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Antinociceptive Effects of Grape Seed Oil with Use of Formalin Test in Male Rats

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Abstract: The aim of this study is the evaluation of antinociceptive effects of grape seed oil in male rats. In this study, 72 adult male wistar rats, weighing 200-250g were used. All the animals were divided into 9 groups of eight and were evaluated by formalin test. Control group received 50µl formalin 2.5% and sham group received 2/5ml/kg morphine before formalin test. Experimental groups, once a day for 1 week sequentially received 1, 2, 4, 8, 16 ml/kg and underwent the test on the eighth day. At first, they received 2/5ml/kg morphine intraperitoneally and after 30 minute formalin test was performed. The sixth experimental group received 8ml/kg of grape seed oil and on the eighth day, at first received 2/5ml/kg morphine intraperitoneally and after 30 minute formalin test was performed. The seventh experimental group once a day for 1 week received grape seed oil and on the eighth day, at first received 10ml/kg Naloxane intraperitoneally and after one hour formalin test was performed on them. After recording the behavioral responses, the means of acute and chronic pain score were evaluated using ANOVA test between experimental groups and control . Means of acute and chronic pain score in all of the experimental groups except the first and seventh experimental groups in acute and chronic phase of formalin test, were significantly reduced from that in control and sham groups($p<0.05$).
Based on the results of the present study, grape seed oil in acute and chronic phase has an antinociceptive effects. This effect maybe due to essential fatty acids, procyanidins and flavonoid compound that have an antinociceptive effect. Also, grape seed oil strengthens the antinociceptive effect of morphine .

KEY WORDS: Grape seed oil, Pain , Formalin test, Rat.

1. Introduction

Since the existing antinociceptive drugs have wide range of side effects, finding new antinociceptive compounds was a priority for researchers(1). Today, researchers believe that plants could be a significant resource of chemical compound with powerful therapeutic effects. So, study of plant species traditionally used for reducing pains, and producing analgesics look necessary(2). Grape is a climbing plant with a depth and branching roots and a wooden trunk. Trunk has node and branching crust(3).

All of the parts of this tree from leaf, fruit to plant juice are used(4). Grape seed forms 2.5 percent of the grape weight, and 10-20 percent of grape seed weight is made of oil(5). Studies shows that because grape seed oil includes high amount of essential fatty acid such as, linoleic acid (69-78%), palmitic acid (5-11%), oleic acid(15-20%) , and stearic acid(3-6%), it is essential for the production of prostaglandins and is effective for preventing blood clotting in vessels and their inflammation(6). Grape seed oil also, contains a high amount of phenolic compounds including galic acid and procyanidins (7). Procyanidins are colorless flavonoids and with biological activities including antioxidant property, anticancer and have stabled effect

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for the collagen of the skin(8). Proantocyanidins inhibits the activity of some of the proteolytic enzymes (collagenase , elastase), which play a role in the destruction of essential construction part of extra vessel matrix collagen , elastin and hyaloronic acid(9).

Castilo and his colleagues in 2000 showed that antioxidant property and biological activity of grape seed oil are 50 times greater than that vitamin E and C (9) . Some studies have also shown that grape seed oil inhibits aromatase enzyme activity (10). Evidences shown that grap seed oil inhibits the activity of protein kinase of epidermal growth factor receptor and also destruction of brain cell due to oxidative stress(11). Stefanie Bail and his colleagues in 2007, studied the phenolic compound , antioxidant capacity and triglyceride of grape seed oil (Vitis vinifera) (6) .

The results of their experiments revealed that antioxidant capacity of this oil is within 1.16mg/g and 0.09mg/g(6). Also, other researchers studied the effects and amount of antioxidant phenolic in this oil(7). With respect to the importance of the therapeutic plants in traditional medicine and their protective effects and since there is no study performed about the antinociceptive effect of grape seed oil and with regard to the fact that the use of medicinal plants in human for the relief of pain may have a few side effects, in this study, the effect of grape seed oil on the acute and chronic pains due to formalin in male rat was investigated.

2. Materials and Methods

2.1. Experimental animals

In this experimental study, we used adult male wistar rats belonging weighing 200-250g. All of the animals were kept in standard cages and in the same condition (20-22°c temperature) and with 12 hour light-darkness period. All of the animals had access to food and water and except for the experimentation time.

2.2. Formalin test and Pain Score

Formalin test box is composed of a plexi glass with 30×30×30cm dimensions with a mirror with 45 degree behind the box in order to monitor the position of their soles. Forming pain in this test have two distinct phases, the first is the result of direct stimulation of pain receptor in the claw of animals , whereas the second phase is due to inflammation process. Only 50µl of formalin 2.5% was injected subepidermally into the foot claws of the controls. Sham group received 2.5ml/kg morphine intraperitoneally 30 minutes before formalin test. First, second, third, forth and fifth experimental groups, once a day for 1 week received 1,2,4,8 and16 ml/kg of grape seed oil and then formalin test was done on the eighth day. As for the sixth group, the amount of grape seed oil that showed the best reflex (8ml/kg), was given once a day for a week and on the at eighth day they initially received 2.5 ml/kg morphine intraperitoneally and after 30 minutes formalin was injected into their claws. . The seventh experimental group, once a day for 1 week received grape seed oil and on eighth day initially received 10mg/kg naloxane intraperitoneally and formalin test was performed. In all of the above- mentioned groups, in order to familiarize the animals with the experimental environment and remove their stress, three days before doing the experiment every time for 30 minutes, they were placed in formalin test box and after injection of 50µl formalin 2.5%t into their right soles, they were transferred to the plexi glass box and every 15 seconds their motor reflexes were recorded, according to Dubuisson Dennis method in the form of 0,1,2,3, as follows (12): Number 0 for the cases that animal during movement has a complete balance and sitting without regard to the injected foot. Number 1, for the times that animal fall more weight body on the injected feet and have difficulty with moving. Number 2, for the times that animal raised painful injected foot from the box floor. Number 3, for the times that animal sucked painful injected feet intensely or shake it. Number of theses quantitative data were counted in the form of 12 blocks per 15 minutes and recorded based on the pain score on the every time section. Data recording continued for 60 minutes after formalin injection and pain score average for each block was calculated as follows:

$$Pain\ score = \frac{0T_0 + 2T_1 + 3T_2 + 4T_3}{300\ s}$$

T_0,T_1,T_2,T_3 in the pain score average, refers to the number of 15 second periods that the rats showed 0,1,2,3 behaviors, respectively. In all the groups, 0 to 5 minutes was taken as acute phase and 16-60 minutes as chronic phase. For comparative statistical analysis of the results between control and experimental groups, we used ANOVA and Excell, SPSS software. All the results were expressed in (X±SEM) and significant level was taken at ($P<0.05$).

3. Results

Based on the results of present study grape seed oil in doses of 2,4,8,16(ml/kg) cause a significant decrease of pain score ($P<0.05$) in acute phase (0-5minute) and chronic phase (16-60) of the formalin test in the experimental groups, in comparison with sham and control groups(Fig 1,2). The results also indicate that grape seed oil combined with morphine, increase antinociceptive effects of morphine and use of naloxane suppresses the antinociceptive effects of the oil.

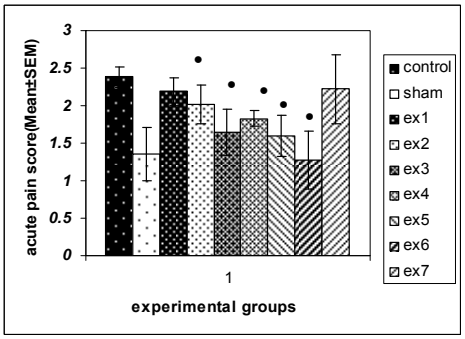

Fig1. Comparison of pain score means in acute phase (0-5 minute), in the experimental group receiving different doses of grape seed oil ,sham and control groups. The figure is based on Mean ±SD .

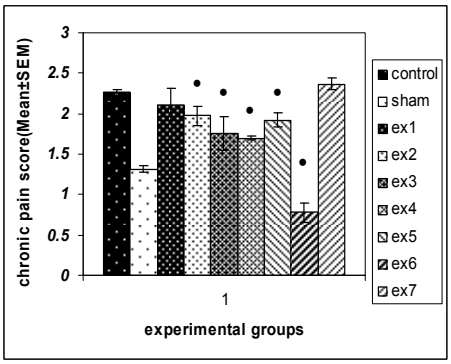

Fig2. Comparison of pain score means in chronic phase (16-60minute) in the experimental groups receiving different doses of grape seed oil with sham and control group. The figure is based on the Mean± SD .

4. Discussion

As the results show, using of grape seed oil alone and accompanied by morphine can decrease the pain score in the acute and chronic phases. Some studies have shown that substance P and Brady Kinin are involved in the appearance of first pain phase and histamine, serotonin and prostaglandin in the second pain phase. Drugs like opioids inhibit both phases (13), whereas drugs like aspirin only inhibit the latent phase(14,15). The studies by other researchers have shown that grape seed oil contain linoleic acid (omega 6), oleic acid (omega 9), palmitic acid, stearic acid, α-linoleic acid (omega 3), procyanidins, vitamins E and C, tannans, some of which effective as analgesiics.

Linoleic acid is one of the unsaturated fatty acids and by activation proxyzom proliferators activated receptor (PPARs) cause decrease in the amount of potassium transmission in peripheral nervous system and finally decrease pain in acute phase. There is much evidence supporting the increased level of two inhibitory compounds in central nervous system(Adenosine A1, GABA A, GABAB) after using linoleic acid (16). Studies also show that linoleic acid block voltage gated calcium channel L(VGCCL) in neurons of hippocampus and in doing so, can bring about analgesic effects (17). α-linoleic acid is one of the essential fatty acids and by increasing oxidative phosphorylation and changing glutamate balance:aminotransferase aspartat , play an important role in analgesic effects(18) . Other studies have shown that different protein kinases cause peripheral neuron's activation ,α-linoleics acid ,have a modulatory role on protein kinase dependent on cAMP , protein kinase C (19). Oleic acid, inhibit releasing calcium from intracellular resources. Studies have shown that IL-1B causes a change in releasing neurotransmitter in ecombense nucleus. Oleic acid protect the brain against inflammation cytokins injuries (TNF-α, IL-6, IL-1β) and inflammation eicosanoids (PGE2) (19). Palmitic acid (hexadecanoic acid) is the first product fatty acid during lipogenes. Palmitic acid by the geranulation in mast cells causes decrease in histamine releasing from mast cells(20). Palmitic acid also, causes a decrease in releasing NO and inhibit expression of cyclooxygenase-2 enzyme and consequently, cause analgesic effects(20). Studies have shown that procyanidins could be effective in the production and releasing NO, PGE2(21). Procyanidins causes a decrease in PGE2 production and in doing so causes analgesic effects (22,23). Studies have also shown that flavonoids by the opioids system and adrenergic system also, can be involved in pain regulation. Flavonoids are one of the Nitric oxide synthase inhibitions and prevent NO production. Some studies have shown that flavonoids by inhibition the activation of N-methyl-D-aspartate (NMDA) receptors cause a decrease in intracellular calcium and after that cause a decrease in the activity of nitric oxide synthase enzyme and phospholipaseA_2, with decrease in NO and prostaglandin analgesic effects appeared .In this experiment, pretreatment with naloxan, opioid receptor antagonist caused a decrease in antinociceptive effects of the grape seed oil, and grape seed oil by the activation of opioid receptors in pathway of pain causes analgesia effects. Considering the results of present study and studies by other researchers, it seems that we can relate the antinociceptive effects of grape seed oil to the essential fatty acids, procyanidins , flavonoids and vitamin E.

5. Conclusion

Also, the present results indicate that grape seed oil strengthens the atinociceptive effects of morphine. To consolidate the present findings, further studies into the analgesic effects of grape seed oil is warranted.

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