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WOUND HEALING ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRCTS OF FRUITS OF *ZIZYPHUS OENOPLIA*

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ABSTRACT

The wound healing activity of aqueous and alcoholic extracts of fruits of *Zizyphus Oenoplia* was evaluated by excision, Incision and dead space wound models on rats. The study was carried out by topical application of 5% w/w ointment of aqueous and alcoholic extract was prepared in 2% sodium alginate. The study includes measurement of parameters like rate of wound contraction, period of epithelialization, tensile strength of tissue and formation of granulation on tissue. The results revealed that the significant decrease in time of epithelialization, significant increase in tensile strength, and granuloma tensile strength in the animals treated with aqueous and alcoholic extracts of fruits of *Zizyphus Oenoplia*. Significant activity was found in alcoholic followed by aqueous extracts when compared to control. From the study it was concluded that both the extracts were found to posses significant wound healing activity. The results were compared with the Framycetin sulphate cream as reference standard drug.

Key words: *Zizyphus Oenoplia*, Wound healing activity, Extracts.

INTRODUCTION

A wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Wound healing can be defined as a complex dynamic process that results in the restoration of anatomic continuity and function. Many herbal plants have an important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way. The healing process can be physically monitored by assessing the rate of contraction of the wound.

Zizyphus Oenoplia belonging to the family Rhamnaceae has been used both for its food as well as for medicinal value. It is usually grown in dry deciduous forests and in waste

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lands region. This fruits have been found to be believed some of the pharmacological properties and commonly used as blood purifier, febrifuge, abdominal pain, etc.[1-3] The earlier results shows that the chloroform and methanol extracts have been used to show antibacterial activity against few bacterial strains[4]. Preliminary phytochemical studies revealed that both the extracts have the presence of phytochemical constituents like sterols, carbohydrates, saponins and flavonoids, where as in alcoholic extract there are some traces of alkaloids. Literature survey revealed that no systemic work has been carried out to establish the wound healing activity on the *Zizyphus Oenoplia* fruits. In view of this, the present study was aimed to evaluate the wound healing activity of aqueous and alcoholic extracts of *Zizyphus Oenoplia* fruits.

EXPERIMENTAL

Preparation of extracts

Zizyphus Oenoplia fruits were collected from the surrounding of Shivamogga, Karnataka, India. The fruits were shade dried and powdered mechanically. About 250 g of the powdered material was subjected for extraction with alcohol (EtOH) using soxhlet apparatus and the other 250 g of powder was macerated with distilled water which gives aqueous extract. The percentage yield of alcoholic and aqueous extract was found to be 26% w/w and 19% w/w, respectively. Both the extracts were subjected to preliminary phytochemical study according to the standard procedures[5] and the extracts were screened for pharmacological activities.

Formulations

The drug formulations were prepared from each of the extract. For topical application 5% w/w ointment was prepared in sodium alginate (2%). For oral administration, suspensions in the concentration 35 and 20 mg/mL were prepared by in tragacanth (1%).

Pharmacological screening

Acute toxicity studies[6]

The experiment was initiated only after approval Institutional Animal Ethical Committee. Acute toxicity study was carried out for both the extracts by stair case or Up and down method. The LD_{50} of aqueous and alcoholic extracts of fruits was found to be 350 mg/Kg b.w and 200 mg/Kg b.w, respectively. One tenth of LD_{50} was taken for the evaluation of wound healing activity.

Wound healing activity

Albino rats of either sex of Wistar strain weighing 150-200 g were procured from Central animal house, National College of Pharmacy, Shivamogga. The animals were housed in polypropylene cages and were maintained at 27 ± 2^{o}, relative humidity 60 ± 5% and 12 h light/dark cycle; they were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water *adlibitum*. Under light ether anesthesia wounding was performed aseptically.

Three models in albino rats i.e. (i) excision wound (ii) incision wound and (iii) dead space wound, were used for accessing wound healing activity. Here the rats were anaesthetized prior to excision and other surgical procedures and they are finally sacrificed by exposing them to higher doses of anesthetic ether prior to the determination of tensile strength of the resutured wounds and the removal of granuloma tissues.

Excision wound[7]

The rats were inflicted with excision wounds under light ether anesthesia. A circular wound of about 500 mm^2 was made on depilated ethanol sterilized dorsal thoracic region. The animals were divided into four groups of six each. The 1st group was considered as the control, IInd was served as reference standard and treated with Framycetin sulphate cream (1% w/w) (FSC), IIIrd and IVth group animals were treated with ointment prepared from aqueous and alcoholic extracts of fruits of *Zizyphus Oenoplia*, respectively. The ointment was topically applied once in a day, till the complete epithelialisation takes place starting from the day of the experiment. The parameters studied were wound closure, epithelialisation time and tensile strength of tissue. The wounds were traced on mm^2 graph paper on 4th, 8th, 12th and 16th day and there after daily until healing was complete. The percentage of wound closure was calculated. The period of epithelialisation was calculated as the number of day's required for filling of the dead tissue without any residual raw wound.

Incision wound model

The method of Ehrlich and Hunt was used where under light ether anesthesia rats were wounded and two- Para vertebral incisions of 6 cm were made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp blade. The incisions were sutured using 4-0 silk thread with the help of straight rounded bodied needle. The sutured were removed on the 8th post wounding day and the tensile strength was determined on 10th post wounding by continuous constant water flow technique[8-10].

Table 1: Effect of topical application of aqueous and alcoholic extracts of fruits of Zizyphus *Oenoplia* on excision wound models.

Group (N)	4th Day	8th Day	12th Day	16th Day	Period of epithelialization
Control	15.33 ± 0.29	30.54 ± 0.43	44.19 ± 0.42	66.67 ± 0.51	23.67 ± 0.21
Framycetin sulphate	33.59 ± 0.51***	59.59 ± 0.43***	88.87 ± 0.20***	96.29 ± 0.36***	17.67 ± 0.21***
Ethanol extract	34.06 ± 0.31***	58.55 ± 0.37***	87.07 ± 0.30***	97.29 ± 0.29***	17.33 ± 0.21***
Aqueous extract	28.37 ± 0.24***	52.66 ± 0.30***	78.74 ± 0.36***	91.15 ± 0.41***	19.83 ± 0.40***

N = 6 animals in each group

*** = $p \leq o.001$ indicates significant when compared to control values are expressed as mean ± SEM

Table 2: Mean tensile strength (grams) of resutured incision wound on 10th post wounding day of alcoholic and aqueous extract by Zizyphus *Oenoplia* fruits

Extract/fraction	Dose mg/Kg	Grams ± S.E.M
Control	-------	313.5 ± 2.172
Standard (FSC)	1% w/w	460.16 ± 4.285 ***
Alcoholic	200	438.83 ± 3.167***
Aqueous	350	380.50 ± 5.214***

*** = $p \leq .001$indicates significant when compared to control values are expressed as mean ± SEM

Dead space wounds (Granuloma studies)[11]

In dead space wound model, the animals were divided in to three groups of 6 rats in each. The I[st] group was served as the control which received 1 mL of tragacanth (1%) /Kg body weight (b.w.) the II and III groups received oral suspensions of aqueous and alcoholic extracts of *Zizyphus Oenoplia* fruit (35 and 20 mg/Kg b.w.) respectively. Under light anesthesia, dead space wounds were created by subcutaneous implementation of sterilized

cylindrical grass piths (2.5 cm x 0.3), one on either side of the dorsal paravertebral surface of each rat. The granulation tissues formed on the grass piths were excised on 10^{th} post wounding day and the tensile strength was determined on 10^{th} post wounding by continuous constant water flow technique.

Table 3: Mean granuloma tensile strengths (grams) on post wounding day by Zizyphus *Oenoplia* of alcoholic and aqueous extract

Extract/fraction	Dose mg/Kg	Grams ± S.E.M
Control	-------	328.5 ± 3.766
Alcoholic	200	557.66 ± 4.745 ***
Aqueous	350	523.33 ± 2.704***

*** = $p \leq .001$indicates significant when compared to control values are expressed as mean ± SEM.

Statistical analysis

The data was subjected to one way ANOVA followed by Tukey's multiple comparison test and values of $p \leq .001$ were considered as statistically significant.

RESULTS AND DISCUSSION

The doses of the extracts were determined by "Up and Down" method and were found to be 350 mg/Kg body weight and 200 mg/Kg body weight for aqueous and alcoholic fruit extracts, respectively. The results of excision wound on the 4^{th}, 8^{th}, 12^{th} and 16^{th} day, the period of epithelialization was summarized in Table 1. Contraction of excision wound was promoted from 4^{th} day of treatment to 16^{th} day. In case of rats treated with fractions of aqueous and alcoholic extract of fruits the epithelialisation of wound was found to be quite earlier as compared to control. Alcoholic extract was found to be having more significant wound healing activity than aqueous extract. The results of incision wound model and granuloma studies are reported in Table 2 and Table 3, respectively. A significant increase in the tensile strength of the tests as compared to control suggests that the extracts promote wound healing activity. Results of excision, incision and granuloma models showed significant wound healing property of the fruit extracts of Zizyphus *Oenoplia.*

The recent studies on wound healing activity claim that flavonoids promote significant wound healing property. The well known property of almost every group of flavonoids is their capacity to act as antioxidants. Flavonoids protect the body against

reactive oxygen species. The body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism[12,13]. The increased production of reactive oxygen species during injury results in consumption and depletion of endogenous scavenging compounds and flavonoids may have an additive effect to the endogenous Scavenging compounds. Here flavonoids can increase the function of endogenous antioxidants. It is also found that hydroxyl groups of flavonoids make the radical inactive by combining with radical, Flavonoids [OH] + R → Flavonoids [O] + RH. Phytochemical screening revealed the presence of flavonoids in *Zizyphus Oenoplia* fruit extracts. The enhanced wound contraction effect and epithelialization may be due to the presence of flavonoids. However confirmation of this suggestion will need well designed clinical evaluation.

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