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Potential wound healing activity of hydroalcoholic extract of *Ficus religiosa* root in albino rats

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Abstract
The hydroalcoholic extract of leaves of *Ficus religiosa* (Family: Moraceae) was assessed for their wound recuperating action in rats. It was carried out in rats by utilizing excision wound models taking after topical application. The qualitative primary phytochemical investigation of ME shown the presence of tannins, triterpenoids, alkaloids and steroids. All healthy rats of either sex were gathered into 4groups and each group contains six rats. The rats of treatment (5 and 10% w/w) of methanol extract were compared to povidone-iodine treatment old reference data treated as a standard. The outcomes about research work showed that the treatment of *Ficus religiosa* formulations treated wounds appeared critical lessening of wound compression region as compared to control and demonstrated quick epithelialisation. The concurrent and free different natural handle granulation, collagenation, collagen development and scar development play critical part in wound mending handle. At last concluded that test treated bunch essentially upgrades the wound withdrawal rate with regard to standard, control gather and epithelialization.

Keywords: *Ficus religiosa*, wound healing, albino rats

Introduction
Wound healing is an intricate immunological response to restore the structural and functional integrity of tissue following an injury. It is a complex physiological process involving coordinated interactions between diverse immunological and biological system. It involves a cascade of carefully, precisely regulated biochemical events that are correlated with the appearance of various immune cells, inflammatory mediators and extracellular matrix components in the wound bed [1]. Wound healing is triggered immediately following an injury to tissues and initiates its four overlapping sequential time dependent phases. These phases are (1) Hemostasis, (2) Inflammatory, (3) Proliferative and (4) Remodeling.

The normal physiological response that prevents significant blood loss by blood clotting following vascular injury and then subsequent dissolution of clot to keep the blood intact in damaged vasculatures termed as homeostasis. Immediately upon vascular damage, triggering cascade of events to prevent excessive blood loss by thrombus formation and protecting tissue from pathogen invasion and allowing tissue to repair are the main aims of hemostasis. During this phase, damaged and dead cells are cleared out and acute inflammatory response with exudation of plasma, neutrophils and some monocytes along with bacteria and other pathogens or debris occur within few hours. The inflammatory phase is an important and crucial phase of wound healing. Soon after injury, setting up the hemostatic plug formationto stop bleeding by innate system. This hemostatic phase is overlapped by inflammatory phase of healing, which is characterized by increased vascular permeability, release of chemotactic substances from platelets, chemotaxis of cells from circulation to wound site, local release of cytokines, growth factor and activation of migrating cells. The main objectives of inflammatory phase are clearance of invading microbes, cellular debris or invaded tissues and neutralization of injury causing agent [2-3]. The proliferative phase is a second reparative phase of wound healing next to inflammatory phase. It occurs for about 4 days to 20 days depending on the size of the wound. This reparative phase involves migration and proliferation of keratinocytes, reepithelialization, angiogenesis, restoration of basement membrane, wound contraction and formation of epidermis. The proliferations of keratinocytes towards wound site restore the barrier function of epithelium. The collagen IV

is the abundant component in basement membrane. The remodeling phase is a final stage of wound healing, which can last for years. This phase starts from hemostasis phase by replacement of fibrin matrix to granulation tissue and later enters to proliferative phase of angiogenesis by the formation of collagen type-III from fibroblast. Soon after the formation of new blood vessels by angiogenesis, vascular density of the wound site is reduced to normal condition and next enters to remodeling phase by deposition of ECM. The ECM synthesized mainly by fibroblast is deposited in the wound site [4]. The Evaluation of antioxidant, wound healing, and anti-inflammatory activity of ethanol extract of leaves of *Ficus religiosa*. He concluded that the obtained results it can be concluded that *Ficus religiosa* extract has significant wound healing activity and initial healing may be due to presence of glycosides and tannins. The extract shows prominent anti-inflammatory activity as compared to that of standard. The extract showed good anti-inflammatory activity on carageenan induced rat paw odema method [5-7].

Material and Methods

Authentication of plant material

The plant material (Root) was collected from local area of Jaipur in November 2023 and was authenticated at the Department of Botany, Janta PG collage APS University Rewa. A voucher specimen number or the herbarium number is J/Not/FR-R/763, Dated 15/11/2023 has been deposited.

Preparation of crude powder: The collected plant material will be manually cleaned to remove course impurities and then air-dried in shade at a well-ventilated place in the laboratory. Further drying will be done in the incubator to remove moisture at a temperature of 40^{O}C. The dried bark will be crushed and grounded in electric mixer-grinder to form crude powder and stored in air tight container.

Preparation of Hydroalcoholic extracts: 50 g crude powder of each plant material will be soaked in 400 ml of analytical grade methanol in a glass flask and then will be covered with aluminum foil followed by stirring at hourly intervals at room temperature. Soaking was done for a period of 72 hours. The soaked crude powder will be filtered through Whatman filter paper No.1 with separating funnels. The filtrates will be concentrated by evaporation at 50-55 °C in a rotator vacuum evaporator.

Preparation of aqueous extracts: 50 g of crude powder of each plant material will be soaked in 400 ml of triple glass distilled water in a glass flask will be stirred at hourly intervals initially for 2-3 times followed by 8 hours of undisturbed activity at room temperature. Soaking was done for a period of 12 hours. The soaked crude powder will be filtered through Whatman filter paper No. 1 with separating funnels. The obtained filtrate will be concentrated by using rotator vacuum evaporator at 45-50 °C.

Phytochemical & Physico-chemical study

The physico-chemical study, behavioral study of powder drug towards different chemical reagents and fluorescence analysis of powder plant materials were carried out using standard methods and UV visible spectroscopy also.

Antibacterial study

Bacteria used for the study

The Gram positive bacterial strains of *S. aureus*, *A. bumannii* and Gram nergative bacterial strains *E. Aerogenes*, *E. coli* were obtained from patient culture to diagnosed and identified in Dept. of Microbiology, Jayoti Vidyapeeth Women's University, Jaipur Rajasthan.

Media used for the culture of bacteria

Nutrient agar and broth were used for antibacterial activity of leaves & seeds Extract & assay.

Qualitative test

In this disc diffusion method the bacterial lawn was made on Nutrient agar plates from 10^3 CFU/ml of respective bacterial cultures. The mthanolic leaves & seeds extract of *Ficus religiosa* impregnated paper discs (Whatman filter paper No.1) were put in triplicate. The plates were incubated at 30 ^{0}C for 24 hours. The zones of inhibition depicted by the activity of drug were measured in mm. The Linezolid 30µ g/ml and Imipenem 10 µg/ml were taken as standard drug and DMSO as solvent control to test against gram positive bacterial and gram nergative bacterial respectively.

Quantitative Assay

The tube dilution method was followed to determine the minimum inhibitory concentration of potent leaves & seeds extract of *Ficus religiosa*. The lowest concentration of drug inhibiting the bacterial growth was treated as MIC of the drug. For this purpose, a range of concentrations 0.5 to 10.0 mg/ml of hydroalcoholic extract was taken.

Animal's studies

Albino rats of either sex (150-200 g) were obtained from IIRT Ghaziabad UP. The animals were kept under controlled environmental conditions at 25±2 ºC temperature and 45% relative humidity with natural light/dark cycle and allowed free access to food (Standard pellet diet, Hindustan Lever Ltd., India) and dist 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).

Ethical issue

The study protocol was approved by Institutional Animal Ethics Committee (Registration No. CPCSEA IAEC/2024/22/018) IIRT, Ghaziabad.

Excision Wound Model

The animals will be randomly divided into four groups of 5 animals for extract. Group I as a control, Groups II (low dose of extract), Group III (High dose extract), Group IV (Highest dose).

Evaluation of wound healing activity of methanol extract of *Ficus religiosa*

The Animals were depilated and wounded under light ether anesthesia, semi- aseptically. Then they were divided into 4 groups of 5 animals each.

Incision Wound Model

The animals will be randomly divided into four groups of five animals for extract. Group I as a control, Groups II (low dose of extract), Group III (High dose extract), Group IV

standard drug. The rats will be anesthetized before and throughout the wound infliction.

Results

Antimicrobial activities of *Ficus religiosa* hydroalcoholic extracts: The hydroalcoholic root extract of *Ficus religiosa observed* antimicrobial activity against all the tested microrganisms *viz C. albicans, E. coli, S. aureus, P. aeruginosa, and r*esults are represented in Table 4 & figure 2. Maximum antimicrobial activity was found against *C. albicans* with the mean zone of inhibition of 16.5±0.45 mm whereas minimum antimicrobial activity was observed against *S. aureus* with the mean of zone of inhibition 13.67±0.57 mm. The effective antimicrobial effect was observed against all tested pathogens with the Streptomycin. The zones of inhibition (mean) against various microbes are as follows: Candida albicans- 24.5 ±0.47, *E. coli* - 27.7±0.51 mm, *S. aureus*- 29.833±0.288 mm and *P. aeroginosa*- 30.667±0.57 mm.

Table 1: Antimicrobial activity of hydroalcoholic *Ficus religiosa*

S. No.	MIC (mg/ml)	Microorganism	Zone of inhibition mm ± SD
1.	2.36	*E. coli*	15.5 ± 0.5
2.	7.78	*S. aureus*	13.67 ± 0.57
3.	3.22	*P aeruginosa*	14.97 ± 0.45
4.	1.83	*C. albicans*	16.5 ± 0.5

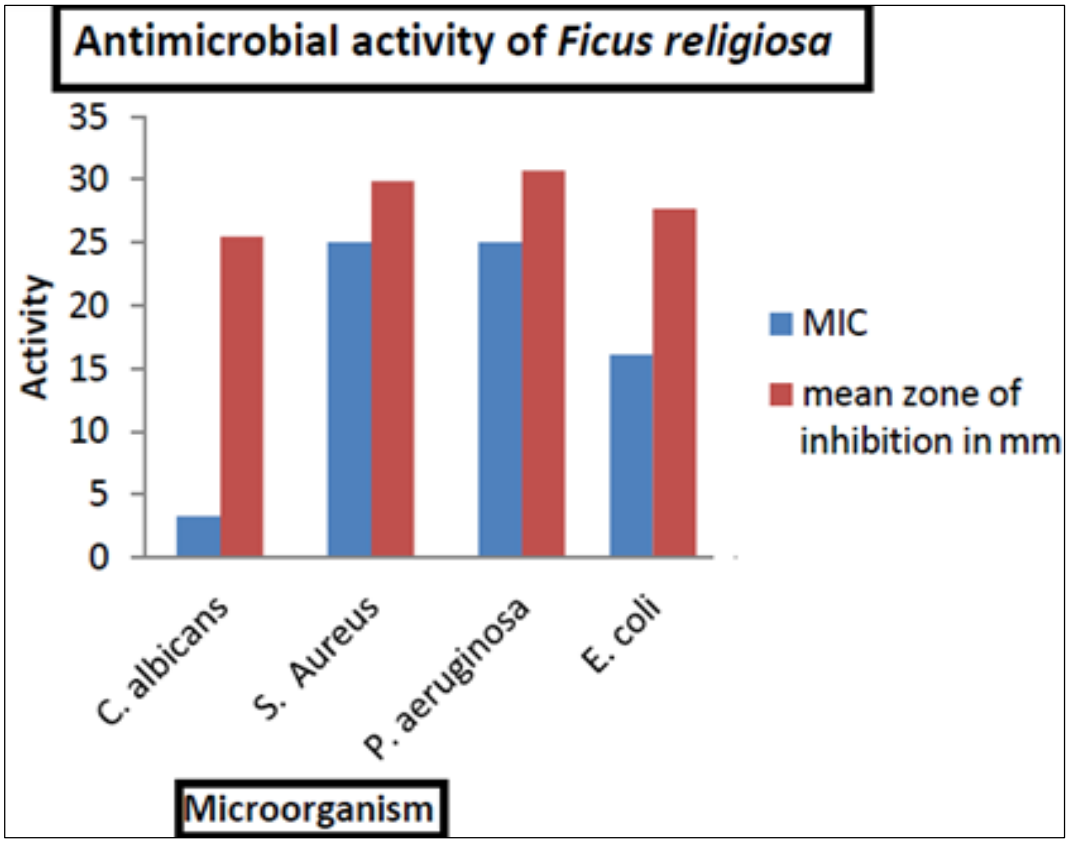

Fig 1: Antimicrobial activity of hydroalcoholic *Ficus religiosa*

Association of microbial infections with wounds, which is a common occurrence, deteriorates and further delays wound healing. Wound healing is particularly impaired in diabetic patients having foot ulcers due to peripheral neuropathy and poly microbial infections, and may lead to amputation of limb extremities in chronic condition. The increasing popularity of multidrug resistant (MDR) strains of bacteria and the current appearance of strains with decreased susceptibility to antibiotics increase the specter of non-treatable bacterial infections and add of urgency to the search for novel infection-fighting policies. In recent years, large body of evidences has accumulated to demonstrate the promising potentials of medicinal plants as anti-microbial agents.

Plants growing in wild are rich in wide variety of secondary metabolites such as tannins, terpenoids, and flavonoids etc. which have been found *in vitro* to have antimicrobial properties. Plants are more potent, safe and valuable healers because they promote the repair mechanisms in the natural way.

Phytochemical analysis of extract using UV-visible spectroscopy

The preliminary phytochemical screening and UV spectroscopy of the *Ficus religiosa* showed the presence of saponins, flavonoids, (310 nm) phenolic compounds, fixed oil and fats,(292 nm) alkaloids, phytosterol and tannins (385 nm) [9].

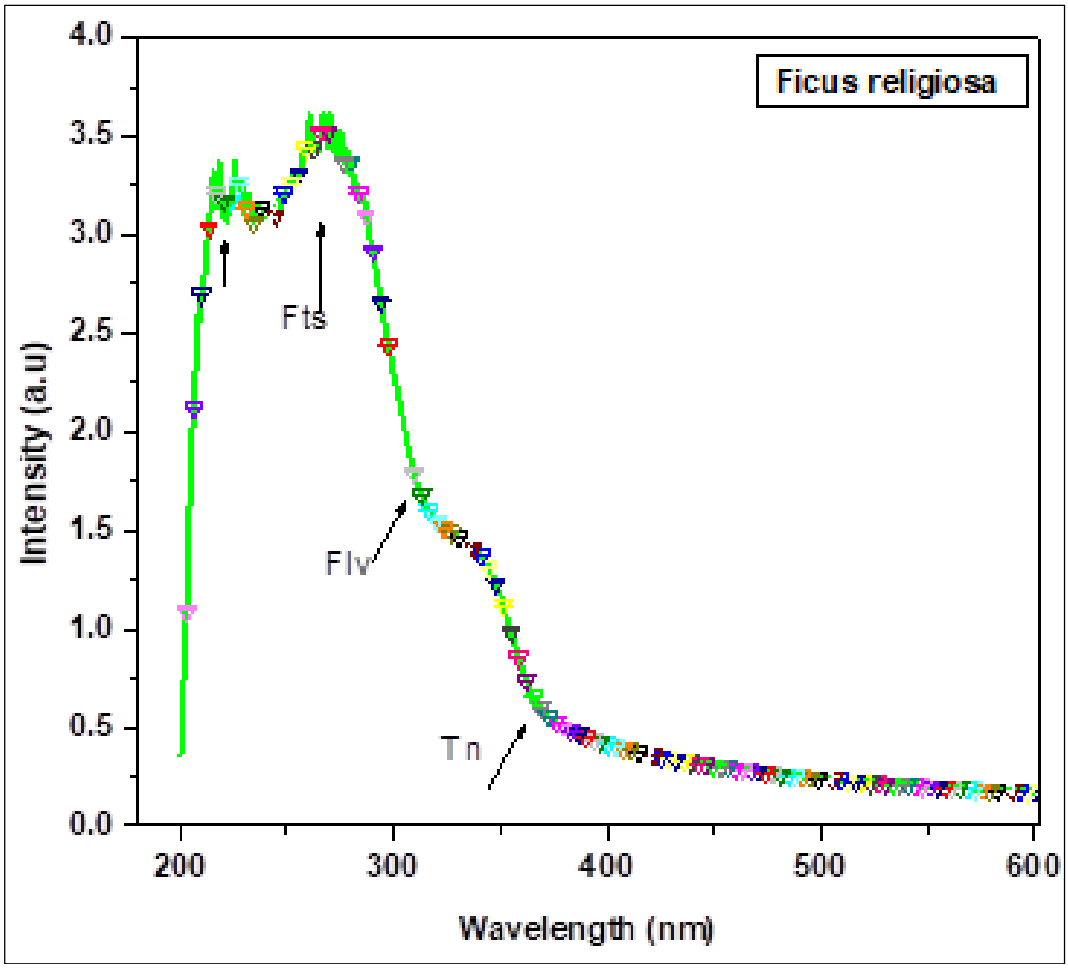

Fig 2: UV visible spectra of *Ficus religiosa* for phytochemical studies

Animal Models

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, 1.5 cm 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 7th post-wounding day.

Excision wound model

Each animal was anesthetized by open mask method with mild anesthetic ether. The rats were depilated on the back and a pre-determined area of 500 mm^2 full thickness skin was excised in the dorsal inter scapular region. The areas of the wounds were measured (sq.mm) immediately placing a transparent polythene graph paper over the wound and then tracing the area of wound on it. This was taken as the initial wound area reading. All the test samples were applied once daily. The wound area of each animal was measured on days 0, 3, 6, 9, 12, 15, 18 after inflicting the wound Excision wounds were created in rats to study the epithelialization period, scar area and rate of wound contraction. F1 (200 mg/kg) GIII, F2 (500 mg/kg) GIV, the measurement of progress of wound healing induced by reference drug, two herbal test formulations *viz*., formulation I *Ficus religiosa* (Low dose ethanol extract 5% aelextract), formulation II *Ficus religiosa* (High dose extract 15%), control group treated by simple 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 epithelialization 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, 15day and then up to 18th day or till complete epithelialization period of study. In the excision wound model has been shown in the Table no.2 and Figure no. 4&5 [10].

Table 2: Excision wound model, effect of sample formulation on epithelialization period in Rats

S. No.	Treatment Group (mg/kg, od)	Epithelialization period (Days)	Scar Area (mm^2)
1.	Normal Control Group I	16.5±0.43	80.1±2.3
2.	Group II	14.5±0.53	60.8±4.5
3.	Group III	16.1±0.45	65.50±401
4.	Group IV	16.2±0.42	65.4.±4.2

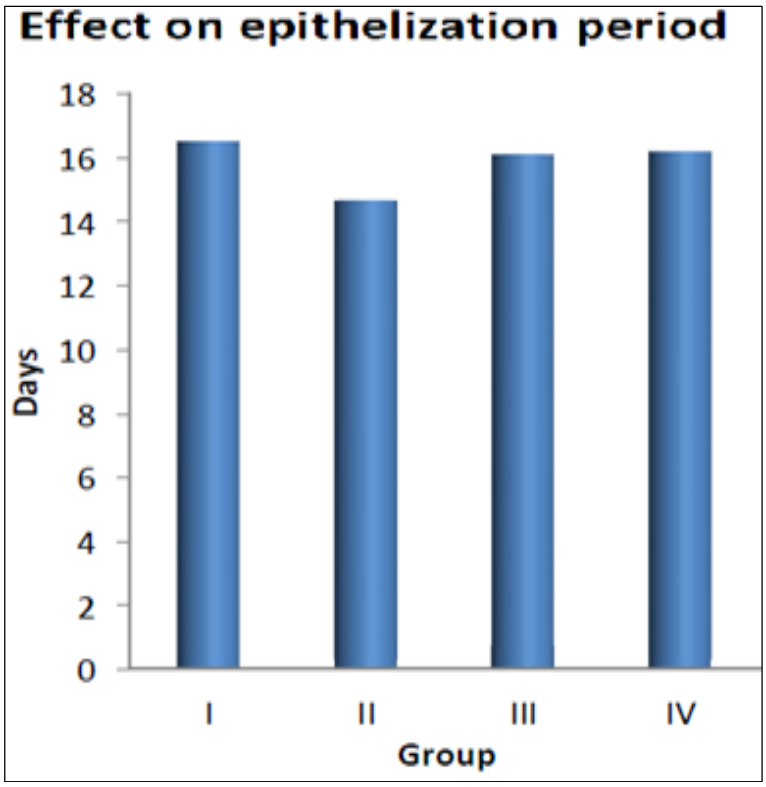

Fig 3: Effect of Sample formulation on epithelialization period in Rats

The Animals were depilated and wounded under light ether anesthesia, semi-aseptically. Then they were divided into 4 groups of 5 animals each & treated according groups.

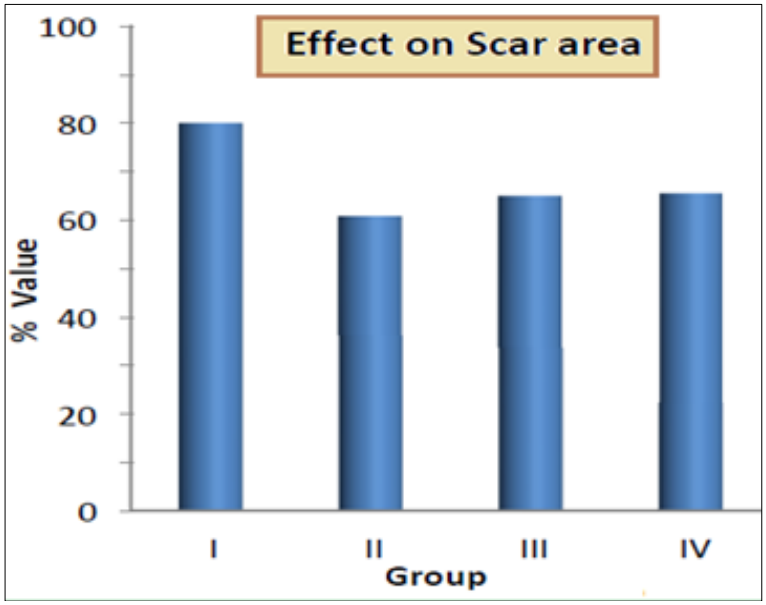

Fig 4: Effect of sample formulation on Scar area in Rats

Results are mean ± SEM of 6 animals in each group. Value indicates percent from their respective NR control group. P values: *<0.05, **<0.01, ***<0.001 compared to respective NR rats (Statistical analysis was done by one way ANOVA followed by Dunnett's test for multiple comparisons).

Incision Wound (Albino rats) 10 days study

Incision wound was produced in Albino 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 7th day and Wound Breaking Strength (WBS) was measured on 10th post wounding day. The 50% ethanol extracts of dried roots powder of *Ficus religiosa* (Formulation 1) and dry powder roots of *Ficus religiosa* (Formulation 2) were suspended in 0.5% carboxy methyl cellulose (CMC) and were administered orally, once daily for 10 days. Graded doses of formulation 1 and formulation 2 when given in orally form for 10 days as above to rats, showed a dose-dependent increase in WBS both in rats. On the basis of our above experiment and our earlier reported studies on wound healing effects of extracts of F1 (200 mg) & F2 (500 mg) respectively were selected for future studies [11].

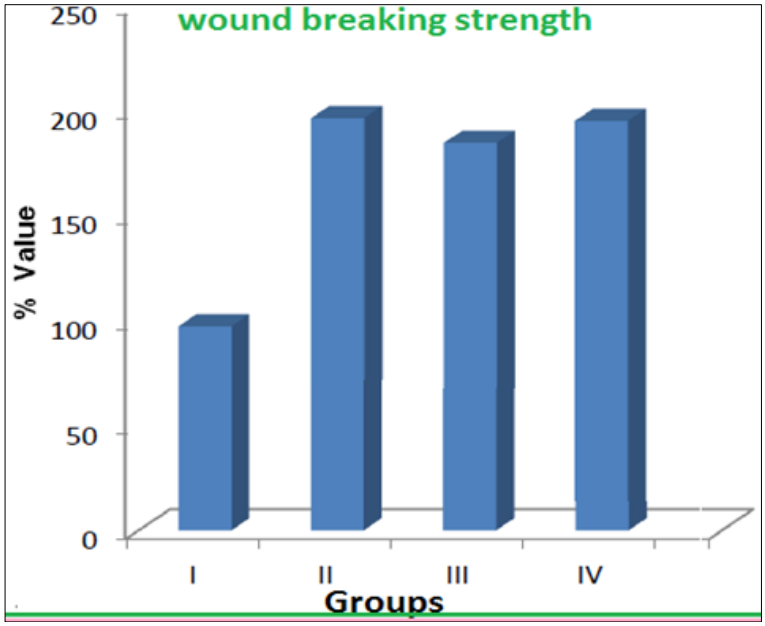

Fig 5: Effect of graded doses of Formulation 1 (200 mg/kg) & Formulation 2 (500 mg/kg) on wound breaking strength in Albino rats (Group I -IV)

Discussion

This study ascertained antimicrobial activities of hydroalcoholic extracts of selected plants parts and the natural products *viz*., honey and alovera gel. The hydroalcoholic extracts of *Ficus religiosa* and Streptomycin were screened *in vitro* for anti-microbial properties against five microbes which are reported to be associated commonly with diabetic wounds *viz Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Candida albicans* etc. employing conventional methods. The agar well diffusion method was used to evaluate the anti-micorbial activities of hydroalcoholic extracts of selected plants by measuring the inhibition zone against the microorganisms. The extracts exhibited varying degrees of antimicrobial effects against the microbes tested. The tested plant extract exhibited significant anti-microbial activity against the microbes with a highest mean zone of inhibition of 25.5±0.5 mm against *E. coli* whereas, the least antimicrobial effect was shown by with a mean zone of inhibition 20.667±0.57 mm *C. albicans*.

The plants extracts which showed promising antimicrobial activity were further used for the development of antimicrobial and wound healing herbal formulations and their pharmacological effect(s) with respect to wound healing functions were evaluated in experimental animal models. The wound healing activities of two formulations designed were evaluated by assessing rate and degree of wound contraction and duration of epithelialization, following topical application of a given formulation in excision wound model. Higher percentage of wound concentration coupled with minimum time of epithelialization was indicative of better healing. The formulation-1 exhibited maximum wound healing activity similar to that of reference formulation with epithelialization time of 16±0 days. The examination of affected tissues provided consistent results of healing parameters i.e., varying status of deposition of collagen fibers, lesser, moderate or higher number of inflammatory cells, and various grades of fibrosis, vascularity and hair follicles for the formulation 2 [12].

Conclusions

Ethanol Extracts of *Ficus religiosa* formulation 1 and formulation 2 indicated significant wound healing as evidenced by enhanced wound contraction, epithelialization period and wound breaking strength in normal and diabetic rats. Histology study revealed decreased inflammatory reactions and enhanced healing, angiogenesis and collagen tissue formations while, biochemical investigations indicated decrease in free radicals, MPO and cytokines and increase in antioxidant status, growth factors and collagen synthesis in NR rats. The research studies revealed perpetuated inflammatory reactions and decrease in angiogenesis and collagen tissue formations while, biochemical investigations indicated enhancement of free radicals, MPO and cytokines and decrease in antioxidant status, growth factors and collagen synthesis when compared with normal rats [12]. Formulation 1 and Formulation were found safe and showed antibacterial activity which helped in decreasing bacterial load and better wound healing and found to reverse the histological and biochemical parameters in NR rats by virtue of wound healing properties. Therefore, it can be concluded that *Ficus religiosa* Formulation 1 (200 mg/kg) & formulation 2 (500 mg/kg) have good potential to be used as a product for faster and better wound healing in diabetic condition. However, further work is required in the field of molecular research to authenticate their use.

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