International Standard Serial Number : 0976-139X

Deccan J. Pharmacology 2(2): April-June 2011

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

www.deccanpharmajournals.com

(Research Article)

Received; accepted

WOUND HEALING ACTIVITY OF HEARTWOOD EXTRACTS OF *CAESALPINIA SAPPAN* LINN. (CAESALPINIACEAE)

K. Hemalatha [*], N. Shivaji[1], D. Satyanarayana [2]

[*1] Department of Pharmaceutical Chemistry, Mall Reddy College of pharmacy .Dhullapally, Secundrabad, Hyderabad-500014. India

[2] Department of Pharmaceutical Chemistry, N.G.S.M.Institute of Pharmaceutical Science, Paneer, Deralakatte- 574 160, Mangalore, Karnataka, India

Keywords:

Caesalpinia sappan wound healing**,** Framycetin sulphate cream

For Correspondence:

K. Hemalatha

Mall Reddy College of pharmacy .Dhullapally, Secundrabad, Hyderabad-500014. India

E-mail:

kamurthy18@gmail.com

ABSTRACT

Purpose: The aim of the present study was to investigate the wound healing activity of the selected plant ***Caesalpinia sappan* Linn.** ***Method:*** Aqueous and ethanolic extracts of heartwood of ***Caesalpinia sappan*** were studied for its effect wound healing models in rats, i.e by excision, incision and dead space wound models and as well as histopathology of granulation tissue of model. ***Result:*** Aqueous extract shows significant wound healing activity which was evidenced by significant decrease in the period of epithelialization 18.04 ± 0.06 in excision wound model and increase in wound contraction rate, skin breaking strength 576.45 ± 4.94 in incision wound model and granulation tissue breaking strength 523.80±5.08 dry weight of granulation tissue 49.23 ± 0.10 and elevated concentration of hydroxpyroline 2122.43 ± 8.49 on dead space wound model. Whereas the histopathology of granulation tissue of the aqueous extract treated animals showed few macrophages with increase in collagenation indicating the potency of the aqueous extract in promoting the process of the wound healing.***Conclusion:*** Hence, the wound healing promoting activity of *C. sappan* may also be attributed to the anti-oxidant and anti-bacterial potency of the active constituents present in it. The present studies revealed that, the aqueous extract possess significant wound healing promoting activity compared to ethanol extract.The present findings provide scientific evidence to the ethanomedicinal properties of *C. sappan* in healing the wounds.

INTRODUCTION:

The plant *Caesalpinia sappan* (Caesalpninaceae) commonly known as sappan wood is abundantly distributed throughout India, China Malaysia, Indonesia and Taiwan. The heartwood is being used in some parts of Kerala as herbal drinking water for its anti-thirst, heapatoprotective activity [1], blood purifying, anti-inflammatory [2], anti-oxidants [3], anti-convulsant activity [4], and other healing properties. The plant is reported to contain homoflavonoids and bioflavonoid [5], unidentified terpenoids and small amounts of sterols [6].

According to Ayurveda, the heartwood is useful in vitiated conditions of pitta, burning sensation, wounds, skin disease etc and other traditional Ayurvedic formulation[7]. We are unable to find any information on the wound healing properties of this plant. Thus, the present study is designed to fill up the lacunae in the literature for its wound healing on modern lines.

MATERIAL AND METHODS:

Plant material

The heartwood of *C. sappan* was collected from the local area of Kerala and voucher specimen (HK-235) was authenticated by referring the specimen department of Drava Guna, SDM College of Ayurveda, Udupi. Voucher specimen is kept in NGSM Institute of Pharmaceutical Science. Derralakatte, Mangalore for future reference.

Preparation of extracts of C. sappan

The heartwood pieces were shade dried and powered mechanically (Sieve size 10/45). About 250 g of powered material was exhaustively extracted with 70 % ethanol for 48 hrs in soxhlet extractor. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator. (yield: 18.5 % w/w).

For aqueous extract 250 g of powered material was macerated with 1000 ml of distilled water for three days with intermittent stirring, filtered and concentrated (yield: 20.8 % w/w).Both the extracts were subjected to preliminary phytochemical tests [8].

Preparation of different formulation

Two types of drug formulation were prepared from the extracts. For topical administration, 5 % w/w ointment was prepared in 2 % sodium alginate. For oral administration, suspension of 30 mg /ml and 20 mg/ml of aqueous and ethanol extract were prepared in 1 % gum tragacanth.

Animals:

Wister rats of either sex weighing 150-200 g were used for the study and were maintained at standard housing conditions. The animals were fed with commercial diet and water *ad libitum* during the experiments. The study was approved Institutional Animal Ethical committee KSHMA Medical college, Derralakatte, Mangalore (Ref: KSHMA/AEC/034/2005).

Excision wound model

The rats were inflicted with excision wound as described by Morton and Malone [9], under light ether anesthesia. A circular wound of about 500 mm^2 was made on duplicated ethanol sterilized dorsal thoracic region of rats. The animals were divided into 4 groups of 6 each. The animals of group I were left untreated and considered as the control, the group II served as reference standard and treated with 1 % Framycetin sulphate cream (FSC), the group III and IV animals were treated with 50 mg ointment prepared from aqueous and ethanolic extracts of *C. sappan*. The ointment was tropically applied once a day till epithelialisation was complete, starting from the day of operation. The wounds were traced on graph paper on 4^{th}, 8^{th}, 12^{th} and 16^{th} day. The 16^{th} post wound days and there after daily until healing was complete. The parameters studied were percentage of wound closer and period of epithelialisation.

Incision wound model

In the incision wound model, 6 cm long paravertebral incisions were made through full thickness of the skin on either side of vertebral column of the rat as described by Ehrlich and Hunt [10]. The wounds were closed with interpreted sutures of 1cm apart. The grouping of animals was similar to excision wound model. The ointment was tropically applied once in a day. The sutures were removed on 8^{th} post wound day. The skin breaking strength of the wounds were measured on 10^{th} day as described by the method of Lee[11].

Under light ether anesthesia, dead space wound were created by subcutaneous implantation of sterilized cylindrical grass piths (2.5 cm X 0.3 cm), one on either side of the dorsal paravertebral surface of rat [12]. The animals were divided into 3 groups of 6 rats in each group. The group I served as control, which received 1 ml of 1 % gum tragacanth / kg, P.O. The animals of group II and III received oral suspensions of aqueous and ethanolic extracts, respectively (30 and 20 mg/kg P.O. respectively). The granulation tissues formed on the grass piths were excised on 10^{th} post wound day and the tissue breaking strength was measured. Simultaneously, granulation tissue so

harvested was subjected to hydroxyproline estimation following the method a Woessner [13] and histopathological study was carried out to evaluate the effect of the extract on collagen formation. The data were subjected to ANOVA followed by Turkey's multiple comparison test and the values of $P < 0.01$ were considered statistically significant.

Table 1: Effect of topical application of both the extracts of *C. sappan* on excision wound model.

Groups	4th day	8th day	12th day	16th day	Mean time of epithelisation in days
Control	13.9±0.31	26.35±0.19	58.58±0.18	76.52±0.91	22.61±0.15
Framycetin Sulphate cream (std)	38.58±0.15	75.29±0.25*	83.58±0.16	95.5±0.27*	17.37±0.17*
Aqueous extract	35.59±0.09	69.90±0.14*	81.23±0.07*	92.27±0.14*	18.04±0.06*
Ethanolic extract	33.27±0.23	62.01±0.22	74.22±0.77*	85.23±0.02*	20.08±0.01*

*P<0.01 indicates significant to compared to control values are expressed as mean ± S.E.

N=6 animals in each group

Table 2: Effect of tropical application of both the extracts of *C. sappan* on incision wound mode.

Groups	Tissue breaking strength (g)
Control	408.98±7.39
Framycetin (std)	655.70±13.03*
Aqueous extract	576.45±4.98*
Ethanolic extract	504.62±6.33

*P<0.01 indicates significant to compared to control values are expressed as mean ± S.E.

N=6 animals in each group

Table 3: Effect of tropical application both the extracts of *C. sappan* on dead wound space model

Groups	Granulation tissue dry weight (mg/100mg)	Breaking strength (g)	Hydroxyproline (µg/100mg)
Control	33.45±0.15	399.20±8.39	1285.00±21.98
Aqueous extract	49.23±0.10*	532.80±5.08*	2122.43±8.49*
Ethanolic extract	39.66±0.23	492.18±5.59*	1983.09±16.4*

*P<0.01 indicates significant to compared to control values are expressed as mean ± S.E.

N=6 animals in each group

Histopathological profile:

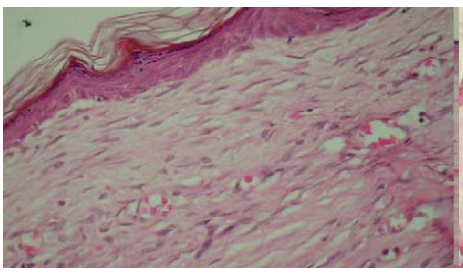

Fig:1

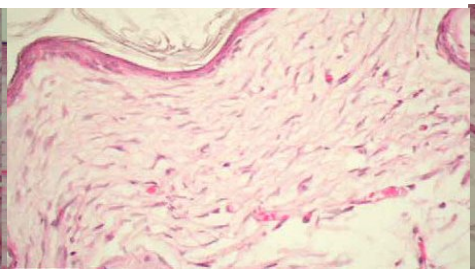

Fig:2

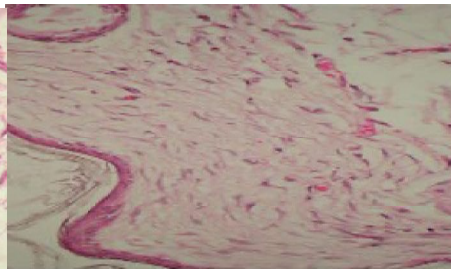

Fig:3

Fig 1 : Histological section of granulation tissue in control animal.

Fig 2: Animals treated with aqueous extract of C. *sappan* showing well formed but thick granular cell layer, more deposited collagen fiber and macrophages

Fig 3: Animals treated with ethanolic extract of C. *sappan* showing well formed but thick granular cell layer, the underlying dermis contains deposited collagen fiber with minimal inflammation

RESULT AND DISCUSSION:

Preliminary phytochemical analysis of aqueous extract revealed that, presence of flavonoids, saponins, sterols, glycosides, triterpnoids and tannins. Whereas ethanol showed positive test to flavonoids, saponins, glycosides, triterpnoids and tannins.

In excision wound model, the aqueous extract treated animals showed faster epithelialization of wound 18.04 ± 0.06 which is more of less similar to the values of standard drug treated group 17.37 ± 0.17. While the period of epithelisation was 22.61 ± 0.15 in case of control and 20.08 ± 0.01 in case of animal treated with ethanolic extract (Table 1).

In incision wound model, the mean skin breaking strength was significant in animal tested with aqueous and ethanolic extracts 576.45 ± 4.98 and 504.62 ± 6.33 respectively when compared to control 408.98 ± 7.39 (Table 2).

Dead space wound:

In dead space wound model also, aqueous extract treated animals showed significant increase in dry weight of granulation tissue 49.23 ± 0.01 and tissue breaking strength 532.80 ± 5.08 followed by ethanolic extract treated group of animals (Table 3). Estimation of hydroxyproline content in the granulation tissue revealed that the higher concentration of hydroxyproline was noticed in aqueous and ethanolic extract treated groups (2122.43 ± 8.49 and 1983.09 ± 16.45 respectively).

The histopathological profile of granulation tissue in control group of animals revealed clumping of macrophages with poor collagenation if observed (fig 1), which in aqueous extract treated animals increased collagen fibers with few macrophages (fig 2) indicating the effect of aqueous on collagen maturation. However in the animals treated with ethanolic extract showed moderate collagen depositions with scattered macrophages have been noticed (fig 3).

Discussion:

Collagen is a major protein of the extracellular matrix and is a component that ultimately contributes to wound strength. Increase in breaking strength of granulation tissue indicates the enhanced collagen maturation by increased cross linking. In addition, increase in dry granulation tissue weight indicates the presence of higher protein content[14]. Breakdown of collagen liberates free hydroxyproline and measurement of the hydroxyproline could be used as an index for collagen turnover [15].The results revealed that, animals treated with aqueous and ethanol extracts showed faster rate of epithelialization in excision wound model which may be attributed to the

phytoconstituents like flavonoids [16], tannins [17] and triterpenoids [18] which are known to promote the wound healing process mainly due to their antimicrobial property. Increase in skin breaking strength and tissue breaking strength incision and dead space wound model respectively indicated enhanced collagen maturation. Increase in the granulation tissue dry weight and hydroxyproline content which may be due to the activity of some phytoconstituents like flavonoids which are known to reduce liquid peroxidation not only by preventing or showing onset of cell necrosis but also by improving vascularity.

Hence, any drug that inhibits liquid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers, by preventing the cell damage and by promoting the DNA synthesis [19]. Hence, the wound healing promoting activity of *C. sappan* may also be attributed to the anti-oxidant and anti-bacterial potency of the active constituents present in it. The present studies revealed that, the aqueous extract possess significant wound healing promoting activity compared to ethanolic extract. The present finding provides scientific evidence to the ethanomedicinal properties of *Caesalpinia sappan* in healing the wounds.

REFERENCES:

1. Kirtikar and Basu BD, Indian Medicinal Plants Blatter E, Gaiue J.F and Mhaskar KZ (Edn), Allahabad Publication.1989; p 847-848.
2. Hinkino H, Taguchi T, Hiramatru Y. Anti-inflammatory effects of Chinese Herbal Medicines on human sperm. J Formos Medical Association. 1990; 6:466-469.
3. Badami S, Moorkoth S, Raj SR, Elango K, Bhojraj S. Antioxidant activity of *Caesalpinia sappan* heartwood. Biol Pharma Bulletin. 2003; 11: 1534-1537.
4. Beak NI, Jeon SG, Aln EM, Hahn JT, Bahn JH, Jang JS, Park JK, Choi SY. Anticonvulsant compound from the heartwood of *Caesalpinia sappan*. Archive Pharmacol Research. 2000; 4: 344-348.
5. Niranjan Reddy VL, Ravi Kanth V, Jansi Lakshmi VVNS, Suryanarayan Murthy V, Venkateswaralu Y. Inhibitory activity of Homoisoflavanoids from *Caesalpinia sappan* against *Beauvesia bassiana*. Fitoterpeia. 2003; 74: 600-602.

6. Oswal VB, Garg SC. Unsaponifiable Matter of fixed oil from the seeds of *Caesalpinia sappan*. Asian J of Chem 1993; 5(3): 676-678.
7. Warriers Pk, Nambair VPS, Ramankutty C, Vaidhyarathanam, Varriers PS. India Medicinal Plants, Compendium of 500species, Orient Logman Ltd, Chennai. 1993; p 291-294.
8. Kokatae CK, Purohit Ap, Gokhale SB. In:Pharmacognosy. Nirali Prakashan, Pune. 1990; p 120-123.
9. Morton JP and Malone MH. Evaluation of vulnerary activity by open wound procedure in rats, Arch Int Pharmacodyn. 1972; 196: 117-119.
10. Ehrlish HP and Hunt TK. Effect of cortisone and vitamin A on wound healing. Ann Surg. 1968; 167: 324-328..
11. Lee KH and Tong G. Study on the mechanism of action of salicylates, retardation of wound healing by Asprin. J Pharm Sci. 1968; 57: 1042-1046.
12. Patil MB, Jalapurae SS, Nagoor VS. Wound Healing Activity of *Eclipta alba*. Indian Drugs. 2004; 41: 40-43.
13. Woessner JF. Catabolism of collagen and non-catabolism of collagen. Archiev Biomed and Biophysics. 1963; 93: 440-447.
14. Azad S. In: Essentials of Surgery. Paras Medical Publishers, 1st Edn, Hyderabad. 2002; p 1-5.
15. Rane Madhura M and Mengi Sushma A. Comparative effect of oral administration and topical application of alcoholic extract of *Terminalia arjuna* bark on incision and excision wound in rats. Fitoterpia. 2003; 74: 553-558.
16. Ya C, Gaffiney SH, Lilley TH, Hashan E, In: Hemingway RW and Karchery J, Eds. Chemistry and significance of condensed tannins. Plenm Press. New York. 1998; 553-559.
17. Tsuchiya H, Sao M, Miyazaki Fujiwara S, Tanigaki S, Ohayama M, Tanaka T. Innuma M. Phytochemical flavones isolated from *Scutellaria barbata* and antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. JEthanopharmacology.1996; 509: 27-34.
18. Scortichini M., Rossi M. P. Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylo*V*ora* (Burrill) Winslow et al.

19. J. Appl. Bacteriol. 1991; 71: 109-112.

20. Gitie M. Gebre Marrian T, Reitz R, Neubert RH. Pharmazie. 2002; 57: 320.