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Natural Cumin Seeds for Wound Healing Activity in Albino Rats

[1]D.N. Patil, [2]A.R. Kulkarni, [1]A.A. Shahapurkar and [1]B.C. Hatappakki
[1]Department of Pharmacognosy and Phytochemistry,
KLES's College of Pharmacy, Gadag-582101, Karnataka, India
[2]Department of Pharmacology, KLES's College of Pharmacy,
Belgaum-10 Karnataka, India

Abstract: To screen the wound healing activity of extract and different fractions of cumin seeds on excision, incision, granuloma wound models in albino rats. The cumin seeds obtained from the plant known as Cuminum cyminum were subjected to solvent extraction with 90% ethanol, its successive fractionation by petroleum ether (40-60°) and ethyl acetate. Extract and fractions were screened for wound healing properties on excision, incision and granuloma wound models in albino rats. The exactly weighed quantity of (250 mg kg^{-1} b.wt.) alcoholic extract and its petroleum ether fraction showed better epithelisation ($p<0.001$) as compared to control in resutured incision and granuloma wound models. It is concluded that, alcoholic extract and its petroleum ether fraction of seeds of cumin showed promoted wound healing activity on excision, incision and granuloma wound models. However, ethyl acetate fraction failed to show significant wound healing activity.

Key words: Cumin seed, LD_{50}, excision wound, incision wound, granuloma studies

INTRODUCTION

Wound healing is a process that is fundamentally a connective tissue response initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other intracellular macromolecules, which are later remodeled to form a scar. The dried ripe seeds of *Cuminum cyminum* are commonly known as cumin seeds in English, Jeera in Hindi and Jeeragi in Kannada is a daily used spice in India. Cumin seeds belong to family Umbelliferae. The review of literature reveals that a cumin seed has a potent medicinal value. Volatile oil extract from cumin is known to possess 49 compounds there are 16 hydrocarbon and 32 oxygenated compounds. The main compounds are cuminal and safranal. The other 9 compounds are monoterpenes, sesquiterpenes, aromatic aldehydes and aromatic oxides etc (Hiruma-lima *et al*., 2002; Favier *et al*., 2005). It contains 2.5 to 4.5% volatile oil, 10% fixed oil and proteins. Volatile oil mainly consists of 30 to 50% cuminaldehyde, small quantities of alpha pinene, beta pinene, phellandrene, cuminic alcohol, hydrated cuminaldehyde, hydrocuminine and terpenes mainly monoterpenes and sesquiterpenes. The presence of sesquiterpenes in the plant material is known to possess a potent antiulcer activity (Yesilada *et al*., 2004). It is known to possess anticarcinogenic (Nalini *et al*., 2006), hepatoprotective (Kode *et al*., 2005), antidiabetic (Dhandapani, 2002), antibacterial (Iacobellis and Cantore, 2005), antiepileptic (Janahmadi *et al*., 2006) and antioxidant

Corresponding Author: D.N. Patil, Department of Pharmacognosy and Phytochemistry,
KLES's College of Pharmacy, Gadag-582101, Karnataka, India

(Satyanarayana *et al*., 2004) activities. One of the main constituents of the seeds of cumin if terpenoids. The free terpenoids present in tridax procumbens have been reported to pro-healing activity (Udupa and Kulkarni, 1991; Bairy, 2002). In view of these, has been designed the present work to study the possible effect of extract and different fractions of cumin seeds on wound healing activity. The Literature survey revealed that, no scientific investigation has been made regarding the wound healing activity of seeds of cumin. In the present investigation, an attempt was made to screen the seeds of cumin for wound healing activity.

MATERIALS AND METHODS

The shade dried seeds of cumin were collected from local area of Gadag district and was authenticated by Prof M.M. Mamadapur, J.T. Science College, Gadag. This research work was conducted in between December 2008 to August 2009.

Successive Solvent Extraction

The authenticated shade dried seeds were reduced to fine powder (partical size # 40) and were subjected to hot continuous extraction (Soxhlet) with 90% alcohol. After the complete extraction, extract was concentrated on rotary vacuum flash evaporator to obtain alcoholic extract. The concentrated alcoholic extract was subjected to fractionation using petroleum ether (40-60°) and ethyl acetate in succession. The fractions were again concentrated on rotary vacuum flash evaporator to obtain respective residues.

Acute Toxicity Study

Four groups, each consisting of 6 mice were used. Animals were fasted for 24 h with water *ad libitum* and then administered by oral route with alcoholic extract and its fractions (500 to 2500 mg kg^{-1}), suspended in Tween-80. Animals were observed for clinical signs and mortality continuously for the first 2 h and then frequently for 4th. The number of dead or survived after 24 h was recorded and calculated LD_{50} (Miller and Tainter, 1965).

Wound Healing Activity

Healthy albino rats of either sex, weighing between 150-200 g, were used for study. All the animals were grouped into 4 groups of 6 animals each. Animals were depilated at the desired site before wounding. They were housed individually with free access to food and water, the basal food intake and body weights to the nearest gram were noted. The animals were starved for 12 h prior to wounding under light ether anaesthesia.

Wound Models

Excision wound, icision wound and ganuloma studies were performed as follows.

Excision Wound

For the excision wound study (Ehrlich and Hunt, 1969), each group containing 6 animals were selected. The animals were starved for 12 h prior to the wounding. A circular wound of about 2-5 cm diameter was made on depilated dorsal thoracic region of rats under light ether anaesthesia in semi-aseptic condition and observed throughout the study. The animals were housed individually. The oral dose was given once a day to the rats. The percentage of wound closure was made on 4th, 8th, 12th, 16th and 18th post wounding day.

Incision Wound

Under light ether anaesthesia the animal was secured to operation table in its natural position. Two para vertebral straight incisions of 6 cm were made through the entire

thickness of the skin, on either side of the vertebral column with the help of a sharp blade (Ehrlich and Hunt, 1969), care was taken to see that the incisions were at least 1 cm apart, using 4-zero silk thread and straight round bodies needle wounds were then mopped with cotton swabs soaked in 70% alcohol. The animals were caged individually. Removal of the sutures was done on 8th post wounding day. The tensile strength was determined on 10th post wounding day by continuous, constant water flow technique (Lee, 1968).

Granuloma Studies (Dad Sace Wounds)

Physical changes in the granuloma tissue were studied in this model. Under light ether anaesthesia, subcutaneous dead space wounds were inflicted in the region of the axilla and groin, by making a pouch through a small nick in the skin, granuloma formation was induced by implanting grass pith. Cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in diameter were introduced in to the pouch similarly. Each animal received 2 grass piths in different locations. The wounds were sutured and mopped with an alcoholic swab. Animals were placed into their individual cages after recovery from anaesthesia. Granuloma surrounding the gross piths were excised and slit open. The tensile strength of piece measuring about 15 mm in length and 8 m in width (obtained by trimming the rectangular strip of granuloma tissue) was determined on 10th post wounding day by adopting continuous water flow technique of Lee.

Statistical Analysis

The data was calculated as Man±SE, evaluated by unpaired one-way ANOVA test. Values of $p<0.001$ were considered statistically significant (Morton and Malone, 1972).

RESULTS

When administered orally, alcoholic extract and different fractions were found to be practically non-toxic up to 2500 mg kg^{-1} b.wt. of the mice, therefore 1/10th of this dose 250 mg kg^{-1} b.wt. was taken as therapeutic dose. The result of excision wound model are shown on 18th day as control 81.12±0.92 sq mm, alcoholic extract 96.10±0.55 sq mm, petroleum ether 99.50±0.20 sq mm and ethyl acetate 82.50±0.35 sq mm. The results indicate that alcoholic extract and petroleum ether fraction have shown the complete epithelization on an average 19.52±0.17 and 19.48±0.15 days, respectively when compared to control 22.00±0.28 days. The results also indicated least scar for alcoholic extract 12.87±0.28 sq mm% and petroleum ether fraction 12.00±0.10 sq mm% (Table 1). The results of incision wound model are shown as animals treated with alcoholic extract 298.06±1076 g and its petroleum fraction 299.08±1.48 g showed significant increase in the tensile strength as compared to

Table 1: Mean percentage closure of excision wound area on the following post wounding days

	Percentage of wound contraction in sq mm±SE						
	4th	8th	12th	16th	18th	Period of	Mean size of
Groups	(day)					epithelization (days)	scar area (mm^2)
Control	16.50±1.65	41.40±1.50	62.71±1.31	76.02±1.27	81.12±0.92	22.00±0.28	16.90±0.13
Alcoholic extract	25.36±0.62*	69.32±0.17*	81.82±1.90*	93.60±0.80*	96.10±0.55*	19.52±0.17*	12.87±0.15*
Petroleum ether fraction	26.33±0.65*	76.53±3.10*	88.00±1.35*	98.70±0.75*	99.50±0.20*	19.48±0.15*	12.00±0.10*
Ethyl ether fraction	16.70±1.00	44.00±2.51	63.18±1.70	77.01±1.02	82.50±0.35	21.68±0.16	16.98±0.12

*Indicates significant activity at $p<0.001$ compared to control. All values are Mean±SE of sample size of 6, all treatments are given orally

Table 2: Mean tensile strength of resutured incision wound and granuloma studies

Groups	Tensile strength (g) of incision wound on 10th day	Tensile strength (g) of granulama studies
Control	154.30±8.95	160.22±2.82
Alcoholic extract	298.06±1.76*	256.26±1.69*
Petroleum ether fraction	299.08±1.48*	280.10±1.02*
Ethyl acetate fraction	160.64±1.35	167.18±1.21

*Indicates significant activity at $p<0.001$ compared to control. All values are Mean±SE of sample size of 6, all treatments are given orally

control group 154.30±8095 g. The results of granuloma wound model are also shown that, alcoholic extract 256.26±1.69 g and its petroleum fraction 280.10±1.02 g showed significant ($p<0.0010$) increased in breaking strength in grass pith granuloma model as compared to control group 160.22±2.82 g (Table 2).

DISCUSSION

Triterpenes are the main responsible constituent for wound healing (Aljancic *et al*., 1996; Oksuz *et al*., 1991). The course of searching an ethnopharmacologically active plant extract down to a single active principal may result in a defeat of biological activity for a number of reasons, for instance, a special compound might be unstable during extraction, fractionation or in the purified form, or, the fundamental basis for ethnopharmacology does not always exist in a single active compound but rather is a result of the interaction of more than one active compounds found in the extract (Savelev *et al*., 2003). Orally administered, alcoholic extract and different fractions were found to be practically non-toxic. The alcoholic extract and petroleum ether fraction exhibited significant ($p<0.001$) wound healing activity as compared to control. The contraction of excision wound was promoted from 8th day of treatment till 18th day. The alcoholic extract and petroleum ether fraction have shown the complete epithelization on 19th days and increase in the tensile strength as compared to control group in incision wound model. In the granuloma wound model significant increased in breaking strength as compared to control group but, ethyl acetate fraction failed to show the significant wound healing activity in all models. From the results obtained in study, it can be stated that the terpenoids in the extract and petroleum ether fraction were responsible for wound healing activity (Bairy and Rao, 2001).

CONCLUSION

The alcoholic extract and its petroleum ether fraction of seeds of cumin showed promoted wound healing activity on excision, incision and granuloma wound models. However, ethyl acetate fraction failed to show significant wound healing activity.

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