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Original Article

Evaluation of Wound Healing Activity of *Ichnocarpus frutescens l.*

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ABSTRACT

Wounds may be defined as loss or breakings of cellular and anatomic or functional continuity of living tissue .Wounds are inescapable event in life. Wounds may arise due to physical, chemical or microbial agents.
Wound healing is the restoration of integrity of injured tissues. It is a dynamic process, involving sequence of events which takes place in an orderly way, i.e. inflammatory repair, closure, remodelling and final healing. Wound healing refers to replacement of dead tissue by visible tissue.
Plants have been used for health and medical purpose from several thousand of years. They are one of the rich and important sources of medicine since human civilization.
The plant *Ichnocarpus frutescens* L. is a plant from the family Apocynaceae is extensively cultivated in most region of the world and common avenue tree, commonly called as black creeper in English
Young stems and leaves contain triterpenoids, α –amyrin and its acetalupeol and its acetates, friedelin, epifriedelinol and β-sitisterol. Flowers and fruits contains flavonoids-Quercetin, Kaepferol-3-glucoside, Sorbopyranoside and flavonoids and is used as a wound healing agent in household remedies and the recent studies on wound healing activity claims that flavonoids promote significant wound healing property .
Key word: Wound healing, *Ichnocarpus frutescens* L.

1. INTRODUCTION

Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissues[1] .Wounds are inescapable event in life. Wounds may arise due to physical, chemical or microbial agents.

Wound healing is the restoration of integrity of injured tissues. It is a dynamic process, involving sequence of events which takes place in an orderly way, i.e. inflammatory repair, closure, remodelling and final healing. Wound

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healing refers to replacement of dead tissue by visible tissue[2].

PHASES OF WOUND HELING[3]

1) Inflammatory Phase
2) Fibroblast proliferation phase
3) Maturation phase
4) Epithelization
5) Contraction phase
6) Granulation tissue production

Plants have been used for health and medical purpose from several thousand of years. They are one of the rich and important sources of medicine since human civilization. Now a day's person prefers plant based medicines over synthetic medication for the treatment of different disease because of their safety as well as economy. Herbal medicines are particularly used by the traditional practitioners since the ancient time but they do not have scientific data.

SCREENING METHODS FOR WOUND HEALING AGENTS

The process and phases of wound healing are almost similar in mammalians. For studying wound healing activity various animals are utilized includes rabbits, rats, guinea pigs, goat and other large veterinary animals. Basically the models used includes excision, incision and dead space wound which usually cover almost all kind of possible wounds one can encounter in clinical practice.

EXCISION WOUND:

In this model a standard wound is made by cutting a circular skin in dorsal thoracic region of the experimental animals. Usually a wound of 500 mm^2 areas is made with the help of marking of margin on pre-shaved area with an indelible ink and rubber seal. However the wound measurement can also be carried out by putting sutures on the margin of the wound or after tattooing or after marking the margin with indelible ink. Morton and Malone have described the evaluation of vulnerary activity of open wounds in rats by planimetric measurements. It is also possible to study the phase of the wound healing in this model.

This is done by taking photographs of the wounds at different time intervals in the process of wound healing. By this method it is possible to differentiate the process of construction from epithelization[4].

Arthur and Ehrlich have modified the technique to study the wound causes by the burn and freeze injury. They have used novel parameter to study the process of wound healing apart from rate and extend of contraction. The histological studies of wound will give more insight into the phases and process of wound healing[5].

INCISION WOUND:

Incision wounds are caused by making two paravertebral straight incision of 6 cm length each through the entire thickness of pre-shaved skin of the experimental animals with the help of a sharp blade as directed by Ehrlich and Hunt.[6] These incision should be 1 cm away laterally to the vertebral column. After hemostasis wounds are cleaned of blood with sterile wet cottons. The wounds are closed with sutures made of '0' no. silk thread. They are placed at equidistant points at least one cm apart from each suture. The wound are mopped with cotton swabs soaked in 70% alcohol. Animals are housed individually. On 8^{th} day the sutures are removed and wound tensile strength are measured by constant water flow technique of Lee. This method indirectly estimates the extent of wound healing that is more the wound tensile strength more is the deposition of collagen in the wound area.[7]

Estimation of bursting strength is another parameter to measure wound strength.[8] The incision wound can be studied in rabbits, guinea pigs, rats and mice. This method is more convenient as the animals cannot infect the wounds from self biting.[9]For distinct results the sutures are removed two days earlier to bursting strength measurement.

DEAD SPACE WOUND:

Deposition of granulation is a vital step in wound healing. The rate of granulation can be directly correlated to the wound healing. Hence measurement of extent of granuloma deposition in dead space wound serves as a meaningful parameter. The granulation tissue may be grown on foreign bodies like sterile cotton pellets, grass piths or polyvinyl sponges.[10,11] These materials are implanted subcutaneously, on which granulation tissue grows. These studies can be extended for histological studies and wound tensile strength measurement.

Objectives

This study is aimed to investigate and validate anti-diabetic and wound healing property of *Ichnocarpus frutescens* L.flowers and the extensive traditional applications in the medical practice and also pharmacological screening and evaluation with the background of *Ichnocarpus frutescens* L.

The scheme of proposed work was:

1. To prepare different extracts of dried flowers of *Ichnocarpus frutescens* L.

2. Wound healing activity:

Preparation of different strains of ointments (5%w/w and 10%w/w of extract in simple ointment).

1. Excision wound model

A) Percentage of wound contraction

B) Period of epithelisation

2. Incision wound model

Measurement of breaking strength

Genus *Ichnocarpus* [12]

Plants scramblers or woody lianas, with latex. Leaves – opposite. Inflorescences – cymose, terminal and/ or axillary. Flowers- small. Calyx- with basal glands inside, lobes- free. Corolla- White, yellowish, or red, salver form; tube widened near base, throat hairy; lobes- oblong, falcate, overlapping to right, in bud with inflexed distal halves. Stamen included, inserted at or below middle of corolla tube; filaments very short; anthers sagittate, adherent to pistal head, cells spurred at base; disc entire, 5-crenate or 5- dentate, or deeply divided

in to 5 erect segment. Ovaries adnate basally to disc, pubescent; ovules numerous. Pistal head ovoid or cup shape. Follicles to spreading or divaricate. Seeds numerous, linear, compressed, not or hardly beaked, comose; endosperm copious; cotyledons long, flat, radical superior.

Habitat- typically found at an altitude of 0 to 2,108meters (0 to 6,916feet).

Plant monograph[13]

The plant *Ichnocarpus frutescens* L. belongs to the family Apocynaceae, is a large evergreen, lactiferous, woody creeper with red appearance, found throughout India, ascending up to an altitude of 4000feets. The roots of the plants are used as medicines in as a substitute for Indian sarsaparilla (*Hemidesmus indicus)*and are often mixed with the later; neither their therapeutic properties or their suitability for use as sarsaparilla substitute have been established.

It is known by several names in the vernacular language, they are

Synonyms[14]

- Ben-Dudhi
- English**:** Black Creeper
- Hindi: Kalidudhi, Shyamalata
- Kannada: Gorwiballi, gouriballi, kappunamadaberu
- Malayalam: nannari, naruninti, palvalli
- Marathi: Dudhbel, Krishna-sarwa, Kante-bhouri
- Sanskrit: Ananta, bhadra, Chandanagopa
- Tamil: Paravalli, udargodi, udarkkoti
- Telugu: Illukkatti, karampala, muntagajjanamu
- Oriya: Bhotinoi

Habit: Shrub, Family- Apocynaceae

Distribution[15]

Common on bushes and hedges in deciduous forests .On iron netted fences around deer park in 1st ghat road (tirumala), S.V. dairy farm in Tirupati on high way sides from Erravaripalem to Nerabailu.

Morphology[15]

Climbing shrub with slender branches; branch lets pubescent.

Leaves elliptical- obviate or oblong, entire, acute apiculate, base attenuate, glabrous above, pubescent beneath, lateral nerves 4-6 pairs. Flowers white, in auxiliary and terminal paniculate cymes. Calyx copular. Lobes 5 subequal, ovate, alternating with 5 glandular scales, acute. Corolla salver-form, widened from below the middle, included.

Chemical constituents[16]

Young stems and leaves contain triterpenoids, α-amyrin and its acetalupeol and its acetate, friedelin, epifriedelinol and β-sitosterol.

Flowers and fruits contain flavonoids-quercetin kaempferol-3-glucoside, sorbopyranoside.

Roots contains flavonoids, sterols, terpenoids[17].The root portion shows the presence of phenyl propanoids, phenolic acid, coumarins, pent acyclic terpenoids[13]

Medicinal uses:

The literature survey revel that *Ichnocarpus frutescens* L. roots has been used as tonic, diuretic, demulcent, diaphoretic, dyspepsia, stones in gall bladder, skin troubles and diabetic[16].Young stems and leaves are also used for diabetes[16].

It is used as a hepatoprotective and antioxidant agent[18]

2. METHODOLOGY

Wound healing activity:

Preparation of Ointments:

Simple ointment was prepared by taking weighed quantities of white bee's wax (2%), hard paraffin (3%), cetosteryl alcohol (5%) and white soft paraffin (90%) and melted. Weighed quantities of 70% ethanol and aqueous flower extract of *Ichnocarpus frutescens* L. (5% and 10%) were mixed with simple ointment by using ointment slab and spatula. The formulated ointments were preserved in a refrigerator .

Models used in screening of wound healing activity

No.	Wound models	Observation	Made on post wounding day
1.	Excision	Wound tracing Epithelization	4,8,12 and 16 was noted on falling of escar without raw wound area
2.	Incision	Sutures removal Tensile strength measurement	8 10

Excision wound model:

The animals were divided into 6 groups each with 6 animals.

Group A: Control, applied topically (0.5 g), simple ointment.

Group B: Standard, applied topically (0.5g), 5% w/w Povidine ointment.

Group C: Treated with ethanolic extract of *Ichnocarpus frutescens* L. flowers 5% w/w ointment (0.5g), topically.

Group D: Treated with ethanolic extract of *Ichnocarpus frutescens* L. flowers 10% w/w ointment (0.5g), topically.

Group E: Treated with aqueous extract of *Ichnocarpus frutescens* L. flowers 5% w/w ointment (0.5g), topically.

Group F: Treated with aqueous extract of *Ichnocarpus frutescens* L. flowers 10% w/w ointment 0.5g, topically.

Animals were under light ether anesthesia throughout the surgical procedure. An impression of 2.5 cm diameter (500 sq mm) as described by Morton and Malone[19]was made after leaving at least 5 mm complete space from the ears. The skin of the impressed area was excised carefully to the complete thickness and a wound of 500 sq mm was formed. Homeostasis was achieved by application of normal saline solution. The rats were kept individually in separate cages. The physical attributes of wound healing via wound closure (contraction) and epithelization were recorded. The wound contraction was studied by tracing the raw wound area on a transparent paper on 4th, 8th, 12th and 16th day. The criterion for complete epithelization was fixed as formation of scar with absence of raw wound area. The wound area was

measured planimetrically with the help of sq mm scale graph paper. The percentage wound closure was calculated by using the following formula:

Percentage closure= $1-A_d / A_o X 100$ ……..(1)

Where,

Ao = wound area on day zero (500 sq mm)

A_d = Wound area on corresponding days.

The number of days for complete epithelization was noted. In the present study no animals showed visible signs of infection.

The results were tabulated in table no-1 and figure 1

Incision wound model:

The animals were divided into 6 groups each with 6 animals.

Group A: Control, treated orally with normal saline.

Group B: Standard, (0.5g), Povidine.

Group C: Ethanolic extract of *Ichnocarpus frutescens* L. flowers, treated orally 250 mg/kg b.w.

Group D: Ethanolic extract of *Ichnocarpus frutescens* L. flowers, treated orally 500 mg/kg b.w.

Group E: Aqueous extract of *Ichnocarpus frutescens* L. flowers, treated orally 250 mg/kg b.w.

Group F: Aqueous extract of *Ichnocarpus frutescens* L. flowers, treated orally 500 mg/kg b.w.

The rats were given plant extract daily up to 10th post wound day.

The incision wound model was studied as described by Ehrilch and Hunt[158-20].

The results are tabulated in table no-2 figure 2

3. RESULTS AND DISCUSSION

a) Excision wound healing:

Effect of topical application of alcoholic and aqueous flower extract ointment of *Ichnocarpus frutescens* L. flowers in excision wound model:

Excision wounds heal by contraction (wound closure) and epithalization, the percentage of wound closure or closure rate includes by recording the changes in wound area at a fixed intervals of time, viz. 4th, 8th, 12th and 16th day after treated with aqueous and ethanolic extract. The maximum percentage of wound closure (100) on the 16th day was observed with standard drug, povidine iodine and (99.5± 0.9) with 10% aqueous extract .With 10% ethanolic extract the percentage of wound closure was (92.4±0.64).The percentage of wound closure with 5% aqueous and ethanolic extracts were 98.8±0.35 and 89.8±0.60 respectively.

The results are tabulated in table no.1

b) Incision wound healing:

Effect of ethanolic and aqueous extract (250 mg/kg p.o.and 500 mg/kg p.o) of *Ichnocarpus frutescens L* flowers in incision wound model.

Incision wounds heal by granulation and collagenation.the mean wound breaking strength or tensile strength of wound in control group was 298.6±8.48.while ,in the case of ethanolic extracts(250 and 500mg/kg)were 346.0±6.51 and 380.0±8.56 respectively and its aqueous extracts(250 and 500 mg/kg)were 412.0±11.37 and 479±10.85 respectively, it was found that the mean time for epithelization and mean scar area were reduced significantly , thereby increasing mean tensile strength compared to control group.

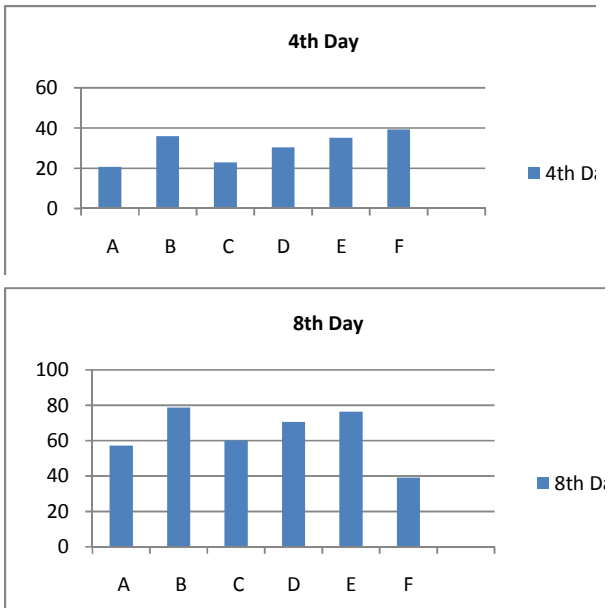
The results are tabulated in table no.2

Table 1: Effect of Ethanolic and Aqueous extract of *Ichnocarpus frutescens* L. flowers On Excision wound parameters

Group	% wound contraction on 4th day	8th day	12th day	16th day	Epithelization time (Days)
A Control	20.8 ± 0.34	57.23 ± 0.67	67.4 ± 0.52	85.8 ± 0.69	20.0 ± 0.86
B Standard	36.0 ± 0.52**	78.8 ± 0.39**	92.0 ± 0.61**	100.0**	15.00 ± 0.26**
C 5% Ethanolic	23.0 ± 0.45	60.2 ± 0.82**	75.7 ± 0.52**	89.8 ± 0.60**	17.8 ± 0.31*
D 10% Ethanolic	30.4 ± 0.59**	70.6 ± 0.47**	84.8 ± 0.42**	92.4 ± 0.64**	16.6 ± 0.33**
E 5% Aqueous	35.2 ± 0.73**	76.5 ± 0.54**	90.6 ± 0.54**	98.8 ± 0.35**	14.0 ± 0.26**
F 10% Aqueous	39.2 ± 0.63*	77.0 ± 0.6*	90.8 ± 0.53*	99.5 ± 0.9**	16.2 ± 0.58**

Animals: Albino rats Route: 0.5 g of extract as ointment applied locally once a day Control: 0.5 g of simple ointment applied locally

The values are expressed as Mean ± SEM, n=6 in each group. If *P>0.05, **P>0.01 vs. control.



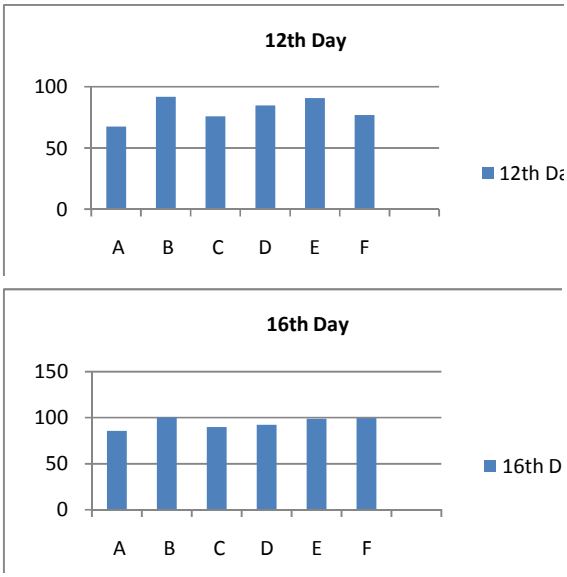
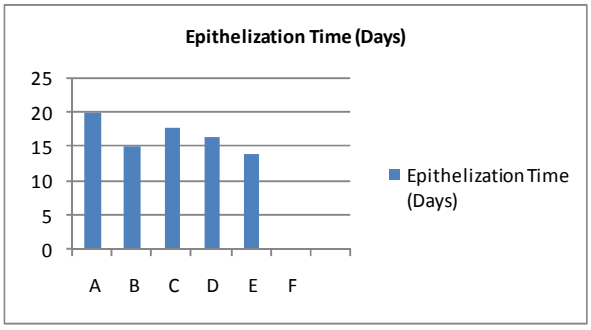


Fig 1: Effect of topical application of flower extract ointment of *Ichnocarpus frutescens* L. on percentage wound contraction on 4th day.

A = Control
D = Ethanolic extract 10% w/w
B = Standard (Povidine iodine ointment 5%)
E = Aqueous extract 5% w/w
C = Ethanolic extract 5% w/w
F = Aqueous extract 10% w/w



A = Control
D = Ethanolic extract 10% w/w
B = Standard (Povidine iodine ointment 5%)
E = Aqueous extract 5% w/w
C = Ethanolic extract 5% w/w
F = Aqueous extract 10% w/w

Fig 2: Effect of topical application of *Ichnocarpus frutescens* L. flower extract ointment on Epithelization time (Days).

Effect of Ethanolic and Aqueous extract of *Ichnocarpus frutescens* L. flower on Incision wound parameters

Animals: Albino rat **Route:** 250 mg/kg body weight orally once a day

Control: 1 ml of 2% gum acacia orally.

Group	Dose	Breaking Strength(g)
A-Control	1 ml of 2% gum acacia	298.6± 8.48
B-Standard	1ml of Povidine	420.0 ± 6.35**
C-Ethanolic extract	250 mg/kg body weight	346.0 ± 6.51**
D-Ethanolic extract	500 mg/kg body weight	380.0 ± 8.56**
E-Aqueous extract	250 mg/kg body weight	412.0 ± 11.37**
F-Aqueous extract	500 mg/kg body weight	479± 10.85**

The values are expressed as Mean ± SEM, n=5 in each group. **P<0.01 compared with the normal control

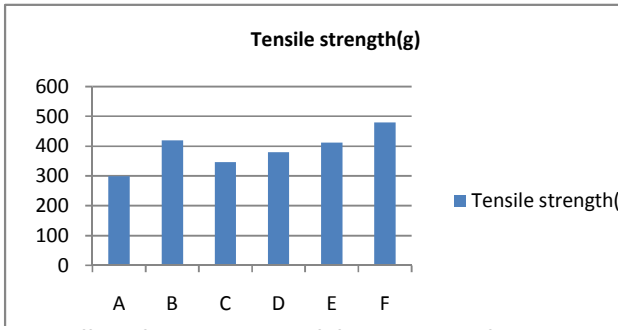


Fig 3: Effect of oral treatment of flower extract of *Ichnocarpus frutescens* L. on tensile strength (g) of resutured incision wound on 10th post wounding day.

A = Control
B =Standard
C= Ethanolic extract (250 mg/kg p.o.)
D= Ethanolic extract (500 mg/kg p.o.)
E = Aqueous extract (250 mg/kg p.o.)
F = Aqueous extract (500 mg/kg p.o.)

4. CONCLUSION

Ethanolic extract of *Ichnocarpus frutescens* L. flowers have shown a dose dependent significant wound healing activity in both, excision and incision wound model. Whereas, the aqueous extract of *Ichnocarpus frutescens* L. flowers have shown significant wound healing property in both models.

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