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EFFECT OF HYDROALCOHOLIC EXTRACT OF CICHORIUM INTYBUS L. ON PASSIVE AVOIDANCE LEARNING IN MALE WISTAR RATS

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ABSTRACT

Learning and memory is characteristically human to survive, living a normal life and scientific progress is inevitable. Many systems are involved in memory and learning Due to the use of herbal medicine. So, in this study aim was effect of Cichorium intybus than difference case on male Wistar rat..

Methods: In this study used adult Wistar rats' weight of 230 ±20. In control group (saline) group of (receiving half an hour before the test), Group acquisition (receiving half an hour before training), consolidated group (receiving a half hours of instruction) (n = 8). All injections are intraperitoneal and was injected doses of 10.25 mg/kg. Shuttle box was used to assess the passive avoidance learning and the delay in entering the dark room and the time spent were considered as measures in a dark room. The results showed that a dose of 25 mg per kg of chicory extract increased significant compared to controls in a dark room tardiness show in all groups of animals, acquisition, and consolidation. These results are probably due to antioxidant properties of chicory extract.

Keywords: passive avoidance, chicory extract, shuttle box, memory, rat

INTRODUCTION

Several neurotransmitter systems such as cholinergic and GABAergic system are involved in learning and memory process. Studies have also shown that the cholinergic system plays an important role in learning, memory, and attention. A consistent neuropathological occurrence associated

with memory loss is a cholinergic deficit [1]. It has been reported that degeneration of the cholinergic system, changed distribution of cholinoceptors and decreased acetylcholine (ACh) transferase levels in the brains of patients with Alzheimer's disease [2].

The effects of cholinomimetic drugs and cholinergic receptor antagonists on learning and memory tasks have been investigated [3]. Scopolamine, a blocker of muscarinic acetylcholine receptors, induces amnesia. This model of amnesia has been widely used to provide a pharmacological model of memory dysfunction [4].

One other candidate is the inhibitory neurotransmitter, γ-aminobutyric acid (GABA). Several lines of evidence indicate that GABAergic system and GABA type A ($GABA_A$) receptors regulate memory consolidation [5]. The muscimol as a $GABA_A$ receptor agonist impairs memory performance of behavioral tasks [6].

An ethnopharmacological approach has provided leads to identifying plants and potential new drugs that are relevant for the treatment of cognitive disorders, including AD [7].

Numerous plants are used in traditional medicine to enhance cognitive function [8]. Cichorium intybus is one of the most numerous genera within the family Asteraceae, which grows in many parts of the world [9-11]. The species of Cichorium genus (sage) has been reported to enhance memory and has been used in the treatment of memory disorders such as Alzheimer's disease [12]. Cichorium intybus. (Asteraceae) is one species of Salvia genus that geographically grows in some eastern regions of Iran. This plant was introduced in Flora Iranica in 1982 [13]. Some parts of C. intybus, especially leaf and root, are rich in saponins, tannins [14, 15], flavonoids and diterpenes [16]. Recently, studies have shown that C. intybus generally has multiple pharmacological effects, including neuroprotective, affecting morphine dependence [17], hypoglycemic [18], anti-inflammatory, analgesic and antioxidant activities [19, 20].

The present study examined the effects of ethanolic extract of C. intybus on memory in rat.

Material and Methods

2-1) Plant Material

Leaves of C. intybus were collected in May 2012 from city of Qom Province and approved in the Department of Pharmacognosy of the Qm Islamic Azad University (Voucher number 158-1932-08, deposited in Haddad Herbarium, with Mrs. Heidary as the director).

2-2) Extract preparation

To prepare 80% ethanolic extract of C. intybus, dried leaves were powdered at room temperature and 100 g of the powder was mixed with 1 L of ethanol (80%) at 1:10 (w/v) ratio for 72 hours [21]. The ethanolic extraction was conducted by percolation procedure in three steps. The extract was filtered and concentrated by using a rotary evaporator and dried in vacuum at 45°C. The dried extract was kept in a refrigerator (4°C) [22].

2-3) Animals

Forty male Wistar rats weighing 230±20 g were obtained from Pasteur Institute of Iran. The animals were housed five per cage in a regulated environment (23±1°C), with a 12-hour light/dark cycles (08:00 to 20:00 o'clock), under the relative humidity of 55%±15% and free access to food and water. The animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23; revised 1978). The protocol of the study was approved by the Islamic Azad University Institutional Animal Care and Use Committee.

2-4) Inhibitory avoidance apparatus

The step-through passive avoidance apparatus consists of a two-compartment acrylic box (20 × 20 × 30 cm) with a lightened compartment connected to a darkened compartment separated by a guillotine door (7 cm × 9 cm). Electric shock was delivered to the grid floor of both compartments, made of stainless steel rods (a diameter of 3 mm) spaced 1cm apart, by an isolated shock generator (Borj Sanat, Iran).

2-5) Behavioral procedure

The passive avoidance test was basically performed according to the step-through method described previously [23, 24]. All training and testing were done between 10:00 AM and 16:00 PM. The rats were allowed to habituate to the laboratory environment 30m before each training or testing session. The rats were subjected to acquisition test trials in the apparatus. In this trial, rat was placed in a lightened compartment for 30 seconds, and then the guillotine door was opened. Rats have a native preference to the dark environment. Immediately upon entering the dark compartment, the door was closed. The acquisition test recorded the latencies times for entering the dark compartment. After 30 minutes, the rats were again placed in the lightened compartment. After the rats had spontaneously entered the dark compartment, the guillotine door was closed and a mild electrical shock (50 Hz, 0.5 mA,

3 s) was applied. Exactly 24 hours after the acquisition trial for training, the retention test was performed. The retention test measured the step-through latency (STL) for entering the dark compartment in the same method with acquisition test and the time spent in the dark compartment (TDC). STL was shown before stepping into the dark compartment and the maximum entry latency allowed in the retention test was 300 seconds.

2-6) Experimental Protocol

2-6-1) Effect of C. intybus on PA memory

The aim of this experiment was determine the effect of C. intybus of different doses of C. intybus extract on memory and its most effective dose. Three doses 10 and 25 mg/kg of C. intybus ethanolic extract, were considered. Animals were randomly divided into three groups (n=12 in each group) including: the control group received saline and three extract-treatment groups received different doses (10 and 25 mg/kg, i.p.) of extract. The doses were chosen on the basis of previous reports (21).

2-7) Data analysis

Data were reported as mean ± standard error of the mean (SEM). Analyses were conducted using one-way ANOVA, followed by post hoc Tukey tests. A significance level of 0.05 or less ($p<0.05$) was considered statistically different.

1) Results

3-1) Effect of C. intybus on PA memory

One-way ANOVA indicated that the time spent in the dark compartment (TDC) was decreased significantly by C. intybus ethanolic extract at concentrations of 25 mg/kg in comparison to the control group ($p<0.01$) (Table 1),(Figure1). Also, latencies for entering the dark compartment (STL) showed an increase at doses of 25 ($p<0.001$) and 10 mg/kg ($p<0.05$), with no difference at compared to the control (Table 1),(Figure2).

The maximum effects of C. intybus were exhibited by 25 mg/kg dose and this dose was the effective dose of C. intybus. At 25 mg/kg dose of ethanolic extract STL was increased and TDC was reduced significantly compared to the control and other extract doses, significantly.

There were no dose-dependent effects of C. intybus ethanolic extract but a significant difference was observed between 10 and 25 mg/kg doses of the extract in STL ($p<0.05$) and TDC ($p<0.01$) parameters (Table 1).

Table 1. Effect of Cichorium intybus ethanolic extract on passive avoidance memory

Group	Dose mg/kg	Parameter STL (Seconds)	TDC (Seconds)
Control	-	207.0 ± 9.9	173.0 ± 7.5
Ethanolic extract	10	287.5 ± 26.5	138.43 ± 7.2 [a]
	25	586.4 ±21.5 [b]	112.50 ± 3.3 [b]

Each value represents the mean ± SEM for eight rats. [a] P<0.05 and [b] P<0.001, versus saline controlgroup; [c] P<0.001,versus extract 200 mg/kg group

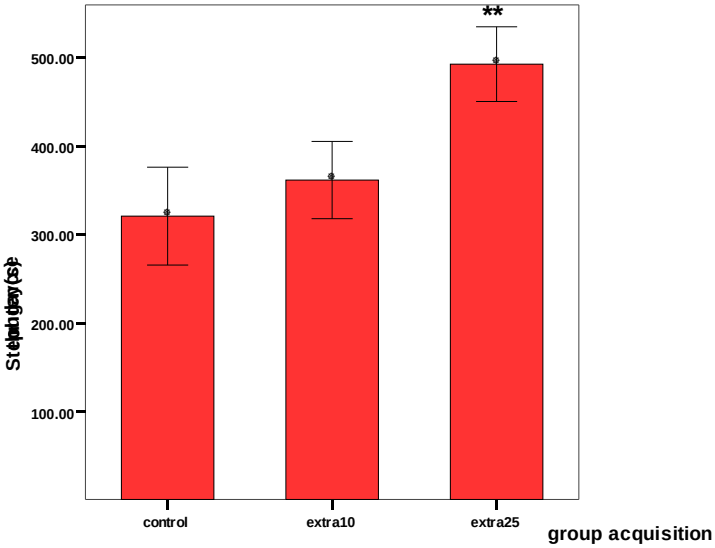

Figure1) Effect of Cichorium intybus ethanolic extract on STL

Each value represents the mean ± SEM for eight ratsand ** P<0.01, versus saline control group; ** P<0.01,versus extract 25 mg/kg group

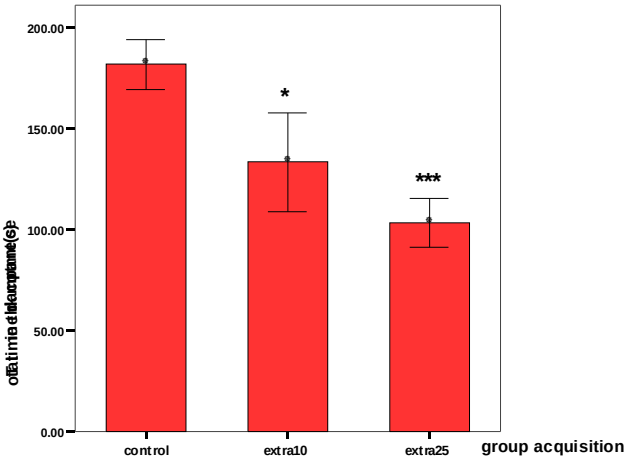

Figure2) Effect of Cichorium intybus ethanolic extract on TDC
Each value represents the mean ± SEM for eight rats* P<0.05 and ** P<0.001, versus saline control group; [c] P<0.001,versus extract 25 mg/kg group

2) Discussion

In this study, the effects of C. intybus ethanolic extract on the PA memory were evaluated in rats.

The step-through avoidance test is used to evaluate the learning and memory capabilities as well as maturation of the inhibitory process [25].

The results of this study show, memory-enhancing activity of C. intybus ethanolic extract at doses of 10 and 25 mg/kg. The dose of 25 mg/kg was significantly more effective than the other doses of extract. Therefore, this dose of ethanolic extract was considered the effective dose of C. intybus for administration to the drugs.

The well-replicated amnesic effect of cholinergic system anatagonist, scopolamine, has been interpreted as a principal consequence of the blockade of post-synaptic muscarinic M1 transmission [4]. In this study, treatment with scopolamine increased TDC more significantly than the control group and latency time exhibited a significant reduction than the control group. These results show properties of memory impairment of scopolamine, which have also been reported.

Decrease or increase in levels of the neurotransmitters or activation or blockade of different receptors related to the neurotransmitters indicates that other mechanisms may alter learning and memory [26]. It has been reported that the GABAergic system plays a role in the consolidation of inhibitory avoidance memory performance [27-29]. Post-training intraperitoneal injection of the GABA agonist, muscimol, impaired memory [30]. The results of the present study showed that the time spent in the dark compartment increased and latency time decreased 24 hours after training.

Traditional medicine systems have long included a number of Lamiaceous and Asteraceous plants for use in the treatment of a variety of disorders [31]. Among these, C. intybus has shown its reputed beneficial effects on memory and cognition disorders [7, 32, 33]. Members of the Salvia genus, such as C. intybus L. and Salvia lavandulaefolia Vahl. Have enhancing effects on memory [34]. It has been reported that essential oil and ethanolic extract of C. intybus enhance memory in rats [31, 35].

In the present study, ethanolic extract of C. intybus exhibited memory enhancing activity and potentiated memory retrieval, consistent with some other reports. The compounds of C. intybus such as tanshinone and flavonoid, maybe contribute to its

health properties and thus used as a popular folk medicine for the treatment of various ailments [36]. In the aerial parts of C. intybus diterpenes and flavonoids, which are also found in other species of this genus, contain 5-hydroxy 4, 6, 7- trimethoxy flavon (I, a flavonoid) and a new labdane diterpene (II) [16]. Therefore, memory enhancing effects of C. intybus is maybe due to these compounds.

Members of the sage family have a long history of use as memory enhancing agents coupled with cholinergic properties that may be relevant to amelioration of the cognitive deficits associated with Alzheimer's disease [36] and some sage genera have memory enhancing activity coupled with GABAergic properties [37].

It has been shown that some C. intybus, have memory-improving activity on memory impairment induced by scopolamine in rats [34, 38]. Same as these in the present study, C. intybus extract ameliorated effects of memory impairment on rats.

Recently $GABA_A$ receptor modulation has been shown by Salvia triloba. S. triloba has cognition enhancing properties that are likely to occur via different modulatory sites on $GABA_A$ receptor complexes [37].

Our results showed that ethanolic extract of C. intybus ameliorates the effects on memory in the passive avoidance test. C. intybus improved amnesia and memory process impairment induced by agonist of GABAergic system in rats.

3) Conclusion

It concluded that C. intybus possess a memory enhancement effect in rat. Ethanolic extract of C. intybus. Therefore, the traditional use of this plant may be validated by this study.

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