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Acute effects of triterpene compounds on locomotor performance and Morris water maze tasks in Spraque-Dawley rats.

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Abstract

***Centella asiatica* has been recognized as one of the plants that can be used to surge cognitive function in animals and humans. Currently, several compounds isolated from *C. asiatica* have been proven to enhance cognitive function via learning and memory properties. The present study investigated the acute effects of administration of triterpene compounds isolated from *C. asiatica* on memory and learning in male Spraque-Dawley rats via Morris water maze and their influences on motor activity in locomotor performance. The five-week old Spraque-Dawley rats were intraperitoneally administered with 10 mg/kg concentration of triterpene compounds; asiatic acid, madecassic acid, madecassoside and the control groups with scopolamine and saline. The rats were then evaluated via Morris water maze tasks and locomotor activity. The administration of 10 mg/kg of asiatic acid resulted in significantly improved memory (p<0.5) with increased escaped latency compared to saline and scopolamine. Furthermore, the administration of 10 mg/kg of madecassic acid and madecassoside significantly induced higher escape latency compared to scopolamine (p<0.5) but there was no significant difference compared to saline (p<0.5). For spontaneous locomotor activity, the three triterpene compounds showed no effect on locomotor activity as compared to saline group. However, the triterpene compounds had significant effect on locomotor activity as compared to scopolamine group (p<0.5). The administration of acute asiatic acid facilitated escaped latency which can be translated as having the properties of enhancement on memory and learning. Hence, it may serve to be useful on improving memory and learning with no sedative effect on the locomotor performance.**

Keywords: Asiatic Acid, Madecassic Acid, Madecassoside, Morris Water Maze, Locomotor Activity

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Introduction

Natural products such as herbal medicines have been used for centuries as an alternative medicine in treating and preventing neurodegenerative diseases including Alzheimer's disease (AD). There is an unambiguous relationship between AD and natural products. The most consistent features of AD are cholinergic deficits [1], decreased acetylcholine (ACh) production and also enhanced levels of acetylcholinesterase (AChE). Due to that, treatment strategies have been focusing on replacement therapy for deficits in central cholinergic neurotransmission [2, 3] which result in loss of ability to recall memory and process of formation of new memory and learning.

Over the past decade most research in AD has emphasized the use of natural products including *Centella asiatica* (L.) Urb. (Apiaceae). *C. asiatica* is a slender and creeping perennial herbal plant with weak aroma used by diverse ancient people from different cultures in Malaysia and other Asian countries. *C. asiatica* is considered as a vital herb in Ayurveda, the traditional science of health in India, used to stimulate learning and memory [4]. Previous studies demonstrated that whole plants of *C. asiatica* have been shown to be beneficial in improving memory [5]. These results support that *C. asiatica* can enhance memory and learning. *C. asiatica* contains triterpenes, essential oils, amino acids and other compounds, such as vellarin. The triterpenes include asiaticoside, centelloside, madecassoside, brahmoside, brahminoside, thankuniside, centellose, brahmic, centellic, madecasic acids and asiatic acid [6] as shown in Figure 1.

A recent study reported that asiatic acid has been shown to improve memory via passive avoidance study and learning capabilities via active avoidance [7]. One the one hand, madecassoside has been indicated as causing neuroprotective effect in a rat's brain [8] and most impairment observed there will affect a significant cognitive learning and memory deficit [9]. On the other hand, antioxidant activity has been proven to cause cognitive-enhancing activities [10] and *C. asiatica* is consistent with higher corresponding to antioxidant activity [11]. Thus far, however, most studies in the field of *C. asiatica* have only focused on the extracts rather than the compounds. Little is known about the compounds of *C. asiatica* and it is not clear what factors on the learning and memory. Therefore, the aims of the present study were to investigate the effects of intraperitoneal (I.P) administration of asiatic acid, madecassic acid and madecasosside on memory and learning along with the spontaneous locomotor activity in male rats.

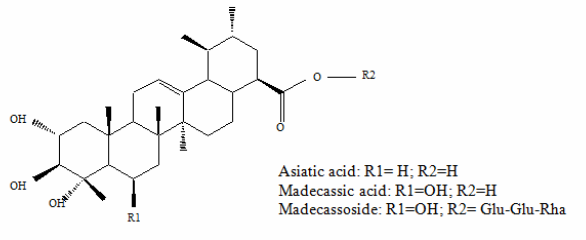


Figure 1. *Structure of triterpene compounds of C. asiatica (Glu:Glucose, Rha: Rhamnose)*

Materials and methods

Sample preparation and animals and drugs preparation

Sample preparation

C. asiatica whole plants were obtained from a local producer in Kuala Terengganu, Terengganu Darul Iman, Malaysia. Then, the samples were subjected to correct botanical identification by a herbarium expert from Universiti Sultan Zainal Abidin (UniSZA). A voucher specimen is deposited at Biochemistry Laboratory, Faculty of Medicine and Health Science as CA 100. The samples were collected and weighted. Thereafter, the whole plants were grounded with an electric blender (Blender Waring Commercial) to obtain powder form. The grounded samples then underwent an extraction phase.

Extraction and solvent partitioning

The powder samples were extracted with methanol (MeOH) using hot extraction with Soxhlett method. Thereafter, the extractions were repeated three times to ensure the entire bioactive compound was extracted from the sample as previously reported [12].

The extracts then underwent solvent partitioning technique with n-hexane and chloroform. All soluble fractions in two different solvent systems were screened and subjected to thin layer chromatography (TLC) profiling with reference compound to determine the prominent soluble fraction. p-Anisaldehyde–sulfuric acid mixture were used as visualizing agent in detecting triterpene compounds.

Column chromatography

Preparation of column chromatography (CC) was performed as previously described [13]. Briefly, the column (35.0 x 6.5 cm) was filled with silica gel (Merck 9385). Silica gel was mixed with solvent to obtain a slurry mixture. The mixture was stirred slowly to eliminate air bubbles and filled into the column. The column was tapped constantly while packing with the slurry mixture of silica gel. The column was stabilized with the lowest polar of the desired solvent system. The samples were impregnated to silica gel (1:1) and were introduced to the top of the silica gel bed.

The elution process was carried out in increasing polarity (step gradient). The fractions were collected in 100 ml each (first CC process) and 5 ml each (for repeated CC process). The fraction of interest was monitored by TLC. Fractions with the same TLC pattern/profile were combined. The fraction containing the same chemical compound was purified by repeating CC (70.0 x 1.0 cm). The compounds were identified using Infrared spectroscopy (IR spectroscopy) and co-TLC with standard.

Animals

Male Spraque-Dawley rats (4-5 weeks) weighing 180 ± 30 g were obtained from Laboratory Animal Unit, Universiti Sains Malaysia and were bred at the animal house, Faculty of Medicine and Health Sciences. The Spraque-Dawley rats were used to analyze the acute effect of treatment with asiatic acid, madecassic acid and madecassoside via I.P injection. It was observed that male and female animals differ in their memory capacities. Estrogen has been found to enhance memory in female mice and women [14]. Therefore, male animals were employed to eliminate estrogen-related beneficial effects on memory. The rats were kept four per cage at room temperature of 22–24 ^{0}C and with a 12-h light 12-h dark cycle light period, 0700–1900 h. The animals had free access to food and water during the experiments. The animals were divided randomly into five subgroups of seven animals each namely negative control group, positive control and treated group. Negative control group were given 1 ml/kg saline whereas treated groups animals were given (10 mg/kg) via I.P of three triterpene compounds. The 10 mg/kg administrations were chosen due to the limited compound samples available and as starting point of the comparison of the three compounds. For positive control, the animals were given 3 mg/kg scopolamine to induce amnesia and hyperactivity in locomotor activity. Previous study indicated that 100 to 1000 mg/kg *C.asiatica* did not produce toxicity effects; breathing, cutaneous effects, sensory nervous system responses [15]. All experiments were conducted at the same time of the day to minimize circadian influence. The experimental protocols were approved by Ethical Committee on the Use and Care of Animals (Animal Ethics Committee, Universiti Sultan Zainal Abidin, UniSZA: UniSZA/AEC/2013/001).

Drugs

Asiatic acid, madecassic acid and madecassoside were isolated from *C. asiatica* extract; scopolamine was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Drugs preparation and administration

Asiatic acid, madecassic acid and madecassoside were dissolved in saline with some modification [16] and scopolamine was dissolved in saline and vortex in 10 minutes. The three triterpene compounds were injected via I.P as well as scopolamine [17, 18]. The I.P administration was chosen in view that it allows quite long longer period of absorption as rapid as intravenous route and it is a common route in animal studies [19].

Spontaneous locomotors activity

The open-field test was used for estimation of locomotor activity of rats using the SMART video tracking software (3.0.03) from Panlab, Spain. The locomotor activity was determined by performing the method as described by [16]. Briefly, the apparatus consisted of a square of 100 cm × 100 cm black floor. A single rat was placed inside the apparatus for 1 min of adaptation. The total length of crossings was 5 min. Asiatic acid, madecassic acid and madecassoside, scopolamine and saline were administered 30 min before the test.

Morris water maze

The Morris water maze task was performed as previously described [20, 21]. The Morris water maze is a black circular pool (150cm in diameter and 65cm in height) with a featureless inner surface. The circular pool was filled with water to a height of 30 cm (20±1 ◦C). The pool was divided into four quadrants of equal area. The hidden circular platform (10 cm in diameter), made of Plexiglas, is located in the center of the southeast quadrant, submerged 1.5 cm beneath the surface of the water. It has been previously shown that the Plexiglas is invisible for the rats. The continuous location of each swimming mouse, from the start position to the platform, was monitored by a SMART video tracking software (3.0.03).

In the water maze experiments, the day prior to the experiment was dedicated to swim training for 60 s in the absence of the platform. During the following days, the rats were given two trial sessions each day for 4 consecutive days. During each trial, the time taken to swim to the platform (escape latency) was recorded. This parameter was averaged for each session of trials and for each rat. Once a rat located the platform, it was permitted to remain on it for 15 s. If the rat did not locate the platform within 120 s, it was placed on the platform for 15 s and then removed from the pool by the experimenter (trial 1). The rat was given second trial (trial 2) with an inter-trial interval of 20 min for 4 consecutive days. The point of entry of the rat into the pool and the location of the platform for escape remained unchanged between trial 1 and trail 2 but was changed each day thereafter.

The decrease in escape latency from day to day in trial 1 represents long-term memory or reference memory, while the decrease from trial 1 to trial 2 represents short-term memory or working memory. Seven rats were used per treatment. The rats were treated with Asiatic acid, madecassic acid and madecassoside (10 mg/kg body weight) given before the training trial. After 90 min, amnesia was induced in rats with scopolamine (3 mg/kg body weight) given subcutaneously. All rats were tested for spatial memory 30 min after the administration of scopolamine.

Statistical analysis

Results were expressed as means ± SEM of seven animals per group. The data was analyzed with Kruskall-Wallis and followed by Mann-Whitney and 1-way ANOVA using the SPSS 11.0 software. In all the tests, the criterion for statistical significance was $p < 0.05$.

Results

Extraction and isolation of triterpene compounds

The dried and finely ground plants of *C. asiatica* (15 kg) were extracted at room temperature with MeOH for three days and were concentrated under vacuum to afford 345 g of crude extract. The extract (200 g) was dissolved in water and successively extracted with n-hexane and chloroform to yield, respectively, 85 g and 70 g of fraction.

The hexane and chloroform fractions were compared via TLC and n-hexane fraction was chosen due to more desired terpenoids were concentrated in this fraction. The n-hexane fraction were subjected to a CC filled with silica gel (63–200, 60 A°) and eluted with a gradient of n-hexane in dichloromethane and dichloromethane in methanol. Eighty-eight fractions of 100 ml were collected and regrouped on the basis of analytical (TLC) in fifty fractions. The main active fraction of 12 g was subjected to repeated column chromatography (60 cm×3 cm) filled with silica gel (32–63, 60 A°) eluted with a gradient of methanol in chloroform to yield 3 compounds.

IR spectroscopy spectrum for asiatic acid, madecassic acid and madecassoside were done using Perkin Elmer spectrum 100 FTIR spectrometer and the data were compared [22]. The compounds were isolated as a white amorphous powder and identified as asiatic acid (80 mg), madecassic acid (65 mg) and madecassoside (54 mg). The identity of compound was also determined by co-TLC with standard.

Spontaneous locomotors activity

Spontaneous locomotor activity results are shown in Figure 2. Based on the results, the rats from scopolamine group showed statistically significant hyperactivity compared to saline group. For asiatic acid, madecassic acid and madecassoside with each 10 mg/kg the groups showed no effect on locomotor activity as compared to saline group.

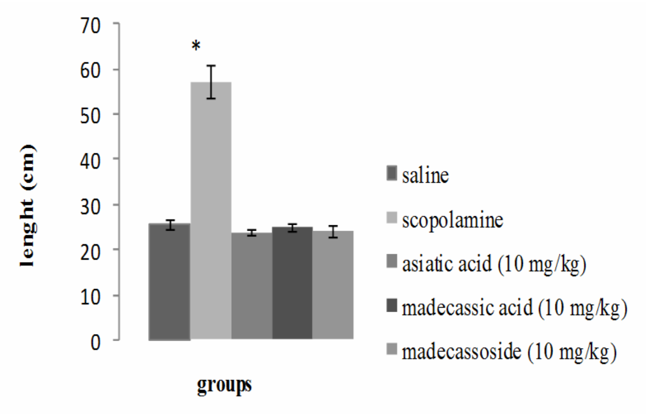
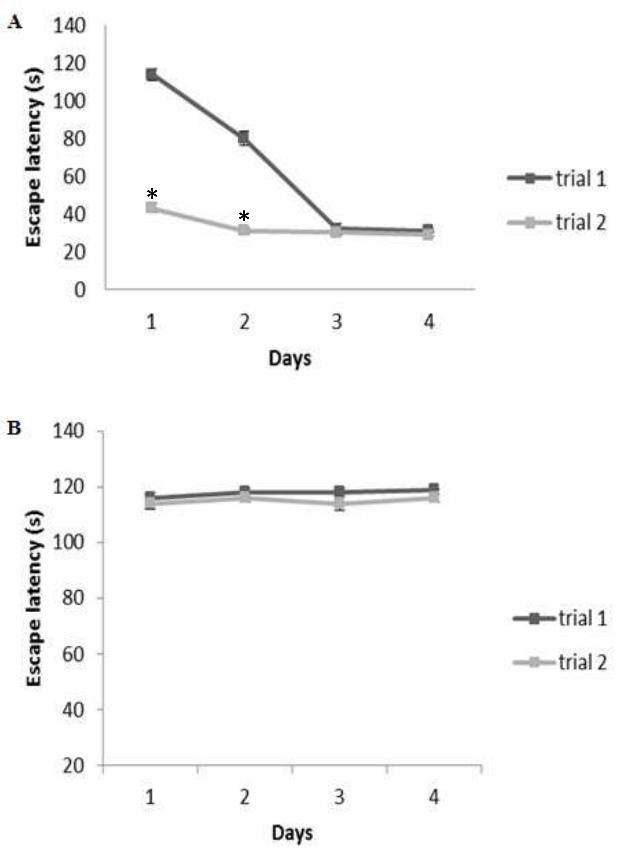
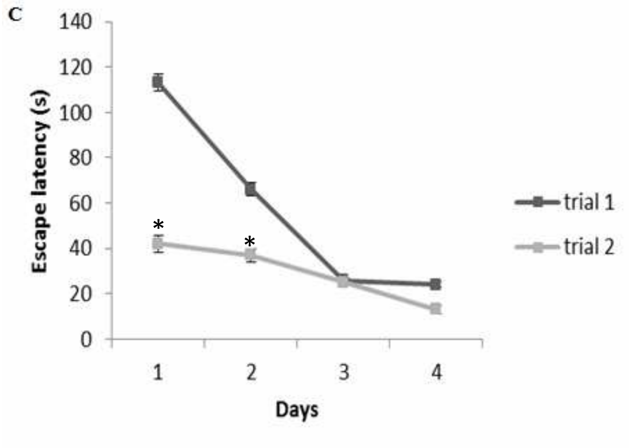
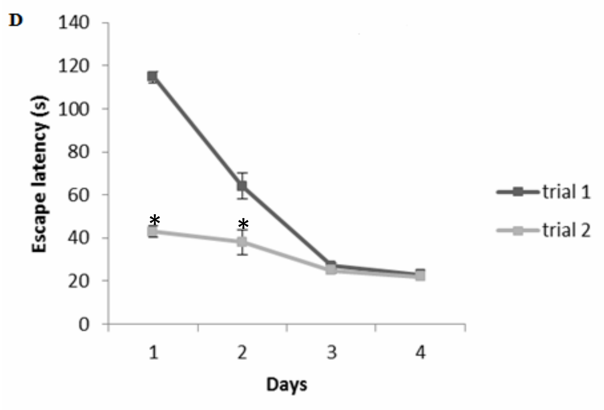


Figure 2. *Effects of asiatic acid, madecassic acid and madecassoside on spontaneous locomotor activity in Spraque-Dawley rats. Each group comprised of a maximum of seven rats. The * indicates significant value compared to saline group with P < 0.05. Ordinate shows the mean ±SEM.*







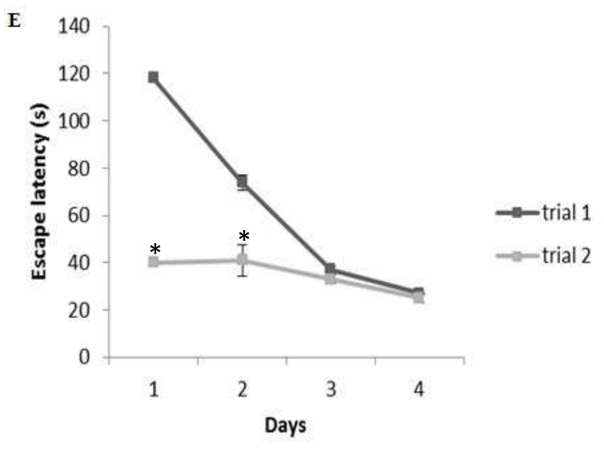


Figure 3. *Effects of saline (A), scopolamine (B), asiatic acid(C), madecassic acid(D), and madecassoside (E) on Morris water maze in Spraque-Dawley rats. Rats were given two sessions of trial each day for 4 consecutive days. The swimming time required for the rats to escape to platform was recorded in each day. Each group comprised a maximum of seven rat. The * indicated significant value with P < 0.05 and the ** indicate the significant value compared with saline (day 4). Ordinate shows the mean ±SEM*

Morris water maze

The Morris water maze test results were shown in Figure 3. Based on the results, the escape latency to find a platform of normal control, asiatic acid, madecassic acid and madecassoside were rapidly dwindling after day 1 (Fig. 3A, 3C, 3D, and 3E). These four groups also exhibited a marked reduction in escape latencies as compared to trial 1 and trial 2 and reached stable latencies within 13 to 35s in four days. By contrast, the scopolamine group did not significantly change during the 4 days training session (Fig 3B). The asiatic group detected the lowest escape latencies (14s) as compared to saline group on day four (Fig. 3C). The escape latencies were shortened until four days except for scopolamine group exhibiting the formation of reference memory in rats.

Discussion

In this experiment, the effect of acute administration of three triterpene compounds as well as saline and scopolamine were investigated in male Spraque-Dawley rats. The results of locomotor activity experiment showed that asiatic acid, madecassic acid and madecassoside can enhance learning and memory behavioral performance via Morris water maze and this study confirmed that the increase in performance was not due to the influences of the motor activity in locomotor activity. The results of the locomotor activity indicate that asiatic acid, madecassic acid and madecassoside administrations did not alter the locomotor activity as compared with the saline group.

On the other hand, scopolamine administrations induced hyperactivity of locomotor in rats parallel with previous study done by [23], who demonstrated that scopolamine dependently induced hyperactivity locomotor in rats. Another triterpene group, asiaticoside which is of almost similar structure did not alter the total locomotor activity of the animals [24]. Furthermore, an aqueous extract of *C. asiatica* (100-300 mg/kg) did not alter spontaneous locomotor activity in these rats thus excluding the possibility that the CNS depressant/stimulant activity of the herb had contributed to the changes in the passive avoidance and elevated plus maze tests [5].

The results of Morris water maze experiment showed that asiatic acid, madecassic acid and madecassoside improved the spatial memory similar to the saline group. The asiatic acid had the most prominent effect on spatial memory which can be translated as having significantly lowest escape latency compared to saline in day 4. Our previous data showed that asiatic acid acts as AChE inhibitor properties [25]. This is parallel with previous study that the drugs that increased cholinergic levels facilitated behavioral performance in some rodent models of cognitive impairment [26-28].

The ACh-GABAergic feedback mechanisms were also affected by AChE inhibitor; physostigmine via increasing behavioral performance in rats. When the compound is activated, ACh is released via pyramidal neuron, thus the hippocampus GABAergic interneurons were also activated and sent the feedback to the medial septal region which is located at the basal ganglia that project cholinergic axons [29]. Furthermore, increasing the available amount of ACh in hippocampus, by preventing its breakdown via physostigmine, eliminates the behavioral impairment observed after Pyrithiamine-induced thiamine deficiency (PTD) treatment. However, in normal rats, excessive activation of cholinergic activity can become dysfunctional [30, 31].

According to [32] the standardized *C. asiatica* extracts significantly improved behavioural alterations in Morris water maze and attenuated increased AChE in mice. On the other hand, *C. asiatica* leaf extract has been demonstrated to improve spatial learning performance in neonatal rats during growth spurt period and increased the hippocampal cornu ammonis area 3 neuronal dendritic arborisation in rats [33, 34]. According to [35] the ethanol extracts of *C. asiatica* leaf increases memory performance in rats after chronic stress. ACh levels played an excitatory role in the hippocampus area. Beyond exciting hippocampal pyramidal neurons, pharmacological activation of hippocampal muscarinic ACh receptors also directly excites GABAergic interneurons [29, 36]. For the positive control, scopolamine groups showed impairment in Morris water maze and this is parallel with the previous study [37] which showed that scopolamine induced amnesic syndrome in a rat.

Conclusions

Asiatic acid administration is effective in enhancing spatial memory as well as with madecassic acid and madecassoside with no sedative effect on locomotor activity. Hence, asiatic acid administration might be useful in slowing down age-related deficits in memory and learning. The rapid development in the field of animal models of learning and memory processes may hopefully lead to an improved understanding of pathophysiology of cognitive disorders, and finally permit a rationale designing of therapeutic strategies for distinct cognitive dysfunctions through drug discoveries.

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