

Effect of Aqueous Extract of *Trigonella foenum-graecum* on Pain and Inflammation Induced by Formalin in Male Mice

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Abstract

Background: Studies have emphasized the effect of *Trigonella foenum-graecum* extract on the reduction of pain and inflammation.

Objective: In this research we investigated the mechanisms of *Trigonella foenum-graecum* extract in reducing pain and inflammation induced by formalin.

Methods: Male Albino mice (weight 20 - 25 g) were evaluated through the injection of 2 microliters of formalin to the plantar part of right foot. Following this, the rate of animal foot pain and inflammation were measured using Dubbison-Dennis and immersion in mercury. *Trigonella foenum-graecum* extract was injected 30 minutes before administration of formalin to the animals intraperitoneally. In addition, blood samples were taken from animals and corticosterone concentrations were measured. In an *in vitro* study the effect of extract on the activity of cyclooxygenase type 1 and 2 was assessed.

Results: Our results showed that *Trigonella foenum-graecum* extract inhibits the first and second phase of pain induced by formalin, while inflammation is slightly reduced. Also the effect of *Trigonella foenum-graecum* extract is reversible with naloxone or memantine administration. Also *Trigonella foenum-graecum* extract could not increase plasma corticosterone level and was ineffective in activity of cyclooxygenase type 1 and 2 enzyme.

Conclusion: Although *Trigonella foenum-graecum* extract can inhibit pain induced by formalin administration, but it seems that the reduction of pain is due to the possible interaction of components of *Trigonella foenum-graecum* extract with opioid and/or glutamate systems which occurs in the body and the mechanisms of inflammation reduction are not activated by the extract.

Keywords: *Trigonella foenum-graecum* extract, Formalin test, Pain, Inflammation, Cyclooxygenase 1 and 2 enzyme, Corticosterone

Introduction

Pain is an unpleasant feeling and a defense mechanism for the body that can serve as a sign of tissue damage [1, 2]. Pain is created by various types of stimuli that have nothing in common, such as tissue mechanical twist, high temperature, low pH, chemicals (such as neural activator materials, which are released during injury) and solutions hyper osmotic [1]. Pain is a warning factor that in case of probability or existence of risk shows itself as acute or chronic and is the most common problems that people face and always seek for a way to achieve the cause and the methods for overcoming [3]. Chronic pain is usually associated with tissue destruction. Such pain can lead to unbearable and long suffering. This pain can almost occur in any visceral, deep or surface tissues [3]. At present opioid drugs [4] and no steroid anti-inflammatory drugs (NSAIDs) are mainly used to relieve pain [5]. Mechanisms of NSAIDs are inhibition of cyclooxygenase enzyme activity and then stop prostaglandins to be produced [6, 7, 8]. Cyclooxygenase type 2 mediates inflammatory response and is largely released with delay [9]. Many compounds of conventional NSAID inhibit both cyclooxygenase 1 and 2 enzymes [10]. But these drugs may not be useful in all cases because of side effects and other problems [5].

Medicinal plants have been among the first facilities which have been used in treatment at first, and based on the experience, those plants that have treated various diseases effectively, have been identified as healing herbs or medicinal plants.

Herbal medicine which are usually essential oils or plant extract existing in nature, are usually among the drugs that do not cause severe side effects while using, for this reason investigating these drugs has spread [11].

Trigonella foenum-graecum is a plant that has different alkaloids, glycosides, sapogenin, minerals, flavonoids, nicotinic acid and tannin [11, 12]. The extract of this plant contains proteins rich in Lysine, Tryptophan, Trigoniline, Trigonilic acid and Trigomarine [12].

Many therapeutic effects of this extract have been reported in traditional medicine. Plant's seed reduces fever [13], increases milk secretion [14], stimulates uterine contractions and facilitates delivery [12], shortens recovery period [12], improves breathing disorders [12], digestion [12] and is also used as laxative and diuretic [12], sputum production and anti-parasitic [12] and anti-tumor [15]. In Chinese traditional medicine this plant has been used for abdominal pain and improving dysfunction related to renal weakness [12] as well as vaginal suppositories of the plant for the treatment of uterus cancer [15]. Using seeds of this plant has had good results in patients with tuberculosis and osteomyelitis [12] and plant tea can be used as gargle in tonsillitis and sore throat, as enema in gastrointestinal inflammation and swelling, simple diarrhea, hemorrhoids, rectal prolapsed and as lotion in aphthous and as hot compress in lip fissure [12]. Seed and its extract decrease blood sugar and cholesterol [16, 17]. The extract along with Vitamin B6 has been used in the preparation of a kind of formulations as a stimulator of hair growth. Trigoniline or Trigonilic acid increase length, thickness and pigmentation of the hair shaft through blood vessels dilation and increasing feeding hair follicles [12].

Plant's seed and leaf is useful for analgesia [18], and anti-inflammatory [19].

The present study has evaluated the effect of aqueous extract of *Trigonella foenum-graecum* on reducing pain and inflammation through formalin test in male mice.

Materials and Methods

Animals

Male Swiss Webster strain of laboratory rat with mean weight of 25 ± 2 g was purchased from Pasteur Institute (Tehran, Iran). The animals were exposed to 12 hours of daylight and fed with standard rat food and tap water (environment temperature, $23 \pm 2^\circ\text{C}$). In each group of animal experiments, 6 mice were studied. This study was conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah (a.s.) University of Medical Committee on the Use and Care of Animals, 87/381, July 25, 2009).

Preparation of extract

100 g of dry powder of *Trigonella foenum-graecum* leaves was soaked in 1000^{CC} distilled water for 24 hours at 30°C and then the supernatant liquid was placed at 35°C to be evaporated in order to obtain the dry extract. Through 23.5 g dry extract was obtained from every 100 g of powder. This extract was dissolving in 10 ml saline and injected intrapretonealy to the animals.

Drugs

Morphine sulfate (Temad - Iran), dexamethasone, indomethacin, naloxone hydrochloride (Sigma-USA) and memantine bromide (TOCRIS-UK) were used in this work. Drugs were dissolved in saline and injected intrapretoneally to the animals in volumes of 10 ml/kg except for morphine which was given subcutaneously.

Experimental Procedure

Deniss Dubisson (1977) method was used for formalin test with modifications [20]. Briefly, each animal was placed inside a Plexiglas box with the dimensions of $30 \times 30 \times 30$ cm (length

\times width \times height) after injection of formalin in plantar part of right foot. Foot position and how animals respond to formalin injection were evaluated by observer as 0 to 3 score depending on the animal's foot condition. Score zero means no pain, score 1 was determined by not putting body weight on the injected foot (claudicating), score 2 was avoidance of animals from contacting injected foot with the bottom of box, Score 3 was documented when animal bit or lick the injected foot. Extract of the *Trigonella foenum-graecum*, morphine, dexamethasone and indomethacin were injected to the animals 30 min before formalin. Naloxone and memantine were injected 30 minutes before the extract injection.

Determining the of inflammation

Fereidoni et al., method was used [21] for inflammation study. In this method the animal's left foot was considered as the control foot, in which saline was injection. Animal's left foot was placed in a container that contained mercury whose exact weight was determined and the mercury weight change was calculated. By calculating the weight change of mercury due to inhalant of left foot (control) and right foot (test), foot weight changes were determined after formalin injection and this weight change shifts to the volume change by dividing in to 13.6 (density of mercury).

Determination of plasma corticosterone concentration

30 minutes after injection of extract to the animals' eye 0.3 CC blood was taken from the retro-orbital sinus and was added to 1 cc Eppendorf pipes containing 0.7 CC sodium citrate solution %2.0. The samples were centrifuged at 3000 rpm at 4°C and the

supernatants were used for determination of plasma concentration of corticosterone. Plasma corticosterone concentration was determined by ELISA kit (Rat Corticosterone ELISA kit; EIA-4164; DRG Instruments GmbH, Germany) in 450 nm wave lengths.

Determining activity of the enzymes cyclooxygenase type 1 and 2 (Cox 1, 2)

The ELISA kit was used for activity of cyclooxygenase type 1 and 2 enzymes. Each extract was poured in three well and one of two kits related to cyclooxygenase type 1 and 2 enzymes was added to the environment and in vitro enzyme activity was measured by ELISA method.

Statistical Analysis

Data expressed as Mean \pm S.E.M of pain score or hind paw volume. One-way ANOVA followed by Tukey post hoc test was used for statistical analysis. $P<0.05$ was considered significant.

Results

Effects of different doses of *Trigonella foenum-graecum* extract on formalin-induced pain

Different doses of *Trigonella foenum-graecum* extract (10, 20, 30, 40 and 50 mg/kg; i.p.) were injected to the animals 30 min before formalin. Animals' responses were evaluated 30 min later. Data indicated that the extract suppress the acute phase of formalin-induced pain [$F(8, 49) = 6.21, p<0.001$] (Fig. 1A). More over, comparing to morphine, dexamethasone and indomethacin, *Trigonella foenum-graecum* extract demonstrated a comparable effect on suppressing pain in phase 2 in formalin test [$F(8, 49) = 12.45, p<0.0001$] (Fig. 1B).

Effects of *Trigonella foenum-graecum* extract on formalin-induced inflammation

The results showed that *Trigonella foenum-graecum* extract (10, 20, 30, 40 and 50 mg/kg, i.p.) can not suppress the inflammation induced by formalin in animals [$F(8, 49) = 0.098, p>0.05$] (Fig. 2).

Effects of opioid and glutamate receptor inhibition on *Trigonella foenum-graecum* extract-induced analgesia

The effect of naloxone and memantine on *Trigonella foenum-graecum* extract induced analgesia in phases 1 and 2 of formalin test is shown in figure 3A and B respectively. Pretreatment of the animals with naloxone and memantine inhibit the extract effects on phase one and two of the formalin test [$F(8, 49) = 11.23, p<0.001$] (Fig. 3A) and [$F(8, 49) = 9.36, p<0.001$] (Fig. 3B).

Effects of *Trigonella foenum-graecum* extract on suppressing cyclooxygenase enzyme type 1 and 2

The obtained results from *invitro* study showed that, *Trigonella foenum-graecum* extract is not able to suppress cyclooxygenase enzymes type 1 [$F(6, 49) = 1.02, p>0.05$] (Fig. 4A) and type 2 [$F(6, 49) = 0.83, p>0.05$] (Fig. 4B).

Effects of intraperitoneal administration of *Trigonella foenum - graecum* extract on the plasma corticosterone level

The effect of *Trigonella foenum-graecum* extract on plasma corticosterone concentration is shown in Fig. 5. The extract did not increase plasma corticosterone level in the experimental groups [$F(5, 43) = 0.563, p>0.05$] (Fig. 5).

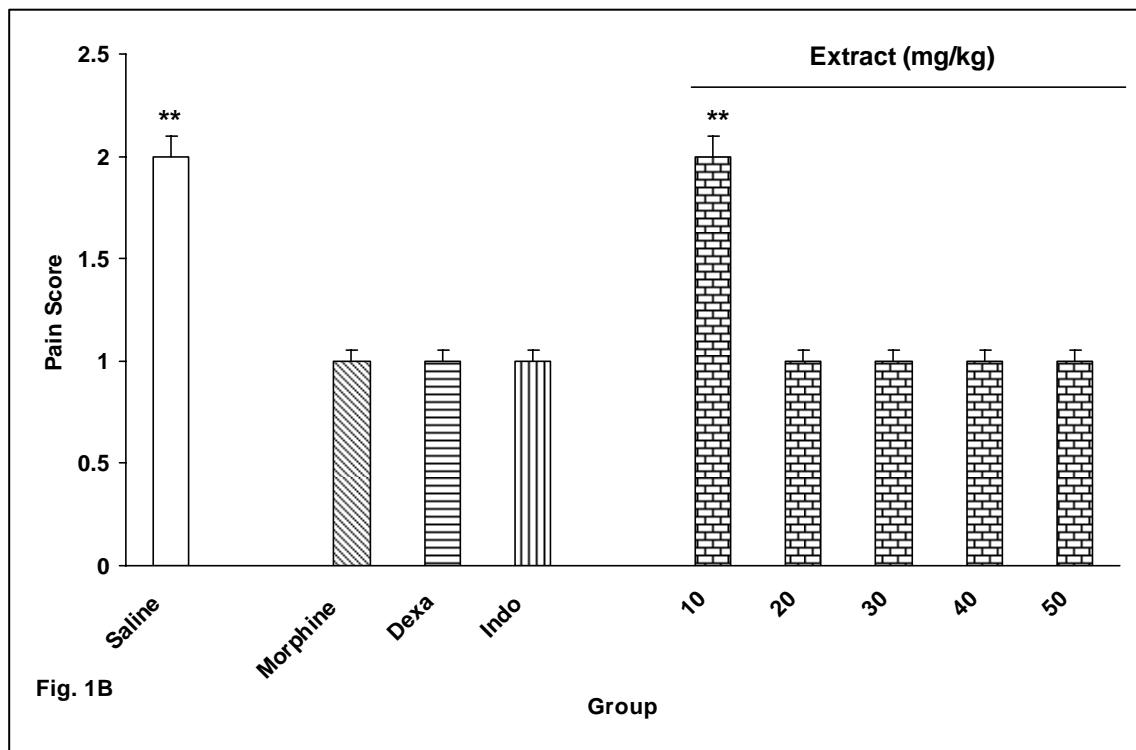
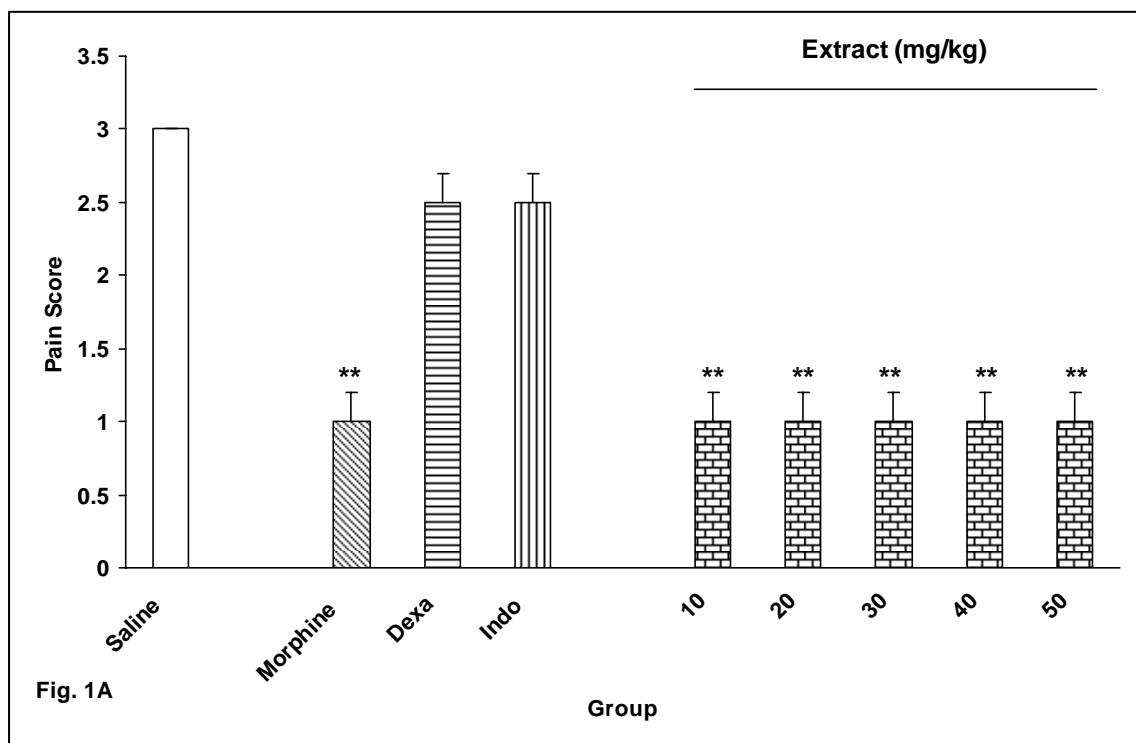


Fig. 1A and B - Effects of *Trigonella foenum-graecum* extract on phase 1 (A) and 2 (B) of formalin test in mice. The extract inhibit both phase 1 and 2 of the formalin test. Data showed as mean \pm SEM, for 6 mice, **p<0.01 different from saline control group.

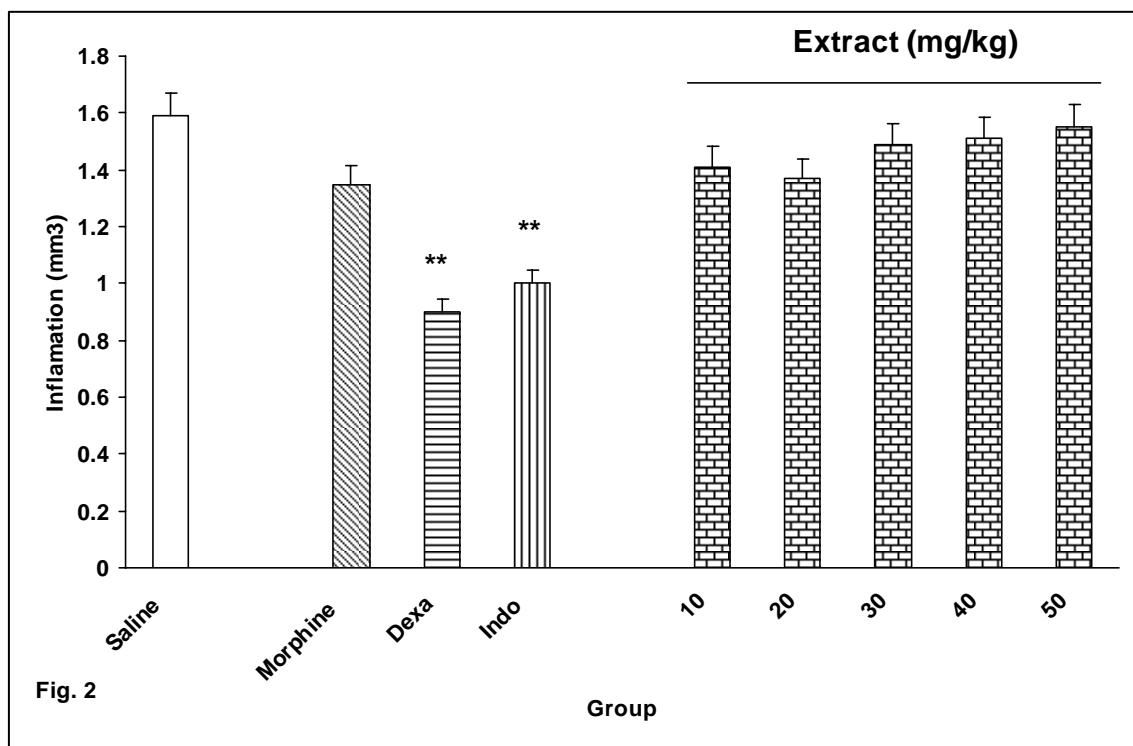
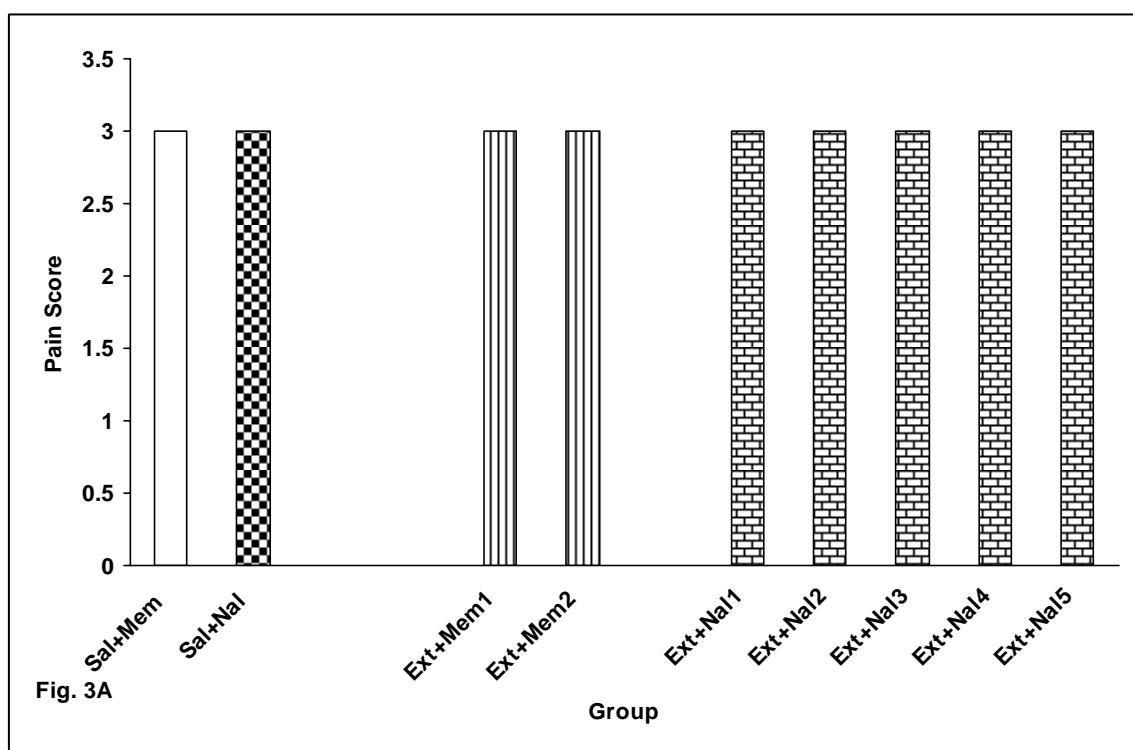


Fig. 2- Effect of *Trigonella foenum-graecum* extract on inflammation induced by formalin in mice. The inflammation did not reduced by the extract and morphine but it was reduced by dexamethasone and indomethacin. Data showed as mean \pm SEM, for 6 mice, **p<0.01 different from Saline group



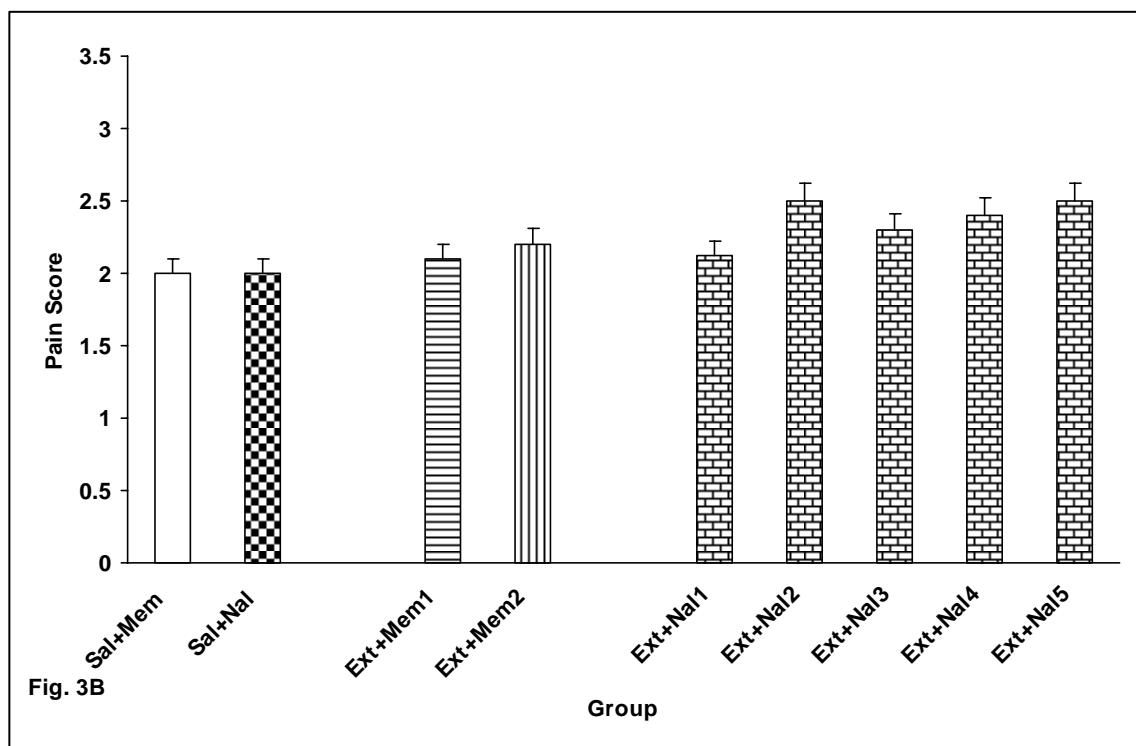


Fig. 3A and B - Effect of opioid and NMDA receptor inhibition on *Trigonella foenum-graecum* extract pain reduction in mice treated by formalin. As it is clear, both drugs can abolish the extract effects on phase 1 (A) and 2 (B) of the formalin test. Data showed as mean \pm SEM, for 6 mice

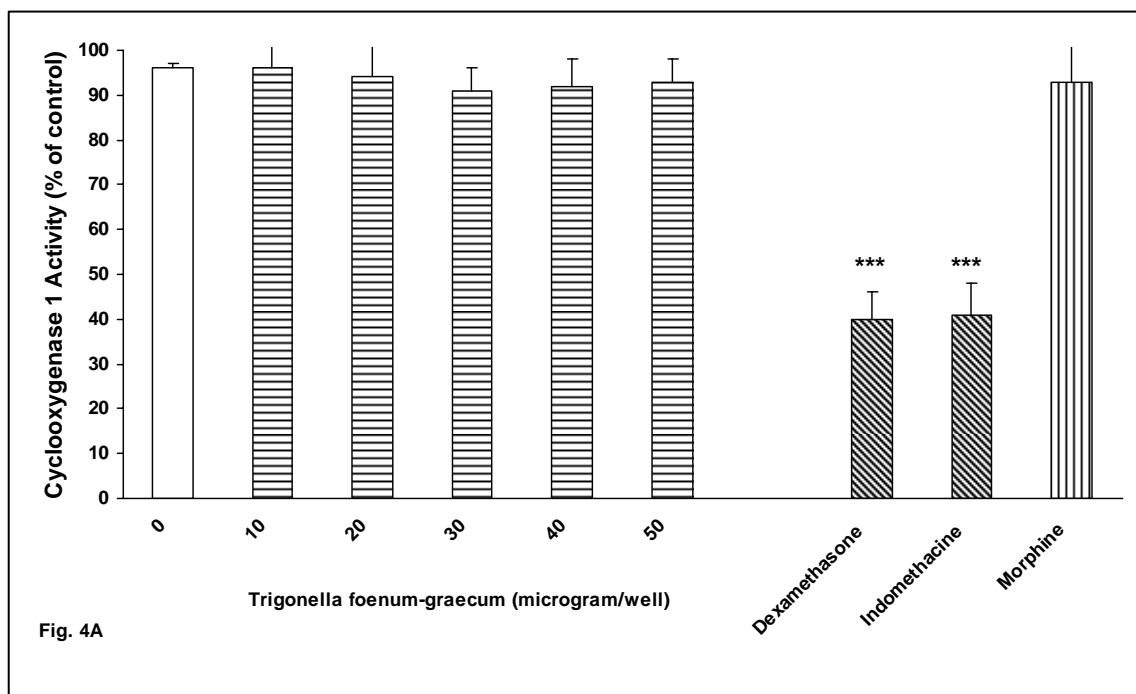


Fig. 4A

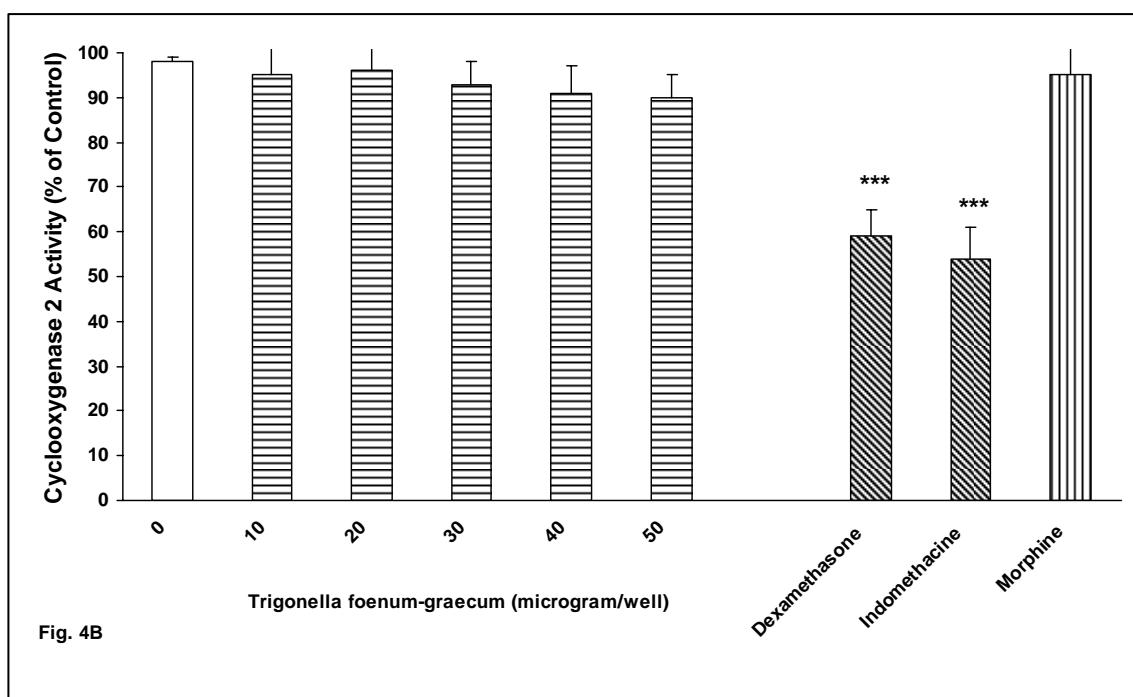


Fig. 4A and B - Inhibition of the enzyme cyclooxygenase type 1 (A) and 2 (B) (Cox 1 and Cox 2) by the extract of *Trigonella foenum-graecum* *in vitro*. Data showed as mean \pm SEM, for 3×3 well, * $p < 0.001$ different from control group**

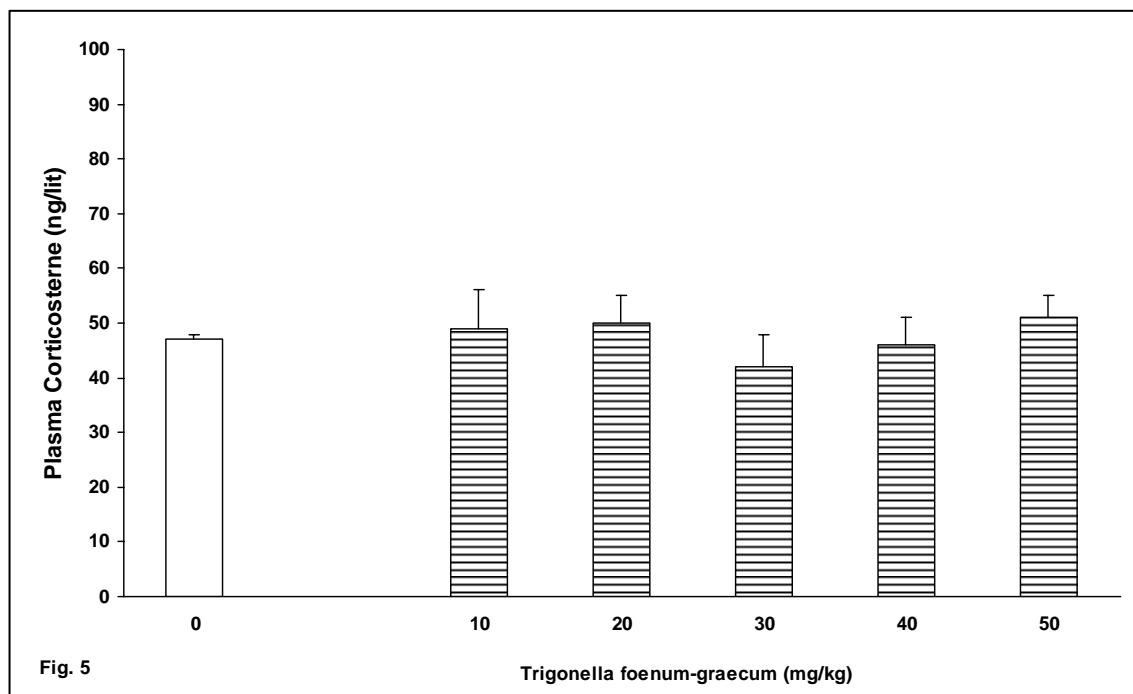


Fig. 5 - Effects of *Trigonella foenum-graecum* extract on corticosterone plasma level in mice. As showed in the figure, the extract can not induce corticosterone level increment in the mice. Data showed as mean \pm SEM, for 6 mice

Discussion

Pain has been known as an unpleasant and very disgusting phenomenon that can quite affect individual's function in the society [1]. This phenomenon has been in the focal point of researchers, especially neurology and psychology researchers in recent years and so far the definitive solution is not found for the treatment and relief [1]. Previous studies have shown that pain transmission pathways can be blocked using narcotic analgesic drugs such as morphine [22] and no steroid anti-inflammatory drugs like aspirin can inhibit producing inflammation and pain mediators such as prostaglandins [5]. But these issues are faced with other risks. For example, administration of opioids can lead to dependence and tolerance [23], as well as high consumption of no steroid anti-inflammatory drugs causes problems such as gastrointestinal bleeding [5]. For this reason, research on drugs with the ability to inhibit pain transmission pathways or mediators of inflammation is continuing. Our research showed that administration of *Trigonella foenum-graecum* extract can inhibit formalin-induced pain in the first and second phase. While this was inhibited together with administration of naloxone (opioid receptor antagonist) [24] and memantine (NMDA receptor antagonist) [25]. In previous investigations that have been conducted on *Trigonella foenum-graecum* extract, the results showed that inhibition of serotonin and opioid receptors inhibit performance of *Trigonella foenum-graecum* extract in rat [18, 19]. Our research shows that inhibition of opioid receptor in mice is done through a similar results and the function of *Trigonella foenum-graecum* extract is inhibited. On the other hand, our study showed that administration of NMDA receptor antagonist is effective to

inhibit the function of the *Trigonella foenum-graecum* extract and considering the previous results it can be stated that *Trigonella foenum-graecum* extract inhibit formalin-induced pain through opioid, serotonin and glutamate NMDA mechanisms. On the other hand administration of the extract had no effect on the inhibition of inflammation induced by formalin and the inflammation continued after the injection of the extract. This experiment shows that probably *Trigonella foenum-graecum* extract is not effective in inhibiting stimulation of inflammatory pathways (stimulating release of corticosteroid hormones from adrenal glands) and also production of inflammation mediators such as prostaglandin (by inhibiting cyclooxygenase enzyme). In order to test this part of the theories we investigated the changes in concentrations of corticosterone hormone in the blood of laboratory rats after administration of different doses of extract.

Conclusion

Our research showed that the extract does not have the ability to stimulate Corticosterone hormone release and concentration of hormone in mice blood does not increase after administration of extract. In this study the in vitro effect of extract on changes of cyclooxygenase type 1 and 2 enzyme was investigated. Our research showed that the activity of this enzyme does not change in the presence of different concentrations of the extract. For this reason, we can conclude that *Trigonella foenum-graecum* extract could inhibit pain induced by formalin in phase 1 and 2 with opioid and glutamate-dependent mechanisms and does not activate none of the control mechanisms of inflammation.

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