Original Article

Evaluation of the Negative Effects of Opium Tincture on Memory and Hippocampal Neurons in the Presence of Chicory Extract

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Abstract

Background: Due to the high prevalence of addiction in society and the need to its attention, various methods are used for addiction withdrawal. The side effects of some methods restrict their use and increase the risk of recurrence. One of the Iranian useful methods is consumption of opium tincture (OT) that may cause brain structure and memory defects. Hence, this study aimed the effects of different doses of OT on memory and hippocampal neurons with the use of an antioxidant such as various concentrations chicory.

Materials and Methods: In the present study, 70 Wistar rats were randomly divided into 10 groups and the effect of various doses of chicory extract and OT were assessed on memory by the passive avoidance test. The neurons and astrocyte cells numbers in dentate gyrus were investigated, using histological examination.

Results: In passive avoidance test, the total time in dark compartment was significantly more in groups with 100 and 75 µl OT compared with control and normal saline groups ($P < 0.001$). Traffic number results showed that there was a significant difference between T100 and control groups ($P > 0.05$). Moreover, initial latency time was significantly shorter in groups with 75 and 100 µl of OT compared with control and normal saline groups ($P < 0.05$). However, the presence 250 mg/kg of chicory increases granular layer thickness of dentate gyrus and number of neurons.

Conclusion: The use of 250 mg/kg of chicory extract may be promising strategy for inducing neurogenesis and this dose could prevent neural damage.

Keywords: Chicory, hippocampus, memory, opium tincture

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Introduction

Opioids are used as pain reliever of thousands of years ago.[1] There are receptors for binding of opioids in different regions of the brain.[2] Opium is one of the common opioids that were extracted from *Papaver somniferum* and this material has beneficial pain-relieving properties.[3] Morphine is one of most important the opium alkaloid that has analgesic effects on the central or peripheral nervous systems. Furthermore, other constituents of opium are noscapine, papaverine, codeine, and thebaine.[4] While the opium compounds apply in the production of most common analgesic in many countries, opium abuse has many side effects on different organs in the body[5] and creates serious social problems.[6]

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It seems that the use of opium has capable to induce oxidative stress and injury to antioxidant system.[7] Furthermore, the opium compounds such as morphine may provide functional and structural changes in the central nervous system (CNS).[8,9] Morphine can directly act through neuroadaptive changes in glutamatergic and dopaminergic neurotransmission on CNS. The administration of morphine increases dopamine release and changes dopaminergic receptors in the different areas of CNS including the striatum, ventral tegmental area, hippocampus, and the prefrontal cortex.[10,11]

There are several methods for treatment of addiction that tincture of opium is one of alternative pharmacotherapies in opioid withdrawal.[12] Opium tincture (OT) is a liquid that forms of opium in alcohol and water as it contains 1% morphine.[13]

Chicory intybus (chicory) is a membrane of the *Asteraceae* family that called Kasni in Persian and it has many applications in human diets and preparation of traditional and herbal medicines. Chicory is major crop in northwestern Europe, India, South Africa, Chile, and grows widely in Iran.[14]

This plant has extensive properties such as antidiabetic, antioxidant, antibacterial, immunotoxic, antihepatotoxic, and cardioprotective.[15] Due to these unique features, this plant plays critical roles in reproductive, sedative, the wound healing without adverse effects.[16]

Chicory has high levels of anthocyanin and polyphenolic antioxidants and it can exert a inhibitory effect on reactive oxygen species formation.[17] Furthermore, leaves of chicory are a rich source of other antioxidants such as phenols, potassium, calcium, vitamins A and C.[18] The roots of Chicory are used as a coffee surrogate while it is rich of polysaccharide inulin.[19] In addition to the root of the plant is usually consumed in the community, the stem, leaves, and flowers of the plant should be used in terms of features of its effectiveness. Leaves of chicory are applied as wound healing and regulation of blood pressure.[20]

According to negative effects of morphine on CNS, the use of antioxidants such as chicory with OT may reduce the morphine side effects during opioid withdrawal. Hence, the present study aimed to evaluate the effect of Chicory extract with different concentrations on hippocampus structure, working, and short-term memories following the use of various doses of OT in rats.

Materials and Methods

Plant material and extraction

Chicory leaves were purchased from a reliable center of medicinal plants in Qom and received a confirmation code by Herbarium of Qom Agriculture Education and Research Center with the number CH-IN: 97-567. Dried plant of approximately 50 g was extracted with 80% ethanol using soxhlet equipment. The balloon of the device was placed on the heater. The solvent is gradually evaporated by heat and guided by a tube to a glass tube on top of the device during 24 h. To complete the extraction of the sample, the process of extraction was carried out several times, and finally, the extract was filtered using Whatman filter paper and then evaporated by rotary evaporator. The green extract was prepared and then, it was completely dried and turned into a brown powder. The extracts were kept in sterile bottles and put in refrigerator at 2°C–4°C until use.[21]

Animals and animal treatment

All procedures of this study were carried out in accordance with Ethics Committee of the Qom University of Medical Sciences (Ethical code: IR.MUQ.REC.1397.21). Male Wistar rats (n = 70) with weighing ~200–250 g were obtained from the animal department of the Qom University of Medical Sciences. Animal room temperature and relative humidity were 22°C ± 2°C and 40%–60%, respectively, and there was a 12 h light/dark cycle. The rats were randomly divided into 10 groups (7 rats in each group): (1) control group (without injection), (2) receive normal saline (3) receive 100 µl of OT, (4) 75 µl of OT, (5) 30 µl of OT, (6) 75 µl of OT with 100 mg/kg of chicory extract, (7) 75 µl of OT with 250 mg/kg of chicory extract, (8) 75 µl of OT with 400 mg/kg of chicory extract, (9) 100 µl of OT with 250 mg/kg of chicory extract, (10) 30 µl of OT with 250 mg/kg of chicory extract. We determined lethal dose to choose of different concentrations OT. Furthermore, the different concentrations of chicory were chosen based on previous studies.[22]

Determination of lethal dose

Lethal dose (LD50) was estimated when a dose required to kill 50% of experimental animals.[23] Approximately 18 h before experiment, the animals were fasted. Following intraperitoneal injection of OT with different concentrations of 0.5 cc (10 mg of morphine), 0.3 cc (6 mg of morphine), and 0.1 cc (2 mg of morphine), animals were placed in individual cages, allowed to eat and drink. All rats seizure and died after 5 min of 10 mg morphine injection while 3 rats died during the first 30 min period after receiving of 6 mg of morphine. Finally, all rats survived after 2 mg of morphine injection. Hence, 0.1 cc of OT (2 mg of morphine) was considered as maximum dose in rats. According to the treatment protocol of addiction in human and calculation the maximum dose, we injected the 30, 75, and 100 µl concentrations of OT to rats with weighing ~200–250 g in different group during 21 days.

Passive avoidance task

This test was performed 17 days after start treatment for the evaluation of the short and long memories based on previous studies.[24,25] The animals were used for passive avoidance task through a shuttle box which included identical light and dark Plexiglas square boxes (30 cm × 20 cm × 20 cm) which were separated by a guillotine door. Floor of light and dark compartments were composed of 2 mm steel rods spaced 1 cm apart and the light compartment contained a 50 W bulb while dark compartment is without bulb. The animals underwent two stages of trials: a training trial and a test trial.

During the training trial, each rat was placed in the lighted compartment, as soon as it entered the dark compartment, an electric shock was provided (0.5 mA, 1s). In the testing trial, 1 day after the training trial, the rat was again placed in the lighted compartment and finally, time took the animal to enter the dark compartment (initial latency time), time spent in the dark compartment (TDC), and traffic number was measured.

Histological examination

After the behavioral tests, the animals were perfused with 4% paraformaldehyde in 0.1 mol/L phosphate buffer solution (pH: 7.4). Then, the brains were placed in the postfix solution overnight. The samples were processed and embedded in the paraffin. Coronal sections (4 μm thickness) were prepared by a microtome rotary (LEICA RM 2235) and were stained with Nissl staining (cresyl violet acetate: 0.01%). The thickness of granular layer in the dentate gyrus region of the hippocampus, number of astrocyte cells, mature and immature neurons were evaluated in 3 sections for each sample.

Statistical analysis

All data are presented as mean ± standard deviation and analyzed by Statistical Package for the Social Sciences 24 (SPSS) software. Differences between groups were evaluated by one-way analysis of variance. $P < 0.05$ was considered as statistically significant.

Results

Passive avoidance test

Effect of various concentrations of chicory extract on memory retrieval assessed following analysis of TDC, traffic number, and initial latency in the passive avoidance test. Figure 1a shows that TDC was increased in groups treated with 100 and 75 μg/kg of OT compared to control and normal saline groups ($P < 0.001$). However, there was no significant difference between T30 group and control group ($P > 0.05$). In addition, the use of high dose of chicory extract (400 mg/kg) significantly increased TDC compared to control and normal saline groups ($P < 0.001$).

The behavioral data indicated that traffic number significantly increased in animals which were under treatment of 100 μl concentration of OT compared to control and normal saline groups ($P < 0.001$) [Figure 1b]. The passive avoidance test revealed no significant difference in traffic number between 100 and 250 mg/kg of chicory extract receiving groups with normal saline group ($P > 0.05$).

There was a significant reduction of initial latency time in the groups treated with 75 or 100 μl of alone OT compared to control and normal saline groups ($P < 0.001$). Moreover, receive of high dose of chicory extract resulted in a decrease in initial latency time compared to control and normal saline groups ($P < 0.001$) [Figure 1c].

Histological results

Figure 2A shows the light microscope images of hippocampus in various groups. The histological evaluation showed that 100 and 75 μl concentration of OT can decrease granular layer thickness of dentate gyrus compared with control and normal saline groups ($P < 0.001$). However, there is no significant difference between groups which received 250 mg/kg or 100 mg/kg chicory extract, compared with control and normal saline groups ($P > 0.05$) [Figure 2B].

The number of mature and immature neurons in the presence of 100 and 75 μl of OT significantly is decreased compared with control and normal saline groups ($P < 0.005$) [Figure 3a, b, d and e]. A significant reduction of neurons number is found in T75+ C400 group compared with control and normal saline groups ($P < 0.001$). Yet, the use of 250 mg/kg chicory extract with 75 μl of OT improved number of immature and mature neurons compared to T75 and T100 groups ($P < 0.001$). Our data also showed that there is no significant difference in astrocytes number between control group and other groups ($P > 0.05$) [Figure 3c and f].

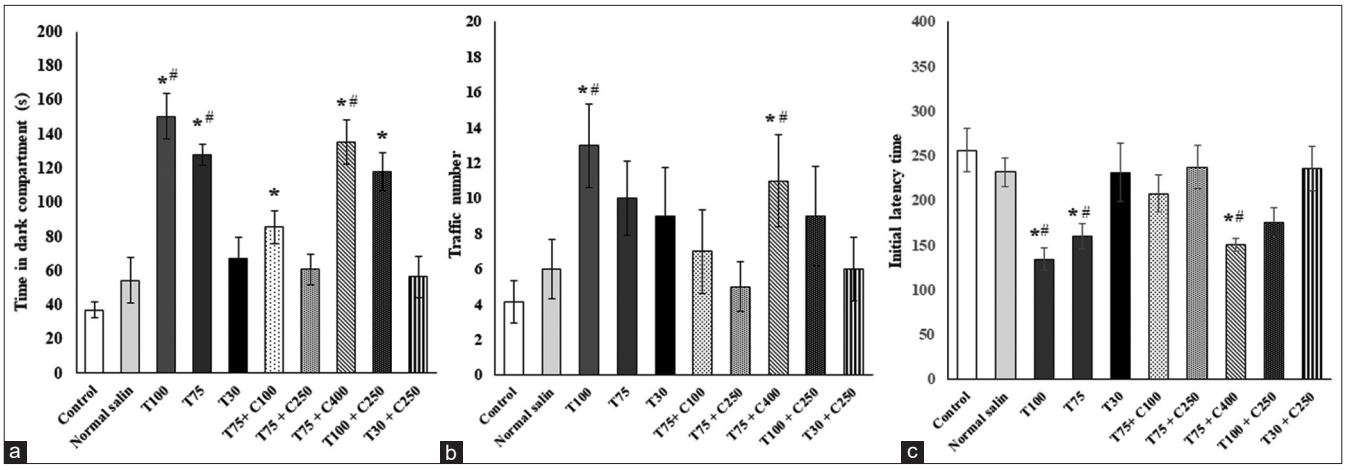

Figure 1: (a) The comparison of time dark compartment among experimental groups. (b) The comparison of traffic number among different groups. (c) The comparison of initial latency time in different groups. (c) *$P < 0.001$: Significant difference between control and normal saline groups with other groups. #Significant difference at $P < 0.001$ compared to T75+ C250 group

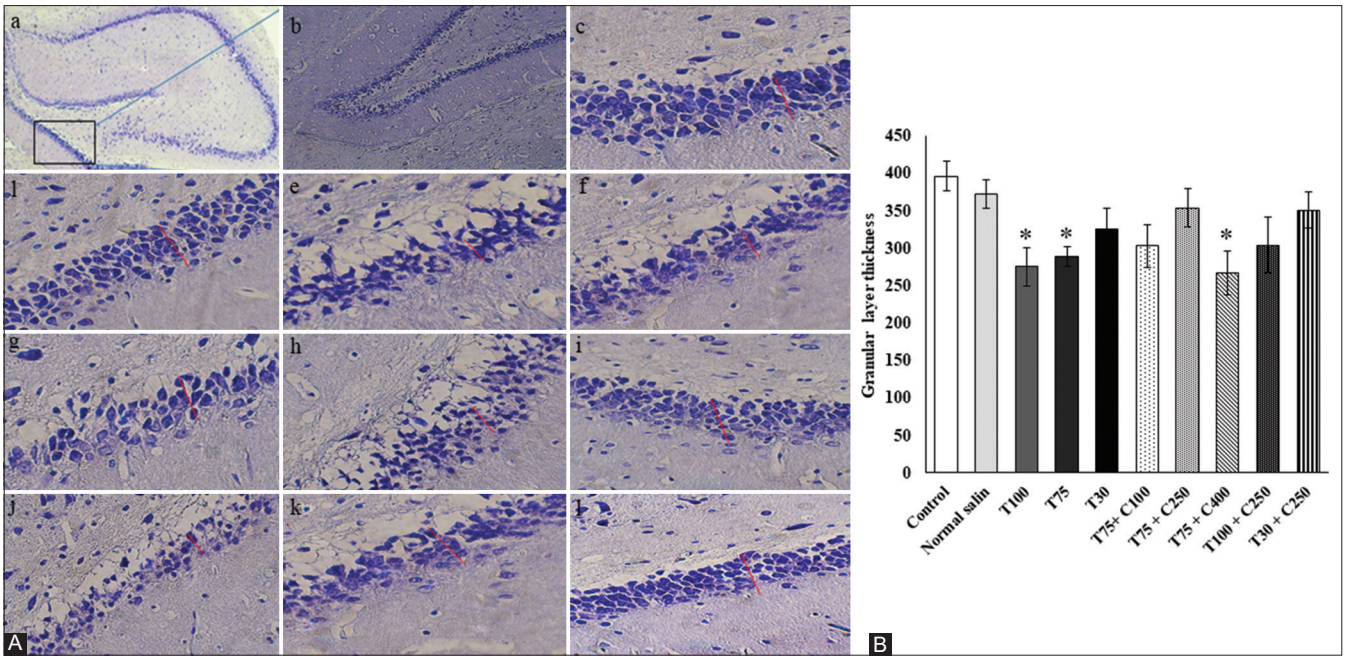

Figure 2: (A) (a and b) The photomicrographs of coronal section of the hippocampus and the granular layer thickness of dentate gyrus region (red arrow) in 10 groups (c) Control group, (d) Normal saline group, (e) T100 group, (f) T75 group, (g) T 100 group, (h) T75+ C100 group, (i) T75+ C250 group, (j) T75+ C400 group, (k) T100+ C250 group, (l) T30+ C250 group. H and E, X 4 (a, b), X 40 (c-l). (B) The granular layer thickness of dentate gyrus region in different groups, *Significant differences at $P < 0.001$ from control and normal saline groups

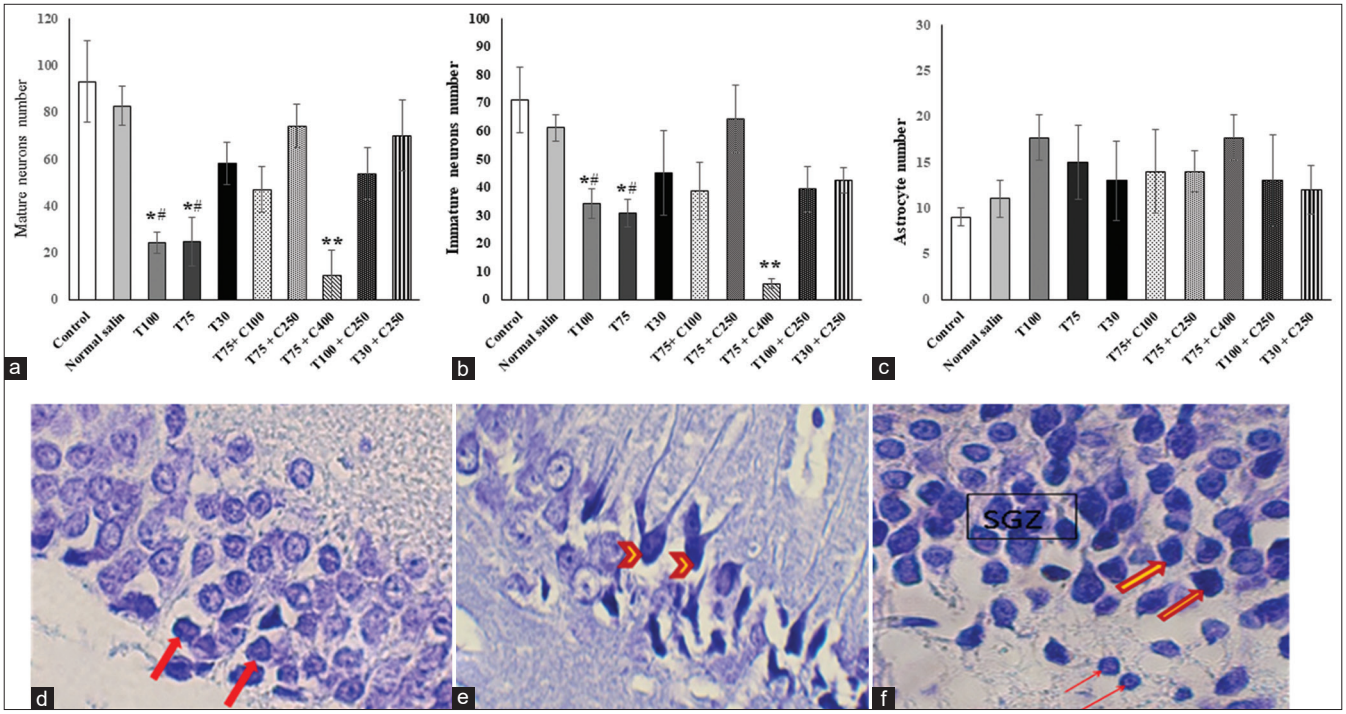

Figure 3: (a) The comparison of the mean number of mature neurons in different groups, (b) The mean number of immature neurons in different groups, (c) The comparison of mean number of astrocyte cells in different groups. (d) The red arrows point to immature neurons in T75+ C250 group (e) The mature neurons are shown by red arrow in T30+ C250 group (f) Astrocyte cells (thin arrow) and immature neurons (thick arrow) were identified in T75+ C250 group. The mature neurons have large nucleus with clear nucleolus and euchromatin. Immature neurons have a medium size of approximately condensing nucleus without euchromatin. Astrocyte cells have small pale nucleus and sometimes clear nucleolus. H and E, ×40 (*$P < 0.05$, **$P < 0.001$ compared with control and normal saline groups, #$P < 0.001$ compared with T75+ C250 group)

Discussion

Few areas of the adult mammalian brain including subventricular zone and the dentate gyrus (DG) of the hippocampal formation are capable for neurogenesis.[26] Neurogenesis process is regulated by several agents such as growth factors, neurotransmitters, and corticosteroid hormones.

Morphine as one of the compounds of OT affects cognition function and learning and it may inhibit neurogenesis in the hippocampus. Furthermore, morphine can reduces dendritic branching and the density of dendritic spines of neurons in the brain.[27] Morphine can strongly induce the release of corticosteroids that have a inhibitory effect on neurogenesis. Furthermore, the opioids can affect on the hippocampal neurogenesis by reducing the number of glial cells and immature neurons in the hippocampus.[28] A study evaluated the effect of morphine on the cell proliferation and death in the adult rats DG that showed that morphine injection plays a negative impact on neurogenesis and cell proliferation.[29] Another study showed that chronic consumption of morphine in different concentrations had no effect on learning ability and short-term memory (working memory), moreover, dependence on morphine with high dose may impaired long-term memory (reference memory) in rats.[30] Our study evaluated the effect of OT various concentrations on short-term memory (working memory) and long-term memory (reference memory) by passive avoidance test proving that short and long-term memories can be diminished after injection of 75 and 100 µl of OT in rats.

Today, many studies were assessed the effect different antioxidants on memory and hippocampus functions. It proved that antioxidants can improve cognitive functions and prevents neuronal damages in the brain.[30] The chicory extract plays neuroprotective role by reducing of the oxidative stress, restoring glutathione levels, and increasing of the activity of the catalase enzyme.[18]

Our results suggested that 100 and 250 mg/kg of chicory extract has positive effect on learning and memory. Furthermore, we observed not only the high dose of chicory extract provide no positive effect on memory but also it caused memory impairment.

It was evidenced that the 75 and 100 µl doses of OT significantly decreased number of neurons compared with control and saline groups, while the presence of chicory extract with 250 mg/kg could remove adverse effects of OT the thickness of the granular layer and prevented the destruction of mature neurons. Yet, it seems that the use of 400 mg/kg chicory extract has toxicity effects on granular layer thickness of dentate gyrus. Therefore, effect of chicory extract is dose dependent and the dose of 250 mg/kg could reduce the negative effects of the OT on brain.

As expected, results of histology studies were consistent with behavioral experiments and it approved that 250 mg/kg concentration of chicory extract may be suitable antioxidant for decreasing of side effects of OT. A study showed that the use of chicory leaf extract stimulates neuroprotective by increasing of acetylcholinesterase activity during 10 days.[31] Another study evaluated the effect of chicoric acid extracted from chicory on amyloidogenesis and cognitive impairment. They reported that chicoric acid can prevent neuroinflammation by inhibition inflammatory mediators and it as a neuroprotective agent can be used for the treatment brain injuries.[32]

Conclusion

It seems that the use of OT with high concentrations causes disorders in learning, working, and reference memories. Furthermore, it reduces the number of neurons and the thickness of the granular layer of dentate gyrus. The use of appropriate plant extracts like chicory extract as antioxidant with optimal concentration is able to improve side effects of opium.

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Conflicts of interest

There are no conflicts of interest.

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