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Analgesic Activity of the Extract of Aerial Parts of Colza (Brassica Napus) in Mice

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ABSTRACT

BACKGROUND AND OBJECTIVE: It has been reported that Cruciferous family plants such as *Brassica napus* (Colza) are abundant sources of flavonoid compounds that involve in prostaglandin synthesis and may show analgesic and anti-inflammatory effects. The purpose of this study was to investigate the analgesic effect of hydroalcoholic extract of aerial parts of Colza in comparison with morphine, with or without naloxone in mice.

METHODS: The male mice weighing 18-26 g were divided into experimental groups (6 mice in each group) and received i.p. injections of 50, 100, 250 and 500 mg/kg of the hydroalcoholic extract of colza and morphine with or without naloxone, respectively. Normal saline was used as control. The hot-plate test was performed to evaluate the analgesic effects of all treatments and pain latency was measured at 15, 30, 45, 60, 75, and 90 minutes after injection of the drugs.

FINDINGS: Pain tolerance of the mice receiving various doses of colza extract was significantly increased compared to the control. Moreover, simultaneous injection of morphine (20 mg/kg) and colza extract (250 mg/kg) increased pain tolerance compared to morphine alone. Also, simultaneous injection of colza extract and morphine at 10 minutes after naloxone (1 mg/kg), increased analgesia in the animals. The highest analgesia was observed after treatment with morphine and colza extract (250 mg/kg) at 30 minutes after the injection (32±2 seconds).

CONCLUSION: According to the results, the hydroalcoholic extract of the aerial parts of colza induced analgesia In mice during the hot-plate test. This effect may be attributed to the presence of flavonoid compounds in the extract, which confirms the analgesic properties of colza.

KEY WORDS: *Colza (Brassica napus), Flavonoids, Morphine, Naloxone, Inflammation, Analgesic.*

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Introduction

Pain as an unpleasant feeling, is the reaction of the body to deleterious stimulants and works as a protective mechanism. Many medications have been introduced to fulfill the goal of pain relief but the adverse drug reactions can limit their administration. It is believed that complications are rare when natural plants are used instead of chemical medications, therefore, they are in the center of scientists' attention as future analgesics.

The plants of Brassica family including *brassica Napus* are known for their anticancer activities (1-9). They have various chemical compounds such as polyphenols that have anti-inflammatory properties. It has been shown that *brassica oleracea* can be used in treating gout, jaundice, rheumatoid arthritis, obesity, and kidney diseases (10). Analgesic and anti-inflammatory effects of *brassica oleracea* were previously reported (11). It was reported *brassica juncea* can reduce gastric pain induced by acetic acid (12). Some other characteristics of this family have been studied by investigators but analgesic properties of *brassica napus* (Colza in Farsi) have not been studied before. Colza contains thiol, indol, and flavonoid compounds (11, 12).

It also contains phenolic compounds which can reduce inflammation and oleic and linoleic acids which regulate steroid's metabolism (7, 13). This plant is broadly cultivated in the north of Iran. The oil of colza seeds has been used in food industries. This study was done to evaluate the analgesic effects of the different doses of hydroalcoholic extract of Colza aerial parts in mice

Methods

The research protocol was approved by the ethics committee of Babol University of Medical Sciences and no animal was hurt during this study. Male albino mice weighing 18 to 26 g were used. The mice were divided into experimental groups and different doses of colza, morphine, or naloxone were intraperitoneally injected (i. p.). Control groups were only injected with normal saline. At least, six mice were used in each treatment or control groups. To prepare Colza extract, the plants were collected in spring from the farms in northern areas of Iran. The aerial parts were washed, dried, and then chopped and powdered. Ethyl alcohol 80% was then added to this powder and filtered after 72 hours. The filtered liquid was left for 72 hours for evaporation the ethanol and Colza extract to remain. Colza has been administered in four different doses (50, 100, 250 and 500 mg/kg) and the dose of 250 mg/kg of colza extract was selected to use with morphing and naloxone.

Naloxone (1mg/kg) as opioid antagonist was injected 10 min before morphine or doses of colza. Hot-plate latency times were measured for each animal before any injection and 15, 30, 45, 60, 75 and 90 minutes after drug or saline treatments. Hot plate device was set to 54 ºC and end point was defined as the moment when the animal started to lick his legs. Cut-off point was set at 40 seconds. If each animal stayed on hot plate without any reactions for more than 40 seconds, the mouse was excluded from the experiment. ANOVA post-post hoc Tukey test was used to compare the data. P-value $\leq$ 0.05 was considered significant.

Results

Table 1 summarizes data of the treatments groups and presents results of pain latency time in hot-plate test as mean and standard deviation. The pain latency time of colza (50, 100, 250, and 500 mg/kg) groups and saline groups is presented in figure 1. Group receiving colza 500 mg/kg shows a significant pain latency time compared to saline at 15 minute after injection (p=0.004). Evaluation of the concomitant use of morphine and colza (250 mg/kg) showed that injection with morphine 20 mg/kg can significantly increase pain latency at times 15 (p=0.001), 60 (0.01), and 90 (p=0.018) minutes after injection in comparison with the injection of the same concentration of morphine alone (fig 2). It seems that combination of colza and morphine has better analgesic effects than single morphine injection. In figure 2, the slope of the curve of the effect of Colza (250 mg/kg) + morphine (20 mg/kg) versus time is ascending after 90 minutes while the morphine (20 mg/kg) alone is descending at the same time. Naloxone (1 mg/kg) was used as a pretreatment of colza (250 mg/kg) and morphine (20 mg/kg) the results showed significant differences at 15(p<0.001), 30(p=0.006), 60(p<0.001), and 90 (p=0.002) minutes after injection compared to group receiving colza 250 mg/kg or morphine 20mg/kg(fig 3).

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Table 1. Mean (SD) of hot-plate pain latency time (sec) in mice receiving doses of colza in comparison to saline and morphine. Pre-treatment naloxone (1 mg/kg) was used as opioid antagonist (the number of animals in each group were at least 6 mice)

Time / Treatment groups	Baseline	15 min	30 min	45 min	60 min	75 min	90 min
Saline	10.52(5.56)	13.04(8.11)	14.52(5.51)	12.20(8.19)	10.56(6.16)	11.16(5.56)	11.40(7.66)
Colza 250 mg/kg	12.08(9.11)	14.00(7.48)	11.25(4.92)	13.83(7.72)	12.00(6.67)	12.50(6.74)	9.50(2.78)
Colza 500 mg/kg	10.93(4.98)	18.00(9.20)	13.56(6.28)	12.20(5.90)	11.53(7.33)	13.40(6.40)	9.83(4.03)
Morphine (20 mg/kg)	15.22(8.66)	23.72(8.66)	22.38(9.97)	20.50(11.65)	16.39(8.85)	17.67(10.20)	15.72(5.65)
Colza 250 + Morphine (20 mg/kg)	14.08(6.92)	30.92(8.70)	23.58(11.73)	20.83(11.14)	25.42(11.28)	19.25(9.72)	21.33(7.89)
Naloxone+Colza250+Morphine20	17.67(12.19)	29.00(11.21)	32.33(10.52)	17.83(11.58)	22.33(14.45)	23.00(13.94)	27.33(14.08)
Naloxone+Colza500+Morphine20	16.67(2.42)	28.50(9.16)	28.50(24.83)	24.83(9.22)	23.67(8.21)	18.50(7.69)	19.67(4.08)

*Pretreatment with naloxone (1 mg/kg) used as morphine antagonist (minimum of six animals in each group); **Animals receiving colza extract (500 mg/kg) had a significant difference with normal saline group at 15 minutes after injection (p=0.004);***No significant differences observed in other groups

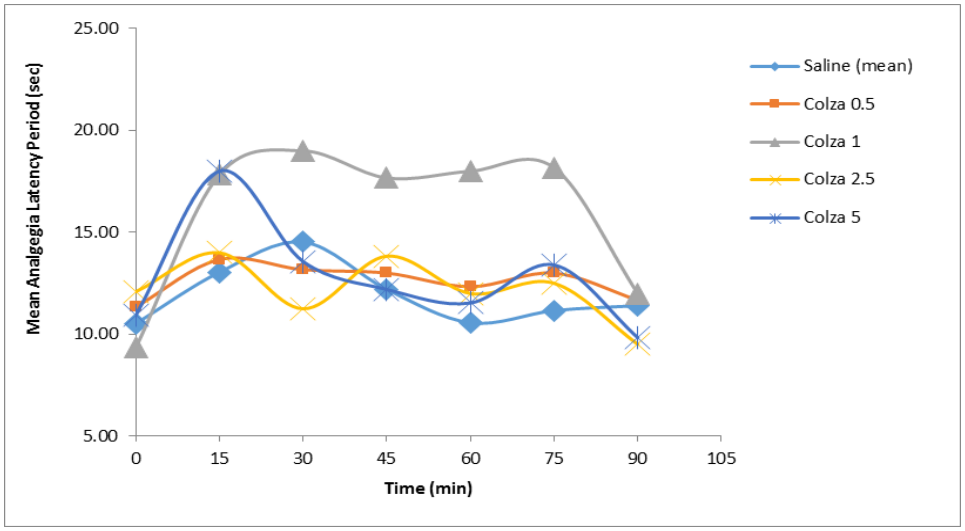

Figure 1. Mean of pain threshold in hot-plate test (sec) in mice receiving colza extract at different doses (50, 100, 250 and 500 mg/kg) compared to control group.

*Animals administered with colza extract (500 mg/kg) had a significant difference with control group at 15 minutes after injection (p<0.001).

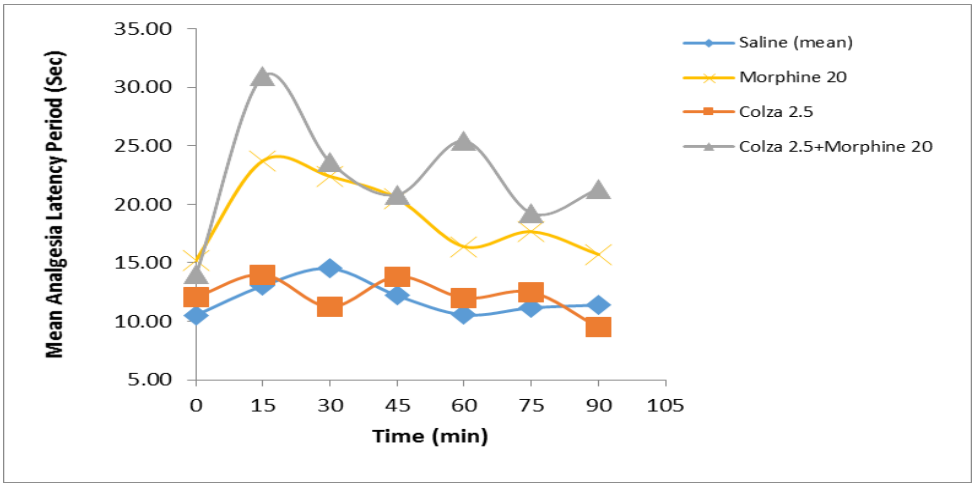

Figure 2. Mean of pain threshold in hot-plate test (sec) in mice receiving colza extract (250 mg/kg) and morphine (20 mg/kg) at different times (simultaneous and separate administration).*Simultaneous administration of morphine (20 mg/kg) and colza extract (250 mg/kg) had a significant difference with morphine only (p<0.001).

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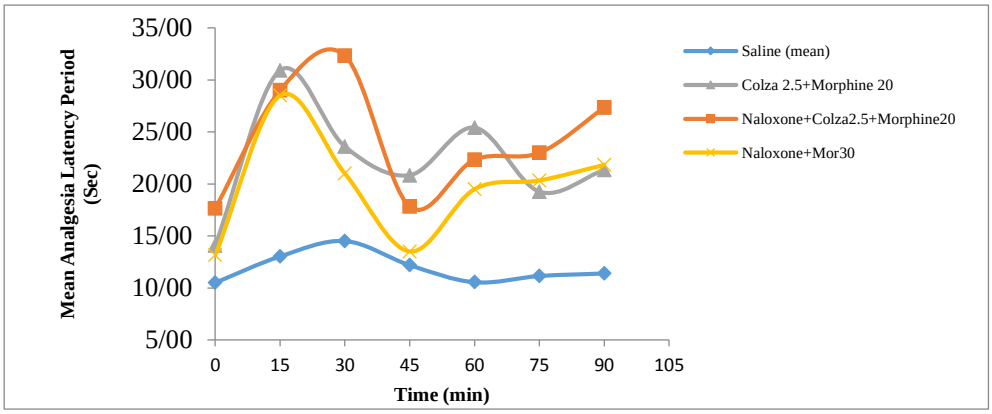

Figure 3. Mean of pain threshold in hot-plate test (sec) at different times in mice receiving colza extract (250 mg/kg) with morphine (20 mg/kg) and naloxone (1 mg/kg) (10 minutes before injection) and mice receiving naloxone and morphine (30 mg/kg)

*Animals administered with naloxone, colza extract (250 mg/kg) and morphine (20 mg/kg) had a significant difference with mice receiving colza extract (250 mg/kg) and morphine (P<0.001);**Significant differences between mice receiving colza extract and morphine with normal saline group.

Discussion

Medicinal plants have been used for relieving pain for a long time (11, 14). In this study, analgesic effects of hydroalcoholic extract of aerial parts of colza were investigated using hot-plate test in mice. This study confirmed that colza, in comparison to saline, shows analgesic activity in high dose at 15 minutes after i.p. injection. On the other hand, colza can increase analgesic activity of morphine in almost all times of the measurements. Naloxone as a specific opioid antidote could not fully antagonize the morphine effect when colza was added. It seems that the mechanism of action of colza may be different and opioid involvement in its action cannot be confirmed (11). In addition, it has been found that colza can maintain analgesia for more than 90 minutes. Antioxidants have various functions in the body (15, 16), and flavonoid compounds have been shown to have remarkable anti-inflammatory and analgesic properties (17, 18). These compounds are abundantly found in the leaves and roots of the species of the Brassica family (19). Flavonoids exert their analgesic effects by crossing the blood-brain barrier and influencing the alpha-2 adrenergic and GABA receptors. Moreover, these compounds inhibit the enzymatic activity of cyclooxygenase-2 in damaged tissues preventing the production of prostaglandins (20, 21). Several studies have suggested that through inhibiting the NMDA receptors, flavonoids reduce calcium concentration in the cells, which diminishes the enzymatic activity of nitric oxide synthase and phospholipase A2. This mechanism might explain the analgesic properties of flavonoids through decreasing the activity of nitric oxide and prostaglandins (22). Previous studies have confirmed that flavonoids are able to decrease metabolism of arachidonic acid and production of prostaglandin E through degradation of tumor necrosis factor (TNF-alpha) and inhibition of cyclooxygenase (23). Furthermore, some flavonoids, such as apigenin, prevent the imbalance of lipid signaling pathways (24, 25). Previous studies have shown the analgesic properties of some species of the Brassica family, such as *Brassica juncea*, which may exert anti-inflammatory effects through the decrease of prostaglandin synthesis (26, 27). However, there is lack of adequate data regarding the exact analgesic effects of colza. According to the literature, the most important compounds in *Brassica oleracea* are phenols and flavonoids, which are known to have anti-inflammatory and analgesic properties (28, 29). In conclusion, findings of the present study indicated that the effect of *Brassica napus* on the analgesic properties of morphine was due to the presence of flavonoids in this medicinal plant. Based on the results, the hydroalcoholic extract of *Brassica napus* could be an effective complementary medicine for pain relief. Nevertheless, it is recommended that further investigations be conducted in this regard in order to clarify the exact mechanism of action of colza.

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