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Wound Healing Activity of Extract of Medicinal Plant Pistacia Integrrima

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ABSTRACT

The provided text discusses the wound healing potential of Pistacia integerrima plant extract in the context of addressing chronic wounds. The study focuses on evaluating the wound healing activity of the plant material through in vitro and in vivo analyses. Key findings include the presence of various phytochemicals in the plant, such as carbohydrates, proteins, saponins, flavonoids, tannins, and phenols.

The total phenol and flavonoid content were quantified as 0.316 and 0.244, respectively. In the in vivo setting, the hydroalcoholic extract of Pistacia integerrima at doses of 200 mg/kg and 400 mg/kg demonstrated notable tensile strength, with values of 1.92±0.32 kg and 2.15±0.14 kg, respectively. Comparatively, the standard drug Cipladine exhibited a tensile strength of 1.89±0.15 kg.

Wound contraction on the 15th day showed significant improvement for both the standard drug Cipladine (6.3±6.45) and HAPI-treated groups (200mg/kg & 400mg/kg) with wound diameters of 17±3.65 and 4.5±3.47, respectively. Biochemical analysis revealed a substantial increase in hydroxyproline content in the HAPI-treated groups (80.25 & 86.65 µg/gm for 200mg/kg & 400mg/kg, respectively) compared to the disease control and standard drug-treated groups (43.58 & 67.78 µg/gm, respectively). Collagen content was higher in the HAPI-treated groups (601.74 & 635.85 µg/gm for 200mg/kg & 400mg/kg, respectively) compared to the control and standard drug groups (315.47 & 502.32 µg/gm, respectively). Additionally, hexosamine levels in animal tissues were elevated in the HAPI-treated groups (26.65 & 28.74 mg/gm for 200mg/kg & 400mg/kg, respectively) compared to the disease control and standard drug-treated groups (9.65 & 21.45 mg/gm, respectively). In conclusion, the study's results strongly indicate that Pistacia integerrima possesses effective wound healing activity, showcasing its potential as a natural remedy for promoting the recovery process in chronic wounds.

KEYWORDS:

Wound healing, Medicinal plants, Pistacia integerrima, Phytochemicals, Cipladine, hexosamine, hydroxyproline, collagen

1. Introduction:

The skin serves as the body's protective outer layer, regulating temperature, repelling water, synthesizing beneficial substances like vitamin D, and acting as a crucial barrier between the external environment and internal tissues. In medical terms, abrasion of the skin or underlying tissue can occur due to accidents, acts of violence, or other causes. When the skin is wounded, the complex process of wound healing is initiated.

This process involves various substances, including soluble mediators, blood cells, extracellular matrix, and parenchymal cells. Wound healing is typically categorized into three stages: inflammation, tissue creation, and tissue remodeling. The inflammatory phase involves platelet accumulation, coagulation, and leukocyte migration. Tissue creation encompasses stages such as re-epithelialization, angiogenesis, fibroplasia, and wound contraction. The remodeling phase may extend for about a month, during which the dermis adapts to the injury by producing collagen and matrix proteins before returning to its pre-injury state (Velnar et al., 2009; Enoch and Leaper, 2005).

Current estimates suggest that approximately 6 million people worldwide suffer from chronic wounds. Despite this significant health concern, there are relatively few Indian studies on wound epidemiology.

The investigated population showed a wound prevalence of 15.03 per 1000, with chronic wound prevalence reported to be 4.5 per 1000 individuals. Interestingly, acute wound prevalence was nearly twice as high at 10.5 per 1000 population. Contemporary topical wound therapy focuses on key principles, including the removal of necrotic tissue, control of bacterial loads, management of wound exudates, preservation of open proliferative wound edges, and the provision of a moist and protected wound surface (Gupta et al., 2004; James and Rosso, 2011).

In the effort to facilitate wound repair, medical treatments for wounds encompass the administration of medications either locally (topically) or systemically (via oral or parenteral routes), or a combination of both. Topically applied antimicrobial dressings, including disinfectants, antiseptics, and antibiotics, exhibit a broad spectrum of non-selective antibacterial action (Krahwinkel and Boothe, 2006).

Commonly used antiseptic products in clinical practice include the Povidone-iodine complex, chlorhexidine, alcohol, triclosan, hydrogen peroxide, boric acid, silver nitrate, silver sulfadiazine, and sodium hypochlorite. Topical antibiotics are frequently administered in clinical settings for wounds, cuts, and burns, aiming to address localized cutaneous infections. However, the regular and excessive use of topical antibiotics can result in complications such as the development of resistant organisms (Bolton and Fattu, 1994).

Consequently, there is a growing need for more effective wound healing therapies. Many medicinal plants play a crucial role in the wound healing process. Plants are particularly effective healers as they naturally contribute to the recuperation process.

Plant-based treatments not only expedite regeneration but also contribute to maintaining the aesthetics of the skin. A substantial portion, more than 70%, of prepared wound therapy medications are derived from plants, while 20% are mineral-based, and the remainder contain ingredients sourced from animals. Antiseptic coagulants and wound wash often utilize plant-based ingredients.

Therapeutic herbs exhibit wound healing effects through various mechanisms, including modulation of wound healing, reduction in bacterial counts, refinement of collagen deposition, and increased presence of fibrocytes and fibroblasts (Firdous and Sautya, 2018; Budovsky et al., 2015).

Pistacia integerrima, belonging to the Anacardiaceae family and commonly known as kakarsinghi, is a well-established medicinal herb. It is a medium-sized deciduous tree capable of reaching a height of 40 feet, and it is native to the eastern Himalayan range, spanning from the Indus to Kumaon. In traditional folk medicine, P. integerrima is utilized to address a diverse array of health conditions, including hepatitis, liver disease, anti-inflammatory issues, diabetes, blood purification, gastrointestinal disorders, cough expectoration, jaundice, stomach ailments, fever, and diarrhea (Bibi et al., 2015; Uddin et al., 2011). Recognizing its potential benefits, this study is dedicated to investigating the wound healing activity of extracts derived from the Pistacia integrrima plant.

Materials & Methods:

Collection of Plant Materials:

The fruits of the selected plant, namely Pistacia integerrima, were identified and gathered from various areas in Bhopal based on geographical availability.

Extraction of Plant Materials by Maceration Method:

Powdered plant material weighing 34 grams, specifically the fruits of Pistacia integerrima, was packed into an extraction bottle. The defatted plant material underwent extraction using a hydroalcoholic solvent (ethanol: water; 70:30). The liquid extracts were collected in a tarred conical flask, and the solvent was removed through distillation. Any remaining traces of solvent were evacuated under vacuum. The extracts obtained with each solvent were weighed to a constant weight, and the percentage w/w basis was calculated.

In Vivo Wound Healing Activity:

Animals:

A total of 24 adult Wistar rats (180-200 grams) were divided into four groups (control, standard, Hydroalcoholic extract of Pistacia integerrima at 200mg/kg and 400mg/kg) with six animals in each group. The animals were housed under standard environmental conditions of temperature (23°C) and a 12-hour light-dark cycle. All animals had access to food and water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee and conducted following the guidelines of CPCSEA.

Acute Dermal Toxicity:

Swiss albino female mice weighing 18-22 grams and aged 90 days were utilized to assess the dermal toxicity of test extracts. The toxicological study was conducted to determine the therapeutic dose of the Hydroalcoholic extract of Pistacia integerrima as per OECD guidelines.

The extract was applied at two different concentrations on the shaved dorsal sides of the rats, and it was observed that the dose was safe, with the lower dose considered for further study (Korani et al., 2011).

Animal Testing:

For the in vivo wound experiment, incision and excision wound models were employed. Test extracts were prepared, diluted in double-distilled water, and applied at doses of 200 mg/kg and 400mg/kg.

The test extract was topically applied to the wounded site immediately after creating circular wounds with a surgical blade. The control group of animals received no treatment, and wounds were kept open. In contrast, the standard drug-treated group of animals was administered the reference drug cipladine (10% W/W) (Sami et al., 2019).

Linear Incision Wound Model:

All animals were anesthetized with a 1:1 ketamine hydrochloride and xylazine mixture. The back hair of the rats was shaved, and an impression was made on the dorsal region, 1cm away from the vertebral column and 5cm away from the ear. A linear paravertebral incision of 5cm in length was made through the full thickness of the skin.

Wounds were closed with interrupted sutures, which were removed on the 10th day after wound creation. Incision wounds were treated with the extracts daily for 14 days. The wounds in the control group of animals were kept open and allowed to heal naturally. On the 14th day after wound formation, the breaking strength of the wound (in kilograms) was measured using a tensiometer (Suguna et al., 2002).

Excision Wound Model:

The animals were anesthetized by injecting ketamine hydrochloride and xylazine in a 1:1 concentration. The dorsal fur of the animals was shaved, and an impression was made on the dorsal region. The area of the wound to be created was marked on the back of the animals using picric acid and a circular stainless stencil.

Circular excision wounds of 300 to 400 mm2 were created to full thickness along the markings using toothed forceps and pointed scissors. Wound areas were measured by tracing the wound on a transparency sheet with a permanent marker, using millimeter-based graph paper on days 0, 3rd, 6th, 9th, 12th, and 15th for all groups (Diwan et al., 1983; Gupta and Jain 2011).

Preparation of Test Samples for Bioassay:

The extracts, the reference drug, and the vehicle were applied topically once a day until the 15th day. At intervals of every three days, changes in wound area were monitored, and the wound area was evaluated using graph paper.

The percentage reduction in the wounded area was calculated from wound contraction. Histopathological examination and biochemical parameters were carried out using tissue specimens isolated from the healed skin of each group of rats (Akdemir et al., 2011).

Biochemical Parameters:

Circular wound areas were excised and evaluated for various biochemical parameters at the end of the study. Specifically, collagen content, hydroxyproline, and hexosamine were estimated to evaluate the healing properties of the Hydroalcoholic extract of Pistacia integerrima (Dwivedi et al., 2017).

Statistical Analysis:

Results obtained from the two wound healing models were expressed as Mean ± SD and compared with the corresponding control group by a one-way ANOVA test for assessing statistical significance (Rojas et al., 2002).

Results & Discussion:

The preliminary qualitative phytochemical tests indicated the presence of carbohydrates, proteins, saponin, flavonoids, tannins, and phenols in the plant extract. Terpenoids, flavonoids, and vitamin C, known for their antioxidant and antibacterial activities, have been associated with promoting wound healing. Additionally, triterpenoids have been shown to enhance collagen content, a crucial component in the wound healing process.

The total phenol and flavonoid content were measured to be 0.316 and 0.244, respectively. In the incision wound model, the tensile strength of the wound was evaluated. The tensile strength in the hydroalcoholic extract of Pistacia integerrima at doses of 200 mg/kg and 400 mg/kg was found to be 1.92±0.32 kg and 2.15±0.14 kg, respectively.

The group treated with the standard drug Cipladine exhibited a tensile strength of 1.89±0.15 kg. The control group showed very poor tensile strength at 1.35±0.25 kg. The increase in tensile strength may be attributed to the promotion of collagen synthesis, maturation, stabilization, and angiogenesis by the crude extract.

In the excision wound model, wound contraction on the 15th day for each group was assessed. Wound contraction reflects the rate at which the unhealed region shrinks after treatment. The wound contraction on the 15th day was 6.3±6.45 for the standard drug Cipladine, while for HAPI (200 mg/kg & 400 mg/kg), the wound diameter was found to be 17±3.65** & 4.5±3.47, respectively.

There was a significant increase in hydroxyproline content, measuring 80.25 and 86.65 µg/gm in the HAPI 200 mg/kg and 400 mg/kg groups, respectively. This increase was significantly higher than the disease control and standard drug-treated groups, which had values of 43.58 and 67.78µg/gm, respectively. The rise in hydroxyproline content is indicative of an increase in collagen levels.

In the current investigation, control and standard medication-treated animals exhibited substantially lower collagen content at 315.47 and 502.32 µg/gm, respectively, compared to the HAPI 200 mg/kg and 400 mg/kg treated groups, which had collagen concentrations of 601.74 and 635.85 µg/gm, respectively.

The hexosamine level in animal tissues was determined to be 26.65–28.74 mg/gm in the HAPI 200 mg/kg and 400 mg/kg treated groups, respectively, while it ranged from 9.65 to 21.45 mg/gm in the disease control and conventional medicine-treated groups.

Table 1: Preliminary qualitative phytochemical tests for *Pistacia integerrima* extract

Phytoconstituents	***Pistacia integerrima* extract**
i)Primary Metabolites	
Carbohydrates	(+)
Amino acids	(-)
Proteins	(+)
Fats and oils	(-)
ii)Secondary metabolites	
Steroids	(-)
Triterpenoids	(-)
Volatile oils	(-)
Gums and mucilage	(-)
Glycosides	(-)
Saponins	(+)
Flavonoids	(+)
Tannins & Phenol	(+)
Alkaloids	(-)
HE = Hydroalcoholic extract; '+' = Present; '-' = Absent	

Table 2: Total bioactive constituents' content of *Pistacia integerrima*

Sr. No.	**Extract**	**Total phenol**	**Total Flavonoid**
1	Hydroalcoholic extract	0.316	0.244

The result of study revealed that hydroalcoholic extract of *Pistacia integerrima* contain 0.316 mg/100mg of phenol & 0.244 mg/100mg of flavonoids.

Results of In Vivo Wound Healing Activity:

Incision Wound Model:

Table 3: Results of Incision wound model Tensile Strength (kg)

Sr. No.	Groups	Tensile Strength (kg)
1.	Control	1.35±0.25
2.	Standard Cipladine	1.89±0.15
3.	HAPI (200mg/kg)	1.92±0.32
4.	HAPI (400 mg/kg)	2.15±0.14

Excision Wound Model:

Table 4: Effect of Hydroalcoholic extract of *Pistacia integerrima* (HAPI) in excision wound contraction

Group	0 Day	3rd Day	6th Day	9th Day	12th Day	15th Day
Control	386.5±0.28	315.0±4.25	289.0±3.65	138.0±2.85**	60.4±3.6**5	45.5±7.9**
Standard Cipladine	405.30±0.85	236.7±3.26***	149.8±2.85*	61.8±3.45***	23.3±3.47*	6.3±6.45**
HAPI (200mg/kg)	404.8±2.85	259.7±2.15***	187.3±3.41*	75.2±3.65**	53.5±2.58**	17±3.65**
HAPI (400 mg/kg)	398±3.65	202.3±3.54***	90.3±3.26**	38.2±3.47**	18.7±3.45**	4.5±3.47**

Data: Mean± SD *** P<0.05 when compared with control group.

Table 5: Effect of Hydroalcoholic extract of *Pistacia integerrima* on biochemical parameters of wound healing

Group	Hydroxyproline (µg/gm)	Collagen (µg/gm)	Hexosamine (mg/gm)
Control	43.58	315.47	9.65
Standard	67.78	502.32	21.45
HAPI (200mg/kg)	80.25	601.74	26.65
HAPI (400 mg/kg)	86.65	635.85	28.74

Data: Mean± SD *** P<0.05 when compared with control group.

Conclusion:

The findings of this study demonstrate that Hydroalcoholic Extract of *Pistacia integerrima* (HAPI) possesses the capability to enhance wound healing. This observation aligns with its traditional medicinal use in treating wounds. The observed efficacy may be attributed to the presence of various chemical compounds in the extracts known to contribute to the plant's wound healing properties. Further fractionation and isolation experiments are recommended to identify the active compound(s) in *Pistacia integerrima* responsible for its wound healing activity.

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