# Evaluation of Wound Healing Activity of Ethanolic Extract of *Ficus Religiosa and Trigonella Foenum Graecum* in Experimentally Induced Diabetic Rat.

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## ABSTRACT

**Objective:** The present study was designed to evaluate wound healing activity of ethanolic extract of *Ficus religiosa and Trigonella Foenum Graecum*

**Methods:** Leaves of *Ficus religiosa* and seeds of *Trigonella Foenum Graecum* was extracted using ethanol as solvent by soxhlet apparatus. The evaluation of wound healing activity was done using Incision models in Male Wistar albino rats.

**Results:** Ethanolic extract of leaves of *Ficus religiosa* and seeds of *Trigonella Foenum Graecum* has shown increase in Wound Breaking Strenth (330.6 ± 13.5, 320.5 ± 12.3, 315.8 ± 14.5, 310.8 ± 14.7) compared to Diabetic animals (281.3 ± 14.1) and also increase the Tensile Strength (185.33±15.22, 207.83±19.18, 195.16±11.13, 217.84±22.6) for Incision Model compared to Diabetic animals (95.20±30.56) confirming their Wound healing property.

**Conclusion:** Ethanolic extract of leaves of *Ficus religiosa* and seeds of *Trigonella Foenum Graecum* has shown increase in Wound Breaking Strenth and Tensile Strength in comparison to standard drug Povidone iodine in Diabetic rat.

Keywords : Wound healing, *Ficus religiosa* (FR) *and seeds of Trigonella Foenum Graecum*, (TF) Wound Breaking Strenth and Tensile Strength, ethanolic extract etc.

## INTRODUCTION

Wound may be defined as a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection and is produced due to any accident or cut with sharp edged things. It may be produced due to physical, chemical, thermal, microbial or immunological exploitation to the tissues (Lazarus et al 1994).

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chemical, physical, microbial or immunological exploitation of the tissues (Sabale et al 2012). If it is not treated immediately, it can lead to microbial infections (Pandian et al 2013). The wound infections are most common in developing countries due to poor hygienic condition. The microorganisms like gram positive and gram negative bacteria are the principal pathogen of wound infections. Diabetic patients are at increased risk of developing infection (Edan et al 2010). This is due to impaired leukocyte function associated vascular diseases, poor glucose control and altered immune response (McMahon et al 1995).

Wound healing is a complex phenomenon for the regain or restoration of disrupted anatomical continuity and disturbed functional status of the skin (Begum et al 2000), accomplished by several processes which involve different phases including inflammation, granulation, fibro genesis, neo-vascularization, wound contraction and epithelisation (Clark et al 1996). The basic principle of optimal wound healing is to minimize tissue damage, provide adequate tissue perfusion, oxygenation, proper nutrition, moist wound healing environment (Pierce et al 1995). The main aim of wound therapy is to enhance wound healing in the shortest time possible, with minimal pain, discomfort and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration (Blower et al 2001).

The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatment of diabetic wounds. Antimicrobial screening of plant extracts and presence of active phytochemicals represent a starting point for new antimicrobial drug discovery. Medicinal plants that possess immunomodulatry and antioxidant properties also leading to antibacterial activities are known to have versatile immunomodulatory activity by stimulating both nonspecific and specific immunity (Pandey and Mishra, 2011).

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F. religiosa is a sacred tree native to India where it grows up to elevations of 5,000 ft (1,524 m). It is said to be the tree that Buddha was born under and also where he sat for six years of meditation and enlightenment. Elsewhere in the world and in Hawai'i, trees are occasionally cultivated and are most often seen planted near temples.

## MATERIAL AND METHOD

The plant material was collected from local area of Bhopal and was authenticated at the Department of Botany, Dr. Hari Singh Gour University Sagar. The preparation of extract was carried out according to the

method of. Briefly, the leaves and seeds of *Ficus religiosa* and *Trigonella foenum graecum* was shade dried after collection for 5 days and was powdered. Approximately 0.95 kg of powdered drug material was extracted using 99% pure ethanol in the ratio of 1:2 (w/v) in a air tight container. The extract obtained was dried in a steam bath and the dried mass was weighed and recorded. The percentage of yield was calculated. The weight of dried crude extract obtained calculated and the dried extracts were stored in air tight containers for further studies.

**Phytochemical Screening**

The ethanolic extracts of both the plants were tested qualitatively for different phytoconstituents analysis using various chemical tests. Total phenolic content (TPC) was determined using the various chemical method and total flavonoid content , tannins, steroids, terpenoids, amino acids, proteins and trace elements was determined. The result indicated the presence of flavonoids (kaempeferol, quercetin, myricetin) in *Ficus religiosa*, and phenolic compound (chlorogenic acid rutin and quercetin). in *Trigonella foenum graecum* (Roopashree, 2008).

**In vitro Antioxidant activity**

**Estimation of nitric oxide** As nitrite and nitrate are formed as end products of the reactive nitrogen intermediates, the measurement of nitrite by using the Griess is generally employed as a marker for formation of NO. 0.4 ml of granulation tissue homogenate (100 mg/ml) is mixed with 0.4 ml of absolute alcohol and then centrifuged at 4°C at 14000 rpm for 1 hr. 0.5ml of supernatant was taken, mixed with 0.5 ml of vanadium (III) chloride and 0.5 ml of freshly prepared Griess reagent, and incubated at 37°C for 30 min. The absorbance was measured at 540 nm spectro-photometrically, against blank prepared by using distilled water. Nitrite content was determined from standard curve prepared by using sodium nitrite and expressed as nM/mg protein. (Miranda *et al*., 2001)

**Estimation of myeloperoxidase, MPO:** For MPO estimation, granulation tissue (5% w/v) was homogenized in 0.5% HTAB with 50mM potassiumphosphate buffer (pH 6). The above homogenate was freeze-thawed three times and sonicated for 10 seconds and then centrifuged at 14000 ×g for 45 minutes at 4$^{o}$C and the resulting supernatant was used for estimation of MPO. A unit of MPO activity is defined as that converting 1 µmol of $H_2O_2$ to water in 1min at 25$^{o}$C. The results were expressed as nM/mg protein. (Bradley et al., 1982)

**Inhibition of lipid peroxidation:** LPO level is estimated in terms of malondialdehyde (MDA). To 0.2 ml of 100 mg/ml tissue homogenate was added 0.1 ml of 8.1 % SDS, 0.75 ml of 20 % acetic acid solution (pH 3.5) and 0.75 ml of 0.8 % aqueous solution of TBA in stoppered tubes. The mixture was made up to 2 ml with distilled water, and then heated in an oil bath at 95°C for 60 minutes. After cooling with tap water, 0.5 ml of distilled water and 2.5 ml of mixture of n-butanol and pyridine (15:1, v/v) were added and shaken vigorously. After centrifugation at 3000 rpm for 10 min the organic layer was taken and its absorbance at 532 nm was

measured against blank containing 0.2 ml of distilled water in place of sample. 1, 1, 3, 3- tetra-methoxypropane was used as external standard and the level of LPO was expressed as nM/mg protein. (Ohkawa *et al*., 1979)

**In vivo experiments:**

**Experimental animals**: Male Albino rats of either sex (150-200 g) were obtained from School of Pharmaceutical Sciences, Bhopal. The animals were kept under controlled environmental conditions at 25±2º C temperature and 45 -55 % relative humidity with natural light/dark cycle and allowed free access to food (Standard pellet diet, Hindustan Lever Ltd., India) and water. The animals were acclimatized for a week before the commencement of experimental study. All the experimental procedure and protocols used in this study were in accordance with the guidelines of CPCSEA (Committee for the Purpose of Control of Supervision of Experiments on Animals).

**Acute skin irritation test:** An area measuring about 500 mm2 on the dorsal fur of animals was shaved. The prepared ointments were applied separately to different groups of animals. After 4 hour, the skin of the animals was observed for signs of inflammation (Sanwal and Chaudhary, 2011).

**Wound healing activity:**

**Incision wound model**

Mid-dorsal part of the paravertebral region of rats was prepared before the experiment by cleaning and shaving the part. Incision wound was produced both in NR and DM anesthetized (ketamine 50 mg/kg, ip) rats by two parallel paravertebral incisions, 1.5cm long, made through the full thickness of the skin, 1 cm lateral to the midline of vertebral column. Wounds were closed with interrupted sutures, 1 cm apart, with surgical suture (1.0 Silk). The sutures were removed on the 7$^{th}$ post-wounding day.

**Wound breaking strength (WBS)**: Wound breaking strength (WBS) was measured on the 10$^{th}$ post-wounding day in anaesthetized rats secured on to the operation table. A line was drawn on either side of the incision line 3 mm away from the wound. Two Allis forceps were firmly applied on to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Standard weights were put slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. As and when the wound just opened up, the weight was stopped and noted. Three readings were recorded for a given incision wound at three different sites and the procedure was repeated on the wound on the contra lateral side. The average reading of the group was taken as an individual value of WBS. Mean value gives the breaking strength for a given group. (Ehrlich and Hunt, 1969)

**Determination of tensile strength**

The tensile strength of wound represents the effectiveness of wound healing. Usually wound healing agents promote the gaining of tensile strength. Tensile strength (the force required to open the healing skin) is used to measure the completeness of the healing. The tensile strength was measured by using tensiometer on 18 post wounding day (Lee et al 1968)

## RESULTS AND DISCUSSION

**Phytochemical screening:** The Phytochemical analysis of the leaf extracts of both the plants showed the presence of alkaloids, flavonoids, saponins, phenols, tannins, diterpenes, phytosterols, and proteins in common. The result indicated the presence of flavonoids (kaempeferol, quercetin, myricetin) in *Ficus religiosa*, and phenolic compound (chlorogenic acid rutin and quercetin). in *Trigonella foenum graecum* (Table.1).

***In vitro* Antioxidant activity:**

The results of the present study on free radicals, myeloperoxidase and antioxidants indicate that DM rats showed increase in free radicals, myeloperoxidase and decrease in antioxidant status thereby enhancing oxidative stress and delayed wound healing. FREEO, FREET, TFEEO, FREET & PI (by virtue of their effects on decreasing oxidative stress) and PI (effects on decreasing oxidative stress) enhanced the wound healing in DM rats (Table.2).

**Acute skin irritation test:** In the skin irritation study, the tested (5% w/w) ointment did not show any sign of irritation and also don't have any type of inflammation on the skin.

**Incision wound (DM rats).**

Incision wound was produced in DM rats by two paravertebral incisions (6 cm long), made through the full thickness of skin on either side of vertebral column. Wounds were closed with suture. Suture was removed on $7^{th}$ day and Wound Breaking Strength (WBS) was measured on $10^{th}$ post wounding day. 50% ethanol extracts of dried leaves powder of *Ficus religiosa* (FR) and dry powder seeds of *Trigonella foenum-graecum* (TF) were suspended in 0.5% carboxy methyl cellulose (CMC) and were administered orally and topically, once daily for 10 days. Blood was collected from the retrobulbar plexus of rats and blood glucose was estimated in all the groups following the standard procedure mentioned earlier before the production of incision wound while the first dose of the extracts was given 4 hour after induction of incision wounds to 18 h fasted rats on day 1. The test extracts were given once daily for 10 days and the last dose was given on $10^{th}$ day of experiment to 18 h fasted rats, one hour prior the experiment to normal and Diabetic rats while the control normal and Diabetic rats received CMC alone during the same period.

**Effect on wound breaking strength (WBS)**

Graded doses of FREE AND TFEE when given in orally as well as in ointment form for 10 days as above to DM rats, showed a dose-dependent increase in WBS both in DM (17.0 to 37.5%, P<0.05 to P<0.001) rats (Table.3).

On the basis of our above experiment and our earlier reported studies on wound healing effects of extracts of FREE (200mg) & TFEE (500mg) respectively were selected for future studies.

**Tensile strength**

In infected incision wound model, there is significant increase in skin tensile strength (p<0.01) by 5% w/w FREET & 5% w/w TFEET ointment of and standard as compared to diabetic control group animals (Table.4).

**Table.1: Data showing the preliminary phytochemical screening of *Ficus religiosa* & *Trigonella foenum graecum*.**

| Active principles | Trigonella foenum graecum | Ficus reiligiosa |
|---|---|---|
| Saponins | +ve | +ve |
| Flavonoids | +ve | +ve |
| Phenolic compounds | +ve | +ve |
| Fixed oil & fats | -ve | +ve |
| Alkaloids | +ve | +ve |
| Phytosterol | -ve | +ve |
| Tannins | +ve | +ve |

**Table.2: Effect of *Ficus religiosa* & *Trigonella foenum graecum* on wet granulation tissue myeloperoxidase (MPO) and free radicals, nitric oxide (NO) and lipid peroxidation (LPO) in DM rats**

| Treatment (mg/kg, od) | DM rats | | |
|---|---|---|---|
| | MPO | NO | LPO |
| | nM/mg protein | | |
| (I) NC (0.5% CMC) | 28.7 ±0.43 (100.0) | 49.0 ±1.19 (100.0) | 7.72 ±0.30 (100.0) |
| (II) DC (0.5% CMC) | 33.6 ±0.76 [c] (100.0) | 64.0 ±2.31 [c] (100.0) | 9.79 ±0.41 [c] (100.0) |
| (III) PI (5% w/w) | 29.9 ±1.23 [x] (87.3) | 55.7 ±2.03 [x] (84.0) | 8.84 ±0.45 [x] (80.8) |
| (IV) FREEO (200 mg) | 24.5 ±0.76 [z] (68.7) | 47.5 ±2.04 [z] (69.2) | 8.03 ±0.47 [z] (58.6) |
| (V) FREET (5% w/w) | 25.9 ±0.94 [z] (76.4) | 51.1 ±2.03 [y] (74.4) | 7.99 ±0.43 [z] (66.3) |
| (VI) TFEEO (500 mg) | 27.8 ±1.05 [z] (75.4) | 45.1 ±2.08 [z] (65.1) | 7.65 ±0.31 [z] (62.0) |
| (VII) TFEET (5% w/w) | 28.0 ±0.89 [z] (79.2) | 39.2 ±2.51 [y] (72.7) | 7.90 ±0.52 [z] (61.3) |

Results are mean ± SEM of 6 rats in each group. Values indicate percent of respective control value.

P values: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to respective NR control group and [x]<0.05, [y]< 0.01 and [z]< 0.001 compared to respective DM control group (Statistical analysis was done by one way analysis of variance followed by Dunnett's test for multiple comparisons). FREEO = Ethanolic extract of *Ficus religiosa* oral, FREET = Ethanolic extract of *Ficus religiosa* topical, TFEEO = Ethanolic extract of *Trigonella foenum graecum* oral, TFEET = Ethanolic extract of *Trigonella foenum graecum* topical, PI = Povidone iodine

**Table.3: Effect of graded doses of *Ficus religiosa* & *Trigonella foenum graecum* on wound breaking strength (WBS) in streptozotocin (STZ)-induced DM rats**

| Group/Treatment | WBS (g) DM rats |
|---|---|
| (I) Normal Control (1% CMC) | 245.3 ± 10.4[c] (100.0 ± 4.1) |
| (II) Diabetic Control (1%CMC) | 281.3 ± 14.1 (114.7 ± 5.7) |
| (III) Povidone Iodine (5% w/w) | 301.3 ± 15.6[x] (122.8 ± 6.4) |
| (IV) FREEO (200mg) | 330.6 ± 13.5[z] (125.9 ± 6.0) |
| (V) FREET (5% w/w) | 320.5 ± 12.3[y] (121.5 ± 5.4) |
| (VI) TFEEO (500mg) | 315.8 ± 14.5[z] (1444.9 ± 5.5) |
| (VII) TFEET (5% w/w) | 310.8 ± 14.7[z] (134.9 ± 6.0) |

Results are mean ± SEM of 6 animals in each group. Value indicates percent from their respective NR control and DM control group.

P values: [a]<0.05, [b]<0.01, [c]<0.001 compared to respective NR control group and [x]<0.05, [y]<0.01, [z]<0.001 compared to respective DM control group (Statistical analysis was done by one way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons). FREEO = Ethanolic extract of *Ficus religiosa* oral, FREET = Ethanolic extract of *Ficus religiosa* topical, TFEEO = Ethanolic extract of *Trigonella foenum graecum* oral, TFEET = Ethanolic extract of *Trigonella foenum graecum* topical, PI = Povidone iodine

**Table.4- Effect of ethanolic leaves extract of *Ficus religiosa* & *Trigonella foenum graecum* once daily on tensile strength of incised wound in diabetic rats.**

| Groups/Treatment | Tensile strength |
|---|---|

| | **(g/cm$^2$), mean±SEM** |
|---|---|
| Group-I (1% CMC) (Normal control) | 97.50±15.15 |
| Group-II (1% CMC) (Diabetic control) | 95.20±30.56 |
| Group-III (PI 5% w/w) | 220.50±10.41** |
| Group-IV (FREEO 200mg) | 185.33±15.22* |
| Group-V (FEEET 5% w/w) | 207.83±19.18** |
| Group-VI (TFEEO 500mg) | 195.16±11.13** |
| Group-VII (TFEEO 5%w/w) | 217.84±22.6*** |

n=6; p value: *p<0.05, **p<0.01 values are expressed as mean ± SEM. One-way ANOVA followed by Dunnet's t-test, all the groups are compared with control. *SEM: Standard error of mean.* FREEO = Ethanolic extract of *Ficus religiosa* oral, FREET = Ethanolic extract of *Ficus religiosa* topical, TFEEO = Ethanolic extract of *Trigonella foenum graecum* oral, TFEET = Ethanolic extract of *Trigonella foenum graecum* topical, PI = Povidone iodine.

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