Evaluation of Antioxidant Activity of *Ficus Religiosa* and *Trigonella Foenum Graecum* in the Management of Diabetic Wound Healing in Experimental Rat.

CHOURASIA VIVEK[a], NAYAK AMIT[a], JAIN ALOK PAL[a],GUPTA SHAILESH[b]

a-RKDF College of Pharmacy, SRK University, Bhopal, MP, India,

b- Millenium College of Pharmacy, Bhopal, MP, India.

ABSTRACT

Ficus is an evergreen tree of family Moraceae. Ficus religiosa and Trigonella foenum graecum has been traditionally claimed that the leaves of Ficus religiosa and seeds of Trigonella foenum graecum are useful in the management of diabetic wound healing. The leaves of Ficus religiosa and seeds of Trigonella foenum graecum are reported to have anti-inflammatory, antioxidant, and antimicrobial properties. In the present study, diabetic wound healing activity of ethanolic extracts of Ficus religiosa and Trigonella foenum graecum was evaluated in rats using excision, and dead space model along with estimation of biochemical parameters. In the excision wound model, the period of epithelisation, scar area, percent wound contraction or closure was evaluated respectively. While in the dead space model, the efficacy of drugs against the proliferative and remodeling phases of wound healing was evaluated. In addition, GSH, Catalase and MDA estimation studies were employed to assess the efficacy of both the plants in vivo. The treatment of diabetic wound with oral and ointment containing ethanolic extracts of Ficus religiosa and Trigonella foenum graecum exhibited significant diabetic wound healing. The results were comparable to standard drug povidone iodine ointment, in terms of wound contraction, period of epithelisation and histopathological parameters. A significant antioxidant activity was observed as evidenced from decreased level of MDA and increased the level of GSH, catalase in extract treated animals as compared to control animals.

Keywords: Wound healing, Ficus religiosa (FR), Trigonella Foenum Graecum, (TF) antioxidant, excision wound, dead space wound.

INTRODUCTION

Wound is 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 occur due to thermal, chemical, physical, microbial or immunological exploitation of the tissues [1]. If it is not treated immediately, it can lead to microbial infections [2]. 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 [3]. This is due to impaired leukocyte function associated vascular diseases, poor glucose control and altered immune response [4].

Wound healing in patients with diabetes mellitus is characterized by reduced tensile strength of wounds when compared with controls, suggesting either defective matrix production or deposition [5]. Diabetic wounds are slow, non-healing wounds that can last for weeks despite adequate and appropriate care. Such wounds are difficult and frustrating to manage [6]. Diabetic wound healing is an enigmatic and debilitating complication and poses a serious challenge in clinical practice. The exact pathogenesis of poor wound healing of the diabetic wounds is not clearly understood, but evidence from studies involving both human and animal models revealed several abnormalities in the various phases of wound healing process [7].

The chronic condition of high blood sugar may lead to serious complications such as micro and macrovascular complications including delayed wound healing following injury. Physiological changes in the tissue and cells may delay healing. Furthermore, complications due to diabetes also have an impact that directly or indirectly affects healing of wounds. Impaired or delayed wound healing is one of the major and

serious complications of diabetes mellitus that still remains a challenging clinical problem. It leads to several end results such as diabetic foot ulcer, loosing limb. Therefore correct, efficient wound management is essential in diabetes patients [8].

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 [9].

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 [10].

In vitro Antioxidant activity

Glutathione

1 ml of tissue homogenate (100 mg/ml) was mixed in 15 ml test tube with 0.8 ml of distilled water and 0.2 ml of 50 % TCA. The tubes were shaken intermittently for 10-15 min and centrifuged for 15 min at 3000 rpm for 10 min. 0.6 ml of supernatant was mixed with 0.8 ml of 0.4M Tris buffer (pH 8.9) and 20 µl of 0.1M DTNB in absolute methanol, and the sample was shaken. The absorbance was read within 5 min of the addition of 40 µl DTNB at 412 nm against a reagent blank with no homogenate. The results were expressed as nmol/mg protein and were calculated from the standard curve prepared by using standard glutathione [11].

Superoxide dismutase

The inhibition of reduction of nitro-blue tetrazolium (NBT) to blue colored formozan in presence of phenazine metha sulphate (PMS) and Nicotineamide adenine dinucleotide (NADH) was measured at 560 nm using n-butanol as blank. To 0.2 ml of tissue homogenate was added 0.6 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3), 50 µl of 186 µM of PMS, 150 µl of 300 µM NBT and 0.4 ml of distilled water to make up the volume up to 1.5 ml including with 0.1 ml of 780 µM NADH. Reaction was started by the addition of NADH. After incubation at 30°C for 60 sec, the reaction was stopped by the addition of 0.5 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 2 ml of n-Butanol. The mixture was allowed to stand for 10 min, centrifuged at 3000 rpm for 10 min and butanol layer was taken out. Colour intensity of the chromogen in the butanol was measured at 560 nm in spectrophotometer against n-Butanol, a system devoid of enzyme served as control. One unit of enzyme activity is defined as enzyme concentration required inhibiting the optical density at 560 nm of chromogen protection by 50% in one min under the assay conditions, and the results have been expressed as mU/mg protein [12].

Catalase

Gastric mucosal scrap was homogenized (5 %) in M/150 phosphate buffer at 1-4° C and centrifuge. Stirr the sediment with cold phosphate buffer allow standing in the cold with occasional shaking and then repeat the extraction twice use the combined supernatant for the assay. The supernatant was diluted 1:10 with water

and before the assay and 0.04ml was taken for the assay. Decomposition of H_2O_2 in presence of catalase was followed at 240 nm. Catalase activity was calculated by using the formula given below [13].

Dead Space Wound

Dorso-lumbar part of the back, on either sides, of the rats was prepared before the experiment by cleaning and shaving. Rats were anesthetized and 1 cm incision was made on dorso-lumbar part of the back. Two polypropylene tubes (0.5 x 2.5 cm^2 each) were placed in the dead space of lumbar region of rat on each side, and wounds were closed with a suture material. On the 18^{th} post-wounding day, the animals were sacrificed and granulation tissue formed on and around the implanted tubes was carefully dissected out, weighed and processed for the estimation of free radicals, antioxidants and collagen tissue parameters. Throughout the tissue preparation, tissue was stored at freezing temperature [14].

Estimation of connective tissue parameters To each tube containing 40 mg of the dried granulation tissue, 1 ml of 6N HCl is added. The tubes were kept on boiling water bath for 24 hours (12 hours each day for two days or 8 hours each day for 3 days) for hydrolysis. The hydrolysate was then cooled and excess of acid was neutralized by 10N NaOH using phenolphthalein as indicator. The volume of neutral hydrolysate was diluted to a concentration of 20 mg/ml of dried granulation tissue in the final hydrolysate with distilled water. The hydrolysate was used for the estimation of hydroxyproline, hexosamine and hexuronic acid following the standard procedures.

Estimation of protein

5 mg dry tissue dissolved in 2.5 ml of 0.1N NaOH. To 0.4 ml of hydrolysate was added 4 ml of alkaline reagent and kept for 10 minutes. Then, 0.4 ml of the phenol reagent was added and again 10 minutes were allowed for color development. Readings were taken immediately, against the blank prepared with water, at 610 nm. The protein content was calculated from the standard curve prepared with bovine albumin and expressed in terms of mg/g dry tissue [15].

Estimation of hydroxyproline content

50 mg of each tissue with 1 ml of 6N HCl were taken in respective screw cap glass vials and autoclaved for 3 hrs at 15 lb pressure to prepare protein hydrolysates. Then the hydrolysates were neutralized to pH 7.0 with 0.1 M NaOH solution. One ml of each hydrolysate was taken in respective test tube to which added 1 ml of 0.01 M $CuSO_4$ solution, 1 ml of 2.5 N NaOH solutions and 1 ml of 6% H_2O_2 solution. The mixture was allowed to mix for 5 minutes by occasional shaking and kept for 5 minutes on water bath at 80^0C. Then the tubes were cooled in ice-cold water and added 4 ml of 3N H_2SO_4 solutions. 2 ml of p-dimethylamino benzaldehyde solution was added to the preparation and heated on water bath at 70^0C for 15 minutes. Similar preparation was made for blank by taking 1 ml of distilled water instead of protein hydrolysate. Then the absorbance was taken at 560nm against reagent blank [16,17].

Estimation of Hexosamine

0.05 ml of hydrolyzed fraction (1 mg of dry granulation tissue) was diluted to 0.5 ml with distilled water. To this was added 0.5 ml of acetyl acetone reagent. The mixture was heated in boiling water bath for 20 minutes then cooled under tap water. To this 1.5 ml of 95 % alcohol was added, followed by an addition of 0.5 ml of Ehrlich's reagent. The reaction was allowed for 30 minutes to complete. Color intensity was measured at 530 nm against the blank prepared by using distilled water. Hexosamine contents of the samples were determined from the standard curve prepared by using D (+) glucosamine hydrochloride. The Hexosamine content was expressed as µg/mg protein [18].

Estimation of total collagen in wound tissue

The total collagen content of the wound tissues was determined by the estimation of hydroxyproline, a characteristic indicator (imino acid) of collagen. The samples were washed in physiological saline and cut into small pieces, defatted with the mixture of chloroform and methanol in the ratio of 2:1 (v/v) and lyophilized. 5 mg of lyophilized tissue was weighed and hydrolyzed with 5 ml of 6 N HCl at 110°C for 20 h in sealed tubes. After hydrolysis, the acid was removed through evaporation by keeping the samples in a water bath. The residue obtained after repeated washing was dissolved in water and made up to a known volume. The hydroxyproline present in the residue was estimated by standard a method [19].

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.

Excision wound model

Epithelisation period and scar area

Excision wounds were created in rats to study the epithelization period, scar area and rate of wound contraction. FREEO (200 mg/kg), TFEEO (400 mg/kg), the measurement of progress of wound healing induced by reference drug, (5% povidone iodine ointment), two herbal test formulations viz., formulation I (5% Ficus religiosa gel extract ointment), formulation II (5% Trigonella foenum graecum extract ointment), diabetic control group treated by simple ointment base were administered once daily, orally and topically till the day of complete wound healing while the control rats received CMC. Day of fall of eschar indicated the epithelization period and scar area was noted on the same day. Wound area was noted from the day of wounding and subsequently at regular time intervals, i.e. 3rd , 6th, 9th, day and then up to 18th day or till complete epithelization period of study.

Percent Wound Contraction

The values calculated for percentage reduction of wound area for excision wound model in different treatment groups have been given in Table 6.7 According to it, 100% wound closure was observed for test formulation (5% Polyherbal extract ointment treated group) and reference drug (povidone iodine ointment treated group) on the15th day while 98.56%, and 93.59%% wound closures were observed with test formulation I (Ficus religiosa ethanolic extract treated group) and test formulation II (Trigonella Foenum ethanolic extract treated group) respectively on the 15th day . Whereas, 100% wound closure was observed on 17th & 18th day. However, animals of the normal control group and diabetic control group were observed for the complete wound closure for more than 18 days. It was observed that both the above mentioned groups exhibited 72.34% and 60.56% wound closure respectively on the 18th day. These findings reveal that the wound healing effect observed in herbal test formulation V & VII (Polyherbal ointment) treated group and the reference drug (Povidone iodine ointment) treated group animals have shown excellent wound healing effect with 100% wound closure within 18 days. The other two test formulations, formulation IV and VI treated groups have shown almost similar effects, which were even lower than test formulation V & VII (Polyherbal ointment) and reference drug (5% Povidone iodine ointment). However, the effects were significant and comparable to both reference ointment and formulation VII observed on the day 15th. In these two herbal test formulations, best effect was observed with formulation VII (5% Trigonella foenum extract ointment).

Histopathology

The histopathology study of the skin (newly formed on the wounds) was carried out on 18th post wounding day by fixing the skin in 10% formalin. Paraffin sections (5-10µ) were prepared stained with haematoxylin and eosin, and finally mounted in DPX (Di-N-Butyle Phthalate in xylene) medium. Histopathological examination were performed to study the process of epithelisation on the excised wound and to find out evidence of granuloma, dysplasia, oedema and malignancy in the skin under examination [20].

RESULTS AND DISCUSSION

In vitro Antioxidant activity:

The results of the present study on antioxidant, **SOD, CAT and GSH in wet tissue.** DM rats a significant decrease in wet tissue antioxidants level (50.6 to 65.5% decrease, P<0.01 to P<0.001) compared to NR control rats. Treatments of FREE & TFEE to DM rats showed an increase in all the oxidants (59.6 to 210.5.0% increase, P<0.05 to P<0.001) when compared with DM control group.DM rats showed an increase in all the antioxidants, **SOD, CAT and GSH** and decrease in antioxidant status there by enhancing oxidative stress and delayed wound healing. FREEO, FREET, TFEEO, FREET & PI by virtue of their effects on decreasing oxidative stress and enhanced the wound healing in DM rats (Table.1).

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.

Dead Space Wound

Tissue Protein

DM rats a significant decrease in dry tissue weight (16.8%, P<0.01) and protein content (15.2%, P<0.05) compared to NR control rats. Treatments of AIE, OSE, AME, VTE and GBL to DM rats caused an increase in dry weight of granulation tissue (17.8 to 40.0% increase, P<0.05 to P<0.001) and protein (15.1 to 26.9% increase, P<0.05 to P<0.01) compared with DM control group. Thus, all the above parameters near to their NR control values affected in DM rats (Table. 2).

Hexosamine & Total Collagen Content

However, in case of DM rats FREEO, FREET, TFEEO, TFEET and PI treatments showed increase in Hexosamine **(**HXU) (17.9 to 87.7% increase, P<0.05 to P<0.001) and TCC (24.7 to 58.6% increase, P<0.001) when expressed in terms of µg per mg protein in the above parameters in the dry granulation tissues compared with Diabetic control group (Table.2).

In vivo experiments

Epithelisation period and scar area

The mean epithelization period observed with Group II and VI was 14.0 and 13.6 days and scar area was 70.2 and 65.0 mm^2 respectively. Results of Period of epithelialization of different treatement groups is presented in (table 3). It was observed that the epithelialization period of Group III treated by reference drug were found to be almost similar i.e test formulation Group VII was 12.8 ± 0.53 and 13.4 ± 0.51 days respectively. The epithelialization times were found to be 13.7 ± 0.45 and 13.5 ± 0.40 days in diabetic experimental group treated by Group IV and V respectively. Average number of days that took for the shedding of scar without leaving any residual raw wound in these rats was 13.5 days and mean of scar area was 50.1 mm^2. The mean epithelization period observed with Group IV, V, VI and VII was 13.7, 13.5 and 13.6, 13.4 days respectively while, scar area was 65.4. 64.6, 65.0, 64.2 mm^2 respectively (table. 3).

Percent Wound Contraction

The values calculated for percentage reduction of wound area for excision wound model in different treatment groups have been given. According to it, 100% wound closure was observed for test formulation (5% Polyherbal extract ointment treated group) and reference drug (povidone iodine ointment treated group) on the15th day while 98.56%, and 93.59%% wound closures were observed with test formulation I (*Ficus religiosa* ethanolic extract treated group) and test formulation II (*Trigonella Foenum* ethanolic extract treated group) respectively on the 15th day . Whereas, 100% wound closure was observed on 17th & 18th day. However, animals of the normal control group and diabetic control group were observed for the complete wound closure for more than 18 days. It was observed that both the above mentioned groups exhibited 72.34% and 60.56% wound closure respectively on the 18^{th} day. These findings reveal that the wound healing effect observed in herbal test formulation V & VII (Polyherbal ointment) treated group and the reference drug (Povidone iodine ointment) treated group animals have shown excellent wound healing effect with 100% wound closure within 18 days. The other two test formulations, formulation IV and VI treated groups have shown almost similar effects, which were even lower than test formulation V & VII (Polyherbal ointment) and reference drug (5% Povidone iodine ointment). However, the effects were

significant and comparable to both reference ointment and formulation VII observed on the day 15th. In these two herbal test formulations, best effect was observed with formulation VII (5% *Trigonella foenum* extract ointment)(table. 4).

Table.1: Effect of FREEO, FREET, TFEEO, FREET & PI on wet granulation tissue antioxidants, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in DM rats.

Treatment mg/kg, od	SOD	CAT	GSH
	mU/mg protein	mU/mg protein	nM/mg protein
(I) Normal Control (0.5% CMC)	450.1 ±43.4 (100.0)	51.1 ±1.23 (100.0)	25.5 ±0.97 (100.0)
(II) Diabetic Control (0.5% CMC)	250.8 ±48.6 ^{b} (100.0)	20.1 ±2.05 ^{c} (100.0)	12.5 ±2.56 ^{c} (100.0)
(III) PI (5% w/w)	390.2 ±78.5 ^{x} (154.9)	40.7 ±3.15 ^{y} (195.5)	22.5 ±2.07 ^{x} (180.6)
(IV) FREEO (200mg)	450.3 ±63.2 ^{y} (220.3)	55.2 ±5.04 ^{z} (310.4)	28.8 ±2.89 ^{y} (220.9)
(V) FREET (5% w/w)	421.4 ±49.7 ^{y} (163.5)	48.1 ±4.07 ^{z} (268.6)	27.7 ±2.34 ^{x} (200.0)
(VI) TFEEO (500 mg)	441.5 ±48.4 ^{y} (160.4)	59.9 ±2.16 ^{z} (315.6)	32.7 ±2.23 ^{x} (250.5)
(VII) TFEE (5% w/w)	400.3 ±45.6 ^{x} (168.5)	45.4 ±3.09 ^{z} (251.8)	25.8 ±2.17 ^{y} (225.7)

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.2: Effect of FREEO, FREET, TFEEO, FREET & PI on dry granulation tissue determinants, protein, hexosamine (HXA) and total collagen content (TCC) in DM rats

Oral treatment od (mg/kg)	Hexosamine, Tissue Protein & Total Collagen Content			
	HXA	Weigth	Protein	TCC
	µg/mg protein			
(I) NC (0.5% CMC)	105.3±5.3 ^{a} (100.0)	55.9±2.3 ^{b} (100.0)	208.4±8.4 ^{a} (100.0)	196.9±6.56^{c} (100.0)
(II) DC (0.5% CMC)	108.67±5.3 ^{a} (108.0)	63.7±2.6 ^{x} (112.9)	215.1±6.4 ^{x} (115.2)	201.5±5.61^{c} (100.0)

(III) PI (5% w/w)	124.2±5.1 [x] (117.9)	67.5±3.6 [x] (120.8)	240.1±8.3 [x] (120.7)	248.2±8.73[y] (135.5)
(IV) FREEO (200mg)	139.7±4.1 [z] (132.7)	80.5±5.8 [z] (145.0)	258.2±11.2 [y] (126.9)	290.8±9.62[z] (149.5)
(V) FREET 5% w/w)	136.8±4.1 [z] (128.0)	73.2±3.6 [z] (135.6)	250.7±14.4 [x] (126.3)	286.6±13.4[z] (152.2)
(VI) TFEEO (500mg)	133.1±3.2 [y] (125.4)	71.8±2.9 [y] (126.0)	251.4±12.2 [x] (126.7)	280.5±10.5[z] (140.5)
(VII) TFEET (5% w/w)	130.5±5.5 [y] (120.6)	68.5±2.6 [y] (111.3)	247.6±10.1 [x] (118.8)	264.4±9.93[z] (152.1)

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 PI, FREEO, FREET, TFEEO and TFEET on epithelization period and scar area in DM rats

Treatment Group (mg/kg, od)	Epithelization period (Days)	Scar area (mm^2)
	DM rats	DM rats
(I) NC (1% CMC)	13.5 ± 0.43 (100.0 ± 2.8)	50.1 ± 2.3 (96.0 ± 4.4)
(II) DC (1% CMC)	14.0 ± 0.39 [x] (91.7 ± 2.3)	70.2 ± 3.1 [x] (83.9 ± 3.7)
(III) PI (5% w/w)	12.8 ± 0.53 [x] (85.9 ± 3.2)	62.8 ± 4.5 [x] (75.3 ± 5.5)
(IV) FREEO (200mg)	13.7 ± 0.45 [y] (87.5 ± 3.8)	65.4 ± 4.1[x] (77.0 ± 4.7)
(V) FREET (5% w/w)	13.5 ± 0.40 [x] (86.9 ± 3.8)	64.6 ± 4.5 [x] (76.6 ± 5.6)
(VI) TFEEO (500mg)	13.6 ± 0.42 [x] (87.0 ± 3.2)	65.0 ± 4.2 [x] (70.0 ± 5.3)
(VII) TFEE (5% w/w)	13.4 ± 0.51 [y] (86.9 ± 3.2)	64.2 ± 4.3 [x] (76.5 ± 4.9)

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.4: Percent Wound Area Contraction

Group No.	Post Wounding Days					
	3	6	9	12	15	18
	%Wound Area Contraction / Closure					
I NC (1% CMC)	9.35%	15.78%	30.67%	45.05%	60.54%	72.34%
II DC (1% CMC)	5.67%	12.34%	23.45%	36.67%	48.78%	60.56%
III PI (5% w/w)	15.56%	30.56%	50.45%	75.90%	100%%	100%
IV FREEO (200 mg)	10.36%	23.78%	40.83%	65.45%	90.89%	100%
V FREET (5% w/w)	13.34%	26.73%	45.42%	69.38%	93.50%	100%
VI TFEEO (500 mg)	12.59%	24.00%	43.67%	66.98%	93.78%	100%
VII TFEET (5% w/w)	14.67%	28.59%	50.67%	75.98%	98.56%	100%

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

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

CONCLUSION

The phytochemical studies have clearly understood that both the plants are a rich source of phenolics and flavonoids. Therefore, the presence of these compounds in the plant extracts has exhibited strong antioxidant and pharmacological activities. The activities of ethanolic extracts of Ficus religiosa and Trigonella foenum graecum, in vivo, have clearly shown that both the plants possess antioxidant and diabetic wound healing potential and among both the plants Ficus religiosa and Trigonella foenum graecum is found to be stronger in displaying the abilities of free radical scavenging and wound healing properties. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of Ficus religiosa and Trigonella foenum graecum.

ACKNOLEDGEMENT

I express my profound thanks to my Guide Dr. Alok Pal Jain (Principle), RKDF College of Pharmacy, Bhopal, (M.P.), for his valuable guidance, constructive criticism, supervision, encouragement and providing all the necessary facilities for my work and teaching me lots of things regarding my project without his encouragement and constant guidance

REFRENCES

1. Sabale P, Bhargav B, Chirag P, Vidya S. 2012. An overview of medicinal plants as wound healers. Journal of Applied Pharmaceutical Science. 2, 143-150.
2. Pandian C, Srinivasan A, Indira C, Pelapolu. 2013. Evaluation of wound healing activity of hydroalcoholic extract of leaves of Stachytarpheta jamaicensis in streptozotocin induced diabetic rats. Der Pharmacia Letter. 5, 193-200.
3. Edan I, Alsaimary A. 2010. Bacterial wound infections in diabetic patients and therapeutic implications. Medical practice and review. 1, 12-15.
4. McMahon MM, Bistrian BR. 1995. Host defences and susceptibility to infection in patients with diabetes mellitus. Infectious Disease Clinic of North America. 9, 1-7.
5. Ferguson MW, Herrick SE, Spencer MJ, Shaw JE, Boulton AJ, Sloan P. 1996. The histology of diabetic foot ulcers. Diabet Med 13:S30-S33.
6. Singer AJ, Clark RA. 1999. Cutaneous wound healing. N Engl J Med 341:738-746.
7. Goodson W.H., Hunt T. K. "Deficient collagen formation by obese mice in a standard wound model" *Am J Surg.*vol. 29, 1979, pp.692-94.
8. Coffman J.D., "*Impaired wound healing* is one of the common and serious complications of diabetes mellitus" *New Engl J Med* vol. 311, *1984, pp.* 1615–1619.
9. Pandey A. and Mishra R, "Antibacterial properties of Aegle marmelos leaves, fruits and peels against various pathogen" Journal of Pharmaceutical and Biomedical Sciences vol.13 (13), 2011, pp.1-6.
10. Roopashree TS, Raman D, Shobha RRH, Narendra C, Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. Int J Appl Res Nat Prod, 1, 2008, 20-8.
11. Sedlak J and Lindsay RH. Estimation of total protein bound and non-protien sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 25, 192-205(1968).
12. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophy* 21, 130-132(1984).
13. Aebi H, Scherz B, Ben-Yoseph Y, Wyss SR. 1975. Dissociation of erythrocyte catalase into subunits and their re-association. Experientia 31:397-399.
14. Diwan PV, Tilloo LD, Kulkarni DR. Influence of *Tridax procumbens* on wound healing. *Indian J Med Res* 75, 460-467(1982).
15. Lowry OH, Rosenborough NJ, Farr AL, Randal RJ. Protein measurement with folin phenol reagent. *J Biol chem* 193, 265-275(1951).
16. Nayak BS, Sandiford S, Maxwell A. 2009. Evaluation of the wound-healing activity of ethanolic extractof *Morinda citrifolia* L. Leaf. Evidence Based complementary and Alternative Medicine. 6, 351-356.
17. Ramachandra YL, Sudeep HV, Padmalatha SR, Ramadas K. 2012. Evaluation of wound healing activity of leaf extract of *Alstonia scholaris* Linn in rats. International Journal of Pharmacy and Pharmaceutical Sciences. 4, 390-393.
18. Dische Z and Borenfreund E. A spectroscopic method for the micro determination of hexosamine. *J Biological Chem* 184, 517-522(1950).
19. Woessner JF. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 93:440-447.
20. Vidinsky B, Gal P, Toprcer T, Longauer F, Lenhardt L, Bobrov N. 2006. Histological study of the first seven days of skin wound healing in rats. Acta Veterinaria Brno. 75,197-202.