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Evaluation of *Cucurbita pepo* L. seeds used in folk medicine for their anti-inflammatory and wound healing activity

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Abstract

Since *Cucurbita pepo* L. seeds have been reported to have beneficial effects in folk medicine for many years, in this study, in vivo wound healing activities of diethyl ether, chloroform and ethyl acetate extracts prepared from *C. pepo* seeds were evaluated by linear incision wound model, and hydroxyproline content. Furthermore, the extracts were screened for anti-inflammatory activity based on the inhibition of acetic acid-induced capillary permeability. The ethyl acetate extract had the highest inhibitory effect on acetic acid-induced capillary permeability, but the rest of the extracts didn't display significant anti-inflammatory activity. On the other hand, according to the activity using linear incision wound model as well as hydroxyproline content of the treated tissue, all three extracts did not indicate wound healing activity.

Keywords: *Cucurbita pepo* L, anti-inflammatory activity, wound healing activity

Introduction

Most medicines, currently, contain one or more active ingredients of herbal origin in the form of herbal extracts. One of them, *Cucurbita pepo* L. (Cucurbitaceae), has been traditionally used in many countries in the treatment of different diseases such as an anti-inflammatory, antiviral, analgesic, antiulcer, antidiabetic and antioxidant [1]. Seeds, the most preferred parts of *C. pepo* have been used to treat urinary and bladder problems, bronchitis, fever and hypertension, alleviate prostate diseases and used as an anthelmintic, diuretic, tonic agent [2-4]. In addition, fruits have been recorded as cooling, astringent to the bowels, laxative, good for teeth, sore throat and eye infections [4]. In particular, the seeds of the plant have become popular as a modern therapeutic agent in phytotherapy because of their beneficial effects against benign prostatic hyperplasia (BPH) [5]. It has been approved by German Pharmacopoeia, German Standard License Monographs, British Herbal Pharmacopoeia, and American Pharmacopoeia and has also been included in Commission E monographs for its uses in irritable bladder and prostate complaints [6].

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Therapeutically, *C. pepo* is also effective in different activities such as anti-hypercholesterolemic, anti-hypertensive, anti-parasitic, anti-tumoral, anti-carcinogenic and antibacterial [7, 8]. These activities of *C. pepo* might be due to the presence of certain phytoconstituents such as fatty acids; linoleic, oleic, stearic and palmitic acids, as well, secondary metabolites such as tocopherols, alkaloids, phenolic acids, flavonoids, carotenoids may be responsible for its medicinal properties [1, 9-12]. The aim of the study was to investigate the extracts of *C. pepo* for its in vivo wound healing and anti-inflammatory activities.

Materials and Methods

Plant Materials

The pumpkin seeds used in this study were collected from a farm in Kayseri (Turkey), The seeds were selected by hand to eliminate any damaged ones in any way. Diethyl ether (5.2g), chloroform (4.9g), and ethyl acetate (5.6g) extracts were prepared by successive extraction of the air-dried and powdered seeds of *C. pepo* (each 50g) with the corresponding solvents (150 mL x 3, each) using a rotary extractor without vacuum under 40˚C.

Biological activity experiments

Animals

Male Sprague–Dawley rats (160–180g) and Swiss albino mice (20–25g) were obtained from Kobay Experimental Animals Laboratory (Ankara, Turkey). The animals could acclimate into animal room conditions for 3 days, kept on standard pellet diet and water ad libitum. At least six rats were used in each group. This study was conducted in accordance with the suggested European ethical guidelines for the care of laboratory animals.

Preparation of test samples

A linear incision wound model was used to evaluate wound-healing activity. The test ointments prepared by mixing diethyl ether, chloroform and ethyl acetate extracts of *C. pepo* (each 1% in the ointments), separately, with a mixture of ointment base consisting of glycol stearate: propylene glycol and liquid paraffin (3:6:1) in a mortar thoroughly were topically applied as 5 mg of each onto the injured area on the dorsal part of test animals. Right after the formation of the wound, treatment was started by daily usage of the test ointments on the injured zone. The control group animals were topically treated with blank vehicle base containing glycol stearate: liquid paraffin: propylene glycol: (3:1:6) mixture, while the negative control group were not treated with any product. Madecassol® (0.5 g) was used as the standard drug.

For evaluation of anti-inflammatory activity, test samples suspended in a mixture of distilled water and 0.5% sodium carboxymethyl cellulose (CMC) were administrated orally to the test animals. The control group animals subjected to the same experimental treatment as those of the test groups excluding the drug treatment. Indomethacin (10 mg/kg) in 0.5% CMC was used as standard drug

Wound healing activity

Linear incision wound model

As previously described by Lodhi et al. 2006 [13], and Suguna et al. 2002 [14], a linear incision wound model was performed on the rats to determine the effectiveness of the test samples. The tensile strength of two 5 cm-length linear-paravertebral incisions made previously, and then treated by the samples were measured using a tensiometer Zwick/Roell Z0.5, Germany.

Hydroxyproline estimation

Tissues were dried in hot air oven at 60–70˚C until consistent weight was obtained. Afterward, the samples were then hydrolyzed with 6 N HCl for 4 h at 130˚C. The hydrolyzed samples were adjusted to pH 7 and subjected to chloramine T oxidation. The colored adduct formed with Ehrlich reagent at 60˚C was read at 557 nm. Standard hydroxyproline was also run and values reported as g/mg dry weight of tissue [15].

Histopathology

At the end of the experiment, skin samples were collected from each group. Samples were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 micrometer sections and stained with hematoxylin & eosin (HE). Tissues were examined by light microscope. Re-epithelization or ulceration of the epidermis; polymorphonuclear cells and neo-vascularization in dermis were analyzed for epidermal or dermal re-modeling

Anti-inflammatory activity

Acetic acid-induced increase in capillary permeability

The efficacy of the test samples on the increase of acetic acid-induced vascular permeability in mice was determined according to Whittle method [16] with some modifications [17]. Each test sample was orally administered to a group of 10 mice in 0.2 ml/20 g body weight. Thirty minutes after the administration, the tail of each animal was injected with 0.1 ml of 4% Evans blue in saline solution (i.v.) and allowed to wait for 10 min. Subsequently, 0.4 ml of 0.5% (v/v) AcOH was injected i.p. After 20 min. incubation, the mice were sacrificed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 ml volumetric flasks through glass wool. Each flask was made up to 10 ml with distilled water, 0.1 ml of 0.1 N NaOH solution was added to the flask, and the absorbance of the final solution was measured at 590 nm (Beckmann Dual Spectrometer; Beckman, Fullerton, CA, USA). A mixture of distilled water and 0.5% CMC were given orally to control animals, and they were treated in the same manner as described above.

Statistical analysis

The data on percentage anti-inflammatory and wound healing were statistically analyzed using one-way analysis of variance (ANOVA). The values of $p \leq 0.05$ were considered statistically significant. Since histopathological data were nonparametric, no statistical tests were performed.

Results

Wound healing and anti-inflammatory activities of diethyl ether, chloroform, and ethyl acetate extracts from *C. pepo* were tested in the current study. A linear incision wound model was used to assess the tensile strength of the treated tissues [13] Skin samples collected from animals were evaluated for hydroxyproline content. Collagen is an important extracellular matrix protein that provides strength and integrity to the tissue and plays an important role in wound healing process [18] and high levels of hydroxyproline in regenerated tissue suggest enhanced collagen synthesis [19, 20]. A linear incision wound model and estimation of hydroxyproline content were used together to evaluate the wound healing properties of tested extracts. As shown in Tables 1-3, three extracts did not indicate any wound healing activity on the treated tissue according to the assays applied. According to the results of histopathological analysis presented in Figure 1, the re-modeling was detected only in the reference group. None of the tested extracts, vehicle and negative control groups showed re-modeling or re-epithelization. These results are consistent with the wound healing assay results.

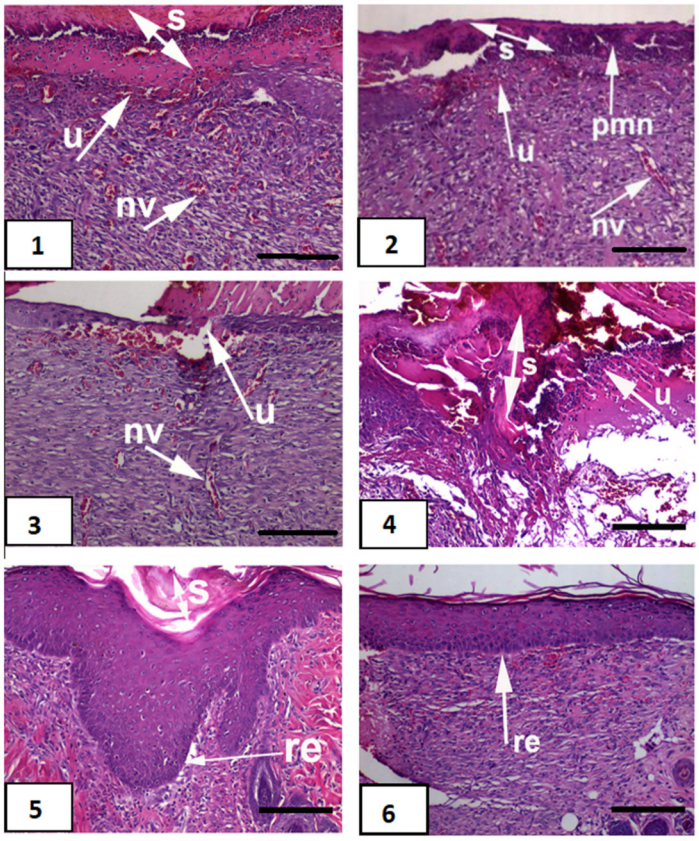

Figure 1. Histopathological view of wound healing and epidermal/dermal re-modeling in the vehicle, negative control, oils and reference ointment Madecassol® administered animals. Skin sections show the hematoxylin & eosin (HE) stained epidermis and dermis. The original magnification was x 100 and the scale bars represent 120 µm for figures. Data are representative of 6 animal per group. 1) Vehicle group, 2) Negative control group, 3) Diethyl ether extract 4) Chloroform extract group, 5) Ethyl acetate extract group, 6) Reference (Madecassol®) group. pmn: polymorphonuclear cells; nv: neovascularization; re: re-epithelization; s: scab; u: ulcus

Table 1. Effects of the extracts on the linear incision wound model

Material	Statistical mean ± .E.M.	Increase in tensile strength %
Vehicle	10.12 ± 2.01	2.5
Negative control	9.87 ± 1.84	-
Diethyl ether	9.54 ± 1.90	-
Chloroform	10.06 ± 1.53	-
Ethyl acetate	12.33 ± 1.02	17.9
Madecassol®	14.92 ± 0.97	47.4***

*** : p < 0.001; S.E.M.: Standard error of the mean

Table 2. Effects of the extracts on the hydroxyproline content

Material	Hydoxproline (µg/mg) ± S.E.M.
Vehicle	7.1 ± 1.04
Negative control	6.8 ± 0.98
Diethyl ether	9.2 ± 1.03
Chloroform	8.0± 2.36
Ethyl acetate	10.2 ± 1.05
Madecassol®	39.5 ± 0.94***

*** : p < 0.001; S.E.M.: Standard error of the mean

Table 3. Inhibitory effect of the extracts on acetic acid-induced capillary permeabilityl

Material	Dose (mg/kg)	Evans blue concentration (µ g/ml) ± SEM	Inhibition (%)
Control		9.32 ± 2.73	
Diethyl ether	100	8.85 ± 2.26	5.0
Chloroform	100	10.04 ± 1.98	-
Ethyl acetate	100	7.13 ± 1.81	**23.5***
Indomethacin	10	5.47 ± 0.93	**41.3*****

* : p<0.05, *** p<0.001; S.E.M.: Standard error of the mean

Discussion

Wound healing is comprised of several cellular and biochemical events of which inflammation is an important stage. Even though, inflammation is an immune system response to harmful stimuli [21], anti-inflammatory activity is important for recovery since prolonged inflammation causes severe healing disturbances and scarring [22]. In this study, anti-inflammatory activities of the extracts were tested by measuring acetic acid-induced capillary permeability while the ethyl acetate extract demonstrated the highest inhibitory effects on acetic acid-induced capillary permeability (23.5% inhibition), as shown in Table 2, the rest of the extracts displayed no significant anti-inflammatory activity.

Conclusion

Clinical data were collected with conventional pumpkin seed preparations, including pumpkin seed powders, for example, seeds milled into powder or pumpkin seed oil, or a whole-seed extract produced from the seeds by extraction with almost pure ethanol. This study is in contrast to the proprietary pumpkin seed oil investigated in the previous studies. Although researchers have focused on the pumpkin seed oil because of the composition of fatty acids, tocopherols and sterols, and their positive health effects, we investigated the effects of crude seeds because of traditional uses and consumption of pumpkin seeds in Anatolia. As well, the intended use of pumpkin seeds in pharmacopeia and monographs and their anti-inflammatory activity suggested these seeds as some valuable candidates to investigate wound healing properties; however, no significant effect of the three tested extracts was detected. According to the comparison of studies, while three tested extracts didn't show any wound healing activities in our study, the oil of pumpkin seeds displayed the promising effects on wound healing activities in a research managed by Barda et al. [23]. In this point, the pumpkin seed oil and cold press technique that the way to obtain the oil from *C. pepo*, as well as the chemical composition, are very important in terms of the activities, but in another study, to present the effects of the oil-free pumpkin seed extract, the single-arm pilot study was conducted by Leibbrand group [24]. From the data evaluated, it was determined that the oil-free pumpkin seed extract appears to be a suitable plant extract to support the provision of health treatment in collective suffering from BPH-related symptoms without the need for medical treatment. Therefore, the effects need to be documented for each form of *Cucurbita* seeds.

Conflict interests

The authors declare that there is no conflict of interest.

Financial Disclosure
This study received no financial support.

Ethical approval
The present study was performed according to the suggested European ethical guidelines for the care of laboratory animals.

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