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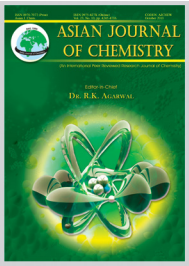

NOTE

Wound Healing Activity of *Phyllanthus niruri* in Albino Wistar Rats

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The entire wound healing process is a complex series of events that begins at the moment of injury and can continue for months to years. The objective of present study is to investigate wound healing activity of the chloroform leaf extract of *Phyllanthus niruri* in albino rats using excision and incision wound models. 200 mg/kg/day of leaf extract of *Phyllanthus niruri* was evaluated for its wound healing activity and compared with neosporin (standard). The present investigation may be concluded that the plant *Phyllanthus niruri* is endowed with significantly as followed the 0, 4th, 8th, 12th, 16th, days *i.e.* 232.21 ± 5.8 (0 %), 197.8 ± 4.5 (14.85 %), 80.7 ± 4.8 (65.5 %), 15.03 ± 2.9 (91.9 %), 2.9 ± 1.04 (98.8 %) wound healing activity due to the presence active constituents, there by justifying its use in the indigenous system of medicine.

Key Words: *Phyllanthus niruri*, Wound healing, Excision wound, Incision wound, Povidone iodine ointment.

The wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues. Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation. Extracts of this herb have shown promise in treating a wide range of human diseases. Some of the medicinal properties suggested by numerous preclinical trials are anti-hepatotoxic[1], antilithic, antihypertensive, antiHIV and antihepatitis B[2,3]. However, human trials have yet to show efficacy against hepatitis B virus[4]. The plant has long been used in Brazil and Peru as an herbal remedy for kidney stones. Research among sufferers of kidney stones has shown that, while intake of *Phyllanthus niruri* didn't lead to a significant difference in either stone voiding or pain levels, it may reduce urinary calcium, a contributing factor to stone growth[5]. In addition, one study conducted on rats showed that an aqueous solution of *Phyllanthus niruri* may inhibit kidney stone growth and formation[6]. This plant is used various diseases like eye infections, skin diseases, cough, hikups, mensural cycle inflammation, mensural cycle cessation *etc*.[7].

Collection of plant materials: The leaves of *Phyllanthus niruri* were collected from Appanapall village region in mahabubnagar District of Andhra Pradesh, India. The plant was authenticated by expert from the Department of Botany, Kakatiya University, Warangal, A.P., India. The collected leaves were dried at room temperature and powdered.

Preparation of extracts: The extracts of *Phyllanthus niruri* were prepared by maceration with methanol as a solvent. The shade dried leaf powder was kept in the macerator apparatus and extraction was allowed to run successively using the solvent chloroform. Extract was concentrated and were weighed.

Animals used: Healthy wistar albino rats of either sex and of approximately the same age, weighing about 180-230 g were used for the study. They were fed with standard diet and water *ad libitum*. They were housed in polypropylene cages maintained under standard conditions like 12 h light and 12 h dark cycle at 27 ± 3 ºC temperature. Animal experiments were carried out following the guidelines of the animal ethics committee of the institute.

Wound healing activity: Screening for wound healing activity was performed by excision wound model[8,9]. Adult albino rats of either sex were divided into three groups, each containing six animals. They were depilated at the desired site and wounding was performed under light ether anesthesia. A circular wound of approximately 2.5 cm diameter was impressed on the skin from the demarked area. The skin was excised to get a wound measuring approximately 300 mm^2 and 2 mm depth. After achieving full haemostasis by blotting the wound with cotton swabs soaked in saline, the animals were placed in their individual cages. The animals were treated

TABLE-1
WOUND HEALING ACTIVITY OF OINTMENT OF *Phyllanthus niruri* EXTRACT

Groups	Wound area (mm^2) ± SEM and (% of wound contraction) post wounding days				
	0 day	4th day	8th day	12th day	16th day
Control	236.84 ± 4.11 (0 %)	225.90 ± 9.81 (4.66 %)	149.06 ± 5.68 (36.86 %)	129.54 ± 1.02 (45.33 %)	83.81 ± 2.11 (64.8 %)
Standard (povidone iodine)	236.02 ± 5.9 (0 %)	210.6 ± 3.89 (10.76 %)	85.36 ± 5.4 (63.98 %)	24.4 ± 0.88 (89.83 %)	4.50 ± 1.23 (98.48 %)
Chloroform extract	232.21 ± 5.8 (0 %)	197.8 ± 4.5 (14.85 %)	80.7 ± 4.8 (65.5 %)	15.03 ± 2.9 (91.9 %)	2.9 ± 1.04 (98.8%)

daily as follows, from 0 to 16th post-wounding day. Group I was treated with control (ointment base), group II with standard (povidone iodine ointment) and groups III were treated with 5 % ointments of leaf extracts (200 mg/kg). The wound contraction rate was monitored by planimetric measurement of wound area of each animal on 0, 4th, 8th, 12th and 16th post wounding day. This was achieved by tracing the wound area on a graph paper. Reduction in the wound area was expressed as percentage of the original wound size.

$$\text{Wound closure}\,(\%) = \frac{\text{Initial area of wound - Nth day area of wound}}{\text{Initial area of wound}} \times 100$$

Incision wound model[10]: The incision wound model was studied under light ether anesthesia. The animal was secured to operation table in its natural position. Paravertebral straight incision of approximately 6 cm diameter was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70 % alcohol soaked with cotton swabs. They were kept in separate cages. Group I was treated with control (ointment base), group II with standard (povidone iodine ointment) and groups III were treated with ointments of leaf extracts (200 mg/kg) for 10 days. The sutures were removed after 8 days, on 10th day the tensile strength was measured by continuous constant water supply technique.

Statistical analysis: The means of wound area measurement and wound breaking strength between groups at different time intervals were compared using values are Mean ± SEM of six animals in each group. Results are expressed as percentage of wound contraction from 6 animals. Statistical significance tested with control group and done by student *t*-test.

The chloroform extract of leaf of *Phyllanthus niruri* were selected for wound healing activity. For initial screening, all the extracts were prepared as 5 % simple ointment by using hydrocarbon base. The Table-1 shows the results of the wound healing activity of leaf chloroform extract ointment. The results are expressed as mean percentage closure of excision wound area. The studies on excision wound healing model reveal that all the tested groups showed a decrease in wound area from 1st day to 16th day as shown Table-1 on 16th day complete healing of wound was observed with standard marketed ointment and ointment of chloroform extract of leaf plant. The chloroform extract treated animals showed faster epitheliasation of wounds (2.9 + 1.04) than the animals treated other remaining. Chloroform extract of leaf plant produced 98.8 % healing of wound as compared to control (ointment base) has shown 64.8 % healing. In incision model, the tensile strength of the animals were measured on the 10th wounding day. The extracts showed better wound healing property compared to control. The tensile strength of chloroform extract treated groups (740 ± 7.8).

Conclusion

It may be concluded that the plant *Phyllanthus niruri* is endowed with significant wound healing activity due to the presence active constituents, there by justifying its use in the indigenous system of medicine.

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