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Research Paper

Wound healing activity of aqueous and methanolic bark extracts of *Vernonia arborea* Buch.-Ham. in Wistar rats

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Abstract

Excision, incision and dead space wound models were used to evaluate the wound healing activity of ***Vernonia arborea* Buch.-Ham.** on Wistar rats of either sex. In excision wound model, treatment was continued till the complete healing of the wound whereas in incision and dead space wound models the treatment was continued for 10 days. For topical application, 5% w/w ointment of aqueous and methanol barks extracts were prepared in 2% sodium alginate and for oral administration suspensions containing 30 mg/ml of each of the extracts in 1% gum tragacanth were prepared. In excision and incision wound models, the control group of animals was left untreated and in dead space wound models the animals were treated with 1 ml of 1% gum tragacanth/kg b.w. The healing of the wound was assessed by the rate of wound contraction, period of epithelialisation, skin breaking strength, granulation strength, dry granulation tissue weight, hydroxyproline estimation and histopathology of the granulation tissue. Aqueous and methanol barks extracts promoted the wound healing activity significantly in all the wound models studied. High rate of wound contraction, decrease in the period for epithelialisation, high skin breaking strength and granulation strength, increase in dry granulation tissue weight, elevated hydroxyproline content and increased collagenation in histopathological section were also observed when compared to the control group of animals. Methanol extract possesses better wound healing property than the aqueous extract.

Keywords: *Vernonia arborea,* Asteraceae, Wound healing, Bark extract.

IPC code; Int. cl.[8]— A61K 36/00, A61P 17/02

Introduction

The plant ***Vernonia arborea* Buch.-Ham.** is a moderate sized tree belonging to the family Asteraceae. It is distributed in the coastal districts of Orissa and available wildly in the South Canara[1]. The plant has many medicinal properties, viz. barks juice is used to treat worms, infusion of roots or decoction of bark is given in fever. In Southern Sumatra, the bark is chewed at the first sign on sprue. It is chewed as a substitute for pan by Nagas[2]. This plant contains sesquiterpene 'zaluzanin D', which is a potent antifungal agent[3].

Tribal groups residing in the Western Ghat region of Chikmagalur district, Karnataka state and coastal area in Orissa use the bark extract for septic wounds, in treating jaundice fever and rheumatic pains (Personal communication). A review of the literature revealed that the wound-healing property of this plant has not been subjected to scientific evaluation. Therefore, the wound-healing property of this plant was taken up for the validation of its folklore use in septic wound.

Materials and Methods

Plant material

Bark of ***V. arborea*** was collected from the Forest park, Bhubaneswar during December and identified by the first author. The voucher specimens (BKM-533, BKM-554) are deposited in the U.D.P.S, Utkal University, VaniVihar, Bhubaneswar.

Extraction

Bark was shade dried and powdered mechanically. About 250g of powder was subjected to Soxhlet extraction with 70% methanol for about 48 hours. The extract was filtered and concentrated in vacuum under reduced pressure using a rotary flash evaporator (Buchi, Flawil, Switzerland) and dried in a desiccator (yield 22.6% w/w). For aqueous extract, 250g of powdered barks was macerated with 1000ml of distilled water for three days with intermittent stirring, filtered and concentrated (yield 18% w/w). Both the extracts were subjected to preliminary phytochemical tests[4].

Drug formulations

Two types of drug formulations were prepared from each of the extracts. For topical administration, 5% w/w ointment was prepared in 2% sodium alginate. For oral administration, 30 mg/ml

of aqueous and methanol suspensions of bark extracts were prepared in 1% gum tragacanth.

Animals

Wistar rats of either sex weighing 150-200 g were procured from the O.U.A.T, Bhubaneswar, Orissa and were maintained at standard housing conditions. On arrival they are randomly divided into various treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature 24±2ºC at relative humidity of 30.70%. A 12:12, light: dark cycle was followed. All animals had free access to water filtered through aquaguard and standard pelleted laboratory animal diet. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) Regd. No 990, U.D.P.S, Utkal University and were in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Ministry of Forests and Environment, Government of India. The animals were fed with a commercial diet (Lipton India, Mumbai) and water *ad-libitum* during the experiment. Acute toxicity study was conducted for both the extracts by the stair-case method[5].

Wound healing activity

Excision, incision and dead space wound models were used to evaluate the wound healing activity.

Excision wound

The rats were inflicted with excision wounds as described by Morton and Malone (1972)[6] under light ether anaesthesia. A circular wound of about 500sq mm was made on depilated ethanol sterilized dorsal thoracic region of the rats. The animals were divided into four groups of six each. The animals of group I were left untreated and considered as the control, group II served as reference standard and treated with 1% w/w Framycetin Sulphate Cream (FSC) which is a broad spectrum aminoglycoside antibiotics and usually used as bactericidal agent, animals of group III and IV were treated with 50 mg of ointment prepared from aqueous and methanol bark extract. The ointment was topically applied once a day, starting from the day of the operation, till complete epithelialisation. The parameters studied were wound closure and epithelialisation time. The wound were traced on mm2 graph paper on days 3, 6, 9, 12, 15 and 18 and thereafter on alternate days until healing was complete. The percentage of wound closure was calculated. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound.

Incision wound

In incision wound model, 6cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat as described by Ehrlich and Hunt *et al*[7]. The wounds were closed with interrupted sutures of 1cm apart. The animals were divided into four groups of six animals each. The animals of group I were left untreated and considered as the control, the group II served as reference standard and received 1% w/w Framycetin Sulphate Cream ointment, animals in groups III and IV were treated with 50mg of ointment prepared from aqueous and methanol barks extract. The ointment was topically applied once in a day. The sutures were removed on the 8th post wound day. The skin breaking strength of the wounds was measured on the 10th day as described in the method of Lee *et al*[8].

Dead space wound

The animals were divided into three groups of 6 rats in each group. Group-I served as the control, which received 1ml of 1% gum tragacanth/kg, b.w., p.o. The animals of group-II and III received oral suspensions of aqueous and methanol lead extracts, respectively (30 mg/kg, b.w., p.o). Under light ether anaesthesia, dead space wounds were created by subcutaneous implantation of sterilized cylindrical grass piths (2.5 cm $\times$ 0.3 cm), one on either side of the dorsal paravertebral surface of the rat[9]. The granulation tissues formed on the grass piths were excised on the 10th post wounding day and the breaking strength was measured. Simultaneously, granulation tissue so harvested was subjected to hydroxyproline estimation following the method of Woessner *et al*[10] and histopathological study to evaluate the effect of the extracts on collagen formation.

Statistical analysis

The data were subjected to ANOVA followed by Dunnett's test and the values of $P \leq 0.001$ were considered statistically significant.

Results

The LD_{50} of aqueous and methanol bark extracts were found to be

300 mg/kg, b.w. One tenth of the dose was selected[5] for the evaluation of wound-healing activity i.e., 30 mg/kg, b.w. Significant promotion of wound-healing activity was observed in both aqueous and methanol barks extracts in all the three wound models such as excision, incision and dead space wound. In excision wound model, the mean percentage closure of wound area was calculated on the 3, 6, 9, 12, 15 and 18 post wounding days as shown in Table 1 and Fig. 1. The methanol bark extract treated animals showed faster epithelialisation of wound (17.86±0.19) than the animals treated with aqueous bark extract (19.03± 0.59). The period of epithelialisation was 16.15 ± 0.21 in the case of standard drug 1% w/w Framycetin Sulphate Cream ointment, Fig. 4.

In incision wound model, methanol and aqueous barks extract treated animals showed increase in breaking strength (496.45 ± 4.30), (463.74 ± 3.53), respectively when compared to the control (230.46 ± 2.57). The mean breaking strength was also significant in animals treated with standard drug FSC (564.03 ± 3.35) in Table 2 and Fig. 2 & 5.

In dead space wound model, histological studies of the granulation tissue of the control group of animals showed more aggregation of macrophages with few collagen fibres. In the case of aqueous bark extract treated animal groups, moderate collagen deposition, macrophages and fibroblasts were noticed whereas the methanol barks extract treated animal group evidenced significant increase in collagen deposition showing lesser macrophages and fibroblasts. Compared to the control group of animals, methanol bark extract treated animals showed significant increase in dry weight of granulation tissue (184.46 ± 0.49) and breaking strength (387.72 ± 3.25) followed by aqueous bark extract treated

Table 1: Effect of topical application of aqueous and methanol bark extracts of *Vernonia arborea* on healing of excision wound model

Group	Post wounding days							Period of epithelia-lisation
	0-day	3rd day	6th day	9th day	12th day	15th day	18th day	
Control	510.91 ± 0.46 (0.00)	483.53 ± 1.49 (5.34)	403.21 ± 1.14 (21.03)	356.62 ± 0.58 (30.14)	277.85 ± 0.72 (45.72)	190.16 ± 0.54 (62.65)	87.32 ± 0.50 (82.74)	24.29 ± 0.23
Standard	511 ± 1.48* (0.00)	408.30 ± 0.61* (20.13)	324.59 ± 1.31* (36.35)	252.37 ± 0.53* (50.51)	136.35 ± 0.47* (73.26)	9.32 ± 0.44* (98.17)	0* (100)	16.15 ± 0.21*
Aqueous extract	505.68 ± 2.12* (0.00)	464.69 ± 1.49* (8.48)	343.40 ± 0.54* (32.22)	271.26 ± 0.54* (46.46)	148.46 ± 0.57* (70.69)	66.22 ± 0.60* (86.93)	7.50 ± 0.43* (98.51)	19.03 ± 0.59*
Methanol extract	507.81 ± 1.51* (0.00)	443.20 ± 1.18* (12.89)	331.64 ± 0.58* (34.82)	268.25 ± 0.55* (47.27)	141.40 ± 0.43* (72.28)	18.50 ± 0.43* (96.36)	0* (100)	17.86 ± 0.19*
One-way ANOVA	F 12.87 P <0.001	25.19 <0.001	15.54 <0.001	12.70 <0.001	10.52 <0.001	12.31 <0.001	15.43 <0.001	11.59 <0.001

Values are expressed as mean ± SEM; df =3, 20; n =6 animals in each group; Numbers in parenthesis indicate percentage of wound contraction; *$P \leq 0.001$ when compared to control

Table 2: Effect of aqueous and methanol bark extracts of *Vernonia arborea* on healing of incision wound model

Group	Granulation tissue dry weight (mg/100 g)	Breaking strength (g)	Hydroxyproline (mg/100g)
Control (1ml of 1% gum tragacanth/kg, b.w.)	89.94 ± 0.61	230.46 ± 2.57	1390.66 ± 1.02
Standard(FSC)	192.25±0.65	564.03±3.35	2278±0.45
Aqueous extract	156.34 ± 0.61*	463.74 ± 3.53*	1975.33 ± 0.80*
Methanol extract	188.46 ± 0.49*	496.45 ± 4.30*	2256 ± 0.57*
One – way F ANOVA *P*	10.02 <0.001	11.89 <0.001	14.85 <0.001

Values are expressed as mean ± SEM; df = 2, 15; n = 6 animals in each group; * $P \leq 0.001$ when compared to control

Table 3 : Effect of aqueous and methanol bark extracts of *Vernonia arborea* on healing of dead space wound model

Group	Granulation tissue dry weight (mg/100 g)	Breaking strength (g)	Hydroxyproline (mg/100g)
Control (1ml of 1% gum tragacanth/kg, b.w.)	87.94 ± 0.61	235.76 ± 2.57	1398.66 ± 1.02
Aqueous extract	146.34 ± 0.61*	347.12 ± 3.53*	1979.33 ± 0.80*
Methanol extract	184.46 ± 0.49*	387.72 ± 3.25*	2250.00 ± 0.57*
One – way F	11.02	10.89	15.03
ANOVA *P*	<0.001	<0.001	<0.001

Values are expressed as mean ± SEM; df = 2, 15; n = 6 animals in each group; * $P \leq 0.001$ when compared to control

group of animals in Table 3 and Fig. 3. Estimation of hydroxyproline content in the granulation tissue revealed that the animal groups treated with methanol barks extract had high hydroxyproline content (2250.00 ± 0.57) followed by the aqueous bark extract treated group (1979.33 ± 0.80). However, the control group showed less hydroxyproline content (1398.66 ± 1.02).

Discussion

Wound healing is a fundamental response to tissue injury that results in restoration not tissue integrity, which is due to the synthesis of the connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of the hydroxyproline could be used as an index for collagen turnover. The data depicted in Table 3 reveal that the hydroxyproline content of the granulation tissue of the animals treated with methanol and aqueous bark extract was significantly increased when compared to the control group, indicating increased collagen turnover. Increase in breaking strength of granulation tissue of methanol and aqueous bark extracts treated animals indicated the enhanced collagen maturation by increased cross linking. In addition, increase in dry granulation tissue weight also indicated the presence of higher protein content[11]. In the present investigation, preliminary phytochemical analysis of aqueous bark extracts revealed the presence of flavonoids, saponins, tannins and glycosides whereas methanol extract showed positive test to flavonoids, saponins, tannins, glycosides, sesquiterpenes and triterpenoids. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by

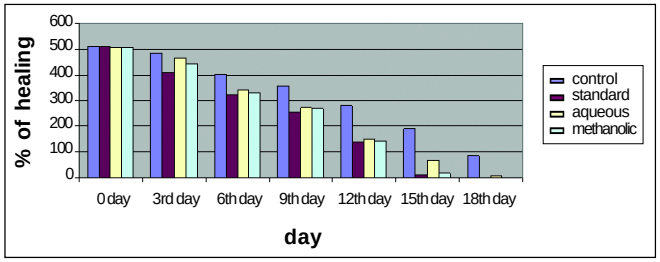

Fig. 1 : Post wound healing of excision wound model

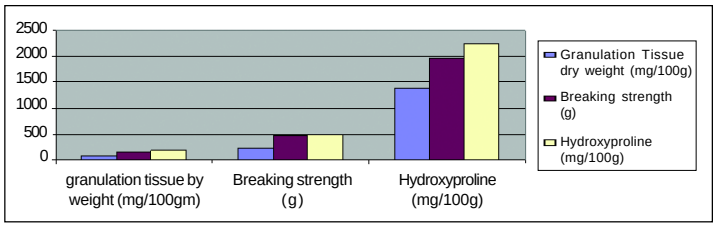

Fig. 2 : Healing of incision wound model

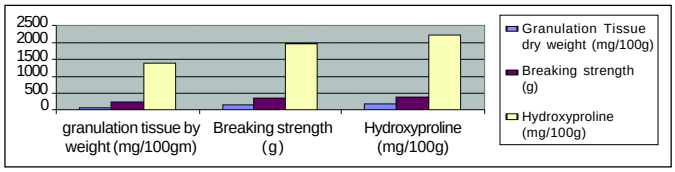

Fig. 3 : Healing of dead space wound model

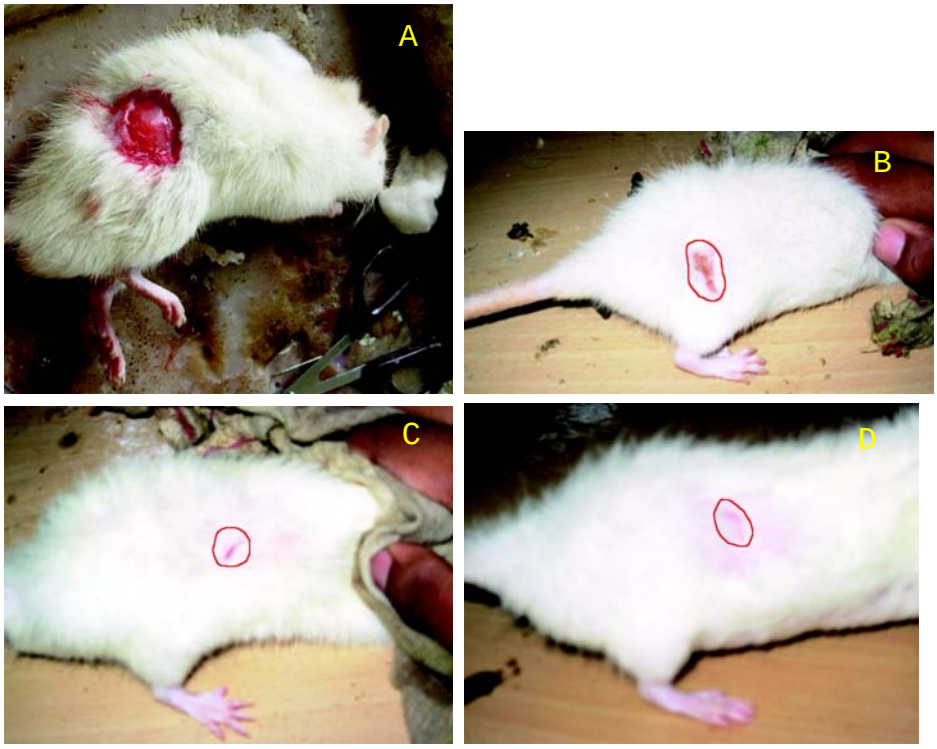

Fig. 4: Excision wound model — A. Day 0 (control); B. Standard (F.S.C, after18 days); C. Aqueous extract (after18 days); D. Methanolic extract (after18 days)

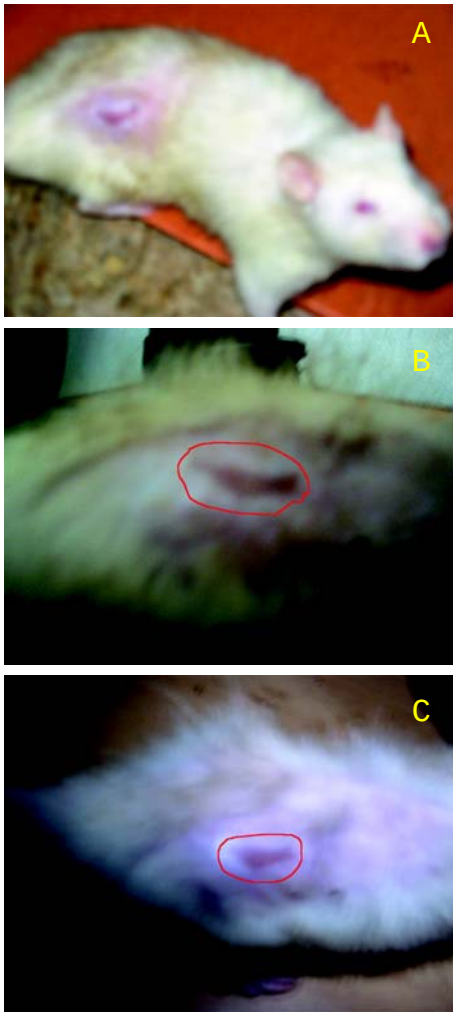

Fig. 5: Incision wound model — A. Control; B. Aqueous extract; C. Methanolic extract

promoting the DNA synthesis[12]. Tannins, flavonoids, triterpenoids and sesquiterpenes are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation[13-16]. The sesquiterpene lactones are known to possess antioxidant

property[17, 18] which may also contribute to the wound healing process.

Conclusion

Thus, wound healing potency of ***V. arborea*** may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that fastens the process of wound healing. Between the two extracts studied, the methanol barks extract was found to possess better wound healing property. Which component(s) of the extract is responsible for this effect, however, was not investigated. Further, phytochemical studies are in progress where the methanol extract will be subjected to further fractionation and purification to identify and to isolate the active compound(s) responsible for these pharmacological activities. The present findings provide scientific evidence to some of the ethnomedicinal properties of this plant.

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