

Full Length Research Paper

Evaluation of analgesic and anti-inflammatory effects of fresh onion juice in experimental animals

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Onion is a well known traditional medicinal plant that has been consumed for its putative nutritional and health benefits for centuries. This study was carried out to determine the possible analgesic and anti-inflammatory effects of fresh onion juice in experimental animals. Hot plate and formalin tests were used to study the analgesic effect of fresh onion juice in mice during acute and chronic pain stages modeling, respectively. The anti-inflammatory effect of fresh onion juice was assessed by applying carrageenan sub plantar injection to Sprague-Dawley rats. The obtained results illustrated a significant analgesic property for fresh onion juice in both pain phases compared with positive control group ($P \leq 0.05$); the effects were similar to that of morphine (5 mg/kg) as the standard treatment. In inflammation assessment, fresh onion juice was able to decrease the hind paw thickness significantly in comparison with control group ($P \leq 0.001$). In the mean time, it also demonstrated better results than the standard treatment, diclofenac with a 10 mg/kg dosage, ($P \leq 0.05$). It can be concluded that fresh juice of onion is capable of inhibiting both acute and chronic pain as well as inflammation, with a more strong effect towards inflammation.

Key words: *Allium cepa*, analgesic, anti-inflammatory, hot plate test, formalin test, carrageenan test.

INTRODUCTION

Onion (*Allium cepa*) is among the oldest cultivated plants, and it is used both as a food and for medicinal application (Lanzotti, 2006). *A. cepa* is a member of the family Liliaceae, which consist of over 250 genera and 3700 species. The origin of *A. cepa* may be the region between the rivers Euphrates and Tigris, which is the former Mesopotamia and actually Iraq (Hegi, 1939). Nowadays onion is cultivated all over the world, especially in moderate climates (Muhlbauer et al., 2003).

Onion bulbs consist of water, carbohydrate, fibre, protein, fat, vitamins (C, E) and minerals. This plant is a rich source of several phytonutrients with interesting

pharmacological properties such as thiosulphinate, volatile sulfur compounds and more polar compounds of phenolic or steroidal origin like flavonoids (Lanzotti, 2006). Therefore, onion is among those useful plants for treatment or prevention of a number of diseases, including cancers (Shenoy and Choughuley, 1992; Shutenko et al., 1999), coronary heart diseases (Lanzotti, 2006), diabetes (Sheela et al., 1995) and cataract (Sanderson et al., 1999). Besides, many scientific researchers showed that onion extract has significant antioxidant activity because of its high amount of flavonoids such as quercetin. (Nuutila et al., 2003; El-Sayed and Rizk, 2009).

A study in the year 2003 showed that hydrophilic ethanolic extract of onion inhibited osteoclast activity and increased the bone formation process (Muhlbauer et al., 2003). In addition, it has been shown recently that the oral intake of fresh onion juice had both spermatogenesis and anti-protozoal effects in *Toxoplasma gondii* infected rats (Khaki et al., 2011; Gharadaghi et al., 2012). Also,

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Abbreviations: LT, Latency time; IP, intra-peritoneal; FOJ, fresh onion juice.

there was an evidence of possible anti-inflammatory effect for onion extract (Alpsoy et al., 2011). Inflammation is as a result of increase in the number of leukocytes and some other complex mediator molecules. One of the most important ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation are prostaglandins (Gupta et al., 2006).

Among the most widely used medications for analgesia and inflammation are the non-steroidal anti-inflammatory drugs (NSAIDs) and their worldwide use demonstrated their efficacy in reducing pain and inflammation (Laine, 2001). The NSAIDs consist of traditional non-selective NSAIDs which inhibit both COX-1 and COX-2 and selective COX-2 inhibitors (Ulrich et al., 2006). Although they are effective at relieving pain and inflammation, both types of NSAIDs are associated with serious adverse events specially when used chronically (Herndon et al., 2011). The traditional NSAIDs are associated with an increased risk of gastrointestinal ulcers, including gastrointestinal hemorrhage, perforation and obstruction (Dhikav et al., 2003). The selective COX-2 inhibitors have an improved gastrointestinal tolerability profile; however, serious cardiovascular effects emerged from clinical studies in recent years (Ong et al., 2007). Thus, many researchers have dedicated their efforts to search for safer drugs as well as natural products with less adverse effects.

In this study, the analgesic effect of fresh onion juice in both chronic and acute pain induction model with hot plate and formalin test respectively in mice, as well as its anti-inflammatory effect using carrageenan induced paw edema in rats were investigated.

MATERIALS AND METHODS

Experimental animals

Male albino mice (25 to 30 g) and male Sprague-Dawley rats (220 to 250 g) were used in this study. Animals were obtained from Tehran University of Medical Sciences and housed in the animal holding unit of the School of Pharmacy at Zanjan University of Medical Sciences with a 12 h light-darkness cycle, air-conditioning ($22 \pm 2^\circ\text{C}$ 45 to 55% humidity) in plexi-glass cages and free access to food and water. All animals received human care according to the guidelines published by the National Institutes of Health (NIH, 2000). The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by The Ethics Committee of Payame Noor University. All animals were given three days time to get acclimatized with laboratory conditions before experiments begin.

Chemicals

Carrageenan lambda type I and formalin 35% solution were purchased from Sigma-Aldrich Co. (Hamburg, Germany). Sodium chloride solution 0.09% was obtained from Soha Helal Pharmaceutical Hygienic (Tehran, Iran), while morphine and diclofenac ampoules were bought from Darou Pakhsh Pharmaceuticals Mfg. Co. (Tehran, Iran) and Tolid Darou Co. (Tehran, Iran) respectively.

Preparation of fresh onion juice

Fresh white onions (*A. cepa.*) was purchased from a retail store (Zanjan, Iran) and identified by botanists in the herbarium of School of Pharmacy, Zanjan University of Medical Sciences. On the day of experiments the onions were peeled, weighed and crushed well in an electrical mill. Then the crushed product was filtered using sterile filter papers with 40 micrometers mesh size. The transparent liquid obtained was used freshly within 2 h after preparation to investigate the possible analgesic and anti-inflammatory effects.

Carrageenan-induced paw edema in rats

Male Sprague-Dawley (S.D) rats ($n=36$) were divided into six groups with six rats in each. Paw edema was induced by sub-plantar injection of 50 μl 1% (w/v) solution of sterile carrageenan in saline to the right hind paw (Zhang et al., 2011). Three groups of animals received different doses of fresh onion juice (5, 7.5 and 10 ml/kg) intraperitoneally (I.P) half an hour before carrageenan injection. Animals in the negative control group received normal saline, while animals in the positive control group were administered 10 mg/kg of diclofenac (Darou Pakhsh, Iran), the standard anti-inflammatory drug and another group given 5 mg/kg of morphine (Tolid Darou, Iran) intraperitoneally. Paw edema was measured according to Olajide et al. (1999); before and at 1, 2, 3 and 4 h after induction of inflammation using a caliper vernier (scale 0.1 mm).

Hot plate method

The hot plate test described by Eddy and Leimback (1953) was used to assess the analgesic effects of fresh onion juice (Kumar et al., 2009). The animals were divided into six groups with eight mice in each. Three groups were treated with different doses of fresh onion juice (5, 7.5 and 10 ml/kg) via I.P injection; one group received normal saline (7.5 ml/kg) as negative control group, another group, received morphine (5 mg/kg) as positive control and the final group of mice received naloxone (4 mg/kg) 10 min before fresh onion juice. Moreover, 15 min after the administration of fresh onion juice; the animals were placed on a hot plate with $50 \pm 0.5^\circ\text{C}$.

A cut-off time period (40 s) was considered as maximal latency at which the animal was picked up from the hot plate by the examiner to avoid injury to mice tissues. Licking or picking up the hind paw was recorded as the reaction time and measured at time points of 0, 15, 30, 45 and 60 min (Kumar et al., 2009). Morphine (5 mg/kg) was used as a reference drug (Abbas et al., 2011).

Formalin test and the pain score

In order to perform this test, 32 male S.D rats were rendered in 4 groups with 8 rats in each as follows: Negative control group received 7.5 ml/kg of normal saline, while positive control group treated with 5 mg/kg morphine as the standard drug intraperitoneally 15 min before formalin injection. The test group received 7.5 ml/kg fresh onion juice (as an optimum dose) and one group received I.P injection of naloxone (4 mg/kg) 15 min before formalin injection. Formalin test chamber includes a plexi glass box with a 45° mirror at the bottom of the box in order to monitor the position of the animal in the chamber for accurate observation. All of the animals received formalin as the standard stimulant of both acute and chronic phase of inflammatory pain (Kim et al., 2007). Prior to formalin injection, the animals were placed in another cone shaped chamber for 30 min. After injection of 50 μL formalin (2.5%) into their right paw, they were transferred to the plexi glass box and were observed by recording the reflexes every 15 s based on

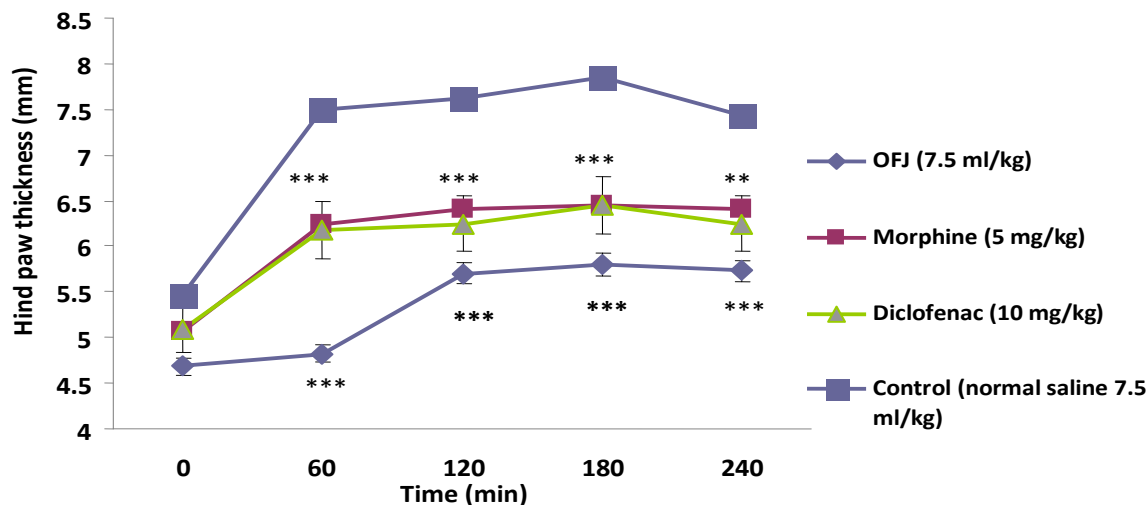


Figure 1. The effects of fresh onion juice, morphine and diclofenac towards hind paw edema in carrageenan induced acute inflammation. A vernier caliper was applied to measure the hind paws thickness right before and at four time points with 60 min intervals. Each measurement was repeated three times and the average was recorded for that animal. The diagram shows that FOJ was even more effective than both diclofenac and morphine. All values represent mean \pm S.E.M and are at least from 8 independent animals. **Shows significant difference ($p \leq 0.01$) with control group; ***Shows significant difference ($p \leq 0.001$) with control group.

original Dubuisson Dennis method with the score of 0, 1, 2, 3 as follows (Abbott et al., 1995): zero (0) score mentions that animal has a complete balance and is walking normally regardless to the injected foot; score (1) applied for the time points that the animal has a moderate imbalance while moving because of pushing the body weight towards the injected foot; score (2) was correlated to the time points when animal was not only walking with a high imbalance but also raising the painful injected foot from the box floor; score (3) is given to the animal licking the painful injected feet intensely or shaking it. The quantitative data was counted per 5 min and recorded based on the pain score on each time interval. The data gathered within 60 min after formalin injection was calculated as follows:

$$\text{Pain score: } [0T_0 + 1T_1 + 2T_2 + 3T_3] / 20$$

Where T_0 , T_1 , T_2 and T_3 refer to the frequency for 15 s that animals expressed behaviors related to 0, 1, 2 and 3 scores, respectively. The first 5 min after injection for all groups was taken as acute phase 0 to 5 min and 16 to 60 min as chronic phase.

Statistical analysis

In order to perform comparative statistical analysis of the results between different groups, SPSS 17.0 software was applied. All the results were expressed as (mean \pm SEM) and P values equal or less than 0.05 ($P_v \leq 0.05$) were determined as significant levels of difference.

RESULTS

Effects of morphine and fresh onion juice on carrageenan-induced inflammation

As shown in Figure 1, the thickness of hind paw was

measured after a sub-plantar injection of carrageenan while rats were received diclofenac, morphine, fresh onion juice and normal saline as positive control, morphine test, fresh juice test and negative control groups, respectively. Interestingly, fresh onion juice illustrated the best response in this test and showed a significant difference not only with negative control group ($P_v \leq 0.001$), but also with the group which received both morphine and diclofenac ($P_v \leq 0.05$); the later one was selected as our standard treatment for anti inflammatory assessments.

Effects of morphine and fresh onion juice on hot plate acute pain model

Using this method, the sensitivity of mice towards pain stimulates received via a standard hot plate, was measured. In order to access the least bias with control group, the ratio of latency time changes were measured applying the formula below for each mouse:

$$(L.T_t - L.T_0) / L.T_0$$

$L.T_t$ expresses the latency time for each mouse in the specific time intervals after receiving treatments, while $L.T_0$ shows the latency time at the zero point just prior to receiving any treatment.

According to the dose-response curve results, the 7.5 ml/kg dosage had the best latency time responses. Therefore, this dose has been used later on, in other protocols and tests. It can be deduced from Figure 2

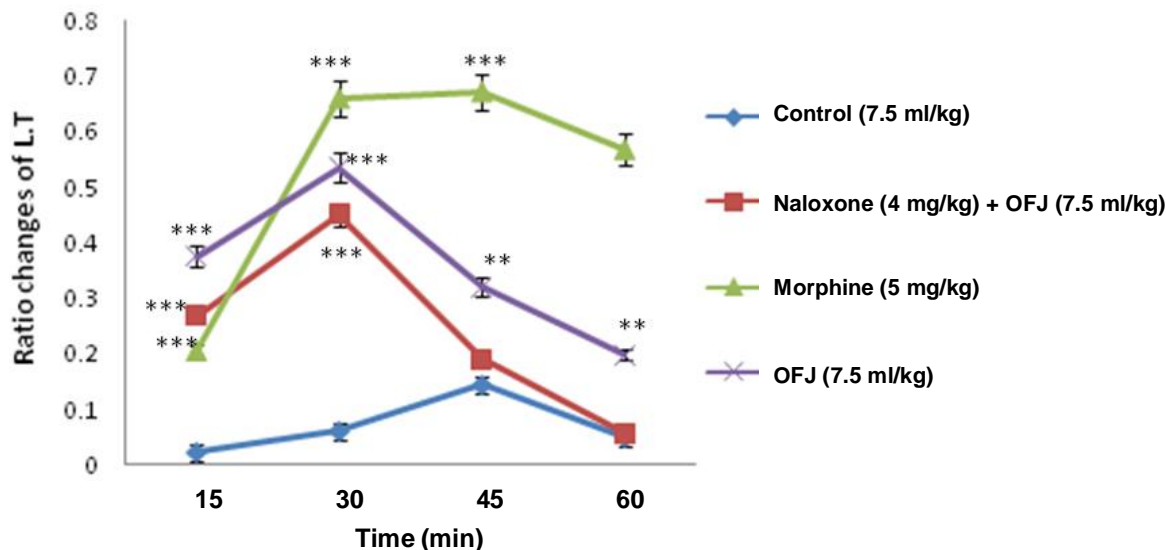


Figure 2. The analgesic effects of fresh onion juice in comparison with that of morphine on hot plate test. The latency time for animals' response to hot plate stimulus was measured within 15 min intervals during 60 min. The FOJ's injection moment was equal to 0 time point. The results were calculated as $(L.T_t - L.T_0) / L.T_0$. naloxone was used as an antagonist for opioid receptors in order to determine if the extract show its effect via opioid receptors. All values represent Mean \pm S.E.M and are at least from 8 independent animals. **Shows significant difference ($p \leq 0.01$) with control group; ***Shows significant difference ($p \leq 0.001$) with control group.

that morphine has the best analgesic effect with a noticeable significant difference ($P_v \leq 0.001$) which was almost the same for the fresh juice especially in first 30 min. This result expresses that the fresh juice plays its best role as an analgesic agent in first 30 min with a high significant difference according to control group ($P_v \leq 0.05$). Although the diagram for morphine illustrates a sharp increase in the first 30 min followed by a plateau phase; adding naloxone to the morphine treated ones, changed the shape of the diagram and makes it similar to the one for fresh juice.

Effects of morphine and fresh onion juice on formalin-induced pain

During this method, the pain score was calculated according to original Dubuisson Dennis method which has been lately modified (Mokhtari, 2011) during 60 min. The results obtained from this method indicate that our treatments were useful in the first 45 min in depriving the pain score significantly ($P_v \leq 0.05$). According to Figure 3, no significant difference was observed between morphine and fresh juice ($P_v \geq 0.05$) in controlling both acute and chronic pain phase which were determined at 0 to 5 min for acute pain phase, and 16 to 60 for chronic one.

On the other hand, Figure 3 indicates the possible relationship between the effects of morphine and fresh juice while we used naloxone together with the fresh onion juice; as it can be seen there were observed no significant differences between two groups in all time

intervals. Adding naloxone to morphine received animals made the diagram more similar to control group, hence the results for that group was not illustrated in Figure 3.

DISCUSSION

In this study, three different methods have been applied in order to identify the possible analgesic as well as anti-inflammatory effects of fresh onion juice in mice and rats. According to the well known models for both hot plate and carrageenan test, it has been suggested in current protocols in pharmacology as well as some articles to apply mice for hot-plate test investigations and rats for carrageenan induced inflammation (Buadonpri et al., 2009; Bannon and Malmberg, 2007) Results obtained from carrageenan test indicates a strong anti-inflammatory effect with a significant difference not only with the negative control group ($P_v \leq 0.001$), but also with the positive control one ($P_v \leq 0.05$). This finding illustrates a possible potent COX inhibitory effect of onion juice in its therapeutic dosage because it was as effective as the standard treatment (diclofenac) towards inflammation. The odd finding of this step was the similar anti-inflammatory response for both morphine and diclofenac. This finding is a subsidiary for the very recent finding of a research group in Denmark proving a noticeable anti-inflammatory characteristic for opioids in general, as well as morphine in particular (Lindegaard et al., 2010) with an unknown mechanism.

According to some studies, fresh onion juice is capable

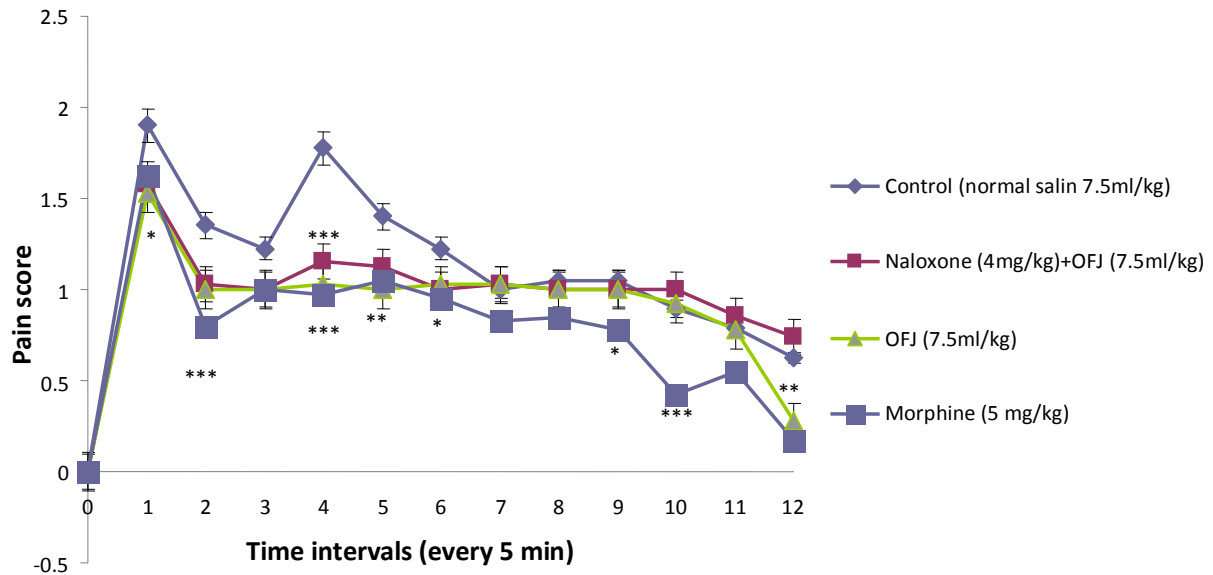


Figure 3. The effects of fresh onion juice, morphine and naloxone plus FOJ on both acute and chronic pain phases induced by formalin in male mice. Right after formalin injection, the pain scores were calculated during one hour and because of the biphasic nature of pain, the acute phase was allocated the first 5 min after formalin injection, while chronic pain arose between 20 to 25 min and lasted for 10 min. This figure illustrates the significant differences between treated groups and control group which received normal saline. All values represent Mean \pm S.E.M and are at least from 8 independent animals. * Shows significant difference ($p \leq 0.05$) with control group; ** shows significant difference ($p \leq 0.01$) with control group; *** shows significant difference ($p \leq 0.001$) with control group.

of inhibiting arachidonic acid metabolism (Dorsch et al., 1988) and so it can prevent formation of leukotrienes and thromboxanes, via inhibiting COX and LOX (lipoxygenase) pathways responsible for its anti apoptotic effect (Alpsoy et al., 2011). On the other hand, it was proven that flavonoids express anti-inflammatory properties by which they inhibit the proliferation and activity of lymphocytes (Recio et al., 1995). According to high content of flavonoids such as quercetin (Lanzotti, 2006) in onion juice and extract, it can be claimed that this potent anti-inflammatory effect might be because of quercetin as would need to be investigated in further studied. Moreover, one paper suggested that onion can cause analgesia as well as local anesthesia via mitochondria (Nouette-Gaulain et al., 2011); which encouraged us towards assessment of the analgesic capability for fresh onion juice.

The analgesic effect of fresh onion juice was studied via two different processes. The hot plate test results indicated that the fresh juice was helpful in reducing acute pain in comparison with the negative control group that received mere normal saline ($P_v \leq 0.05$). This effect was not as potent as morphine (the standard treatment), with a high significant difference ($P_v \leq 0.001$) compared with negative control group. Moreover, in order to examine if fresh onion juice penetrates the CNS system and affect the same receptors as for morphine, naloxone was injected to one group simultaneously with fresh onion juice. The results indicated that there might not be

any interactions between these two treatments (morphine and fresh onion juice). Hence, more studies are needed to determine the possible analgesic pathway by which fresh onion juice attenuates pain; which led us to set up another test using formalin in order to compare the acute and chronic pain and the effects of both treatments on these two phases of pain.

Formalin test results showed that fresh juice illustrated significant analgesic effect both in acute and chronic pain phases which were assumed as the first 5 min after formalin sub-plantar injection and the time period between 16 to 50 min, respectively. It has been assumed that these two stages represent two different type of pain related to direct nerve stimulation (acute pain) followed by an inflammatory process (chronic stage). Diverse studies proved that opioids such as morphine affect both stages with the highest inhibitory response presumably via mu receptors in the central nervous system (Abbas et al., 2011); on the other hand, there are NSAIDs such as indomethacine with the most inhibition towards the chronic stage via COX inhibition (Randolph and Peters, 1997).

According to the literature, reduction in pain, inflammation and the signal transduction pathway(s) responsible for both phenomena mentioned above, results in a decline in plasticity at dorsal root of spinal cord via deprivation in P substance and/or stimulant amino acids such as glycine and glutamate from nerve endings (Willis, 2001; Hunter and Singh, 1994; Terayama

et al., 2000). In addition, there is a substance called ajoene found both in garlic and onion which has been proposed to inhibit the pain receptors at dorsal root of spinal cord, thus resulting in an inhibition of pain signal transduction (Yassaka et al., 2010).

Conclusion

It can be concluded that the fresh onion juice is capable of inhibiting pain and inflammation, the later most especially, and both the exact mechanism(s) for this effect via receptor purification as well as the main fraction responsible for, should be studied in the near future.

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