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Original Article

In vivo Performance of a Hydrogel of *Tribulus terrestris* in Alloxan induced Diabetic Rats

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ABSTRACT

Background: Diabetes is one of the most important factors in chronic injuries. The gold standard for treating diabetic foot ulcers includes debridement of wounds, management of all infections, revascularization when indicated, and non-loading of ulcers. Traditionally, the aerial parts of *Tribulus terrestris* L. (Zygophyllaceae) are used in the folklore for the treatment of various kinds of wounds**. Materials and Methods:** Based on the evaluation of phytochemicals, a hydrogel of the hydroalcoholic and the aqueous of *T. terrestris* were prepared in this study. It was subjected to evaluation of the different parameters. The hydrogel of hydroalcoholic extract and aqueous extract was brown in color, uniform and non-irritant. It was further evaluated for pH, spreadability and viscosity. Furthermore, the *in vivo* performance of the hydrogel was evaluated in alloxan monohydrate induced diabetic rats. **Results:** In excision wound model and incision wound model topical application of 5% hydroalcoholic extract hydrogel in diabetic Wistar albino rat showed remarkable repair effects. It significantly (***$p<0.001$) enhanced the rate of % wound contraction. It also exhibited significant (***$p<0.001$) content of hydroxyproline and extremely high tensile strength (***$p<0.001$) as compared to the diabetic control group. It may be due to enhanced collagen synthesis. The histopathological examination of 5% hydroalcoholic extract hydrogel of *T. terrestris* L. treated rats showed skin architecture which was comparable to the normal group. The diabetes-induced group rats' wounds failed to heal even 25 days post the infliction of the wounds. Our model precisely reproduced the pathophysiology of diabetic ulcers and can serve as a valid alternative model for diabetic foot ulcer and to explore new therapies. **Conclusion:** The results strongly support that the hydrogel of the hydroalcoholic extract of *T. terrestris* seems to be a novel and promising biomaterial used for wound healing applications.

Keywords: Diabetes, *Tribulus terrestris*, Wound healing, Alloxan, Hydroxyproline, Hydrogel.

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INTRODUCTION

The prevalence of diabetes is rising worldwide.[1] In 2017, 425 million people were diagnosed with diabetes and are expected to reach 629 million in 2045.[2] Several organ failures are the end of long-term hyperglycemia.[3] Diabetes is the main cause of diseases and deaths in developing and developed countries, and the burden on health services is heavy. Among the different complications, those related to diabetes are the most serious and fatal diseases.[4] Foot ulceration is the most frequently recognized complication of Diabetes 2 and 6% of people with diabetes have the tendency to multiple complications such as diabetic foot ulcer (DFU). Approximately 25 per cent of diabetics develop foot ulcers throughout their lives.[5]

Foot ulcers are the most common serious problems for diabetic patients. The main etiological factors involving foot ulcers are neuropathy and peripheral vascular diseases. Both factors either work together or work together with combinations of other factors, such as biomechanical abnormalities, microvascular disease, limited joint mobility, and increased susceptibility to infection. Due to diabetic foot ulcers, major medical, social and economic problems worldwide are caused.[6,7] The problem of foot ulcers in diabetic patients increases the morbidity level. The risk of diabetic foot ulcers increases with age, duration, and the most shocking impact is amputation at low extremity.[8]

Foot ulcers healing are complex process that is interrupted by many local factors such as moisture, infection, and the dressing method along with systemic factors such also. The effective management

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of Diabetic foot ulcer starts with physical examination and selection of an appropriate wound care intervention.[9]

The treatment strategies for diabetes mellitus ulcers include metabolic control of diabetes mellitus, elimination of infection, promotion of ulcer healing and elimination of pressure from ulcers.[10,11] Now-a-days antibiotics, anti-inflammatory agents and exosomes also use to counter chronic inflammation and accelerate the healing process, but unforeseen problems, such as drug resistance, negative side-effects, and high costs, have been observed.[12,13]Approximately 20% of moderate or severe diabetes foot infections lead to a certain degree of amputation. Mortality after amputation related to diabetes exceeds 70% in 5 years for all diabetes patients.[2]

Since ancient times, herbal remedies have been widely used for prophylaxis and treatment. Herbal medicine offers different advantages related to low cost, safety and availability.[14]

Traditional herbal medicine is more inclined to holistic thinking than most modern pharmacological research. The multiple components and targets of traditional medicine have become a stumbling block in the study of drug action mechanisms in the life sciences. Traditional herbal therapy is more oriented on the concept of wholeness than the majority of current pharmaceutical studies. The interaction of herbal products and drugs is an important safety issue for drugs with narrow therapeutic indices or for patients receiving drugs for chronic diseases and diseases. A chronic wound is typified by excessive inflammation and a retardation of subsequent phases of repair with no significant progress towards healing for at least 4 weeks.

T. terrestris L. is a traditional herbal medicine. Through the literature survey it was revealed that the diabetic wound healing property of *T. terrestris* L. in excision and incision wound models was not performed. The selection of plant *T. terrestris* L. was based on various activities like wound healing,[15] antihyperglycaemic,[16] antioxidant,[17] antimicrobial,[18] and anti-inflammatory activities, etc.[19]

In the current study, we inflicted wounds on diabetic rats manually by using excision and incision wound model. Hence, this study was aimed to evaluate the wound healing property of *T. terrestris* L. in diabetic rats. To evaluate diabetic wound healing activity, hydrogel of hydroalcoholic and aqueous extract of dried aerial parts was prepared and applied on excision and incision wounds on Wistar albino rats. The present investigation provides a novel medicated herbal hydrogel formulation comprising of pharmacologically potent extract, from the plant *T. terrestris* L. effective for patients in which wound healing is delayed due to type-2 diabetes.

MATERIALS AND METHODS

Plant Material

The dried crude aerial parts of selected plant *T. terrestris* L. were collected from local market of Bhopal. The dried aerial parts were identified and authenticated by a Botanist, Dr. Zia Ul Hassan, at Safia College of science, Bhopal (Voucher specimen no. 460/Bot/Safia/18). The dried crude material was dried under shade and materials were grounded to a coarse powder and stored in air tight container.

Extraction and Phytochemical Analysis

The coarse powdered aerial parts (372gm) of *T. terrestris* L. were subjected to successive extraction with different solvents i.e., petroleum ether (40-60°C), ethyl acetate, hydroalcoholic (50:50), and distilled water by Soxhlet apparatus. Each extract was concentrated also by Soxhlet apparatus and evaporated to dryness on water bath. Percentage yield of each extract was calculated. The yield was found to be 8.23gm (2.21%) of petroleum ether extract, 13.28 gm (3.69%) of ethyl acetate extract, 37.23 gm (10.82%) of hydroalcoholic extract and 29.96 gm (9.79%) of aqueous extract.

Phytochemical analysis

Each dried extract of the aerial parts was analyzed for the presence of phytoconstituents such as carbohydrates, proteins and amino acids, saponins, flavonoids, glycosides, tannins, steroids and alkaloids.[20]

Preparation of hydrogel

To prepare the hydrogel of hydroalcoholic and aqueous extract, sodium metabisulfite, methyl paraben sodium, and propyl paraben sodium were dissolved in water.[21] A gelling agent (Carbopol 934) was added, and the mixture was stirred continuously until it became swollen completely. https://www.sciencedirect.com/topics/medicine-and-dentistry/triethanolamine was slowly added to the dispersion with continuous stirring that resulted in a stiff gel. Next hydroalcoholic extract (5g for 5% and 2.5gm for 2.5% hydrogel) and aqueous extract (5gm for 5% and 2.5gm for 2.5% hydrogel) was added, and stirring continued for 15 min. Volume was made up with water, and the mixture was stirred continuously until uniform two different hydrogel preparations were obtained.

Evaluation of hydrogel

pH measurement

1% solution of hydrogel was prepared in distilled water and pH was determined by digital pH meter (Laquatwin pH meter, Horiba).[22]

Viscosity

The viscosity of hydrogel was determined as such without dilution by R/S CPS plus Rheometer (Brookfield viscometer) using spindle 07 at RPM 100https://www.sciencedirect.com/topics/medicine-and-dentistry/triethanolamine.[23]

Homogeneity

It was tested by visual inspection. It was performed for physical appearance and presence of any aggregates in hydrogel.[24]

Spreadability

To determine the spreadability of formulation, 0.5 g of gel was placed within a circle of 1 cm diameter pre-marked on a glass plate of 20 × 20 cm, over which a second glass plate was placed. A weight of 500 gm was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted.[25]

Test of skin irritancy

The six Wistar albino rats of either sex weighing 150-250gm were used for this test. The intact skin was used. The hair was removed from the dorsal area of rats 3 days before the experiment. The test preparation was applied on the specified area. The Wistar albino rats was treated daily up to seven days and the treated skin area was examined visually for any visible reaction or erythema.[26]

Animals

Healthy Wistar albino rats of either sex 150 to 250 gm weight or 12 to 18 weeks age were selected for the study. The Wistar albino rats kept under 12:12 hr day and night schedules with temperature between 18 to 20°C. They were housed in large spacious hygienic cages during experimental period. They were fed with standard rat pellet diet and free access to water. Animal study was performed in pharmacology division with due permission from Institutional Animal Ethical Committee (CPCSEA protocol no. PH/IAEC/VNS/2K14/006).

Acute Skin toxicity

Following the Guideline number 434 (2004) of Organization for Economic Co-operation and Development (OECD), an acute skin toxicity test of the hydrogels was carried out in albino rats. The rats were divided into groups comprising 6 animals in each group. The hydroalcoholic and aqueous extracts (2000 mg/kg b.w. of rats) were tested in each group for 24 hr. All the rats were observed for dermal reactions for the initial 30 min., 4 hr, 24 hr and later daily for next 15 days.[27]

Experiment protocol

The study was performed using two wound models i.e., excision wound model and incision wound model.

Induction of diabetes by using alloxan monohydrate

Diabetes was induced by single intraperitoneal injection of 120mg/kg alloxan monohydrate in saline to overnight fasted Wistar albino rats. After dosing Wistar albino rats were kept for the next 24 hr on 10% fructose solution bottle, in their cases to prevent hypoglycemia then after 72 hr of injection fasting blood glucose level was measured. Wistar albino rats have an elevated blood glucose (>250 mg/dL) was selected for creation of wounds model.[28]

In both the models, Wistar albino rats were divided into six groups and each group contained six animals.

Group I-Normal control group rats were treated with hydrogel base. Group II-Diabetic control group rats were treated with hydrogel base. Group III- Diabetic rats treated with 2.5% aqueous extract hydrogel of *T. terrestris*. Group IV-Diabetic rats treated with 2.5% hydroalcoholic extract hydrogel of *T. terrestris*. Group V-Diabetic rats treated with 5% hydroalcoholic extract hydrogel of *T. terrestris*. Group VI-Diabetic rats treated with 5% aqueous extract hydrogel of *T. terrestris*.

Animals were treated once daily with the hydrogel and the healing property was assessed in terms of physical, biochemical, and histopathological studies.

Excision wound model

Excision wounds were created after confirmation of diabetes (fasting blood glucose level >250 mg/dL on the 3rd day. All Wistar albino rat were anaesthetized prior to the development of the wounds via intraperitoneal injection of ketamine (50 mg/kg body weight) and xylazine (5 mg/kg body weight). The dorsal skin area was shaved 1 day prior to the induction of wounds. The wounds were inflicted by cutting away 7 mm^2 full thickness of skin from a predetermined shaved area by using toothed forceps, a surgical blade and pointed scissors.

Wounds were left undressed to the open environment and they were treated once daily with the drugs from day 0 (day of wounding) to 22nd post wound day. In this model, % wound contraction, epithelialization period, and hydroxyproline content was evaluated, and histopathological examination was done. The % wound contraction was measured every 2nd day, and the period of epithelialization was calculated on the complete removal of scar tissue. Hydroxyproline content evaluation was estimated by using tissue sample on a 0th day and 22nd post wounding day and histopathology were performed by using tissue sample on 22nd post wounding day of experimental rats.[29]

Incision wound model

Incision wound model was created after confirmation of fasting blood glucose level >250 mg/dL by on the 3rd day. All rats were anaesthetized prior to the development of the incision wounds via intraperitoneal injection of ketamine (50 mg/kg body weight) and xylazine (5 mg/kg b. w.). The dorsal area of rats was shaved 1 day prior to the induction of wound and 3 cm long full thickness paravertebral straight incision was made by a surgical blade. Wounds were closed with intermittent sutures, 1 cm apart, with black silk thread and the curved needle. Animals were treated once daily with the drugs from day 0 (day of wounding) to 11th day after post wounding. Sutures were removed on day 10. On day 11, tensile strength of the wound was measured by tensiometer.[30]

Wound healing evaluation parameters

% Wound contraction

% Wound contraction was measured by digital vernier calliper every two days interval of each animal of control and treatment group. The rate of wound healing was calculated % wound contraction formula.[30]

Epithelization period

Epithelization period was monitored by noting the number of days required for Escher to fall away, leaving no raw wound behind.[30]

Hydroxyproline estimation

For the determination of Hydroxyproline content in tissue, wounds tissue was excised from the Wistar albino rats at the day of wound creation and on 22nd post wounding day. Excised tissues were vacuum dried at 50°C for 24 hr and transfered into test tube then add 6*N* Hydrochloric acid. The tubes were then kept on boiling water bath for 24 hr (12 hr each day for two days) for hydrolysis. The hydrolysate was cooled and neutralized by 10*N* Sodium hydroxide. Chloramine solution was added and stands for 20 min for oxidation. Oxidation reaction was terminated by the addition of 3.15M perchloric acid solution. P-Dimethylbenzaldehyde solution was added and mixed thoroughly. Heated on water bath at 60°C for 15 min. and cooled under running tap water for 5 min. The absorbency of solution was determined spectrophotometrically at 557 nm.[30]

Tensile strength

Tensile strength was measured on 11th post wounding day by using tensiometer. For the measurement of tensile strength, the Wistar albino rats were anesthetised using diethyl ether. The sutures of wound were removed. The Wistar albino rat was placed on the centre of tensiometer board. One side of wound tied with thread and other side of wound adjusted with empty weighing balance pan hanging with thread. One by one weights were put on pan until wound began to open up. Total weight on pan was considered as the tensile strength of the wound.

$$\% \text{ Wound contraction} = \frac{\text{Initial wound size-specific wound size}}{\text{Initial wound size}} \times 100$$

Histopathology

Wound tissue sample was isolated from each group on day 22nd after wounding day for histopathological examination. The tissue sample was immediately fixed in 10% neutral buffered formalin solution. Fixative solution was replaced every two hours until the tissue hardened. Each sample was embedded in the paraffin block and thin section (3µm) was prepared. Then each slide stains with hematoxylin and eosin (H&E) for general morphological examination.[30]

Statistical analysis

The result was represented as mean±SEM. The statistical significance was evaluated by Dunnett's test method of One-way ANOVA.

RESULTS AND DISCUSSION

Phytochemical studies

Phytochemical studies disclose the presence of essential phytoconstituents which is considered as active ingredients.

It was noted that petroleum ether fractionate of *T. terrestris* contains sterols, fats and oils; ethyl acetate extract consists of sterols, phenolic compounds, alkaloids; hydroalcoholic extract gave affirmative tests for carbohydrates, alkaloids, flavonoids, phenolic compounds, saponins and tannins and aqueous extracts showed the presence of alkaloids, phenolic compounds, carbohydrates, proteins, flavonoids, tannins and saponins.

Acute skin toxicity

The application of hydroalcoholic and aqueous hydrogel preparations at 2000 mg/kg body weight of rats showed no signs of toxicity and mortality after observing for 14 days in both the treated and control animals.

Hydrogel Formulation and Evaluation

The hydrogels of hydroalcoholic and aqueous extracts were prepared were homogeneous and brownish in appearance. The results of other evaluation parameters are presented in Table 1.

% Wound contraction

Topical application of 5% hydroalcoholic enhanced the rate of % wound contraction on 10th day extract hydrogel in diabetic Wistar albino rats significantly (***p<0.001) whereas 2.5% hydroalcoholic extract hydrogel, 5% aqueous extract hydrogel and normal wound control groups showed significant (**p<0.01)

Table 1: Evaluation of hydrogels of hydroalcoholic and aqueous extracts.

Sl. No.	Hydrogel	pH	Viscosity (cps)	Spreadability (cm^2)
1	2.5% Aqueous extract	6.5±0.02	212645	7.6±0.06
2	5% Hydroalcoholic extract	6.9±0.05	243350	7.9±0.15
3	2.5% Hydroalcoholic extract	6.6±0.03	26240	8.1± 0.20
4	5% Aqueous extract	6.6±0.02	198654	7.3±0.10

Table 2: % Wound contraction in different groups.

Post wounding Day	Group I	Group II	Group III	Group IV	Group V	Group VI
0	0	0	0	0	0	0
5	19.1±0.7	17.2±1.8	17.3±1.3	21.2±2.5	20.2±2.0	23.1 ± 4.1
10	46.281.8	36.4±1.6	38.7±2.0	49.3±2.5*	55.6±1.9**	49.8 ± 2.8*
15	79.5±2.4*	65.1±2.2	72.7±4.2	81.6±2.2*	86.2±2.1**	79.6 ± 2.7*
20	100±0**	83.6±5.1	95.6±4.2*	87.2±1.1**	100 ± 0**	100 ± 0**
25	-	86.4±1.8	100±0*	100±0** -	-	-

n=6 Wistar albino rat per group; values are represents Mean ± SEM, * = *P* <0.05, ** = *p*<0.01, *** = *p*<0.001 by Dunnett's test method of One-way ANOVA (comparison of group II with Group I, Group III, Group IV, Group V and Group VI).

result on 15th day and 2.5% aqueous extract hydrogel showed significant (*p<0.05) result on 20th day as compared to diabetic control group which showed poor wound contraction. The observations of % wound contraction represented on Table 2, Figure 1.

Period of epithelization

5% Hydroalcoholic extract hydrogel treated group and normal control group showed shortened (**p<0.01) period of epithelization results whereas 2.5% aqueous extract hydrogel, 5% aqueous extract hydrogel, 2.5% hydroalcoholic extract hydrogel treated group showed less significant (*p<0.05) period of epithelization results in comparison with the diabetic control group which showed poor epithelization. (Table 3)

Hydroxyproline content

Diabetic Wistar albino rats treated with 5% hydroalcoholic extract hydrogel (Group V) and normal wound control group exhibited most significant (***p<0.001) quantity of hydroxyproline as compared to the diabetic control group (Figure 2).

n=3; Wistar albino rats from each group, values are represented in mean ± SEM, *= p<0.05, **= p<0.01, ***= p<0.001 by Dunnett's test method of One-way ANOVA (Comparison of Group II with Group I, Group III, Group IV, Group V, and Group VI).

Tensile Strength

Group V (5% Hydroalcoholic extract hydrogel) exhibited extremely high tensile strength (***p<0.001) compared with diabetic wound control which exhibited poor tensile strength. Figure 3.

n=3; Wistar albino rats from each group, values are represented in mean ± SEM, *= p<0.05, **= p<0.01, ***= p<0.001 by Dunnett's test method of One-way ANOVA (Comparison of Group II with Group I, Group III, Group IV, Group V, and Group VI).

Histopathological study

The histopathological examination was done on day 22nd after wounding in various groups. The normal wound control group showed normal epithelium with large quantity of collagen than diabetic wound control. Group I rats showed normal collagen fibers, fibroblasts cells, and new blood vesicles formation. Group II rats showed ruptured blood vessels, incomplete epithelium and less collagen and fibroblasts Though, groups III, IV, V and VI rats showed more collagen and fibroblast cells and complete epithelium compared to diabetic wound control. Group V rats showed normal architecture of skin at 22nd day of wounding (Figure 4).

Diabetes mellitus is a chronic disorder that leads to delay wound healing. A chronic wound is typified by excessive inflammation and a retardation of subsequent phases of repair with no significant progress towards healing for at least 4 weeks.[31] The application of

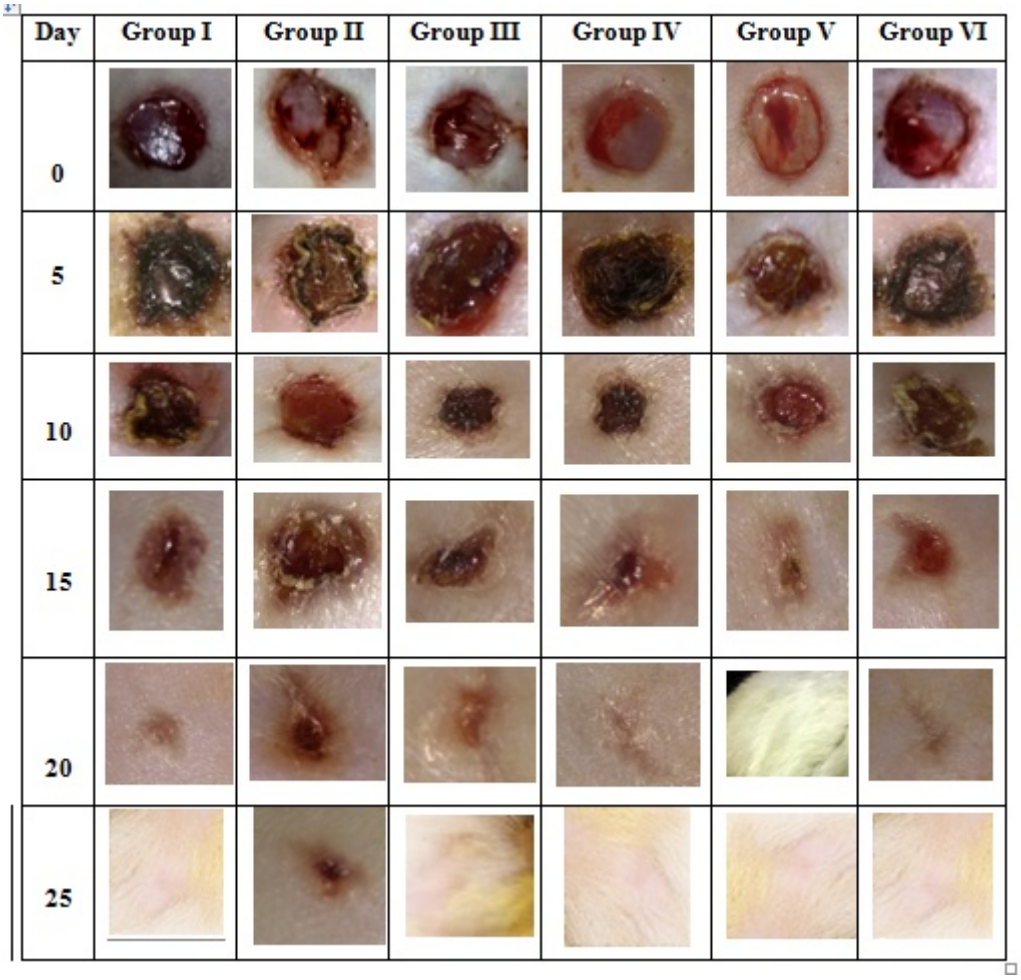

Figure 1: Photographs of wound contraction in different groups.

Table 3: Period of Epithelization.

Group	Treatment given	Period of Epithelization (mean time in day)
I	Hydrogel base (Normal rats)	14.3±0.3**
II	Hydrogel base (Diabetic rats)	18.0±1.1
III	2.5% Aqueous extract hydrogel (Diabetic rats)	15.0 ±0.5 *
IV	2.5% Hydroalcoholic extract hydrogel (Diabetic rats)	13.7 ±0.8*
V	5% Hydroalcoholic extract hydrogel (Diabetic rats)	10.7 ±0.3**
VI	5% Aqueous extract hydrogel (Diabetic rats)	14.0 ± 0.5 *

n=3 Wistar albino rat from each group, values are in mean ± SEM, *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$ by Dunnett's test method of One-way ANOVA (Comparison of Group II with Group I, Group III, Group IV, Group V, and Group VI).

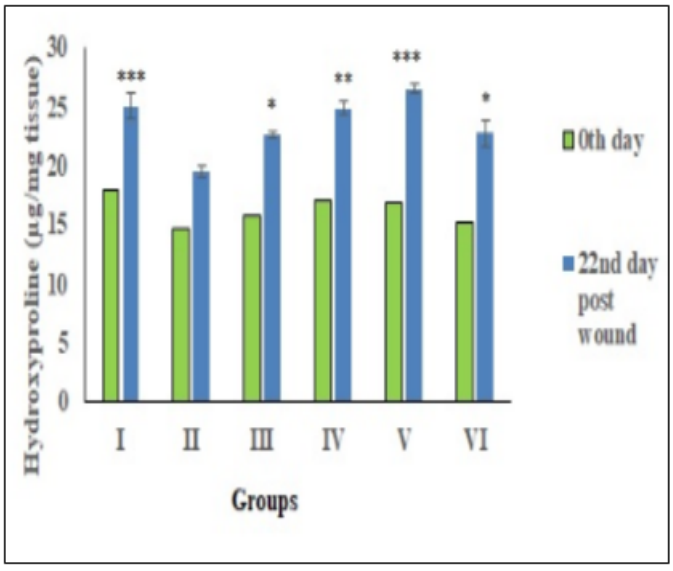

Figure 2: Hydroxyproline estimation in each group on 0th day of wound creation and 22nd day post wound creation using excision wound model.

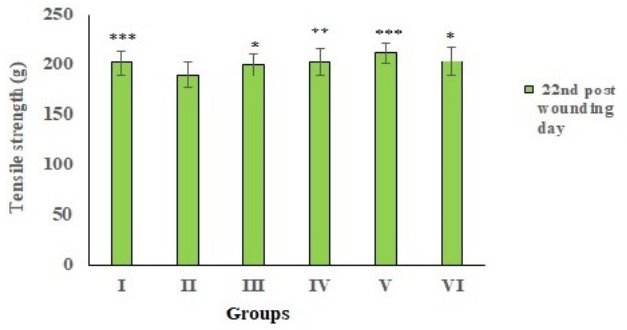

Figure 3: Estimation of tensile strength in various groups using incision wound mode.

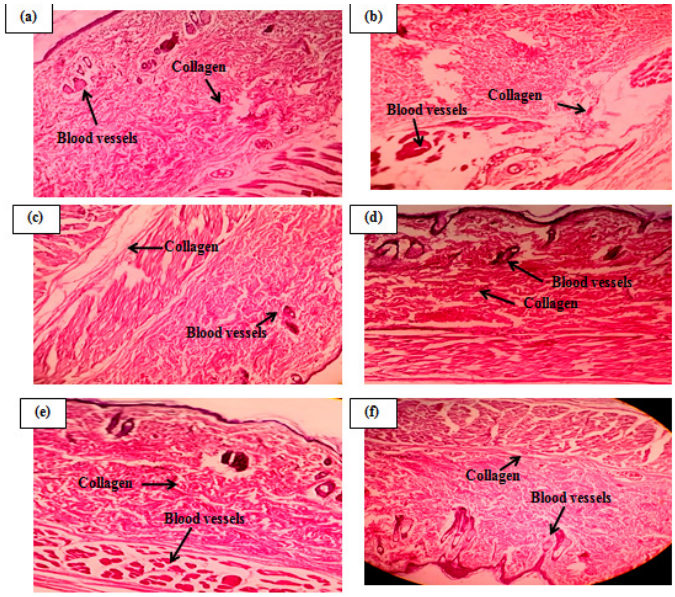

Figure 4: Histopathological examination of Group I, Group II, Group III, Group IV, Group V and Group VI rats.

herbal drug could be a beneficial approach to improve the wound healing in diabetes. A balanced moisture environment accelerate wound repair. Formulations prepared with hydrogels have this advantage over conventional wound dressing materials because of their intrinsic water content and medications can be easily incorporated in them.[32]

As evident from the results, topical application of 5% hydroalcoholic extract hydrogel in diabetic Wistar albino rats showed remarkable repair effects in excision wound model and incision wound models. It significantly (***p<0.001) enhanced the rate of % wound contraction on 14th day by shortening the period of epithelization. Group VI exhibited significant (***p<0.001) contentof hydroxyproline and exhibited significantly (***p<0.001) extremely high tensile strength as compared to the diabetic control group. It may due to enhanced the collagen synthesis. The histopathological examination also indicates 5% hydroalcoholic extract hydrogel of *T. terrestris* L. treated rats showed more profound epithelial cells growth with more collagen than diabetic wound control. The diabetes-induced group rats' wounds failed to heal even 25 days post the infliction of the wounds.

The qualitative analysis showed the presence of various pharmacologically potent phytoconstituents, (antioxidants, radical scavenging, antimicrobial, and anti-inflammatory effects) which may act individually or synergetically in wound healing. *T. terrestris* L. Even though the exact mechanism was not completely understood, it is evident that delayed epithelialization, reduced collagen deposition, decreased production of hydroxyproline and reduced tensile strength are some of the factors that show evidence of the delayed wound healing in diabetes induced rats. Our model precisely reproduced the pathophysiology of diabetic ulcers and can serve as a valid alternative model for diabetic foot ulcer and to explore new therapies.

CONCLUSION

The results obtained in this study clearly indicates that the hydrogel of hydroalcoholic extract of dried aerial parts of *T. terrestris* L. possess significant diabetic wound healing activity in Wistar albino rats. The present invention provides an effective and low-cost method for treatment of diabetic wounds and sores.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

L.: Linnaeus; **SEM:** Standard error of the mean; **ANOVA:** Analysis of variance; ***T. terrestris*:** *Tribulus terrestris.*

SUMMARY

The dried crude aerial parts of selected plant *Tribulus terrestris* L. was collected from local market of Bhopal. The coarsely powdered aerial parts of *T. terrestris* L. was subjected to successive extraction with different solvents i.e., petroleum ether (40-600°C), ethyl acetate, hydroalcoholic (50:50), and distilled water by Soxhlet apparatus. Each extract was concentrated and evaporated to dryness on water bath. Percentage yield of each extract was calculated. Each dried extract of the aerial parts was analyzed for the presence of pharmavcologically potent phytoconstituents. On observation we concluded that petroleum ether (40-60°C) extract was found to contain steroid and some traces of saponins and glycoside. Ethyl acetate extract contained steroids, flavonoids and some traces of proteins and glycosides. Hydroalcoholic extract contained carbohydrates, proteins, saponins, flavonoids, glycosides, alkaloids and tannins. Aqueous extract contained carbohydrates, s, amino acid, saponins, and flavonoids. Medicated hydrogel preparations of hydroalcoholic extract (5g for 5% and 2.5gm for 2.5% hydrogel) and aqueous extract (5gm for 5% and 2.5gm for 2.5% hydrogel) were prepared.

The hydrogels were evaluated for pH, viscosity, homogeneity, spreadability, test of skin irritancy etc. to ensure quality and stability. The hydrogels of hydroalcoholic and aqueous extracts were brown in color, uniform and non-irritant. pH value in the close range of neutral pH 6.4 to 7.4. The dermal irritation studies revealed that formulations were free from noticeable reactions (erythema and edema) upto 72 hr following their application.

Animal study was performed in pharmacology division with due permission from Institutional Animal Ethical Committee (CPCSEA protocol no. PH/IAEC/VNS/2K14/006) on two wound healing models on selected animals i.e., excision and incision wound models. Diabetes was Induced by using alloxan monohydrate in healthy Wistar albino rats of either sex 150 to 250 gm weight.

In excision wound model and incision wound model topical application of 5% hydroalcoholic extract hydrogel in diabetic Wistar albino rat showed remarkable repair effects. It significantly (***p<0.001) enhanced the rate of % wound contraction on 14th dayby shortening the period of epithelization. Diabetic Wistar albino rats treated with 5% hydroalcoholic extract hydrogel and normal wound control group exhibited significant (***p<0.001) content of hydroxyproline and exhibited significantly (***p<0.001) extremely high tensile strength as compared to the diabetic control group. It may due to enhanced the collagen synthesis. The histopathological examination also indicates 5% hydroalcoholic extract hydrogel of *T. terrestris* L. treated rats showed more profound epithelial cells growth with more collagen than diabetic wound control. The diabetes-induced group rats' wounds failed to heal even 25 days post the infliction of the wounds. The present invention provides an effective and low-cost method for treatment of diabetic wounds and sores.

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