done using one-way ANOVA and compared with control and disease control were ***p<0.001, **p<0.01 and *p<0.05 when compared to the normal control; ### p < 0.001, ## p < 0.01, # p <0.05 when compared to disease control were taken statistically significant.

DISCUSSION

Dementia is a set of symptoms arising in brain and is associated with the impairment of learning capacity, memory, thinking, orientation, language and judgment. Many medicinal plants have been claimed to possess learning and memory enhancing activity by different mechanisms like inhibition of ache, antioxidant effect, induction of neurotropic factors and cell death mechanisms.

The use of natural product based drugs, nutraceuticals or lifestyle changes for controlling age related neurodegenerative disorders. In traditional practice of medicines, many plants have been used as nootropic agents

Nootropics are also known as smart drugs that are being developed for over three decades and are the predominantly used method for treating cognitive deficits

Nootropic drugs have a place with the class of psychotropic operators with a particular facilitatory impact on learning and memory. (18) Memory impairment has developed as one of the main health threats in some memory disease conditions. (19)

In the present study, Scopolamine was used to induce amnesia to evaluate the nootropic potential of the ethanolic extract of leaf of *Anacardium occidentale* by using two different exteroceptive models that are EPM and Y-maze model.

The results obtained from the present study revealed the memory-enhancing activity of *Anacardium occidentale* ethanolic leaf extract and also the standard nootropic drug i.e. Piracetam, showed similar results through 2 different exteroceptive models having different parameters and methods of assessment, thus providing enough scientific promise to legalize the claims on their nootropic potentials.

The transfer latencies in the EPM, the percentage alteration and the arm entries criterion in the Y-maze model showed analogous results. Piracetam, the standard drug gave the most prominent results in all the models, followed by the plant extract dosed at 400mg/kg and 100mg/kg. The highest dose of the test drug is being able to produce better results and even giving a closer comparison with the standard drug. Treatment groups V, VI were efficient to overcome the amnesia effect created by scopolamine [20]; presenting an efficient learning response than the negative control explained better responses of learning acquisition, retention and retrieval as compared to the normal control.

The results of acetylcholinesterase [21] enzyme estimation support the plus maze and Y-maze tests by statistically reducing AChE activity in the rat brain resulting in increased acetylcholine (ACh) levels [22, 23], which aid memory efficiency. The current study shows that the ethanolic extract of *A. occidentale* improves memory output in rats by enhancing the ACh level.

CONCLUSION:

Ethanolic extract of *Anacardium occidentale* decreased the transfer latency in Elevated Plus

Maze and the percentage alteration and arm entries criterion in the Y-maze model showed analogous results, in a dose-dependent manner. Pre-treatment with extract showed the extract might be mediating its effect via modulating acetylcholine level in the brain. However, further research is wanted to determine the specific mode of its nootropic activity.

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