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Original Article

Cognitive enhancing of pineapple extract and juice in scopolamine-induced amnesia in mice

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Abstract

The objective of the present study was to evaluate the cognitive enhancing of pineapple juice and ethanolic extract in scopolamine-induced cognitive deficit mice. The ethanolic extract of pineapple (*Ananas comosus* (L.) Merr.) was prepared by maceration method and its juice was obtained by a homogenizer. Object recognition task was used to evaluate the mice memory. Exploration time in the first and second trial was recorded. The differences in exploration time between a familiar and a novel object in the second trial were taken as a memory index. Animals were randomly assigned into 15 groups of 6 each including: control group (normal saline + vehicle), positive control group (scopolamine + rivastigmine), seven experimental groups (received scopolamine alone or scopolamine + ethanolic extract of pineapple in different doses), six other experimental groups were treated by ethanolic extract or juice of pineapple in different doses. Scopolamine (100 µL, 1 mg/kg, i.p.) and pineapple juice or extract (50, 75 and 100 mg/kg, i.p.) were administered 40 and 30 min before starting the second trial in the experimental groups. Object discrimination was impaired after scopolamine administration. Results showed that juice and ethanolic extract of pineapple significantly restored object recognition ability in mice treated with scopolamine. These finding suggested that pineapple had a protective role against scopolamine-induced amnesia, indicating its ability in management of cognitive disorders.

Keywords: Alzheimer's disease; Amnesia; Object recognition task; Pineapple; Scopolamine

INTRODUCTION

Pineapple (*Ananas comosus*, Bromeliaceae) native to central and south America, is grown in several tropical and sub-tropical countries including Hawaii, India, China, Kenya, South Africa, Malaysia, Philippines and Thailand (1,2). Pineapple has been used as a medicinal plant in several native cultures. It has the characteristics such as interference with growth of malignant cells, inhibition of platelet aggregation, fibrinolytic activity, anti-inflammatory action and skin debridement properties (3-6). Literature survey indicated that experimental evidence on the effect of pineapple on the learning and memory processes are scarce. Previous studies have shown that pineapple contains respectable amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, malic acid, water and different minerals (7-11). Moreover, as revealed by phytochemical studies, bioactive compounds including anthocyanins, β-carotene, polyphenols such as flavonoids, and phenolic compounds are present in pineapple's pulp (11-13).

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Some therapeutic effects of pineapple can be due to antioxidant activity attributed to its vitamin C, β-carotene, and flavonoids content (8,13,14). Furthermore, bromelain as a proteolysis enzyme is one of the major components of pineapple extract.

Alzheimer's disease (AD) is a neurodegenerative disease causing memory loss and dementia, which mostly affects the elderly population (15). Acetylcholine is a neurotransmitter which plays a vital role in learning and memory processes (16,17). Acetylcholinesterase (AchE) was found to be the most viable therapeutic target for symptomatic improvement in AD because cholinergic insufficiency is a consistent and early finding in this disease (18,19). Currently, AchE inhibitor drugs such as rivastigmine (20), galantamine and donepezil (21-25) are used for the treatments of AD, which increase the availability of acetylcholine at cholinergic synapses. Narrow therapeutic index and short half-life are the major disadvantages of the above mentioned drugs. They also have many side effects such as nausea, vomiting, headaches, diarrhea, and dizziness (26,27). Naturally derived AchE inhibitors are also a promising area of interest (28). Amongst numerous examples of drugs originating from plants that act as an AchE inhibitors are huperzine A and B (lycopodium alkaloid isolated from *Huperzia serrate*) (29-31), galangin (flavonoid isolated from *Rhizoma Alpiniae Officinarum*) (32), and also *Himatanthus lancifolius* extract (33).

Considering the presence of bioactive compounds such as flavonoids and proteolytic enzymes like bromelain in pineapple and also their therapeutic effects particularly in refreshment of memory, as it could be seen for kallikrein-8 in inhibition of AD (34), the aim of the present study was to clarify the effect of pineapple extract and juice in learning and memory processes.

Here we evaluated cognitive enhancing potential of pineapple in scopolamine-induced amnesia in mice.

In this study, the object recognition task, a non-rewarded paradigm, based on the spontaneous exploratory behavior of mice was applied (35,36).

MATERIAL AND METHODS

Plant material

Pineapple fruit (Prima) was purchased from a local market in Isfahan, Iran, and was verified by Natural Resources Research Center of Isfahan. Pineapples were stored at 7 to 12 °C up to 2 weeks at 95% of relative humidity.

Juice and extract preparation

Purely ripped pineapple fruits (about 900 g each) were cleaned, cut skin off and cut into small slices and weighed. Juice was collected from the fresh pineapple flesh part by homogenization and filtered (500 mL).

Extraction was performed using 1400 g of fresh pineapple flesh in 500 mL of 70% ethanol at room temperature (25–28 °C) conform to maceration method (3 × 24 h). Subsequently, the ethanol extract was filtered, concentrated by a rotary evaporator (Steroglass, Italy) at low temperature and then freeze dried using a freeze dryer (Zirbus, Germany). Samples were stored at -20 °C before use.

Animals

NMRI male mice (Pasteur Institute of Iran, Tehran) weighing 25–30 g were housed under standard conditions in a 12 h light/dark cycle. Tap water and standard food pellets were available *ad libitum*. Tests were performed after the mice had acclimated to the above environment for at least 2 days. All experiments were conducted between 08:00 and 12:00 h in a noise-free room with controlled illumination. A minimum of six mice were used for each treatment group. All procedures were approved by the ethical committee of the Isfahan University of Medical Sciences and conducted in accordance with the internationally accepted principles for laboratory animal use and care.

Object recognition task

The object recognition task was used to evaluate cognition as described by Bertaina-Anglade, *et al* (15). The apparatus was made of a square wooden open field (35 × 35 × 40 cm) painted inside with black color and a white floor. The open field was placed in a dark

room illuminated only by a halogen lamp oriented towards the ceiling. The open field and the objects, Lego toys with different shapes and colors, were cleaned with water between each trial. Animals were placed in the experimental room at least 30 min before testing. Each animal was submitted to a habituation session in the open field for 15 min and allowed to freely explore the arena in the absence of two objects 24 h before the test. On the experimental day, animals were submitted to two trials in 30-min intervals. During the first trial (acquisition trial, T1), the animals were placed in the arena containing two identical objects for recording the time necessary to explore the objects for 20 s. Any animal that did not explore the objects for 20 s within the 12 min was excluded from the experiments. Exploration is defined as the animal directing the nose within 2 cm of the object while looking at, sniffing or touching it. For the second trial (test trial, T2), which was performed 20 min after T1, one of the objects presented in the first trial was replaced by a new object different in shape and color and the animal was placed back in the arena for 5 min. Time spent on the exploration of new and old objects was determined. Animal behavior was recorded using a web camera placed above the experimental apparatus. Recognition index (RI) was defined as following equation:

RI = (time exploring the new objects- time exploring the familiar objects/ time exploring the new objects + time exploring the familiar objects) × 100

Tested compounds

Scopolamine (Daru Pakhsh, Iran) was used as a standard drug to induce amnesia. Rivastigmine (Daru Pakhsh, Iran) was considered as the positive control. Ethanolic extract and juice of pineapple were used in the treatment protocol. All tested materials were dissolved in 0.9% normal saline (vehicle) just before the experiment.

Treatment schedule

Mice were randomly assigned into 15 groups of 6 each. The treatment of each group was begun by i.p. injection of test materials 30 min before T1. Amnesia was induced only in experimental groups by scopolamine (1 mg/kg). Normal saline (0.9%) was used as vehicle and injected in control group. The positive control group was treated by scopolamine (1 mg/kg) + rivastigmine (0.6 mg/kg). Seven experimental groups received: scopolamine (1 mg/kg), scopolamine (1 mg/kg) + ethanolic extract of pineapple (50, 75 and 100 mg/kg), scopolamine + pineapple juice (50, 75 and 100 mg/kg). Six other experimental groups were treated by: ethanolic extract of pineapple (50, 75 and 100 mg/kg) or pineapple juice (50, 75 and 100 mg/kg). All drugs or extracts were injected in a constant volume of 100 µL.

Data processing and statistical analysis

The time required achieving 20 s of object exploration on the T1, and time required to recognize the familiar and new objects on T2 were determined. Recognition memory was assessed using a RI for each animal as explained earlier. Three independent experiments are presented as the mean values ± standard deviation of the mean (SD). Analysis-of-variance (ANOVA) followed by LSD test (as the Post-Hoc) was used to assess significance between the test sample and solvent control. P-value < 0.05 was considered to be statistically significant. SPSS 16.0 software was used for all statistical analysis.

RESULTS

The effect of scopolamine on memory performance

During the first trial (Figs. 1 and 3), T1 in scopolamine-injected and vehicle group mice was 408 and 200 s, respectively. As shown in Fig. 2 and 4, the RI score was found to be 13 and 50 percent for scopolamine-injected and vehicle group mice, respectively. The post-hoc analysis revealed that T1 and RI were significantly increased and decreased, respectively, by scopolamine compared to vehicle ($P < 0.05$).

The effect of ethanolic extract of pineapple on memory performance

As shown in Fig. 1, T1 for mice treated with ethanolic extract of pineapple at the doses of 50, 75 and 100 mg/kg was 185, 150 and 140 s, respectively. The RI values were found to be 45, 51 and 60 percent for mice treated with 50, 75 and 100 mg/kg of the extract, respectively (Fig. 2).

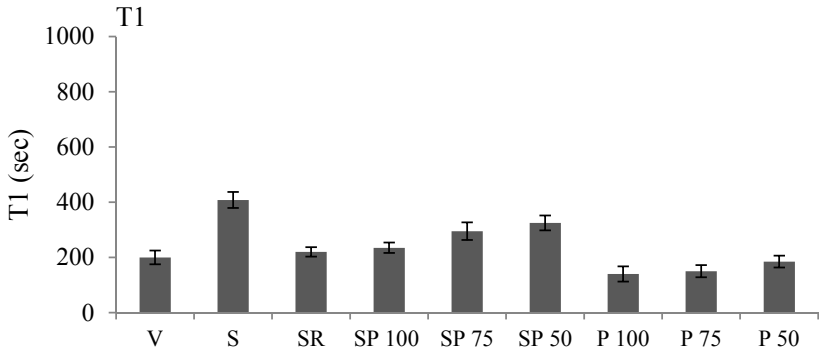

Fig. 1. The effect of ethanolic extract of pineapple (50, 75 and 100 mg/kg) on T1 of the scopolamine-induced amnesic mice, in the object recognition task. Time required to explore 20 s of object exploration on trial 1 (duration of T1), compared with control values in the object recognition tasks. Results are expressed as mean ± SD. Data was statistically significant at $P < 0.05$ compared with scopolamine alone values (n = 6 in each group). (V) vehicle group, (S) scopolamine 1 mg/kg, (SR) scopolamine + rivastigmine, (SP 50, 75 or 100) scopolamine + ethanolic extract of pineapple 50, 75 or 100 mg/kg, (P 50, 75 or 100) ethanolic extract of pineapple 50, 75 or 100 mg/kg.

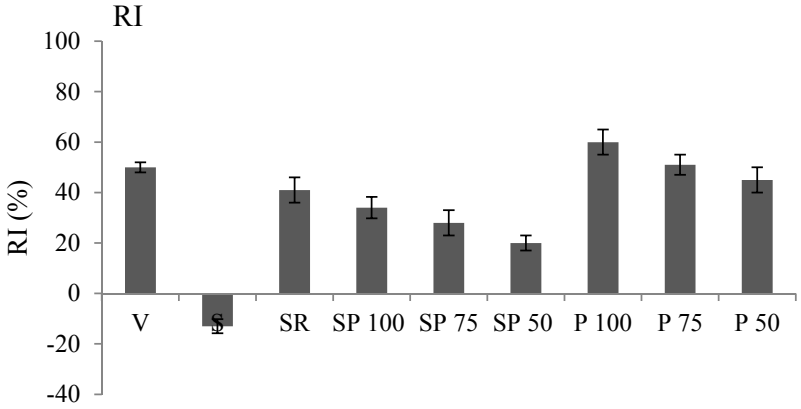

Fig. 2. The effect of ethanolic extract of pineapple (100 mg/kg) on recognition index of the non-treated healthy and scopolamine-induced amnesic mice, in the object recognition task. Data are expressed as mean ± SD. Data was statistically significant at $P < 0.05$ compared with scopolamine alone values (n = 6 in each group). (V) Vehicle, (S): scopolamine 1 mg/kg, (SR) scopolamine + rivastigmine 0.6 mg/kg, (SP 50, SP 75 or SP 100) scopolamine + pineapple at 50, 75 or 100 mg/kg, (P 50, P 75 or P 100) pineapple 50, 75 or 100 mg/kg.

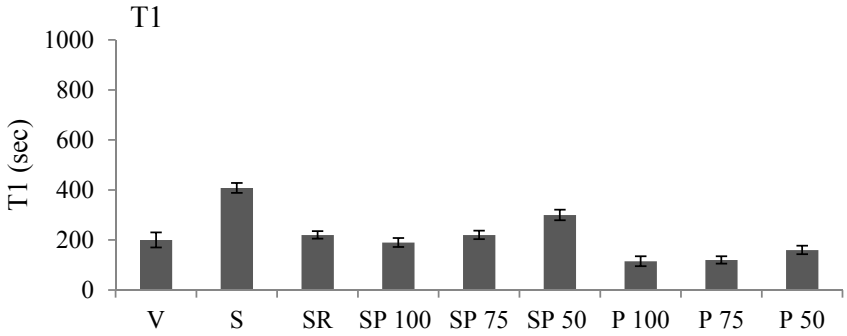

Fig. 3. The effect of pineapple juice (50, 75 and 100 mg/kg) on T1 of non-treated healthy and scopolamine-induced amnesic mice, in the object recognition task. Time required to explore 20 s of object exploration on trial 1 (duration of T1), compared with control values in the object recognition tasks. Results are expressed as mean ± SD. Data was statistically significant at $P < 0.05$ compared with scopolamine alone values (n = 6 in each group). (V) vehicle, (S) scopolamine 1 mg/kg, (SR) scopolamine 1 mg/kg + rivastigmine 0.6 mg/kg, (SP 50, SP 75 or SP 100) scopolamine + pineapple juice 50, 75 or 100 mg/kg, (P 50, P 75 or P 100) pineapple juice 50, 75 or 100 mg/kg.

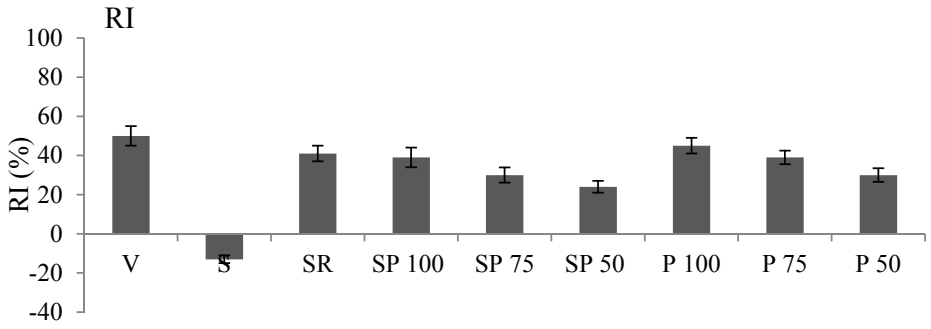

Fig. 4. The **e**ffect of pineapple juice (100 mg/kg) on recognition index (RI) of non-treated healthy and scopolamine-induced amnesic mice, in the object recognition task. Results are expressed as mean ± SD. Data was statistically significant at $P < 0.05$ compared with scopolamine alone (n = 6 in each group). (V) Vehicle, (S) scopolamine 1 mg/kg, (SR) scopolamine + rivastigmine 0.6 mg/kg, (SP 50, SP 75 or SP 100) scopolamine + pineapple 50, 75 or 100 mg/kg, (P 50, P 75 or P 100) pineapple 50, 75 or 100 mg/kg.

The post-hoc analysis showed that the extract at doses of 75 and 100 mg/kg significantly decreased and increased T1 and RI, respectively, in comparison with the vehicle group ($P < 0.05$).

The effect of pineapple juice on memory performance

As shown in Fig. 3, T1 for mice treated with pineapple juice at doses of 50, 75 and 100 mg/kg was determined to be 160, 120 and 115 s, respectively. The RI values were found to be 30, 39 and 45 percent for mice treated with applied doses of pineapple juice, respectively (Fig. 4). The post-hoc analysis showed that the juice at 75 and 100 mg/kg significantly decreased T1 and increased RI, in comparison with the vehicle group ($P < 0.05$).

The effect of ethanolic extract of pineapple on scopolamine-induced amnesic mice

T1 for scopolamine-induced amnesia mice treated with 50, 75 and 100 mg/kg of ethanolic extract was 325, 295 and 235 s, respectively (Fig. 1). The RI values were found to be 20, 28 and 38 percent for scopolamine-induced amnesic mice treated the same doses of ethanolic extract (Fig. 2). As revealed by the Post-hoc analysis, T1 and RI values respectively were significantly decreased and increased after treatment with all doses of ethanolic extract compared to the scopolamine and the performance returned to the normal value ($P < 0.05$). In comparison with vehicle and positive control, the extract showed no significant effect on T1 and RI values at 100 mg/kg dose ($P < 0.05$).

The effect of pineapple juice on scopolamine-induced amnesic mice

T1 for scopolamine-induced amnesic mice treated with 50, 75 and100 mg/kg of pineapple juice was 325, 295 and 235 s, respectively (Fig. 3). As shown in Fig. 4, the RI values were found to be 20, 28 and 38 percent for scopolamine-induced amnesic mice treated by the same doses of pineapple juice, respectively. The Post-hoc analysis revealed that T1 and RI values were significantly decreased and increased after treatment by pineapple juice compared to the scopolamine and the performance returned to the normal value ($P < 0.05$). As compared with vehicle and positive control, pineapple juice showed no significant effect on T1 and RI values at 100 mg/kg dose ($P < 0.05$).

DISCUSSION

The effects of juice and ethanolic extract of pineapple for the first time were evaluated on scopolamine disrupted cognitive deficit using object recognition paradigm. The data suggested that pineapple could reverse the cognitive deficit. Scopolamine, a non-selective muscarinic antagonist blocks cholinergic signaling and produces memory deficit that are similar to those found in age-related senile central nervous system dysfunction. Scopolamine interferes with memory and

cognitive function and subsequently causes impairment of long and short term memories (37,38). Amnesic properties of scopolamine are well known in humans and animals, and in fact, scopolamine amnesia has been proposed as a pharmacological model for human dementia and AD (39). It has been verified that some AchE inhibitors such as donepezil, rivastigmine and galantamine could reverse cognitive deficits induced by scopolamine (40).

In this study, mice were given scopolamine at a dose of 1 mg/kg to induce memory impairment. The object recognition task allows rapid evaluation of memory performance in mice and rats (41). In this method no rewarding or aversive stimulation was used during training, and the learning occurs under normal condition and relatively low stress or arousal (42). The effect of scopolamine on the mice performance was examined with a single injection of 100 μL of scopolamine with a concentration of 1 mg/kg 30 min before T1 which caused amnesia. As shown in Figs. 1-4, at the first and second trial, T1 was high while RI was very low in scopolamine-injected animals. These animals could not discriminate between the new object and the familiar one indicating that recognition was significantly deficit. Using the object recognition task, juice or ethanolic extract of pineapple (50, 75 and 100 mg/kg) significantly improved the cognitive deficit and performance returned to normal in scopolamine-induced amnesic mice compared to the control (scopolamine) group. When scopolamine-induced amnesic mice treated by juice or extract were compared to vehicle and positive control, it was revealed that the dosage of 100 mg/kg in amnesic mice could return memory performance to normal values. In addition, cognition and memory performance were found to be profoundly improved in normal mice treated with 75 and 100 mg/kg juice or extract of pineapple compared with vehicle-injected group. It can be concluded that pineapple not only ameliorates scopolamine-induced amnesia but also improves memory performance in normal mice.

As reported by other studies, pineapple is rich in bioactive compounds such as flavonoids (11-13). Several lines of evidence have shown that plant extracts containing flavonoids could ameliorate cognitive deficits through inhibiting AchE. *In vitro* studies by Orhan, *et al.* revealed that among different flavonoid derivatives quercetin showed the most inhibitory effect against AchE (43). In other study, Jung, *et al.* showed that flavonoid fractions of ethyl acetate extract of *Agrimonia pilosa ledeb* profoundly inhibited AchE (44). As reported by Moyo, *et al.* flavonoids from *Sclerocarya birrea* and *Harpephyllum caffrum* methanolic extracts showed high dose-dependent anti AchE activity, *in vitro* (45). Perry, *et al.* reported that the essential oil extracted from *Centella asiatica* leaf contains some constituents which can inhibit acetyl cholinesterase (46). As shown by Jung, *et al*., the stamens of *Nelumbo nucifera* fed to rats improved memory through AchE inhibition (47). *Himatanthus lancifolius* that contains several indole alkaloids has also shown significant AchE inhibiting properties (33). Various other plant species have also shown significant anti-acetyl cholinesterase activity in memory deficit induced by scopolamine (40). In accordance with these studies, the memory improving activity of pineapple may be attributed to its pro-cholinergic and anti-AchE activity of flavonoids contain, suggesting pineapple might have chemical constituents which possess neurotropic activity and may be as promising as drugs for the treatment of amnesia.

CONCLUSION

Juice and ethanolic extract of pineapple significantly alleviated the scopolamine-induced memory impairment in the object recognition task. Considering the lack and need of drugs with proven effectiveness in improving learning and memory, the specific memory improving effects of pineapple reported here is of enormous interest and deserves further investigations using more experimental paradigms for further confirmation of memory improving potential of pineapple in the treatment of various cognitive disorders.

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