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WOUND HEALING ACTIVITY OF METHANOLIC EXTRACT OF CISSUS QUADRANGULARIS ON ALBINO RAT: A RESEARCH

***[1]A. Mohanty, [2]P.K. Sahu, [1]C.Das.**

***[1]Arnabaditya Mohanty**

Associate Professor, Department of Pharmacology, The Pharmaceutical College, Barpali-768 029, Orissa, India.

[2]Pratap kumar Sahu.

Professor, Department of Pharmacology, School of Pharmaceutical Sciences, SOA University. Bhubaneswar.

[1]Chandan Das.

Assistant Professor, Department of Pharmacognosy, The Pharmaceutical College, Barpali-768 029, Orissa, India

ABSTRACT: Aim: To evaluate the wound healing activity of *C.quadrangularis* on excision and incision wound models in albino rats.

Materials and methods: The different extracts of *C.quadrangularis* are obtained by successive soxhlet extraction with petroleum ether, chloroform and methanol were subjected to acute toxicity studies. The extracts were screened for wound healing properties in the excision and incision wound models in albino rats of either sex under light ether anaesthesia.

Results: All the three extracts showed significant increase in wound contraction and formation of scar in incision wound model. These extracts showed significant increase in the breaking strength of resutured incision wound as compared to control group($p<0.001$). Phytosterol, triterpenes, glycosides, phenols, flavonoids, saponins, tannins and coumarin have been reported to be present in *C.quadrangularis*.

Conclusion: The result of the present study indicate that methanol extract of *C.quadrangularis* has more significant wound healing property than the petroleum ether and chloroform extracts in excision and incision wound models.

KEY WORDS: *C.quadrangularis*, excision and incision wound models.

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Introduction

Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wound tissue in a special manner. Healing of wounds involves the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration normal condition of the injured skin or tissue [1]. The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient and must occur in a in a physiologic environment conducive to repair and regeneration [2]. Some medicinal plants have been employed in folk medicine for wound care.

Cissus quadrangularis Linn (Syn: *Vitis quadrangularis* Wall: Family: Vitaceae) is a fleshy, cactus-like, jointed climber, distributed throughout India, particularly in the hotter part; also cultivated in gardens. Stem slender dichotomously branched, sub-angular, glabrous, brown, fleshy, fibrous, with 4-winged internodes, aerial roots developing during the rainy season, leafless when old, 5-15 cm long; leaves cordate, broadly ovate or reniform, crenate-serrate, some times 3-7 lobed, glabrous, 2.5-7.5 cm x 3-9 cm; flowers small, greenish white, in short umbellate cymes; berries obovoid or globose, succulent, very acrid, peasized, 1-seeded. The herb is fed to cattle to induce flow of milk. The leaf-extract shows anti-fungal activity. The stem is useful in piles and its juice is beneficial as alterative in scurvy and irregular menstruation, and in diseases of the ear and in nose-bleeding. A paste of the stem is given asthma and may be useful for muscular pains, burns and wounds, bites of poisonous insects and for saddle-sores of horses and camels. The powder of dry shoots is given in digestive troubles. A decoction of the shoots with dry ginger and black pepper is given for body pains. The infusion of the plant is anthelmintic [3]. It contains 3-ketosteroid, a steroidal principle, has acetylocholine like action analoguous to muscarine and nicotine; in atropinised hearts, it shows cardiotonic activity, contains calcium oxalate, carotene, and ascorbic acid [4]. The present study was undertaken to find out the wound healing activity of *C.quadrangulris.*

Materials and Methods

Plant material : The whole plant of *Cissus quadrangularis* was collected in the locality of Barpali of Orissa, India, during the month of December, identified and authenticated by Prof.P.Jayaraman, PARC Chennai, voucher specimen No. PARC/2008/97.

Preparation of extract

The whole fresh plant were washed, air-dried under shade at room temperature and then crushed into coarse powder (sieve no.10/40). The dried powder(250gm) was extracted successively with petroleum ether(60-80˚C), chloroform, and methanol by using a Soxhlet apparatus for 16-18 hrs at temperature 37°C.The solvent was removed under pressure and the extract were concentrated under vacuum at 40-60°C and weight of the dried mass was recorded. (Yield- pet.ether-3.07%, chloroform-4.6%, and methanol-6.45%) [5]. The dried extract was incorporated in simple ointment base (5% w/w) and used for excision wound model externally. 2% Gum acacia was used as a vehicle to suspend the extract for incision wound model internally.

Animals

Healthy adult Wister strain of albino rats weighing approximately 180 to 250 gms were procured from an in-house animal facility Ghosh Enterprise, Kolkota. The animals were maintained under controlled conditions of temperature (23±2°C), humidity (50±75%), with a 10-14hr light dark cycle. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. All animals were allowed free access to distilled water and fed on a commercial diet (Lipton India Limited, Banglore). All the studies conducted were approved by the Institutional Animal Ethical Committee (1171/c/08/CPCSEA), School of Pharmaceutical Sciences, Bhubaneswar, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Drugs and Chemicals

All the chemicals used were of analytical grade obtained from standard suppliers of Loba Chemie Pvt, Ltd Mumbai.

Phytochemical analysis

Preliminary phytochemical tests of different extracts were performed by using specific reagents through standard procedures [5].

Toxicity studies

An acute toxicity study was carried out for determination of LD_{50} for different extracts, in Wister strain of albino rats weighing 180 to 250g.The animals were fasted over night prior to acute experimental procedure.

Wound models

Excision wound

For the excision wound study, animals were divided into 4 groups of six rats in each group. Group-I served as control and Group-II, Group-III, Group-IV, were treated with petroleum ether, chloroform, and methanol extracts respectively.

An impression was made on the dorsal thoracic central region 5mm away from the ears, by using a round seal of 2.5 cm diameter as described by Morton and Malone [6]. The skin of the impressed area was excised to the full thickness to obtained area of about 500 mm^2 under light ether anaesthesia in aseptic condition. The animals were housed individually.

The petroleum ether, chloroform and methanol extracts in simple ointment base (5% w/w) were applied on the wound once a day for 18days starting from the day of wounding. The percentage wound closure was observed on 4^{th}, 8^{th}, 12th, 16th, 18^{th} post wounding day. Epithelization time (in days) and size of the scar area was noted.

Incision wound

Incision wound model was performed according to Ehrlich and Hunt [7]. The animals were divided into 5 groups of six rats in each group, and kept in separate cage. Group-I served as control, received only 2% gum acacia suspension (1 ml/kg,p.o) and petroleum ether, chloroform, and methanol extracts (250 mg/kg) were given orally once a day to group-II,III and IV respectively for 10 days.

Under light ether anesthesia, the animals were secured to operation table in its natural position. Two paravertebral straight incisions of 6 cm each were made through the entire thickness of the skin, on either side of the vertebral column with help of sharp blade. Removal of the sutures was done on 8^{th} post wounding day. Tensile strength was determined on both wounds by continuous constant water flow technique of Lee [8].

Statistical analysis

The results are reported as mean±SE. Statistical analysis was done using ANOVA (Tukey's-Krammer Multiple Comparison Test).When probability (p) was less than 0.05 the difference were considered as significant.

Results

Phytochemical analysis

The preliminary phytochemical studies of different extracts showed the presence of alkaloids, carbohydrates, glycosides, saponins, sterols, phenolic compounds and flavonoids. The acute toxicity studies of petroleum ether, chloroform and methanol of *C.quadrangle* were found to be non-lethal up to a dose of 2500mg/kg body weight of the animals. Hence 1/10 of the highest safer dose, i.e. 250mg/kg body weight was selected for evaluation of wound healing. In the study using wound healing model, animals treated with petroleum ether, chloroform, and methanol extracts of *C.quadrangle* showed significant decrease in epithelization period as evidenced by shorter period for fall of eschar as compared to control group($p<0.001$). The extracts also facilitated the increase in rate of wound contraction significantly on 12^{th} day ($p<0.001$) than the control group as shown in the Table-1.The result of present study reveals that extracts of *C.quadrangularis* possess a prominent prohealing activity in incision wound model. This was demonstrated by significant increase in the skin tensile strength in petroleum ether, chloroform and methanol extract treated groups($p<0.001$) on 10^{th} post wounding day are presented in Table-2.

Table-1. Effect of extracts of *C. quardarangularis* on the excision wound parameters.

Sl. No	Group	% wound contraction					period of Epithelization (days)	Mean size of scar area (in mm^2)
		4^{th} day	8^{th} day	12^{th} day	16^{th} day	18^{th} day		
1.	Control	40.53± 2.59	58.74± 3.56	89.75± 1.15	91.53± 0.64	94.55± 0.79	23.88± 0.57	58.93± 2.27
2.	Petroleum ether	62.55± 1.56**	72.44± 2.11**	93.21± 0.89***	98.45± 0.47***	99.30± 0.53***	19.25± 0.35***	37.87± 2.84***
3.	Chloroform	59.55± 1.57	68.45± 1.53*	93.24± 0.84**	98.47± 0.22***	98.47± 0.55***	19.22± 0.75***	44.21± 3.26**
4.	Methanol	67.33±	74.21±	95.47±	97.21±	97.55±	17.38±	33.75±

2.45**	2.87***	0.97***	0.68***	0.35***	0.47***	2.89***

Values are in mean ± SE (n = 6) *p<0.05, **p<0.01, ***p<0.001vs Control.

Table-2. Effect of the extracts of *C.quadrangularis* on the breaking strength in incision wounds.

S. No.	Group	Breaking strength (g)
1.	Control	288.19 ± 21.55
2.	Petroleum Ether	421.53 ± 12.54
3.	Chloroform	392.84 ± 13.54
4.	Methanol	447.87 ± 19.45*

Values are mean ± SE (n = 6) * p<0.001 vs Control

Discussion

In the present study, wound healing activity of *C.quadrangularis* was studied and the results of the present study suggest that local application and systemic administration of methanol extract of the plant has shown more significant wound healing activity in excision and incision wound models as compared to petroleum ether and chloroform extract respectively and support the popular use of plant to open wound in folk medicine.

The complex process of healing involves various phenomena like wound contraction, granuloma formation etc. The contribution for healing by these events depends upon the type of wound. Wound contraction plays a significant role in healing of excision wound, while granuloma formation contributes in healing of resutured incision wounds. The plant contain protein, fat and wax, fibre, carbohydrates, mucilage, pectin and ash. The ash contain mostly carbonates and to a smaller extent phosphates of sodium, potassium, magnesium and calcium. Presence of potassium tartarate is also reported. The plant is rich in vitamin C [9]. Some lipid constituents have been isolated from *C.quadrangularis*. The lipid constituents are 4-hydroxy-2-methyl-tricos-2-ene-22-one, 9-methyl-octade-9-ene, heptadecyl octadecanoate, icosanyl

icosanoate, 31-methyl-tritriacontan-1-ol, 7-hydroxy-20-oxo-docosa-nyl cyclohexane and 31-methyl tritriacontacontanoic acid [10]. Two new unsymmetric tetracyclic triterpenoids onocer-7-ene-3ά, 21β-diol and onocer-7-ene-3 β, 21ά-diol together with sitosterol, δ-amyrin and δ-amyron have been isolated from *C. quadrangularis* [11]. Two new iridoids 6-0-(2,3-dimethoxy)-trans-cinnamoyl catalpol(1) and 6-0-meta-methoxy-benzoyl catalpol(2) along with a known iridoid picroside 1 (3), two stilbenes quadrangularin A (4) and pallidol(5), quercitin(6), quercitrin (7), beta-sitosterol(8) and beta-sitosterol glycoside (9) were isolated from *C.quadrangularis*[12]. Quercitin, a biflavonoid has been reported to posses antioxidant and anti-inflamatory activities and it has also found to inhibit membrane lipid peroxidation. The inflammatory phase of wound healing is considered a necessity for successful healing. The ability of the skin to regenerate and heal wounds in a scar less manner in the absence of inflammation have been shown, in fetal healing studies. Wilgus TA etal have shown that cox-2 inhibitor celecoxib markedly reduced the inflammatory phase wound healing with reduction in scar tissue formation without disrupting re-epithelization or decrease in tensile strength[13]. Quercetin present in the plant C.quadrangularis with its anti-inflammatory properties mighty has helped the wound to heal with minimum scarring. Quercetin synthesized by many plants facilitates wound healing by limiting inflammation and tissue degradation, improving local circulation and also help in formation of strong collagen matrix. Apart from stimulatory effects on vascular smooth muscle, quercetin is known to have stimulatory action on myofibroblasts, to contract at the wound edge, thus promoting healing [14]. Vitamin C is needed to make collagen (connective tissue) that strengthens skin muscles and blood vessels and to insure proper wound healing. Sever injury appears to increase vit C requirements and its deficiency causes delayed healing. Ascorbic acid is required for the synthesis of collagen as it is a known stimulator of collagen production [15] and vit C is essential in the conversion of proline to hydroxyproline. The wound healing property of *Cissus quadrangularis* has been attributed to its antimicrobial effects [16].

The presence of phytoconstituents like phytosterol, triterpene, glycosides, phenols, flavonoids, saponins, tannins and coumarin either individually or combined together may exhibit the synergistic effect towards healing of wounds. However, further investigation employing isolation of constituents and screening models are needed for further confirmation of wound healing potential of *C.quadrangularis.*

Conclusions

The result of the present study indicate that methanol extract of *C.quadrangularis* has more significant wound healing property than the petroleum ether and chloroform extracts in excision and incision wound models. The presence of phytosterols, triterpenes, glycosides, phenols, flavonoids, saponins, tannins, coumarin,ascorbic acid and quercetin in the plant may be responsible for wound healing activity.

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