ISSN: 0974-2115
Journal of Chemical and Pharmaceutical sciences

WOUND HEALING ACTIVITY OF HYDROGEL OBTAINED FROM PIGEON PEA (*CAJANUS CAJAN*) SEED HUSK

PATIL B.S. [1*] AND MASTIHOLIMATH V. S.[2]
[1]SET's College of Pharmacy, SR Nagar, Dharwad 580 002
[2]KLE University's College of Pharmacy, JNMC Belgaum 590 010
***Corresponding author**: E mail: keertibpatil@yahoo.co.in, fax:+91-836-2467190

ABSTRACT

In the present study, wound healing activity of hydrogel obtained from pigeon pea (*Cajanus cajan*) seed husk was carried out in albino rats. Pigeon pea seed husk polysaccharide was successfully extracted and utilized in the preparation of gel formulation. Gel formulation showed significant antibacterial activity against both gram positive and gram negative selected bacteria. Rat excision wound model was used to screen the wound healing activity. Percentage closure of original wound area was calculated on various days and results indicated that the percentage wound closer and epithelialization for the gel formulation treated group was comparable with those of standard group treated with Band aid®.

KEY WORDS: Wound healing activity, anti-microbial activity, pigeon pea seed husk.

1. INTRODUCTION

Wound is a condition which is characterized by disturbance in continuity of skin resulting in death of cell and tissue damage caused by various injuries. Various formulations are being used for wound healing and wound dressing and necessarily consisting of one or more ingredients with different properties (Kulkarni, 2005). Dressing material used in healing of wound may cause disturbance during removal leading to further injury. In this direction, attempts have been made to incorporate polymers and semi-synthetic polymers, usually hydro gels, which will become soft when touches the body fluids. If the dressing material used has antibacterial activity along with its soft nature is an added advantage. In view of this, we are testing wound healing and antibacterial activity of some newly developed gel formulation based on the pigeon pea (*Cajanus cajan*) seed husk.

Cajanus Cajan (L.) *Millsp* (Fabaceae) commonly known as pigeon pea perennial shrub or small tree is widespread and cultivated throughout the tropics and subtropics. It is most important species of tree, that seeds are used as food in all over world. The seeds are traditionally used as anthelmintic, piles and biliousness. The leaves are used in inflammation and wounds. The literature reviews that the plant, has been reported for various treatment of laxative, diabetes, dysentery, hepatitis, measles, as a febrifuge, hypoglycemic activity and to stabilize menstrual period (Abbiw, 1990; Amalraj and Ignacimuthu, 1998; Tang, 1999). For the treatment of wounds, bedsores and malaria, relieve pain and kill worms as well as diet-induced hypercholesterolemia (Luo, 2008). Protective effects of the leaf extracts against hypoxic-ischemic brain damage and alcohol induced liver damage have also been reported (Huang, 2006) and also the seeds have been reported antioxidant and anti-hyperglycemic activity (Wu, 2009). The chemical studies show the presence of polysaccharide like a purified resistant starch and xyloglucon, amino acids like methionine, lysnine and tryptophan.

In this research, pigeon pea (*Cajanus cajan*) seed husk polysaccharide was successfully isolated by hot water extraction process and gel formulations were prepared and evaluated for wound healing activity in rats. Results were compared with control group as well as with standard group treated with Band aid® marketed product.

2. MATERIALS AND METHODS

Materials: Acetone, alcohol, hydrochloric acid and sodium hydroxide were purchased from S. D. Fine chemicals, Mumbai, India. Nutrient agar medium was purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India. Gram positive and gram negative bacterial culture samples were obtained from Karnataka Institute of Medical Sciences, Hubli, India. Albino wistar rats were purchased from Venkateshwara enterprises, Bangalore, India. Animals were maintained under conventional laboratory conditions, at temperature 25±2°C and a 12 h natural light period. Commercial pellet diet (Lipton India) and drinking water were provided *ad libitum*. For the present study, clearance from institutional animal ethical committee was obtained prior to the animal activity.

Methods

Extraction of Polymer: Dried seed husk of pigeon pea was crushed in mortar and pestle for size reduction and weighed quantity of fine powder was mixed with distilled water to get slurry. The slurry was poured into sufficient quantity of boiling distilled water and kept to cool to room temperature. The supernatant was separated by centrifugation and the residue was successively washed with petroleum ether, diethyl ether and acetone. Further, precipitate was dried at

50-60°C under vacuum to remove volatile matter and moisture. The dried material was further ground and sieved to obtain uniform particle size range used for preparation of gel formulation.

Antimicrobial Study: Whatman filter papers were cut into circular pieces with diameter of 10 mm and were dipped into gel formulations. These gel treated films were placed on nutrient agar medium containing standard bacterial inoculum. Anti-microbial activity was carried out against *Escherischia coli*, *Shigella dysentrae*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The anti-microbial activity was measured by measuring the zone of inhibition using a standard technique.

Wound Healing Studies: Male albino rats weighing in between 175-250 grams were selected for the study and divided into three groups of five animals each. On the 0^{th} day, animals were anaesthetized and secured to the operation table on its natural position. An ink impression was made on the dorsal thoracic central region 5 mm away from the ears by using round seal of 2.5 cm diameter as described by Morton and Malone (1972). The skin of the impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2. Wounds of the animals in the control groups were kept open without any treatment whereas wounds of the animals in standard groups were applied with a Band-aid® and wounds of the animals in test groups were applied with gel formulation. The physical attributes of healing which mainly contributes for wound closure in the first three weeks were studied by tracing the raw wound area on the tracing paper till complete epithelialization occurred (Lee & Tong , 1968).

3. RESULTS AND DISCUSSION

Gel formulation was prepared as per the details mentioned in the Table 1. Seed husk of pigeon pea was dispersed into water and other ingredients were added. The polymer extracted is not completely soluble in water but dispersed in water. In order to avoid the microbial contamination methylparaben was used as preservative. Results of antibacterial activities of gel formulations are summarized in Table 2. The activities were investigated against four different pathogenic bacteria usually found in wounds. *P. aeruginosa* is one of the causes of delayed healing and infection in both acute and chronic wounds. The antibacterial gel formulations were compared with standard medicated wound dressing material (WDM). It was observed that the zone of inhibition for gel formulations were 23, 28, 33 and 27 mm where as for the band-aid® films were 30, 27, 19 and 32 mm respectively for *Escherischia coli, Shigella dysentrae, Pseudomonas aeruginosa* and *Bacillus subtilis*. The diffusive permeability of ingredients from the gel formulations resulted the antimicrobial activity. Present formulations show good antimicrobial activity as compared with standard group.

Gel formulation was applied on the excision wounds using filter paper wick such that it does not disturb the open wound. Excision wounds were created on 0^{th} day and the average diameters for different groups were noted. Percentage closure of original wound area was calculated in mm^2 at different time intervals. In the control group the percentage wound closure at 0, 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} day were 0, 0.51, 23.11, 63.00, 83.4 and 95.01 % respectively. In the gel treated group the percentage wound closure at 0, 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} day were 0, 8.11, 68.01, 90.47, 100 and 100 % respectively. In the Band aid® treated group the percentage wound closure at 0, 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} day were 0, 3, 75, 94, 99 and 100% respectively. Results clearly indicate that the percentage wound closure and epithelialization for the gel treated group were comparable with Band aid® treated group.

4. ACKNOWLEDGMENTS

Authors thank SET College of Pharmacy, Dharwad and KLE University, Belgaum for providing necessary facilities.

Table1: Formulation details of gel formulations based on Seed husk of pigeon pea

Ingredients	Quantity in %w/w
Seed husk of pigeon pea	4
Methyl paraben	0.2
Purified water	q.s. 100

Table2: Antibacterial activities of gel formulations based on Seed husk of pigeon pea

Organism	Zone of inhibition in mm	
	Gel formulation	Band-aid
Escherischia coli	23	30
Shigella dysentrae	28	27
Pseudomonas aeruginosa	33	19
Bacillus subtilis	27	32

*Average of three readings

REFERENCES

Abbiw DK, Useful Plants of Ghana, Richmond Intermediate Technology Publications and RoyalBotanic Gardens, Kew, London, UK, 1990.

Amalraj T, Ignacimuthu S, Evaluation of the hypoglycaemic effect of *Cajanus cajan* (seeds) in mice, Indian J.Exp.Biol., 36, 1998, 1032-1033.

Huang G.Y, Liao X.Z, Liao H F, Studies on water-soluble extracts from *Cajanus cajan* leaf against hypoxic-ischemic brain damage, Tradit. Chin. Drug Res. Clin. Pharmacol., 17, 2006, 172-174.

Kulkarni AR, Kulkarni VH, Keshavayya J, Hukkeri VI, Sung HW, Anti-microbial activity and film characterization of thiazolidinone derivatives of chitosan, Macromol. Biosci., 5, 2005, 490-493.

Lee KH, Tong TG, Study on the mechanism of action of salicylates, retardation of wound healing by aspirin, J.Pharm.Sci., 57, 1968, 1042–1046.

Luo QF, Sun L, Si JY, Hypocholesterolemic effect of stilbenes containing extract-fraction from *Cajanus cajan* L. on diet-induced hypercholesterolemia in mice, Phytomedicine, 15, 2008, 932-939.

Morton JJP, Malon MH, Evaluation of vulnerary activity by an open wound procedure in rats, Arch.Int.Pharmacondyn, 176, 1972, 117 – 26.

Tang Y, Wang B, Zhou X.J, Effect of external application of herbal cajani preparation on the fibronection content during healing process of open wound, J.Guangzhou U.Tradit. Chin.Med., 1,1999, 302-304.

Wu N, Fu K, Fu YJ et al., Antioxidant activities of extracts and main components of Pigeon pea [*Cajanus cajan* (L.) Millsp.] leaves. Molecules, 14, 2009, 1032-1043.