EVALUATION OF WOUND HEALING ACTIVITY OF THE METHANOLIC EXTRACT ON *RIVEA HYPOCRATERIFORMIS* (Desr.) Choisy.

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ABSTRACT

Wound is defined as a disruption of cellular, anatomical and functional continuity of a living tissue. Several drugs of plants, minerals and animals origins are described in ayurvedic for their wound healing potential. Thus, the methanol extract of *Rivea hypocrateriformis* (whole plant) was investigated for the evaluation of their wound healing potential on excision wound model in wistar rats. The present study consequence the ointment of plant mixture has significant effects on wound contraction, wound closure time, tensile strength and regeneration of tissues on wound site, among other effects. All of these effects were comparable to those of the standard drug Povidone iodine ointment 5%. Thus, this wound healing evaluation study demonstrates the *R. hypocrateriformis* is effective in stimulating the closure of wounds.

KEY WORDS

Rivea hypocrateriformis, methanol extract, povidone iodine ointment, wistar rats.

INTRODUCTION

Wounds are the inescapable events of life, which arise due to physical or chemical injury or microbial infections. In most of the developing world plants or herbal products play an important role in the treatment of wounds (Phillipson, 2001 ; Mensah *et al*., 2006). Wounds may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue (Kokane, 2009). Several drugs obtained from plant sources are known to increase the healing of different types of wounds (Biswas and Mukherjee, 2003).

Herbal preparations can be more effective than conventional medicines and their non-toxic nature means that can be administrated over long periods (Vinothapooshan and Sundar, 2010). Medicinal plants have invariably been a rich source of new drugs and many drugs in use today were obtained from plants have been used in the management and treatment of wounds over the past years (Deepa *et al*., 2019). Recently many plant extracts have been reported for wound healing activity and their cellular mechanism of wound healing has been studied extensively (Mirmalek *et al*., 2015). Wound healing is an important biological process described as "a break in the continuity of tissue, from violence or trauma" (Karan *et al*., 2011).Wound healing is a highly dynamic and complex process that involves cellular, physiological and biochemical events, lead into re-establishment of structural integrity and functional restoration of injured tissue (Hiren *et al*., 2017). There is no previous report on wound-healing activities of *R.hypocrateriformis* in literature to the best of our knowledge and in this paper; we report the efficacy of *R. hypocrateriformis* barks extract in the treatment of wounds.

MATERIALS AND METHODS

Source of the plant sample

Rivea hypocrateriformis (Desr.) Choisy. collected from Kottaram. It is a village located in Kanyakumari district of Tamil Nadu. This village is used to be a resting place for the Travancore Maharajas in the past history. It is very close to Kanyakumari, Vattakottai and Marunthuvazh Malai. It is a mesmerizing beautiful place.

Preparation of the plant extract

The various parts of the plant such as root, stem and leaves were shade dried and made into coarse powder. 250 g of whole plant was extracted with methanol and made in the form of ointment with two different concentrations (5% and 10% w/w ointment of extract in simple ointment base). This extract was used for the evaluation of wound healing activity.

Experimental Animals

Female wistar rats of 6-8 weeks of approximately weighing 160-180gms were used as experimental animals. All rats were kept at room temperature and allowed in standard conditions

at 12-hr light and 12-hr dark cycle in the animal house. Animals were fed with commercial pellet diet and water and add libitum freely throughout the study.

Wound Healing studies

The animals were seperated into four groups for the study of wound healing effect of the extracts. Each group consisted of six rats. Group I is control and was untreated (only wound). Group II is standard and received Povidone iodine 5%. Group III contains extract 1% and received extract 1% simple ointment base and Group IV contains extract 2% and received extract 2% simple ointment base.

Experimental Models

Rats were divided into 5 groups each containing 6 animals

GROUP	GROUP	INTERVENTION
Group I	Control	Untreated
Group II	Standard	Siver sulfadiazine cream
Group III	Test	EXTRACT 1%
Group IV	Test	EXTRACT 2%

Induction of Infected Excision Wound

On wounding day the Rats were anesthetized with ketamine (30mg/kg, ip) prior to creation of the wounds. The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 1.5cm in width (circular area 2.25cm2) created along the markings using toothed forceps, a surgical blade and pointed scissors. All the surgical interventions were carried out under sterile condition. After 24h of wound creation, the standard ointment and EXTRACT 1% and 2% were applied gently to cover the wounded area. Wounds were traced on 1mm^{2}graph paper on the day of wounding and subsequently at a gap period of 3 days till 12th day. 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. Wound area and wound contraction, and hydroxyproline content were monitored.

% wound contraction = healed area/total wound area × 100

Where, healed area = original wound area - present wound area.

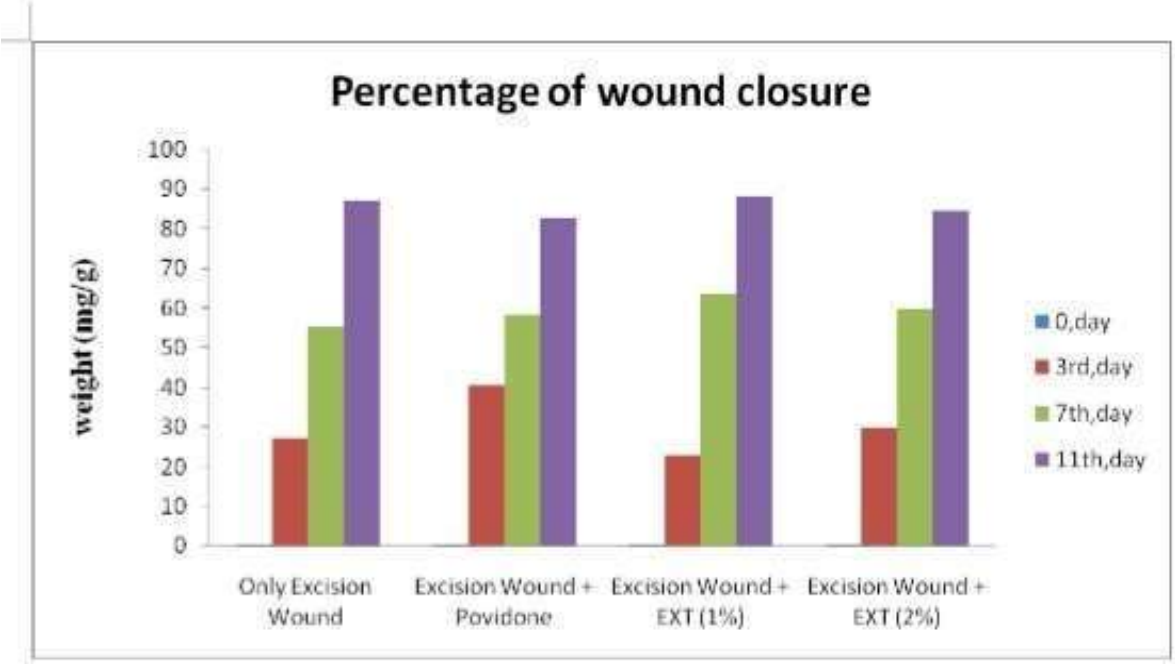
RESULTS AND DISCUSSION

TABLE : 1. EFFECT OF EXTRACT ON WOUND CONTRACTION AND EPITHELIZATION PERIOD IN EXCISION WOUND.

Percentage of wound closure (wound area in mm^2/rat(% contraction)

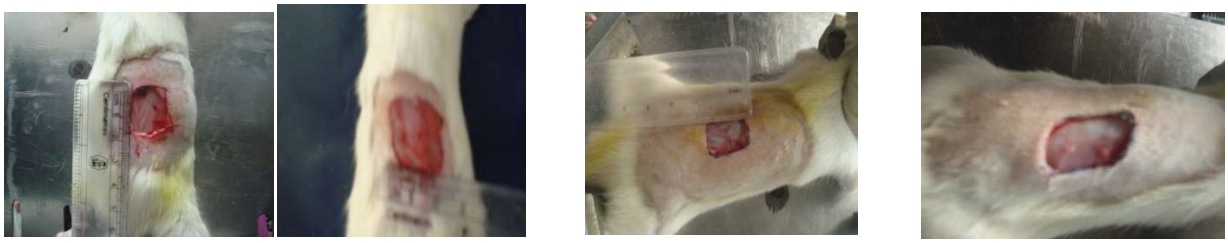
GROUP	Only Excision Wound	Excision Wound + Povidone iodine ointment	Excision Wound + EXT (1%)	Excision Wound + EXT (2%)
0,day	0.38±0	0.38±0	0.38±0	0.38±0
3rd,day	27.23±0.96605	40.534±2.4803**	22.836±2.3225^{ns}	29.778±3.8363^{ns}
7th,day	55.406±1.1138^{ns}	58.392±0.38402^{ns}	63.61±1.2638***	59.8±1.3902*
11th,day	87.118±0.56727^{ns}	82.63±0.45299***	88.228±0.80662^{ns}	84.472±0.71792*

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing only wound group with EXT 1%, 2% group.



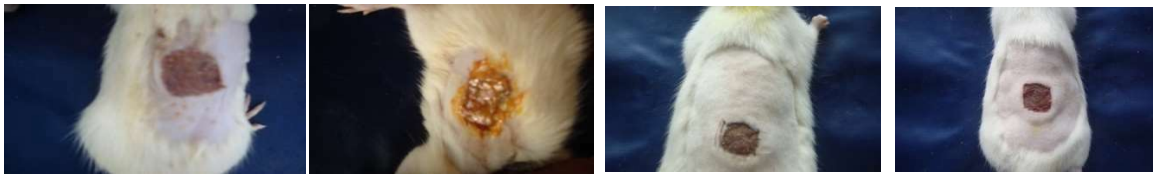
PHOTOGRAPHIC REPRESENTATION OF CONTRACTION RATE SHOWING PERCENT WOUND CONTRACTION AREA ON DIFFERENT POST EXCISION DAYS OF CONTROL, STD OINTMENT AND EXTRACT TREATED RATS.

EXCISION WOUND ON- 0 DAY



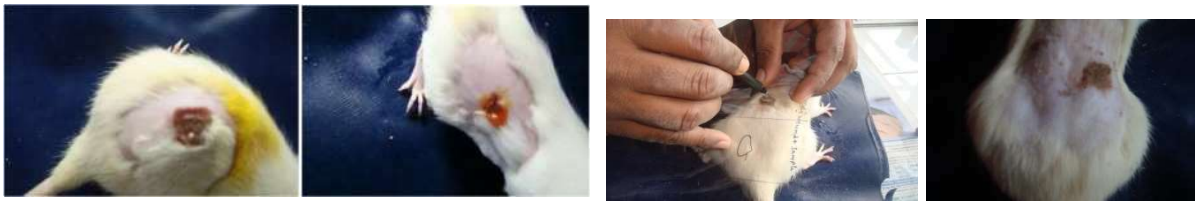
ONLY WOUND **WOUND + STD** **WOUND + EXT 1%** **WOUND + EXT 2%**

EXCISION WOUND ON- 3 rd DAY



ONLY WOUND **WOUND + STD** **WOUND + EXT 1%** **WOUND + EXT 2%**

EXCISION WOUND ON- 7th DAY



ONLY WOUND **WOUND + STD** **WOUND + EXT 1%** **WOUND + EXT 2%**

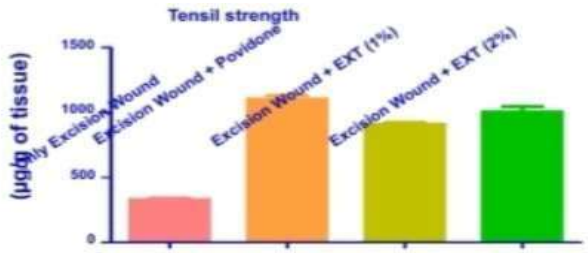
EXCISION WOUND ON- 11th DAY

ONLY WOUND **WOUND + STD** **WOUND + EXT 1%** **WOUND + EXT 2%**

TABLE:2 EFFECT OFEXTRACTON TENSIL STRENGTH OF WOUND IN EXCISION WOUND

GROUP	Only Excision Wound	Excision Wound + Povidone iodine ointment	Excision Wound + EXT (1%)	Excision Wound + EXT (2%)
Tensile strength (µg/g of tissue)	332.1±3.966	1105±14.08***	910.5±7.107***	1007±23.24***

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing only wound group with EXT 1%, 2% group.



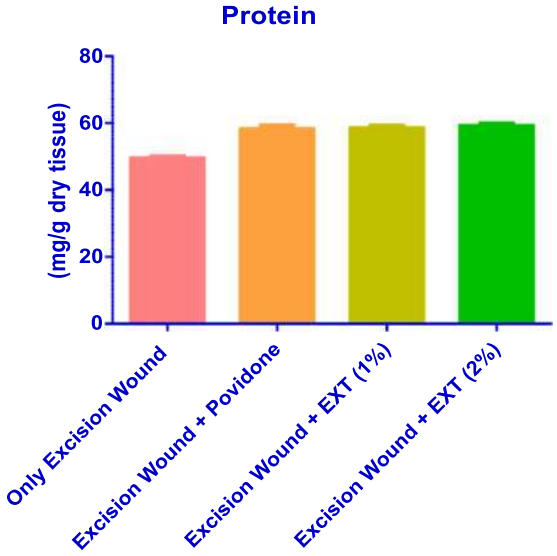
EFFECT OF EXTRACT ON TENSIL STRENGTH OF WOUND IN EXCISION WOUND

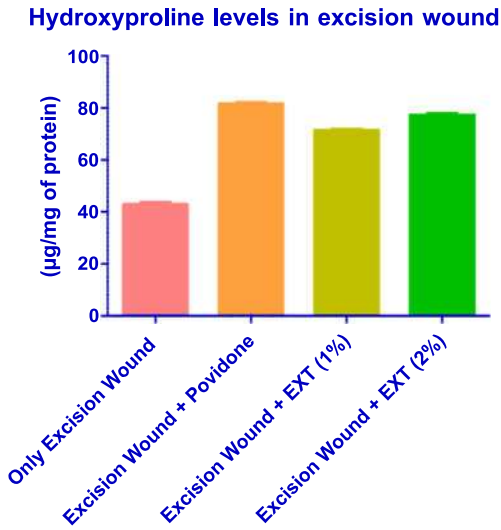
TABLE 3 - EFFECT OFEXTRACTON PROTEIN,HYDROXYPROLINE,HEXOSAMINE,HEXURONIC ACID LEVELS IN EXCISION WOUND

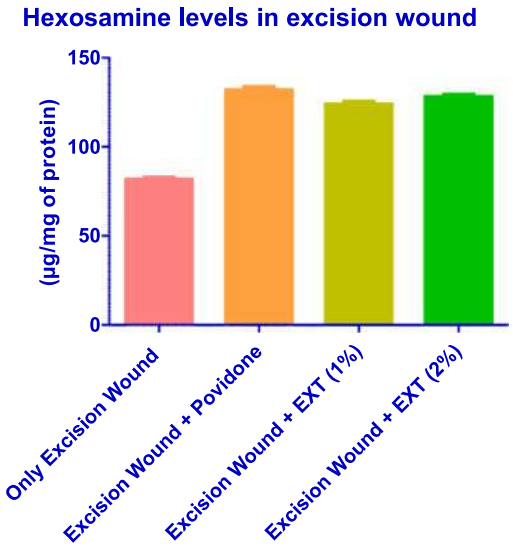
GROUP	Protein (mg/g dry tissue)	Hydroxyproline (µg/mg of protein)	Hexosamine (µg/mg of protein)	Hexuronic Acid (µg/mg of protein)
Only Excision Wound	49.54±0.2951	42.84±0.4101	81.82±0.5845	17.86±0.3855
Excision Wound + Povidone iodine ointment	58.32±0.5797***	81.55±0.2979***	132±0.9576***	56.37±0.4875***

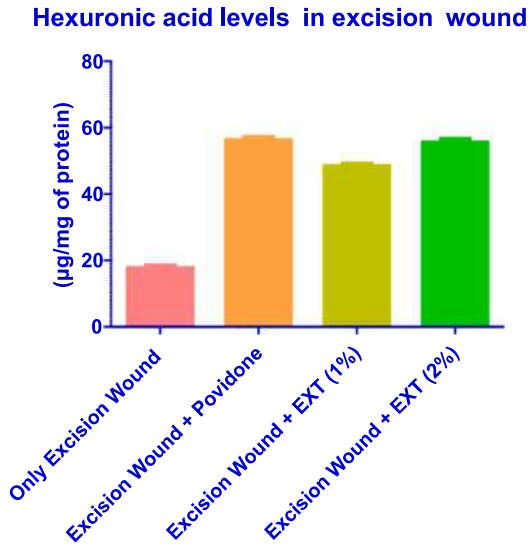
Excision Wound + EXT (1%)	58.58±0.3736***	71.37±0.2085**	124±0.9185**	48.59±0.3586***
Excision Wound + EXT (2%)	59.26±0.336***	77.2±0.3313**	128.2±0.6477***	55.62±0.6099***

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing only wound group with EXT 1%, 2% group.









Plants generally produce a viable wound healing potentials because of their non-toxicity, absence of harmful side effects and their throughout distribution. The wound healing activity of various concentrations of the methanol extract of *Rivea hypocrateriformis* (Desr.) Choisy. have been reported in the wistar rats. The application of the drug over the infected wound is more effective for wound healing activity. The application of the extract ointment at different concentrations (5% and 10%) shows a significant reducion in the wound area as represented in table-1.

In this study of wound healing activity, the percentage of wound closure is higher in standard (40.534±2.4803**) than the control (27.23±0.96605), extract 1% (22.836±2.3225) and extract 2% (29.778±3.8363) on the 3rd day. On the 7th day and 11th day extract 1% is more effective for wound closure activity (63.61±1.2638***) and (88.228±0.80662) respectively. Control on 7th day (55.406±1.1138) and standard on 11th day (82.63±o.45299***) shows less wound closure activity and epithelization stages as represented in the table-1.

The tensile strength of a healing skin wound indicates the degree of wound healing. It represent how much the healed tissue resists to breaking under tension and may identify the quality of healing. On the 12th day, all the animals were anesthetized by injectig ketamine hydrochloride, the healed tissue was excised from all animals. The tensile strength of the sample was tested using DAK SYSTEM BENCH as showed in table-2. The effect of extract on tensile strength of wound are control (332.1±3.996), standard (1105±14.08***), extract 1%

(910.5±7.107***) and extract 2% (1007±23.24***. The protein, hydroxyproline, hexosamine and hexuronic acid content were also monitored in the wound area.(Table.3). The results revealed the methanol extract of Rivea hypocrateriformis have excellent wound healing activity.

Pushparani *et al*., (2018) focuses the role of herbal extracts in the wound healing processess. The methanolic extracts of *Aspidium cicutarium* and *Spathodea campanulata* find a potential as wound healing agents and can be formulated into suitable dosage forms (Maushumi *et al*., 2014). The wound healing evaluation study demonstrates that *Carmona retusa* is effective in stimulating the closure of wounds (Mageswari *et al*., 1999). Evaluation of the wound healing activity of an ethanolic extract of Ceylon cinnamon in mice may be effective in stimulating the enclosure of wounds (Farahpour and Habibi, 2012). Nidhi *et al*., (2014) revealed a significant antioxidant activity evidenced from decreased level of MDA and increased the level of GSH, catalase in extract treated animals as compared to control animals.Yogesh Sharma *et al*., (2013) discussed wound healing potential of plants, which are helpful for researcher to develop new wound healing formulations for human use.

Phytoconstituents present in the hydro-alcoholic extract of aerial parts of *Ocimum basilicum* are responsible for wound contraction and increased rate of epithelialization (Gaurav *et al*., 2017). Evaluation of wound healing effect of *Ziziphus mauritiana* leaf extract in rats increased the rate of wound contraction, decreased period of epithelisation, increasedskin breaking strength and dry granulation tissue weight, elevated hydroxyproline content (Meera Sumanth and Bhargavi (2014).

CONCLUSION

The wound healing studies in methanolic extract of *Rivea hypocrateriformis* exhibited a potential wound healing activity. The wound-healing property of *R. hypocrateriformis* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. The present study revealed that the methanolic extract was found to possess better wound healing property.

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