EFFECT OF DIFFERENT DOSES OF PUMPKIN SEED OIL AS AN ANTI-INFLAMMATORY AND ANALGESIC ON MICE

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ABSTRACT

To evaluate the role of anti-inflammatory and analgesicof Iraqi pumpkin seed oil. The oil seed (25-100 mg / kg) were investigated using various experimental models for analgesic and anti-inflammatory benefit. Acetic acid and thermal induced models of pain were used to examine the anti-nociceptive property. Though models of oedema induced by carragenin were used to evaluate anti-inflammation. Results were reported from these studies that the extract prepared from Iraqi pumpkin seeds possess potential anti-inflammatory and analgesic activity when compared with standard drug Diclofenac. Even though all the concentrations showed varying degree of inflammatory and analgesic activity, 100mg/kg bw showed better anti inflammatory and analgesic activity

Keywords: Extract, Inflammation, Paine,

تأثير جرعات مختلفة من زيت بذور اليقطين كمضاد للالتهابات ومسكن للالم على الفئران

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المستخلص

لتقييم النشاط المضاد للالتهابات والمسكن للالم لمستخلص بذور اليقطين العراقي. تم اختيار جرعات مختلفة من من الزيت المستخلص من البذور (25–100 ملغم / كغم) للتأثير المسكن والمضاد للالتهابات باستخدام نماذج تجريبية مختلفة. تم تقييم الخاصية المضادة للألم باستخدام حمض الأسيتيك ، واحداث الام بالحرارة. في حين تم استخدام نموذج وذمة كاراجينين المستحث لتقييم النشاط المضاد للالتهابات. اظهرت النتائج ان لزيت بذور اليقطين واعتمادا على الجرع قد قلل من الاحساس بالالم وكذلك التقليل من الالتهاب المستحدث. نستنتج من هذه الدراسة ان زيت بذور اليقطين ذا تأثيرات مسكنة ومضادة للالتهابات من خلال المكون الكيميائي النباتي للقرع.

كلمات مفتاحية: المستخلص, الالتهاب, الالم

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INTRODUCTION

Inflammation is a natural defense mechanism t hat defends the host from infection an other thr eats. This involves interactions between variou s cell varieties and chemical mediators to resto re physiological condition.(30).Such mediators included lipid- derived mediators, peptide mediators, enzymes and adherence molecules, depending upon the cell type and thus the source of the injurious stimulation (6, 16, 20). Nevertheless, once they are formed, they will cause harm to host tissues in associated degree of an unregulated fashion, they can cause damage to host tissues, leading to disease, Although it is a protection, the advanced events and mediators involved in inflammatory reactions stimulate, sustain and aggravate various diseases.(30).Furthermore, research on inflammatory diseases are continued and therefore the side effect of the anti-inflammatory drug treatment currently in use causes serious disadvantages throughout its clinical usage.(11).Therefore, it is important to develop new and additional effective anti-inflammatory drugs with lower-aspect effects. Inflammation is either chronic or acute. Medicines for anti-inflammatory medications refer to substances that reduce inflammation and pain. (12). Several bioactive molecules are being intensively investigated because of these risks. alternative bioactive molecules are being intensively investigated. fatty acids are highlighted as important effectors and regulators molecules in the immune-inflammatory response (15) most new pharmaceutical products derived from medicinal plants. (21). The pumpkin (*Cucurbita spp*.), One of the world's most popular vegetables, recently recognized as a functional food(30-18-16).Usually, pumpkin seeds from agro-industrial waste are a truly wealthy source of bioactive compounds with interesting nutraceutical properties (2,21). Numerous studies(17)have highlighted the health characteristics of pumpkin seed oil against various diseases in recent years, as well as obesity, diabetes and cancer. It also has antibacterial, antioxidant and anti-inflammatory effects. (27).The pumpkin seed oil is dark green in color that contains a high quantity of free fatty contain fatty acids (FAs); recognize namely oleic, linoleic, and palmitic acids. The oils were opulent in δ-tocopherol, β-sitosterol and syringic acid (25). A high poly unsaturated FA value and lower free FA content makes it extremely appropriate be used in edible functions (23). Pumpkin seeds contain remarkably high ratio of essential amino acids together with different components like K, Cr, Na, Mg, Zn, Cu, Mo and Seacids including four dominant fatty acids (oleic, linoleic, palmitic and stearic) (4). The aim of this study is to ivestigation the role effects of Pumpkin seeds oil as palliation and inflammation in mice.

MATERIALS AND METHODS

Plant materials

Iraqi pumpkin seeds (Cucurbitamoshata, L. Family (*Curcubitaceae*) were purchased from the local market Baghdad,Iraq. Preparation of plant materials Pumpkin seeds were dried in an air- sawing oven at 40 °C to get a little bit of dried powder. (1).

Animals

Swiss albino mice wrwr had (25-30gm) of both sex from obtined from the animal house of department of Physiology and Pharmacology's of Veterinary Medicine's.Seven days before the tests, they were kept in metal steel cages and acclimatized inside the laboratory. They had free access to water and fed with growers mash bought from the native market.

Preparation of pumpkin seeds oil

The dried powder of the seeds was placed careful in a soxhlet and extracted by utilizing petroleum ether (40–60 °C) to get the oil. The solvent was utterly removed by evaporation beneath reduced pressure at a temperature not override 40 °C.

Preparation of dosage form

Each oil emulsion was formed using gum acacia to easily manage the mice oral dose. The same gum acacia concentrationwas prepared for control groups of mice in water (the vehicle).

Acute toxicity study

The PSO's acute toxicity test was carried out according to Lorke'smethod (17). The range of lethal dose and safe dose for the PSO was listed here. For the method, twenty-four Swiss albino mice were famished with18h but permitted access to water. have been classified (three mice per groupplus

control) orally treated with PSO at different dose levels (200, 400, 800, 1600, 3200, 6400, and 12800 mg/kg).The animals were then watching for nervousness, dullness, in-coordination and or mortality for 24 h.

I- Evaluation of analgesic potential of the PSO

I.1. Thermally induced pain in mice

The impact of PSO on hot plate induced pain was achieved in adult mice. The hot plate was used to measure the response time depend on the method pointed out by (8) In these experiments, the hotplate was fixed at (45±2) ^{o}C, each animal was placed on the heated surface of a glass beaker with a diameter of 50 cm and the time (s) between placing the animal on the hot plate and starting to shake or lick the paws jumping was recorded as the index of response time. An automatic 30-sec cut-off was used to prevent tissue damage. The animals are divided into 5 groups of 6 mice each group at random and fasted for 24 h but, the access water was allowed. Group 1 administered the oral dose of the vehicle10 mL / kg to be negative control Groups 2, 3 and 4 were orally pretreated with 25, 75 and 100 mg / kg doses of PSO, respectively, while group 5 animals were administered 1,4 mg / kg of Diclofenac orally 30 min before test

I.2. Acetic acid induced writhing in mice

The abdominal constrictions performed by intr aperitoneal(i.p.) injection of 3% acetic acid consisting of the contraction of the abdominal muscles and thestretching of the hind limbs was performed depending on th e procedure described by Nwafor *et al* (21). The animals were divided into 5 groups of 6 mice for each group. Group 1 considered as negative control and injects 10 mL/kg of normal saline, but groups 2, 3 and 4 were pre-treated with 25, 75 and 100 mg/kg doses of PSO intraperitoneally, and group 5 gave 1.4 mg/kg of Diclofenac. After 30 min, 0.2 mL of 3% acetic acid was inject intraperitoneally (i.p.). The amount of wiggling movements was counted for thirty min. Antinociception (analgesia) was expressed because the lowering of the amount of abdominal constrictions between control animals and mice pretreated with PSO.

I. Evaluation of Anti-inflammatory activity

11.1 Carrageenin-induced mice hind paw oedema

Increased linear circumference of the mice hin d paw resulting from the

phlogistic agent's plantar injection was used to examine acute inflammation (18). Upon 24 h fasting adult albino mice were used and deprived of water only during experiment. Hind paw inflammation was accomplished by injecting 0.1 mL of freshly prepared carrageenin suspension into the hind paw's subplantarlayer in normal saline. The injected paw's linear circumference were assessed before and after phlogistic administration 0.5, 1, 2, 3, 4 and 5 h (18,20).Increase in mice hind paw circumference 0.5, 1, 2, 3, 4 and 5 hr after administration of carrageenin (21).as parameter of measuring inflammation. Different groups of mice received PSO (25,75 and 100 mg / kg).1h before inflammation events. Control mice gave carrageenin while reference group received Diclofenac(1.4 mg/kg). The average (mean) oedema was assessed by measuring with vernier calipers.

Statistical analysis

The data were expressed as Mean ±SEM. Statistical analysis was performed using one way ANOVA test The pumpkin seed oil is dark green in color

Acute toxicity

The results showed that its average lethal dose (DL50) is more than 5000 mg/kg It was occurred after 24 hours there was no mortality or general signs of toxicity did not produce death in the mice, this suggests that PSO is very low toxic.

Effect of PSO on thermally-induced pain in mice

Showed significant increas ($P<0.001$) in pain redaction time all treated group with different dose of PSO compared to control on (Table 1).but when compart with Diclofenac showed treated group significant decreased ($P<0.001$)

RESULT AND DISCUSSION

Table 1. Effect of the oilseed extract of pumpkinon on hot plate test (mean±SEM).

Treatment	Dose (mg/kg/d)	Reaction time (sec)	% inhibition
(Control)	1 mL/kg	3.25± 0.15	_
pumpkinSeed oil	25	4.13± 0.16***	127.07
pumpkin Seed oil	75	6.21± 0.04***	191.07
pumpkin Seed oil	100	9.75± 0.21***	300
Diclofenac	1.4	18.15± 0.16***	558.46

Level of significance ***P<0.001, **P<0.01, *P<0.05. Percentage reduction of pain

Effect of PSO on acetic acid-induced writhing in mice

The PSO (25-100 mg/kg). Dose-dependent reduction in acetic acid writhing in mice. The reductions are statistically significant (P<0.05) with respect to control and comparab le with the highest dose of the standard drug Diclofenac, (Figure 1).

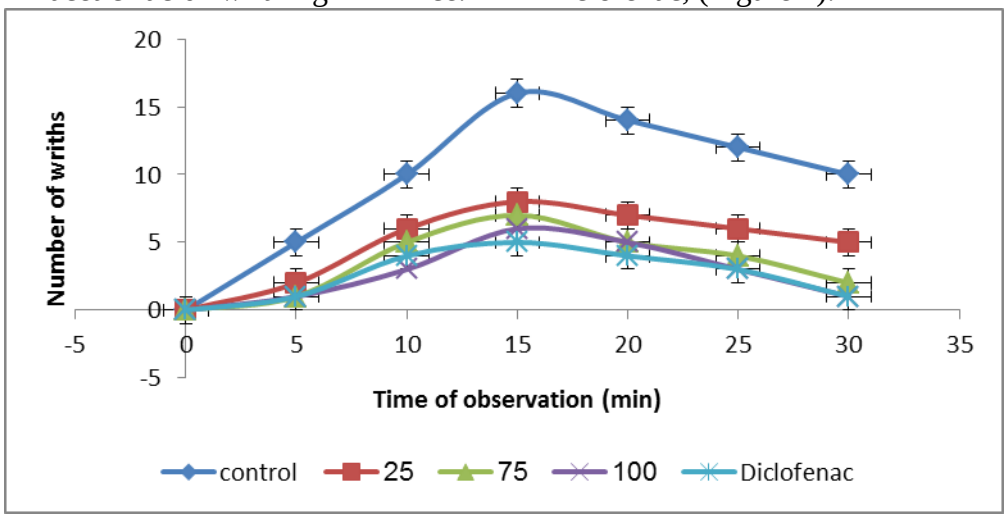

Figure 1. Effect of PSO of on acetic acid induced writhing in mice

Carragenin-induced oedema in mice

The effect of pumpkin seed oil extract on carragenin-induced oedema was shown in Table 2. The seed oil exerted a dose-dependent anti-inflammatory effect. This effect was significant (P<0.05) when compared with control and comparable to that of the standard drug Diclofenac (1.4 mg/kg).

Table 2. Effect of pumpkin seed oil extract on carrageenin-induced mice hind paw oedema (mean±SEM) (n=6).

Treatment (mg/kg)	Time intervals (h)						
	0	0.5	1	2	3	4	5
Control	0.24±0.01 D a	0.36±0.01 A a	0.36±0.01 A a	0.35±0.01 B a	0.34±0.01 B a	0.34±0.01 B a	0.33±0.01 C a
pumpkin Seed oil (25)	0.25±0.01 E a	0.36±0.01 A a	0.35±0.01 A b	0.33±0.01 B b	0.31±0.01 C b	0.28±0.01 D b	0.26±0.01 E b
pumpkin Seed oil (75)	0.26±0.01 D a	0.35±0.01 A a	0.34±0.01 A b	0.33±0.01 B b	0.30±0.01 C b	0.29±0.01 C b	0.26±0.01 D b
pumpkin Seed oil (100)	0.25±0.01 E a	0.34±0.01 A b	0.33±0.01 A c	0.30±0.01 B c	0.28±0.01 C c	0.23±0.01 D c	0.25±0.01 E b
Diclofenac (1.4)	0.26±0.01 D a	0.35±0.01 A a	0.34±0.01 A b	0.32±0.01 B b	0.31±0.01 B b	0.28±0.01 C b	0.26±0.01 D b

Different capital letters mean significant (p<0.05) results between different groups.

Different small letters mean significant (p<0.05) results between

Concentration within group

Pumpkin has been thought- about as helpful to health as result of it contains varied biologically active ingredient.In the present study, the analgesic and anti-inflammatory activity of Iraqi PSO, was studied in an inflammatory model in mice. PSO expected to be wealthy in bioactive ingredients which could has antioxidant, free radical scavenging capacity and anti-inflammatory effects. Throughout 24-h duration of experiment, no deaths occurred in any of the groups. These results indicate that median lethal dose (LD50) was specific to be mor than (5000) mg/kg and presented a large safety margin (29-34). The study additionally shows that the extract

significantly prolongd the reaction time of thermally-induced (hot plate) test. This method is selective for central analgesics and suggests the presence of narcotics (28) with opioid receptors. Often due to the presence of secondary metabolites such as beta-carotene, saponins, tannin, flavonoids, and terpenes which may have the antinocieptive activities carried out. By inhibiting the cyclooxygenase pathway, beta-caroteneand flavonoids are anti-inflammatory properties(24) The seed oil maight be prevent neurogenic and non-neurogenic pains as well narcotic pains may in portion explainthe mechanisms of its action and these effects are due to the existence of phytochemical components in the oil seed extract.The extract significantly reduced writhing caused by acetic acid as well as thermally induce pain.Acetic acid induces inflammatory pain by increasing capillary permeability and part from peritoneal fluid concentration of PGE2 and PGF2 (9) via local peritoneal receptors. This test alone is not specify whether central or peripheral activity involved (32) Thus. Hot plate method commonly achieved in as well as to the above to differentiate between peripheral and centeral pain. centrally acting medication inhibit each abdominal constriction test and thermally induce pain (28). Whereas peripheral drugs only prevent abdominal constriction (7). The phenolic content and also the high concentration of each PSO tocopherol and β-carotene that explain antioxidant and anti-inflammatory effects. Safe and secure (27) In the carragenin-induced oedema, the PSO (25-100 mg/kg) was observed to have exerted significant vital impact at the first stage of inflammation (1-2 h) indicating effect probablyon histamine, serotonin and kinnins that are involved in the early stage of carragenin induced oedema (13,3). The PSO may be inhibited inflammation at later stage to its ability to inhibit prostaglandin synthesis, which is known to mediate in flammation in the second phase of carragenin modele (10). In the present study, PSO induced a significant elevation of antioxidant levels in blood serum inhibitors and a marked inhibition of paw edema that could be attributed to its high content of the antioxidants previously mentioned. Nevertheless, Non steroidal anti-inflammatory model Diclofenic (1.4 mg / kg), a cyclooxygenase inhibitor whose action mechanism involves prostaglandin inhibition, significantly inhibited paw swellin (33). Pumpkin seed oil significantly inhibited adjuvant induced arthritis in rats, similar to a well-known anti-inflammatory substance called indomethacin (1). The beta-carotene in pumpkin seeds has anti-inflammatory properties and orderly consuming of pumpkin seeds can keep against joint inflammation (5).

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