Research Article

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WOUND HEALING POTENTIAL OF *ZIZIPHUS JUJUBA* BARK EXTRACT ON ALBINO RATS

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ABSTRACT
The wound healing activity of topically applied extract of bark of *Ziziphus jujuba* was evaluated in albino rat by excision wound model for a period of 24 days. In this study, the rats were divided into four groups, each groups continued healing process with 5 and 10% w/w methanolic extract of *Ziziphus jujuba* mg/cm^2. Test duration was 24 days, wound diameter measurement using digital image analysis on days 0, 8th, 16th , 21st and 24th with biopsy of two rats from each group were taken. The results revealed that treatment with low-dose group of methanolic extract of *Ziziphus jujuba* (5%) less shrinkage of wound area and also from the perspective of Histopathological change. In the second and third week, tissue had better organization than most other groups ($p<0.01$), but the group treated with high doses of methanolic extract of *Ziziphus jujuba* (10%) had most wound shrinkage and organization.
Keywords: excision wound model, biopsy, healing and shrinkage.

INTRODUCTION

A wound which is disrupted state of tissue caused by physical, chemical, microbial or immunological insult ultimately heals either by regeneration or fibroplasias[1]. Wound healing is a complex process that result in the contraction and closure of the wound and restoration of a functional barrier

i. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue
ii. Repair of injured tissues includes inflammation, proliferation, and migration of different cell types
iii. Inflammation, which constitutes a part of the acute response, result in a coordinated influx of neutrophils at the wound site[2]. There are large numbers of traditional medicinal uses that are not necessarily based on knowledge of the constituents. According to Ayurveda, the root of *Z. jujuba is* bitter and cooling, and cures coughs, biliousness and headache[4]. The bark cures boils and is good for the treatment of dysentery and diarrhea[5]. The leaves are antipyretic and reduce obesity. The fruit is cooling, digestible, tonic, aphrodisiac, and laxative and removes burning sensations, thirst, vomiting[3] and is also good in treating tuberculosis and blood diseases. The seeds cure eye diseases and are also useful in leucorrhoea[6]. The traditional healers of Bastar region use the dried leaves and powdered bark to dress wounds[5]. The fresh leaves are also used for the same purpose. The aqueous paste of the leaves is applied externally to relieve a burning sensation. Roots are used to treat dysentery; they are given with cow's milk until the patient is cured. Senior citizens used the fresh leaf juice with buffalo's milk to reduce the intensity of smallpox. Similarly, in the early days, the use of seeds to treat eye troubles was common. To treat hoarseness of the throat, traditional healers advise patients to keep the fresh roots of this plant inside their mouth. The traditional healers use the fresh leaves of this plant with cumin to treat urinary infections[6]. The fruit is employed as an antidote to aconite poisoning, abdominal pain in pregnancy and externally in poultice and applications for wounds. The kernels increase flesh and strength and are sedative in activity[7]. *Ziziphus jujuba* fruits are very rich in vitamins C and B1 (thiamine) and B2 (riboflavin)[8]. Compared with other edible fruits, one fruit of ber per day would meet the diet requirements for Vitamin C and Vitamin B complex for an adult man as recommended by FAO/WHO. It is also known to have a high Vitamin P (bioflavonoid) content. It enhances the action of Vitamin C. Antibacterial, anti inflammatory and antioxidant are some medicinal properties. It is also known to stimulate bile production, promote circulation and prevent allergies. Presence of Pectin-A in *Z. jujuba* fruit is also reported[9]. Chemically it contains 2, 3, 6-tri-o-acetyl D lactose units. Pectin has a number of pharmaceutical properties such as binding bile acid, lowering plasma cholesterol and anti diarrhoeal properties[10].

MATERIAL AND METHODS

Plant Material

The fresh bark of *Ziziphus jujuba* (Rhamnaceae) was collected from the kothapalli-Village, Peddapalli-Mandal, a local area of the Karimnagar: Dist; Andhrapradesh: state, India in the month of January 2010. The plant was identified and authenticated by Head, Dept. of Botany (Voucher No: DOB/OU/1422) and a specimen was kept for record at Osmania University of Hyderabad, Andhrapradesh, India. The bark of the plant was separated from adulterants, shade dried and powered coarsely.

Extraction

About 400 gm of air dried coarse powdered bark was soaked with petroleum ether for 2 days. At the end of second day the powder was taken out and it was dried.

After drying it was packed in 1000ml Soxhlet apparatus and extracted by using methanol as solvent, till colour disappeared. The temperature was maintained at 55-65^{0}C. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield (10.45% w/v) of methanolic extract were noted.

Ointment preparation for topical application

Methanolic extract of fresh bark of *Ziziphus jujuba* was used for the preparation of ointments for topical application. These ointments for the assessment of excision wound healing activity of the extracts were formulated by using simple ointment BP as base. 10% (w/w) ointment was applied where 10g of extracts were incorporated in 100g of simple ointment base BP. 0.5g of each of extract ointment and povidone iodine ointment was applied twice daily to treat different groups of animals respectively.

Animals

Albino rats of either sex (weight 150- 200 g), procured from National Institute of Nutrition, Hyderabad, India were used for the present study. The study protocol was approved by the institutional ethical committee. Animals were housed in polypropylene cages (4 per cage) with dust free rice husk as a bedding material under laboratory conditions (under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (Mhaveer enterprises146/1999/CPCSEA)) with control environment of temperature 28±2^{0}C, humidity (60%±10%) and 12 light/dark cycle. They were fed *ad libitum* with rodents chow and given free access to drinking water. Before subjecting them to experimentation, the animals were given a week time to get acclimatized with laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee (Reg no:TCPS-wgl /2012/006).

Wound healing Activity

Excision wound model

The animals were divided into four groups each group contains six animals.

- Group I served as control
- Group II served as standard treated with povidone iodine ointment topically
- Group III served as test group treated with methanolic extract of *Z. jujuba* (5%w/w)
- Group IV served as test group treated with methanolic extract of *Z. jujuba* (10%w/w)

Then, for induction of anesthesia in rats in order to create a separate surgical wounds, Zylazine 2% to pre-anesthesia with 10mg/kg dose and then 10% ketamine drug with 40 mg/kg dosage were used for total anesthesia (I.M injection).The back of animals were shaved and sterilized with 70% ethanol before 7x7mm excision wound was created by a surgical blade from a pre-determined shaved area on the back of each animal[11].The wound was left undressed to the open environment and no local or systemic antimicrobial agents were used. This model was used to monitor the rate of wound contraction. The experimental groups were topically applied with the methanolic extracts of *Z. jujuba* twice daily for consecutive 24 days. The group treated with povidone iodine drug served as a reference. A progressive decrease in the wound area was monitored periodically at every 8th day interval. The wound contractions were measured by a tracing paper on the wounded margin and calculated as percentage reduction in wound area .The actual value was converted into percentage value taking the size of the wound at the time of wounding as 100%. The area of the wound contraction was measured in different treated and control group on 8th, 16thand 24th day.

The granulation tissues were removed on the 16th and 24th post wound days and analyzed for protein content (collagen).

Estimation of total protein

Total protein concentration on the regenerated tissues from the healed lesions of wound sample was determined[12].

Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Comparison between control and drug treated groups were considered to be significant. All values are expressed as mean ± SEM.

Table 1: Effect of methanol bark extract of *Z. jujuba* on wound surface area of experimental group of rats

Groups	Treatment	Wound surface area (mm^2)			
		0day	8th day	16thday	24thday
I	Control	360.12 ± 0.21	275.56 ± 0.46	193.75 ± 0.28	92.35 ± 0.63
II	Povidone iodine10%W/W	254.64 ± 0.38***	138.47 ± 0.81***	12.34 ± 0.43***	0***
III	*Z.jujuba* 5%W/W	261.59 ± 0.71***	146.28 ± 0.45***	62.35 ± 0.89***	5.66 ± 0.48***
IV	*Z.jujuba* 10%W/W	258.29 ± 0.69***	141.38 ± 0.93***	49.46 ± 0.68***	0***

Each value represents mean ± S.E (n=6) and was analysed by ANOVA Tukey-Kramer multiple comparison test.* P<0.05, **P<0.01, ***P<0.001. *Z.jujuba* treated groups (III and IV) and standard group (II) were compared with control group (I).

Table 2: Effect of methanol bark extract of *Z. jujuba* on percentage of wound contraction of experimental group of rats

Group	Treatment	Percentage of wound contraction (original wound area 500mm^2)				
		2nd Day	4th Day	8th Day	16th Day	24th Day
I	Control	5.23 ± 0.35	29.83 ± 0.12	46.30 ± 0.65	62.24 ± 0.37	82.01 ± 0.71
II	Povidone iodine10%w/w	20.83 ± 0.46	49.89 ± 0.49	72.75 ± 0.09**	87.57 ± 0.29***	100 ±0.0***
III	*Z.jujuba* 5%w/w	13.77 ±0.63	48.35 ± 0.79	71.12 ± 0.39**	80.69 ± 0.17**	89.88±0.27***
IV	*Z.jujuba* 10%w/w	16.87 ±0.75	49.09 ± 0.42	72.13 ± 0.89**	85.25 ± 0.68***	98.09 ±0.0***

Each value represents mean ± S.E (n=6) and was analyzed by ANOVA Tukey-Kramer multiple comparison test.* P<0.05, **P<0.01, ***P<0.001. *Z. jujuba* treated groups (III and IV) standard group (II) were compared with control group (I).

Table 3: Effect of the methanol bark extract of *Z. jujuba* on wound protein concentration in rats

Groups/treatment	% protein concentrations after days		
	8th day	16th day	24th day
Wound control	17.63±0.76*	20.10±0.25	ND
Povidone iodine	18.13±0.17*	30.17±0.13	ND
Z.jujuba extract (5%w/w)	31.03±0.07	35.05±0.07	ND
Z.jujuba extract(10%w/w)	34.00±0.26	37.02±0.23	ND

Each value represents Mean±S.D, Mean with the same alphabet in the column are not statistically significant (P<0.05), ND =Not determined –wound completely closed.

RESULTS

In the present study, the rate of wound contraction by excision wound model was studied and the area of wound healing in mm^2 is given in table 1. The percentage of wound contraction includes by recording the changes in wound area at fixed intervals of time, (Table 2) Viz. 2nd, 4th, 8th, 16th, and 24th day after treated with methanolic extract. However, on 16th post wounding day, Group I animal showed 62.24% of healing, which may be due to self immunity of animal whereas the extract treated group (Group IV) showed 85.25% healing, and on 24th day 82.01%, 98.09% respectively. When obtained results were compared with control, the activity of the extract was found to be highly significant (P<0.001). The extracts' healing activity is comparable to the reference standard (Povidone iodine).

The observed increase in protein (collagen) (Table 3), an important constituent of extracellular matrix in the treated animals confirmed that the extracts had positive effects towards cellular proliferation, granulation tissue formation and epithelization. The increase in protein content in the treated group is predominantly due to enhanced collagen synthesis in the methanol bark extract of *Z. jujuba* treated groups.

DISCUSSION

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissues as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In the maturational phase, the final phase of wound healing, the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema and new small blood vessels. The methanol bark extract of *Z. jujuba* demonstrated a significant increase in protein and wound closure. Any one of the phytochemical constituents present in methanol bark extract of *Z. jujuba* may be responsible for the wound-healing activity. Recent studies with other plant extracts have shown that phytochemical constituents like flavonoids[13] are known to promote wound healing process mainly due to their astringent and antimicrobial properties, which appears to be responsible for wound contraction and increased rate of epithelization. The wound-healing property of methanol bark extract of *Z. jujuba* may be attributed to the phytoconstituents present in the plant and the faster process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. Tannins for instance ranked third in quantity among the Phytochemical estimated in *Z. jujuba*. Tannins are astringent and antimicrobial in property, hence it can be inferred that the wound healing activity of the methanol bark extract of *Z. jujuba* observed is due partly to its tannin and flavonoids contents, which seems to be responsible for wound contraction and increased rate of epithelization.

CONCLUSION

From the results of the present study it can be inferred that methanolic extract of *Ziziphus jujuba* bark is an effective wound healing agents. While comparing the *Ziziphus jujuba* bark extract 5%w/w, the 10%w/w showed significant wound healing activity when topically administered on rats. These results offer pharmacological evidence on the traditional use of *Ziziphus jujuba* bark for healing wounds. Further studies are needed to better assess the potential value of *Ziziphus jujuba* methanolic bark extracts as wound healing agents.

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