Potential wound healing activity of the ethanolic extract of *Solanum xanthocarpum* schrad and wendl leaves

Hiranand Dewangan[1], Manju Bais[2], Vinay Jaiswal[1] and Vinod Kumar Verma[1]*
[1]Royal College of Pharmacy Sciences Raipur, (C.G.) India
[2]Department of Life Science Mats University Raipur, (C.G.) India

Abstract: The present work objective are to investigate indigenous plants used in wound healing in India, we hereby reported our findings related to wound healing activities of plant *Solanum xanthocarpum* Schrad and Wendl (Solanaceae) from some *in vitro* and *in vivo* model studies. The plant ethanolic extracts of the leaves of *Solanum xanthocarpum* was identified qualitative phytochemical constituents and tested wound healing activity. The important secondary metabolites alkaloid, glycosides, saponins, carbohydrates, tannins, phenolic compounds, protein, and fats were identified in extracts. *Solanum xathocarpum* leaves ethanolic extract showed wound healing activity significantly in the excision and incision wound model in rats on topical application. The animals were divided into five groups with six rats in each group. Topically applied 10% w/v of plant *Solanum xanthocarpum* leaves extracts in saline taking Silver Sulphadizine ointment as standard. The results showed that ethanolic extract of *Solanum xathocarpum* leaves on topical application was reduced the epithelization period from 25.30±0.23 to 19.75±0.28 days control and ethanol extract respectively along with a marked decrease in the scar area from 53.88±0.42 to 37.76±0.17 mm^2 control and ethanol extract respectively. Significant increase in tensile strength and hydroxyproline content of plant extract were also observed and compared to the control and silver sulphadiazine. The above result revealed that the ethanol extract has remarkable wound healing potency and appear to justify the traditional use of *Solanum xanthocarpum* in wound healing in India and offer a scientific support to the treatment of traditional healers.

Keywords: Wound healing activity; *Solanum xanthocarpu;* excision.

INTRODUCTION

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions (Senthil Kumar *et al.,* 2006). Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser known hitherto unknown wild plants for treating wounds and burns (Kumar *et al.,* 2007).

Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine. These plant remedies (both single plant and multiherbal reparations) are used since ancient times even if the mechanism of action and efficacy of very few of them have been evaluated scientifically (Nagappa *et al.,* 2001). There are several reports stating that the extracts of several plants, used for wound healing properties (Diwan *et al.,* 1982; Udupa *et al.,* 1989; Suguna *et al.,* 1996; Saha *et al.,* 1997; Sunilkumar *et al.,* 1998; Govindarajan *et al.,* 2004; Stephen *et al.,* 2010; Rasik *et al.,* 1999; Mukherjee and Suresh, 2000; Park and Chun, 2001; Nagappa and Cheriyan, 2001; Perez Gutierrez and Vargas, 2006).

Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (Meenakshi *et al.,* 2006). In other words wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, haematoma, laceration or an abrasion (Enoch and John Leaper, 2005). Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue (Sumitra *et al.,* 2005). Wound healing process holds several steps which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and aquisation of wound strength (Suresh Reddy *et al.,* 2002). A lot of research work has been carried out to develop better healing agents and it has been a challenging task to the researchers to keep up the pace with problems encountered. Presently scientists are keen to evaluate drugs from plant origin. It is due to their specific healing property and nontoxic action.

Solanum xanthocarpum Schrad. and Wendl commonly known as the Indian nightshade or Yellow berried night

**Corresponding author:* e-mail: vinodmod@yahoo.com

shade, is a prickly, diffusely bright-green, perennial herb somewhat woody at base which grows abundantly in arid areas of India. Villagers have traditionally used the poultice prepared from the plant leaves of *Solanum xanthocarpum* to treat a variety of skin ailments including wound. (Govindan *et al.,* 1999, 2004) Traditionally the plant has been used for curing various ailments. Fruit juice is useful in sore throats and rheumatism; decoction of the plant is used in gonorrhea; paste of leaves is applied to relieve pains; seeds act as expectorant in cough and asthma; roots are expectorant and diuretic, useful in the treatment of catarrhal fever, coughs, asthma and chest pain. Literature survey revealed that the wound healing property of species has not been evaluated so for. Therefore aim of the present work was undertaken to investigating the folkloric claims of plant therapeutic wound healing activity of different solvents plant extracts on open wounds and incision wounds in rats.

MATERIALS AND METHODS

Plant material

The plant species for the proposed study material is *Solanum xanthocarpum* have been collected in the month of Nov.-Dec. from the roadside and waste land of Raipur District, Chhattisgarh, India. The plant species was identified and authenticated as a *Solanum xanthocarpum* Schrad and Wendl by Department of Botany, Govt. Science College Raipur (C.G.). The plant authentication No.-N/S/C-057 and plant specimen sheet was deposited in department.

Preparation of plant extract and phytochemical study

The shade dried leaves was cut and powdered by means of wood grinder and were sieved through sieve no. 60 to get the coarse powder and was extracted with petroleum ether, chloroform, ethyl acetate and ethanol successively in soxhlet extractor apparatus. The solvents are distilled off and extracts were concentrated on water bath .The percent yield was found to be 1.65%w/w (petroleum ether extract), 0.99%w/w (chloroform extract), 1.15% (ethyl acetate) and 6.5%w/w (ethanolic extract) respectively. Phytochemical screening give positive tests for alkaloids, glycosides, saponins, Flavonoids, carbohydrates, tannins, phenolic compounds, protein, and fats using the procedures outlined by Wall *et al.,* 1952 and Harbon 1973. All the extracts of plant leaves were prepared 10% w/v in normal saline consisting of 0.1% propylene glycol.

Animals and animal grouping

Animals

Wistar albino rats of either sex (150-200 g) were purchased from M/S Chakroborty Enterprises, 3/1 D, Grish Vidyaratan Lane Kolkata (WB) India. They were kept at 28±2ºC and relative humidity of 44-56%, light and dark cycles of 12 and 12 h, respectively, for 7 days before the experiment. Animal were given the rodent commercial diet and water *ad libitum*. All the experimental studies were performed in accordance with the National Institute of Health's guideline for Survival Rodent Surgery (1985) after approval from institutional animal ethical committee, Oriental college of pharmacy, Bhopal (approval No. 918/02/C/CPCSEA). Animal were allowed to recover and housed individually in a plastic cages containing sterilized paper cutting.

Animal grouping

In the experiment, the rats were divided into five groups and in each group consisting of six animals, where group-I was kept as control, group-II animals were locally applied with silver sulphadizine ointment, group-III were locally applied with 10% w/v ethyl acetate extract of *Solanum xanthocarpum* in saline, group-IV animals with 10% w/v chloroform extract of *Solanum xanthocarpum* in saline and group-V animal were locally applied 10% w/v ethanolic extract of *Solanum xanthocarpum* in saline. The 10% w/v plant extracts were given to compare the same concentration as given in standard silver sulphadizine ointment. The entire animal was housed in standard condition of temperature and maintained at a well ventilated. The animals are fed with commercial diet and water *ad libitum* during experimental period.

Wound healing activity

The excision wound model and incision wound model were used for study of wound healing activity of plant extracts. Mainly these two models were used for study of the potential wound healing activity.

Excision wound model

An excision wound model was used for studying wound healing activity as describes according to Rashed *et al.,* 2003 and Nagappa *et al.,* 2001 with some modification. Animals were anesthetized prior to and during creation of the wounds with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body wt). Hair was removed by shaving the nape of the back of all the rats. Ethanol (70%) was used as antiseptic for the shaved region before making the wound. A full thickness of the excision wound of uniform 2 cm. diameter circular area was created along the markings using toothed forceps, scalpel and pointed scissors. The wound was left undressed to the open environment and no local or systemic anti-microbial agents were used. The rats were distributed in groups randomly and each rat was placed in a separate cage. The albino rats were divided into groups and extracts were applied once times daily starting from the excisions till complete epithelization. Contractions, which contribute for wound closure in first 2 week was studied by tracing the raw wound. Wound area was measured by retracing the wound on a millimeter scale graph paper. The degree of wound healing was calculated using formula: 1− (wound area on corresponding day/wound area on zero days)×100. The number of days for complete

epithelization was noted (Nagappa *et al.,* 2001). Hydroxiproline constituent of collagen was measured according to Shukla *et. al.,* 1999, healed tissues of the wounds were cut and dried in hot air oven at 65-70°C to constant weights and hydrolyzed in 6 M HCl at 130°C for 4 hrs in sealed tubes. The hydrolyzate was neutralized (upto pH 7.0) and subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 M perchloric acid color was developed with the help of Ehrlich reagent at 60°C and measured at 557 nm using the spectrophotometer.

Incision wound model

The incision wound model used according to Udupa *et al.,* 1995; Govindarajan *et al.,* 2004; Perez Gutierrez *et al.,* 2006; Shivhare *et al.,* 2010 and Stephen *et al.,* 2010 with some modification. The animals were divided into three groups of six rats each and kept in separate cages. Rats were anesthetized and two paravertebral long incisions made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of their depilated back. Aseptic techniques were not applied and no local or systemic antimicrobial was used throughout the experiment. Each of the three groups of animals was treated in the same manner as for the excision wound model. The parted skin was kept together by stitching with a black silk surgical thread (No.000) and curved needle (No.11) and continuous threads on both wound edges were tightened for good wound closure. Group I animals (control) were treated topically with simple ointment base, group II animal with silver sulphadiazine ointment and group III animals received ethanolic extract of *Solanum xanthocarpum* twice a day for 9 days. The tensile strength of a wound represented the degree of wound healing, so wound healing agents usually provide a gain in tensile strength. The tensile strength was calculated from the following equation:

$$\text{Tensile strength} = \frac{\text{total breaking load}}{\text{cross - sectional area}}$$

The ethanolic extract of *Solanum xanthocarpum* treated wounds was compared with the control and silver sulphadizine ointment (as standard) treated wounds tensile strength. Further, after tensile strength determination, also measured the epithelization period and scar area daily for 20 days.

Statistical data analysis

Results were expressed as mean ± SEM. Statistical comparison were made by using Student's t-test analysis and difference were considered statistically significant when P-value were <0.05.

RESULT

Preliminary qualitative phytochemical analysis showed the presence of important secondary metabolites alkaloid, glycosides, saponins, carbohydrates, tannins, phenolic compounds, protein, and fats in *Solanum xanthocarpum* leaves of extracts. The wound healing is an extreme complex phenomenon involving a number of well-orchestrated processes, including regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of extracellular matrix protein, remodeling of connective tissue parenchymal components, collagenization and acquisition of wound strength (Reddy *et al.,* 2002). The ethanolic extract of *Solanum xanthocarpum* leaves possesses good wound healing activity.

The complete wound healing of all extracts of S*olanam xanthocarpum* Schrad and Wendl leaves were studied by counting the number of days and results are presented in tables 1 and 2.

DISCUSSION

The animals treated with ethanolic extract of plant shows highest wound healing potency treatment was continued upto 20^{th} days, no raw wound left after 16^{th} days. The present studied showed ethanolic extract possesses a good wound healing activity, there was a reduction in the epithelization time from 25.30±0.23 to 19.75±0.28 days and the scar area reduced on complete epithelization from 53.88±0.42 to 37.76±0.17 mm^2. The hydroxyproline content and tensile strength of control and ethanolic extract treated group increased from 160.93±5.79 to 335.79±13.20 µg/100mg and from 286.33±1.11 to 391.66±2.44 g respectively, on the 16^{th} day of post healing and results were compared to control and standard group are significant. The single model of wound healing is inadequate because the wound healing process involved various phases and no *in vitro* experiment exists that collectively represent the various components of wound healing. Because of this, *in vivo* assay are highly recommended to confirm the *in vitro* observation. Some of the *in vivo* assays of significance include the determination of hydroxyproline content and tensile strength, which is an indication of quality of the healing (Perez Gutierrez *et al.,* 2006). Collagen is a major protein in the extracellular matrix and is the component that ultimately contributes to wound strength. Breakdown of collagen liberates free hydroxyproline. Measurement of hydroxyproline could be used as an index for the collagen turnover. In present study, a significant increase in the hydroxyproline content of the granulation tissue of the animal treated with S*olanam xanthocarpum* ethanolic extract was recorded compared with control group, thus indicating positive effect of the ethanolic extract on collage synthesis and hence, on wound healing. The increase in tensile strength of the granulation tissue indicated enhanced collagen maturation by increase cross-linking. The increase in tensile strength, as well as the epithelization could be attributed to the increased

Table 1: Effect of topically applied *Solanam xanthocarpum* leaves extracts (10% w/v in Saline) on excision wound model in rats.[a]

Animal Treatment Groups [b]	Contraction of excision wound area (mm^2) after days				
	4^{th} Day	8^{th} Day	12^{th} Day	16^{th} Day	20^{th} Day
Group-I Control	7.86±0.19	8.85±0.18	13.96±0.17	16.8±0.12	18.01±0.17
Group-II Standard (Silver sulphadiazine)	9.91±0.14	12.03±0.29	14.21±0.19	17.65±0.17	19.11±0.21
Group-III Chloroform extract treated	7.80±0.14	7.90±0.17	14.11±0.10	15.88±0.19	17.93±0.10
Group-IV Ethyl acetate extract treated	7.96±0.12	8.70±0.11	12.96±0.08	15.08±0.17	16.11±0.10
Group-V Ethanolic extract treated	10.53±0.13	11.88±0.18	14.98±0.07	18.30±0.14	19.45±0.12

[a] Values are mean ±SEM (n=6) statically significant difference in comparison with control group: $P< 0.05$.
[b] Once a day, for 20 day; control, no treatment.

Table 2: Effect of topically applied *Solanam xanthocarpum* leaves extracts (10% w/v in Saline) on incision wound model in rats.

Animal Treatment Group	Epithelization Period (days) [c]	Scar area (mm^2)	Hydroxyproline content (µg/100mg) [d]	Tensile strength (g)
Control	25.30±0.23	53.88±0.41	160.93±5.79	286.33±1.11
Standard (Silver sulphadiazine)	22.21±0.26	40.91±0.24	274.47±3.71	390.83±3.32
Ethanolic extract treated	19.75±0.28	37.76±0.17	335.79±13.20	391.66±2.44

Values are mean ±SEM (n=6) statically significant difference in comparison with control group: $P<0.05$. [c]On complete epithelization. [d] On day 16^{th} post wound healing.

hydroxyproline content in the wound tissue. (Stephen *et al.,* 2010)

CONCLUSION

On the basis of the results finding in the present investigation, it is concluded that the ethanolic extract of plant *Solanum xanthocarpum* schrad and wendl leaves has highest wound healing activity as compared to other solvent extracts. The wound healing activity of the ethanolic extract may be due to the individual or combined effect of the above phytochemicals. Comprehensive evaluation on the plants with wound healing activity on the basis of traditional medicine may possibly give new compounds that could be used as prominent drugs in wound healing therapy. Further investigations are needed for identification of active principles responsible for the wound healing activity. The present investigation offers a scientific support to the traditional healer account in use of the plant *Solanum xanthocarpum* schrad and wendl for treatment of cuts and wounds.

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