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Exploration of *Ehretia acuminata* for Wound Healing Potential Using *In-Vivo* Animal Model

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ABSTRACT
The skin is recognized as the largest organ in the body. It is the essential component that guards tissues from microbial infection, UV radiation, and very high or low temperatures. In the present research work on wound healing, the leaves of Ehretia acuminata was studied for wound healing in incision and excision models in rats. The phytochemical screening of hydro alcoholic extracts confirms the presence of tannins, saponin glycosides, cardiac glycosides, flavonoids, steroids, proteins, carbohydrates, and alkaloids. The hydro alcoholic extract was administered via topical application to the rats. The wound healing potential was evaluated using various parameters, such as breaking strength, wound contraction, and the time needed for complete epithelialization of the wound area. This suggests that the extract may increase protein cross-linking and encourage collagen formation at the cellular level. The results of our in-vivo investigation show that hydro alcoholic crude extracts from the leaves of Ehretia acuminata favorably influence albino rats ability to repair wounds using different in-vivo wound healing models and substantially decreased lesion area.
Key words: *Wound Healing, Ehretia acuminata, incision and excision wound healing animal model.*

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INTRODUCTION

In terms of surface area, the skin is the largest organ in our body. The key structure protects internal tissues from mechanical damage, bacteriological infection, ultraviolet illumination, and high temperatures. It highly contributes to injury, resulting in substantial repercussions for patients and the healthcare industry. [1,2] A wound refers to a rupture in the skin protective layer known as the epithelium, which can impair the organization and operation of the healthy tissue beneath it. A wound can arise from deliberate tissue disruption through a surgical incision or extensive tissue damage caused by events such as major trauma or burns. A contusion, haematoma, laceration, or abrasion may also cause a wound. Since the skin is important in preserving homeostasis, its continuity must be quickly repaired.[3] Wound healing is an intricate process that can result in pathological scarring, particularly when microorganisms cause infections. [4] The process encompasses the coordination of different cell types with specific functions in the stages of hemostasis, inflammation, proliferation, and remodeling, both in terms of spatial and temporal alignment. [5] During this biological process, an overabundance of oxidative stress biomarkers is generated, ultimately resulting in the onset of inflammation and damage to cellular structures. [6] It is an effective method, assisted by various biological activities that are closely organized to repair injured tissue effectively. As a result of abnormal wound-associated cellular behaviors, diabetes and aging can cause impaired wound healing and the formation of chronic, non-healing lesions. Because of the high occurrence and recurrence of these wounds, there is a significant socioeconomic impact. Therefore, there is a pressing need for greater scientific and clinical comprehension of the processes underlying wound healing. [7]
Their underlying mechanisms and subsequent outcomes determine the categorization of skin wounds into acute and chronic wounds. Acute wounds progress through a sequence of molecular processes that finally lead to restoring structural integrity. Chronic wounds, on the other hand, are not able to heal and are distinguished by pathological mechanisms, including sustained inflammation, infection, and necrosis. [8] Various factors, including external and systemic factors, influence the complete recovery of a wound. External or local causes directly impact the characteristics of a wound. In contrast, systemic elements only impact the person's overall health or sickness status (i.e., healing potential). In addition to oxygenation, inflammation, age, stress, diabetes, nutrition, nicotine, and other factors influence recovery. [9] New therapy modalities and technological advancements are constantly being made to lesion wounds with financial and medical burdens. Simultaneously, studies are conducted to examine the effect of known

traditional approaches to the recovery of wounds, such as botanicals or additional methods like leech treatment. An optimal wound dressing should possess the desirable attributes of being non-toxic and economically viable. Because of its lengthy history of use and easy accessibility, herbal medicine is reflected as non-toxic. [10]

Natural herbs are considered a secure and natural reservoir of various bioactive compounds, which offer potential as novel therapeutic options with negligible adverse effects. Herbal medicine provides a more cost-effective alternative in comparison to synthetic drugs. Several researchers have directed their attention towards exploring the therapeutic properties of medicinal herbs, diligently striving to gain a comprehensive understanding of these herbs through in-depth mechanistic studies. Various studies have been conducted regarding using herbal remedies to facilitate the healing process of skin injuries. [11] Using plant-derived and herbal wound agents has become integral to wound care. Therefore, herbal products have been assessed as topical therapeutic agents to improve wound healing. [12]

The belief that herbs have minimal adverse effects may help to explain why herbal medications remain popular. In the last few years, it has seen a rise in the use of evidence-based medicine with sophisticated scientific methodologies by scientists to demonstrate the effectiveness of herbal remedies and better understand the processes behind their actions. [13] Numerous botanical species possess a documented record of utilization in formulations aimed at facilitating the process of wound healing and addressing various medical conditions. Nevertheless, the effectiveness and mechanisms that underlie the wound-healing properties of these substances have yet to be determined. [14]

This study explores the utilization of incision and excision *in-vivo* animal models for investigating wound healing. These models can be utilized appropriately to comprehend an wound-healing process and accelerate healing by using leaves of *Ehretia acuminata*.

MATERIAL AND METHODS

Plant Material Collection:

The leaves of the *Ehretia acuminata* plant were collected from the Amravati region.

Animal Used:

For this research, normal adult albino Wistar rats weighing between 180 and 250 grams were utilized. The experimental temperature condition was maintained at 25°C with a relative humidity of 30 to 70%. The Institutional Animal Ethics Committee of PWCOP, Yavatmal, approved all protocols utilized in this work. Protocols 650/PO/RE/5-2022/2022/CPCSEA/32 is used on September 24, 2022, for *in-vivo* animal models such as incision and excision wound models.

Extraction of Plant:

The leaves of plants were shade-dried after being rinsed with distilled water to remove contaminants and sand. The plant dried-out leaves were finely crushed and defatted with petroleum ether (60-80^{o}C) before being extracted with hydro alcohol. The extracts were filtered, concentrated by distilling off the solvents, and evaporated to dryness to obtain a pure extract. [15]

Evaluation of Phytochemical screening of the extract:

Hydro alcoholic extract of *Ehretia acuminata* was screened for the presence of tannins, glycosides, steroids, flavonoids, alkaloids, carbohydrates, and proteins using phytochemical analysis. The hydro alcoholic extract was analyzed chemically to determine the presence of various secondary metabolites. [16]

Determination of Irritancy study:

Wistar rats of either sex had a 1 sq. cm patch on their left-hand dorsal surface marked. The designated region was evenly coated with hydro alcoholic extract, and irritation, erythema, and swelling (if any) were noted. [17]

Determination of *In vivo* Wound healing activity:

The wound healing properties of plant was assessed by utilizing excision and incision wound models. Three groups of six animals were utilized for the excision and incision wound models. Experimental animals in group one were treated with a hydro alcoholic extract of ***Ehretia acuminata*** leaves gel applied topically. Animals in group two were treated with Povidone iodine ointment. It is considered the standard. The animals in group III (negative control) were left untreated. [18,19]

In-vivo Incision model:

A 6 cm incision was created in between the epidermis and cutaneous muscle of the depilated rats back by utilizing a surgical blade. The incision was sealed by using interrupted sutures, black surgical thread, and a curved needle. Using the hydro alcoholic extract of ***Ehretia acuminata*** leaves, a gel was produced. Different groups of rats were administered Povidone iodine ointment once daily for injury. Then the sutures were removed. A tensiometer was utilized to measure the breaking strength. [20,21,22]

***In-vivo* Excision model:**
On the shorn backs of the rats, an excision wound of a circular shape (around 500 mm^2) and 2 mm depth was created. Gel prepared with a hydro alcoholic extract of ***Ehretia acuminata*** and standard povidone ointment was administered topically once daily to distinct groups of rats, beginning on day zero and continuing until complete epithelialization. Every two days, the rate of wound contraction was measured as a percentage reduction in wound size. Using transparency paper and a marker, the percentage of wound closure was tracked, and the wound area was visually evaluated to track the percentage of wound closure. The percentage of wound closure reflects the development of new epithelial tissue to cover the wound. To estimate the period of epithelialization, the day's necessary to drop without leaving an open incision was recorded. [23,24]

RESULTS AND DISCUSSION

Phytochemical screening of all solvent extracts of *Ehretia acuminata*
Table No. 1 depicts the results of screening hydro alcoholic extracts of plants for various phytochemical constituents. The hydro alcoholic extract showed the presence of tannin, saponin glycoside, cardiac glycoside, flavonoid, steroid, protein, carbohydrate, and alkaloid.

Irritancy test:
A distinct irritation test revealed that no irritation occurred until 24 hours after application. All the gels were created utilizing hydro alcoholic extracts and are thus safe to use on the skin.

***In- vivo* Wound healing activity:**
In comparison to the reference standard povidone iodine ointment and the untreated negative control group, hydro alcoholic extract demonstrated substantial wound healing effects in the incision and excision models of wound healing.

***In-vivo* Incision model:**
Measuring skin-breaking strength using a tensiometer is a significant criterion for evaluating incision wounds. Compared to the povidone and negative control groups, hydro alcoholic extract significantly increased the breaking strength of rodents (Table 2). The findings suggest that applying hydro alcoholic extract to incision wounds enhances breaking strength. This implies that the extract promotes collagen synthesis at the cellular level and facilitates protein cross-linking. An augmentation in the synthesis of collagen leads to an elevation in the tensile strength of a wound, thereby indirectly enhancing the activity of wound healing.

Table 01: Results of phytochemical investigation of *Ehretia acuminata*

Chemical constituents	Test	Hydro alcoholic Extract
Alkaloids	Dragendorff's Test	+
	Mayer's Test	+
	Hager's Test	+
	Tannic acid Test	+
Phenolic & Tannins	$FeCl_3$ Solution Test	+
	Lead acetate Test	+
	Acetic acid Test	+
Saponin glycoside	Foam Test	+
Cardiac glycoside	Legal's Test	+
	Keller-Kiliani Test	+
Coumarin glycoside	NaOH + Acid Test	-
Flavonoid	Lead AcetateTest	+
	NaOH + acidTest	-
Steroid	SalkowskiTest	+
Protein	Millon'sTest	+
	XanthoproteicTest	-
Carbohydrate	Molish test	+
	Iodine test	+
	Benedict test	+
	Barfoed test	-
	Fehling test	+

+ indicates presence of constituents; **-** indicates absence of constituents.

***In-vivo* Excision model:**

The excision model was used to evaluate the efficacy of chemical constituents in promoting wound healing in trauma-type wounds. This evaluation was based on two parameters such as wound contraction and the duration required for complete epithelialization of the wound area.

Hydro alcoholic extract-treated rats significantly reduced the lesion area in comparison to the untreated negative control group. The hydro alcoholic extract of the plant treated rats demonstrated a significant increase in percent wound contraction in comparison to the negative control group. On day 14, it was observed that there was no substantial difference in the percentage of wound contraction between rats treated with hydro alcoholic extract and standard povidone, indicating that hydro-alcoholic extract had the same wound healing activity as standard povidone. The period of epitheliazation was significantly shortened in rats treated with hydro alcoholic extract and standard povidone than in rats serving as negative controls.

The rate of wound contraction and the days necessary for full epithelialization of the wound area were used to gauge a drug's effectiveness in promoting wound healing in trauma-related wounds.

Table 02: Wound healing effect in an incision wound model

Treatment groups	Skin-breaking strength(g)
Hydro alcoholic extract	704.67±4.1
Positive control	614.33±3.40
Negative control	409.17±3.75

Table: 03 Wound healing effect in the *In-vivo* Excision wound model

Parameters	Wound Area (mm^2)		
Post Wounding Days	**Hydro alcoholic extract**	**Positive control**	**Negative control**
Day 0	655.87 ± 2.287	655.22 ± 2.879	653.62 ± 2. 202
Day 2	593.65 ± 1.255*	578.59 ± 1.362	623.58 ± 2.732
Day 4	519.26 ± 1.959*	489.73 ± 1.225	539.36 ± 2.328
Day 6	436.38 ± 1.7641**	419.81 ± 1.322**	479.33 ± 1.236
Day 8	347.52 ± 1.249**	335.31 ± 1.566*	412.76 ± 1.659
Day 10	203.42 ± 1.503**	150.22 ± 0.976**	355.37 ± 1.572
Day 12	48.25 ± 0.911**	34.88 ± 0.926**	289.83 ± 1.315
Day 14	3.02± 0.311**	00.00± 0.00**	215.96± 1.659

Values are expressed as mean ± SEM; *$P < 0.01$; **$P < 0.001$ significant as compared with Positive control; Hydro alcoholic extract of *Ehretia acuminata*

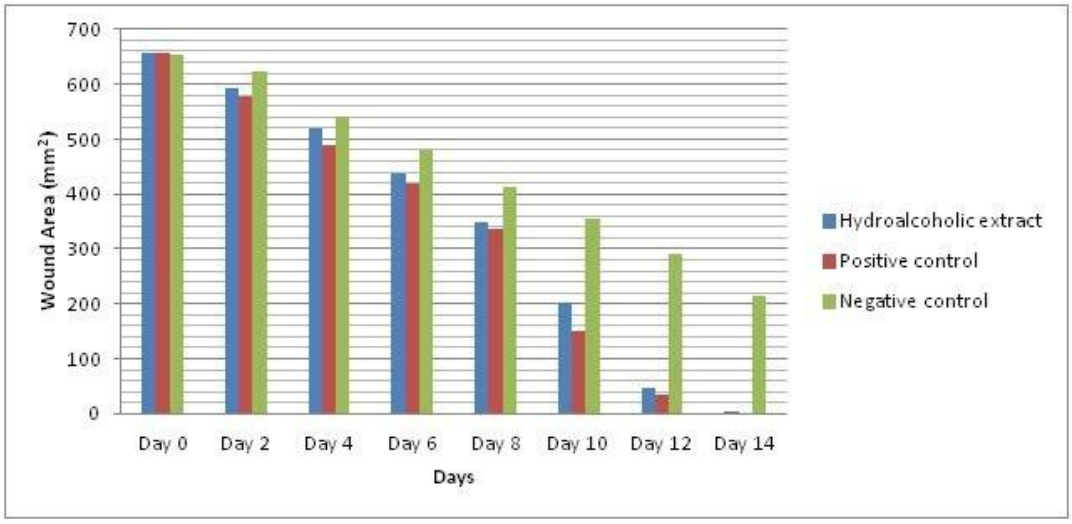

Fig: 01 Wound-healing effect of *In-vivo* excision model

CONCLUSION

The current investigation has provided evidence that the hydro alcoholic extracts derived from *Ehretia acuminata* possess specific properties that facilitate expedited wound healing activity in comparison to

group III (untreated). The utilization of *Ehretia acuminata* in the topical treatment and management of wounds is supported by its breaking strength, rate of wound contraction, and epithelialization of the wound area. A topical preparation including a hydro-alcoholic extract of the leaves of *Ehretia acuminata* may have effective utilization as an external application as a wound healing agent to speed up wound healing.

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Conflict of Interest: Nil

Author's Contribution
"The Concept was discussed by Dr. Nitin Kochar while Mr. Chetan Jain prepared the writing original draft of the article followed by Dr. Anil Chandewar with validation and data analysis, Dr. Nitin Kochar contributes with data curation, then Dr. Anil Chandewar Review and edit the article and finally Mr. Chetan Jain done the formal analysis of the article. All the author read and approved the final manuscript."

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Ethics Statement
The Institutional Animal Ethics Committee of PWCOP, Yavatmal, approved all protocols utilized in this work.

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