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Evaluation of Wound Healing Potential of *Hydnocarpus wightiana* Seed Extract in Alloxan-Induced Diabetic Rats

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Medicinal plants play an important role in Phyto-pharmacological research and the new drug development process. These new drugs are used to treat illnesses and their various illnesses. Hydnocarpus wightiana is one of the most important drugs traditionally used for treating the seas and wounds. During the literature survey, no significant data was found on the wound-healing process of Hydnocarpus wightiana in diabetic rats. Thus, the current study examined the wound-healing potential of Hydnocarpus wightiana seed extract in Alloxan-monohydrate-induced diabetic rats. The crude extract was carried out using ethanol with the help of the Soxhlet apparatus by hot inoculation method. Diabetes was induced by using a single dose of streptozotocin (50mg/kg). The wound healing potential was evaluated with the help of two models, including the excision wound model and the incision wound model. In this study, topical ointment was prepared in three different concentrations: 1%, 2%, & 4%. Various parameters were evaluated during this study, i.e., contraction rate, wound index, period of epithelization, and tensile strength. Our study concluded that the 4% w/w in ointment preparation of ethanolic Hydnocarpus wightiana extract showed better and faster wound healing activity as compared to the 1% and 2% w/w treated groups.

Keywords: Excision; *Hydnocarpus wightiana*; Incision; ointment; Wound Area.

The cellular and anatomical damage to a tissue resulting from a chemical, physical, microbiological, thermal, or immunological rupture of the tissue is referred to as a wound [1]. The process of restoring the structure and functionality of wounded tissue to its presumed pre-wound features is known as wound healing. Effective wound treatment will reduce problems and enable a speedy return to normal function [2]. To meet their healthcare needs and concerns, between 70% and 90% of populations in some industrialized countries and between 70% and 95% of society in the majority of developing countries use routine medicine [3]. Several medicinal plants have been scientifically demonstrated to be effective in the care of wounds, while more therapeutic plants are referenced in Ethiopian folk medicine [4].

The Achariaceae family includes *Hydnocarpus wightiana*, also known as the Chaulmoogra tree. In Indian medicine, Seed oil from *Hydnocarpus wightiana* has been used. Chinese Standard medicine is also used to cure leprosy. It was first used in nineteenth-century Western medicine in its early years, a century before the development of sulphonamides and more antibiotics, to treat a variety of skin conditions

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as well as leprosy. The oil was suggested for leprosy as a combination suspended in gum or an emulsion. With 40 species, Hydnocarpus is an Indo-Malaysian variety that belongs to the Achariaceae family [5]. Out of these five species are reachable in India, specifically *A. alpina, H. kurzii, H.macrocarpa, H.pendulus, and H. wightiana*, and from these species, 4 are reported from Kerala, specifically *H. alpina, H.macrocarpa, H.pinnatum, H.pendulus* [6].

MATERIALS AND METHODS

Collection of Plant Material

The seed was taken in January 2024 from the I.F.T.M. University Botanical Garden.

Authentication of Plant Material

The scientist in charge of the Botanical Survey of India, located in Allahabad, verified the authenticity of the *Hydnocarpus wightiana* plant seed. A reference specimen from the Herbarium file was provided.

Extraction process

Hydnocarpus wightiana seeds were sieved no. 18 after being air dried at room temperature and ground into a coarse powder. Using a Soxhlet apparatus, powdered leaves were defatted with 40–60% Pet. ether to remove fatty particles and other coloring agents. Defatted and dried seeds were then utilized for the hydroalcoholic extraction (70:30). After mixing the concentrations, they were dried in a vacuum burner until there were no more residues of ethanol. The concentrations were kept between 2 and 8 °C in storage.

Phytochemical Screening

The standard screening test of the seed extract was performed for various plant constituents. The qualitative analysis of Phyto-constituents was carried out based on the standard procedure given by Evans [7] for the determination of secondary metabolites.

Experimental animals

Healthy adult (either sex) Wistar rats (250-300 grams) were obtained from the animal house of IFTM University. The animals (n=6) were housed in polypropylene cages at temperature (28± 2°c) with RH (60-70%) and 12:12 h dark/light cycle. The animals had free access to standard pellet chow during the study protocol. The animal had free access to mineral water. The pharmacology and acute toxicity protocols were approved by the Institutional Animal Ethics Committee, IFTM University, Moradabad.

Toxicity Study

Toxicity Studies were done by O.E.C.D. recommendation 402. This rule states that the rat's body must have at least 10% of its surface shaved using a razor or shaving cream. Next, the process or parameters were carried out by OCED 402, and the rats were administered an EEHW dosage of 2000 mg/kg based on their body weight [8].

Ointment Formulation

The ointment was prepared as a formula mentioned in British Pharmacopeia (B.P.). The ethanolic extract of *Hydnocarpus wightiana* seed was used for preparing the formulation. The extract was mixed with the ointment base explained in (Tables 1 and 2)

Induction of diabetes

Animals of various groups are weighed and their level of blood glucose was estimated in fasting condition before inducing diabetes. A single dose of streptozotocin (50mg/kg, sigma) in cold citrate buffer, pH 4.5 (freshly prepared) was used i.p. to induce diabetes. Fasting blood glucose level was measured after three days to confirm the diabetes status of the animals. For blood glucose measurement, blood was withdrawn from the tail vein. The animals selected for the study were having blood glucose levels greater than 200 mg/dl[16]

Wound Healing Activity Testing

Observation of Wound Healing Activity

The animals are isolated into six major groups, each having six Wistar rats with a weight range of 250-300 grams.

Excision wound model

The rats in this study underwent intravenous ketamine Hydrochloride. Treatment at a dosage of 80 mg/kg to produce anesthesia, and the hair on their backs was extracted using a hair removal cream. The rodents' dorsal thoracic region was then marked, and a 300 mm2 circular section was carefully cut away using scissors and a sharp edge. Every animal was housed in a different cage, and it was thought that the injuries would only be 2 mm deep. Until the wound is fully healed, the treatments are applied topically, as shown [in 9]. The animals were arranged in their respective clusters.

Measure of wound area and contraction

The use of tracing paper Measures the rate of wound contraction and the wound area. The alterations were noted on days 0, 4, 8, 12, 16, and 20. A full H.D. camera was used to record all of the changes [10].

After that, the area was calculated as mean ± S.E.M. The formula for wound contraction is as follows-

Percentage wound contraction = [Initial wound area – Nth day of wound area / Initial wound area] × 100

Determine the period of epithelization

When the scab falls off of the wound, it is believed that the damage is healing or that full epithelization has reached its conclusion. The epithelization period is the anticipated number of days for these [11].

Measurement of wound index

The wound index was determined by an arbitrary scoring process, as shown in (Table 4) [12].

Estimation of Bio-chemical Markers

Preparation of hydrolysate

A sample of dried tissue weighing around 150 mg was obtained and ground into a fine powder. Then, the tissue was placed in a glass container with 3 ml of 6N H.C.L. The cylinder or holder was heated to 121°C for 15 to 20 minutes in an autoclave. The hydrolysate was then allowed to cool to room temperature, and any remaining acid was removed using a 10N NaOH solution. Collagen (hydroxyproline) was detected using the final hydrolysate, which was used to test each protein.

Hydroxyproline

The cylinder was marked as a test for the pre-arranged protein hydrolysate test, different from other tests and uniform. Test tubes labeled as tests received 1ml of the test, a test tube labeled as clear received 1ml of double-distilled water, and a test tube labeled as standard received 1ml of an arrangement of hydroxyproline labeled as standard. Finally, 1.0 ml of newly prepared 0.01M copper sulfate solution, each test tube received 1.0 ml of 6% hydrogen peroxide and 2.5N sodium hydroxide. After fully mixing the mixture, it was heated in a water bath set at 80ºC for five minutes. After that, the cylinders were quickly cooled in an ice bath, and each cylinder was then disturbed by filled with 4.0 ml of 3N sulfuric acid. Subsequently, each test tube received 2.0 ml of Ehrlich reagent, and the apparatus was reheated for a further 15 minutes at 70 degrees Celsius in a water bath. A sophisticated colorimeter was used to calculate the optical thickness, which came out to be 540 nm [13].

Estimation of total protein

Protein concentration was determined using the Bovine Serum Albumin (BSA) standard and according to the Lowry and Kandhare method using Bovine Serum Albumin [14].

Histopathological examination

At the conclusion of the investigation, samples were collected from the skin of each group of rats to assess the histological alterations. A formalin solution was used to repair the samples (I.P.I.P. 2007). At 100X magnification, photomicrographs were taken in a 5µm thin segment using hematoxylin and eosin.

Incision wound model

In this paradigm, the rats were anesthetized intraperitoneally (i.p.) with ketamine HCl at a dose of 80 mg per kg body weight, and the back hair was removed using shaving cream or hair remover. After 30 milliseconds of anesthesia, a wound was formed at the entrance and placed on the shaved back of the rodent. With the use of a cautious edge (no. 9), an entrance point of 6 cm in length and 2 mm in profundity was produced on the rodent's skin. A careful needle (no. 32) and careful string (no. 000) were then used to carefully remove the skin, stopping well short of 5 cm stretches. Over ten days, the various mouse groups were treated with medication-infused skin salve. The evaluation of wound healing started on the day the damage occurred and continued until the injury was fully healed. On the eighth day after the injury, the sutures were removed, and using a tensiometer, the elasticity was evaluated on the tenth day [15].

Measurement of tensile strength

Tensile strength was determined when the animals were slaughtered on the tenth day of the model, with the assistance of a high dosage of Ketamine HCl After the rats' sacrifice, A wound stripe equal in length, as well as width, was carefully removed from each animal and placed on the tensiometer at a predetermined distance. The ends of the skin strip were secured with a pair of steel clips. A reweighed polyethylene bottle was

then allowed to dangle and fill with water gently until the wound strip broke down, while one clip was still hanging on the stand. The water content was measured and reported as the wound's tensile strength in grams (g) [16].

Data Management, Processing, and Analysis

All the data were calculated and analyzed by using GraphPad 7. The experimental data was expressed as mean ± S.E.M. Following Tukey's test, P value *P<0.05, **<0.01, ***<0.001 was considered significant.

RESULT AND DISCUSSION

Percentage (%) Yield of Extract

Hydnocarpus wightiana seed crushed = 740gm, Extract (HW) = 40.35gm, Percentage= 6.65%

Preliminary phytochemical screening

The ethanol extract of *Hydnocarpus wightiana* (EEHW) seeds was examined for several chemical tests using the defined methods and was found to contain carbohydrates, flavonoids, glycosides, amino acids, tannins, and Protein (Table 5).

Toxicity study

Based on the acute dermal toxicity, the EEHW did not show any signs and symptoms of toxicity. The dose of EEHW 2000mg/kg was safe on observation. Therefore, 1% w/w, 2% w/w, and 4% w/w were used for the further experimental study.

Pharmacological evaluation of wound healing activity

Incision Wound Study

Effect of EEHW on the wound area and wound contraction

On days 4, 8, 12, and 16 of the control group (basic ointment base), standard (Faramycin sulfate cream 1%w/w, and test drug (EEHW at a concentration of 1%w/w, 2%w/w, and 4%w/w), the wound area (mm^2) was assessed. When EEHW (4% w/w) was compared to control on the fourth day, the wound healing area in the diabetes groups showed a comparable impact (% wound contraction), indicating no significant difference from control. On the eighth day, EEHW (4% w/w) was discovered to be 46.433 ± 0.594 for the control group and 58.780 ± 0.542 for the group. On the twelfth day, the results showed that the control was 70.500 ± 0.146, and the EEHW (4% w/w) was

Table 1. Formula for Ointment Base

Sr. No.	Ingredients	Quantity (g)
1	Yellow Vaseline	8
2	Stearic acid	15
3	White wax	2
4	Propylene Glycol	8
5	Triethanolamine	1
6	Purified Water	q.s. to 100g

Table 2. Formula of Herbal Ointment

Sr. No.	Ingredients	1%w/w (F1)	2%w/w (F2)	4%w/w (F3)
1	EEHW	1g	2g	4g
2	Ointment Base	Q.S. to 100g	Q.S. to 100g	Q.S. to 100g

Table 3. Animals grouping

Group No.	Group Name	Treatment
1	Control	Blank
2	Negative Control	Ointment Base
3	Standard	Soframycin
4	Test-1	F-1
5	Test-2	F-2
6	Test-3	F-3

Table 4. Gross change and score for wound index measuring

No.	Gross Change	Score
1.	Necrosis	4
2.	Area of wound (Healing) but healing not be started	3
3.	Delayed healing	2
4.	Healthy healing	1
5.	Wound Completely close	0
6.	Total	10

Table 5. Preliminary phytochemical Constituents Present in EEHW Seeds

Sr. No.	Chemical Test	Result
1.	Carbohydrates	+
2.	Protein	+
3.	Amino acid	-
4.	Fats and oil	-
5.	Steroids	-
6.	Volatile oil	-
7.	Glycosides	+
8.	Flavonoids	+
9.	Alkaloids	+
10.	Tannin	+

+ Present; - Negative

81.052 ± 0.331, which was significantly different from the control. On the 16th day, EEHW (4% w/w) was found to be 97.487 ± 0.331, and the control was 87.733 ± 0.614, which was again significantly different from the control (Fig 1 and 2)

Effect of EEHW on Period of Epithelization and Wound Index

When the test group's time of epithelization was determined, EEHW (4% w/w) showed the least amount of epithelization, which was the most potent test group overall and statistically different from the control group. The 4% w/w preparation was considerably similar to the standard, indicating that it has the same capacity for wound healing as the standard, as demonstrated by the impact

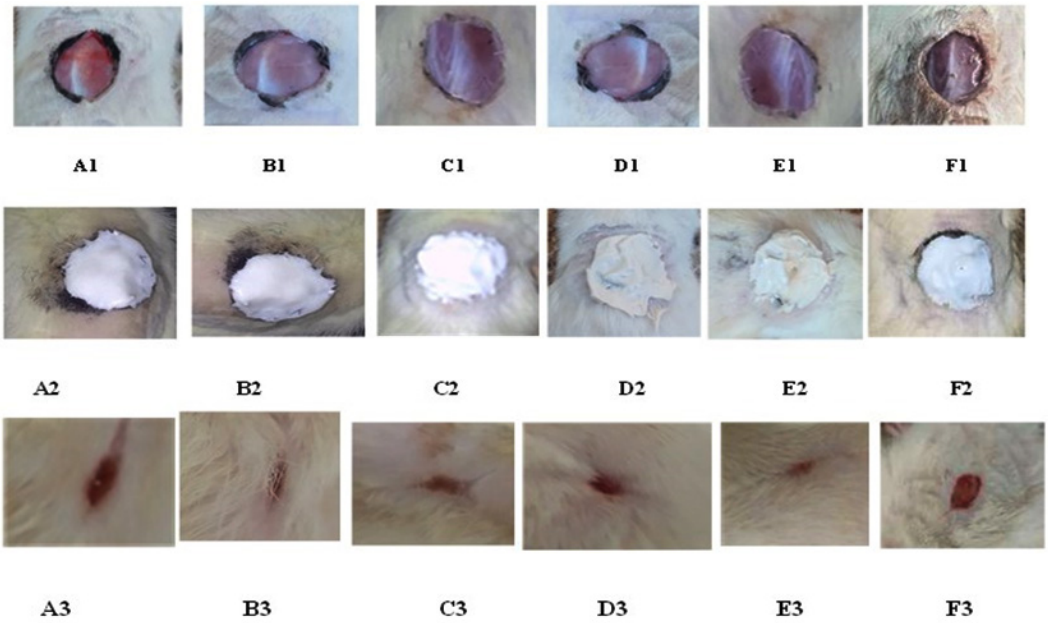

Fig. 1. Wounding day, 2: After applying ointment, 3: After healing of the wound

of the EEHW preparation at 4% weight/weight. The test group's most powerful treatment, EEHW (4% w/w), showed the lowest wound index when the wound index was assessed. This suggests that EEHW can aid in wound healing and was substantially different from the control group. The group's last value indicates that healing is occurring more quickly than it is for other groups. The compound with the lowest wound index value, EEHW (4% w/w), was found to be somewhat comparable to the standard treated groups (Fig 3).

Effect of EEHW on Bio-chemical markers

Estimation of Hydroxyproline Content and Total Protein

The 4% w/w EEHW was the most potent test group among all of them when it came to hydroxyproline and total protein content. This was evident when the content of hydroxyproline and

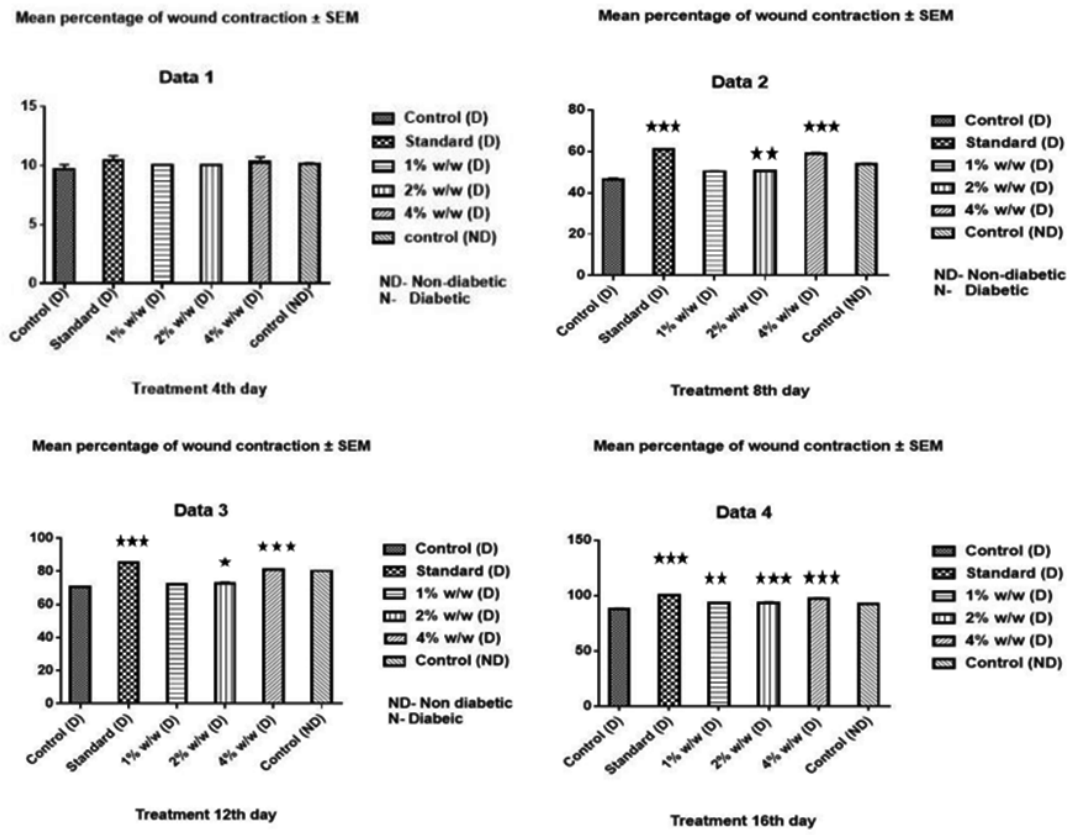

Fig. 2. Effect of EEHW ointment treatment on wound area (mm^2) and rate of wound contraction in rats

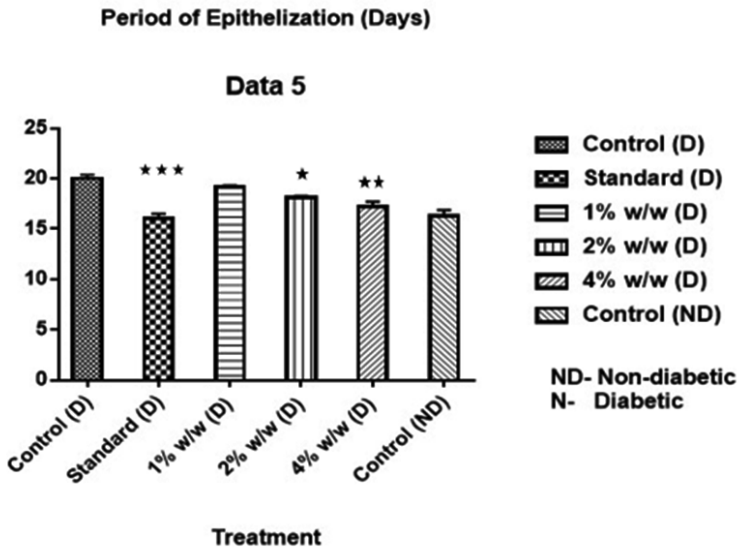

Fig. 3. Effect of EEHW on Period of Epithelization and Wound Index
Data are expressed as mean ± S.E.M from six rats and analyzed by one-way ANOVA followed by Tukey tests.
*P<0.05, **<0.01, ***<0.001 as compared to control group animals

total protein was measured in the treated group, where it was higher than in the control group and marginally similar to the standard groups. (Fig 4) demonstrates that EEHW (4% w/w) exhibited the highest level of hydroxyproline value and total protein among all the compounds, suggesting its potential for wound healing.

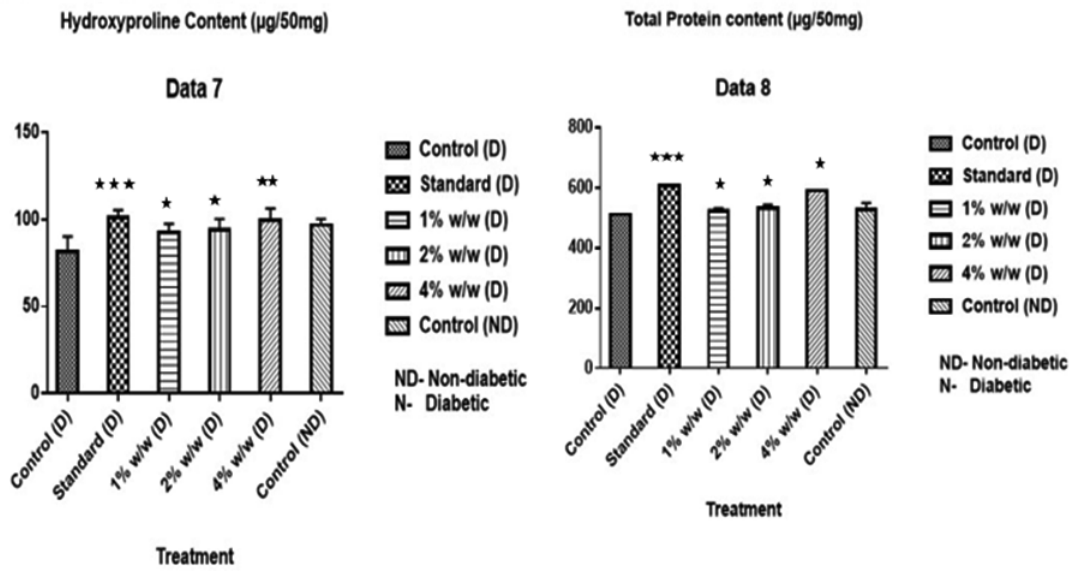

Fig. 4. Estimation of hydroxyproline Content and Total Protein
Data are expressed as mean ± S.E.M from six rats and analyzed by one-way ANOVA followed by Tukey tests. *P<0.05, **<0.01, ***<0.001 as compared to control group animals

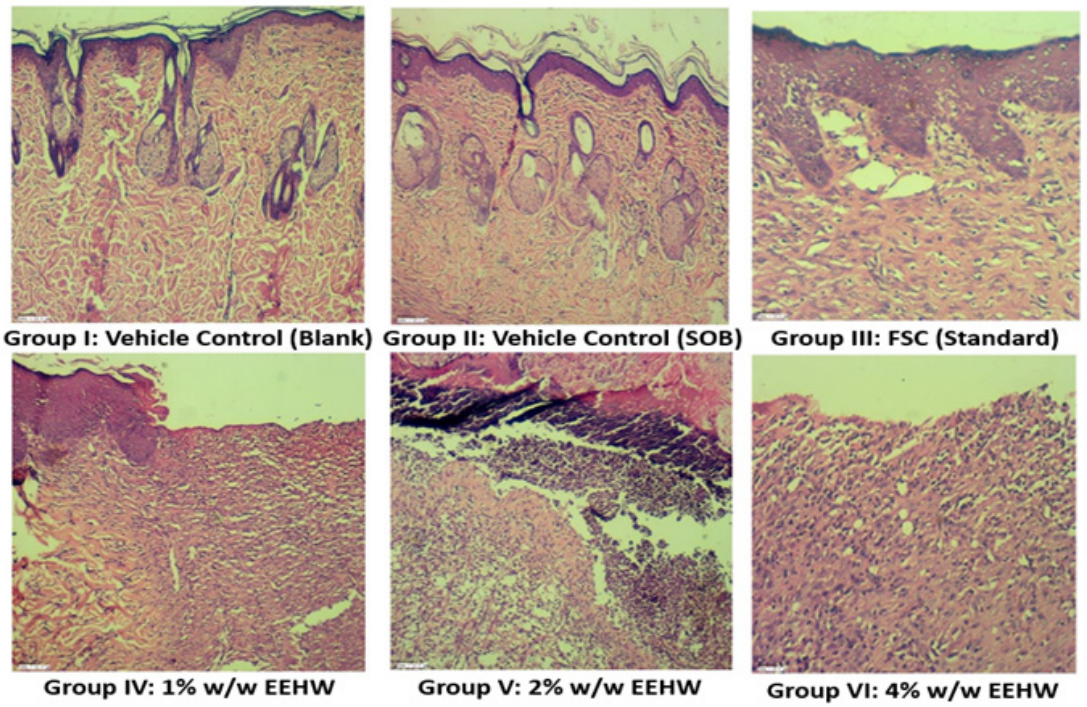

Fig. 5. Histopathological examination

Histopathological examination

Hemostasis, inflammation, proliferative (fibroblast) phase, and wound remodeling are the phases of the wound healing process that were seen in the diabetes groups throughout the experiment. Necrosis, oedema, and monocyte cells were seen in the control group, associated with delays in the healing phase of the lesion. In the control group, a collection of macrophages with little collagen was seen during the histological investigation. Large numbers of fibroblasts were seen in the dermis, and the control group showed reduced new blood vessel creation. When EEHW ointments (1%, 2%, and 4% w/w) were applied to the dermis of both diabetes groups, significant increases in collagen, fibrous tissue development,

Fig. 6. Measurement of tensile strength

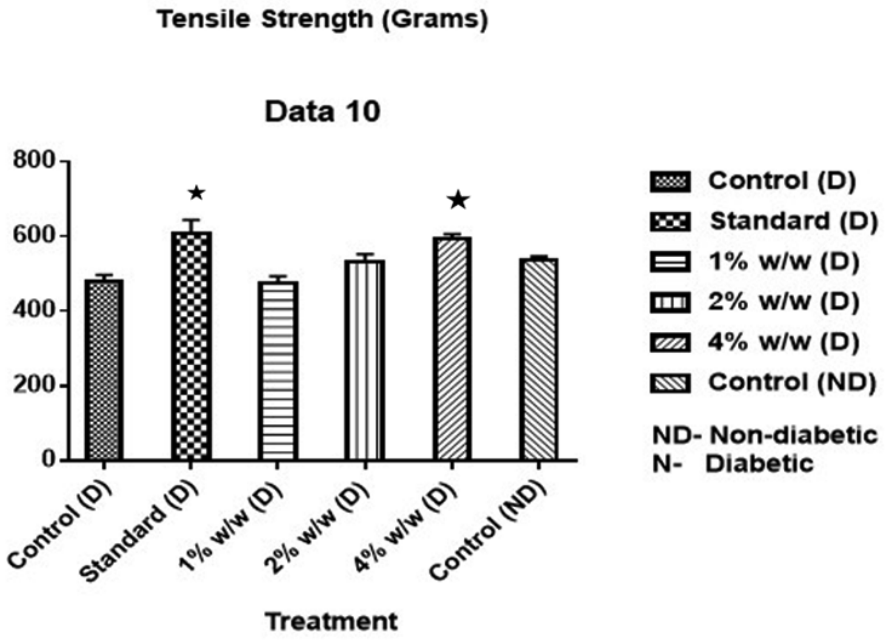

Fig. 7. Effect of EEHW on Tensile Strength
Data are expressed as mean ± S.E.M from six rats and analyzed by one-way ANOVA followed by Tukey tests.
*P<0.05 as compared to control group animals

and neovascularization were seen. In the EEHW ointments treatment group, further observations included reduced inflammation, elevated tissue perfusion and proliferation rates, wound contraction (remodeling), decreased macrophage counts, and increased collagen fibers. (Fig 5)

Excision Wound Study

Effect of EEHW on tensile strength

Tensile strength measurements revealed that 4% EEHW had the highest tensile strength among all test groups and that this difference was significant from a control group. When compared to the standard, EEHW (4% w/w) outperformed all other compounds in terms of tensile strength value, suggesting that it may impact wound healing (Figs. 6 and 7).

CONCLUSION

Following the initial stages of damage, wound healing is a multifaceted and ongoing process that includes phases of homeostasis, blood clotting, inflammation, proliferation, and remodeling. All of these stages can accelerate or delay recovery by affecting internal or external variables such as diet, sex hormones, and infection. A delayed healing process raises the risk of infection, poor healing, and the development of unsightly scars. In this study, an ointment containing extract from *Hydnocarpus wightiana* seeds plant improved, relative to the control group, different phases of wound healing, wound contraction, epithelialization time, and tensile strength in both models. The plant's coarse powder was defatted using ethyl acetate and petroleum ether before being extracted using a soxhlet using ethanol. Furtherly, the ethanolic extract was used for further investigation. In phytochemical screening, a chemical test was carried out, and extract showed the presence of alkaloids, Flavonoids, and Saponins. In the pharmacological study, wound healing activities were performed in alloxan-induced diabetic laboratory animals. In wound healing activity, the formulation 4%w/w showed a significant effect. In the present studies, development formulation has been evaluated for animal activity and can be used for the treatment of wound healing potential.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author contributions

Deepali Singh: Conduct the research; Dr Alankar shrivastav: Experimental work and analysis; Navneet Verma: Design the paper and evaluation the toxicity study

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