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WOUND HEALING ACTIVITY OF ETHANOLIC SEED EXTRACT OF BRASSICA JUNCEA

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ABSTRACT

Wound healing is physiological process, which takes place by body's natural regenerative capacity. Due to various reasons they may delay in healing and this prolonged healing sometimes lead to scar formation. Currently attention has been focused on natural products to prevent infections and to promote the healing. In the present study, *Brassica juncea* has been used since ancient times and it is popularly known as mustards. The aim of present study was to evaluate the wound healing activity of ethanolic extracts of *Brassica juncea* (EEBJ) against incision, excision and burn wound models in rats. Ethanolic extract has shown the wound healing activity against different wound models in rats in a concentration dependent manner and among the two concentrations of gels. 20% gel shows better wound healing activity compared to 10% gel.

Key words: Brassica juncea, Incision, Excision, Burn wound.

INTRODUCTION

Wound healing is the body's natural process of regenerating dermal and epidermal tissue. The healing cascade is activated when platelets come into contact with exposed collagen leading to platelet aggregation and the release of clotting factors resulting in the deposition of a fibrin clot at the site of injury.

The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing[1]. Inflammatory cells also arrive along with the platelets at the injury site providing key signals known as growth factors[2]. The fibroblast is the connective tissues cell responsible for collagen deposition required to repair the tissue injury[3]. Collagen accounts for 30% of the total protein in the human body[4]. In normal tissues, collagen provides strength, integrity and structure. When tissues are disrupted following injury, collagen is required to repair and restore normal structure and function. *Brassica juncea* (Brassicaceae) commonly known as mustard. The plant *Brassica juncea* itself can grow from two to eight feet tall, with racemes of small yellow flowers. These flowers are usually up to 1/3" across, with four petals each. The leaves are covered in small hairs they can wilt on hot days, but recover at night. Crushed seeds have pungent odour[5].

Traditionally *Brassica juncea* was used as anti-inflammatory, anti-oxidant[6], antifungal[7], anti-microbial[8] hypoglycaemic[9] and anti hyper lipidemic[10]. Literature review also indicated that wound healing property of this species in not been clinically evaluated so far. So the present study was aimed to evaluate the wound healing potency of ethanolic extract of *Brassica juncea* against incision, excision and burn wound models in rats.

MATERIALS AND METHODS

Plant material
The dried seeds of *Brassica juncea* belonging to the family *Brassicacea* were collected at the local markets of Bangalore, Karnataka, India. The plant material was identified and authenticated.

Processing of sample
The dried seeds were pulverized into fine powder and used for extraction.

Preparation of extract
Ethanolic extract
The powdered crude drug was loaded into the soxhlet extractor and subjected to extraction with ethanol. After the extraction, the solvent was distilled off and the extracts were concentrated to dryness at room temperature. The yield value, texture and colour obtained were stated in table 1.

Preliminary phytochemical screening:
Qualitative phytochemical analysis of *Brassica juncea* seed extract revealed the presence of carbohydrates, proteins, glycosides, alkaloids, flavonoids, tannins.

Phytochemical Screening Methods
Test for Tannins:
To an aliquot of the extract (dissolved in water) 2 mL of sodium chloride (2%) was added, filtered and mixed with 5 mL 1% gelatin solution. Precipitation indicates the presence of tannins[11].
Test for alkaloids:
Extract (300 mg) was digested with 2 M HCl, and the acidic filtrate was mixed with amyl alcohol at room temperature. Pink colour of the alcoholic layer indicates the presence of alkaloids[12].
Test for flavonoids:
The presence of flavonoids was determined by using 1% aluminum chloride solution in methanol, concentrated HCl, magnesium and potassium hydroxide solution[11].
Test for carbohydrates:
The extracts were treated with Benedict's, Fehling's, Molish's and Barfoed's regents under suitable conditions. Appearance of red, brick red, purple colour in response to the above reagents respectively indicates the presence of carbohydrates.

Test for glycosides:
Small quantity of the extract was subjected to Balijet, Borntrager's, Keller-Killiani, Legal and Modified Borntrager's tests under suitable conditions. Appearance of red, pink –violet brown, pink-red and rose pink-cherry red colour in response to the above tests respectively indicates the presence of glycosides.
Test for proteins:
The extract was subjected to Biuret, Millions, Xanthoproteic and Ninhydrin tests. Appearance of pink-purple, violet, violet, red and blue-purple colour in to the above reactions respectively indicates the presence of proteins.

Drug formulation
The ethanolic extract of *Brassica juncea* is formulated in to gel formulation. Two concentrations of gels were prepared. 10g of ethanolic extract of *Brassica juncea* is incorporated in to 100g of 2% sodium alginate to get 10% w/w gel. To prepare 20 % gel, 20g of ethanolic extract of *Brassica juncea* is incoraporated in to 100g of 2 % sodium alginate to get 20% w/w gel.

Experimental Animals
Male Wistar rats weighing about 150-200 gms were used in the experiments for wound healing activity. The animals were kept in acclimatized environment (25 ± 3°C), with light/dark control each 12 hours. The animals were placed in cages. They were individually housed and maintained on normal food and water *ad libitum*. The rats were anaesthetised prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (100mg/kg body wt.,) and xylazine (16mg/kg). Animals were closely observed for any infection and if they showed signs of infection were separated, excluded from the study and replaced.

Animal groups
The animals were grouped into 4 groups (n=6).
Group-I: Base (2% sodium alginate gel) was supplied and served as normal control.
Group-II: Standard drug Povidone iodine was applied on the wound.
Group-III: 10% gel was applied on the wound.
Group-IV: 20% gel was applied on the wound

Skin irritation study:
The animals does not shown the erythemia and oedema .

Burn wound [13]
Rats were anaesthetized with ketamine (80 mg/kg) and the hair on the back was clipped with electric clippers. Burn wounds were created by pouring hot molten wax at 80°C into a metal cylinder placed on the back of the rat. The metal cylinder has 100 mm area of circular openings and capacity of to hold 4.0 g of wax. On solidification of wax (8 min), the metal

cylinder with wax adhered to the skin was removed, which left distinctly demarked circular wounds of 100 mm^2. After this each animal was placed in a separate cage for full recovery from anaesthesia before being returned to holding rooms. The following parameters were studied.

Epethelization period: It was monitored by noting the number of days required for eschar to fall away, leaving no raw wound behind.

Wound contraction: To monitor this, progressive changes in wound area were followed planimetrically. Leaving the wounding day, wounds were traced on a transparent on an alternate day. The animal was restrained in proper position during tracing. The tracings were then transferred to 1 mm^2 graph sheet. From this, wound areas were read.

Excision wound [14,15]

The animals were anesthetised using ketamine (80mg/kg) and xylazine(16mg/kg).An impression was made on the dorsal thoracic region 1cm away from vertebral column and 5cm away from ear on the anesthetised rat. The particular skin area was shaved one day prior to experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500mm^2. Haemostasis was achieved by blotting the wound with cotton swab soaked with normal saline. Animals were grouped and different formulations were applied to cover entire wound area. Wound area measured by tracing the wound on millimetre scale graph paper on predetermined days i.e., 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 days post wounding.

Incision wound

The Animal were grouped and treated as per the animal groups. The animals were anesthetised by using ketamine (80mg/kg) and xylazine (16 mg/kg). One full thickness paravertibral incision of 6 cm length was made through the skin and cutaneous muscle. Full septic measures were not taken and no local or systemic antimicrobials were used throughout experiment[16]. After the incision was made the parted skin was kept together and stitched with black silk at 1 cm apart. On 9^{th} day sutures were removed and on 10^{th} day braking strength was measured with Tensiometer[17].

Skin irritation study

This study was carried out on rabbits. The skin of the animal was shaved at three different positions on the dorsal side, each about 6 cm in length. The formulation base containing drugs were applied. After 4 hr, the skin was observed for signs of inflammation.

Statistical analysis

Results obtained from three wound models have been expressed as mean $\pm$ SEM. The data was evaluated by one way ANOVA followed by dunnett's *t*-test, $p<0.05$ was considered as significant.

Discussion:

Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent to each other. Hence in this study three different models were used to assess the effect of ethanolic extract of *Brassica juncea* on various phases.

The results of the present study indicate that ethanolic seed extract gel at both concentrations (10%, 20%) exhibited significant wound healing activity. This was demonstrated by a significant increase in the rate of wound contraction and enhanced epithelisation of excision wounds. This may be due to the effect of *Brassica juncea* extract increased collagen synthesis.

Conclusion:

The preliminary phytochemical screening of *Brassica juncea* leaf extract showed the presence of flavonoids, alkaloids, glycosides, proteins, carbohydrates, tannins. These results demonstrated that *Brassica juncea* was showing wound healing property partially by increasing the collagen synthesis, probably due to the presence of mixture of phytoconstituents in the plant. Thus form this study we concluded that the *Brassica juncea* seed extract has a reproducible wound healing potential and hereby justifies its uses in the folk medicinal uses in India.

Table No-1: Extract yield

Plant part	Type of Extract	Colour	Texture	% Yeild
Brassica juncea seeds	Ethanolic extract	Yellowish brown	Gummy	25 %w/w

Table No-2: Phytochemical analysis of *Brassica juncea* seed extract

Phytochemicals in Brassica juncea	ETHANOLIC EXTRACT
Carbohydrates	+
Proteins	+
Amino acids	+
Glycosides	+
Alkaloids	+
Flavonoids	+
Tannins	+

Table No-3: Burn wound

Post wounding days	Control	Standard	10% EEBJ	20%EEBJ
1st	94.50±0.6708	96.33±0.5578	96.17±0.4773	96.17±0.7923
3rd	74.33±1.229	74.67±0.9545	75.67±0.9189	74.67±0.9888
5th	56.33±1.856	47.83±0.6540***	52.50±0.8851	49.67±1.783**
7th	47.00±1.291	34.67±0.8433***	41.17±0.9458**	40.83±1.682**
9th	37.50±1.088	27.00±1.183***	32.83±1.249	28.67±1.687***
11th	27.17±1.537	19.00±0.5774***	23.17±0.6540*	21.83±1.167**
13th	20.33±0.8819	12.17±0.7032***	17.33±0.6667*	15.17±0.6009***
15th	13.33±0.6146	7.333±0.4944***	10.50±0.8851*	9.50±0.670**
17th	6.50±0.4282	3.00±0.2582***	5.667±0.210	4.50±0.5627**
Epithelialisation day	7.500±0.2236	6.667±0.2108	7.167±0.3073	7.167±0.3073

Values are mean ± SEM (n-6) one way ANOVA followed by Dunnet's test where * represents significant at < 0.05, ** represents highly significant at p < 0.01, *** represents very significant at p< 0.001.

Table No-4 Excision wound

Post wounding days	Control	Standard	10% EEBJ	20%EEBJ
0	477.7±2.348	476.0±3.055	479.7±0.954	465.7±5.20
2nd	436.2±3.016	428.7±18.22***	418.3±3.393**	410.7±3.283***
4th	312.8±11.86	347.8±11.46**	347.8±11.46	333.3±8.713
6th	258.8±4.167	202.8±8.479***	240.3±12.87	213.7±7.82**
8th	214.3±4.120	148.8±9.642***	182.8±5.986*	160.7±7.671***
10th	147.8±5.069	85.17±6.554***	95.64±17.19***	102.5±2.029***
12th	92.33±2.654	40.50±5.614***	75.00±5.978	53.17±4.385***
14th	49.33±4.558	18.17±1.922***	39.33±3.565	25.83±2.056***
16th	29.67±3.232	8.833±1.108***	20.0±1.770**	12.0±0.9309***

18th	15.00±3.183	1.167±1.167***	12.50±1.057	3.000±1.366***
20th	5.00±2.280	0.00±0.00*	4.667±1.085	0.00±0.00*
22nd	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Epithelialisation day	9.167±0.4014	8.333±0.2108	8.00±0.3651	7.667±0.4216

Values are mean ± SEM (n-6) one way ANOVA followed by Dunnet's test where * represents significant at < 0.05, ** represents highly significant at p < 0.01, *** represents very significant at p< 0.001.

Table No-5:**Incision model Breaking strength**

Group	Incission model breaking strength
Control	268.7± 6.528
Standard	371.0±15.24***
10% EEBJ	444.0±10.89***
20%EEBJ	487.2±12.33***

Values are mean ± SEM (n-6) one way ANOVA followed by Dunnet's test where * represents significant at < 0.05, ** represents highly significant at p < 0.01, *** represents very significant at p< 0.001.

REFERENCES

1. Iba Y, Shibata A, Kato M, Masukawa T. Possible involvement of mast cells in collagen remodelling in the late phase of cutaneous wound healing in mice. Int Immunopharmacol, 2004; 4(14): 1873-1880.
2. Cross K, Mustoe T. Growth factors in wound healing. Surg Clin N Am, 2003; 83(3): 531-545.
3. Ross R. Wound healing. Sci Am, 1969; 220 (6): 40-50.
4. Prockop DJ, Kivirikko KI. Collagens molecular biology diseases and potentials for therapy. Annu Rev Biochem, 1995; 64(3): 403-434.
5. Post George Edward. 1900. "Mustard".in james Hastings. A dictionary of bible11 Duke,J.A. 1979. Ecosystematic data on economic plants. Quart. J. Crude Drug Res. 17(3–4):91–110.
6. Kirtikar KR, Basu BD, Indian Medicinal Plants. 2nd ed., India; International Book Distributors: 1935.
7. A GRAS Notification submitted by the Canola Council of Canada, Winnipeg, Manitoba to the United States Food and Drug Administration, Washington, D.C. in accordance with proposed Section 170.36 of the Federal Food Drug and Cosmetic Act September, 1999.
8. A *Brassica juncea* chitinase with two-chitin binding domains show anti-microbial properties against phytopathogens and Gram-negative bacteria. Plant Signaling and Behavior, 2008; 3(12): 1103-1105.
9. Thirumalai T, Viviyan therasa S, Elumalai EK, David E. Hypoglycemic effect of *Brassica juncea* seeds on streptozotocin induced diabetic male albino rat. Asia Pac J Trop Biomed, 2011; 1(4): 323-325.
10. Sathya priya R, Siva kumara V. Phytochemical and anti hyper lipidemic activity of brassica juncea L. Int J Plant Sci, 2010; 5(1): 13-15.
11. Kapoor LD, Singh A, Kapoort SL, Strivastava SN. Survey of Indian Medicinal Plants for Saponins Alkaloids and Flavonoids. Lloydia, 1975; 38(3): 221-224.
12. Harborne JB. Photochemical Methods a Guide to Modern Techniques of Plant Analysis. 1st ed., London; Chapman A & Hall: 1973.
13. Nayak BS, Raju SS, Ramsubhag A. Investigation of wound healing activity of *Lantana camara* L. in Sprague dawley rats using a burn wound model. Int J app res nat prod, 2008; 1(1): 15-19.
14. Kamath JV, Rana AC, Chowdary AR. Pro-healing effect of *Cinnamomum zeylanicum* bark. Phytother Res, 2003; 17(8): 970-972.
15. Reddy S, Rao PR, Reddy MS. Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats. J Ethanopharmacol, 2002; 79(2): 249-251.
16. Udupa SL, Udupa AL, Kulkarni DR. Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and *Aegle marmelos.* Fitoterapia, 1994; 65(2): 119-123.
17. Saha K, Mukherjee PK, Das J, Pal M, Saha BP. Wound healing activity of *Leucas lavandulaefolia* Rees. J Ethanopharmacol, 1997; 56(2): 139-144.