

Therapeutic Effect of Pumpkin (*Cucurbita pepo* L.) on Post Burn Injury in White Mice

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ABSTRACT

This investigation aims to shed light on the healing capability of Pumpkin (*Cucurbita pepo* L.) fruit on burn wounds, to do so thirty albino mice were separated into 3 groups: group one treated with cold extract, group two treated with hot extract and group three treated with ointment as control. Each group involved 10 mice, all animals were burned in femur by using solid aluminum bar, and the burned area was (8-10) mm in diameter with second degree burn. Hours after burning process, all animals treated with treatment as mention above (Twice/day) and during treatment; animals were sacrificed and histological study was performed. Result displayed that burn remedial was better among all animals treated with cold *C. pepo* extract comparing with burn-wound animals treated with hot *C. pepo* extract and ointment, by forming a thin epidermal layer. These result indicated

the positive effect of pumpkin on wound healing.

In conclusion it is the first time the therapeutic indication of the healing capability of cold *C. pepo* extract on burn wound, this open the door for clinical application of *C. pepo* fruit.

Key words: *Cucurbita pepo*, extract, burn, therapeutic, histopathology

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INTRODUCTION

Several plants are used as natural healing remedies and widely used to treat burn-wounds; a lot of them have been described treatment skin complaints containing burn-wound (1). Also there are numerous factors such as the insufficient of drugs and the excessive fee of treatment in addition to undesired effect of chemical drugs all of this steered to an enlarged of plant extract as therapeutic products on burn-wounds. As well as recent studies demonstrated that *C. pepo* is a powerful antioxidant, anti-inflammatory and anti-bacterial properties (2-4). Have encourage us to focus on this plant.

C. pepo fruit (*Cucurbita pepo*) popularly known as "Kraa" is one of the broadly grown vegetables exceedingly rich in biological antioxidant, and vitamin. It packed in abundance with vitamins A, C, E, K, thiamine (B1), riboflavin (B2), piridoxine (B6), and polyphenolic flavonoid antioxidants such as lutein, xanthin, and carotenes. Pumpkin in common, feature orange or yellow external skin color; nevertheless, some varieties may show dark color to pale green color, brown color, white color, red color and gray color (5,6). The use of herbal remedies individually or in combination with traditional medicines was used for the treatment of various diseases in various medical treatises. *C. pepo* is one of the well-known edible plants and has significant medical properties. The antidiabetic, antioxidant, anti-carcinogenic, anti-inflammatory, immuno modulative, antibacterial and other medicinal properties have been well documented (7-9).

Burn is a kind of hurt to skin or further tissues produced by heat, cold, electricity, chemicals, friction or radiation and nearby three main forms of burns: first degree, second degree, and third degree. Every degree is based on harshness of injury to the skin with first-degree presence in the most minor while third degree presence in the most sever, second-degree burn are further severe because the damage extend elsewhere the top layer of skin. These type burns cause the skin to blister and become very red and sore (10).

In this contest the objective of this study was to found an investigational protocol for stimulation of deep second-

degree thermal lesion in white mice to evaluate the healing action and therapeutic effect of two kind of *C. pepo* extract (cold and hot).

SUBJECTS, MATERIALS AND METHODS

Animales

The experiment was conducted at Department of Biology, College of Education for Pure Sciences (Ibn-Al Haitham), University of Baghdad. Using albino male and female mice (30 mice at age 8-9 weeks), weighting 25±5 gm were separated into 3 groups: group 1 treated with cold extract, group 2 treated with hot extract, group 3 treated with ointment as control. All treated twice per day and kept in individuals cages of polypropylene and controlled condition temperature (25±2) C° receiving water and food.

Experimental Model and Thermal Burn

Thirty mice were pre-anesthetized by using (diethyl ether) 1 ml of diethyl ether were dropped on cotton put in glass container with cover then put animal in the same container after that thermal injuries were prepared with solid aluminum bar (8-10) mm in diameter electricity fiery, the temperature reached to 100 C° measured with thermometer. The bar is preserved in connection with the animal skin on the (Femur) for 15 seconds. The compression applied on the animal skin used in the burn induction then the animals were lofted for 24 hours to induction the lesion of burn injury.

Preparation of *C. pepo* extracts (Cold and Hot)

Whole *C. pepo* fruit peel and divided into two parts the first (500 mg) placed in blender contains 500 ml distilled water, and then filters the mash to obtain the extract. While the other part put on stainless steel container and placed on hot plate 100 C° for 15 minutes then the mash filtered to obtain the hot extract and both kept in -4C°.

Clinical Evaluation

The clinical progression of skin lesion by burns was estimated established on the following sign swelling, redness, bleeding, secretion, crust, blistering granulation tissue and scar. The wound retraction was estimated via a vernia each 3 days.

Histological analysis

When local anesthesia, a biopsy sample is detached, The foremost methods for eliminating our sample is excisional a biopsy (removed the entire site). The detached sample is fixed directly with 10% formaldehyde to evade secondary deterioration, After 24 hours, the sections were pounded in phosphate buffered saline (pH:7.4), dehydrated in graded ethanol series, cleared via xylene and embedded via paraffin wax. The 4-µm-thick sections taken from the paraffinized liver tissues were placed onto slides; sections were deparaffinized via xylene, rehydrated using a graded ethanol series and stained by hematoxylin-eosin (H and E).

Statistical analysis

Data are given as mean ± standard error (S.E.) and differences between means were evaluated by ANOVA. The analyses were performed using version 13 of the statistical program SPSS.

RESULTS AND DISCUSSION

As shown in table 1, burned area treated with cold *C. pepo* extract recovered faster than burns area treated with hot *C. pepo* extract and ointment and skin was completely cured on the 15 days of the burning as well as animals hair become growing. Also the table showed a significant positive effect (p<0.01) after three days from burning as compared to hot

C. pepo and ointment (5.00±0.31, 6.40±0.50 and 7.20±0.37mm respectively). This study clearly demonstrated the accelerating healing activity of cold *C. pepo* extract, in comparison with the treating by hot extract and ointment. The most arresting effect in presentation of cold *C. pepo* was its capability to stimulate reinstating of the normal skin construction subsequent in the lack of the scar tissue afterward healing of the second degree burns. A significant reduce in healing time was observed in cold extract treatment group compared with other groups, from the first day of burning to the day 15 after it. All burn wounds are characterized by the formation of blood clot. Thrombin, protease elaborate in blood coagulation, indications to acute inflammatory reactions that start the blood clotting sequence (11). As the coagulated dermal mass well dry, it turn to scab, formed by the damaged or necrotic tissues, the scab usually protect the wound surface from external irritants, beneath these scabs, granulated tissues formed and macrophage remove large amount of dead cells remnants, as well as neutrophils, and reduce the inflammation and prepare the tissue to healing process (12). These findings may be assign to the therapeutic ability of *C. pipo* (13,14,15). The scab is start to fall through the last week of our treatment with *C. pipo* compared with the other groups, while the granulation tissue was wholly formed the healing pathway happens through both immediate wound reduction and re-epithelialization. The contribution of healing process requires a harmonize interaction among the cells, development factors and intercellular proteins (16,17). The epithelial layer appearance was better when treated with *C. pipo* pads than other treated groups that showed a slower epithelialization and wound contraction (18)

Table 1: Illustrate stages of burn healing

Groups/Day	Type of Therapy (Twice / day)			P≤
	Ointment	Cold Extract	Hot Extract	
	Burn Diameter (mm) / (Mean ± S.E.)			
0	9.30±0.36	9.20±0.37	9.40±0.40	N.S.
7	7.20±0.37	5.00±0.31	6.40±0.50	0.01
14	6.80±0.35	3.80±0.36	5.20±0.20	0.001
21	5.00±0.44	2.20±0.37	4.20±0.37	0.001
28	3.60±0.40	0.60±0.18	2.00±0.31	0.001
35	1.90±0.33	H.	0.30±0.20	0.001
42	H.		H.	-

P values are significant at p< 0.05. H= Burn healing

Histopathology evaluation (Skin)

On day1, after hours from burning process, local anesthesia, a biopsy specimen is removed all the animals showed damage of the dermis of the burning wound, with epidermal detachment, scabies formation of necrotic tissue Residues, no hair follicles or sebaceous glands, and phagocytic cell infiltration (Figure 1). The wounds of all the groups had no edema or congestion. While on day 10 the group that daily twice treated with cold *C. pepo* extract showed epithelialization, fibroblast infiltration, and angiogenesis more than in the group treated with hot *C. pepo* extract (Figure 1). On day 15 all the daily treated

animals with cold *C. pepo* extract showed clearly developed epithelialization, angiogenesis, and hair follicles, more than the other groups. Extensive fibroblast proliferation was observed (Figure 3).

The histopathological findings displayed that the burn-healing was improved among all the animals that cured with cold *C. pipo* comparing with the burn-wound biopsies from mice cured with hot *C. pipo* and reference drug, by forming a thin epidermal layer. These results showed the positive possessions of *C. pipo* on wound healing, regarding to the antioxidant and antibacterial effects and besides to its anti-inflammatory activities

(19,20), and to the high aggregate of polyunsaturated fatty acids mostly oleic acid that showing a strong protagonist in the recruitment of inflammatory cells at the inflammation location and reduce the healing time (21, 22). Conclusion the effect of the current study shows, for the first time the

therapeutic confirmation of the healing capability of cold *C. pepo* pads on burn wounds, therefore these results opening the manner for prospect clinical presentations and support the traditional use of *C. pepo* fruit.

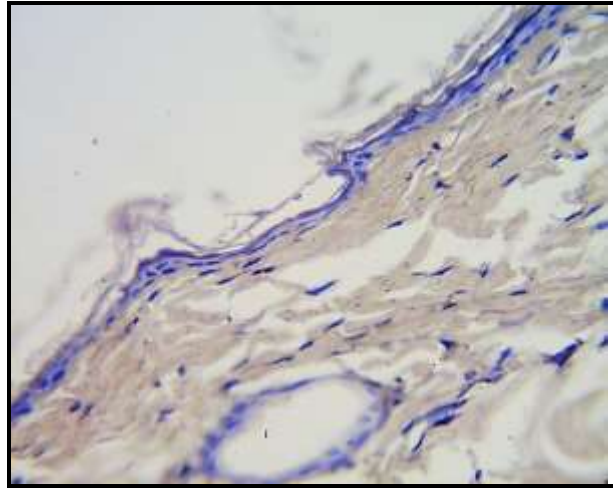


Figure 1: Epidermal detachment, scabies formation of necrotic tissue Residues (arrow) stained with H and E, $\times 40$ (After hours of the burning)

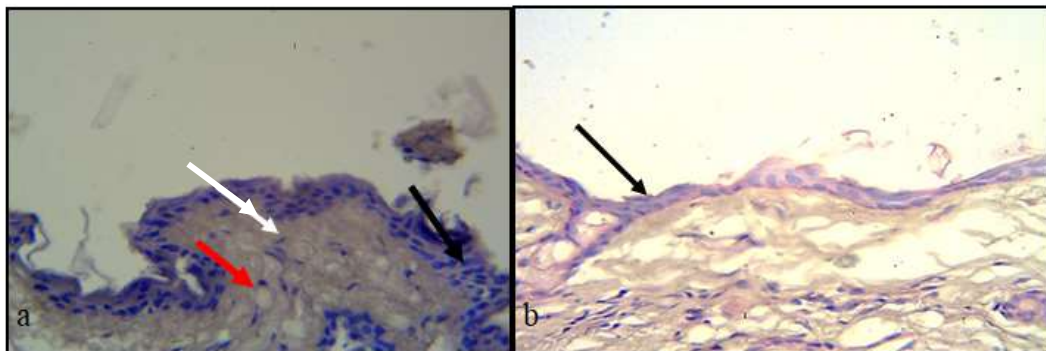


Figure 2 a and b: Histological observation of wound skin sections, cold *C. pepo* extract treated (a), hot *C. pepo* extract treated (b), showed epithelialization (Black arrow), fibroblast infiltration (White arrow), and angiogenesis (Red arrow). Stained with H and E, $\times 40$ (during treatment)

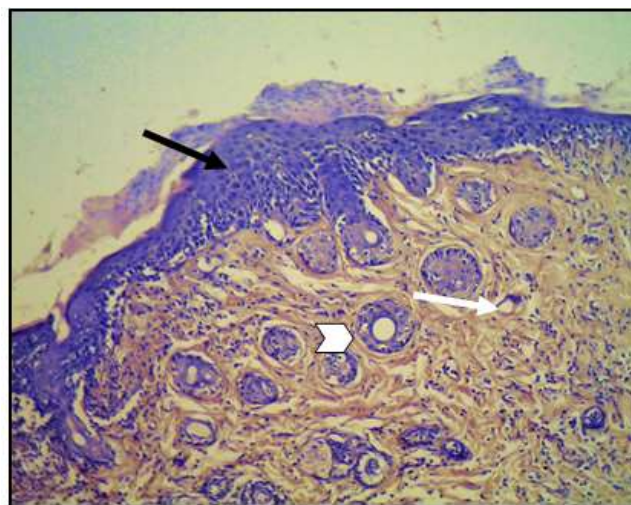


Figure 3: Histological observation of wound skin sections, treated with cold *C. pepo* extract, showing well developed epithelialization (Black arrow), angiogenesis (Red arrow), and hair follicles (Arrow head). Stained with H and E, $\times 10$ (Healing)



Figure 4: Stages of burned skin healing

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