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Wound healing activity of the galls of *Quercus infectoria* olivier

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Abstract

Objective: To screen the wound healing activity of different extracts of galls of *Quercus infectoria* on incision, excision and dead space (granulation) wound models in albino rats. Materials and method: Crude aqueous extract of *Quercus infectoria* galls and its three different crude fractions, petroleum ether (40º-60ºC), solvent ether, ethyl acetate were tested for various preliminary phytoconstituents and were screened at dose of 100mg/kg for wound healing properties in incision, excision and dead space (granulation) wound models in albino rats. Results: Tannins, flavonoids, steroids and carbohydrates were found to be present in aqueous extract and its various fractions of galls of *Quercus infectoria.* In resutured incision wound models aqueous extract and its fractions showed significant ($P<0.01$) breaking strength compared to control. In excision wound parameters the aqueous extract promotes better wound healing (96.36%) compared to control and organic fractions. Breaking strength of the grass pith induced granuloma studies the results show significant ($P<0.01$) activity in case of aqueous extract along with solvent ether fraction. Conclusion: From the results obtained, it can be observed that aqueous extract and its fractions of galls of *Quercus infectoria* have significant wound healing property. Also it can be concluded that flavonoids may be responsible for wound healing activity.

Key words: *Quercus infectoria,* wound-healing, aqueous extract, soxhlet extraction.

1. Introduction

Galls are pathological outgrowths formed on the twigs of the Dyer's oak, *Quercus infectoria* (Oiliver) Fam. Fagaceae, which arise as a result of deposition of an egg by a small Hymenopterus insect, gall wasp, *Adleria galloetinctorioe* Olivier. Fam: Cynipidae [1]. In the traditional systems of medicine, the gallnut is used in ointments for the treatment

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of piles and also as suppositories [2]. It is used for long-standing gonorrhea, profuse menstruation, for burns, dermatitis eczema, and wounds [3]. The galls have also shown to possess antibacterial activity [4].

One of the main constituents of the galls of the *Quercus infectoria* is flavonoids [5]. The free flavonoids present in *Tridax procumbens* have been reported to have pro-healing activity [6,7,10]. In view of these has been designed the present work to study the possible effects of the different extracts of galls of *Quercus infectoria* on the wound healing process. Petite pharmacological work has been done on galls of *Quercus infectoria* inspite its wide use in folk medicine.

Present study attempt to investigate comprehensive phyto-pharmacological aspects of the drug on the wound healing in albino rats.

2. Materials and methods

The galls of *Quercus infectoria* were procured from a local Ayurvedic drug store, M/s. Pragati Pharma, and were authenticated at the Dept. of Botany, R. L. Science Institute, Belgaum.

2.1 Extraction and Fractionation

Powdered galls were exposed to a moist and warm atmosphere for 4-5 days and 1kg of powdered galls were then subjected to extraction with a mixture of ether and smaller proportion of alcohol and water in a soxhlet extractor.

The extract separates into two layers with upper ethereal layer contains resins, colouring matter and other extractives were separated. The lower aqueous layer was evaporated under reduced pressure and the residue was dried. The aqueous extract was dispersed in distilled water and further extracted with petroleum ether (40º-60ºC), solvent ether and ethyl acetate.

Each fraction was washed with water, then dried over anhydrous sodium sulphite and concentrated to a small volume and then evaporated to dryness. The aqueous extract and its fractions were subjected to qualitative chemical investigation and were taken for pharmacological studies.

2.2 Wound healing studies

Albino rats of either sex weighing 150-200 g were selected, and divided into five groups of six 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 anesthesia, sterilizing the area with ethanol performed wounding. The first group served as control and given the vehicle (gum acacia 2%) orally. Second, third, fourth and fifth groups received the aqueous extract, petroleum ether (40º-60ºC), solvent ether and ethyl acetate fractions respectively by oral route at a dose of 100mg/kg. The suspensions of desired concentrations were prepared in gum acacia (2%) solution.

2.3 Wound Models

2.3.1 Resutured Incision

The method of Ehrlich and Hunt [8] was adopted. Under light-ether anaesthesia, two paravertebral 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. The incisions were sutured using 4-0 silk threads with the help of straight round-bodied needle.

On eighth post wounding day, sutures were removed and the breaking strength was

Table 1.
Effect of the extract of *Quercus infectoria* on the breaking strength of Incision wounds.

Group	Dose (mg/kg oral.)	Breaking Strength (g)
Control	1 ml Gum acacia 2%	110.94 ± 2.86
Petroleum Ether	100	175.52* ± 4.01
Solvent Ether	100	255.55* ± 3.30
Ethyl Acetate	100	201.55* ± 2.74
Aqueous Extract	100	282.44* ± 3.25

All values are in mean ± SEM; * P < 0.01 *vs* control.; n=6.

determined on 10th post wounding day by continuous constant water flow technique of Lee [9].

2.3.2 Excision Wound

For the excision wound study, groups containing six animals were selected. A circular wound of about 2.5 cm diameter was made on depilated ethanol sterilized dorsal thoracic region of rats under light ether anaesthesia and observed throughout the study. To the animals were housed individually, the oral dose was given once a day.

The observations of percentage wound closure were made on 4th, 8th, 12th, and 18th post wounding days and also for epithelization and size of scar.

2.3.3 Dead Space Wounds (Granulation tissue)

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. Cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in diameter were introduced into the pouch to harvest granulation tissue.

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. Excision of the granulomas from the surrounding tissue was performed on the 10th post-wounding day under light-ether anaesthesia.

Granulation tissues surrounding the grass piths were excised and slit open. The breaking strength of piece measuring about 15 mm in length and 8 mm in width (obtained by trimming the rectangular strip of granuloma tissue) was determined on 10th post-wounding day by a continuous constant water flow technique of Lee [9].

2.4 Statistical analysis

All the results were analyzed by student's *t* - test and the level of significance was set at $P<0.05$.

3. Results and discussion

The average percentage yield of aqueous extract of leaves of *Quercus infectoria* was found to be 24% w/w and petroleum ether (40º-60ºC) fraction, solvent ether fraction and ethyl acetate fraction were 9.5%, 2.4% and 4.2% w/w respectively. Tannins, flavonoids, steroids and carbohydrates were found to be present in aqueous extract and its various fractions as observed by the qualitative tests.

Table 2.
Effect of extracts of *Quercus infectoria* on the excision wound parameters.

Group	Dose mg/kg (oral)	4th Day	8th Day	12th Day	18th Day	Period of Epithelization (Days)	Mean size of scar area mm²
		Wound contraction (mm²) on day					
Control	1 ml gum acacia2%	15.61 ± 1.75	38.32 ± 1.75	55.66 ± 0.79	74.98 ± 1.67	22.50 ± 0.42	16.85 ± 0.28
Petroleum Ether	100	15.70 ± 1.09	48.30 ± 1.65	63.21* ± 1.71	91.29* ± 0.68	20.08* ± 0.47	11.65* ± 1.33
Solvent Ether	100	35.17 ± 2.19	55.09 ± 0.85	71.77* ± 0.73	93.31* ± 0.33	18.61* ± 0.47	11.98* ± 1.53
Ethyl Acetate	100	20.42 ± 0.75	35.63 ± 0.71	44.28 ± 1.31	62.26 ± 0.83	21.33 ± 0.44	13.56 ± 0.70
Aqueous	100	38.37 ± 2.91	63.82* ± 1.72	68.25* ± 1.10	96.36* ± 0.40	17.83* ± 0.40	11.60* ± 0.28

All values are in mean ± SEM; * P < 0.01 *vs* control ; n=6.

In resutured incision wound models aqueous extract and its fraction showed increased mean Breaking strength compared to control. The maximum activity was seen with the aqueous extract was 282.44 ± 3.25 g, solvent ether fraction 255.55 ± 3.30 g, ethyl acetate fraction 201.55 ± 2.74 g and petroleum ether 175.52 ± 4.00 g, which were highly significant (Table-1). The excision wound heals by contraction and epithelization.

The study of parameters includes wound closure, time of epithelization (days) and size of scar (mm^2). The percentage wound closure for different fractions were made at the different intervals of days of post wounding. The results specify that the aqueous extract promote better wound healing (96.36%) compared to control and organic fractions.

The results also indicate that aqueous extract, solvent ether, petroleum ether and ethyl acetate fractions have shown the complete epithelization on an average 17.83, 18.61, 20.08 and 21.33 days respectively when compared to control (22.50 days).

This very well signifies better wound healing activity with aqueous extract. The results also indicated least scar for aqueous extract ($11.60mm^2$) followed by other organic fractions, when compared with control ($16.85mm^2$) (Table-2).

Similar results were obtained for breaking strength of the grass pith induced granuloma studies (Table-3). The results show significant activity in case of aqueous extract along with solvent ether fraction.

The present investigation reveals that aqueous extract of galls of *Quercus infectoria* has shown significant pharmacological activity towards wound healing in albino rats. This plant finds mention in Ayurvedic literature and has been used in folk medicine. There have been scanty reports of study of pharmacological activity of this plant.

From the results obtained in the study, it can be stated that the flavonoids in aqueous extract, were responsible for wound healing activity [6,7,10]. Other organic solvent fractions have been also

Table 3.
Mean granulation tissue Breaking strength (g) of dead space wound on 10^{th} Post Wounding day.

Group	Dose (mg/kg) oral	Breaking Strength (g)
Control	1 ml. of Gum Acacia 2%	113.33 ± 2.43
Petroleum ether	100	210.13 ± 1.43
Solvent Ether	100	249.08* ± 5.79
Ethyl Acetate	100	189.81 ± 3.10
Aqueous extract	100	279.25* ± 2.46

All values are in mean ± SEM; * P < 0.01 *vs* control.; n=6.

shown to possess wound-healing activity as indicated in the tables but to lesser extent.

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