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Research Article

WOUND HEALING ACTIVITY OF FLAVONOID FRACTION OF *CYNODON DACTYLON* IN SWISS ALBINO MICE

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ABSTRACT
The aim of the present study is to evaluate the wound healing activity of flavonoid fraction of *Cynodon dactylon* in swiss albino mice. The wound healing property of the fraction was studied in excision wound which was inflicted by cutting away 0.5cm standard full thickness of wound was created on the predetermined dorsal area. The flavonoid fraction of *Cynodon dactylon* were applied externally daily on the excised wound area for 8 days. This fraction facilitates the healing process as evidenced by increase in collagen and protein and decrease in lipid peroxide in granulation tissue. Thus, the enhanced wound healing may be due to free radical scavenging activity of the flavonoid fraction.
Key words: *Cynodon dactylon*, flavonoid fraction, collagen, protein, swiss albino mice

INTRODUCTION

Wound can be defined as cut or break in the continuity of any tissue which may arise due to physical, chemical or microbial agents[1]. Healing of wound is a fundamental response to tissue injury occurs by a process of connective tissue repair, the end product of which is a scar. The phases of normal wound healing include homeostasis, inflammation, proliferation and remodeling [2,3] All these steps are orchestrated in controlled manner by a variety of cytokines including growth factors like platelet derived growth factor(PGDF), transforming growth factor B(TGF-B), fibroplast growth factor (FGF) and epidermal growth factor (EGF) have been identified in self healing wounds . Wound healing requires adequate blood supply and nutrients to be supplied to the site of damage [4]

Cynodon dactylon (Poaceae) commonly known as arugampul, is a perennial grass, forming thick mats by means of stolen and rhizomes. It is an important medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine. It is bitter, sharp, hot taste, good odour, laxative, brain and heart tonic, emetic, expectorant, carminative and useful against pains, inflammation and tooth ache [5].It is traditionally used as an agent to control diabetes in India [6]. The present study is to evaluate the wound healing efficacy of flavonoid fraction of *Cynodon dactylon* in Swiss albino mice.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Cynodon dactylon* was collected in area free of pesticides and other contaminants from the area surrounding of Coimbatore, Tamilnadu. The collected leaves were washed thoroughly and blotted dry with filter paper and used for the flavonoid fraction preperation.

Preparation of flavonoid fraction

In the preliminary screening, the direct ethyl acetate extract of *Cynodon dactylon* with powdered magnesium + conc.HCl developed an orange to magenta color indicated the presence of flavonoid showed a characteristic color reaction in shinoda test. The color is due to the reductive conversion of the flavone into the corresponding anthocyanin pigment [7]. Knowing the presence of flavonoid in ethyl acetate extract, the extraction was undertaken with 20 g of powdered plant material and 200ml of light petroleum ether (b.p. $40^0 - 60^0$ C) in a Soxhlet apparatus for 18 hours to remove the chlorophyll, non flavonoid components and lipid de waxing.[8] The treated material was dried and extracted with ethyl acetate using Soxhlet apparatus.[9]

Experimental animals

Swiss albino mice 2-3 weeks old of either Sex weighing 25-30 gms were kept in cage under standard conditions of temperature (22±3°c) and relative humidity (R_H 30-70%) with free access to food and water. All protocols in this study were conducted in accordance with internationally accepted principles for use and care of experimental animals.

Experimental procedure

A Circular skin piece of 0.5 cm standard full thickness wound was created on the pre determined dorsal area under ether as anaesthesia. The animals were randomly divided into 4 groups and each group consisted of six animals.

Group 1. Control mice wound was untreated for 8 days

Group 2.Mice were treated with sodium alginate (2%) for 8 days

Group 3. Mice were treated with povidone-iodine ointment (1%) for 8 days

Group 4 Mice were treated with extract (2.5%w/w) of *Cynodon dactylon* for 8 days

Assessment of wound healing

The granulation tissue formed on day 8 of wound creation was used for further biochemical Studies. The Samples were washed in physiological saline and cut into pieces, defatted with Chloroform: methanol (2:1) and Lyophilized.5 mg of lyophilized tissue was hydrolyzed with 5 ml of 6N Hydrochloric acid for 20 hours in sealed tubes. After hydrolysis, the sample was evaporated to dryness; the residue

was dissolved in water and made up to 3 ml which was used for the estimation of total collagen [10], protein, [11] and lipid peroxide [12].

Statistical Analysis

The results were presented as the mean ± standard deviation of 6 animals

RESULTS AND DISCUSSION

Table 1 shows the effect of flavonoid fraction of *Cynodon dactylon* on wound healing activity. Collagen, protein were increased in group 2, 3 & 4 when compared to control group and lipid peroxide was decreased in group 2, 3 & 4 when compared to control. The increase hydroxyproline content which was a reflection of increased collagen levels by increased cross linking of collagen fibres.[13] The breakdown of collagen liberates free hydroxyl proline and its peptides and elevated level of hydroxyl proline is the index of increased collagen turnover. This growth was accompanied by concomitant increase in total Protein. The potential decrease in lipid peroxide in granulation tissue of treated mice clearly indicates the antioxidant nature of *Cynodon dactylon* has higher wound healing capacity when compared to control.Flavonoids have been documented [14] to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing. Tannins and flavanoids are known to promote wound healing process due to their astringent and antimicrobial activity, which is responsible for wound contraction and faster epithelisation period [15]. Thus, the enhanced wound healing may be due to free radical scavenging action and the antibacterial property of the phytoconstituents present in it which either due to their individual or additive effect fastens the process of wound healing. Attempt is also being made to isolate the active principle.

CONCLUSION

Thus, wound-healing property of *Cynodon dactylon* may be attributed to the flavonoid content in it, which may be either due to their individual or additive effect that fastens the process of wound healing. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

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Table 1. Wound healing activity of flavonoid fraction of *Cynodon dactylon* in swiss albino mice

Groups	Total Collagen mg / 100 mg tissue	Protein mg / 100 mg tissue	Hexosamine mg / 100 mg tissue	Lipidperoxide n moles / 100 mg tissue
Group 1 control	3.338 ± 0.042	12.033 ± 0.350	649.333 ± 5.785	1065.167 ± 4.021
Group 2	5.462 ± 0.090	14.133 ± 0.628	728.833 ± 2.317	672.167 ± 5.492
Group 3	7.373 ± 0.060	24.233 ± 0.388	859.000 ± 2.608	223.667 ± 5.574
Group 4	14.110 ± 0.318	31.570 ± 0.699	991.000 ± 3.098	481.333 ± 3.830

Values are mean ± SD of six samples in each group

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