Research Article

Evaluation of Woundhealing Activity of *Bambusa arundinace* Methanolic Extract of Bamboosa arundinaceae Leaves in Rodents

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ABSTRACT

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 our study is to investigate wound healing activity of the methanolic leaf extract of Bambusa arundinace in albino rats using excision and incision wound models. 200 mg/kg/day of leaf extract was evaluated for its wound healing activity and compared with povidone iodine (Standard). The present investigation may be concluded that the plant Bambusa arundinace is endowed with significant wound healing activity due to the presence active constituents, there by justifying its use in the indigenous system of medicine.

Keywords: Wound healing, Excision wound, Incision wound, Povidone iodine.

INTRODUCTION

The exploitation of plants for medicines has a long and interesting history, since at one time all drugs were obtained from natural sources. During the least few decades, there has been a resurgence of interest in plants as source of medicines and of novel molecules for use in the elucidation of physiological phenomena. Plants have been mostly exploited from the day, when humans realized their effectiveness and versatility as medicines. There is a genuine expectation in developing countries that their drug problems can be alleviated through a sensible scientific exploitation. Medicinal plants some of which have been used for generation by indigenous populations. Then there is the worldwide green revolution that is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Further underlying this upsurge of interests in plants is the facts that many important drugs in use were derived from plants. Plants have also yielded molecules, which are extremely valuable tools in the medicine. Some scientists thus expect that the plant kingdom holds the key to the understanding of complex human pathology and the cure of man's perplexing diseases. Traditional systems followed in ancient civilization have been passed on through generations. Systems like Ayurveda, Siddha, Chinese system, Tibetan system, indigenous African medicine utilized plant source to maximum. The initial optimism, engendered by the idea that a sophisticated understanding of plants by our traditional healers would pave the way to predictable drug development has not been realized. Therefore laboratories around the world are engaged in the screening of plants for biological activity with the therapeutic potential. One major criterion for the selection of a plant for such study is traditional healer's claim for its therapeutic usefulness. The number of species of higher plants on the planet is estimated between 370,000 and 500,000. All higher plants elaborate chemical secondary metabolites that are of potential medical interest. Therefore, the determination of the criteria for selecting plants for phytotherapeutic investigation is perhaps as important an exercise as the investigation itself. Selection is mainly based on traditional usage, chemical composition and screening for a specific biological activity.

In medicine, a wound is a type of injury in which skin is torn, cut or punctured

contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin.(an open wound),or where blunt force trauma causes.

Types of Wounds

a) Open

Open wounds can be classified according to the object that caused the wound. The types of open wounds are: Incisions or incised wounds, caused by a clean, sharp-edged object such as a knife, a razor or a glass splinter. Lacerations– Injury where tissue is cut or torn. For treatment, tissue is first cleansed of any blood clots and foreign material, debrided and irrigated. Local anesthetic is administered and a traumatic technique of wound closure is employed, where wound margins are realigned with careful regard to prevention of any further crush injury to tissues. Sterile dressings are applied and immobilization is recommended for complex extremity wounds. Abrasions – Injury where a superficial layer of tissue is removed, as seen with 1st degree burns. The wound is cleansed of any foreign material, sometimes employing a scrub brush to prevent traumatic tattooing by dirt and gravel, and should be performed within the first day of injury. Local anesthetic can be used for pain; however treatment of the wound is non- surgical, using moist dressings and a topical antibiotic to protect the wound and aid healing.

b) Puncture wounds

caused by an object puncturing the skin, such as a nail or needle. Penetration wounds, caused by an object such as a knife entering and coming out from the skin.Gunshot wounds, caused by a bullet or similar projectile driving into or through the body. There may be two wounds, one at the site of entry and one at the site of exit, generally referred to as a "through-and-through."

c) Closed

Closed wounds have fewer categories, but are just as dangerous as open wounds.
The types of closed wounds are:

Contusions – Injuries resulting from a forceful blow to the skin and soft tissue, however leaving the outer layer of skin intact. These injuries generally require minimal care as there is no open wound. However, contusions should be evaluated for possible hematoma deep to the surface or other tissue injuries that may indicate more severe morbidity. An expanding hematoma can damage overlying skin and demands evacuation. Avulsions – Injuries, where a section of tissue is torn off, either partially or in total. In partial avulsions, the tissue is elevated but remains attached to the body. A total avulsion means that the tissue is completely torn from the body with no point of attachment. In the case of a partial avulsion, where the torn tissue is still well-vascularized and viable, the tissue is gently cleansed and irrigated and the flap is reattached to its anatomical position with a few nsutures. If the torn tissue is non-viable, it is often excised and the wound closed using a skin graft or local flap. In the case of a total avulsion, the tissue is often very thick and demands de-bulking and de-fattening methods before it can be re-grafted. Major avulsions describe amputation of extremities, fingers, ears, nose, scalp or eyelids and require treatment by a replant team. Hematomas, also called a blood tumor, caused by damage to a blood vessel that in turn causes blood to collect under the skin. Crush injury, caused by a great or extreme amount of force applied over a long period of time. Chronic and Acute or traumatic wounds are the result of injuries that disrupt the tissue. Chronic wounds are those that are caused by a relatively slow process that leads to tissue damage. Chronic wounds include pressure, venous, and diabetic ulcers. Typically, an insufficiency in the circulation or other systemic support of the tissue causes it to fail and disintegrate. Infection then takes hold of the site and becomes a chronic abscess. Once the infection hits a critical point, it can spread locally or become systemic (sepsis).

MATERIALS AND METHODS

The leaves were collected and washed with water and dried in shade. Leaves were powdered by means of wood- grinder, and passed through the sieve no.80 and stored in airtight container at room temperature (300±200 C).

Extraction of Crude drug

The powdered material of dried leaves of Bambusa arundinace(200gm) was extract with methanol (70%methanol apparatus for 24 hrs at temperature 400 C and pressure to dryness .The dried was properly stored in the desiccators for further studies. and 30% water) in a soxhlet

Table 1: Preparation of 5%w/w Bambusa arundinace

Ingredients	Quantity
WHITE BEES WAX	10gm
HARD PARAFFIN	15gm
CETOTOSTERYL ALCOHOL	25gm
WHITE SOFT PARAFFIN	450gm
Bambusa arundinace EXTRACT	2.5gm

transferred into a china dish and melted in a water bath, 25gm of Cetosteryl alcohol was added followed by 10gm of white bees wax and 450gm of white soft paraffin were mixed at a temperature of 700c till it solidify.

Identification of plant active constituents by Phytochemical Tests

The methanolic extract of leaves of Bambusa arundinace was subjected to preliminary phytochemical tests for the identification active constituents

PHARMACOLOGICAL EAVALUATION

Animal care and handling as per CPCSEA guideline

Male Wister albino rats of weight 150-250 grams were selected, procured from the Laboratory Animal House, Padmavathi College of Pharmacy. They were kept in polypropylene cages and acclimatized to the standard laboratory conditions in well cross ventilated animal house at temperature 25±2°C relative humidity 44 – 56% and light and dark cycles of 10 and 14 hours respectively for 1 week before and during the experiments. The animals were fed with standard diet and water ad-libitum. The experiments were approved by CPCSEA and the institutional ethics committee.

STRAIN: Albino wistar rats

AGE: 4-5 month

GENDER: either sex

BODY WEIGHT: -150-250gm.

Selection of albino species

Wister albino rats were selected for the study as they develop epithelium within one hour when compared to other species; they are widely used as experimental animals for the significant development of epithelial tissue.

Experimental design

i) Excision model: (n=6)

Group I: Control, treated with ointment base,

Group II: Test, treated with 5% w/w ointment leaf extract,

Group III: Standard, treated with 5 % w/w Povidone iodine ointment

ii) Incision model: (n=6)

Group I: Control, treated with ointment base.

Group II: Test treated with 5% w/w ointment leaf extract

Group III: Standard, treated with 5 % w/w povidone iodine ointment.

Methods

i) Excision wound model

The skin of the impressed area was excised to full thickness on the dorsal thoracic region of the rats to obtain a wound area of about 500 mm2 under light ether anesthesia. The drugs were topically applied daily till the complete epithelialization starting from the day of operation. The parameters studied were wound closure and time of epithelialization.The wounds were traced on mm2 graph paper on the days of 4th, 8th, and 16th day of post wounding day (2). The wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelialization time that indicates the formation of new epithelial tissue to cover the wound. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization (1).

a) Percentage of Wound contraction

The Percentage of Wound contraction was calculated with the use of the following formula. % wound healing=Total wound area –Reduction wound areax100Total wound area

b) Epithelialisation period

The epithelialisation time that indicates the formation of new epithelial tissue to cover the wound. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization (1).

c) Estimation of protein content

Total protein was estimated by the method of Lowry et al.,1951 using as the standard

Principle: The aromatic amino acid residue of protein react with alkaline copper reagent to form a copper protein complex, which reduces the phosphor molybdic-phosphor tungstic acid(folins reagent) to develop a blue color, which is read at 640 nm . Bovine serum albumin was used as standard.

Regents

1. Alkaline copper reagent

Solution A:2% sodium carbonate in 100 ml of 0.1 sodium hydroxide solution .

Solution B:0.5% copper sulphate in 1% sodium potassium tartarate.l ml of solution A and 1ml of solution B were mixed just before use .

2. Folin-Ciocalteau reagent

It was diluted with equal volume of double distilled water in the ratio of1:1.

Standard protein solution

20mg of crystalline bovine serum albumin was dissolved in 1000ml of distilled water to give a standard containing200 µg/ml.

Procedure

Sample containing 50-100 µg protein were mixed with 4.5 ml of alkaline copper solution and kept for 10 minutes at room temperature . It was then mixed with 1ml of folin – ciocalteau reagent and allowed to stand for 30minutes and the color developed was read at 640 nm after 20 minutes. The total protein content was expressed as mg/100mg wet tissue.

ii) Incision wound model

The incision wound model was studied under light ether anesthesia. 6 cm long paravertebral incisions were made through full thickness of the skin on either side of the vertebral column of the rat. Care was taken to see that incision was at least 1 cm lateral to vertebral column. The wounds were closed with interrupted sutures of 1 cm apart using a surgical thread (No. 000) and curved needle (No. 11). The wounds were left undressed and drugs were topically applied to the wound once a day, till complete healing. The sutures were removed on 8th day, on 10th day the skin breaking strength was measured by continuous constant water technique (64).The skin breaking strength is expressed as the minimum weight (in grams) of water necessary to bring about the gapping of the wound (63).

a) Determination of wound breaking strength

Rats were secured to the operation table and a line was drawn on either side of the

wound 3mm away from the wound. Two Alice forceps were firmly applied on to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated bottle through a string run over the pulley. Water was allowed to flow from the reservoir slowly and steadily into the bottle. As the water level rise in the graduated bottle ,the increasing weight of the bottle was transmitted to the wound site pulling apart the wound edges .Water flow was arrested when the wound was just opened and the volume of water collected in the bottle (approximately equal to its weight) was noted. Three readings were recorded for a given incision wound and the procedure was repeated on the incision wound on the contra lateral side. The average of six readings in one animal was taken as an individual value of breaking strength in that animal. Mean value of breaking strength of six animals gives the breaking strength of a given group. (65)

Statistical analysis

The results were expressed as Mean ±SEM. The data were analyzed by one way analysis of variance (ANOVA) followed by Trukey multiple comparisons test and $p < 0.01$*,0.001** was considered significant.

DISCUSSION

The topical application of Bambusa arundinacea ointment increased the percentage of wound contraction and this indicates rapid epithelizaton and collagenation. Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent of each other. It is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as close as possible to its normal state. Wound contracture is a process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. Collagen, the major component which strengthens and supports extracellular tissue is composed of amino acids, hydroxyproline, which has been used as a biochemical marker for tissue collagen. Hence in this investigation two models were used to assess the effect of the methanolic extract of Bambusa arundinacea leaves. The result of the present investigation showed that Bambusa arundinace a posses a definite pro- healing action. The methanolic leaf extract was screened for wound healing activity. Table IV shows the results of the wound healing activity of extract ointment formulations by excision method. The results were expressed as mean percentage closure of excision wound area. The studies on excision wound healing model reveal that the test group showed a decrease in wound area from 1st day to 16th day. Ointment prepared from methanolic leaf extract has shown significant wound healing activity, which was comparable to that of standard marketed preparation.

CONCLUSION

The methanolic extract of the leaves of Bambusa arundinacea ointment significantly reduces the wound area on the 4th, 8th and 16th day, which is less significant compared to the standard (5%povidine ointment) and more significant compared to the control (ointment base).The epithelization period decreases which is less significant when compared to the standard (Povidine ointment) and shows more significance compared to the control. Protein content increases on the 16th day compared to the control. In Incision model the breaking strength is more significant when compared to the standard and Flavonoids reduce inflammation and promote circulation and inhibit allergic reactions. Phenols can scavenge a wide range of reactive oxygen and nitrogen because of their scavenging activity owing to the presence of hydroxyl group. Bambusa extract have electron or hydrogen donor to scavenge free radicals and convert into more stable products by terminating the radical chain These results provide strong evidence that the methanolic extract of Bambusa arundinacea leaves

have wound healing activity due to the presence of flavonoids and phenols.

PHARMACOLOGICAL EVALUATIONS

Excision wound model

i) Determination of percentage of wound contraction

Table 2: Effect of Bambusa arundinacea leaf extract on excision wound model (% wound clouser)

Days	Group I (Control)	GROUP II (Extract)	Group III (Standard)
0th day	0	0	0
4th day	12.01%	20.94%	24.88%
8th day	24.86%	38.68%	38.68%
16th day	76.86%	90.98%	95.13%

ii) Epthelialisation period

Table 3: Epthelialisation period

GROUPS	Period of Epthelialisation
Group I (Control)	26.26±0.40
Group II (Extract)	23.17±0.54*
Group III (Standard)	20.03±0.39**

Values are expressed as Mean ± S.E.M.*p<0.01 significant, **p<0.001 highly significant

iii) Estimation of protein content

Table 4: Effect of Bambusa arundinacea ointment on biochemical parameter protein estimation

GROUPS	protein estimation		
	4th day	8th day	16th day
Group I(Control)	3.04±0.2	7.35±0.57	11.31±3.22
Group II(Extract)	5.23±1.01*	8.60±2.09*	12.66±3.10*
Group III(Standard)	7.18±0.06**	12.45±1.42**	15.75±2.71**

Values are expressed as Mean ± S.E.M.*p<0.01 significant, **p<0.001 highly significant

Table 5: Effect of leaves extract of Bambusa arundinaceaon incision wound (breaking strength in grams)

Groups	Incision wound breaking strength (g)
Group I (Control)	283.17±31.9
Group II (Extract)	465.17±34.64**
Group III(Standard)	411±81.14*

Values are expressed as Mean ± S.E.M. *p < 0.01 significant, **p<0.001 highly significant

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