



# Frankincense improves memory retrieval in rats treated with Lipopolysaccharide

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## ABSTRACT

**Introduction:** Frankincense has been shown to possess anti-inflammatory activity. In this study the effect of pretreatment with the hydro-alcoholic extract of frankincense on memory retrieval was assessed in lipopolysaccharide (LPS) treated rats.

**Methods:** Forty-two adult male Wistar rats were distributed into 7 groups of 6 each. One group received LPS (1 mg/kg; i.p) pre-test. The control group received saline (1 ml/kg; i.p). 2 groups of animals received frankincense (50 mg/kg; P.O) or DMSO 5% (1 ml/kg; P.O) and 30 minutes later LPS (1 mg/kg; i.p). Two other groups of animals received frankincense (50 mg/kg; P.O) or DMSO 5% (1 ml/kg; P.O) and 30 minutes later saline (1 ml/kg; i.p). Another group of rats received LPS (1 mg/kg; i.p) and 30 minutes later Ibuprofen (100 mg/kg; P.O). In all the experimental groups, memory retrieval was assessed 4 hours following the last injection, using a passive avoidance task (PAT). Hippocampal TNF- $\alpha$  levels were measured by ELISA as an index of LPS-induced neuroinflammation.

**Results:** LPS impaired memory retrieval by decreasing step-through latency (STL), significantly. LPS also increased levels of TNF- $\alpha$  in the hippocampus as compared to the control group. Administration of frankincense (50 mg/kg; P.O) before LPS (1 mg/kg; i.p) improved memory retrieval as compared to the control group. Frankincense reduced hippocampal TNF- $\alpha$  level in the LPS treated rats, significantly, compared to the control group.

**Conclusion:** The results indicate that the hydro-alcoholic extract of frankincense has the potential to improve memory retrieval in LPS treated rats, possibly via an anti-neuroinflammatory activity.

### Implication for health policy/practice/research/medical education:

Frankincense, a gum resin produced from *Boswellia* species, has the potential to improve memory and might be beneficial in Alzheimer disease.

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## Introduction

Inflammation is a protective mechanism of the body which leads to the loss of function of the affected area. Neuroinflammation is implicated in impairments in neuronal function due to aging, trauma, and/or disease and is involved in the pathology of various acute and chronic brain diseases, including Alzheimer disease (1). It is well known that neuroinflammation impairs memory processing during consolidation and retrieval stages and induce severe cognitive disturbances (2,3). The current treatment of inflammation includes compounds which inhibit prostaglandin synthesis, named nonsteroidal anti-inflammatory drugs (NSAIDs). These anti-inflammatory drugs are associated with a variety of severe side effects (4). There

is currently a high demand for natural therapies to treat inflammation and reduce the side effects of drugs used in the clinic (5). The *Boswellia* species (Burseraceae) are trees native to Ethiopia, Somalia, India, and the Arabic peninsula. They produce a gum resin that is known as frankincense (olibanum). The putative anti-inflammatory activity of frankincense has been approved using different models of inflammation (6-8). The resin of *Boswellia carteri* has been used for the treatment of inflammatory diseases in the traditional medicine in several countries (7). In addition, no significant side effects or toxicity have been reported from frankincense and one of its major constituents, boswellic acids (9).

The beneficial effects of frankincense have been shown

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on memory, both in normal and impaired memory conditions. For example, frankincense was shown to enhance the retention phase of spatial memory in the Morris water maze (10). It could prevent hypothyroidism induced spatial memory impairment in rats (11) and induced significant improvement in visuospatial memory in multiple sclerosis patients (12).

Lipopolysaccharide (LPS) is the active fragment of gram-negative bacteria. Systemic administration of LPS has been extensively used to induce neuroinflammation. Peripheral inflammation induced by LPS produces a neuroinflammatory response involving blood-brain barrier, glia and neurons (1).

Despite the fact that frankincense has potential anti-inflammatory activity and beneficial effects on memory formation, the impact of frankincense on neuroinflammation induced memory impairment have not been studied. Therefore, the aim of the present study was to examine whether acute administration of the hydro-alcoholic extract of frankincense from *Boswellia carteri* could improve memory loss induced by LPS.

## Materials and methods

LPS was purchased from Sigma (USA). LPS was dissolved in sterile saline. Frankincense from *Boswellia carteri* was donated by Raha Pharmaceutical Company (Isfahan, Iran). Protease inhibitor cocktail was purchased from Roche (Germany). Rat TNF- $\alpha$  ELISA kit was purchased from Boster (USA).

### Preparation of the hydro-alcoholic extract of frankincense

The species origin of frankincense was identified by an expert botanist. An appropriate amount of the resin of *Boswellia carteri* was pulverized and soaked in 96% ethanol. After 24 hours the ethanol was evaporated in rotary equipment. The remaining crude was dissolved in DMSO 5% (V/V in saline) and filtered before injection, then was warm-heated on a 50°C water bath for 60 minutes and cooled at room temperature before injection. The volume of the extract for gavage injection was 1 ml/kg. The dose of frankincense (50 mg/kg; P.O) was selected according to previous reports indicating the effectiveness of it on cognitive functions (13).

### Animals

Totally 42 male Wistar rats weighing 230-280 g were obtained from the breeding colony of Department of Biology, University of Isfahan and randomly distributed into 7 groups of 6 each. Rats were housed four per cage in a temperature (24°C) controlled room that was maintained on a 12:12 light cycle (light on at 07:00 AM). Rats had unrestricted access to food and water in their home cage. All experiments were executed in accordance with the guidelines for the care and use of laboratory animals (National Institute of Health publication No. 80-23, revised 1996) and were approved by the graduate studies committee of

the Department of Biology, University of Isfahan.

### Passive avoidance task

The step-through passive avoidance paradigm was performed as previously described (14). Briefly, each rat was placed in the white chamber of the passive avoidance task (PAT) apparatus facing the sliding door. After 5 seconds the door was raised. When the animal stepped into the dark chamber with all four paws, the door was closed and the rat remained there for 20 seconds. Then the animal was removed to be placed in a temporary cage. 30 minutes later, the rat was again placed in the white chamber for 5 seconds, then the door was raised to let the animal enter the dark chamber and following entrance, the door was closed, but this time a controlled electrical shock of 0.3 mA lasting for 1 second was delivered. After 20 seconds, the rat was placed into the temporary cage. Two minutes later, the same testing procedure was repeated. When the rat remained in the white compartment for a 2-minute times period, the training was terminated. On the second day, a retrieval test was performed to evaluate long-term memory. Each animal was placed in the white start chamber for 20 seconds, then the door was raised and the step-through latency (STL), was recorded, up to 600 seconds.

### ELISA assay

Immediately following completion of the behavioral tests, rats were euthanized and their hippocampi were removed and frozen in liquid nitrogen, then were stored in -80°C freezer. Each sample of hippocampus was homogenized in 320  $\mu$ l of cold buffer (Tris-HCL 50mM, NaCl 150mM, Sodiumdeoxycholate 0.25%, SDS 0.1%, EDTA 1mM, protease inhibitor cocktail 5%; pH=7.2) and centrifuged at 12000 rpm for 30 minutes at 4°C. Total protein concentration of supernatant was determined by Bradford assay (15). TNF- $\alpha$  levels were measured by ELISA kit according to the manufacturer's instruction (Boster Biological Technology Co., Ltd.). Concentration of the cytokine was quantified as pg of antigen per 10  $\mu$ g of total protein.

### Experiment 1

Six animals were used in each experimental group. In this experiment, 2 groups of animals received LPS (1 mg/kg; i.p) or saline (1 ml/kg; i.p) on the test day and 4 hours later memory retrieval was assessed.

### Experiment 2

In this experiment, 2 groups of animals received the hydro-alcoholic extract of frankincense (50 mg/kg; P.O) or DMSO 5% (1 ml/kg; P.O) and 30 minutes later saline (1 ml/kg; i.p) pre-test (24 hours after training). Two other groups of animals received the hydro-alcoholic extract of frankincense (50 mg/kg; P.O) or DMSO 5% (1 ml/kg; P.O) and 30 minutes later LPS (1 mg/kg; i.p) pre-test. Another group of animals received ibuprofen (100 mg/kg; P.O) and 30 minutes later LPS (1 mg/kg; i.p). Four hours after the

last injections, memory retrieval was assessed.

### Statistical analysis

The data are presented as mean  $\pm$  SEM. One-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons post hoc test or unpaired *t* test were performed for data analysis. In all the experiments,  $P < 0.05$  was considered statistically significant.

### Results

#### The effect of systemic administration of LPS on memory retrieval

In the PAT, the decrease in STL indicates loss of memory. The intra-peritoneal administration of LPS (1 mg/kg) 4 hours pre-test, impaired memory retrieval. Un-paired *t* test indicated that LPS decreased STL in a significant manner ( $P < 0.05$ ). The ELISA assay results showed that LPS increased TNF- $\alpha$  levels in the hippocampus of rats, significantly compared to the control group ( $P < 0.05$ ).

#### The effect of the hydro-alcoholic extract of frankincense and Ibuprofen on memory retrieval in rats treated with LPS

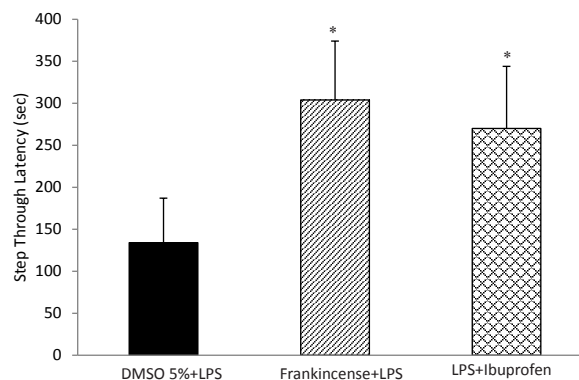
One-way ANOVA indicated significant main effect of STL in animals treated with the hydro-alcoholic extract of frankincense (50 mg/kg) before LPS (1 mg/kg) [ $F(2, 17) = 4.135$ ;  $P = 0.03$ ]. Post-hoc comparison showed that frankincense significantly increased STL as compared with the group received DMSO 5% and LPS (Figure 1;  $P < 0.05$ ). Injection of Ibuprofen (100 mg/kg) after LPS (1 mg/kg) increased STL, significantly as compared with the group received DMSO 5% and LPS (Figure 1;  $P < 0.05$ ).

One-way ANOVA indicated significant decrease of TNF- $\alpha$  levels in the hippocampus of rats [ $F(2, 17) = 12.32$ ;  $P = 0.0007$ ]. Post-hoc comparisons showed that injection of frankincense (50 mg/kg) and ibuprofen (100 mg/kg) before or after LPS, respectively, decreased TNF- $\alpha$  levels in the hippocampus of rats significantly, compared with the group received DMSO 5% and LPS (Figure 2;  $P < 0.01$  and  $P < 0.001$ ).

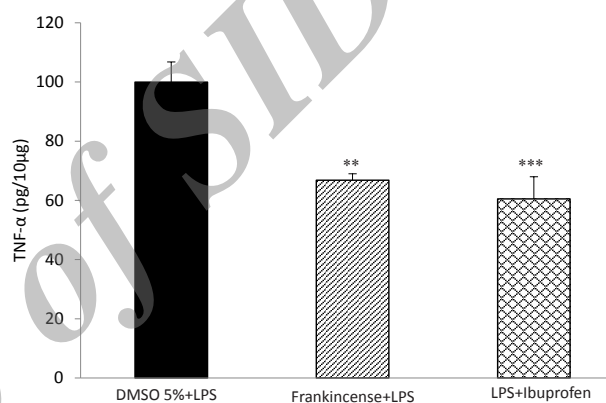
### Discussion

We found that systemic administration of LPS 4 hours pre-test increased the levels of TNF- $\alpha$  in the hippocampus, which indicated that it had induced neuroinflammation. It also impaired memory retrieval in the passive avoidance paradigm. These findings are in accordance with previous reports, showing the effect of LPS induced neuroinflammation on memory impairment (4).

The main outcome of the present study was that the hydro-alcoholic extract of frankincense could improve memory retrieval in rats treated with acute intra-peritoneal injection of LPS. More than 200 different compounds have been identified in the oleo-gum resin of different *Boswellia* species (16). These include monoterpenes, diterpenes, triterpenes, pentacyclic triterpenic acids (boswellic



**Figure 1.** The effects of pre-test administration of the hydro alcoholic extract of frankincense (50 mg/kg; P.O) and ibuprofen (100 mg/kg; P.O) before and after administering LPS (1 mg/kg; i.p), respectively, on STL. Data are expressed as means  $\pm$  S.E.M. \* $P < 0.05$ .



**Figure 2.** The effects of pre-test administration of the hydro alcoholic extract of frankincense (50 mg/kg; P.O) and ibuprofen (100 mg/kg; P.O) before and after administering LPS (1 mg/kg; i.p), respectively, on hippocampal TNF- $\alpha$  levels. Data are expressed as means  $\pm$  SEM. \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

acids) and tetracyclic triterpenic acids. It was reported that pure compound of frankincense exhibit anti-inflammatory property in human peripheral blood mononuclear cells and mouse macrophages through inhibition of tumor necrosis factor-alpha, interleukin-1 beta, NO and mitogen activated protein kinases (9). Boswellic acids are the main active components of the resin of *Boswellia carteri* which deal with the ethnomedicinal use for the treatment of inflammatory diseases (7). Boswellic acids were shown to specifically inhibit the synthesis of pro-inflammatory enzyme, 5-lipoxygenase (5-LO). 5-LO generates inflammatory leukotrienes. Leukotrienes cause inflammation by promoting free radical damage, cell adhesion and migration of inflammation-producing cells to the inflamed zone. Boswellic acids are specific inhibitors of leukotrienes, which interact directly with 5-LO or block its translocation. Boswellic acids were also shown to downregulate the pro-inflammatory cytokines including TNF- $\alpha$ , IL-1 $\beta$ , IL-2, IL-6 and INF- $\gamma$  by interacting with the production/release of these cytokines (8). Another active compo-

ment of frankincense, incensole acetate, was also shown to inhibit activation of nuclear factor-kappa B (NF- $\kappa$ B), a transcription factor which is crucial for inflammatory responses (17).

In accordance to the anti-inflammatory activities of frankincense, the results of the present study indicated that the hydro-alcoholic extract of frankincense decreased the levels of TNF- $\alpha$  in the hippocampus of rats treated with LPS, significantly. TNF- $\alpha$  is involved in inflammatory reactions. Inhibition of TNF- $\alpha$  and its signaling is a highly successful strategy for the treatment of inflammatory diseases (16). Our finding showed that the hydro-alcoholic extract of frankincense could induce an anti-neuroinflammatory activity, which was comparable with the putative NSAID, Ibuprofen. In support of this finding, different doses of frankincense have also inhibited generation of TNF- $\alpha$  by human monocytes stimulated with LPS by a direct inhibitory action on I $\kappa$ B kinases (IKK), conveyed inhibition of NF- $\kappa$ B and subsequent down-regulation of TNF- $\alpha$  (18). Also, chronic administration of a methanolic extract of frankincense possessed anti-inflammatory effect against neuroinflammation characterizing A $\beta$ 1-42 Alzheimer disease in rats (19). Therefore, owing to an evident effect of frankincense on decreasing an important pro-inflammatory cytokine (TNF- $\alpha$ ) in the hippocampus of rats, it appears that frankincense was able to improve cognitive functions probably due to anti-neuroinflammatory property.

In the present study, we administered frankincense 30 minutes before LPS injection. This was because it was reported that boswellic acids from frankincense directly target LPS rather than unspecifically interacting with other molecules in downstream LPS signaling (20). Accordingly, it is probable that boswellic acids of frankincense might even have prevented initiation of neuroinflammation.

A number of previous studies have indicated that frankincense has beneficial effects on memory, both on normal brain (10) and on impaired memory conditions (11,13). It was argued that the effect of frankincense on memory is mainly due to structural alterations in the brain neuronal circuits (21). For example, chronic maternal administration of *Boswellia* gum resin in gestational period induced significant volumetric alteration in the layers of the CA3 hippocampal field in young male rats (22). Also, different doses of frankincense was shown to increase the number of neurons and dendritic spines in hippocampal region CA<sub>1</sub> in rats (23). For this reason, in most of the studies assessing the effect of frankincense on memory, the extracts were administered chronically. In the present study, we administered the hydro-alcoholic extract of frankincense, acutely and 4.30 hours pre-test. Our results indicated that frankincense, showed a tendency towards improving memory parameters (data not shown), but failed to be statistically significant. Therefore, it is unlikely that the direct effect of frankincense on improvement of memory in LPS treated rats were involved in the observed results. We hypothesize that the major effect of frankincense on

improvement of memory retrieval observed in the present study might be due to its anti-neuroinflammatory property rather than its direct beneficial effects on memory. Further studies are required to clarify whether the acutely administered frankincense could also induce functional and structural changes related to the improvement of memory, in brain areas related to memory processing.

## Conclusions

In conclusion, our results indicated that the hydro-alcoholic extract of frankincense could prevent memory loss caused by systemic administration of LPS. The anti-neuroinflammatory activity of frankincense which was comparable with the putative NSAID, Ibuprofen, is suggested to be involved in the observed results.

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## Authors' contributions

SB contributed to the conception and design of the work, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. BK contributed to conducting the study.

## Conflict of interests

We wish to confirm that there are no known conflicts of interest associated with this publication.

## Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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