Asian Journal of Chemistry; Vol. 25, No. 9 (2013), 4991-4993

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14199B

Wound Healing Activity of Ethanolic Extract of *Bryophyllum calycinum* Salisb

PRAVEEN KUMAR* and LAXMI TRIPATHI

Department of Pharmaceutical Chemistry, S.D. College of Pharmacy and Vocational Studies, Muzaffarnagar-251 001, India

*Corresponding author: E-mail: praveensha77@yahoo.co.in

(*Received*: 18 June 2012; *Accepted*: 13 March 2013) AJC-13120

In light of the Nigerian traditional claim to use *Bryophyllum calycinum* Salisb in the treatment of wounds, an investigation was carried out to evaluate the wound healing activity of ethanolic extract obtained from the dried whole plant of *B. calycinum*. The wound healing activity was evaluated using excision, incision and dead space wound models and acute toxicity study was also performed. The study demonstrated that the ethanolic extract of *B. calycinum* accelerates wound healing process as compared with placebo controls. Wound contraction, increased tensile strength and increased hydroxyproline content support the above finding.

Key Words: *Bryophyllum calycinum* Salisb, Wound healing.

INTRODUCTION

Bryophyllum calycinum Salisb (*Crassulaceae*) a shrub mainly found in the tropical parts of Bengal and in southern African and American continents. It is locally known as Patharkuchiì and for long has been used in ayurvedic medicine[1]. It has been reported for its hepatoprotective[2], neuropharmacological[3], antimutagenic[4], antihistaminic[4], antibacterial[5], antinociceptive[6], antidiabetic[6] and antiinflammatory[6,7] activities.

Besides these activities, it has been traditionally reported that *B. calycinum* is useful for the treatment of wounds in Nigerian herbal medicines[8]. The leaf extract of *B. calycinum* has been reported for its wound healing activity[9]. The present study aims to evaluate the wound healing potential of the whole plant of *B. calycinum* as traditional medicine healers in south-eastern Nigeria often use the entire shurb in treating wounds and burns[8]. The whole plant of *B. calycinum* was extracted with ethanol and subjected to chemical characterization. The dried extract was evaluated for its acute toxicity and wound healing activity in excision, incision and dead space wound models.

EXPERIMENTAL

The whole plant of *B. calycinum* was collected from district Muzaffarnagar, India in the month of April and was authenticated in the Department of Pharmacognosy, S.D. College of Pharmacy and Vocational Studies, Muzaffarnagar, India. A voucher specimen (No. PK-126) has been deposited.

Extraction: The dried and powdered whole plant of *B. calycinum* was soaked in ethanol overnight and extracted in a Soxhlet's apparatus for 24 h (ethanolic extract of *B. calycinum*: EBC, 4.5 % w/w). The crude extract was subjected to chemical characterization and evaluation of wound healing activity and acute toxicity.

Chemical characterization: The dried crude extract EBC was subjected to qualitative chemical tests for the presence of alkaloids, glycosides, tannins, flavanoids, triterpenoids and saponins.

Animals used: The study was approved by Institutional Animal Ethical Committee, S.D. College of Pharmacy and Vocational Studies, Muzaffarnagar, India. Healthy male sprague dawley rats weighing 200-220 g were used for the study. They were individually housed and maintained on normal food and water ad libitum. The rats were anaesthetized prior to and during infliction of the experimental wounds. The surgical intervention was carried out under sterile conditions using ketamine anaesthetic (120 mg/kg).

Acute toxicity study: In acute toxicity test the rats were fed with increasing doses (1,2, 4 and 8 g/kg body weight) of EBC for 14 days. The animals were allowed access to food and water and behaviour changes were observed for a period of 72 h for any sign of acute toxicity.

Wound healing activity: Excision, incision and dead space wound models were used to evaluate the wound-healing activity of *B. calycinum*.

Excision wound model: Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malon[10]. The animals were divided into two groups of 6 each. Group 1

animals were topically treated with the carboxymethyl cellulose (100 mg/kg/day) as a placebo control. The animals of group 2 were topically treated with the EBC at a dose of 100 mg/kg/day till complete epithelization. The wound closure rate was assessed by tracing the wound on days 1, 5 and 15 post-wounding using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelization.

Incision wound model: A longitudinal paravertebral incision, six centimeters in length was made through the skin and cutaneous muscle on the back as described by Ehrlich and Hunt *et al.*[11]. After the incision, surgical sutures were applied to the parted skin at intervals of one centimetre. The wounds were left undressed. The rats were given EBC (dissolved in drinking water) orally at a dose of 100 mg kg^{-1} day^{-1}. The controls were given with normal saline. The sutures were removed on the 8th post wound day and the treatment was continued. The skin-breaking strength was measured on the 10th day by the method described by Lee[12].

Dead space wound model: Dead space wounds were inflicted by implanting two sterilized cotton pellets (10 mg), one on either side of in the lumbar region on the ventral surface of each rat. On the 10th postwounding day, the granulation tissue formed on the implanted cotton pellet was carefully removed. The wet weight of the granulation tissue was noted. These granulation tissues were dried at 60 ºC for 12 h and weighed and the weight was recorded. To the dried tissue 5 mL of 6 N HCl was added and kept at 110 ºC for 24 h. The neutralized acid hydrolyzate of the dry tissue was used for the determination of hydroxyproline[13].

Determination of wound breaking strength: The anesthetized animal was secured to the table and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound and the procedure was repeated on the contralateral wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group.

Estimation of hydroxyproline: Hydroxyproline present in the acid hydrolyzate of granulation tissue oxidized by sodium peroxide in the presence of copper sulfate, when complexed with *para*-dimethylamino bezaldehyde, develops a pink colour that was measured at 540 nm using colorimetry.

Statistical analysis: Results, expressed as mean ± SD were evaluated using student's *t*-test.

RESULTS AND DISCUSSION

The qualitative tests used to identify phytochemical constituents of the *B. calycinum* showed the presence of triterpenoids, tannins, saponins and flavonoids.

In acute toxicity studies of ethanolic extract of *B. calycinum*, the animals did not produce any signs of toxicity and mortality at the highest administered dose.

The significant increase in the wound-healing activity was observed in the animals treated with the ethanolic extract of whole plant of *B. calycinum* compared with those who received the placebo control treatments. Table-1 shows the effects of the ethanolic extract of *B. calycinum* administered orally at a dose of 100 mg kg^{-1} day^{-1} for 10 days on wound healing activity in rats inflicted with incision wound. In the incision wound model, a significant increase in the wound breaking strength (432.2 ± 2.61 g) was observed when compared with the controls. In the excision wound model, *B. calycinum* treated animals showed a significant reduction in the wound area and epithelization period (Table-2). In the dead space wound model (Table-3), the ethanol extract-treated animals showed significantly increased levels of hydroxyproline content as compared with the control group of animals. A significant increase was observed in the weight of the granulation tissue in the animals treated with the extract.

TABLE 1
WOUND HEALING EFFECT OF *B. calycinum* IN INCISION WOUND MODEL

Parameter	Placebo control	Experimental
Skin breaking strength	309.82 ± 4.37	432.2 ± 2.61

N = 6, Values are expressed as mean ± SD

TABLE 2
WOUND HEALING EFFECT OF *B. calycinum* IN EXCISION WOUND MODEL

Parameter	Placebo control	Experimental
Wound area (mm^2)		
Day 1	203.1 ± 2.98	211.7 ± 1.04
Day 5	153.0 ± 1.93	143.3 ± 1.89
Day 15	124.4 ± 1.33	64.3 ± 2.7
Period of epithelization (day)	14 ± 2.3	10.1 ± 0.75

N = 6, Values are expressed as mean ± SD

Any one of the observed phytochemical constituents present in ethanolic extract of *B. calycinum* may be responsible for the wound healing activity of the plant. The quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents.

TABLE 3
WOUND HEALING EFFECT OF *B. calycinum* IN DEAD SPACE WOUND MODEL

Parameter	Placebo control	Experimental
Wet weight of granulation tissue (mg/100 g rat)	72.91 ± 2.5	99.9 ± 2.39
Dry weight of granulation tissue (mg/100 g rat)	6.73 ± 0.23	15.08 ± 2.34
Hydroxyproline (mg/g tissue)	22.6 ± 1.27	68.13 ± 1.79

N = 6, Values are expressed as mean ± SD

Conclusion

The present study has demonstrated that an ethanolic extract of whole plant of *B. calycinum* has properties that render it capable of promoting accelerated wound healing activity compared with placebo controls. Wound contraction, increased tensile strength and increased hydroxyproline content support further evaluation of *B. calycinum* in the topical treatment and management of wounds.

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