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Original Article

WOUND HEALING POTENTIAL OF YOUNG LEAVES OF *TRITICUM AESTIVUM* ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Objective: Wheatgrass is known to have several medicinal properties to treat various disorders. *Triticum aestivum* Linn. is a traditional medicinal plant apparently used for bowel disorders, skin inflammation, infection, mouth sores and wound healing. It is also used for conditions as varied as headache to a folk remedy for diabetes. No data was found for the simultaneous use of it for wound healing and diabetes. The present study was designed to study the wound healing activity with the complications of diabetes. The objective is to help those people suffering from diabetes and not getting relief from pain and wounds.

Methods: The 5% methanolic extract ointment of *Triticum aestivum* young leaves were evaluated for its wound healing activity in alloxan induced diabetic rats using excision wound model. The percent wound closure and their epitelialisation periods were observed for 16 days.

Result: The wound treated with *Triticum aestivum* grass extract was healed in very efficient manner and the healing was very close to the standard intadine.

Conclusion: The *Triticum aestivum* young leaves extract has shown similar wound healing property as compared with intadine. The quercetine and other grass factors present in the extract may be responsible for promoting the wound healing activity.

Keywords: Diabetic induced wound healing, Excision wound model, Alloxan, *Triticum aestivum,* Wheatgrass.

INTRODUCTION

Wheatgrass has been used from ancient times as a medicine for the betterment of human health. Apart from being used as a food and nowadays gaining importance as medicine for many dreaded diseases. It has been shown to reduce blood transfusion requirements in patients with β thalassemia [1]. Fernandes and Donovan reported the beneficial effects of wheatgrass juice in patients with hemolytic anaemia [2]. Wheatgrass extract induces the production of fetal hemoglobin [3]. Improvement in hemoglobin, total protein and performance were observed in terminally ill 400 cancer patients after administration of its juice [4]. The treatments of suppurative wounds burn, hepatocellular carcinoma and ulcerative colitis through wheatgrass have been reported [5-8]. CN Lai used wheat sprout extract in inhibiting the metabolic activation of carcinogens *in vitro* [9]. Wheatgrass was also used as a potent antibacterial and anti-inflammatory agent. However, by far the most important attribute of wheatgrass is its ability to generate a layer of new epithelial cells to cover the wound surface within 24-36 hours. Wheatgrass have been known to contain "growth factors" capable of promoting rapid re-epithelialisation of acute wounds and burns [10-15]. Hypoglycemic effect of wheatgrass juice in alloxan induced diabetic rats has also been reported by Shaikh *et al* [16]. Wheatgrass has also been studied for its oxidative stress in Type 2 diabetes [17]. Wheatgrass juice has been proven over many years to benefit people in many ways as the formation of blood, restoring balance in body, removing toxic material from cells, nourishing the liver and kidney and restoring vitality [18]. Based on these evidences the present study was designed, to evaluate ninth day *Triticum aestivum* young leaves for its wound healing potential with the complications of diabetes which itself is a dreaded disease.

MATERIALS AND METHODS

Procurement of seeds and authentication of the plant material

The Wheatgrass seeds for the research were purchased from Breeder Seed Production Unit Field crops, Department of Plant Breeding and Genetics, Jawahar Lal Nehru Krishi Vishwavidyalaya, Krishinagar, Jabalpur M.P. and the release order number was obtained. The whole plant of *Triticum aestivum* was collected in the month of December and authenticated at Safia college of Science Bhopal, Madhya Pradesh. The herbarium of the plant was prepared and the voucher specimen number 236/BOT/SAFIA/2011 was obtained.

Preparation of *Triticum aestivum* powder

The leaves of *Triticum aestivum* was cultivated, collected at the ninth day. It was dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve no.40 and stored in a labeled air tight container for further studies.

Preparation of extract

Maceration process involves separation of medicinally active portions of the crude drugs. It is based on the immersion of the crude drugs in the bulk of solvent or menstrum. Solid drug material was taken in a stoppered container with about 750 ml of the methanol and allowed to stand for seven days in a warm place with frequent shaking. The mixture of crude drug containing solvent was filtered until most of the liquid drains off. The filtrate and the washing were combined to produce 1000 ml of the solution. This solution was kept aside for solidification and was dried on hot air oven for 30 mins at 40ºC. The extract is then collected in a dark colored bottle [19].

Animal care and handling

The wound healing activity was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 100-150 gm. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hrs, feed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the Institutional Ethics Committee and as per CPCSEA guidelines (approval no. 1413/PO/a/11/CPCSEA).

Formulation of the ointment

5% w/w ointment of methanolic extract of *Triticum aestivum* was prepared by using Carbopol 934P. Carbopol 934P is high cross link

water swellable acrylic polymer. 25 gm of carbopol was allowed to swell in distilled water over night at room temperature then added 0.5 gm of methanolic extract of *Triticum aestivum* and mixed vigorously**.** Sufficient amount of water was then added for better cream like preparation. Finally ointments were prepared and stored in cool place.

Induction of experimental diabetes

Overnight fasted albino rats were made diabetic by injecting alloxan monohydrate (150mg/kg i.p.)**.** Blood was drawn after 72 hrs from the eye and the glucose level was estimated using glucometer (Gluco chek). Wounds were made on the rats showing elevated blood glucose (> 190 mg/dl). Blood glucose levels were estimated at the time of creation of the wounds [20].

Experimentally induced excision wounds

Wounds were created on the 3rd day after induction of diabetes in all rats. The animals were anesthetized by diethyl ether. The skin shaved by electrical clipper, disinfected with 70% alcohol and injected with 1 ml of Lignocaine HCl (2%, 100mg/5 ml). An area of uniform wound 2.00 cm in diameter (circular area = 3.14 cm^2) using circular stamp, was excised from the nape of the dorsal neck of all rats with the aid of round seal as described by Morton and Melone (1972) with slight modification[20]. Incision of the muscle layer was avoided and tension of skin was kept constant during the procedure. The wound area was measured immediately under light diethyl ether anesthesia by placing a transparent tracing paper over the wound and traced out. The tracing paper was placed on 1 mm^2 graph sheet and traced out. The wound area and epithelialisation period was recorded as described by Chah *et al*. (2006) with slight modification [21].

Grouping: In the experiment, a total of 24 rats were used. The rats were divided into 4 groups comprising of 6 animals in each group as follows:

Group I: Left untreated and considered as control (No diabetes).

Group II: Served as negative control, diabetes + wound, Left untreated.

Group III: Served as standard, diabetes + wound, treated with 5%w/w ointment

Intadine applied once a day.

Group IV: Served as test, diabetes + wound, treated with 5%w/w ointment of

Triticum aestivum extract applied once a day.

Statistical analysis

All the values are expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and Dunnett's multiple comparison test by employing statistical software, Graph Pad In Stat 3. Differences between groups were considered significant at $P < 0.05$ levels

Table 1: Effect of *Triticum aestivum* extract on rats wound

Groups	Treatment	Percent Wound Closure (mm)^2				Epithelisation period (Days)
		4thday	8thday	12thday	16thday	
I. Control	Untreated	20.43±0.8	56.17±0.32	65.51±0.67	82.32±0.62	19.29±0.43
II. Negative control	Diabetic wound	13.57±0.36 a*	30.26±0.33 a*	48.34±0.62 a*	74.53±0.76 a*	21.24±0.37a*
III. Standard	Intadine ointment	35.93±0.24 a*,b**	77.47±0.32 a*,b**	92.14±0.35 a**,b**	100.00±0.00 a*,b**	13.66±0.25 a**,b**
IV. Test	*Triticum aestivum* extract ointment	28.23±0.14 a*,b**,c*	68.67±0.26 a*,b**,c*	82.64±0.43 a*,b**,c*	91.33±0.47 a*,b**	16.41±0.26 a*,b**,c*

Data are expressed in mean ± SEM, n= 6 (no. of six animals) in each groups,*p<0.05, **p<0.01, compared with multiple group using One-way ANOVA followed by Dunnett's multiple comparison test.

a = significant difference in compared with untreated group.

b= significance difference in compared with negative control (Diabetic wound) group.

c= significance difference in compared with standard drug treated group.

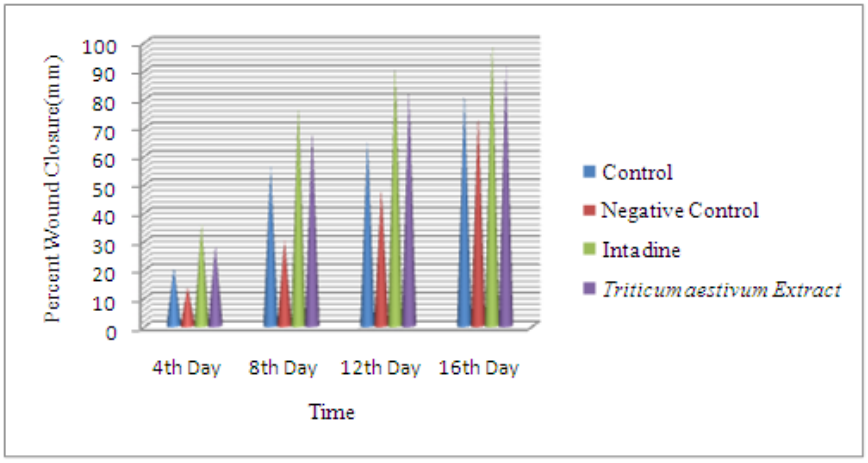

Fig. 1: Effect of *Triticum aestivum* extract ointment on rats wound

Fig. 2: 0day

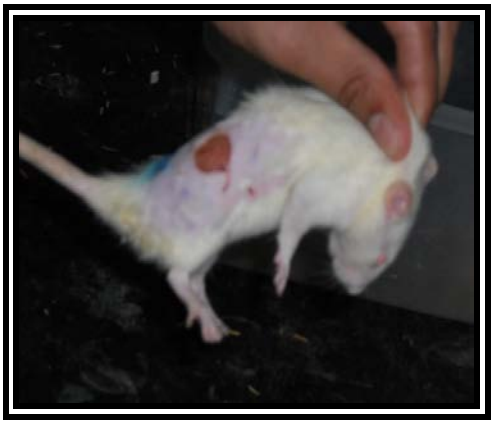

Fig. 3: 4th day

Fig. 4: 8th day

Fig. 5: 12th day

Fig. 6: 16th day

Fig. 2-6 Untreated Rats with Wound

Fig. 7: 0day

Fig. 8: 4th day

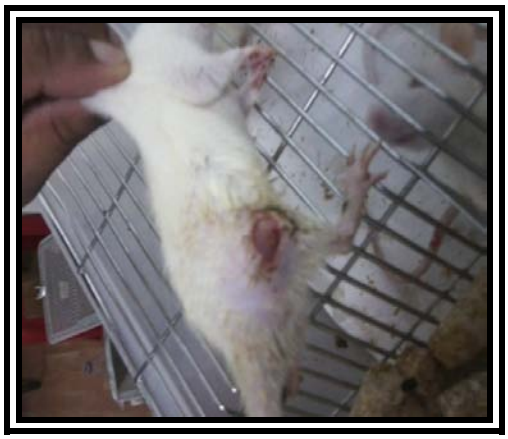

Fig. 9: 8th day

Fig. 10: 12th day

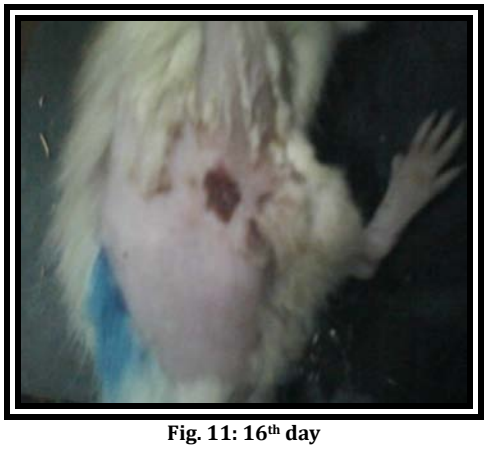

Fig. 11: 16th day

Fig. 7-11 Diabetic Wound Rats.

Fig. 12: 0day

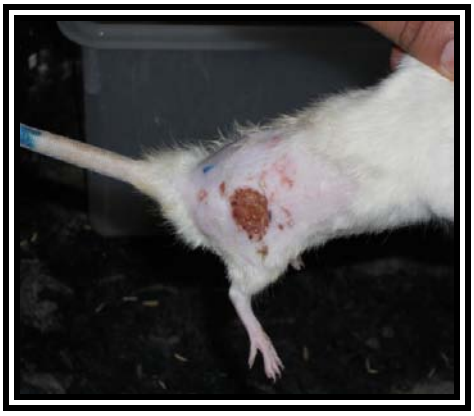

Fig. 13: 4th day

Fig. 14: 8th day

Fig. 15: 12th day

Fig. 16: 16th day

Fig.12-16 Intadine Ointment Treated Rats with Wound.

Fig. 17: 0day

Fig. 18: 4th day

Fig. 19: 8th day

Fig. 20: 12th day

Fig. 21: 16th day

Fig. 17-21 *Triticum aestivum* Extract Ointment Treated Rats with Wound.

RESULTS AND DISCUSSION

The excision wound model was used to evaluate the wound healing potential of topically applied 5% ointment of *Triticum aestivum* extract. The extract significantly increased the rate of wound closure and rate of epithelialisation. The mean percentage wound closure was calculated on the 4th, 8th, 12th and16th wounding days as shown in Table-1 & Figure 1. The extract treated animals showed faster epithelialisation of wound (16.41±0.26) then the diabetic wound (21.24±0.37). The period of epithelialisation was almost similar (13.66±0.25) to that of standard drug 5% intadine ointment.

From the results of these animal studies it is apparent that significant increase in the wound healing was observed in *Triticum aestivum* extract treated rats. In excision wound model, animals of groups III and IV showed a decrease in the epithelialization period and increased rate of wound closer when compared with the animals of groups I and II (Table-1 & Figure 2-21). The faster rate of wound closure by *Triticum aestivum* extract may be possibly due to the presence of the quercetine and other grass factors. The antibacterial, immunomodulator and antioxidant potential of extract may also support in faster wound healing. The enhancement of collagen formation by extract may also increase the rate of wound closure.

CONCLUSION

The *Triticum aestivum* extract has shown similar wound healing property as compared with intadine. The quercetine present in the *Triticum aestivum* extract may be responsible for promoting the wound healing activity. The topically applied 5% ointment of wheatgrass extract significantly increased the rate of wound closure and rate of epithelialisation. Marked increase in thickness of granulation tissue in *Triticum aestivum* extract treated animals in present study showed that *Triticum aestivum* has definite wound healing properties. The diabeties induced wound healing model proved that it can be a strong candidate for the treatment of both diseases diabetes and wound healing as well as for the patients suffering from wound previously having diabetes.

CONFLICT OF INTERESTS

Declared None

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