WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 20, 1036-1048. Research Article ISSN 2277–7105

THE EVALUATION OF EFFECT OF *BOERHAVIA DIFFUSA* (L) ON WOUND HEALING ACTIVITY ON MICE

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Article Received on 15 Sept. 2026,
Article Revised on 05 Oct. 2026,
Article Published on 16 Oct. 2026,
https://doi.org/10.5281/zenodo.23387455

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How to cite this Article: Kashiram Prajapati*[1], Dr. Gudur Pavan Kumar[2], Ramu Kaushal[1], Nitin Goel[1], Davinder Kaur[3]. (2026). The Evaluation of Effect of Boerhavia Diffusa (L) on Wound Healing Activity On Mice. World Journal of Pharmaceutical Research, 15(20), 1036–1048.
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ABSTRACT

The study aimed to investigate the phytochemical profile and wound healing efficacy of Boerhavia diffusa leaves. Plant material was collected from Deori, Sagar district, and authenticated by the Department of Botany, Dr. H.S. Gour University, Sagar (M.P.). Soxhlet extraction was employed, yielding 7.3% with petroleum ether and 13.6% with ethanol, confirming ethanol as the more effective solvent. Phytochemical screening of the ethanolic extract revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, proteins, and amino acids, indicating a rich composition of bioactive compounds. The wound healing potential was evaluated using incision and excision wound models in rats. In the incision model, the extract showed a dose-dependent increase in breaking strength, granulation tissue formation, and hydroxyproline content, with the 200 mg/kg dose comparable to the standard drug, povidone iodine. In the excision model, the ethanolic extract significantly enhanced wound contraction, particularly at 200 mg/kg, which nearly paralleled the standard treatment. These findings suggest that the phytoconstituents of Boerhavia diffusa contribute to collagen synthesis, extracellular matrix formation, and tissue remodeling, thereby accelerating wound healing.

KEYWORDS: Boerhavia diffusa, Soxhlet extraction, Phytochemical screening, Wound

healing, Incision model, Excision model.

INTRODUCTION

Wounds are physical injuries that result in an opening or breaking of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical stability and disturbed functional status of the skin. Repair of injured tissues occurs as a sequence of events, which includes inflammation, proliferation, and migration of different cell types.[1] The inflammation stage begins immediately after injury, first with vasoconstriction that favours homeostasis and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process.

The remodeling stage is characterized by reformulations and improvement in the components of the collagen fiber that increases the tensile strength.[2] Factors that contribute to causation and perpetuation of the chronicity of wounds include repeated trauma, poor perfusion or oxygenation, and excessive inflammation.[3] Imbalance in free radical generations and antioxidants has been observed to induce oxidative stress and tissue damage and delayed wound healing. Therefore, elimination of ROS could be an important strategy in healing chronic wounds.[4]

Wound healing is an important but complicated process in human or animal, containing a multifaceted process governed by sequential yet overlapping phases, including hemostasis/ inflammation phase, proliferation phase, and remodeling phase.[5] After an injury to skin, the exposed sub-endothelium, collagen and tissue factor will activate platelet aggregation, which results in degranulation and releasing chemotactic factors (chemokines) and growth factors (GFs) to form the clot, and all above-mentioned procedures will achieve successful hemostasis. Neutrophil the first cells to appear at the injury site, cleanse debris and bacteria to provide a good environment for wound healing. In following, macrophages accumulate and facilitate phagocytosis of bacteria and damage tissue.[6]

Boerhavia Diffusa Linn. (Nyctaginaceae) is a well-known medicinal plant in traditional Indian medicine as well as other parts of world, for example, Southern American and African continent. Its various parts and especially roots have been used for gastrointestinal, hepatoprotective, and gynaecological indications in above mentioned parts of the world and also throughout India. In ayurvedic texts, more than 35 formulations of different types contain it as major ingredient.

In Ayurveda, *Boerhavia Diffusa* has been classified as "rasayana" herb which is said to possess properties like antiaging, re-establishing youth, strengthening life and brain power, and disease prevention, all of which imply that they increase the resistance of the body against any onslaught, in other words, providing hepatoprotection and immunomodulation.[7]

The wound healing is an intricate process in which the skin or another organ-tissue repairs itself after injury. The wound healing process is a complex series of the events that begins at the moment of injury and can continuously for months and years. The basis of literature survey the *Boerhavia Diffusa* showed different activities. Antioxidant as a therapy, can promote the process of wound healing. The *Boerhavia Diffusa* extract has different antioxidant components. In this studied are evaluated *Boerhavia Diffusa* extract for its possible wound healing properties.

The aim of the study is to screen the evaluation of the effect of *Boerhavia Diffusa* (L.) on wound healing on rodent animals.

METHOD AND MATERIALS

Identification, collection and authentication: Leaves were purchased form Deori of Sagar district. The leaves of *Boerhavia Diffusa* were authentication by Department of Botany, Dr. H.S. Gour University, Sagar (M.P.).

Extraction Method: Generally three methods are used in extraction of plant material. Soxhlet extraction cannot be used for extraction of thermo labile substances. Due to various advantages of Soxhlet extraction this method were selected for this experimental study.

Pharmacological Screening: Selection of Experimental Animal experimentation was conducted after getting approval from Institutional Animal Ethics Committee, which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (SIPS/EC/2013/21).Animals (mice) were obtained from Animal House Of Sips Sagar (M.P). Albino rats weighing 180-250g were selected and obtained from the animal house. The animals were grouped and housed in polyacrylic cages (38x23x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25+2ºC). They were allowed free access to standard dry pellet diet and water ad libitum. The mice were acclimatized to laboratory condition for seven days before commencement of experiment. The experimental protocol was approved by Institutional

Animal Ethical Committee (IAEC) constituted under CPCSEA.

Animal model used for wound healing activity

(a) Excision model

(b) Incision model

- Breaking strength
- Dry wet of granulation tissue
- Hydroxyproline content

(a) Incision wound model: The animals were divided into 4 groups of five mice each:

Group-I: Vehicle control,

Group-II: Negative control(disease induced)

Group-III: Positive control (standard marketed drug treated with povidone iodine ointment topically)

Group-IV

- Test group-1ethanolic extract of *Boerhavia Diffusa* 100mg/kg, topical,
- Test group-2 ethanolic extract of *Boerhavia Diffusa* 200mg/kg,topical

Procedure Incision wound model: Four groups of animals containing in each group were taken. The animals were anaesthetised under light ether anaesthesia. One full thickness paravertebral incision of 6 cm length was made including the cutaneous muscles of the depilated back of each rat. Full septic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. After the incision was made, the parted skin was kept together and stitched with sutures, 1 cm apart[8]. The continuous threads on both wound edges were tightened for good adaptation of wound and it was left undressed. The ointment of the leaf extract, standard drug was applied to the wound twice daily, until complete recovery to the related groups of animals. Following parameters in incision wound model were calculated.

Breaking /Tensile Strength: Tensile strength of wound represents the effectiveness of wound healing. Usually wound-healing agents promote the gaining of tensile strength. Tensile strength (the force required to open the healing skin) is used to measure the completeness of healing.

Dry weight of granulation tissue and protein content were increased by 19.4% and 18.1% ($P < 0.05$), respectively, in BME treated groups compared with control group. HPR, HXA, and HUA were significantly increased In BME treated group by 59.8%, 59.1%, and 153%, respectively, compared to control group. The results with BME were comparable with VTE on the previous connective tissue parameters.

Hydroxyproline Content (HPR): To each tube, 0.3 mL each of hydrolysate, 2.5 N NaOH, 0.01 M CuSO4, and 6% H2O2 were added. Tubes were shaken vigorously and placed immediately in water bath at 80∘ C. After 15 minutes, tubes were removed and cooled for 5 minutes in cold water. 0.6 mL of freshly prepared 5% solution of paradimethyl amino-benzaldehyde in n-Propanol and 1.2 mL of 3 N H2SO4 was added. The test tubes were once again placed in a hot water bath at 75∘ C for 15 minutes and then cooled for 5 minutes under running stream of water. Color intensity was measured at 540 nm against the blank. Hydroxyproline content in the tissue was estimated as per standard curve prepared with standard 4-HydroxyL-proline from 75 to 900 μg/0.3 mL using 3 mg/mL working solution.[9]

(b) Excision wound model: The animals were divided into 4 groups of five mice each

Group-I: Vehicle control,

Group-II: Negative control (disease induced)

Group-III: positive control (standard marketed drug treated with povidone iodine ointment topically)

Group-IV

- Test group-1ethanolic extract of *Boerhavia Diffusa* 100mg/kg, topical,
- Test group-2 ethanolic extract of *Boerhavia Diffusa* 200mg/kg, topical,

Procedure Excision wound model: Rats were anesthetized with ketamine and an area of about ≈500 mm2 were marked on the back of the rat by a standard ring. Full thickness of the marked skin was then cut carefully. Wounds were traced on 1 mm2 graph paper on the day of wounding and subsequently at a gap period of 4 days till 12th day, then on the alternate days until healing was complete. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on a graph paper every alternate day. The changes in healing of wound i.e. the measurement of wound area on graph paper were expressed as unit (mm^2).

Wound contraction was expressed as percentage reduction of original wound size. Changes in

wound area were measured regularly and the rate of wound contraction calculated as given in the formula below. Significance in wound healing of the test groups is derived by comparing healed wound area on respective days with healed wound area of control group. The period of epithelization, that is, day of fall of eschar and the scar area, was also noted down.[10]

RESULT AND DISCUSSION

Percentage Yield of different extracts

The table provided highlights the percentage yield of different extracts of *Boerhavia diffusa* using two solvents: petroleum ether (60–80) and absolute ethanol. The extractive values, expressed on a dry weight basis, reveal important insights into the efficiency of each solvent in isolating phytoconstituents. Polar solvent, is more efficient in extracting polar phytochemicals such as flavonoids, glycosides, alkaloids, and tannins. The higher yield indicates that *Boerhavia diffusa* is rich in polar bioactive compounds, which are often associated with medicinal properties.

Table No. 1: percentage Yield of different Extracts of *Boerhavia Diffusa.*

Solvents	Colour	Extractive values in % W/W on dry weight basis
Petroleum ether 60-80	Dark green & semisolid	7.3%
Ethanol absolutes	Dark brown &semisolid	13.6%

Phytochemical testing of ethanol extract of *Boerhvia Diffusa*

The phytochemical screening of the ethanol extract of *Boerhavia diffusa* reveals the presence of a wide range of bioactive compounds. Ethanol, being a polar solvent, efficiently extracts diverse secondary metabolites, which are crucial for the plant's medicinal properties. The presence of alkaloids, flavonoids, tannins, and saponins strongly supports the traditional medicinal use of *Boerhavia diffusa* in treating liver disorders, inflammation, and infections. Overall, the phytochemical profile indicates that ethanol is a highly effective solvent for extracting medicinally important compounds from *Boerhavia diffusa*.

Table No. 2: Phytochemical testing of ethanol extract of Boerhvia Diffusa.

S. No	Test	Ethanol extract
1.	**Alkaloids**	
	Mayer's test	+
	Dragendrffs test	+
	Wagner's test	+
	Hager's test	+
2.	**Carbohydrates**	

	Molischs test	+
	Benedict's test	+
	Felling's test	+
3.	**Glycosides**	
	Killer-killanis test	+
	Modified Bandager's test	+
4.	**Protein and amino acid**	
	Ninhydrin test	+
	Millons test	+
5.	**Phytosterods and triterpenoids**	
	Liebermann's test	+
	Liebermann's burchard test	+
	Salkowill beki test	_
6.	**Phenolic and tannins**	
	Ferric chloride test	+
	Lead acetate test	+
7.	**Flavonoids**	
	Alkaline reagent test	+
8.	**Saponins**	
	Foam test	+

(+) Phytochemical constituents present and (-) phytochemical constituents absent.

The ethanolic extract of *Boerhavia Diffusa* gives positive test for alkaloids, carbohydrates, flavonoids, phenols and tannins, glycosides, protein and amino acids and saponins.

Incision Wound Model

The data presented in the table compares the wound healing efficacy of *Boerhavia diffusa* extract at two doses (100 mg and 200 mg) against a negative control and a standard treatment (povidone iodine). The parameters measured include breaking strength of incision wounds**,** dry weight of granulation tissue, and hydroxyproline content.

Table No. 3: Effect of extract in wound healing in incision breaking strength model.

Treatment Group	Incision Wound	Dead space wound model	
	Breaking Strength (gm)	Dry weight of granulation tissue (mg/100 gm of tissue)	Hydroxyproline content (mg/gm of tissue)
Negative Control	281.33±1.201	8.16±0.601	16.16±0.601
Povidone Iodine	496.66±3.711***	17.33±1.201***	30.16±1.013**
Extract (100mg)	327.27±3.179*	12.96±0.601*	23.48±0.726*
Extract (200mg)	380.33±2.185**	14.33±0.726**	27.29±1.212**

n=6, values are in Mean±SEM, statistically significant test with control using one way ANOVA followed by Dennett Test. *** very significant $p<0.001$, *significant $p<0.05$.

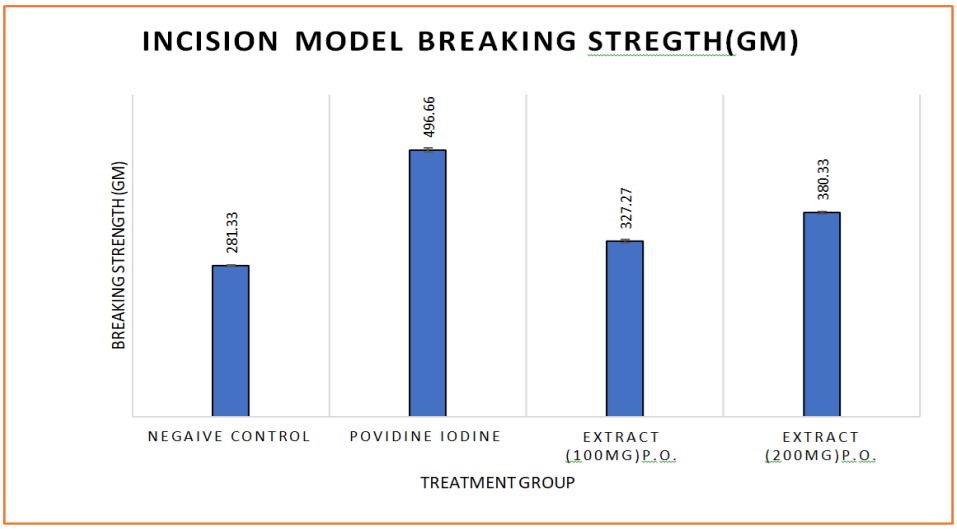


Figure 1: Effect of Ethanol extract in wound healing in incision breaking strength model.

The dose-dependent increase in breaking strength, granulation tissue, and hydroxyproline content indicates that *Boerhavia diffusa* extract enhances wound healing. Hydroxyproline content, a marker of collagen deposition, was notably higher in treated groups, suggesting improved extracellular matrix formation and tissue remodeling. The 200 mg extract group showed results closer to the standard drug, highlighting its potential as a natural wound healing agent. The findings support the traditional use of Boerhavia diffusa in wound management and suggest that its phytoconstituents (alkaloids, flavonoids, tannins, saponins) may contribute to collagen synthesis, angiogenesis, and tissue repair.

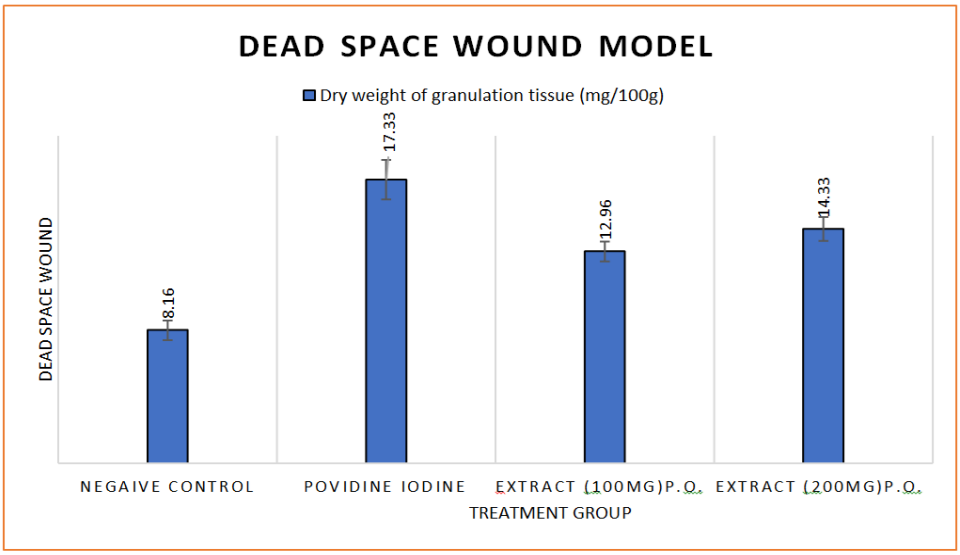


Figure 2: Effect of ethanolic extract in dead space wound model (Dry weight of granulation tissue).

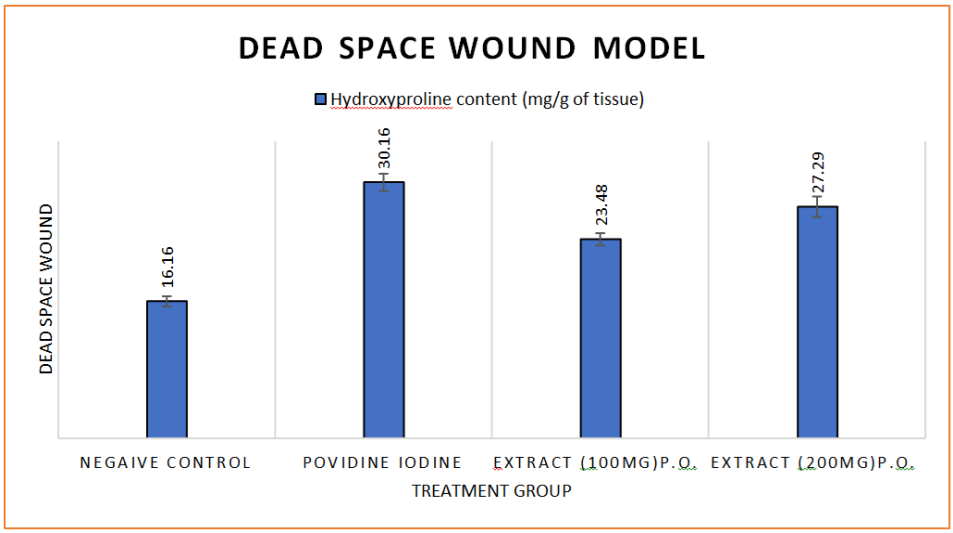


Figure 3: Effect of ethanolic extract in dead space wound model (Hydroxyproline content).

Excision Model Wound

The excision wound model provides valuable insight into the wound contraction and healing potential of *Boerhavia diffusa* ethanolic extract compared to a control and standard treatment (povidone iodine). The wound area was measured at different time intervals (0th, 4th, 8th, 12th, and 16th day).

Table No. 4: Effect of ethanolic extract on Excision wound model.

Group	Parameter wound area (mm^2)				
	0th day	4th day	8th day	12th day	16th day
Control (Vehicle)	263±6.5	196±6.7	153±6.9*	134±6.3*	109±6.2**
Povidone Iodine	256±6.3	153±6.3*	95±5.8**	32±5.9*a***	11±3.9*a***
Extract (100 mg)	261±3.2	189±5.8	142±4.6*	117±4.7**	83±4.8***
Extract (200 mg)	259±6.2	167±5.2*	102±6.2*	79±3.8***	26±5.6*a***

Values are expressed as mean ± SEM n=6 in each group, Statistically significant test with control using one way ANOVA followed by Dennett test, p>0.05, *p<0.05,**p<0.01. a*** = significant compared with vehicle treated control group.

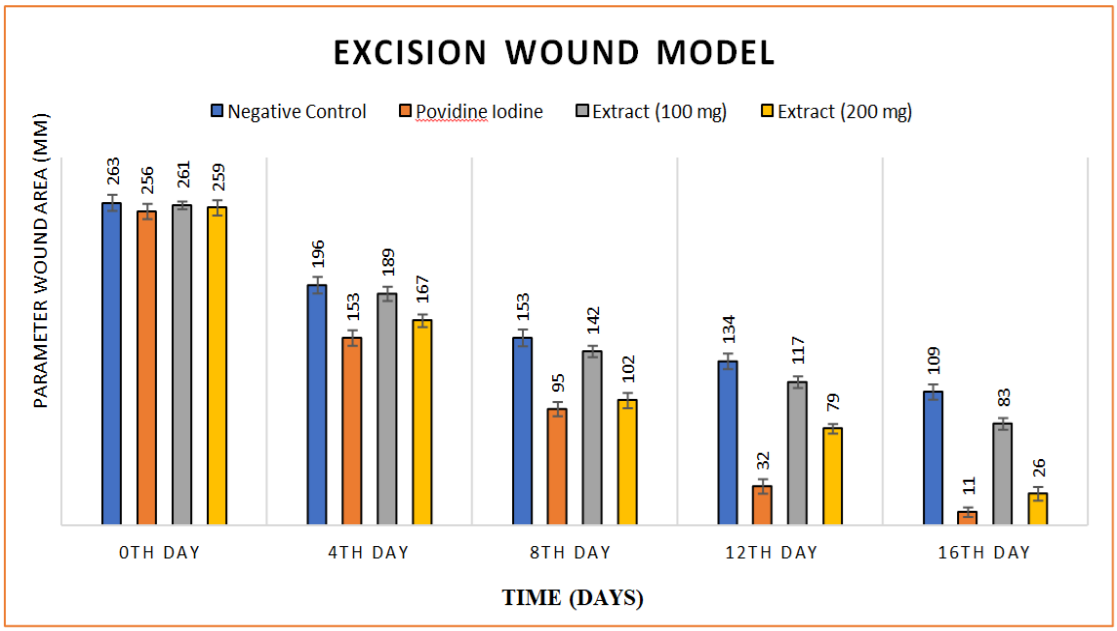


Figure 4: Effect of ethanolic extract on Excision wound model.

The dose-dependent effect of the ethanolic extract is evident, with 200 mg showing superior wound contraction compared to 100 mg. Statistical significance ($p<0.05$, $p<0.01$) confirms that the extract has genuine wound healing activity beyond natural recovery. The 200 mg extract group nearly parallels the standard drug, suggesting that *Boerhavia diffusa* contains potent phytochemicals (flavonoids, tannins, saponins, alkaloids) that accelerate wound contraction and collagen synthesis. The results support the traditional medicinal use of Boerhavia diffusa in wound healing and highlight its potential as a natural therapeutic agent.

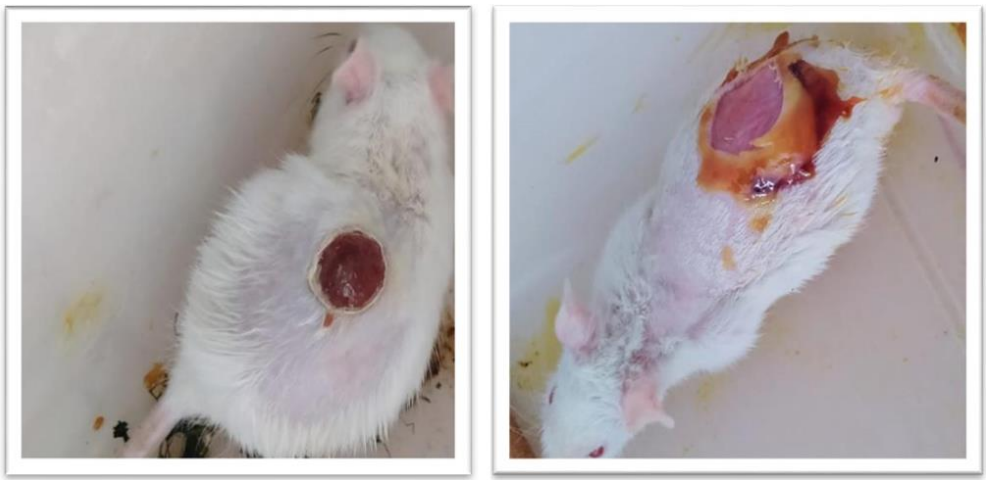
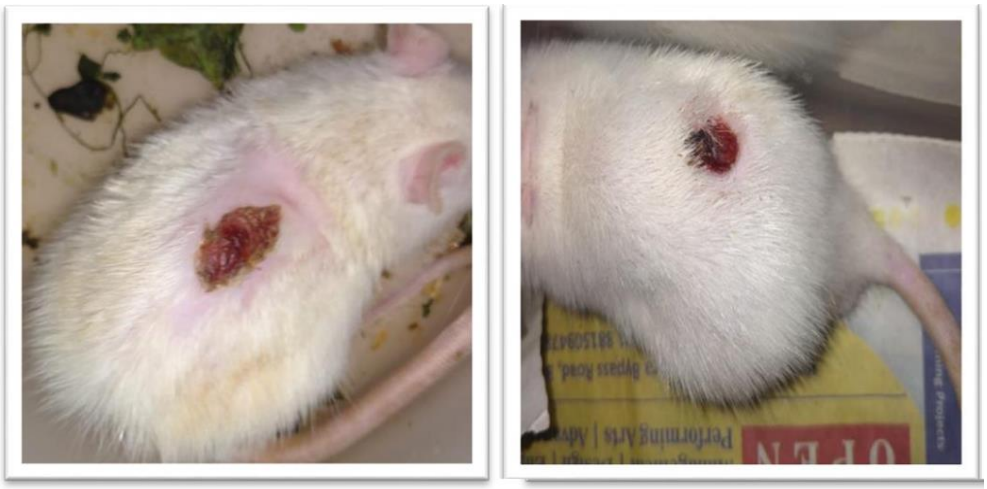


Figure 5: Effect of ethanolic extract on wound area on 0th day in excision wound model.



a) Extract b) Standard povidone iodine

Figure 5: Effect of ethanolic extract on wound area on 16th day in excision wound model.

DISCUSSION

Incision wound model: The effect of extract at different doses (100 and 200 mg/kg) per day for 10 days on wound healing activity in rats inflicted with incision wound. The mean breaking strength in control group were 281.33±1.201gm. The extract at 100 mg/kg dose increased significantly braking strength as compare to control. The extract at 200 mg/kg dose effect was extremely significant and equivalent to standard povidones iodine.

Excision wound model: The result of the studies are Shown the table. The extract (200mg) group showed more significant ($p<0.01$) wound healing from 4th day onward, which were comparable to that of the standard drug. Povidone iodine ointment treated group of animals. Povidone iodine (standard drug) showed more significant ($p<0.01$) wound healing from 8th day and extract showed significant wound healing from 8th day. Extract (100 mg) showed more significant from 12th day. Extract (200 mg) showed more significant from 12th day. All extracts showed more significant in 16th day towards control group.

CONCLUSION

The study on *Boerhavia diffusa* leaves, collected from Deori in Sagar district and authenticated by the Department of Botany, Dr. H.S. Gour University, Sagar (M.P.), provides strong evidence of the plant's medicinal potential. The Soxhlet extraction method was employed due to its efficiency, yielding 7.3% with petroleum ether and 13.6% with ethanol**,** confirming ethanol as the superior solvent for extracting bioactive compounds. Phytochemical screening of the ethanolic extract revealed the presence of diverse secondary

metabolites including alkaloids, flavonoids, tannins, saponins, glycosides, proteins, and amino acids. These constituents are well known for their roles in antioxidant, anti-inflammatory, antimicrobial, and wound healing activities**,** thereby validating the traditional use of the plant in herbal medicine.

The incision wound model demonstrated a dose-dependent increase in breaking strength, granulation tissue formation, and hydroxyproline content, with the 200 mg extract group showing results comparable to the standard drug (povidone iodine). Similarly, the excision wound model confirmed significant wound contraction, particularly at 200 mg, which nearly paralleled the standard treatment. These findings highlight the extract's ability to enhance collagen synthesis, extracellular matrix formation, and tissue remodeling.

Overall, the study establishes that *Boerhavia diffusa* ethanolic extract possesses potent wound healing properties supported by its rich phytochemical profile. The dose-dependent efficacy observed in both incision and excision wound models underscores its potential as a natural therapeutic agent for wound management. This scientific validation strengthens its traditional medicinal use and opens avenues for further pharmacological and clinical exploration.

CONFLICT OF INTEREST

There are no any conflicts of interest.

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