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Wound healing activity of the leaves of *Thespesia populnea*

H. Shivakumar[1]*, T. Prakash[1], R. Nagendra Rao[1], B. H. M. Jayakumar Swamy[1], A. N. Nagappa[2]

1. P. G. Department of Pharmacology, S. C. S. College of Pharmacy, Harapanahalli - 583 131, Karnataka, India.

2. Pharmacy Group B. I. T. S., Pilani - 333 031, Rajasthana, India.

Abstract

Objective: To screen the wound healing activity of petroleum ether, alcohol and aqueous extracts of leaves of *Thespesia populnea* on excision and incision wound models in albino rats. Materials and methods: The different extracts of the leaves of *Thespesia populnea* are obtained by successive soxhlet extraction with petroleum ether, alcohol and aqueous 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 anesthesia. Result: All the three extracts showed significant increase in wound contraction and formation of scar in excision wound model. These extracts showed significant increase in the breaking strength of resutured incision wound as compared to control group ($P<0.001$). Glycosides, flavonoids, alkaloids, and phytosterols have been reported to be present in leaves of *T. populnea*. Conclusion: The results of the present study indicate that aqueous extract of *T. populnea* leaves has more significant wound healing property than the petroleum ether and alcoholic extracts in excision and incision wound models. The presence of flavonoids, alkaloids and phytosterols in the leaves of the plant may be responsible for wound healing activity.

Key words: *Thespesia populnea*, incision and excision wound models.

1. Introduction

Thespesia populnea (L) Soland Ex. Corr (Malvaceae) is locally known as Bagari mara or Arasi in Kannada and Indian Tulip tree in English. It is a common tree found from West Bengal to South India. *T. populnea* is used in folk medicine as an external application made from fruits, flowers and leaves for the treatment of various skin ailments like scabies, psoriasis, wound healing and decoction prepared from bark is employed in ulcer healing [1] and it is one of the ingredient in the marketed Ayurvedic proprietary medicine 'Kamilari' a hepatoprotective agent [2]. The medicinal value of this plant is described in ancient texts of Ayurveda such as Dravyaguna.

The aqueous extract of *T. populnea* fruits has reported to possess significant wound healing activity [3]. The main constituents of the leaves of *T. populnea* are glycosides, flavonoids, alkaloids, phytosterols, quercetin, rutin and lupeal

* Corresponding author
Email : shivkumarhugar@yahoo.com

[4,5]. In the present study the possible effect of different extracts of the leaves of *T. populnea* on healing of excision and resutured incision wounds in albino rats was examined.

2. Materials and methods.

2.1 Plant material

Fresh leaves of *T. populnea* were collected from local areas of Harapanahalli in September and shade-dried at room temperature for one week. The plant was certified as *T. populnea* by Prof. K. Prabhu, Dept. of Pharmacognosy and voucher herbarium specimen (SCSCP/PG/02/ 1997) was preserved in the department.

2.2 Preparation of extract

The dried leaves (250g) were powered and passed through sieve No. 22. The powder was extracted with 500ml of petroleum ether (40-60°C), alcohol (70%) and distilled water in a soxhlet extractor successively. The extracts were dried under vacuum and weight of the dried mass was recorded. (Yield-pet.ether-3.07%, ethanol-6.02% and water-6.45%.) 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.

2.3 Toxicity studies

An acute toxicity study was carried out for determination of LD_{50} for different extracts, in albino mice of either sex weighing 20-25g. The animals were fasted over night prior to acute experimental procedure [6].

2.4 Wound healing studies

Albino rats (Wistar strain) of either sex (150-200g) were obtained from NILAS C/O National Institute of Nutrition (NIN), Hyderabad and were maintained on rat feed (M/S Lipton India Ltd., Bangalore) and water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee of our college as per the requirements of Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), New Delhi (Registration No: 157 / 1999/ CPCSEA).

The animals were starved for 12hrs prior to wounding and were housed under standard environmental conditions. Under light ether anesthesia wounding was performed aseptically.

2.5 Wound models

2.5.1 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 groups II, III and IV were treated with petroleum ether, alcohol and aqueous extracts respectively.

An impression was made on the dorsal thoracic central region 5mm away from the ears, by using a round seal of 2.5cm diameter as described by Morton and Malone [7]. The skin of the impressed area was excised to the full thickness to obtain a wound area of about 500 mm2 under light ether anesthesia in aseptic condition. The animals were housed individually.

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

2.5.2 Resutured Incision wound

Incision wound model was performed according to Ehrlich and Hunt [8]. The animals were divided into four groups of six rats in each group, and kept in separate cage. Group I served as control, received only 2% gum acacia suspension (1ml/kg, P.O) and petroleum ether,

Table 1. Effect of extracts of *T. populnea* leaves on the excision wound parameters

Sl. No	Group	% Wound contraction on					Period of Epithelization (days)	Mean size of scar area (in mm2)
		4th day	8th day	12th day	16th day	18th day		
1	Control	41.73± 3.22	57.46± 3.34	87.10± 0.85	92.80± 0.68	93.53± 0.61	23.50± 0.42	57.83± 2.03
2	Petroleum ether	61.30± 1.36**	69.66± 2.00**	92.93± 0.80***	97.30± 0.45***	99.20± 0.39***	18.33± 0.33***	37.50± 2.72***
3	Alcohol	57.43± 1.75	66.63± 1.33*	91.70± 0.47**	97.13± 0.48***	98.96± 0.34***	18.66± 0.44***	41.83± 3.42**
4	Aqueous	65.26± 2.26**	72.13± 2.66***	94.03± 0.93***	98.33± 0.66***	99.63± 0.25***	16.33± 0.49***	32.66± 2.99***

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

Table 2. Effect of the extracts of *T. populnea* leaves on the breaking strength in incision wounds.

Sl No	Group	Breaking Strength (g)
1	Control	278.16 ± 20.09
2	Petroleum Ether	419.33 ± 11.42*
3	Alcohol	389.83 ± 12.57*
4	Aqueous	446.83 ± 18.56*

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

alcohol and aqueous extracts (200 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 pavavertebral straight incision 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 8th post wounding day. Tensile strength was determined on both wounds by continuous constant water flow technique of Lee [9].

2.6 Statistical analysis

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

3. Results

The acute toxicity studies of petroleum ether, alcohol and aqueous extracts of *T. populnea* leaves were found to be non-lethal up to a dose of 2000mg/kg body weight of the animals. Hence 1/10th of the highest safer dose, i.e. 200mg/kg body weight was selected for evaluation of wound healing.

In the study using excision wound model, animals treated with petroleum ether, alcohol and aqueous extracts of leaves of *T. populnea* 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 12th day ($P < 0.001$) than the control group as shown in the Table – 1.

The result of present study reveals that extracts of leaves of *T. populnea* possess a prominent prohealing activity in incision wound model. This was demonstrated by significant increase in the skin tensile strength in petroleum ether, alcohol and aqueous extract treated groups ($P<0.001$) on 10th post wounding day are represented in Table – 2.

4. Discussion

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

Quercetin, a bioflavonoid has been reported to possess antioxidant and anti - inflammatory activities [10] and it has also found to inhibit membrane lipid peroxidation [11].

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 scarless manner in the absence of inflammation have been shown, in fetal healing studies. Wilgus TA *et.al* have shown that cox-2 inhibitor celecoxib markedly reduced the inflammatory phase of wound healing with reduction in scar tissue formation without disrupting re-epithelization or decrease in tensile strength [12]. Similarly, quercetin present in the leaves of *T. populnea* with its anti-inflammatory properties mighty have helped the wound to heal with minimum scarring.

Bioflavonoids like quercetin synthesized by many plants facilitate 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 [13].

Lipid peroxidation is an important process in several types of injuries like burns, infected wounds, skin ulcers, etc. Hence any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, which in turn results in increase in the strength collagen fibers by increasing the circulation, preventing a cell damage and promoting the DNA synthesis [14]. This is suggested by the fact that there was an significant increase in wound breaking strength after the administration of the aqueous extract of the plant. Further more leaves of *T. populnea* is known to contain lupeol, which possess free radical scavenging activity which may attributed to wound healing activity [15, 16].

The title plant is known contain various phytochemicals like Kaemferal and its glycosides, herbacetin and glycosides, beta-sitosterol and its glycosides and carbohydrates which may be responsible for wound healing activity of aqueous extract of leaves *T. populnea* [17].

Thus it may be concluded that the leaves of *T. populnea* are bestowed with significant prohealing activity justifying its use in traditional medicine. Further studies are in progress to identify the possible bioactive component of the plant extract.

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