EXAMINING THE EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF
MOMORDICA CHARANTIA FRUIT ON AVOIDANCE MEMORY ALTERATIONS IN
MICE USING STEP-THROUGH MODEL

A. Miri, S. F. Askari, E. Shahraki

Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences,
Zabol, Iran

Received: 20 September 2018 / Accepted: 23 November 2018 / Published online: 01 January 2019

ABSTRACT
Medicinal plants have been, and still are, of a particular value and importance for public health provision in terms of both treatment and prevention of diseases. Among the effects of such plants are the impacts on the memory and learning process. In this study, the effect of hydroalcoholic extract of Momordica charantia on the avoidance memory alterations was investigated in mice using the step-through model. The hydroalcoholic extract of the soaked plant was administered to the mice at doses of 10, 25, 50, 100, and 200 mg/kg by gavage (intragastric tube) method. In comparison with the control group receiving only drinking water, the highest memory improvement was observed at a dose of 25 mg/kg. The results of interferential intraperitoneal administration of scopolamine and the extract gavage at a dose of 25 mg/kg indicated the ability to restore scopolamine-induced memory corruption by the total extract of M. charantia.

Key words: Momordica charantia, scopolamine, avoidance memory, step-through, mice.

Author Correspondence, e-mail: el.shahraki@gmail.com
doi: http://dx.doi.org/10.4314/jfas.v11i1.8
1. INTRODUCTION

*Momordica charantia* or bitter melon is an herbaceous plant of the Family Cucurbitacea. Plants of this family are commonly found in tropical regions of the Earth with many edible genera such as *M. charantia* and *M. dioica*.

The fruit of *M. charantia* is used in spices and as pharmaceutical products in many areas such as India. In traditional medicine, this herb has been used as hypoglycemic, anti-worm and anti-malaria, carminative, menstrual stimulant, antipyretics, wound healing, abortion, etc. [1].

Previous studies have reported the properties of genus *Momordica*, namely anti-inflammatory, anticancer, antioxidant, astringent, cholesterol level and blood pressure reducer, gall-inducer, antipyretic, appetizer, tonic, laxative, blood purifier, burns healer, menstrual stimulator as well as anti-depression, vomiting, weight control, leprosy treatment, anti-malaria, anti-gout, respiratory and blood disorders, and liver inflammation attributes [2]. The seeds of plants in this family contain such amino acids as carboxyphenylalanine and methyl asparagine [3]. Some antioxidants have also been observed such as vitamins C and E, carotene, xanthophyll, tannins, and other phenolic compounds [4]. In this study, the effect of different doses of the plant on avoidance memory in male Syrian rats was investigated using step-through model.

2. METHODOLOGY

2.1. The plant

The fruit of *M. charantia* was obtained from the Research Center of Zabol, Iran, and, after fragmentation, dried through air drying method, and then crushed. The shredded plant was extracted by soaking in 80% ethanol at room temperature.

2.2. The animals

This study was conducted on mice weighing 30-35 g. Groups of seven animals were placed in cleaned cages and kept in a photoperiod of 12 h light: 12 h dark at 22-24 °C. All animals were provided with enough food and their drinking water was replaced daily. During the experimental period, the ethics of working with laboratory animals was observed according to the Helsinki Code of Ethics. Each animal was used only once.
2.3. Step-through passive avoidance test

The device was regulated in the control unit according to tests performed at previous studies, including delays in opening the doors for 10 seconds and an electric shock intensity of 2.0 mA for 2 seconds. On training day, after the animals had been adapted to the test environment, they were placed in the light step-through chamber. Following the time delays or 10 seconds (as mentioned above) the door was opened and automatically closed immediately after passing the animal followed by subsequent application of an electric shock of 2.0 mA to the animal for 2 seconds (It should be noted that the animals with no shocking treatment tend instinctively to move from the light to dark chamber). After completing the training and shocking stage, the avoidance memory of all animals was evaluated within one day (24 h), two days (48 h), four days (96 h), and seven days (168 h) in a certain time of the day according to the training time. The animals were placed again in the light chamber on the test day (with similar experimental conditions applied on training days with the difference that the animals were not given an electric shock). If the animal passed through the door, the delay time was automatically recorded on the control display.

A maximum experimental time of 300 s (5 min) was considered for each animal on the test day, which was automatically applied by the machine through the cut-off in the control unit. Therefore, the cut-off time was reported when an animal crossed after 300 s.

2.4. Administration of the hydroalcoholic extract

All mice were selected in groups of seven individuals with doses of 10, 25, 50, 100, and 200 mg/kg (based on previous studies) and gavaged orally with the fruit extract of *M. charantia* for two weeks. The control group received drinking water as gavage containing the aqueous extract of *M. charantia* in the same conditions as the groups receiving the extract.

On Day 14, following a half to one h after gavage, the animals were trained by passive avoidance step-through system. In this stage, the animals were shocked (0.2 mA) for 2 s and the time delays of passing from light to dark chambers were recorded; the memory acquisition was also evaluated in this step.

From this day onward, the animals’ retentional avoidance memories were tested at times of 24, 48, 96, and 168 h (7 days) after training day and the results were recorded. At the times
listed, no shock was applied to the animals by the device when they entered the dark chamber.

2.5. Administration of scopolamine
Scopolamine (1.0 mg/kg) was injected intraperitoneally in 2- and 4-day intervals. The normal saline was used as a solvent in the control group.

2.6. Combined administration of M. charantia extract and scopolamine
To explore the avoidance memory alterations, the animals received orally (as gavage) the fruit extract of *M. charantia* at a dose of 25 mg/kg, which yielded the greatest memory improvement within 14 days. On days 11 to 14, scopolamine (1.0 mg/kg) was also administered intraperitoneally after 15 min of the extract gavage. On the same days, control animals were injected normal saline intraperitoneally (15 min after drinking water gavage) for 2 and 4 days. On Day 14, the animals trained by the electric stimulator (shock) were placed in the step-through device. The animals’ retentional avoidance memories were evaluated in step-through model at 24, 48, 96, and 168 h after the training day.

2.7. Data analysis
The Graphpad Prism 5.0 software was applied in order to analyze behavioral data. Statistical tests used in this study were one-way ANOVA and t-test. The Newman-kules multiple comparison test was employed when statistically significant differences were observed among the data obtained. A significant level of p < 0.05 was considered as the minimum criterion.

3. RESULTS
Animals were treated with different doses of *M. charantia* extract (10, 25, 50, 100, and 200 mg/kg). The results showed that a dose of 25 mg/kg significantly increased the latency time during training (*p < 0.05) and 24 h (**p < 0.01) after training in step-through model compared to the control animals (Table 1).
Table 1. Effects of different doses of feMC on the mice avoidance memory alterations in step-through model

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Treatments (M. charantia extract: mg/kg/day)</th>
<th>Training</th>
<th>24</th>
<th>48</th>
<th>96</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>16.98±1.342</td>
<td>36.90±22.18</td>
<td>104.5±60.89</td>
<td>72.08±55.46</td>
<td>159.3±54.89</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>21.63±3.822</td>
<td>94.42±51.99</td>
<td>142.0±55.53</td>
<td>164.9±58.00</td>
<td>113.1±67.40</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>40.04±6.661**</td>
<td>239.1±24.40*</td>
<td>271.6±26.66</td>
<td>260.9±26.63</td>
<td>247.4±52.63</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>14.22±1.409</td>
<td>140.8±53.75</td>
<td>214.8±44.05</td>
<td>164.2±51.26</td>
<td>172.5±57.17</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16.98±3.469</td>
<td>97.43±41.60</td>
<td>104.2±51.61</td>
<td>148.1±45.90</td>
<td>139.3±48.08</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>11.60±1.744</td>
<td>37.70±21.56</td>
<td>68.48±41.09</td>
<td>36.93±22.44</td>
<td>119.0±37.68</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01 (n = 7). Data are presented as mean ± SEM.

Table 2. The effect of time-course administration of scopolamine and normal saline on training stages (acquisition) and avoidance memory anamnesis at 24, 48, 96, and 168 h (7 days) after training animals in step-through model

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Treatments</th>
<th>Training</th>
<th>24 h</th>
<th>48 h</th>
<th>96 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (2 days)</td>
<td>29.75 ± 2.622</td>
<td>148.4 ± 40.70</td>
<td>190.6 ± 31.21</td>
<td>151.1 ± 33.22</td>
<td>150.8 ± 53.58</td>
</tr>
<tr>
<td></td>
<td>Scopolamine (2 days)</td>
<td>24.68 ± 8.232</td>
<td>96.77 ± 44.03**</td>
<td>127.3 ± 44.99</td>
<td>99.23 ± 52.23</td>
<td>109.8 ± 46.79</td>
</tr>
<tr>
<td></td>
<td>Control (4 days)</td>
<td>51.98 ± 3.495</td>
<td>246.6 ± 26.61</td>
<td>186.2 ± 43.34</td>
<td>215.1 ± 63.84</td>
<td>164.7 ± 35.89</td>
</tr>
<tr>
<td></td>
<td>Scopolamine (4 days)</td>
<td>41.25 ± 7.719</td>
<td>37.73 ± 7.500***</td>
<td>36.38 ± 19.47*</td>
<td>36.02± 22.28*</td>
<td>38.59± 9.094*</td>
</tr>
</tbody>
</table>

Results are reported as mean ± SEM (n = 7).

(*p < 0.05), (**p < 0.01), and (***p < 0.001) compared with control group (normal saline).

The results indicate that hydrochloalcholic extract of M. charanita restored mother impairments included by scopolamine (Fig. 1, A-D).
Latency (sec)

**A**

- Control
- Scopolamine i.p
- Extract of Momordica charantia & Scopolamine i.p

**B**

- Control
- Scopolamine i.p
- Extract of Momordica charantia & Scopolamine i.p

**C**

- Control
- Scopolamine i.p
- Extract of Momordica charantia & Scopolamine i.p
Fig.1. Effect of 14-day oral administration of *M. charantia* hydroalcoholic extract on retentional avoidance memory alterations induced by scopolamine at 24 (A), 48 (B), 96 (C), and 168 (D) h after the training phase in step-through method. (*P < 0.05; **P < 0.001) compared with the control group (normal saline). (#P < 0.05; ## P < 0.01) compared with the groups receiving scopolamine.

4. DISCUSSION

The first step of present study evaluated the effect of *M. charantia* hydroalcoholic extract on the avoidance memory in step-through model compared with the control group. The higher consciousness of animals in step-through system and increased delaying time indicate the boosting effects of the plant. The neuronal protective effects of *M. charantia* have been proven in global cerebral ischemia, nerve damages from diabetes, and hyperlipidemia [5-7]. These impacts are largely dependent upon the antioxidant properties and vitamin C booster [8-9], β-carotene [10-11], phenolic compounds (such as tannic acid, gallic acid, catechin, caffeic acid, paracomic acid, ferulic acid, and benzoic acid) [12], and conjugated structures of linoleic acid such as α-eleostearic and punicic acid contents in the plant [13].

Potassium, sodium, calcium are the minerals contained in *M. charantia*, the effects of which have been documented on memory. Sodium and potassium play important roles in cellular polarization and depolarization as well as in the excitability of nerve cells, eventually leading to the release of neurotransmitters into the synaptic space. Characterized by directing an appropriate flow of potassium, the short-term memory is formed [14].

Calcium ions are very important intracellular signaling factors and changes in their...
concentrations can affect many intracellular activities including ion channel function; in this regard, a number of reports have been presented on calcium-associated potassium channels in neural cell membranes [15]. The voltage-dependent directing effect of flow on calcium and potassium is considered to be an important mechanism in the formation of memory [16]. Overexpression of special calcium receptors, called NCS-1, accelerate the acquisition and, ultimately, facilitate learning creating a memory with the ability of lesser degradation. Calcium signaling through NCS-1 raises a new mechanism in relation to learning and memory [17].

The time-dependent effects of scopolamine or hyoscine hydrobromide intraperitoneal administration on avoidance memory of male Syrian mice were examined in step-through model of this study. The results showed a significant reduction in retentional avoidance memory with increasing administration time compared to the control group. Acetylcholine is an essential neurotransmitter stimulant in the central nervous system that plays a fundamental role in cognitive processes. Thus, many studies have investigated the effects of nicotinic acetylcholine muscarinic receptors on the regulation of synaptic plasticity [18].

The behavioral similarity caused by bilateral destruction of the hippocampus has been proved by the effects of anticholinergic drugs such as atropine and scopolamine such that a delay in recognizing the new environment has been observed in mice receiving scopolamine [19]. Anti-cholinergic effects of low doses of scopolamine on the short-term memory and visual recognitions have also been demonstrated in primates such as monkeys [20]. Scopolamine administration in humans has interfered with learning as well as with verbal and nonverbal and memories [21]. A study by Elrod K et al. has determined the ability of scopolamine in disabling learning and passive avoidance memory, though, the exact nature is not specified [21].

It has been demonstrated that scopolamine has an effect as a central cholinergic muscarinic receptor antagonist associated with a reduced anamnesis in the recipient groups [21-23]. It seems that the effects of scopolamine on the central nervous system to be two-sided such that the prescribed dose may cause drowsiness, malaise and fatigue, or restlessness and excitement. Administration of scopolamine before training causes strengthening or
destruction of anamnesis by affecting the acquisition [22]. Scopolamine improves anamnesis at low doses through better cholinergic antagonists binding to presynaptic receptors. Presynaptic receptors regulate the release of acetylcholine. Low doses of some cholinergic antagonists increases the release of acetylcholine. Another possible mechanism deals with the adjustment of dopamine receptors by muscarinic presynaptic receptors [22]. At high doses, scopolamine binds to both pre- and post-synaptic receptors, resulting in reduced binding of acetylcholine to postsynaptic receptors [22]. Amnesia induced by scopolamine increases the astrocytes’ number of dentate gyrus in step-through model. Astrocytes are a type of glial cells with a number ratio of 10 to 1 to neurons that occupy 25-50 percent of the brain playing a major role in brain functions. All chemical neurotransmitter receptors (amino acids, peptides, monoamines) have been observed on the astrocytes showing that astrocytes support special mechanisms of learning and memory. Astrocytes are effective on learning and synaptic plasticity via glutamate neurotransmitters and increasing intracellular calcium.

Aging is associated with changes in the frontal cortex and the CA1 zone. The increased production of astrocytes due to degradation of cholinergic system by scopolamine results from the prevention of memory corruption due to syntheses of both nerve and basic fibroblast growth factors by astrocytes [24]. Scopolamine has been used in a variety of studies and models to investigate mechanisms influencing learning and memory; the following study can be noted as an instance. Receptor 5-HT6 antagonists have positive effects on cognitive processes in rodents. Such antagonists reverse avoidance and spatial memory defects caused by cholinergic antagonists such as scopolamine in step-through and Morris water maze models. The exact mechanism of this action is to facilitate cholinergic system activity and a modest increase in the extracellular acetylcholine [25]. Scopolamine has remarkably contributed to recognizing the mechanisms of aging and diseases such as Alzheimer’s. In a study, it was found that administering a nicotinic agonist such as choline in the hippocampus reverses the destructive effects of scopolamine. Choline is only able to restore memory in animals received a lower dose of scopolamine. Administering high doses of scopolamine inhibits cholinergic actions, thereby, the process of memory consolidation is halted and information will not be stored. However, it is possible that a very poor memory is
formed, the restore of which would require higher doses of choline or other enhancers of consolidation process. Consequently, mild cholinergic deficits allow the formation of memory, but tracing the restored memory fails in behavioral expression. Therefore, memory deficits observed in mild cholinergic problems, as seen in the early stages of Alzheimer's disease, is actually a sign of defects in the expression of memory and not losing it. Approved medication for the early stages of Alzheimer's disease is to prescribe cholinesterase inhibitors [26]. Our results of behavioral studies indicate that the interferential administration of *M. charantia* hydroalcoholic extract improves retentional avoidance memory corruption caused by intraperitoneal administration of scopolamine in step-through model. Comparing the structure of compounds in *M. charantia* to those of active ingredients in plants with proven effects on memory reveals similarities suggesting that the discussed effect of memory improvement by the plant can be attributed to these compounds. Among the active ingredients *M. charantia*, a steroidal glycoside called charantin could also be noted, which is composed of the glycosides sitosteryl and stigmasteryl being responsible for anti-diabetic properties of the plant [27].

![Structural formulas of some active ingredients in M. charantia: (1) Charantin, (2) stigmasteryl glucoside, (3) sitosteryl glucoside.](image)

The similarity of these glycosides to other steroidal compounds such with confirmed effects as bacoside and ginsenoside Rg1 on memory can endorse the effect of charantin on memory improvement [28].
Vicine is the pyrimidine alkaloid in this plant species [29]. Some studies have investigated the effects of alkaloids on memory. These include the protective and memory-enhancing roles of nerve cells in different behavioral models by the alkaloids dendrobiunm [30], total coptis alkaloids (TCA) [31], Huperzine [32], Xiatianwu total alkaloids, and corydalis type alkaloids [33]. The pharmacological functions of pyrimidine cores such as anti-inflammatory, anti-bacterial, anti-cancer, anti-AIDS, anti-malaria, antihistamine, tranquilizers, hypnotogogic, and anti-seizure have also been found during investigations [34]. Pyrimidine compounds are opioid receptor regulators (δ) [35]. Activation of delta opioid receptors inhibits the excitatory postsynaptic flow through reduced release of presynaptic glutamate [36]; such a mechanism results in soothing, hypnotogogic, and anticonvulsants effects, to which the poor learning and memory at high doses of M. charantia extract gavage are likely to be attributed. The effectiveness of a non-narcotic alkaloid called ibogaine at low compared to high doses has been shown to restore memory [37]. However, it is known that delta receptor antagonists inhibit passive avoidance learning while agonists prevent the action [38] probably confirming the effectiveness of present low-dose extract (25 mg/kg). Considering the overall dual effects of pyrimidine alkaloids on delta receptors causing decreased consciousness and stupefaction when stimulated but enhance excitatory processes and memory when inhibited, and given the effects of plant extract at both high and low doses, the hypothesis of delta opioid receptor partial agonist can be raised regarding vicine composition, which require further studies to be confirmed. In recognition of the fact that the plant in question has the ability to restore damage by scopolamine, other plants such as Ruta graveolens, Lavandula angustifolia, Rosmarinus officinalis, Petroselinum crispum, Mentha spicata and Crinum
spp. can be noted, all of which increase the amount of acetylcholine in the synaptic space through inhibition of acetylcholinesterase activity [39].

Insulin is well known due to the effects on peripheral tissues, including fat cells, muscle, and liver in order to regulate glucose. In the central nervous system, insulin and its receptors have been distributed in the hippocampus and cerebral cortex being involved in cognitive functions. Studies suggest that insulin signaling plays an important role in synaptic plasticity by regulating the excitatory and inhibitory activities of glutamate and GABA receptors. With the launch of insulin signaling cascade, the gene expression changes leading to long-term memory consolidation. In addition, it has been observed that reduction in insulin receptors’ signaling associated with aging because of brain degeneration, as in Alzheimer's disease and cognitive problems, are affected by type II diabetes mellitus in the patients [40]. A study reported that insulin resulted in passive avoidance memory enhancement [41].

The plant *M. charantia* contains natural insulin, a polypeptide with 166 amino acids and a molecular weight of 11 KDa, which can influence memory according to the mechanisms mentioned. Because of beneficial effects on obesity, the plant prevents insulin resistance and, ultimately, account for a form of prevention for Alzheimer's, which have been shown in research with insulin [42].

Thus, according to the active ingredients and all the above cases, *Momordica charantia* is able to restore memory corruption caused by scopolamine through various mechanisms discussed.

5. REFERENCES


33-8.


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**How to cite this article:**