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Research Article

Evaluation of Burn Wound Healing Activity of *Momordica cymbalaria* Fenzl.

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ABSTRACT

The present investigation is undertaken to evaluate the burn wound healing efficacy of *Momordica cymbalaria* Fenzl (family Cucurbitaceae) and to explore its possible mechanism of action on experimental burn wounds in rats. Burn were induced in SD female rat divided into five group as following; Group-I (negative control) received no treatment. Group II, III, IV and V Received SSC (1%w/w), 5% extract of MC, Scafold, 5% of sapaninof MC topically twice daily for 14 days. The efficacy of treatment was evaluated based on the wound contraction, period of epithelization, hydroxyproline content, anti-oxidant activity and histopathology studies. The 5% of saponin of MC treated group show faster reduction in wound area in comparison but lesser then the SSC-treated groups. The topical application of 5% of saponin of MC inceased collagen synthesis and stabilization at wound site by increase in hydroproline and up-regulated collagen type III.Furthermore there was significant increase in level enzymatic and non-enzymatic antioxidant and decresed in lipid peroxide level.*Momordica cymbalaria* roots possess significant healing potential in burn wound and have positive influence on different phases of wound repair.

Keywords: Wound Contraction, Epithelization period, Hrdroxyproline, Angiogenesis, Anti-oxidant, Burn wound, Extracellular Matrix. Momordica Cymbalria Fenzl.

INTRODUCTION

A burn as type injury that can be caused by heat, cold, electricity, chemical, light Radition or friction. Burn can be highly variable in terms of tissue affected, the severity and resultant complication. Muscle, bone, blood vessel and epidermal tissue can all be damaged with subsequent pain due to profound injury to nerves. Number of potentially fatal complication including shock, infection, electrolyte imbalance and respiratory distress. Burn injury produces profound systemic changes such as oligemic shock, anemia, renal failure and metabolic disturbance. It causes direct tissue damage as well as inflammatory reaction. Infection is another major complication of thermal injury. It also lead to increased oxidant stress in the cells as seen by decreased endogenous non enzymatic and enzymatic antioxidant activity. The mediators of burn shock include histamine, serotonin, kinins, oxygen free radical, prostaglandins, thromboxane and interleukins. After a severe burn, the injured surface becomes vulnerable to the bacteria, due to loss of protective skin barrier. High levels of bacteria in the burn wound can decrease the availability of growth factor, which can retard the healing process. Slow healing and non healing wounds, such as ulcers, as well as wounds caused by major or minor injuries, surgery, or burns, represents the most widespread treatable condition encountered by humans and animals. Wound repair is a well highly coordinate process that involves a series of overlapping phases: inflammation, cell a proliferation, matrix deposition and tissue remodeling. Underlying repair is a complex dynamic series of events including clotting, inflammation, cell proliferation, matrix deposition and tissue remodeling. Underlying repair is a complex dynamic series of events including clotting, inflammation, granulation tissue formation, epithelization, revascularization, collagen synthesis and wound contraction. *Momordica cymbalaria* Fenzl(MC) (Cucurbitacea) is a species used locally for the treatment of diabeties mellitus, antiovulatory, abortifacient, anti implantation and cytoprotective activities.MC are also reported to have antimicrobial activity and anti-hyperglycemic activity`other plant aiso having wound healing activity like Ampure, Honey, Chitosan, Liquorice, Turkish propolis, Lantana camara, Hippophae rhamnoides leaf.

MATERIALS AND METHOD

Collection and authentification of plant materials

The fresh root of *Momordica cymbalaria*. Were collected from Gadag district, Karnataka, identified and authentified by Dr. Sreenath, Department of Botony, Banglore university, and Banglore. A specimen sample of the same was preserved in the herbarium section of the department of Botony, Bangalore Univercity, Bangalore with the voucher No.18122003 for future reference

Preparation of Extract

The root of *Momordica cymbalaria*. Were chopped into small pieces and dried under shade room temperature for seven days. The dried roots were powdered and passed

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Table 1: Priliminary Phytochemical Investigation of Saponins of *Momordica cymbalaria*

S. No	Test	Present (+) Or Absent (-)
1.	Alkaloids	Negative
2.	Carbohydrates	Positive
3.	Glycosides	Positive
4.	Fixed oils and fats	Negative
5.	Gums	Negative
6.	Proteins and amino acids	Negative
7.	Saponins	Positive
8.	Tannins	Negative
9.	Phytosterols	Positive
10.	Flavanoids	Negative

Table 2: Effect of methanolic Extract *Momordica cymbalaria* Fenzl.5%w/w/14 days), Saponin and Silver Sulfadiazine cream (1% w/w/14 days) on burn wound on topical application.

Groups	Wound Contractio (%) on 9th day of topical application			Epithilisation period (14 days)
Wound control (Untreated)	1st day	9th day	Wound contracti on	134
Silver Sulfadiazine cream(1%)	0.8	0.7	135	785
5% Extract of MC	0.8	0.4	487	124
Scafold	0.8	0.6	976	654
5% Saponin of MC	0.8	0.6	346	908

through the sieve (Coarse 10/40). The powder was used for the preparation of methanolic extract.

Extraction and Isolation

Dried and powdered roots of Momordica Cymabalaria (1.0 kg) were extracted with boiling 70% MeOH in a reflux condition. After fiteration, the solution was concentrated under vacuum. The methanolic extract of *Momordica cymbalaria* was dissolved in hot water and was saturated n-butanol and a water layer (1:1). The organic layer (n-butanolic layer) was separated and evaporated to get a residue. This n-butanolic residue was dissolved in methanol and was poured in diethyl ether (Et_2O) (1:1) to obtain a flocculent precipitate. This precipitate was separated by using a filter paper and washed with excess of EtOH and dried to yield a crude fraction of saponin. The saponin mixture was dissolved in distilled water and was used for study.

Burn Wound Model (Hot Wax Model)

Healthy adult (Spargue Dawley) femal rats are selected (150-200) were divided into 5 group, each group consisting of 6 rats and each animals kept separately under laboratory condition. They had free access to commercial pallet diet and ad libitium. Each rat will be anesthetized with ketamine Hcl inj. (50 mg/kg) and the hair on the back will be clip with electric clippers. Burn wounds will be created by pouring hot molten wax at80C into a metal cylinder placed on the back of the rat. The metal cylinder has 300mm ares of circular opening and the capacity of to hold 4.0g of the wax. On solidification of the wax (8 min) the metal cylinder with wax adered to the skin will be remove, which left distinctly demarked cicular wounds of 300mm. After this each animal will be placed in a separate cage for full recovery from anesthesia before being returned to holding rooms.

Drug Treatment and Group Division

Group 1: Wound Control (untreated) – Only vehicle

Group 2: Reference standard – SSC (1% w/w) mg/kg 2 times a day

Group 3: Methanolic extract of MC -5 mg/kg 2 times a day

Group 4: 5% of saponin of MC -5mg /kg 2 times a day

Different Parameter Measurement Procedure

Wound Contraction:

The size of the wound will be measured by taking daily photo used digital camera transperant scale.by taking the initial size of the wound contraction using following equation

$$= \frac{\text{Initial wound size-specific day wound size} \times 100}{\text{Initail wound size}}$$

Epithilization period

It is to monitoring by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind.

Estimation of Hydroxyproline Content

Procedure

- Granulation mass, which was dried at 60ºC for about 24hrs was weighed and placed in selected tube containing 10ml of 6 N HCL.The tube were sealed and Autoclave 300ºc for 3 hrs to hydrolyze the tissues.
- The hydolysate was cooled and excess of acid was neutralized with 10N NaoH using methyl red as indicator.
- The volume of neutral hydrolysis was made upto 200ml with distilled water. From this 0.1ml was used to estimate hydroxyproline content.
- 0.1ml of hydrolysate sample was pipette out into clean test tube, volume made up to 0.5 ml distilled water.
- From the stock solution of the standard hydrxyproline 1.6 mi was taken and dilited up to 100 ml. From this 0.5 (8µg) was pipette put into a clean test tube.
- To this 1ml each of 2.5 NaoH 0.01 M Cuso4 and 6% H_2O_2 were added. Immediately, the tubes were placed in a water bath at 80^0c for 1 minute and then cooled for 5 minutes.
- To this 2ml of freshly prepared 5% solution of paradimethylamine benzaldehyde in propanol and 4 ml of 3 N H_2 SO were added. Test tubes were once again placed in hot water bath at 80ºc for I minute and then cool for 5 minutes.
- The optical density (O.D) of the pink color of these test sample were comto that of the standared hydroxyproline of the known concentration samples at 540nm using Beckman Du-64 spectrophotometer for the estimation of hydroxyproline Formula used for calculation is:

Concentration of unknown= $\frac{\text{OD of unknown}}{}$ x concentration x Volume of

Table 3: Effect of Methanolic Extract *Momordica cymbalaria* Fenzl.(5%w/w/14 days),Saponin of MC (5% w/w/14 days) ,Scafold and Silver Sulfadiazine cream (1%w/w/14 days) on Burn wound

Group	Total protein (g/dl)	Hydroxyproline(mcg)
Wound Control(untreated)	0.8483 ± 0.01	2.700 ± 0.02582
Silver Sulfadiazine Cream (1%)	3.103 ± 0.01	4.350 ± 0.74
5% Extract of MC	1.235 ±0.01	3.683± 0.04
Scafold	1.722 ±0.01	4.183 ±4.19
5% Saponin of MC	2.242±0.01	4.783 ±0.03

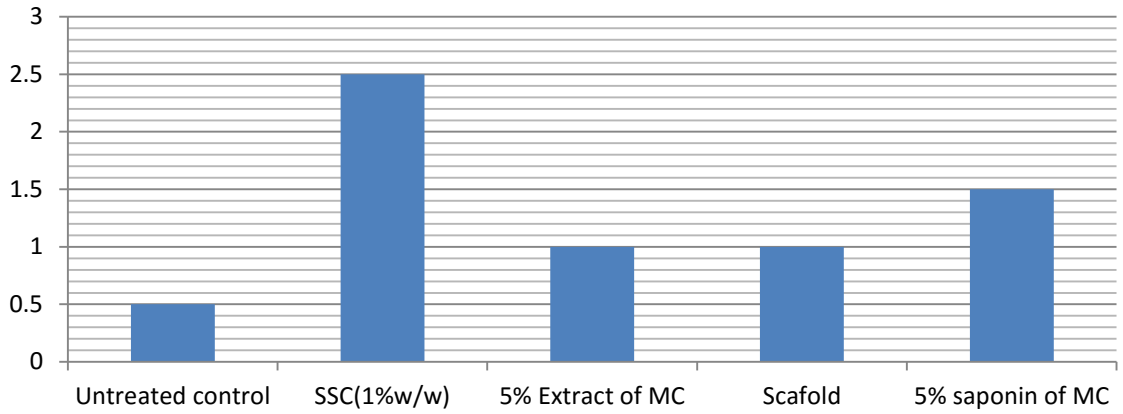

Figure 1:

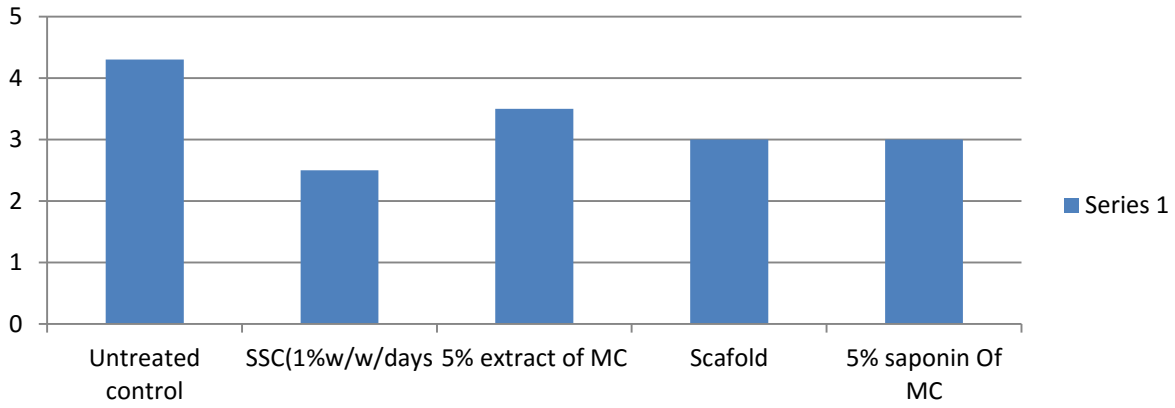

Figure 2: Effect of methanolic Extract *Momordica cymbalaria* Fenzl.5%w/w/14 days),Saponin of MC (5% w/w/14 days) ,Scafold and Silver Sulfadiazine cream (1%w/w/14 days) on Erpithelization period (%days)

(µg) OD of standared of standared hydrolysate

Estimation of MD in tissue for sample

The granulation tissue was homogenized in 0.1M Tris-HCL Buffer (ph 7.4) to produce 1%w/v homogenate. To a sample of 0.2ml of tissue homogenate,0.2 ml of 0. 8% aques solution of TBA were added and mixed thoroughly. The final volume in all the tubes was made up to 5 ml distilled water and the resulting chromigen is extracted with 5 ml mixture of n-butanol and pyridine (15:1v/v) by vigorous shaking. Separation of organic phase was facilitated by centrifugation at 400rpm for 10min. The absorbance of the solution was measured at the wave length of 532nm.

RESULTS AND DISCUSSION

Burn wound healing of *Momordica cymbalaria* Fenzl was demonstrated by a significant increase in the rate of wound contraction, hyroproline content, total protein and Anti-oxidant activity and also decreases in the period of epithelization and lipid peroxidation.the inceased in wound contraction of *Momordica cymbalaria* Fenzl in treated rats might be due to an enhanced activityof fibroblast in regenerated wound tissue. Application of saponinof *Momordica cymbalaria* Fenzl anc Silverdiazine Crem (1%) to the hotwax burn wound produce significant ($p>10$) increase in Catalase (CAT), Superoxide Dismutase (SOD), Glutathion –s-transferase (GST) activity in the granulation tissue when compared to untreated hot wax burn wound rat. Application of Silver Sulfadiazine Cream (1%), Saponin of *Momordica cymbalaria* Fenzl. Scaffold, and Saponin of *Momordica cymbalaria* Fenzl to the hot wax burn wound produced siginificant ($p<0.001$) decrease in MDA activity in the granulation tissue when compared

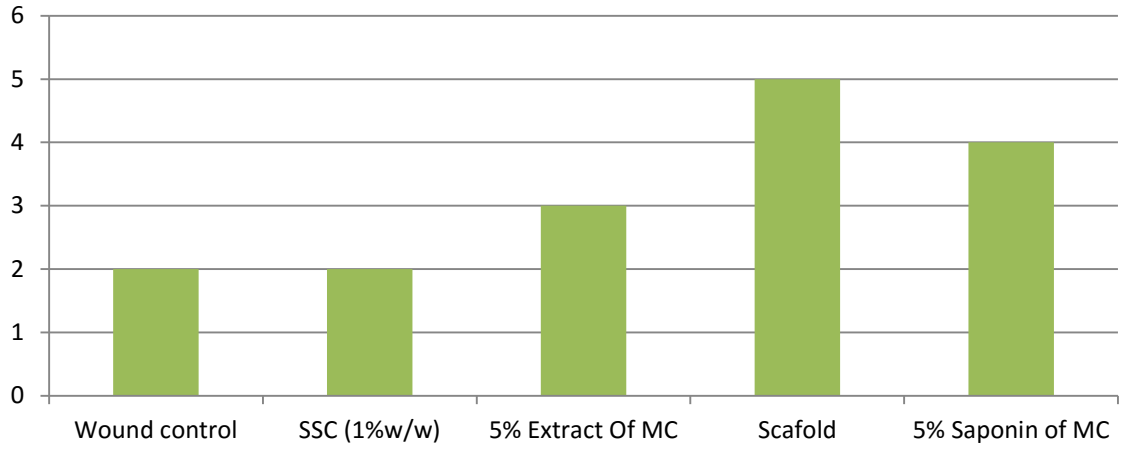

Figure 3: Effect of Methanolic Extract *Momordica cymbalaria* Fenzl. (5%w/w/14 days),Saponin of MC (5% w/w/14 days) ,Scafold and Silver Sulfadiazine cream (1%w/w/14 days) on topical protien

to untreated hot wax burn wound rats. Apart from all *Momordica cymbalaria* Fenzl showing more anti –oxidant activity significant compare others so indicate it is having burn wound healing activity.

CONCLUSION

The result of the present study indicated that the Methanolic extract of *Momordica cymbalaria* Fenzl. Promotes burn wound healing in experimental burn animals. This was demonstrated by a significant in rate of wound contraction, hydroxyproline content, total protein and Anti-oxidant content and also decreased the period of epithelization and lipid peroxidation. Hence *Momordica cymbalaria* Fenzl having burn wound healing activity by using hot molten wax model in burn wound animal rats.

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