



REVIEW ARTICLE

EFFECT OF *MOMORDICA CHARANTIA* L. SEEDS EXTRACT ON BIOPHYSICAL AND BIOCHEMICAL PARAMETERS OF WOUND IN EXPERIMENTALLY INDUCED DIABETES IN RATSAhuja D^{1*}, Mittal A²¹ Ph.D. Research fellow, Faculty of Pharmaceutical Science, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India.² Dean, Faculty of Pharmaceutical Science, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India.

Received 18 November 2013; Revised 05 October 2013; Accepted 15 November 2013

ABSTRACT

Momordica charantia Linn. seed extract in the form of an ointment (20% w/w dried powder in simple ointment base) was evaluated for wound-healing potential in an excision wound model whereas the seed extract given orally was evaluated for incision and dead space wound model in rats. The rats were divided into six groups of diabetic control, diabetic treated, diabetic standard, normal control, normal treated and normal standard in excision wound model whereas the rats were divided into four groups of diabetic control, diabetic treated, normal control and normal treated in incision and dead space wound models, each group consisting of six rats. Wound-contraction ability in excision wound mode was measured at different time intervals on days 4th, 8th, 16th and the study was continued until the wound had completely healed. Tensile strength was measured in 10-day-old incision wound and hydroxyproline content and wet and dry granulation weight of wound was evaluated for dead space wound. The extract treated wounds were found to contract faster as compared to controls, the wet and dry granulation tissue weight and hydroxyproline content was also increased as compared to controls. This suggests that Momordica charantia Linn. Seed extract promotes significant wound healing in diabetic rats.

Key words: Momordica charantia, wound healing, ointment, excision wound, incision wound, dead space wound

INTRODUCTION^{1,2}:

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of diabetes mellitus exist and are caused by a complex interaction of genetics, environmental factors, and life-style choices. Depending on the etiology of diabetes mellitus, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose usage and increased glucose production. The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.

Most commonly diabetic patients have neuropathy, which could be causative. When coupled with an impaired ability to fight infection, these patients become largely unable to mount an adequate inflammatory response. Thus, the Diabetic foot ulcers that may look like a healing wound becomes a portal for infection that can lead to sepsis and require limb amputation. Over 100 known physiologic factors contribute to wound healing

deficiencies in individuals with diabetes. These include decreased or impaired growth factor production, angiogenic response, macrophage function, collagen accumulation, epidermal barrier function, quantity of granulation tissue, keratinocyte and fibroblast migration and proliferation, number of epidermal nerves, bone healing and balance between the accumulation of extracellular matrix components and their remodeling by Matrix metalloproteinases. Wound healing occurs as cellular response to injury and involves activation of keratinocytes, fibroblasts, endothelial cells, macrophages and platelets. Many growth factors and cytokines released by these cell types are needed to coordinate and maintain healing. Molecular analyses of biopsies from the epidermis of patients have identified pathogenic markers that correlate with delayed wound healing. These include overexpression of c-myc gene Coupled with a reduction in and abnormal localization of epidermal growth factor receptor and activation of the glucocorticoid pathway, keratinocyte migration is inhibited. At the nonhealing edge (callus) of Diabetic foot ulcers, keratinocytes show an absence of migration, hyperproliferation, and

incomplete differentiation. Fibroblasts demonstrate a phenotypic change as well as decreased migration and proliferation.

The plant material obtained from dried seeds of *Momordica charantia* Linn belongs to family Cucurbitaceae contains chemicals that lower blood sugar include a mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids. The hypoglycemic effect is more pronounced in the seed of bitter melon where these chemicals are found in greater abundance. Alkaloids, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponin, guanylate cyclase inhibitors, gypsogenin, hydroxytryptamines, karounidiols, lanosterol, lauric acid, linoleic acid, linolenic acid, momorcharasides, momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordicosides, momordin, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid, oxalic acid, pentadecans, peptides, petroselinic acid, polypeptides, proteins, ribosome-inactivating proteins, rosmarinic acid, rubixanthin, spinasterol, steroidal glycosides, stigmastadiols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vacine, v-insulin, verbascoside, vicine, zeatin, zeatin riboside, zeaxanthin, and zeinoxanthin are all found in bitter melon.

Diabetic complications especially diabetic wound have a very low research. Use of *Momordica charantia* has been established in diabetic management the plant has geographical distribution globally in almost every climatic condition. There is no significant work done in management of diabetic wound complication on the plant *Momordica charantia*. Hence study is undertaken to evaluate scientifically the effect of *Momordica charantia* on biophysical and biochemical parameters of wound healing in experimentally induced diabetes in rats.

MATERIALS AND METHODS:³

Procurement and identification of crude drug:

The dried Leaves, Roots & Seeds of *Momordica charantia* were procured from market of Jaipur. The plant material was identified by comparing with voucher specimen no. **SBCP/PCG/H/2010/99** deposited in the herbarium of Sri Balaji College of Pharmacy, Jaipur.

Collection of reagents:

All the reagents used were obtained from central store house JVVU Jaipur.

Extraction:^{4,5}

The dried seeds were coarsely powdered, weighed and filled in Soxhlet apparatus for extraction. The solvent used was hydroalcoholic i.e. 50% ethanol and 50% water. % yield was calculated for each extract after drying, which was found 66%.

Procurement and selection of animals:

Wistar rats of either sex weight between 100 – 150 gm were obtained from B.R.N.C.P. Mandsaur animal house. The animals were stabilized for 1 week; they were maintained in standard condition at room temp; $60 \pm 5\%$ relative humidity and 12 h light dark cycle. They have been given standard pellet diet and water *ad-libitum* throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output. These animals were used for the acute toxicity and diabetic wound healing activity.

Acute toxicity study:^{6,7}

The acute toxicity study was carried out in adult female wistar rats by “fixe dose” method of OECD (Organization for Economic Co-operation and Development) Guideline No.420. Fixed dose method as in Annex 2d: Test procedure with a starting dose of 2000 mg/Kg body weight was adopted. The animals were fasted overnight and next day extracts of the dried seeds of *Momordica charantia* (suspended in 0.5 % w/v sodium CMC) were administered orally at dose level 2000 mg/kg. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days. The observations were tabulated according to ‘Irwin’s Table’.

Acute Dermal Toxicity (Fixed Dose Procedure):⁷

The acute dermal toxicity study was carried out in adult female albino rats by “fix dose” method of OECD (Organization for Economic Co-operation and Development) Guideline No.434. Extracts (heart wood) of the plant *Momordica charantia* were applied topically at dose level 2000 mg/kg.

The test substance was applied uniformly over an area which is approximately 10 % of the total body surface area with the help of a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. Then the animals were observed continuously for pain Changes in skin eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, and somatomotor activity and behaviour pattern, tremors, convulsions

Salivation, diarrhoea, lethargy, sleep and coma. Every 30 min for next three hour and finally for mortality after 24 hour till 14 days.

Selection of doses:

For the assessment of diabetic wound healing activity, dose level was chosen in such a way that, dose was approximately one tenth of the maximum dose taken during acute toxicity studies (200 mg/kg).

Induction of diabetes:³

Wistar rats of either sex weighing 100-150 gm were taken. Animals were fasted for 24 hours then a single intra peritoneal injection of freshly prepared alloxan (120 mg/kg dissolved in 0.9% saline) was injected. After that the animals were left aside for 4 hrs and then 10% glucose solution was placed in the cages for 24 hrs. The diabetes was confirmed by estimation of blood glucose level (BGL) at 3rd day. Rats having BGL more than 250 mg/dl were used for study. After 7 days when animal become diabetic excision wound were made.

Preparation of steric acid ointment

20% extract ointment was prepared with steric acid formulation.

Steps followed for 100gm ointment preparation

1. 0.7 gm KOH dissolved in 60 ml water
 ↓
 Added 20 gm glycerine
 ↓
 Added 10 gm *Momordica charantia* extract

2. Melted 24 gm stearic acid in beaker

3. mixed the preparations of 1st and 2nd steps with constant stirring

Excision wound healing activity in diabetic rats³

Excision space Wounds were created on the 7th day after induction of diabetes. Excision wounds were used for the study of biochemical parameters and the rate of wound contraction All wounds were of full-thickness type extending up to the adipose tissue. The dorsal inter-scapular region of each rat was shaved after anaesthetising the animal with anaesthetic ether. Excision wounds of size 330 mm² were made by cutting out a 1x1 cm piece of skin from the shaved area.

Grouping of animals:

Group I Diabetic wound control treated with simple base ointment.

Group II Diabetic wound topically treated with standard drug ointment (mupirocin ointment.)

Group III Diabetic wound topically treated with ointment of extract.

Group IV Normal wound control treated with simple base ointment.

Group V Normal wound topically treated with standard drug ointment (mupirocin ointment.)

Group VI Normal wound topically treated with ointment of extract.

Incision wound healing activity in diabetic rats:³

A longitudinal paravertebral incision of six centimeters in length was made through the skin and cutaneous muscle on the back in anesthetized rats. After the incision, surgical sutures were applied at intervals of one centimeter. The wounds were left undressed (day 0). The sutures were removed on the 8th post wound day and the application of extract was continued. The skin-breaking strength was measured on the 11th day by tensiometer

Tensiometer structure:

A tensiometer was designed consisting of a 6 – 12 inch wooden board with a 4 inch long arm, fixed on each side of longest possible distance of board and a platform in middle of board.. A pulley with a bearing was mounted on the top of one arm. A co-assembly consisting a reservoir with water and a plastic bottle was also designed.

Method applied for measuring tensile strength:

To measure the tensile strength, The board was placed at the edge of table, the rats were again anesthetized and each rat was placed on wooden platform situated on the middle of the board. The thickness of platform was adjusted in a manner such that the wound was on the same level as the tips of the arms. The clamps were then carefully clamped on the skin of the opposite edges of the wound. The position of the board was adjusted so that the bottle received a rapid and continuous flow of water from a large reservoir, until the wound began to open. The weight of water required to open the wound was measured in gm and considered as tensile strength.

Grouping of animals:

Group I Diabetic wound control treated orally with normal saline.

Group II Diabetic wound treated orally with 400mg/kg extract.

Group III Normal wound control treated orally with normal saline.

Group IV Normal wound treated orally with 400mg/kg extract.

Dead space wound healing activity in diabetic rats:³

Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg each), one on left side in the groin and axilla on the ventral surface of each rat. On the 10th post-wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anesthesia. After noting the weight of the granulation tissue, the tissue was dried at 60°C for 12 hr, and the dry granulation tissue weight was recorded. To the dried tissue 5 ml 6N HCL was added and kept at 110°C for 24 hr. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline.

The assay procedure for hydroxyproline content:^{8,9}

1. Aliquots of standard hydroxyproline(2-20µg) prepared from stock solution and test samples containing hydroxyproline under 10 µg/mL were mixed gently with sodium hydroxide (2N final concentration) in a total volume of 50 µL.
2. The sample were hydrolyzed by autoclaving at 120°C for 20 min.
3. 450 µL of chloramines-T was added to the hydrolyzate, mixed gently, and the oxidation was allowed to proceed for 25 min at room temperature.
4. 500 µL of Ehrlich’s aldehyde reagent was added to each sample, mixed gently and the chromophore was developed by incubating the samples at 65° for 20 min.
5. Absorbance of each sample was read at 550nm using a spectrophotometer to find out concentration of hydroxyproline.

Grouping of animals:

- Group I** Diabetic wound control treated orally with normal saline.
- Group II** Diabetic wound treated orally with 400mg/kg extract.
- Group III** Normal wound control treated orally with normal saline.
- Group IV** Normal wound treated orally with 400mg/kg extract.

Statistical analysis:

The data of activity were analyzed by one way analysis of variance (ANOVA) followed by “Tukey’s test” by using graph pad prism version 4 software and *p* value less than 0.001 was considered as statistically significant.

RESULTS:

Acute Toxicity studies:

Acute Toxicity studies on female rat’s shows no mortality at a dose of 2000 mg/kg, during a time period of 14 days . The Behavioral, Neurological, Autonomic responses were studied for a time period of 6 hrs of toxicity study. During the study no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe.

Wound healing studies (Excision wound model)

(a)Wound area (mm²)

Table 1: Wound area (mm²)

| Group Day | Group-I DC | Group-II DS | Group-III DE | Group-IV NC | Group-V NS | Group-VI NE |
|-----------|---------------|---------------|---------------|---------------|---------------|---------------|
| 0 | 234.17 ± 2.39 | 237.17 ± 2.33 | 283.33 ± 4.41 | 267.33 ± 3.71 | 232.50 ± 3.82 | 215.00 ± 1.06 |
| 4 | 215.33 ± 1.41 | 189.67 ± 2.91 | 242.50 ± 2.35 | 240.00 ± 2.89 | 183.33 ± 3.01 | 174.67 ± 1.54 |
| 8 | 199.50 ± 2.93 | 173.50 ± 0.92 | 220.33 ± 1.52 | 219.50 ± 0.96 | 154.00 ± 1.39 | 162.00 ± 1.65 |
| 16 | 189.33 ± 1.20 | 152.33 ± 1.20 | 194.67 ± 4.37 | 194.17 ± 1.25 | 132.33 ± 0.84 | 140.00 ± 1.51 |

N= 6, P< 0.001, data expressed in mean ± SEM

During study of cutaneous wound healing in normal and diabetic rats following results were obtained:

In diabetic animals the extract treated group III showed significantly greater wound healing as compared to control animals. In normal animals the extract treated

group VI showed significantly greater wound healing as compared to control animals. The standard drug treated animals in both normal and diabetic animals were showed significantly greater wound closure as compared to control and extract treated animals.

(b). Percentage wound closure

$$\text{Percentage wound closure} = \frac{(\text{Initial area of Wound} - N^{\text{TH}} \text{ day area of wound})}{(\text{Initial area of Wound})} \times 100$$

Table 2: Percentage wound closure

| Group Day | Group-I DC | Group-II DS | Group-III DE | Group-IV NC | Group-V NS | Group-VI NE |
|--------------|------------|-------------|-----------------|----------------|------------|----------------|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 8 | 20.1 | 14.4 | 10.22 | 21.1 | 18.7 |
| 8 | 14.1 | 26.8 | 22.2 | 17.89 | 33.76 | 24.6 |
| 16 | 19.1 | 35.7 | 31.3 | 27.1 | 43 | 34.8 |

In diabetic animals the extract treated Group III showed significant greater percentage (74.30%) wound closure as compared to control animals, Group I (25.17%). In normal animals the extract treated Group VI showed greater percentage (85.00%) wound closure as compared to

control animals (76.22%). The standard drug treated Groups in both normal and diabetic animals were showed significantly greater percentage wound closure group II (54.76%) and Group V (87.86%) as compared to control and extract treated animals.

Wound healing studies (Incision wound model):

(a) Tensile strength (gms):

Table 3: Tensile strength (gms)

| Group I DC | Group II DE | Group III NC | Group IV NE |
|---------------|----------------|-----------------|----------------|
| 315.33 ± 0.88 | 396.67 ± 5.45 | 354.33 ± 1.43 | 444.83 ± 2.65 |

N= 6, P< 0.001, data expressed in mean ± SEM

In diabetic animals the extract treated group II showed significantly greater tensile strength as compared to control animals. In normal animals the extract treated

group IV showed significantly greater wound healing as compared to control animals.

Wound healing studies (dead space wound model):

(b)Wound Parameters:

Table 4: Wound Parameters

| Group Parameter | Group I DC | Group II DE | Group III NC | Group IV NE |
|--|---------------|----------------|-----------------|----------------|
| Wet granulation weight (mg/100gm rat) | 99.50 ± 1.43 | 180.00 ± 2.89 | 126.17 ± 2.39 | 162.67 ± 0.95 |
| Dry granulation weight (mg/100gm rat) | 33.50 ± 1.18 | 53.17 ± 0.79 | 40.67 ± 1.52 | 50.50 ± 0.92 |
| Hydroxyproline (mg/gm tissue) | 53.00 ± 1.32 | 62.83 ± 1.14 | 95.50 ± 1.26 | 105.50 ± 1.18 |

N= 6, P< 0.001, data expressed in mean ± SEM

In diabetic animals and normal animals the extract treated groups II and IV showed significantly higher levels of hydroxyproline as compared to control animals. A

significant increase was also observed in the dry and wet weight of the granulation tissue in the extract treated groups II and IV.

DISCUSSION:

Granulation collagen maturation and scar formation are some of phases of wound healing which run concurrently but independent of each other. In excision model of wound healing the *Momordica charantia* seed extract showed faster healing compared with control group and wound contraction rate is significantly higher in both extract and standard drug treated animal in both normal and diabetic group.

Wound contraction, epithilization and fibrosis are the biological response regulated by the body's on cellular defence mechanism. The faster wound contraction rate may be due to stimulation of interleukin (an inflammatory, α -chemokinin). which effect the function of various inflammatory cells, fibroblasts and keratinocytes. It may increases gap junctional intra cellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue.

Molecular oxygen place an important role in pathogenesis and therapy of chronic wounds. Over production of reactive oxygen species results in oxidative stress there by causing cytotoxicity and delayed wound healing.

The *Momordica charantia* seed extract has been shown to possess steroids, flavanoids and phenolic compound on preliminary screening. The wound closure is mainly depend upon formation of collagen and maturation of collagen, this is may be because of flavanoids, steroids which are responsible for free redical scavenging activity and helps to promote most important phase of wound healing.

In diabetic patient delay in wound healing may be due to high blood glucose level, denaturation of proteins and cellular components by high blood glucose and pressure of free redicals, specially oxidative free redicals. The *Momordica charantia* seed extract showed anti hyperglycaemic & anti microbial activity which are due to presence of various active phytochemicals present in the plant extract i.e. steroids phenolic and flavanoids.

Hence on the basis of results obtained and above facts we can stat that the faster wound healing activity of *Momordica charantia* seed extract in diabetic and normal animals is mainly because of presence of phytochemicals and their effect on components of wound healing.

CONCLUSION:

The effect of *Momordica charantia* Seeds, Leaves & Roots extract on wound healing has been studied in diabetic

and normal animals The effect has also been compared with that of antibiotic (mupirocin) application. In the absence of specific animal models for cutaneous diabetic wounds, we have used common model of wound healing (i.e excision wound model) in animals having diabetes (by administration of alloxan monohydrate 120mg/kg I.P).

In the excision wound model the ointment of extract was prepared in stearic acid base and applied topically after creation of wounds in diabetic and normal animals.

Measurement of wound contraction was done 4th, 8th and 16th day of wound creation the result show that application of *Momordica charantia* Seeds, Leaves & Roots extract significantly increases wound healing in both normal and diabetic animals.

On the basis of above it may be concluded that plant extract promotes wound healing in diabetic and normal animals by topical application of extract.

This supports its prevalent use in treatment of diabetes wound healing and infection however, further more specific and controlled biophysical and biochemical studies are required to draw a definite conclusion.

REFERENCE:

1. Braunwald, Fauci, Kasper, Hauser, Longo, Jameson. Principles of internal medicine, 15th ed. New york: Mcgraw-hill; 2001. p. 2019.
2. Harsh Mohan. Textbook of pathology, 5th ed. New Delhi : JP brothers; 2006. p. 843.
3. Nayak BS, Pereira PL, Maharaj Dale, wound healing activity of *Carica papaya* L in experimentally induced diabetic rats. Indian Journal of Experimental Biology, 2007 Aug; 45: 739-743.
4. Kokate CK. Pharmacognosy, 27th ed. Pune: Nirali prakashan; 2004. p. 257.
5. Khandelwal KR. Practical Pharmacognosy, 16th ed. Pune: Nirali Prakashan; 2006. p. 149-157.
6. Ghosh M. Fundamentals of Experimental Pharmacology, 3rd ed. Kolkata: Hilton and Company; 2005. P.190-197.
7. OECD guidelines for testing of chemicals, acute dermal toxicity fixed dose procedure. 2004; 434:1-13
8. Reddy KG, Enwemeka SC, A simplified method for the analysis of hydroxyproline in biological tissues. Clinical Biochemistry, 1996;29: 225-229.
9. Mayer KE, Sagger GC, Ehrlich HP. Effect of IL-8 on granulation tissue maturation. J cell physiology. 2002;193: 173-179.