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Memory enhancement by administration of ginger (*Zingiber officinale*) extract on morphine-induced memory impairment in male rats

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ABSTRACT

To study the chronic treatment with hydroethanolic extract of ginger (50, 100 and 200 mg/kg, p.o) would effect on the passive avoidance learning (PAL) and memory in rat. **Methods:** The rats were divided into eight groups. On the training trial, the mice received an electric shock when the animals were entered into the dark compartment. Twenty-four hours later, 30 min after treatment, the STL (step-through latency) and TDC (total time in dark compartments) was recorded and defined as the retention trial. **Results:** The time latency in morphine-treated group was lower than control ($P<0.001$). Treatment of the animals by 100 and 200 mg/kg of ginger extract before the training trial increased the time latency at 24 hours after the training trial ($P<0.01$ and $P<0.001$). Administration of both 100 and 200 mg/kg doses of the extract in morphine received animal groups before retention trials also increased the time latency than the morphine-treated group groups ($P<0.001$). **Conclusion:** The results revealed that the ginger extract attenuated morphine-induced memory impairment.

1. Introduction

Zingiber officinale (*Z. officinale*) Roscoe (Zingiberaceae), commonly known as 'Ginger', is one of the frequently used spices in the world. Ginger has been cultivated for thousands of years and used safely in cooking, and medicinally in folk and home remedies. It is used extensively in traditional medicine to treat cold, fever, headache, nausea and digestive problems; and is also used in Western herbal medical practices for the treatment of arthritis, rheumatic disorders and muscular discomfort[1]. It has been reported that ginger or its extracts possess some pharmacological activities including antiemesis[2], analgesic effect[3], anti-tumor[4], anti-oxidant[5], anti-inflammatory effect[6,7]and a neuroprotective effect[8,9].

The oleoresin (*i.e.*, oily resin) from the rhizomes (*i.e.*, roots) of Ginger contains many bioactive components, such as 6-gingerol, which is the primary pungent ingredient that is believed to exert a variety of remarkable pharmacological and physiological activities. Ginger has been used for thousands of years for the treatment of numerous ailments, such as colds, arthritis, migraines, and hypertension[10]. It is used in traditional Asian medicine for the treatment of stomach aches[11], nausea, diarrhea, and joint and muscle pain[12].

Antioxidants in ginger include gingerols, shogaols and some phenolic ketone derivatives. The anti-inflammatory and anti-oxidant properties in ginger help relieve various inflammatory disorders like gout, osteoarthritis, and rheumatoid arthritis. It provides substantial relief in pain caused by inflammation and help decrease swelling and morning stiffness[13]. Another study suggests that Ginger can reduce cell death and restore motor function in a rat spinal cord injury[14].

Learning and memory in laboratory animals are known to be affected by opioids and their antagonists[15]. For example, pretraining administration of morphine impairs memory retrieval in passive avoidance tests which will be restored by pretest administration of the same dose of

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morphine[16]. Hippocampus is one of the areas involved in learning and memory in which both opioid peptides and opioid receptors are expressed[17]. Endogenous opioid peptides consider important neuromodulators in the brain, which are rich in the hippocampus and cerebral cortex[18]. Using different animal models, it was shown that repeated administering morphine can impair memory and learning processes[17, 19, 20].

With regard to the possible effects of ginger on learning and memory and its interactions with opioid system, the aim of the present study was to evaluate the effect of hydroethanolic extract of *Z. officinale* on morphine-induced memory impairment in mice using the passive avoidance test.

2. Material and methods

2.1. Plant material

The fresh rhizomes of *Z. officinale* (herbarium code no. 1483) was purchased from the Institute of Medicinal Plants Tehran, Iran. The plant material was dried with room temperature (25 ℃), shade dried and powdered.

2.2. Preparation of Plant Extracts

Approximately 500 g of the dried powder from *Z. officinale* were extracted with 5 L of 80% aqueous ethanol using the percolation method at room temperature. The extracts were filtered through filter paper and evaporated to dryness under reduced pressure at a maximum of 40 ℃ using a rotary evaporator. The hydroethanolic extract of ginger was stored in small samples at −20 ℃ until use. The extract was dissolved in saline and was then applied.

2.3. Drugs

The drugs used in the present study was morphine sulfate (darou pakhsh, Tehran, Iran). Morphine dissolved in physiologic saline solution.

2.4. Animals

Sixty-four locally produced male Wistar rats (250-280 g) from the Iranian Razi Institute, were used in the present experiments. All animals were maintained at a constant temperature (22±2) ℃and on a 12 h light/dark cycle. They had free access to laboratory chow and tap water. Each Experimental group consisted of eight animals that were chosen randomly from different cages. Each rat was used only once.

2.5. Experimental design

The animals were divided into eight equal groups (n=8); (1): Normal control group received normal saline via oral gavage, (2): control group received morphine sulfate at dose 5 mg/kg via i.p injection 30 min before training, (3-5): groups received *Z. officinale* extract at doses 50, 100 and 200 mg/kg via oral gavage 30 min before training, (6-8): groups received morphine at dose 5 mg/kg and *Z. officinale* extract at doses 50, 100 and 200 mg/kg via oral gavage 30 min before training. Thirty minutes after the treatment, learning and memory were evaluated. The operator was unaware of the specific treatment groups to which an animal belonged. Animals were handled in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication 86-23; revised 1985). All the protocols were also approved by the institutional ethics committee of Bu-Ali Sina University.

2.6. Passive avoidance task

The apparatus used for evaluation of the passive avoidance task was two-way shuttle box (Borj Sanaat Co. Iran), which consisted of two adjacent Plexiglas compartments of identical dimensions (27 cm×14.5 cm×14 cm). For the experimental procedure, on the first day (adaptation day) each rat was allowed a 3 min adaptation period and free access to either the light or dark compartment of the box to avoidance training and after being placed in a shuttle box. Following this adaptation period, on the second day (training phase), rats were placed in the illuminated compartment and 30 s later the sliding door was raised. Upon entering the dark compartment, the door was closed and a 1.5 mA constant-current shock was applied for 3 s. After 20 s, the rat was removed from the dark compartment and placed into home cage. In order to test short- and long-term memories, 24 h after receiving foot shock, the rats were placed in illuminated chamber and 30 s later the sliding door was raised and the latency of entering the dark compartment (step-through latency) and the time spent there during 5 min were recorded again, because the maximum time that was considered in this procedure was 300 s[21-24].

2.7. Statistical analysis

The data are expressed as mean values with their standard errors. Following a significant P-value, *post hoc* analysis (Tukey's test) was performed for multiple comparison. Statistical analysis was performed using SPSS (version 21; SPSS Inc., Chicago, IL, USA). A level for P<0.05 was considered to be significant.

3. Result

The retention test which was conducted 24 h after training. It revealed a significant difference in the STL among the groups (Figure 1). There was a significant difference in STL

between saline treated control groups and Ginger treated groups at dose 100 and 200 mg/kg (P<0.01and P<0.001, respectively). But there was no significant difference in STL between extract treated group at dose 50 mg/kg and control group. Specifically, the STL of morphine treated group at dose 5 mg/kg were significantly lower than the saline control group. Administration of ginger at doses 100 and 200 mg/kg to animals that received morphine (5 mg/kg) resulted in longer STL compared to morphine treated animals (P<0.001). There was a significant difference in STL between extract treated at dose 200 mg/kg that received morphine and saline treated control groups (P<0.01).

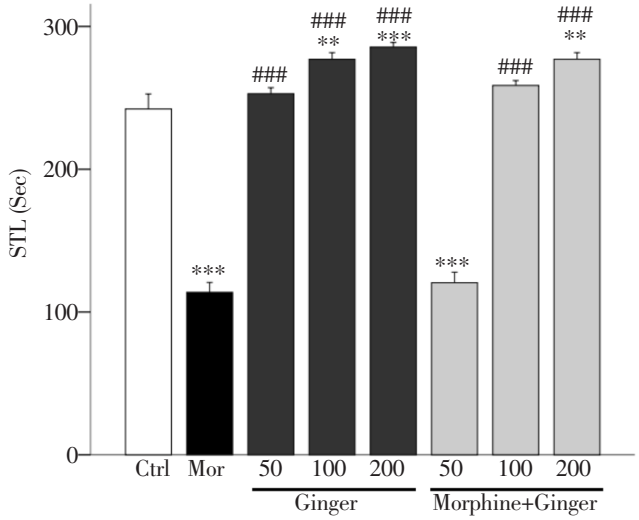

Figure 1. Effect of ginger on the step-through latency in the acquisition trial (STL) of passive avoidance learning (PAL) task in the rats. Columns represent mean ± SEM. **P<0.01, ***P<0.001 as compared with its related Control group. ###P<0.001, as compared with its related Morphine received group.

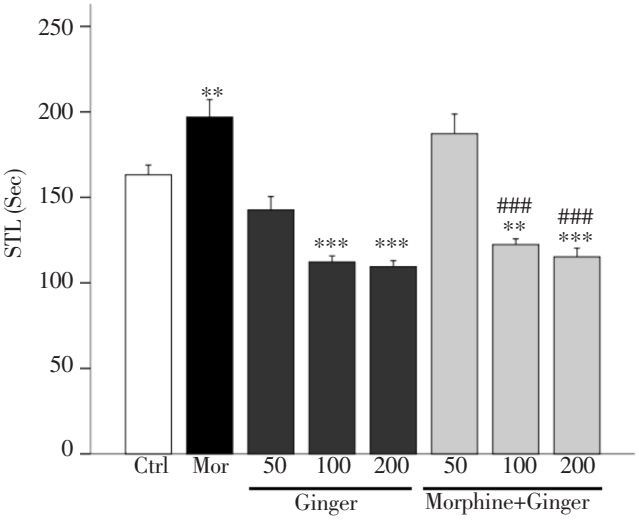

Figure 2. Effect of ginger on the time spent in the dark compartment in the retention trial (TDC) which was carried out 24 h after acquisition of passive avoidance learning (PAL) task in the control and diabetic groups. Columns represent mean ± SEM. **P<0.01, ***P<0.001 as compared with control group. ###P<0.001 as compared with morphine treated group.

Also there was a statistically significant difference in TDC among the experimental groups (Figure 2). Consistent with a cognitive impairment, TDC of the Morphine treated rats was greater than the saline treated control group (P<0.01). Time spent in the dark compartment in the group that treated extract at doses 100 and 200 mg/kg was significantly less than the respective saline treated control group (P<0.001). Also it was a significant difference in TDC between Ginger extract treated at doses 100 and 200 mg/kg that received Morphine and Morphine treated groups (P<0.001). There was a statistically significant difference in TDC between extract treated at dose 50 mg/kg that received Morphine than the Morphine treated group (P<0.01).

4. Discussion

It has long been known that learning and memory are affected by opioids. According to present results, which are in agreement with previous reports, pre-training administration of morphine impaired memory acquisition[25,26]that is partially restored by pretest administration of the same opioid[27, 28]. Some studies have demonstrated that opioid agonists such as morphine and endorphin, possessing higher affinity for μ-opioid receptors, inhibit cholinergic activity in the hippocampus[29]. Moreover, it has been reported that mu and delta opioid receptors located on the cholinergic terminals are normally under tonic inhibition by the opiate system[30]. It has been demonstrated that morphine, at a dose that impairs memory, decreases hippocampal acetylcholine output. Moreover, learning and memory impairment caused by acute administration of morphine is suggested, at least in part, to be related to a decrease in hippocampal acetylcholine release[31]. By using in vivo microdialysis, it has been demonstrated that acute morphine administration significantly decreased the release of acetylcholine in some brain regions[32-34].

It has been shown that the degree of memory impairment is related to the degree of cholinergic loss in early Alzheimer's disease[35]. Computational models linking ACh-related behaviors to the cellular effects of ACh provide additional evidence of the role of ACh in learning and memory[36]. Cholinergic neurotransmission is a central process underlying memory and cognitive function. Cholinergic basal forebrain neurons in the nucleus basalis magnocellularis innervate the cerebral cortex, amygdaloid complex and hippocampus, and are essential for learning and memory formation[37,38]. Hippocampus and cortex are innervated by cholinergic projections. Abnormalities in cholinergic functions lead to impaired processing of the hippocampal region[39].

Ginger (*Z. officinale* Roscoe, Zingiberacae) has been traditionally used for a wide range and unrelated ailments that include fever and infectious diseases, stomachaches, abdominal spasm, nausea and vomiting, motion sickness, arthritis, rheumatism, ulcerative colitis, gingivitis,

hypertension, and diabetes[1,40]. The bioactive components of *Z. officinale* were characterized by spectroscopic analysis as zingerone, gingerdione, dehydrozingerones which exhibited potent antioxidant, shogaols, gingerols and volatile oil[41]. Sesquiterpene lactones (SLs) are natural products responsible for the anti-inflammatory activity[42].

High antioxidant activity of ginger and its compounds has been demonstrated in numerous reports[43,44]. Furthermore, Lee and colleagues showed that 6-Gingerol, a bioactive component of Ginger, efficiently suppresses β-amyloid-induced intracellular accumulation of reactive oxygen and nitrogen species and restores endogenous antioxidant glutathione levels and up-regulates the expression of antioxidant enzymes[45]. 6-shogaol is one of the most bioactive components of Ginger rhizome which has been shown to decrease inducible nitric oxide syntheses (iNOS) levels in LPS-treated astrocytes[46]and macrophages[47]. Recent accumulating lines of evidence show that antioxidants could also improve cognitive performance in healthy elderly subjects[48,49]. According to the cognitive enhancing effects of substances possessing antioxidant activity, the concentration of gingerol and shogaol of the extract, and the antioxidant activity of *Z. officinale*, we suggest that the cognitive enhancing effect of this plant extract on working memory observed in this study might be partly related to its antioxidant effect. However, the precise underlying mechanism and possible active ingredient responsible for the cognitive enhancing effect of *Z. officinale* still require further investigation.

Recent findings also suggest an important role of the hippocampus in spatial working memory[50]. In addition, it was found that dopamine, and norepinephrine play a key role in numeric working memory including word and picture recognition (organized by the lateral PFC), while acetylcholine and serotonin in the hippocampus simultaneously were activated during spatial working memory tasks[51]. Various investigators have previously demonstrated that extract of Ginger and its fractions have anti-5HT3-receptor effects[52-54]. 5-HT3-receptor stimulation contributes to fast excitatory synaptic transmission in the central nervous system[55, 56]and also modulates the release of several neurotransmitters including Acetylcholine, Cholecystokinin (CCK), Dopamine, Glutamate, Norepinephrine and Particularly γ-Aminobutyric Acid (GABA), the exocytosis of which is enhanced by direct Ca2+ influx through the ionophore of presynaptic 5-HT3-receptors[57, 58]. Zingerone and other derivatives from Ginger inhibits the release of most neurotransmitter especially serotonin[59, 60]. 5-HT3-receptor antagonists have multiple pharmacological actions[61]. It has been shown that 5-HT3-receptor antagonists also possess improve cognitive and memory[62]. Moreover, this plant extract and its active component, 6-Gingerol, also inhibited the cholinesterase activity which in turn increased acetylcholine (ACh), a neurotransmitter that plays an important role in learning and memory[63]. Therefore, taking all data together, we suggest that the cognitive enhancing effects of *Z. officinale* might be partly associated with the modulation effect of this plant extract on the alteration of both the monoamine system and the cholinergic system in various brain areas, including the prefrontal cortex and hippocampus.

It is well known that *Z. officinale* and its components have potent anti-inflammatory effects in different animal models of inflammation[3]. Recently, Li *et al*. (2011) demonstrated that the anti-inflammatory effect of Ginger occurs through the inhibition of TNFα and IL-1β gene expression[64]. Cyclooxygenase-2 (COX-2), the inducible isoform of COX, is rapidly up-regulated in damaged tissue, for example in the central nervous system (CNS) damage. COX-2 induction in CNS overall contributes to inflammation and injury mainly by producing prostanoids. Prostaglandin E2 (PGE2), a dominant enzymatic product of COX-2 in the brain[65]. Researchers have hypothesized that the anti-inflammatory effects of Ginger might be related to its ability to inhibit prostaglandin and leukotriene biosynthesis[66]. Some others have showed that Gingerols actively inhibit arachidonate 5-lipoxygenase, an enzyme of leukotriene biosynthesis. 8-Gingerol, was shown to inhibit COX-2 expression, which is induced during inflammation to increase formation of prostaglandins[67]. Therefore, it seems that anti-inflammatory and anti-phospholipase properties of ginger are also the other possible mechanisms for our result in this study. However, those possible issues need to be clarified by further investigation.

Conflict of interest statement

The authors declare that they have no competing interests.

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