



Research Article

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Comparison of the Effect of Lycopene with Ibuprofen on Sensory Threshold of Pain Using Formalin Test in Adult Male Rats

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ABSTRACT

Pain is such an experience that every human being is faced in his/her lifetime. On one hand, it is a warning for awareness from tissue damage and on the other hand it is a bad experience which always attacks the body and the soul of human. Treatment of acute and chronic pain is the subject of many clinical and laboratory researches due to the complexity and multiple protests of pain. Through inhibition of cyclooxygenase-2 and thereby inhibition of prostaglandins, lycopene prevents from sensitization of pain receptors caused by these molecules and reduces the pain that comes with these responses. This study is aimed to evaluate the effect of lycopene with ibuprofen on sensory threshold of pain using formalin test in adult male rats. 32 male Wistar rats were randomly divided in this study into 4 groups including a control group and three experimental groups. 5 and 10 mg of lycopene per kg of body weight and ibuprofen at a dose of 6 mg/kg of body weight were intraperitoneally administered to the experimental groups, respectively. Formalin test was performed 30 minutes after the injection. Data were analyzed using one way analysis of variance (ANOVA) and least significant difference (LSD) test at significance level of 0.05. Intraperitoneal injection of lycopene in a dose dependant manner (only at a dose of 10 mg/kg of body weight) can reduce formalin-induced acute and chronic pains. Because of flavonoid and high antioxidant properties and inhibition of cyclooxygenase and prostaglandins, lycopene can probably reduce formalin-induced analgesic effects and according to hazards of nonsteroidal drugs for clinical use, it can be helpful in the future.

Keywords: Lycopene, Formalin Test, Rat, Ibuprofen

INTRODUCTION

Pain is such an experience that each human is faced in his or her lifetime. In one hand, pain is a warning for awareness from tissue injury and on the other hand is a bad experience which always attacks the body and the soul of human. Treatment of acute and chronic pains has been the subject of many clinical and laboratory researches because of the complexity and multiple protests of pain [1]. Two main categories of anti-pain materials including opioids (narcotics) and analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) (pseudo-aspirins) are currently used that their adverse effects are also considerable despite the abundant use. Pseudo-aspirins, for example, damage to the gastrointestinal tract, kidneys and central nervous system; and more ever they are not effective in some patients and even development of tolerance is reported in some of them. There are also some complications such as drug resistance, addiction, euphoria, nausea, constipation and respiratory depression about opioid analgesic drugs [2]. The above elements have led scientists to seek medicines that are cheap and available; meantime they are free from the mentioned complications. Therefore, the effects of lycopene, a natural pigment, on acute and chronic pains using formalin test will be discussed in this study.

Formalin test is one of the standard tests in measurement of pain responses against painful stimuli [3]. Subcutaneous injection of formalin causes two-phase pain that the first phase or acute stage (neurogenic phase) is the result of direct stimulation of pain receptors in animals' paw, while the second phase or chronic phase is caused by inflammation stage [4]. Analgesic drugs that exert their effects through the central nervous system are able to inhibit both phases of formalin-induced pain, while peripherally acting anti-pain medicines can only inhibit the second phase of pain in the formalin test [5].

Lycopene is the predominant carotenoid available in human blood serum [7, 9] that structurally is a red carotenoid and mainly is available in tomatoes, watermelon, grapefruit and apricot [6, 7]. This carotenoid is not a precursor of vitamin A, but it has very powerful antioxidant properties due to the presence of 11 conjugated double bonds that eliminates free radicals [8, 9]. Researches results show that the ability to absorb free radicals by lycopene is two times more than β - carotene and ten times more than alpha-tocopherol [10]. Inflammation is considered as a productive peripheral process of pain in the second phase of formalin test [15]. Formalin-induced inflammation leads to increased nitric oxide synthase (NOS) [39]. Nitric oxide synthase is an active enzyme in inflammatory lesions [40]. Nitric oxide is a highly active and small molecule that is produced by NOS [41]. Nitric oxide (NO) plays role in many functions of the nervous system as a matter of mediating and moderator [42]. Inflammation stimulating cytokines, including tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) and interleukin-6 (IL-6), lipid mediators and eicosanoids such as prostaglandins are triggers of inflammatory responses. Inflammatory mediators lead to expression of inducible nitric oxide synthase (iNOS) [45]. Microglia cells play an important role in inflammation process and excessive activation of them leads to release of nitric oxide, prostaglandins and cytokines that stimulate inflammation [46]. Lycopene effects on inhibition of inflammatory mediators are known in multiple diseases. Lycopene can prevent from inflammatory response to acute brain stroke and reduce the extent of the affected area in the brain [16]. It was determined in evaluation of lycopene effect on cultured macrophage cells in mouse that the pigment exerts its anti-inflammatory effect through inhibition of iNOS [17]. It has been reported about evaluation of protective effects of lycopene on ischemic injury of the brain in rats that lycopene at doses of 5 and 10 micromolar inhibits nitric oxide (NO) by 61% [18]. Nitric oxide is a full of pain mediator that increases followed by injection of formalin [19], so maybe some parts of analgesic effects of lycopene are associated with inhibition of NO.

Cyclooxygenase-2 (COX-2) enzyme causes the synthesis of prostaglandins from arachidonic acid in response to inflammatory stimuli. Prostaglandins are also famous mediators of pain in the formalin test [20]. Non-steroidal anti-inflammatory drugs are used to inhibit cyclooxygenase isomers (COX-1, COX-2), cyclooxygenase pathways and reduction of prostaglandin synthesis. COX-2 is associated with inflammation and pain [43]. COX-2 expression decreases by pro-inflammatory cytokines and antioxidants and leads to increase of inducible nitric oxide synthase (iNOS) enzyme (44, 47). It has been reported that lycopene prevents from the nerve tissue inflammation in rat by inhibition of COX-2 enzyme in microglial cells [21]. In addition, one of the anti-cancer mechanisms of lycopene is suppression of inflammatory responses that includes inhibition of pro-inflammatory mediators such as reduction of reactive oxygen species (ROS), inhibition of synthesis and release of pro-inflammatory cytokines, changes in the expression of cyclooxygenase and lipoxygenase and adjustment of signaling pathways [22]. So, through inhibition of cyclooxygenase-2 enzyme and thereby inhibition of prostaglandins, lycopene can possibly prevent from sensitization of pain receptors caused by these molecules and reduce the pain sense that comes with these responses. Moreover to assess the effect of lycopene on acute and chronic pains induced by formalin, a comparison will be done between this carotenoid and a common pain reliever medication called ibuprofen in this study. Ibuprofen is a non-steroidal anti-inflammatory drugs and it has shown analgesic effects in the formalin test [23]. This medicine exerts its analgesic effects through inhibition of prostaglandins synthesis [24]. It is known that ibuprofen is a nonspecific inhibitor of COX. On the other hand COX inhibitor medicines exert their significant effect in the second stage of pain [25]. In addition, there are some reports about lack of significant effect of ibuprofen on acute pain or early phase of the formalin test [26].

So the aim of this study is to evaluate the effect of lycopene with ibuprofen on sensory threshold of pain using the formalin test in adult male rats.

EXPERIMENTAL SECTION

This research is an experimental study that was performed in 2015 in the Keeping and Breeding Center of Laboratory Animals in Jahrom University of Medical Sciences. A total of 32 male Wistar rats were randomly selected among 300 male rats. The entire process was done in accordance with international laws on keeping of laboratory animals.

The samples were selected by simple random sampling and the rats were randomly divided between the groups using random numbers table.

Based on papers published in this field, this study was carried out on 32 healthy male Wistar rats in the weight range of 180 to 200 grams. The animals were randomly divided into four groups of eight each.

The total number of required samples was 32 male rats. Based on previous studies, prescribed concentration of lycopene was determined in dose of 5 and 10 mg per kg of body weight [28]. Thus, experimental and control groups in this study are as follows.

Control group: formalin (50 μ l, 2.5%) was subcutaneously injected by insulin syringe into the right hind paw of rats in this group. The animals in this group did not receive any treatment (lycopene or ibuprofen) (n=8).

Experimental 1 group: 30 minutes prior to injection of formalin, the animals of this group were intraperitoneally received 5 mg of lycopene per kg of body weight (n=8).

Experimental 2 group: 30 minutes prior to injection of formalin, the animals of this group were intraperitoneally received 10 mg of lycopene per kg of body weight (n=8).

Experimental 3 group: 30 minutes prior to injection of formalin, the animals of this group were intraperitoneally received 6 mg of ibuprofen (nonsteroidal anti-inflammatory medicine as a positive control) per kg of body weight (n=8) [26].

All ethical issues about quality of working with laboratory animals were considered in this study.

32 male Wistar rats with an average weight of 180-200 g were used to contact this study. The rats were kept in the Animals Breeding Room in Jahrom University of Medical Sciences for a week to adopt. Dark and bright cycle was adjusted for 12 hours of light and 12 hours of darkness and humidity was about 50 to 55 percent. After weighing, the rats were kept in their cages (4 rats per cage). They fed from pellet, the rats' food. Water was provided for them in glass bottles. The cages were cleaned and disinfected 3 times a week.

Formalin test: the most important feature of formalin test is that rodents show two responses to pain, which apparently have two different mechanisms. The first stage (acute pain) appears immediately after injection of formalin and lasts 3 to 5 minutes. It does not show any particular behavior within 5 to 15 minutes after the first five minutes. The second stage (chronic pain) begins from 16 minutes after injection of formalin and lasts 3 to 5 minutes. To do formalin test and record analgesia behaviors, administration of medication or distilled water was performed 30 minutes before beginning of the test and the rats were immediately placed in the formalin test chamber to compromise with environment and eliminate stress. After 30 minutes, 50 μ l formalin (2.5%) was subcutaneously injected by insulin syringe into the right hind paw of the animals and they were immediately placed in plexiglass chambers of formalin test with dimensions of 30 \times 30 \times 30 cm. The animals' behaviors were observed with the help of an embedded mirror at an angle of 45 degrees with horizontal plane in the floor of the chambers and pain motor response were recorded every 15 seconds with the numbers 0, 1, 2 and 3 and according to Dubisson and Dennis Method in 1997.

Zero (0): for the time that the rat has full balance during walking and its body weight is equally distributed on both feet.

One (1): for the time that the rat does not tolerate its body weight on the injected foot and/or when it has no trouble in walking.

Two (2): for the time that the animal raises its painful toes and has no contact with the floor of the chamber.

Three (3): for the time that the animal licks its painful leg and/or intensely shakes it.

Pain score is calculated during 60 minutes as 12 blocks of 5 minutes and the mean pain score is calculated as follows.

$$\frac{3T3 + 2T2 + 1T1 + 0T0}{300S} = \text{Pain Score}$$

Where, T0, T1, T2 and T3 are the number of 15 seconds that the animal respectively shows 0, 1, 2 and 3 behaviors over a period of 5 minutes. The times 0-5 minutes and 16-60 minutes were respectively considered in all the groups as an acute phase and a chronic phase. The study inclusion criteria were healthy male Wistar rats weighing from 180 to 200 grams and exclusion criterion of the study was the animal death.

RESULTS

As shown in Table 1, the group received 5 mg lycopene ($p=0.12$) and ($p=0.19$) did not show a significant decrease in the acute phase (from the time of injection to 15 minutes after that) and chronic phase (15 to 60 minutes after injection). However, the group received 10 mg lycopene did not show a significant decrease compared with the control group both in the acute phase ($p=0.001$) and in the chronic phase ($p=0.03$) of pain. The group received 6 mg ibuprofen showed a significant reduction compared with the control group both in the acute phase ($p=0.004$) and in the chronic phase ($p=0.01$).

Table 1- Mean score of analgesia induced by intraperitoneal injection of lycopene in acute and chronic phases of pain caused by formalin in the different groups

Groups	0-15 (min)	15-60 (min)
Control	1.9 ± 0.12	2.5 ± 0.14
Lycopene (5 mg)	1.7 ± 0.09	2.2 ± 0.18
Lycopene (10 mg)	1.3 ± 0.11 ^a	1.6 ± 0.13 ^a
Ibuprofen (6 mg)	1.1 ± 0.06 ^a	1.4 ± 0.17 ^a

Mean ± standard error of the mean

According to Duncan Test, if there is at least one common letter in each column, those groups do not have significant difference with each other.

DISCUSSION

One of possible solutions to achieve new analgesic medicines with higher usage and fewer side effects is paying attention to herbs and natural substances which extracted from them. Today, carotenoids such as lycopene are widely extracted for human health from fruits and vegetables as food pigments, nutritional supplements and powerful antioxidants [9]. In addition to powerful antioxidant effects [29], previous studies have shown that lycopene shows blood cholesterol-lowering effect [30] and can inhibit TNF- α and peroxy nitrite. In addition, lycopene antioxidant effect causes the cells and chromosomes of human fibroblasts in culture media be protected from damages induced by ionizing radiations.

Given to the results of this study, lycopene at dose of 10 mg can reduce pain in both acute and chronic phase, which is similar to the results obtained from ibuprofen medicine. No study has been done till now on the analgesic effects of lycopene by the formalin test or other chemical models for creation of chronic and acute pains. But the analgesic effects of lycopene on thermal hyperalgesia and cold allodynia have been studied in streptozotocin-induced diabetic rats using hot plate and tail flick models which are usually considered as models for creation of acute pain and show its analgesic effects [27]. Studies have shown that nitric oxide (NO) levels increase in affected area following tissue injuries [34]. NO is a free radical gas which involved in neuropathic pain as a messenger. NO is synthesized from L-arginine amino acid by nitric oxide synthase (NOS). Three NOS types including the main type as neuronal nitric oxide synthase (nNOS, type 1), endothelial type (eNOS, type 2) and immunological type (iNOS, type 3) have been detected till now [35]. nNOS is activated first as a result of tissue injury or inflammation and then NO levels are increased. Studies have shown that all three isoforms of nitric oxide are increased after the increase of neuropathy in the damaged area [36]. In investigation on lycopene effect on cultured macrophage cells of mouse was determined that this pigment exerts its anti-inflammatory effect through inhibition of iNOS [17]. The analgesic activity of lycopene is possibly through inhibition of NO and release of TNF-alpha [38]. On the other hand living organisms have different mechanisms including antioxidant defense system such as flavonoids to counteract the effect of these reactive materials [37]. Studies have shown that analgesic effects and anti-inflammatory activities (acute and chronic) may be due to presence of flavonoids (such as lycopene) [37].

In addition, side effects of lycopene consumption have not been seen in human. In a study that was performed by Mckinley *et al.* red-orange discoloration of the skin and the liver associated with high levels of lycopene have been reported due to excessive consumption of carotenoid in middle-aged women who had had long-term continuous use of tomato product. The discoloration had returned to normal after three weeks on a lycopene-free diet [33]. Therefore, future studies on the use of lycopene as an alone analgesic or in combination with other medicines are recommended.

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