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EVALUATION OF WOUND HEALING ACTIVITY OF METHANOLIC EXTRACT OF STEMS OF IPOMOEA PES-CAPRAE (L.) R.BR ON WISTAR ALBINO RATS

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ABSTRACT

The objective of the study is to evaluate wound healing activity of methanolic extract of stems of Ipomoea Pes-caprae (L.) R.Br on wistar albino rats. Ipomoea Pes-caprae is widespread in coastal areas along the beach. Ipomoea Pes-caprae (L.) R.Br is considered as a wonder plant due to its wide range of therapeutic applications. The aerial parts, seeds and other parts of Ipomoea Pes-caprae (L.) R.B rare traditionally useful in inflammatory conditions such as Rheumatoid arthritis, Ankylosing spondylitis, Osteoarthritis, Gout, etc., and also in conditions such as Pain, Ulcer, Cancer and Wounds. Preliminary phytochemical study of the plant extract revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols, terpenoids and glycosides. The acute dermal toxicity (OECD guideline 434) of the plant extract was found to be non-toxic at a concentration of 2000 mg/kg. The wound healing activity of the plant extracts studied using Excision wound model. The plant extract showed significant activity in an Excision wound model. The research showed possible wound healing activity of the plant extract and confirmed its folkloric claim in the treatment of wounds.

KEY WORDS: ***Wound healing activity, Ipomoea Pes-caprae (L.) R.Br, Rheumatoid arthritis, Preliminary phytochemical study, Acute oral toxicity, Excision wound model.***

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1. Introduction

Virtually all cultures worldwide have relied historically on or continue to rely on medicinal plants for primary health care. Approximately one-third of all traditional medicines are for treatment of wounds or skin disorders, compared to only 1-3% of modern drugs [1]. Wound healing or repair is a natural process of regenerating dermal and epidermal tissue and may be categorized into three phases, viz inflammation, proliferation and remodelling phase. In the inflammation phase, various growth factors such as tumor necrosis factor (TNF) and interleukins (IL) are released to initiate the proliferation phase. The latter is characterized by angiogenesis, collagen deposition, granular tissue, epithelialization and wound contraction. Intervention in any one or more of these phases would eventually lead to the promotion of wound healing [2].

Some drugs like topical steroids in wound healing have been more effective and less time-consuming but adverse effects like local, systemic and psychological side effects are inevitable [3]. Hence phytoconstituents from plants are required to be identified for treatment and management of wounds [4].

Ipomoea is the largest genus in the flowering plant family Convolvulaceae, with over 500 species. Ipomoea pes-caprae (L.) R.Br (IP) is a valuable medicinal plant, distributed in the tropics and subtropics regions and used in folk and tribal medicines [5]. Ipomoea Pes-caprae (L.) R.Br is medicinally a valuable plant which was traditionally used to treat arthritis, pain, ulcer, cancer and wounds [6]. Methanolic stem extracts of IP (MESIP) was selected for testing the in-vivo wound healing activity based on a study which showed a high concentration of active constituents in the methanol extract [7]. A limit test can be performed in situations where there is sufficient information on the non-toxicity of the test material used. All the constituents identified and isolated from IP so far were found to non-toxic. Based on the non-toxic indications in the constituents of IP a limit test of 2000 mg/kg was done straightaway [8].

2.Materials and Methods

2.1.Collection and Authentication of plant material

The whole plant of IP was collected from coastal areas of Kanyakumari district, Tamil Nadu and authenticated by Dr.P.Jayaraman (Botanist), Director PARC, West Tambaram, Chennai with Register number of the certificate "PARC/2013/2110". The Herbarium was kept in C.L. Baid Metha College of Pharmacy.

Figure 1: Whole plant and Stem of Ipomoea Pes-caprae (L.) R.Br

2.2. Preparation of extract

The leaves and stems were segregated, dried, powdered and were extracted separately with methanol using Soxhlet apparatus for 48 hrs. The solvent was distilled at a lower temperature under reduced pressure and concentrated on a water bath to get the crude extract stored in a desiccator for future use. The percentage yield of the stem was found to be 4.15%.

2.2. Preliminary Phytochemical Screening

This study was approved by institutional ethical committee of government siddha medical college for

The preliminary phytochemical screening for MESIP was carried out to ascertain the presence of various phytoconstituents.

Alkaloids

To the extracts, few drops of acetic acid were added, followed by Dragendroff's reagent and shaken well. Formation of an orange-red precipitate indicates the presence of alkaloids.

The substance was mixed with a little amount of dilute hydrochloric acid and Mayer's reagent. Formation of a white precipitate indicates the presence of alkaloids.

Glycosides

The extracts were mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was included and made into a paste, warmed gently over a water bath. The presence of glycosides was identified by dark green colouration.

Anthraquinones

Bontrager's Test

The extracts were macerated with ether and after filtration, aqueous ammonia (or) caustic soda was added pink, red (or) violet colour in the aqueous layer after shaking indicates the presence of anthraquinone.

If present as glycoside then the test should be modified by hydrolyzing with hydrochloric acid as the first step.

Triterpenoids (Noller's test)

The extracts were warmed with tin and thionyl chloride. The presence of triterpenoids was identified by pink colouration.

Flavones (Shinado's Test)

To the extracts, in alcohol, few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes and observed for a red colouration for the presence of flavones.

The extract was mixed with alcohol, after which 10% sodium hydroxide solution and ammonia were added. Dark yellowish colour indicates the presence of flavones.

Steroids (Liebermann - Burchard test)

The extracts were dissolved in a few drops of chloroform, 3 ml acetic anhydride, 3 ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Bluish-green colour confirms the presence of the steroid.

Sugars

The extracts were mixed with Fehling's solution I & II and examined for the appearance of red colouration for the presence of sugars.

Proteins (Biuret Test)

To extracts, a 1% solution of copper sulphate was included followed by a 5% solution of sodium hydroxide, the formation of violet-purple colour indicates the presence of proteins.

Phenol

To the extracts, a few drops of alcohol and ferric chloride solution were added. Bluish-green or red indicates the presence of phenol.

Tannins

The extracts were mixed with basic lead acetate solution. Formation of a white precipitate indicates the presence of tannins.

Saponins

The extracts were shaken with water, and copious lather formation indicates the presence of saponins.

Resin

To the extracts, add 5-10 ml of acetic anhydride gently heat and cool. To this add 0.05 ml of sulphuric acid. Bright purplish-red colour rapidly changing to violet indicates the presence of resins.

Flavonoids

Shinoda test

Four pieces of magnesium fillings (ribbon) are included to the ethanolic extract followed by a few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonoids, crimson to magenta indicated flavanones.

Sodium hydroxide test

About 5 mg of the compound is dissolved in water, warmed and filtered. 10% aqueous sodium hydroxide is added to 2 ml of this solution. This produces a yellow colouration. A change in colour from yellow to colourless on the addition of dilute hydrochloric acid is an indication for the presence of flavonoids [9].

2.3. Preparation of ointment

The semi-solid mass of MESIP was made into ointment using Vaseline as a vehicle (MESIP ointment).

2.4. Animals

Wistar albino rats of either sex (200-250 g weight) were used. The evaluations were carried out with the institutional animal ethical committee clearance (Ref:(IAEC/I/02/CLBMCP/2012 dated 28.08.2012)). All the animals were housed in polypropylene cages using paddy husk bedding at 28 ± 1°C temperature and 50 ± 5% humidity. Animals were fed on laboratory feed and water ad libitum.

2.5. Acute dermal toxicity (OECD guideline 434)

Acute dermal toxicity was carried out by single topical application of EELIP and EESIP (Ethanolic extracts of Leaves and Stems of Ipomoea Pes-caprae (L.) R.Br) at the dose of 2000 mg/kg in a separate study. No signs of toxicity or mortality were observed during the acute dermal toxicity study [10].

2.6. Excision wound model

The wound site was prepared following the excision wound model. Three groups of five animals each were used. The rats were anaesthetized before and during the infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using diethyl ether. The wound of 500 sq. mm on the dorsal thoracic region was produced. Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced. The animals were

observed for wound closure at 0, 5, 10 and 15th day and period of epithelialization [11].

2.7. Animal grouping

The animals were divided into 3 groups of 6 animals each,

Group I: Normal (ointment base treated)

Group II: 5% w/w Standard Povidone Iodine ointment

Group III: 5% w/w MESIP ointment

3.Results

Table 1: Preliminary Phytochemical analysis of MESIP

Experiment	Observation
ALKALOIDS	
Mayer's reagent	-
Dragendorff's reagent	+
Hager's reagent	+
Wagner's reagent	-
CARBOHYDRATES	
Molisch's test	+
Fehling's test	+
Benedict's test	+
GLYCOSIDES	
Anthraquinone	+
Cardiac	-
Cyanogenetic	-
Coumarin	-
PHYTOSTEROLS	
Salkowski test	+
Liberman Burchard's test	+
SAPONINS	+
TANNINS	+
PROTEINS AND FREE AMINO ACIDS	
Millon's test	+
Biuret test	+
GUMS AND MUCILAGE	-
FLAVANOIDS	+

Table 2: Effect of MESIP ointment on wound area (Excision wound model)

GROUP	Wound Area (mm^2) Post wounding days				Period of Epithelialization
	0^{th}	5^{th}	10^{th}	15^{th}	
Group I	503.5 ± 4.79	410.27 ± 14.68	282.30 ± 11.92	179.41 ± 10.83	26.21 ± 0.45
Group II	503.9 ± 5.21	296.7 ± 17.26	180.63 ± 6.63	17.07 ± 0.96	17.08 ± 0.36
Group III	504.3 ± 4.82	321.67 ± 6.88	207.54 ± 7.46	24.74 ± 3.42	20.02 ± 0.51

One-way ANOVA followed by Dunnett's test; significance $p< 0.05$

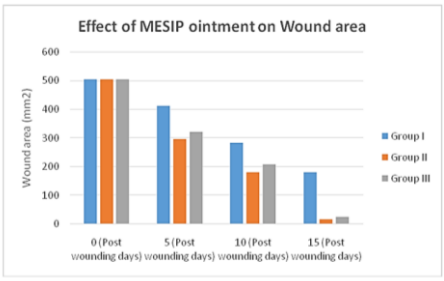

Figure 2: Effect of MESIP ointment on the wound area (Excision wound model)

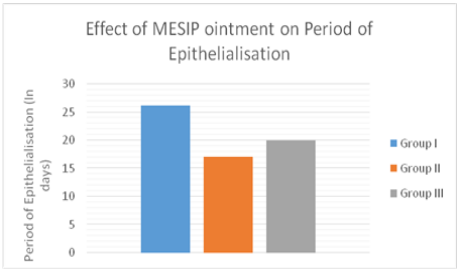

Figure 3: Effect of MESIP ointment on Period of Epithelialization (Excision wound model)

Table 3: Effect of MESIP ointment on Wound contraction (Excision wound model)

Group	Wound contraction (%)			
	0^{th}	5^{th}	10^{th}	15^{th} day
Group I	0.0	18.52	43.93	64.36
Group II	0.0	41.11	64.15	96.61
Group III	0.0	36.22	58.84	95.09

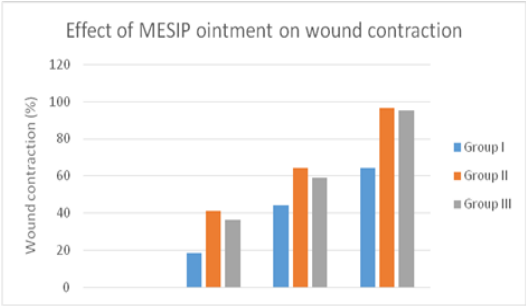

Figure 4: Effect of MESIP ointment on wound contraction

4.Discussion

The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols, terpenoids and glycosides in MESIP (Table 1). Alkaloids and flavonoids have been reported to promote the process of wound healing [12].

The first phase of wound healing or repair is the inflammatory phase2. The significant anti-inflammatory effect of the extracts of IP in cotton-pellet induced granuloma has already been reported. Phytoconstituents such as Flavonoids, tannins, sterols and terpenoids revealed during the phytochemical investigation were found to play a significant role in the inhibition of inflammation [13]. Hence it can be inferred that MESIP exhibits its wound healing activity by interfering with the inflammatory phase of the wound healing process.

Group II (MESIP ointment treated) and Group III (Standard) were found to significantly reduce the wound area whereas in Group I (ointment base treated) reduction in wound area was less (Table 2 and Figure 2). The period of Epithelialization was significantly briefer in Groups II and III when compared to Group I (Table 2 and Figure 3). Groups II and III reported significant wound contraction, on the other hand, Group I showed the least wound contraction (Table 3 and Figure 4).

The proliferative phase which is the second phase in the wound healing process is characterized by re-epithelialization (period of Epithelialization) and wound contraction [14-17]. Thus, MESIP ointment has shown to promote the wound healing process by its action on the proliferative phase.

Remodelling phase or Wound damage repair phase represent the third and terminal phase in the process of wound healing or repair. Oxygen-free radicals mediate the inhibition of wound healing following ischaemia-reperfusion and sepsis [18].IP was reported to be effective in scavenging Hydrogen peroxide radical [19] which is one among the Reactive Oxygen Species assays. Several recent studies are showing that phenolic compounds are commonly found in plants and have been reported to possess several biological activities including powerful antioxidant compounds [20,21]. So, the antioxidant properties of phenolic compounds such as flavonoids and tannins present in the MESIP ointment may have contributed to the wound healing properties by acting on the remodelling phase. The remodelling phase is the step in which damaged/ injured skin is brought back normal. So, the effect of MESIP on wound area, the period of Epithelialization and wound contraction all together can be considered as its action on the remodelling phase.

5.Conclusion

The present study demonstrated that the Methanolic stem extracts of Ipomoea Pes-caprae (L.) R.Br exhibited accelerated wound healing activity compared with the Group I animals treated with only the ointment base. The presence of anti-inflammatory phytoconstituents (Flavonoids, tannins, sterols and terpenoids) and phytoconstituents possessing antioxidant properties (flavonoids and tannins) revealed by the phytochemical analysis may have contributed to the wound healing properties of MESIP ointment. The action of MESIP ointment on all three phases of wound healing viz, inflammation, proliferation and remodelling phase as revealed by parameters like Wound area, Period of Epithelialization and Wound contraction may have led to its significant wound healing activity.

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