Pharmacological Evaluation of Pharmaceutical Dermal Gel Intended for the Management of Diabetic Wound Healing

Manpreet Kaur, Dr Rupinder Kaur Sodhi

Abstract---Kidney stone is a common disease in the world. These stones are present in solid structures which lead to severe pain and discomfort in kidney. This problem is also known as urolithiasis which affects approximately 12 percent of the world's population. Kidney stone is the third most prevalent disease among the various diseases which are affecting the urinary system. The accumulation of crystals / stones, usually made of calcium oxalate, characterizes this disease. Having the stones repeated after treatment is the main challenge. In males there is a 78-81% and in females a 47-60% rate of recurrence. In contrast to women, the occurrence of stones is three times higher in men. Though some products are available on the market, most of them do not deal with reoccurrence problems. Our researches have shown the promising role of diosgenin in stone prevention, so it may address the reoccurrence problem. It is also a drug derived from plants and not artificial, and therefore may have fewer side effects. In this paper, treatment of kidney has been performed with the utilization of diosgenin.

Keywords---diosgenin, kidney, urolithiasis, kidney stones

I. INTRODUCTION

Diabetes is a class of hyperglycemic, metabolic diseases due to the lack of “insulin secretion, insulin action or both”. The long-term injury, disease and degradation of various species, especially the ears, kidney, heart and blood vessels, are related to the chronic hyperglycemia of diabetes [1][2]. Hundreds of millions of people around the world are affected by the diabetes. The prevalence of the patient suffering from the diabetes mellitus (DM) has been increased dramatically over the last two decades from “30 million patients in 1985 to 285 million in 2010 and is estimated to reach up to 360 million by 2030” [3][4]. A diabetes mellitus patient shows a reported deficiency in the healing of acute injury. This group is also pronged to develop chronic diabetic foot ulcers (DFUs) [5][6][7][8] estimated for 15% of people with DM and 84% of all lower leg amputations associated with diabetes.

The development of diabetes includes various pathogenic processes. It involves the autoimmune disruption of the B-cells in pancreas with consequent insulin deficiencies, as well as defects that contribute to the insulin resistance. The insulin has insufficient action on target tissues because of the defects in carbohydrate, fats and protein metabolism in diabetes. Insulin deficiencies arise from insufficient insulin discharge and reduced insulin tissue response or more points in the complex hormonal action processes. Insulin-secretion and insulin-action deficiencies often coexist with a patient and the primary reason for hyperglycemia is often not apparent whether or not the abnormality is alone.
The skin constitutes a physical barrier that is critical to protect against microbial invasions and to preserve temperature and fluid homeostasis. Skin damage temporarily destroys this barrier and thus presents a great challenge for health.

Wounds contribute to tissue degradation and vascularisation loss at the injury site. The cell proliferation [9] and angiogenesis [10] at the site of the injury were natural cure procedures. Each soft tissue wound healing follows a similar and orderly protocol. Wound healing is a well orchestrated reparative reaction after any operation or traumatic injury. Wound healing is a complex procedure that involves inflammation, migration of various types of cells, fibroplasia, and deposition of collagens and wound contraction. Inflammatory cells in the wound site increased significantly during the inflammation phase and produced explosive levels of wound tissue formation of reactive oxygen species (ORSs), which affects the healing process of the wound.

Diabetic wound patients suffer from “impairment in angiogenesis, neovascularisation, failure in keratinocyte formation and fibroblast functions”, which thereby affects the mechanisms of wound healing. Delayed wound healing in diabetic animals occurs due to reduced derangement in tissue repair and impediment in the appearance of new connective tissue matrix. In addition, the formation of reduced collagen and lesser wound contraction have also been reported in surgical diabetic wounds along with impaired sensation in the wound area.

Chronic injuries impact over 8 million people in U.S. a year's health and lifestyles. About 900,000 people per year are affected by diabetic ulcers in the United States, 915,000 are affected by venous stase ulcer and 6.5 million are affected by stress ulcers. Chronic injuries can take years to heal and the risk of injury recurrence is usually very high. Ten to 15 percent of the population suffer from chronic injuries and are refractory to conventional wound healing procedures. Approximately 75,000 people need to be hospitalized in US each year as a result of their injuries.

The healing of skin injuries is a dynamic, complex and well organized process that requires many cell type and cell processes to be orchestrated. The classic wound cure model is divided into three phases: inflammation, proliferation and maturation.

Wounds can be classified into acute and chronic non-healing injuries depending on the period of cure. Once the injuries are not treated within 3 months, chronic, non-healing wounds are referred to. Non-healing wounds are often related to pathology associated with reduction of healing potential and/or increased susceptibility for acquiring wounds (e.g. peripheral neuropathies) (such as in people with arterial / venous insufficiency or diabetes or undergoing systemic steroide treatment). Non-healing wounds also are associated with underlying conditions due to fewer healing abilities (e.g., artery or venous defects or those with diabetes or systemic steroid therapy) and/or increased susceptibility to wounds acquired (e.g. peripheral neuropathy). Nonhealing injuries are commonly divided into “pressure ulcers, venous leg ulcers, arthral ulcers and diabetic foot ulcers (DFUs)”. Patients developing nonhealing lesions are heterogeneous. Not just causing discomfort, distress and an increased risk of amputation, non-curing injuries often involve significant medical expenses.
II. MATERIALS AND METHODOLOGY:

1. Drugs and Reagents:

The reagents used in this study are of analytical grade and are freshly prepared before laboratory use. Phenytoin sodium is purchased from “TCI Chemicals, India”, Quercetin was purchased from “HiMedia Laboratories, India” and Rosemary oil is purchased from “DeveHerbes”. Streptozotocin (STZ) from “Oswal-Scientific Store, India”, DTNB (5,5-dithiobis-2-nitrobenzoic acid), Folin-Ciocalteu Phenol reagent, Bovin serum albumin, n-butanol, Pyridine, reduced glutathione (GSH) from Loba Chemicals Mumbai, India. Trichloroacetic acid is purchased from Nice Chemicals Pvt. Ltd., Chochin, India and Thiobarbituric acid is purchased from Magus Chemicals. Carbopolultrazet, HPMC, PEG1500, glycerin, benzyl alcohol, Propylene glycol, DMSO, EDTA were purchased from Thomas Baker, India.

2. Preparation and characterization of topical gel:

The pharmaceutical topical gel bearing of Phenytoin Sodium, Quercetin and Rosemary oil was prepared in two equal parts by using the “Neutralization” method.

   i) Part 1 (50 gm): In first embodiment, weighed amount of carbopolultrez as mentioned in table 1 was dissolved in small quantity of water. Following which phenytoin sodium was dissolved separately in small quantities of propylene glycol and gradually mixed in the above mixture containing carbopolultrez individually with continuous stirring. PEG 1500 and HPMC were then added in the above obtained mixture. Glycerin and benzyl alcohol were mixed in separate beaker and poured into the final mixture. After mixing of all the ingredients, final pH was maintained with the addition of triethanolamine (TEA).

   ii) Part 2 (50 gm): In second embodiment, weighed amount of carbopolultrez as mentioned in table 1 was dissolved in small quantity of water. Quercetin was dissolved carefully into small quantity of DMSO with continuous stirring. Glycerine and benzyl alcohol were added into mixture. Then PEG 1500 and HPMC were added in the mixture containing quercetine. Propylene glycol was added into above mixture. After mixing of all the ingredients, final pH was maintained with the addition of triethanolamine (TEA).

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Ingredients</th>
<th>Part 1</th>
<th>Part 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbopolultrez</td>
<td>0.6% w/w</td>
<td>0.6% w/w</td>
</tr>
<tr>
<td>2.</td>
<td>PEG 1500</td>
<td>0.2% w/w</td>
<td>0.2% w/w</td>
</tr>
<tr>
<td>3.</td>
<td>HPMC</td>
<td>0.05% w/w</td>
<td>0.05% w/w</td>
</tr>
<tr>
<td>4.</td>
<td>Propylene glycol</td>
<td>10% w/w</td>
<td>5% w/w</td>
</tr>
<tr>
<td>5.</td>
<td>Phenytoin sodium</td>
<td>5% w/w</td>
<td>-</td>
</tr>
</tbody>
</table>
The components of the formulation, such as part 1 and part 2, were mixed together in standard manner by mechanical stirring for 30 minutes to yield a homogenous mixture. After mixing of both the parts, rosemary oil was added into gel and mixed well. The prepared gel was then packed into air tight container.

3. Characterization of topical gel:

Three batches were formulated for the optimization of invented gel formulation and compared with the blank topical gel.

i) Color and homogeneity: The color of formulated gel and marketed gel was noticed. Moreover, the prepared gel and marketed gel were inspected visually for homogeneity, consistency and grittiness.

ii) pH measurement: The pH of novel invented gel and the marketed gel was measured accurately using a calibrated pH meter (Eutech). In brief, 1gm of gel is dissolved in 50 ml of distilled water and a homogenous dispersion is formed. The pH of dispersions was measured in triplicate (n=3).

iii) Measurement of rheology: The viscosity of the tailored gel samples is determined by the Brookfield Rheometer (Brookfield DV-3T + Rheometer) using a LV-4 spindle. However, the gel samples are poured into the adaptor of rheometer and the viscosity of the test samples is determined as a function of shear rate according to standard operating procedure provided by the manufacturer. Each experiment is carried out in triplicate (n=3).

iv) Spreadability: The term "dissemination" refers to the extent to which the topical gel spreads to the infected areas on the application of skin. 1 gm of gel sample has been distributed between two glass slides and compressed for 5 min, by putting 1000 gm of weight on glass slides. Afterwards, its 100 gm weight is added into the pan and the top plate was pulled using a hook string. It was noted that the upper glass slide moves against the lower glass slide to 10 cm. A shorter duration suggests increased propagation. Every triple experiment is performed at n=3. The propagation capacity (S) has been determined with the following formula: 

$$S = \frac{M \times L}{T}$$

where S refers to propagation power; M indicates weight linked to the top glass dial, L is equivalent to the distance covered in the dial, and T refers to the time required.

<table>
<thead>
<tr>
<th></th>
<th>Component</th>
<th>Concentration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Quercitin</td>
<td>-</td>
<td>2 % w/w</td>
</tr>
<tr>
<td>7</td>
<td>Rosemary oil</td>
<td>1% w/w</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Glycerine</td>
<td>0.5% w/w</td>
<td>0.5% w/w</td>
</tr>
<tr>
<td>9</td>
<td>Benzyl alcohol</td>
<td>0.6% w/w</td>
<td>0.6% w/w</td>
</tr>
<tr>
<td>10</td>
<td>DMSO</td>
<td>-</td>
<td>15% w/w</td>
</tr>
<tr>
<td>11</td>
<td>Triethanolamine</td>
<td>0.5-1% w/w(approx.)</td>
<td>0.5-1% w/w(approx.)</td>
</tr>
<tr>
<td>12</td>
<td>Distilled water upto</td>
<td>50ml</td>
<td>50ml</td>
</tr>
</tbody>
</table>
v) **Drug content:**

To ensure the quantity of active pharmaceutical ingredients, formulated gel was assayed for the drug content by gas chromatography.

The *in vivo* evaluation of novel topical gel was performed using Wistar rats (180-220g) of either sex. The animals were classified into four groups of 6 rats each. Group 1- normal control (treated with normal saline), Group 2- disease control (Streptozotocin, 50 mg/kg; *i.p*), Group 3- blank group (Streptozotocin, 50 mg/kg; *i.p* and blank gel), Group 4 – marketed gel group (Streptozotocin, 50 mg/kg; *i.p* and marketed gel), Group 5 - formulated gel group (Streptozotocin, 50 mg/kg; *i.p* and formulated gel). The formulated topical gel was comprised of Phenytoin Sodium, Quercetin and Rosemary oil.

**i) Induction of diabetic wound:**

a) *Induction of Diabetes:*

Diabetes (dissolved in the 0.1 M ice-cold citrate buffer (pH4.5) in overnight fasted animals) is inducted from a single STZ (50mg / kg / *i.p*) injection in the Wistar rats. In order to prevent animals from lethal hyperglycemia, 5% sucrose was added for 24 hours following STZ therapy. The tail vein blood glucose measurement using glucometer is verified one week after the STZ injection. Animals more than 240 mg / dl of blood glucose are considered as the diabetic and were included in the test. After 4 weeks of administration of STZ, pathological changes begin.

b) *Induction of diabetic wound by excision method:*

The animal is given ketamin and xylazine (100 and 3.33 mg / kg body weight respectively *i.p.*) after 4 weeks of STZ administration. After the injury was developed on the lateral side of each back limb, the back region was cleaned and disinfected by sterile 6 mm biosplits. The wounds were left open and then individual animals were held for cannibalism prevention.

c) *Healing parameters:*

i) **% wound contraction and healed area:**

Quantitative measurements of wound size was performed on day 0,4,8,12 and 16 to assess initial wound size before and after debridement, as well as progression towards wound closure.

ii) *Biochemical Estimations:*

a) *Collection of sample:*

At the end of experiment on day 16th, the animals from each group were sacrificed and the granulation/healing tissue was excised out carefully. The collected tissue was immediately divided into two portions. The actual length and width of the extracted tissue were measured. Wound reduction is calculated as a percentage of the original size which had been measured planimetrically by plotting the area of the wound on a transparent graph sheet. During the experiment to estimate biochemical parameters such as total tissues, antioxidants and granulated tissue free radical markers for inflammatory tissue parameters and histopathological evaluation, the samples have been preserved in cold by scratching ice-cold, physiologically sound saline.
1. **Estimation of tissue total proteins:**

The maximum tissue protein has been calculated with generic Bovine Serum Albumin (BSA) modifications by the process of Lowry et al. (1951). Spectrophotometrically at 750 nm the protein content was determined against properly prepared blank. A default curve was drawn using BSA 0.2-2.4 mg / ml. The total protein quantity is given in mg / ml.

2. **Estimation of antioxidants and granulation tissue free radical:**

The phosphate-buffered saline (PBS, pH 7) used to estimate proteins, free radicals and antioxidants were homogenized in wet granulating tissues in a glass Teflon homogenizer (10.0% w / v) at 4% C.

i) **Estimation of superoxide dismutase (SOD):**

SOD activity was estimated in terms of measuring reduced NBT by the method of Wang et al (1998). The absorbance of formazonspectrophotometrically was determined at 540 nm. Result was expressed as reduced NBT picomole/min/mg of wet tissue.

\[ \text{Amount of reduced NBT} = A*V/ T*wt*l*ε \]

\[ A = \text{absorbance} \]

\[ V = \text{volume of pyridine, 1.5ml} \]

\[ T = \text{time for which tissue was incubated with NBT, 1 hr} \]

\[ wt = \text{blotted wet wt of tissue} \]

\[ ε = \text{extinction coefficient (0.72hr/m or mole/min)} \]

\[ l = \text{length of light path (1 cm)} \]

ii) **Estimation of catalase (CAT):**

CAT measurement is done based on the ability of catalase to oxidize hydrogen peroxide. The catalase levels in granulation wound tissue are measured according to the method of Aebi et al., (1983). Amount of H2O2 consumed is determined spectrophotometrically by recording absorbance at 570 nm.

iii) **Estimation of reduced glutathione (GSH):**

The “reduced glutathione levels” are measured according to the method of Beutler et al., (1963). The absorbance of yellow coloured complex is noted spectrophotometrically at 412 nm within 15 minutes. A standard curve was plotted using reduced form of glutathione and the results were expressed as nM/mg of protein.

iv) **Estimation of lipid peroxidation (LPO):**

The thiobarbituric acid reactive substance (TBARS) calculated according to the Niehius and Samuelson (1968) method is used for estimating lipid peroxide peroxidation in the homogeneous tissues. Eventually, the absorption of the protein is measured as the mole per mg of the protein at 532 nm (UV-1800 Spectrophotometer, Shimadzu, Japan). Standard curve (1-10Nmol) of 1.1.3,3- tetramethoxypropane (TMP) is used to calculate TBARS levels.
(MDA Concentrations).

v) Estimation of nitric oxide (NO):

The level of tissue nitrite is estimated by using the Griess reagent according to the method described by Green et al. (1982). The absorbance is measured spectrophotometrically at 540 nm. The standard absorbance from the standard curve is generated with sodium nitrite. The concentration of nitrite in the supernatant is determined from the standard curve (range: 5-50μM) using sodium nitrite and expressed in mM/mg of protein. The results were expressed as micromoles of nitrite per mg of protein.

vi) Estimation of inflammatory markers:

a) Estimation of myeloperoxidase (MPO) activity:

A Krawisz et al. method (1984) was taken to measure the activity of myeloperoxidase (MPO) measured as an index of neutrophile accumulation. The absorption change was measured with a 460 nm spectrophotometer. A unit of MPO activity was expressed as 1 μg H2O2 transferred by 1 unit of MPO activity at room temperature per minute. The formula for calculating MPO activity is:

\[
\text{MPO activity} = \frac{X}{\text{weight of the tissue}}
\]

Where \(X\) = 10 x change in absorbance per minute/volume of supernatant taken in ml.

3. Estimation of connective tissue parameters:

Approximately 250 mg of wet tissue was dried at 50°C for 24 h, and was weighed and kept in the glass stopper test tubes. To each tube containing 40 mg of the dried granulation tissue, 1 mL of 6 N HCl was added. The tubes were then kept on the boiling water bath for 24 h (12 h each day for two days) for hydrolysis. The hydrolysate was then cooled and excess of acid was neutralized by 10N NaOH using phenolphthalein as an indicator. The volume of the neutral hydrolysate was diluted to a concentration of 20 mg/mL with distilled water. The final hydrolysate was used for the estimation of “hydroxyproline, hexosamine, and hexuronic acid” following the standard curve prepared using the proper substrate.

a) Estimation of Hydroxyproline (HPR):

The measurement of color intensity was done against the blank at 540 nm. Hydroxyproline content in the tissue was estimated as per the standard curve prepared with standard “4-HydroxyL-proline (HiMedia Laboratories Pvt. Ltd., Mumbai, India)”, from 75 to 900 μg/0.3 mL using 3 mg/mL working solution.

b) Estimation of Hexosamine (HXA):

The measurement of color intensity was done against the blank at 530 nm. Hexosamine content of the samples was determined from the standard curve prepared with “D (+) glucosamine hydrochloride (HiMedia Laboratories Pvt. Ltd., Mumbai, India)”, from 5 to 50 μg/0.5 mL using 100 μg/mL working solution.

c) Estimation of Hexuronic Acid (HUA):
The measurement of color intensity was done against the blank at 530 nm. The content of the Hexuronic acid of the samples was determined by using the standard curve prepared with “D (+) Glucurono-6, 3- lactone (HiMedia Laboratories Pvt. Ltd., Mumbai, India)”, from 5 to 40 μg/0.5 mL using 100 μg/mL working solution.

4. **Histopathological evaluation:**

The rats were sacrificed and the granulation tissues were removed. These samples were then separately fixed in 10% formalin–saline, dehydrated through the “graded alcohol series” and cleared in the “xylene” and embedded in the “paraffin wax” (melting point 56°C). Serial sections of 5 μm were cut and stained with the “haematoxylin and eosin”. The sections were examined under light microscope and photomicrographs were taken.

i) **Statistical analysis:**

The results were expressed as the (±) standard error of means (S.E.M.). The data from the biochemical results are statistically analyzed using the “one-way ANOVA” followed by “Bonferonni’s post hoc-test” using Graph Pad Prism Version-5.0 software. The p-value < 0.001 was considered to be statistically significant.

III. **RESULTS:**

i) **Characterization of gel:**

The tailored topical gel successfully qualified the official and non official tests of topical gel formulations. The color of tailored gel was shown to be yellow (table 2). Moreover, the gel was homogenous in nature and without lumps. The pH of tailored gel and blank gel was found in the range of 7.60 ±0.01 and 7.4 ±0.03 respectively (table 2), as recommended for topical dermal products. The rheological analysis of prepared gel pointed out that the tailored gel samples followed the “Non-Newtonian flow”. The spreadability of prepared gel and blank gel was estimated to be 66.09 ±7.3 g.cm/sec and 87.60±18.49 g.cm/sec, respectively (table 2). Hence the spreadability of tailored gel samples was comparable to the blank gel formulation. The content of phenytoin sodium oil was estimated to be 4.86 gm per gm of tailored gel. The tailored gel should be stored at cool place or 20°C.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>CHARACTERISATION OF GEL</th>
<th>FORMULATED GEL</th>
<th>MARKETED GEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Yellow</td>
<td>White</td>
</tr>
<tr>
<td>2</td>
<td>Homogenity</td>
<td>Homogenous</td>
<td>Homogenous</td>
</tr>
<tr>
<td>3</td>
<td>pH</td>
<td>7.6 ±0.3</td>
<td>7.5 ±0.2</td>
</tr>
</tbody>
</table>
ii) Effect on % wound contraction and healed area:

Wounds of animals treated with STZ showed minimum wound contraction (21.07%). However, the diabetic animals treated with formulated as well as marketed gel showed significant wound contraction (76.69% and 84.72%). Marked increases in content of the granulation tissues of the treated groups suggest a higher rate of protein synthesis and cellular proliferation which resulted in better healing of diabetic wounds (Figure 1 & 2).

![Figure 1](image-url)
Effect on superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), lipid peroxidation (LPO) and nitric oxide (NO):

Administration of streptozotocin (50 mg/kg i.p.) significantly reduced SOD, CAT and GSH levels (Figure 3, 4 and 5) and enhanced LPO and NO levels (Figure 6 & 7) in comparison to normal untreated animals, indicating oxidative injury. Application of tailored gel (once a day, topically) for 16 days to rats significantly enhanced the levels of all three enzymes (SOD- 0.043±0.001 IU/mg of protein; CAT- 22.85±1.40 mU/mg of protein; GSH- 44.12±1.94 nM/mg of protein) in the wound tissue when compared to the blank gel treated (SOD- 0.019±0.001 IU/mg of protein; CAT- 8.88±1.05 mU/mg of protein; GSH- 20.17±2.37 nM/mg of protein) and non-treated rats (SOD- 0.019±0.001 IU/mg of protein; CAT- 7.27±0.72 mU/mg of protein; GSH- 19.94±1.63 nM/mg of protein). Similarly, application of formulated gel for 16 days significantly decreased the levels of both i.e. nitric oxide and lipid peroxidation (LPO- 0.039±0.004 nM/mg of protein; NO- 3.75±0.32 nM/mg of protein) when compared to STZ treated (LPO- 0.086±0.002 nM/mg of protein; NO- 8.58±0.39 nM/mg of protein) and blank gel treated rats (LPO- 0.082±0.003 nM/mg of protein; NO- 8.35±0.45 nM/mg of protein).
iv) **Effect on myeloperoxidase (MPO):**

Single dose of streptozotocin (50 mg/kg *i.p.* ) significantly enhanced MPO activity when compared to normal untreated and tailored gel treated rats (Figure 8). Application of tailored gel (once a day, topically) for 16 days significantly decreased STZ- induced myeloperoxidase activity in tailored gel treated MPO- 0.41±0.025 mU/mg of protein) in comparison to diabetic control rats (MPO- 0.84±0.04 mU/mg of protein) and blank gel treated rats (MPO- 0.83±0.03 mU/mg of protein).
v) **Effect on connective tissue parameters such as hydroxyproline (HPR), hexosamine (HXA) and hexuronic acid (HUA):**

Administration of streptozotocin (50 mg/kg *i.p.*) significantly reduced HPR, HXA and HUA levels when compared to normal rats and tailored gel treated rats (Figure 9, 10 & 11). The data depicted in study showed application of tailored gel (once a day, topically) 16 days enhanced the hydroxyproline, hexosamine and hexuronic acid contents of the wound tissue of the animals (HPR- 6.44±0.49 μg/mg of protein; HXA- 482.80±9.79 μg/mg of protein; HUA- 38.78±1.47 μg/mg of protein) when compared to the diabetic untreated rats (HPR- 2.89±0.24 μg/mg of protein; HXA- 140.00±15.39 μg/mg of protein; HUA- 13.99±0.80 μg/mg of protein) and blank gel treated rats (HPR- 3.18±0.20 μg/mg of protein; HXA- 150.03±9.98 μg/mg of protein; HUA- 13.15±1.05 μg/mg of protein). Additionally, the group of animals treated with marketed or formulated gel indicated increased collagen turnover.
vi) Effect on tissue histopathology:

H & E stained wound tissue of STZ treated animals showed marked histopathological changes such as incomplete epithelization with thin collagen fiber as well as less fibroblasts and blood capillaries at the wound site as compared to section of normal, marketed and formulated gel treated animals. However, gel treated section illustrated significantly elevated levels of collagen deposition with few fibroblast and more blood capillaries (Figure 12). Additionally, gel treated section showed complete epithelialization, high fibrous tissues with uniform collagen deposition at the wound site (Figure 13). It was more or less equal to the animals treated with marketed gel (at magnification X40).
IV. CONCLUSION:

From this study, we concluded that the dermal gel formulation which is used for diabetic wound healing enhances the cellular proliferation and collagen synthesis at the site of wound for promoting healing. This dermal formulation is used for the treatment and/or prevention of wounds in subjects with diabetes mellitus by using pharmacologically effective and pharmaceutically acceptable salts thereof. In addition, the differences in healing between a normal vascular wound and diabetic wound have been found. Therefore this report made it necessary to formulate a specific treatment in such conditions (diabetes).

REFERENCES