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Full Length Research Paper

Comparative wound healing efficacy of ampucare and becaplermin in diabetic rat

Vivek Kumar Dwivedi* and Manu Chaudhary

Pre-clinical Division, Venus Medicine Research Centre, Baddi, H.P. 173205 India.

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To determine the comparative wound healing efficacy of ampucare and becaplermin drugs in diabetic rats, diabetes was induced by alloxan 75 mg/kg body weight via intraperitonial route. 500 mm^2 wound were created on the dorsal area of all animals. Wound lesions were cleaned with normal saline and treated with respective drug for 14 days. Our results revealed that the catalase activity, reduced glutathione, protein, ascorbic acid and nitrate; hydroxyproline and hexosamine levels were significantly increased along with significant decrease in xanthine oxidase activity, and malonaldialdehyde level in the granulation tissue of ampucare treated group as compared with becaplermin treated group on the 14th day. Wound size and bacterial count of ampucare treated group were also significantly reduced as compared to diabetic controlled and becaplermin treated groups. In the histological examination, well organized fibrous tissue proliferation, epithelization and complete scar formation were observed in ampucare treated group in comparison to becaplermin treated group. So these findings concluded that ampucare showed better wound healing efficacy than becaplermin in diabetic rat model.

Key words: Ampucare, becaplermin, wound healing, diabetes, biochemical and antioxidant, oxidative stress.

INTRODUCTION

Natural products are sources of synthetic and traditional herbal medicine and are still the primary health care system (Blanks et al., 1998). The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infective diseases and management of chronic wounds. The healing cascade begins immediately following injury when the platelets come into contact with exposed collagen. As platelet aggregation proceeds, clotting factors are released, resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing (Clark, 2001).

Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities. These abnormalities contribute to the impaired wound healing observed in diabetes.

The collagen content of the skin is decreased as a result of reduced biosynthesis and/or accelerated degradation result of newly synthesized collagens (Chithra et al., 1998). 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 (Singer and Clark, 1999). Impaired wound healing is an enigmatic and debilitating complication of diabetes and poses a serious challenge in clinical practice. The exact pathogenesis of the poor wound healing in diabetes is not clearly understood, but evidence from studies involving both human and animal models of diabetes reveal several abnormalities in the various phases of wound healing process (Goodson and Hung, 1977; Goodson and Hunt, 1979).

Ampucare is a poly herbal ingredient.The main active components of ampucare are *Azadirachta indica* and *Curcuma longa*. It is an oil base drug which is used topically for the treatment of burn wounds, diabetic wound etc. It has anti-inflammatory and antimicrobial properties, which also improves blood flow of wounds. It has an immunomodulatory action along with tissue regeneration properties (Dwivedi et al., 2010).

Becaplermin is a topical drug used in treating diabetic ulcers of lower limbs (foot, ankle and leg). It belongs to a class of drugs called platelet-derived growth factors

*Corresponding author. E-mail: vivekdwivedi@venusremedies.com. Tel: 91 1795 302127. Fax: 91 1795 302023.

(PDGFs). It promotes the division of cells and the formation of new skin. So, in the present investigation, authors have tried to determine the comparative wound healing efficacy of ampucare and becaplermin drug in diabetic rats.

MATERIALS AND METHODS

Chemicals and reagents

5, 5 diathiobis 2 nitro benzoic acid (DTNB; 55495), Thiobarbaturic acid (TBA; 30231), 2,4- Dinitrophenyl hydrazine (DNPH; 52144), Xanthine (03472), Bovine Serum albumin (BSA; 27423), reduced glutathione (GSH; 13679) and other biochemicals were procured from Himedia laboratories Ltd, Mumbai, India and Sigma, St. Louis, MO, U.S.A. All other chemicals (Ethanol, Formalin, benzene, harris hematoxylin stain, eosin, DPX mount, Xyline) were procured from BDH, SRL, Merck etc.

Drugs

Ampucare is polyherbal ingredients of *Azadirachta indica, Curcuma longa, Glycyrrhiza glabra, Jasminum officinale, Pongamia pinnata, Rubia cordifolia, Terminalia chebula, Trichosanthes dioica, Symplocos racemosa, Ichnocarpus frutescens, Capsicum abbreviata, Nymphaea lotus* etc. These pure ingredients were purchased from authorized dealer for the formulation of ampucare drug. Ampucare is medicated oil base drug and formulated in Venus Remedies Ltd. Baddi, H.P. The drug was obtained from Venus Remedies Ltd, Baddi, H.P. for carrying out this experiment work. Becaplermin was gel and procured from a pharmacy (Chandigarh).

Experimental animals

Total of 30 male wistar albino rats (150 to 200 g) were selected for the present study. They were housed in animal house of Venus Medicine Research Centre, Baddi, H.P. in individual polypropylene cages. Rats were provided with commercially available feed and water ad *libitum*. The experimental room was air conditioned with temperature 22 ± 3°C, humidity 55 ± 5%, and with artificial fluorescent light (12:12 h of light and dark cycle). Experiment was carried out after approval from the institutional animal ethical committee (IAEC). The IAEC approval number for carried out this experiment was IAEC/2011/08.

Induction of diabetes

Diabetes was induced by of Heikkilaa et al.'s (1976) method. 30 wistar male rats were starved over night prior to the day of experiment. Six animals were transferred into another cage and considered as control group, while the rest rats were used for the induction of diabetes. Intraperitonial injection of alloxan (75 mg/kg/body weight) was administered to animals for the induction of diabetes. After 72 h the fasted blood glucose level was measured in all animals for the confirmation of diabetes. Animals with blood glucose levels above 200 mg/dl were considered to be diabetic. Out of 24 animals, 22 rats were diabetic while rest two rats were non-diabetic. Total eighteen rats were randomly selected from 22 diabetic rats and divided into three groups (II-IV) of six rat each as follows:

Group I (n = 6): Normal control group (wound)
Group II (n = 6): Diabetic wound induced group
Group III (n = 6): Diabetic wound + Ampucare treated group
Group IV (n = 6): Diabetic wound + Becaplermin treated group

Excision wound

After induction of diabetes, the dorsal fur of animals was shaved using disposable razor, and the area was cleaned with betadine. A uniform circular of 500 mm^2 and 0.2 cm depth wound was created by a surgical blade from a predetermined shaved area on the back of each animal.

Treatment

Wound lesions of all groups (I to IV) were cleaned twice daily by using moistened cotton swabs with normal saline. Wound lesions of rats belonging to groups III and IV were further treated with topical application of 0.5 ml Ampucare and 50 mg Becaplermin, respectively twice daily for 14 days.

Biochemical and enzymatic measurement

At the end of experiment, 200 mg of granulation tissues were carefully collected from each group and prepared the tissue homogenates in phosphate buffer saline solution (PBS; pH 7.4) for the measurement of biochemical (hydroxyproline, hexosamine and ascorbic) and enzymatic (catalase, reduced glutathione, xanthine oxidase) parameters .

All parameters were measured at 25°C. Xanthine oxidase enzyme activity was carried out according to the method of Roussos (1967). One unit of activity has been defined as change in absorbance at 290 nm in 1 min by 1 ml of granulation tissue sample.

Protein was assayed by the method of Lowery et al. (1951) using bovine serum albumin as standard. Malonaldialdehyde measurement was been utilized as a maker of oxidative stress by the method of Ohkawa et al. (1978), using thio barbituric acid reagent. Reduced glutathione was measured in the granulation tissue by the method of Hissin and Hilf (1976). Ascorbic level was estimated in the granulation tissue sample by the method of Roe (1967). Nitric oxide concentration was determined in the granulation tissue by Green et al.'s (1982) method. Hydroxyproline content was measured according to Woessner's (1962) method. Hexosamine was determined by according to the method of Elson and Morgan (1933).

Measurement of bacterial count in tissue

The bacterial count in the granulation tissue was measured according to method of Hirsch et al. (2008). 100 µl of granulation tissue homogenate was taken for measurement of *Staphylococcus aureus* bacterial colony. Serial dilution was performed, and samples were plated onto trypticase soy agar containing 5% sheep blood agar for total colony counts, mannitol salt agar for *S. aureus* detection. The plates were incubated aerobically at 37°C for 24 h. All colony counts were expressed as log10 colony-forming units (CFU) per gram of tissue. Bacterial counts >1 × 10^5 were considered to denote bacterial infection (O'Meara et al., 2006; Robson et al., 1999).

Histological analysis

At the end of the experiment, the rats were anaesthetized using the

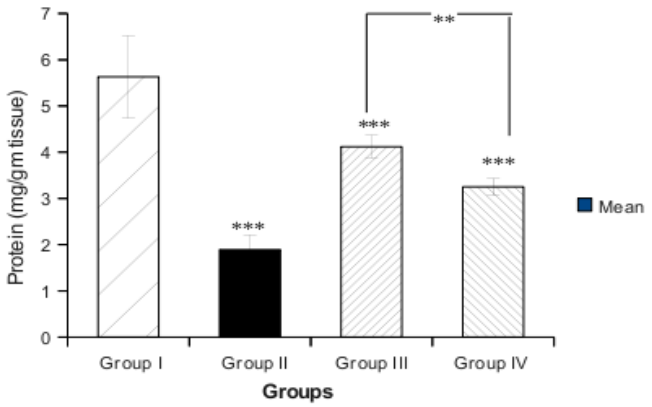

Figure 1. Status of protein level in the granulation tissue of diabetic group as well as treated groups. All data are expresses as mean ± SD. Statistical analysis was analysed between control (group I) vs diabetic group (group II) and diabetic vs both treated groups (groups III and IV). P < 0.001; highly significant (***), p < 0.001; significant (**), p < 0.005; significant (*), p > 0.05; non significant (ns).

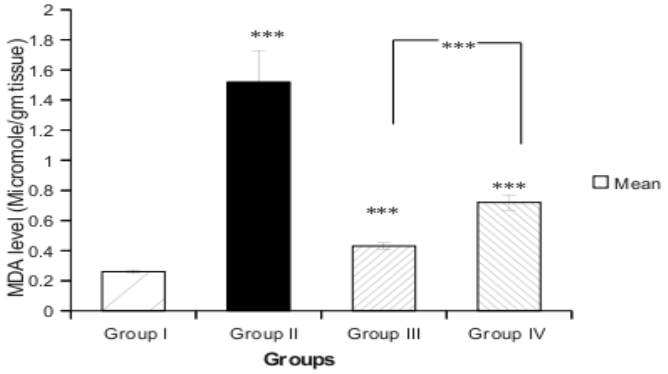

Figure 2. Status of malonaldialdehyde (MDA) level in the granulation tissue of diabetic group as well as treated groups. All data are expressed as mean ± SD. Statistical analysis was analysed between control (group 1) vs diabetic group (group II) and diabetic vs both treated groups (group III and IV). P < 0.001; highly significant (***), p < 0.01; significant (**), p < 0.005; significant (*), p > 0.05; non significant (ns).

combination of xylazine (10 mg/kg) and Ketamine (100 mg/Kg), and skin tissues (0.8 × 0.5 cm) were collected into 10% formalin solution for histological examination. After fixation of skin tissues, the tissue were washed overnight in running tap water, dehydrated in ascending grades of alcohol and cleared in benzene. The 4 to 5 micron thick sections were cut from paraffin embedded tissue and stained with haematoxylin and eosin stain (H&E) method.

Wound area measurement

Wound size of rats belonging to all the groups were measured using graphical planimetry method on days 0, 5, 10 and 14. Wound size was assessed by tracing the outer edge of the wound onto a butter paper using a fine-tipped permanent marker. The butter paper tracings were further traced on graph paper and area was measured manually in square centimetres (sq.cm). Percentage wound contracture or closureness was calculated using following formula:

% Contracture = [Area on day 0 – Area on final day/ Area on day 0] × 100

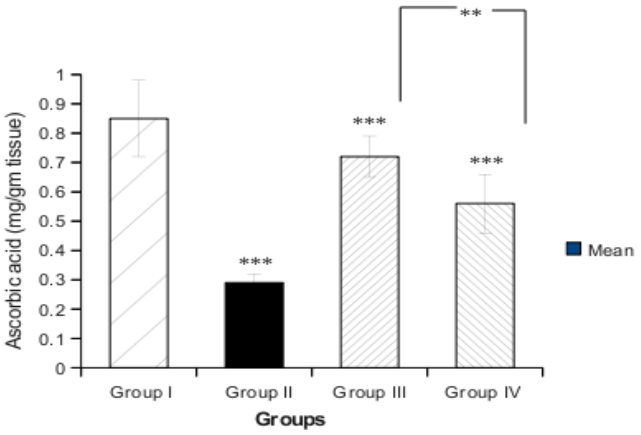

Figure 3. Status of ascorbic acid level in the granulation tissue of diabetic group as well as treated groups. All data are expressed as mean ± SD. Statistical analysis was analysed between control (group I) vs diabetic (group II) and diabetic vs both treated groups (group II and IV). P < 0.001; highly significant (***), p < 0.001; significant (**), p < 0.05; significant (*), p > 0.05; non significant (ns).

Statistical analysis

The resulting data are presented as mean ± SD. Statistical significance was analyzed by One way ANOVA followed by Tukey-Kramer's multiple comparison test using computerized Graph pad InStat version 3.05, Graph pad software, U.S.A. P > 0.05 was considered as insignificant.

RESULTS

In the present study, there were significant (p < 0.001; p < 0.01) decrease in the protein and ascorbic nitrate levels, along with significant (p < 0.001) increased malonaldialdehyde level in diabetic control group as compared with control group. After treatment with ampucare and becaplermin drugs for 14 days, the protein, ascorbic and nitrate levels were increased along with significant decreased MDA level in the both treated groups as compared with diabetic control group. When both treated groups were compared to each other, the protein and ascorbic levels were increased along with significant (p<0.001) decreased malonaldialdehyde level in ampucare treated group in comparison to becaplermin treated group (Figures 1 to 3). Whereas, in the case of nitrate level, the level was insignificantly increased in ampucare treated group compared to becaplermin

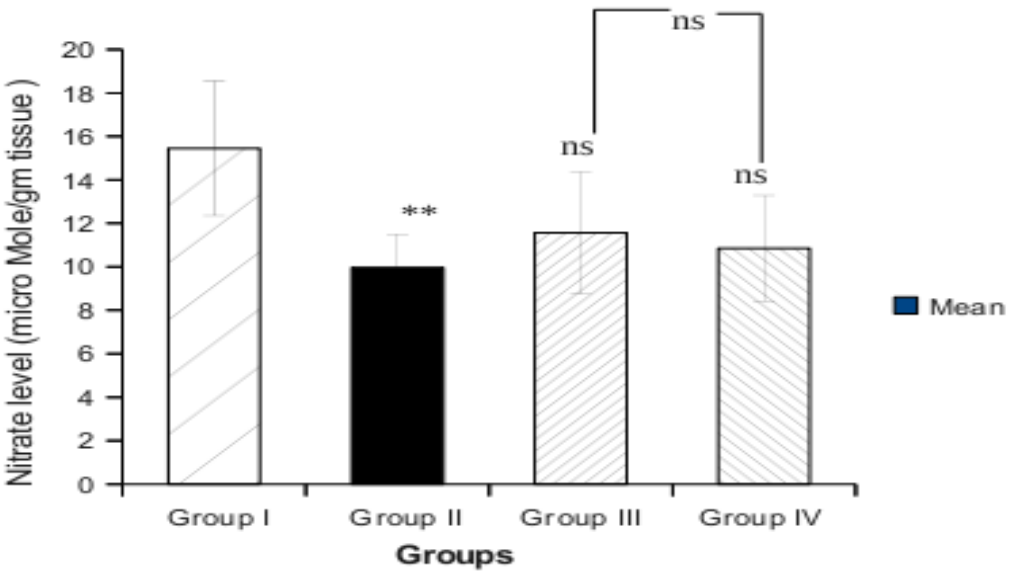

Figure 4. Status of nitrate level in the granulation tissue of diabetic group as well as treated groups. All data are expressed as mean ± SD. Statistical analysis was analysed between control (group I) vs diabetic group (group II) and diabetic vs both related groups (group III and IV). P < 0.001; highly significant (***), p < 0.001; significant (**), p < 0.05; significant (*), p > 0.05; non significant (ns).

Table 1. Status of oxidant and antioxidant enzymatic parameters in diabetic induced and treated groups.

S. no.	Parameter	Group I	Group II	Group III	Group IV	P value: GIII-GIV
1	Xanthine oxidase (µmole/min/gm tissue)	19.77± 5.07	45.94±9.21***	21.55 ± 4.85***	26.82 ± 2.15***	P > 0.05
2	Catalase (mMole/min/gm tissue)	115.2±6.84	76.21±4.50***	92.87 ± 5.88***	87.40 ± 6.96**	P > 0.05
3	Hydroxyproline (mg/gm tissue)	5.12±0.35	1.01±0.19***	3.56 ±0.23***	2.88 ± 0.15***	P < 0.001
4	Hexosamine (µg/gm tissue)	9.35±0.25	1.39±0.11***	7.86 ± 0.08***	7.71 ± 0.12***	P > 0.05
5	Reduced Glutathione (mg/gm tissue)	10.14±0.37	3.91±0.61***	8.83 ± 0.19***	8.06 ± 0.52***	P < 0.01

All data are expressed as Mean ± SD. Statistical analysis was performed between control *vs* diabetic group and diabetic *vs* both treated groups *via*Tukey-Kramer's multiple comparison test. P < 0.001; highly significant (***), p < 0.01; significant (**), p <0.05; significant (*), p > 0.05; non significant (ns).

treated group (Figure 4).

The activity of xanthine oxidase enzyme was significantly (p < 0.001) increased along with significant (p < 0.001) decrease in the activity of catalase enzyme in the diabetic control group as compared with the control group. Both enzyme activities were significantly improved in both treated groups after treatment with ampucare and becaplermin drugs for 14 days. When both treated groups were compared to each other, these enzyme activities were insignificant improved in the ampucare treated group. The reduced glutathione (GSH) was significantly decreased in the granulation tissue of diabetic control group as compared to the control group. After treatment with respective drugs, the GSH level was increased in both treated group as compared with diabetic control group. When both treated groups were compared, the GSH level in ampucare treated group was increased (Table 1).

The hydroxyproline and hexosamine contents were significantly (p < 0.001) decreased in the granulation tissue of diabetic control group as compared to control normal saline group. After treatment with ampucare and becaplermin respective drugs for 14 days, these parameters were significantly increased in both treated groups as compared to diabetic control group. The hydroxyproline content was found to have significantly (p < 0.001) increased in the ampucare treated group in comparison to becaplermin treated group; whereas, hexosamine content was found to have increased but insignificantly (p > 0.05) in ampucare treated group compared to becaplermin treated group (Table 1).

S. aureus bacterial count was found higher (9.23 ×

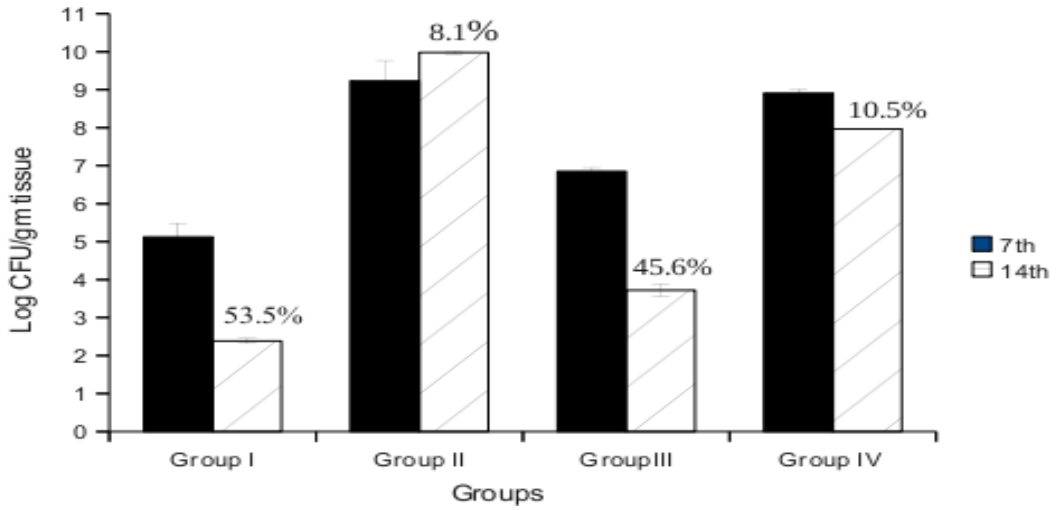

Figure 5. Microbial count on 7th and 14th day in diabetic wound and treated wounding data are mean ± SD. Percentage value was parenthesis between 7th and 14th day of all groups. Bacterial counts > 1×10^5 were considered to denote bacterial infection.

Table 2. Measurement of wound size in cm^2 by graphical planimetry in diabetic wound and treated wound.

Days	0 Day	5th Day	10th Day	14th Day
Control	5.18 ± 0.03	3.22 ± 0.34	1.18 ± 0.24	0.55 ± 0.08
Diabetic control	5.10 ± 0.12	3.71 ± 0.36	2.9 ± 0.89	2.44 ± 0.98
Diabetic wound + Ampucare treatment	5.01 ± 0.09	3.54 ± 0.44	1.81 ± 0.14***	1.00 ± 0.27***
Diabetic wound + Becaplermin treatment	4.96 ± 0.15	3.54 ± 0.44	3.19 ± 0.29*	1.98 ± 0.21

All data are expressed as mean ± SD. Where cm^2 is square centimetres. Statistical analysis was performed between control *vs* diabetic group and diabetic *vs* both treated groups *via* Tukey-Kramer's multiple comparison test. P < 0.001; highly significant (***), p < 0.01; significant (**), p < 0.05; significant (*), p > 0.05; non significant (ns).

10^8; 9.98×10^8 CFU/gm tissue) in diabetic control group on 7th and 14th post wounding day. After treatement with ampucare and becaplermin drugs, the infection was reduced as evidenced by decrease in the bacerial count (6.85×10^6 CFU/gm tissue) in ampucare treated group; whereas, in becapermin treated group, the bacterial count was 8.91×10^8 CFU /gm tissue. When all groups were compared on the 7th and 14th day, the bacterial count was decreased at 53.5% in control group, 45.6% in ampucare treated group and 10.5% in becaplermin treated group; whereas, 8.1% increased bacterial infection in diabetic control group. The infection was significantly decreased in ampucare treated group as compared to becapermin treated group on the 14th day treatment (Figure 5).

The size of wound was significantly (89.38%; p < 0.001) reduced in normal control group from initial day to end day; while in diabetic control group, the size of wound was reduced (at about 52.1%; p < 0.001) from initial day to end day. The wound size of diabetic control group was significantly (p < 0.001) increased in comparison to normal control group on the 14th day. After treatment with ampucare for 14 days, the wound size was (p < 0.001; 80.0%) reduced significantly from initial day to end day; whereas, in becaplermin treated group, the wound size was also significantly reduced (p < 0.001; 60.0%) from initial day to end day. When ampucare and becaplermin treated group was compared with diabetic control group on the 10th and 14th day, the wound size was significantly reduced in ampucare treated group on the 10th and 14th day; whereas, in case of becaplermin treated group the wound size was mildly increased on the 10th day due to formation of pus, but on 14th day the wound size was reduced as compared to diabetic control group. When ampucare treated group was compared with becaplermin treated group, the wound size was same on the 5th day in both treated group, whereas on the 10th and 14th day, the wound size was significantly (P < 0.001) reduced in ampucare treated group compared to becaplermin treated group. Wound size was insignificant (P > 0.05) in the all groups on the 5th day (Table 2).

Wound contracture was significantly (p < 0.001; p <

Table 3. Wound contracture percentage by graphical planimetry.

Groups	Contracture percentage
Control	89.44
Diabetic control	52.28
Diabetic wound + Ampucare treatment	80.05**
Diabetic wound + Becaplermin treatment	60.04

All data are expressed as Mean ± SD. Statistical analysis was performed between control *vs* diabetic group and diabetic *vs* both treated groups *via* Tukey-Kramer's multiple comparison test. P < 0.001; highly significant (***), p < 0.01; significant (**), p < 0.05; significant (*), p > 0.05; non significant (ns).

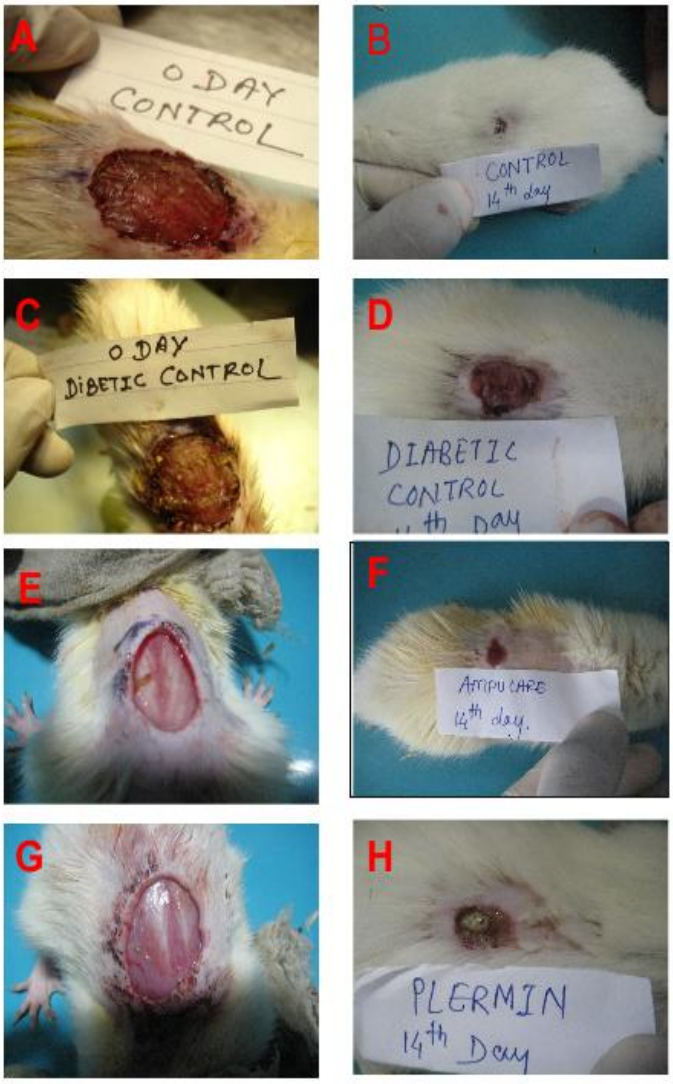

Figure 6. Gross changes in normal control rat, diabetic control and diabetic plus treated rats on 0 and 14th days. (A) Wound of a non-diabetic control rat on day 0; (B) wound of a non-diabetic control on day 14; (C) wound of a diabetic control rat on days on day 0; (D) wound of a diabetic control rat on day 14; (E) wound of a diabetic rat treated with ampucare on day 0; (F) wound of a diabetic rat treated with ampucare on day 14; (G) wound of a diabetic rat treated with becaplermin on day 0; (H) wound of a diabetic rat treated with becaplermin on day 14.

0.01) increased in normal control group (group I) as well as in ampucare treated group (group III) as compared to diabetic control group in 14 days. When becaplermin treated group was compared with diabetic control group, the wound contracture did not altered significant (p > 0.05) increase in becaplermin treated group. When ampucare treated group was compared with becaplermin treated group, the wound contracture was found higher in ampucare treated group on the 14th day, and the % of wound contracture of normal control group was reached (Table 3). Gross photographs of wound on day 1 and day 14 of all groups are shown in Figure 1. Macroscopic evaluation of healing area on day 14 of the experiment showed a very clear beneficial effect of ampucare in group III on the healing process in diabetic wound, which was analogous to normal control group (I). A well demarcated difference in epithelial neoformation was observed in ampucare treated group as compared to diabetic control and becaplermin treated groups. Diabetic rat wounds and Becaplermin treated rat wounds showed pus formation from 9 to 10 days onwards (Figure 6A to H).

The microscopic examination of skin wound tissue transverse sections of normal control rat showed a well organized fibroelastic tissue proliferation, development of stratified squamous epithelium over the fibroelastic tissue, epithelium covered by thin layer of keratinised tissue, and progressive loss of capillaries within the fibroelastic tissue was observed in the healed wound (Figure 7A). The microscopic examination of diabetic control skin wound tissue shown a disorganized fibrous tissue proliferation with incomplete scar formation, accumulation of cellular debris consisting of neutrophils and suspected bacterial colonies were present. Microabscess formation was evident histologically (Figure 7B). The wound in diabetic rats treated with Ampucare revealed a well-organized fibrous tissue proliferation along with complete scar formation. Debris accumulation and neutrophils were not seen in these rat wounds as seen in diabetic control rat wounds. Development of stratified squamous epithelium and constriction of the capillaries within the fibrous tissue was observed in this group (Figure 7C and D). The skin wound tissue sections of diabetic wound rats treated with Becaplermin revealed

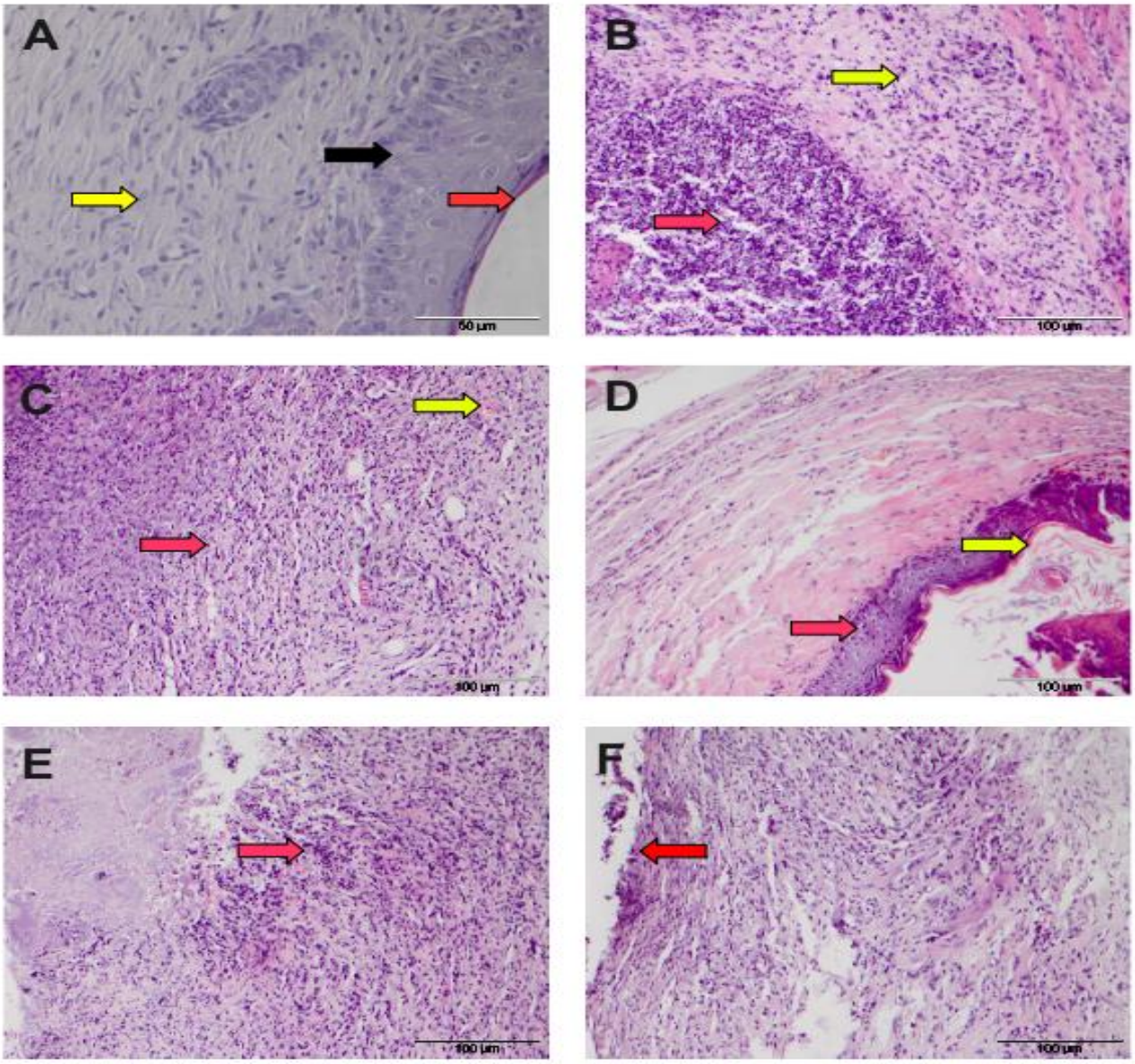

Figure 7. Histopathological changes in normal control rat, diabetic control and diabetic plus treated rats. (A) Section of skin from a non-diabetic control rat showing well organized fibro-elastic tissue proliferation (yellow arrow), development of stratified squamous epithelium (black arrow) and thin layer of keratinized tissue (red arrow). (B) Section of skin from a diabetic control rat showing disorganized fibrous tissue proliferation (yellow arrow) and accumulation of cellular debris consisting of neutrophils (red arrow). (C) Section of skin from a diabetic rat treated with ampucare showing well organized fibrous tissue proliferation (red arrow) along with constriction of the capillaries within the fibrous tissue (yellow arrow). (D) Section of skin from a diabetic rat treated with ampucare showing development of stratified squamous epithelium (red arrow) and thin layer of keratinized tissue (yellow arrow). (E) Section of skin from a diabetic rat treated becaplermin showing disorganized fibrous tissue proliferation (red arrow). Section of skin from diabetic rat treated with becaplermin showing incomplete scar formation (red arrow).

well-organized fibrous tissue proliferation along with complete scar formation. However, three of the six rat wounds showed disorganized fibrous tissue proliferation with incomplete scar formation (Figure 7E and F). Debris accumulation and neutrophil accumulation was seen in these animal's skin tissue sections. On the grading system by histological examination showed that inflammatory cells were found higher in diabetic control group and becaplermin treated group as compared to ampucare treated group. In normal control group, the inflammatory cell was found less. Collagen, angiogenesis, granulation and cell proliferation were

Table 4. Histological base grading system in all groups after 14 days.

Groups	Collagen	Angiogenesis	Inflammation	Granulation	Sq. cell proliferation
Control	++++	+++	+	+++	++++
Diabetic control	++	+	+++	++	++
Diabetic wound + Ampucare treated	+++	++	+	+++	+++
Diabetic wound + Becaplermin treated	++	++	+++	++	++

Degree of grading index ++++, very higher; +++, high; ++, normal; +, low.

measured in the term of grading system (Table 4).

DISCUSSION

Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived protein, all coordinated by an array of cytokines and growth factors (Bhat et al., 2007). Four stages are involved in wound healing namely inflammatory stage, debridement stage, proliferation stage and maturation/remodeling stage (Thomas, 1997). Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodeled to form scar (Shetty et al., 2008). Certain factors which influence the wound healing effects include bacterial infection, nutritional deficiency, drugs, sterility, movement of wound, edges, site of wound and wasting diseases (Karl et al., 1995). Di Girolamo et al. (1993) postulated that defects in wound healing are caused by the hyperglycosylation of the locally synthesized cellular fibronectin. Hyperglycaemia affects the whole range of neutrophil functions, including migration, chemotaxis, adherence and bactericidal activity (Wall et al., 2003). Molecular oxygen also plays a significant role in the chronic wound. Excessive generation of reactive oxygen species (ROS) results in oxidative stress thereby causing delayed wound healing. Therefore, estimation of catalase antioxidant enzyme in the granulation tissue is relevant because this enzyme hastens the process of wound healing by destroying the reactive oxygen species. Collagen is a component of connective tissue, and is the most abundant protein in mammals. It has been reported that diabetes impaired wound healing process and decreases angiogenesis and collagen formation (Joyce, 2010). So in the present investigation, there was insignificant increased catalase activity along with insignificant decreased xanthine oxidase enzyme activity in the granulation tissue of ampucare treated group as compared to becaplermin treated group. The levels of protein, ascorbic, hydroxyproline and GSH were significantly ($p < 0.01$; $p < 0.001$; $p < 0.01$) increased along with significantly ($p < 0.001$) decreased MDA level in the granulation tissue of ampucare group as compared to becaplermin treated group. Nitrate level also insignificantly increased in ampucare treated group as compared to becaplermin treated group. These aforementioned parameters were found to be altered in diabetic group in comparison to normal group. The alteration in the antioxidant profile accompanied by the increased the level of malonaldialdehyde may be attributed to impaired wound healing due to generation of reactive oxygen species (Brem et al., 2003). It is well reported that alteration in antioxidant enzymes may cause impaired wound healing in immunocompromised rat (Gupta et al., 2002). The resulting data suggests that ampucare increases cell proliferation and collagen synthesis at the site of wound, as evidenced by increase the protein level and hydroxyproline content in the granulation tissue. The levels of cellular antioxidant (asorbic, GSH) were decreased in the diabetic group as compared to control. These parameters were improved in the ampucare treated group when compared to becaplermin treated group. However, a depleted level of ascorbic in the granulation tissue of diabetic control group suggesting that the cellular antioxidants are adversely affected during chronic wound. The depleted levels of GSH and ascorbate may be due to oxidation of these by activated neutrophils (Himilia et al., 1984; Thomas et al., 1988). Nitric oxide (NO) plays a pivotal role in the normal wound healing. It promotes processes including angiogenesis, remodeling, migration and proliferation of fibroblasts, epithelial cells and endothelial cells. The level of nitrate was reduced in diabetic control group as compared with control group. The nitrate level was increased in ampucare treated group when compared with becaplermin treated group. The data denotes that ampucare may increase eNOS protein expression level and eNOS activity. The level was increased in granulation tissue which enhance the synthesis of NO, thus promoting the process of wound healing. It has been suggested that poor wound occur due to nutritional deficient and oxidative stress (Guo and Diapietro, 2010). *S. aureus* bacterial infection was found higher in diabetic group as compared to control group. Due to infection, the pus formation occurred along with increased (52.16%) wound size in the diabetic group compared to the control group (89.38%). It means that delay wound healing involved in the diabetic group due to bacterial infection which causes pus formation. The bacterial infection and wound size were higher in the becaplermin treated group

in comparison to ampucare treated group on the 14th day. The increase bacterial infection and wound size occurred in the becaplermin treated group due to formation of pus. Various reports suggested that poor wound healing occurred due to bacterial infection in diabetic wound (Hackl et al., 2011). On the histological score data, it was found that angiogenesis, collagen process were reduced in diabetic control group. When ampucare treated group was compared with becaplermin treated group, the angiogenesis and collagen process were found almost equal increased in both treated group. It has also reported that topical administration of glucose to wound of non diabetic rat inhibits the normal angiogenesis process (Tellechea et al., 2010). Similar result was reported by Teixeira and Andrade (1999) that diabetic control group inhibited the angiogenesis process. Various articles showed that curcumin enhances wound healing in diabetic rats and genetically diabetic mice (Sidhu et al., 1999; Bhagavathula et al., 2009; Gupta and Jain, 2010). The preceding study showed that ampucare has fast wound healing activity than becaplermin. Fast wound healing activity in the ampucare treated group was due to presence of curcumin and galotenin. *C. longa* and *A. indica* (both) have natural medicinal properties, including antibacterial, anti-inflammatory, antineoplastic and analgesic activities (Tang and Eisenbrand, 1992; Fabry et al., 1996; Fang et al., 2003; Swarnakar et al., 2005). *A. indica* also have a significant anti-inflammatory activity (Subapriya and Nagin, 2005). The anti-inflammatory and antioxidant properties of curcumin may exert wound healing property through scavenging reactive oxygen species (Panchatcharam et al., 2006). The role of other ingredients of ampucare have a anti-ulcerogenic, anti-tumor, immuno-modulatory effects and useful in atopic dermatitis (Hussain et al., 2009; Pandey et al., 2007; Otsuki et al., 2010). Various reports have been suggested that curcumin treated wound showed faster healing by modulate collagen, improve rates of epithelialisation, wound contraction, increased tensile strength and decrease reactive oxygen species (Shehzad and Lee, 2010; Thangapazham and Sharma, 2007). From the study, it is concluded that ampucare has better wound healing efficacy than becaplermin drug due to reduce inflammatory response as well as increase antioxidant levels in diabetic wound rat.

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REFERENCES

Bhagavathula N, Warner RL, DaSilva MD, McClintock S, Barron A, Aslam MN, Johnson KJ, Varani J (2009). A combination of curcumin and ginger extract improves abrasion wound healing in corticosteroid-damaged hairless rat skin. Wound. Repair. Regen., 17(3): 360-363.

Bhat RS, Shankrappa J, Shivkumar HG (2007). Formulation and evaluation of polyherbal wound treatments. Asia. J. Pharma. Sci., 2(1): 11-17.

Blanks T, Brown S, Cosgrave B, Woody J, Bentley V, O' Sullivan N (1998). The Body Shop Book of Wellbeing Mind, Body, and Soul. Ebury Press London. pp. 173-192.

Brem H, Jacobs T, Vileikyte L, Weinberger S, Gibber M, Gill K, Tarnovskaya A, Entero H, Boulton AJ (2003). Wound-healing protocols for diabetic foot and pressure ulcers. Surg. Technol. Int., 11: 85-92.

Clark RA (2001). Fibrin and wound healing. Annals of the New York Academy of Sci., 936: 355-367.

Chithra P, Sajithlal GB, Chandrakasan G (1998). Influence of Aloe vera on collagen characteristics in healing dermal wounds in rats. Mol. Cell. Biochem., 181(1-2): 71-76.

Dwivedi VK, Chaudhary M, Ahmad A, Soni A, Naithani V (2010). Comparative Efficacy of ampucare and silversulfadiazine against Burn Wound Rat. J. Appl. Sci. Res., 6(6): 674-682.

Elson LA, Morgan WTJ (1933). Colorimetric method for the determination of glucosamine and chondrosamine. Biochem. J., 27(6): 1824-1828.

Fabry W, Okemo P, Ansorg R (1996). Fungistatic and fungicidal activity of east Africa Medicinal plants. Mycoses, 39: 67-70.

Fang JY, Hung CF, Chiu HC, Wang JJ, Chan TF (2003). Efficacy and irritancy of enhancers on the *in-vitro* and *in-vivo* percutaneous absorption of curcumin. J. Pharm. Pharmacol., 55(5): 593-601.

Girolamo Di N, Underwood A, McCluskey PJ, Wakefield D (1993). Functional activity of plasma fibronectin in patients with diabetes mellitus. Diabetes, 42(11): 1606-1613.

Goodson WH, Hung TK (1977). Studies of wound healing in experimental diabetes mellitus. J. Surg. Res., 22(3): 221-227.

Goodson WH, Hunt TK (1979). Wound healing and the diabetic patient. Surg. Gynecol. Obstet., 149(4): 600A608.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982). Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal. Biochem., 126(1): 131-138.

Guo S, Diapietro LA (2010). Factors Affecting Wound Healing. J. Dental Res., 89(3): 219-229.

Gupta A, Singh RL, Raghubir R (2002). Antioxidant status during cutaneous wound healing in immunocompromised rats. Mole. Cell. Biochem., 241: 1-7.

Hackl F, Kiwanuka E, Nowinski D, Eriksson E (2011). The Diabetic Wound: A New Experimental Wound Healing Model in Large Animals. Adv. Wound Care, 2: 166-170.

Heikkila R, Winston B, Cohen C (1976). Alloxan induced diabetes evidence for hydroxyl radical as a cytotoxic intermediate. Biochem. Pharmacol., 25: 1085-1092.

Himilia H, Roberts P, Wikstrom M (1984). Activated polymorphonucler leucocytes consumes vitamin C. Federal Eur. Biochem. Society, 178: 25-30.

Hirsch T, Spielmann M, Zuhaili B, Koehler T, Fossum M, Steinau HU, Yao F, Steinstraesser L, Onderdonk AB, Eriksson E (2008). Enhanced susceptibility to infections in a diabetic wound healing model. BMC Surg., 8(5): 1-8.

Hissin PJ, Hilf RA (1976). A flurometric method for the determination of oxidised glutathione and reduced glutathione in tissues. Anal. Biochem., 74 (1): 214-226.

Hussain ZG, Amresha SS, Chandana VR (2009). Hepatoprotective and antioxidant activity of *Amaranthus spinosus* against CCl4 induced toxicity. J Ethnopharmacol., 125: 364-366.

Joyce KS (2010). Understanding the Role of Nutrition and Wound Healing. Nut. Clin. Pract., 25(1): 61-68.

Karl M, Lacrix JV, Preston HH (1995). Canine surgery, 4th edition, American Anionic Veterinary Publications, California, 80: 42-45.

Lowery OH, Roseborogugh MJ, Farr AL, Randall RJ (1951). Protein measurement in Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Ohkawa H, Ohishi N, Yagi K (1979). Assay of lipid per-oxidation in animal tissue bythiobarbutric acid reaction. Anal. Biochem., 95(2): 351-358.

O'Meara S, Nelson EA, Golder S, Dalton JE, Craig D, Iglesias C (2006). Systematic review of methods to diagnose infection in foot ulcers in diabetes. Diabetic Med., 23: 341-347.

Otsuki N, Dang NH, Kumagai E, Kondo A, Iwata S, Morimoto C (2010). Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. J. Ethnopharmacol., 127: 760-767.

Panchatcharam M, Miriyala S, Gayathri VS, Suguna L (2006). Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Mol. Cell. Biochem., 290(1-2): 87-96.

Pandey M, Rastogi MS, Rawat AKS (2007). Saussurea costus: Botanical, Chemical and pharmacological review of an ayurvedic medicinal plant. J. Ethnopharmacol., 110: 379-390.

Robson MC, Mannari RJ, Smith PD, Payne WG (1999). Maintenance of wound bacterial balance. Am. J. Surg., 178(5): 399-402.

Roe JH (1967). Determination of ascorbic dehydroascorbic and diketogluconic acids. Meth. Biochem. Anal., 1- GLICK eds, Interscience Publisher, New York. p. 115.

Roussos GG (1967). Xanthine oxidase from bovine small intestine. (Methods in Enzymology; 12 A). In: Grossman L, Moldave K, editors. New York (NY): Academic Press; pp. 5-16.

Shetty S, Udupa S, Udupa L (2008). Evaluation of Antioxidant and Wound Healing Effects of alcoholic and aqueous Extract of *Ocimum sanctum* Linn in Rats. Evidan. Based Compl. Alter. Med., 5(1): 95-101.

Shehzad A, Lee YS (2010). Curcumin: Multiple molecular targets mediate multiple pharmacological actions: A review. Drugs Future, 35(2): 113-119.

Sidhu GS, Mani H, Gaddipati JP, Singh AK, Seth P, Banaudha KK, Patnaik GK, Maheshwari RK (1999). Curcumin enhances wound healing in streptozotocin induced diabetic rats and genetically diabetic mice. Wound. Repair Regen., 7(5): 362-374.

Singer AJ, Clark RAF (1999). Cutaneous wound healing. New Eng. J. Med., 341(10): 738-746.

Subapriya R, Nagini S (2005). Medicinal properties of neem leaves: A review. Curr. Med. Chem. Anticancer Agents, 5(2): 149-160.

Swarnakar S, Ganguly K, Kundu P, Banerjee A, Maity P, Sharma AV (2005). Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer. J. Biol. Chem., 11; 280(10): 9409-9415.

Tang W, Eisenbrand G (1992). Chinese drugs of plant origin: springer-verlag. Berlin and Heidelberg, Germany, pp. 401-415.

Tellechea A, Leal E, Veves A, Carvalho E (2010). Inflammatory and Angiogenic Abnormalities in Diabetic Wound Healing: Role of Neuropeptides and Therapeutic Perspectives. The Open Circu.Vascu. J., 3: 43-55.

Teixeira AS, Andrade SP (1999). Glucose induced inhibition of angiogenesis in the rat sponge granuloma is prevented by aminoguanidine. Life Sci., 64: 655-662.

Thangapazham RL, Sharma A, Maheshwar RK (2007). Beneficial role of curcumin in skin diseases. Adv. Exp. Med. Biol., 595: 343-357.

Thomas EL, Learn DB, Margaret M (1988). Superoxide dependent oxidation of extracellular reducing agents by isolated neutrophil. J. Biol. Chem., 268: 2178-2186.

Thomas JC (1997). Veterinary Pathology. 6th edition, William's and Wilkin, Maryland: USA, pp.150-56.

Wall SJ, Sampson MJ, Levell N, Murphy G (2003). Elevated Matrix metallo proteinase 2 and 3- production from human diabetic dermal fibroblast. Brit. J. Dermatol., 149(1):13-16.

Woessner JF (1961). The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch. Biochem. Biophys., 93(2): 440-470.

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