Journal of Biology and Today's World
ISSN 2322-3308
http://www.journalbio.com

Received: 04 May 2014 • Accepted: 15 June 2014

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doi:10.15412/J.JBTW.01030702

Effects of hydro-alcoholic extract of broccoli (Brassica *oleracea)* on sensory threshold of pain using the formalin test in adult male rats

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ABSTRACT

The study of plant species that have been traditionally used as painkillers is a useful and logical research strategy in the search for new anti-nociceptive compounds. The aim of the present study was to evaluate the effect of oral administration of the *Brassica oleracea*hydroalcoholic extract by formalin test in rats. In this research, 50 male- Wistar rats weighting about 210± 20 grams were divided into 5 groups (n=10). For evaluation of antinociceptive effects, the formalin test induced pain. The nociceptive response develops two phases: First (0-5) min after formalin (first or acute phase) and (16-60) min after formalin injection (second or chronic phase). The animals pre-treated with oral doses of *Brassica oleracea* extracts (500, 1000, 2000 mg/kg), one month before administration formalin. The control group without receiving any drug and the sham group receiving 1ml hydro alcoholic extract one month before administration formalin. A statistical analysis by ANOVA and T-Test used (p< /05). The data shows there is decreased pain in the formalin test in the group that received 2000 mg/kg dose of extract in comparison with the control and sham groups (P< /05). It was concluded that probably, vitamin c, sulforaphane and flavonoids are important compound of the extraction decrease pain. *Brassica oleracea*hydroalcoholic extract by decrease of intracellular ca^{2+} and ca^{2+} - dependently enzyme activity inhibition such as phospholipase A_2 and iNOS that decreased NO and prostaglandins consequently causes that decrease pain.

Key words: Brassica oleracea, Formalin, Nociception, Rat

1. INTRODUCTION

Pain is one of the main and basic problems with different symptom that every man has to face during his or her lifetime. Although several medications for pain have been discovered, the need for new drugs with minimal side effects is still being felt (1). At the present time, there are two main categories of analgesic materials, i.e. Opioid (narcotic) and anti-inflammatory non-steroidal analgesic drugs (aspirin-like drugs) that in spite of usages, they have negative and undesirable effects. For example, aspirin-like drugs caused damage, gastric system, kidney and central nervous system. For opioid analgesic drugs, there are some problems such as resistance to drugs, euphoria, nausea, constipation, weakness of breathing (2). The above components have led scientists to look for drugs that lack the side effects, but cheap and usable. In this regard, the attention was attracted towards the plant. Broccoli *Brassica olearacea* belongs to Crucifer family. Broccoli is rich in vitamins C, A, E, fiber, beta carotene (precursor of vitamin A), and flavonoids and sulforaphane (3, 4). Sulforaphane is the result of enzyme activity on glicofarnin, it is the most important isothiocyanate in vegetables of Crucifer family. Basic and clinical studies have shown that Sulforaphane plays an important role in controlling and blocking different stages of the cancer process reducing harmful oxidative stress, improving blood lipid concentration, protecting cardiovascular disease, and treatment of H.pylori infection (5, 6). Sulforaphane is capable of activating some antioxidant enzymes such as glutathione-S-transferase and Quinone reductase activity and reduce the formation of cancer cells (5). These plants due to high doses of vitamin C, fiber and beta-carotene are also a powerful antioxidant that serves to prevent the destructive effects of free radicals in the body (6, 7). In the

present work, anti-nociceptive effect of hydro-alcoholic extract of broccoli (Brassica Oleracea) was measured by using formalin test. Obtained results would be useful in clinics and treatment centers.

2. MATERIALS AND METHODS

2.1. Animals

This study was performed on 50 adult male Wistar rats weighing approximately 20 ± 220.They were obtained from the Animal House of Shiraz University of Medical Sciences. **A**nimals had free access to food and water under a constant light/ dark (12:12 h) cycle with controlled temperatures (22-20 °C). All experiments were conducted between eight to16 hours.

2.2. Preparation of extract

A Fresh broccoli was purchased from a supermarket in Shiraz in May 2012, identified, and approved by botanist of the biology department of Science College in Shiraz and number 32155 was attributed for each barium.

500 grams of broccoli powder with a ratio of 50:50 to 96% ethylic alcohol and distilled water were mixed together and kept for 48 hours. During this period, the contents of the container were shaken to dissolve the extract in alcohol completely. The extract was centrifuged at 4500 RMP for 8 min. The fluid in an open container was placed in the chamber until the alcohol is evaporated. The concentrated green juice was placed in the oven (50 ° C) until the mixture was dried and a dry matter remained. The amount of dried extract was dissolved in distilled water and different doses of the extract (500, 1000, 2000ml/ kg) were obtained in order to administer orally to animals (8).

2.3. Formalin test

Animals were divided into 5 groups of 10. Analgesic effect was examined using formalin test with Dennis and Dubuisson method (9). It should be noted that the formalin test is a valid and reliable model to assess pain. In this test, a two-phase response appears. Animals were placed in a Plexiglas chamber (30 × 25 cm) that incorporated a mirror with the 45 angles under the chamber until the status of animal، paw is fully determined. The animal was kept for 30 minutes to get used to the conditions. Each animal was tested only once. One-hour oral administration of broccoli extract with values of 500, 1000 and 2000 mg/ kg were given to treatment groups and the hydro-alcoholic solvent to sham group. Later on 50 ml of a 5%, formalin was injected subcutaneously into the right hind paw of each animal and the animal was immediately returned to the formalin test chamber. Formalin was injected into the control group and animals did not receive any extract. Recording of behavioral responses began immediately after formalin injection and this continued every 15 seconds to 1 hour. It was considered as an indicator of the severity of pain in the formalin test. **U**sing this method, the numbers from zero to three for pain scores were obtained at different times. **T**he mean pain score in the first 5 minutes after formalin injection was considered as the primary (fast pain) or acute pain and in minutes 60-16 after a late phase of the formalin injection (slow pain) or chronic pain.

The severity of pain was classified into four levels:

0. The rat walked or stood firmly on the injected paw.
1. The injected paw was favored or partially elevated.
2. The injected paw was clearly lifted off the floor.
3. The rat licked, chewed or shook the injected paw.

Pain score was measured during 60 minutes as 12 blocks 5 minutes. Average of pain score in each block was measured according to formula (1):

(1)

$$\text{Pain Score} = \frac{0T_0 + 1T_1 + 2T_2 + 3T_3}{300S}$$

T_0 , T_1, T_2 , T_3 are the number of 15 seconds that animal in a 5 minutes period shows numbers 0, 1, 2, 3 that was based on behavior.

All results were expressed in mean ± SEM. For statistical analysis between control and experimental groups, the Kruskal - Wallis ANOVA and Tukey tests used to evaluate differences between groups. SPSS software was used for data analysis and the difference $p<0.05$ was considered significant.

3. RESULTS AND DISCUSSION

Based on the results of this study, the doses of 500& 2000 mg/kg broccoli extract decrease in pain score ($P = 0.00034$) in the acute phase of formalin test (5-0 min) compared with the control group (Figure 1).

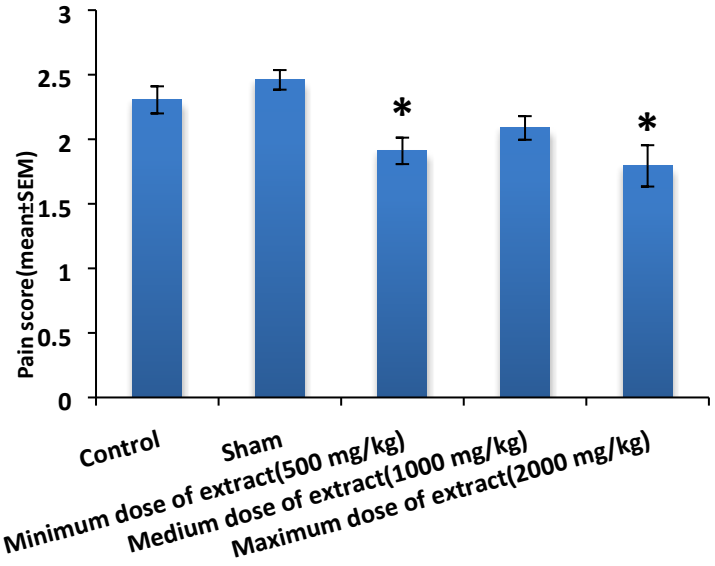

Figure 1. The comparison of pain score (Mean± SEM) of formalin test acute phase (0-5 min) in pre-treated groups with various doses of broccoli hydroalcoholic extract with the controlgroup

The obtained results showed that the highest concentration of broccoli extract 2000 mg/kg decrease in pain score (P = 0.008) in the chronic phase of formalin test (16-60 min) compared with the control group (Figure 2).

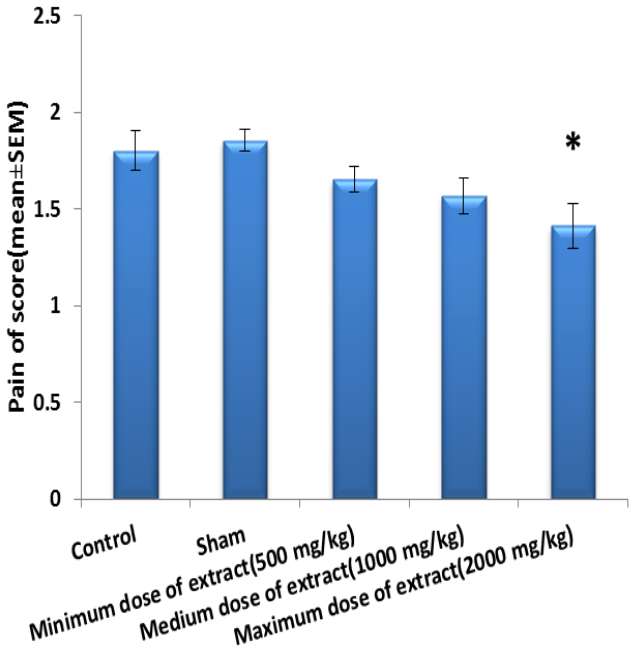

Figure 2. The comparison of pain score (Mean± SEM) of formalin test chronic phase (16-60 min) in pre-treated groups with various doses of broccoli hydroalcoholic extract with the control group

Statistical analysis also showed that the maximum amount of broccoli extract significantly reduced pain severity in both the formalin test phases in comparison to control group (Figure 3) (Table 1).

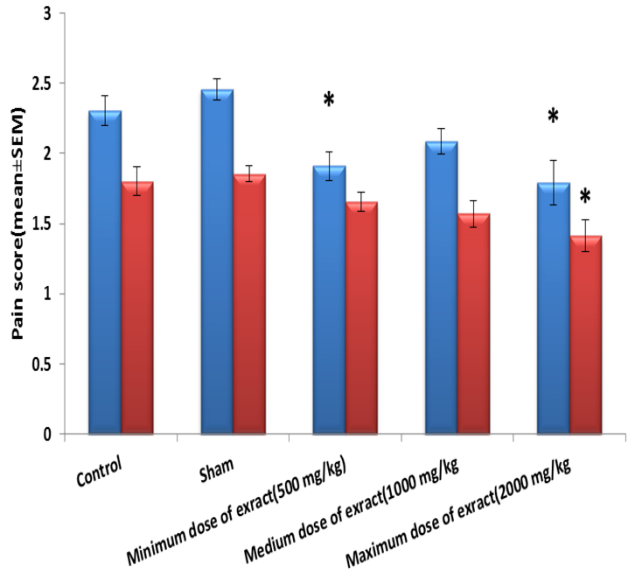

Figure 3: The comparison of pain score (Mean± SEM) of acute and chronic phases formalin test in pre-treated groups with various doses of broccoli hydroalcoholic extract with each other

Table 1. Comparison of the acute and chronic phase of the formalin test in experimental and control groups receiving different doses of hydro-alcoholic extract of broccoli and control

Group	number	Acute phase (Mean± SEM)	P-value Acute phase	Chronic phase (Mean± SEM)	P-value Chronic phase
Control	N=10	2.305±0.105		1.802±102	
Sham	N=10	2.460±0.075	P-Value = 0. 369	1.856±0.055	P-Value = 0.369
Experimental group 1 (500mg/kg)	N=10	1.910±0.102*	P-Value = 0.00034	1.653±0.068	P-Value = 0.331
Experimental group 2 (1000mg/kg)	N=10	2.087±0.091	P-Value = 0.281	1.568±0.094	P-Value = 0.125
Experimental group 3 (2000mg/kg)	N=10	1.793±0.160*	P-Value = 0.00034	1.413±115*	P-Value = 0.008

*P≤ 0.05

The average (Mean±SEM) amounts marked by * have a significant difference with the control group.

The results of the effect of oral administration of different doses of broccoli extract of 2000, 1000 and 500 mg/kg on acute and chronic pain in formalin test shows Minimum value (mg / kg 500) and maximum (mg / kg 2000). Broccoli extract significantly ($p<0.05$) reduced pain severity in the acute phase of the formalin test as compared to control group. Only the maximum dose of Broccoli extract caused a significant reduction ($p<0.05$) in pain intensity compared to control group in chronic phase of the formalin test. Effect of broccoli extracts on the fast and slow phases of the formalin test suggests that the analgesic activity of broccoli is probably due to its central action. On the other hand, the results show the dose-dependent effects of broccoli extract on pain threshold in acute and chronic phases. Broccoli is rich in the powerful antioxidant compounds such as vitamin C, vitamin E, flavonoids (especially Quercetin) Sulforaphane (3-5). The analgesic effect of broccoli extract is the result of vitamins, flavonoids, and Sulforaphane. Vitamin C is one of the major compounds found in broccoli so that the amount of 115 mg/100g broccoli has been reported (10). Vitamin C due to its powerful antioxidant properties can help to lower back pain and pain from bulging discs in the spine (11). Vitamin C also plays an important role in preventing muscle loss because of heavy exercise (12). Intraperitoneal administration of vitamin C, without affecting on fast phase, causes to reduce chronic pain (13). The reason of reducing chronic pain of formalin test by vitamin C may be related to anti-inflammatory effects (14). According to the report of the anti-inflammatory effect of vitamin C on rats, it can be assumed that the effect of vitamin C on reduction of pain may be related to anti-inflammatory effects. (15)

This point can confirm that the vitamin C increases the inhibitory effect of aspirin on the production of prostaglandin E2 by reducing the enzymatic activity of cyclooxygenase II (16) Prostaglandins have an important role in formalin-induced pain (17). It has been reported that vitamin C reduces the pain caused by plantar injection of formalin and glutamate in mice (18). It is reported that antioxidants combined with analgesics may reduce analgesic agents such as morphine (19-21). Nitric oxide (NO), a radical produced by nitric oxide synthase, acts as a second messenger of pain caused by formalin injection. Sulforaphane is one of enzyme inhibitors of nitric oxide synthesis. Therefore, prevents the production of NO and pain in formalin test (7, 22, 23). Quercetin is the major flavonoid in broccoli (24) analgesic properties of Quercetin was noted first by *Rylski and collageues* in 1997 and then by Picq and colleagues noted in 1991 (25, 26). Rylski et al. reported that Quercetin exerted analgesic action in the hot-plate test (25). Filho research on the mechanism of the analgesic action of Quercetin showed that Quercetin significantly inhibited both phases of neurogenic and inflammatory in the formalin test (27, 28). Quercetin increases brain levels of endogenous serotonin or directly reacts with 5HT3 and 5HT2A receptors. Serotonin inhibits both phases of the formalin –induced pain (27). Analgesic effect of Quercetin is associated with the GABA system (27). The analgesic effects of Quercetin are inhibited by faclophen (receptor antagonist GABA) and bicuculline (receptor antagonist of GABAA). Therefore, Quercetin can reduce pain by stimulating GABA receptors. Adrenergic system is involved in the analgesic effect of Quercetin (29). Studies show that Quercetin also decreased the intracellular calcium and inhibits calcium-dependent enzymes (30). Quercetin acts as an antagonist calmodulin (31).Therefore Quercetin decreases intracellular calcium, which is followed by reducing phospholipase A2 and nitric oxide synthesis enzyme activity (27, 32). Thus, Quercetin by decreasing nitric oxide can alleviate pain (27).

4. CONCLUSION

According to the results obtained in this study and other studies, it can be said that the analgesic effects of Broccoli extract are related to Vitamin C, Quercetin and Sulforaphane. Overall, broccoli extract broccoli is related to serotonergic, adrenergic, the GABAergic, glutamatergic and opioid system.

ACKNOWLEDGMENT

No mentioned acknowledgment by any authors.

AUTHORS CONTRIBUTION

This work was borne out in collaboration between all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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