

1 **Effect of Topical Resveratrol Formulation on Healing of Experimental Full Thickness Wound in Diabetic Male**  
2 **Albino Rats: (Histological and Immunohistochemical study)**

3  
4 Wardah Abdullah Alasmari<sup>1</sup> Naser A. ElSawy<sup>2</sup>, Mohammed A.S. Abourehab<sup>3,4</sup>, Eman Mohamed Faruk<sup>5</sup>, Ashwaq  
5 Abdullah Alasmari<sup>6</sup>

6  
7 <sup>1</sup>Department of Anatomy Faculty of Medicine, Umm al Qura University, Saudi Arabia.<sup>2</sup>Department of Anatomy &  
8 Embryology Faculty of Medicine, Zagazig University, Egypt.<sup>3</sup>Department of Pharmaceutics, Faculty of Pharmacy,  
9 Umm Al-Qura University, KSA<sup>4</sup>Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia,  
10 Egypt<sup>5</sup>Department Histology and Cell Biology, Faculty of Medicine, Benha University, Egypt.<sup>6</sup> Faculty of Pharmacy,  
11 Umm Al-Qura University, KSA.

12  
13 Correspondence: Wardah Abdullah Alasmari University of Umm Al Qura, Department of Anatomy, Medicine KSA

14 E-Mail ([naser\\_elsawy@ymail.com](mailto:naser_elsawy@ymail.com)) telephone 0966540889314.

15 Conflict of interest: The authors declare no conflict of interest.

16  
17 **ABSTRACT:** Impaired healing of wounds is one of the most serious diabetes mellitus microvascular complications.  
18 Resveratrol (RSV), a natural polyphenol compound, has antioxidant, anti-inflammatory and antidiabetic activities. **Our**  
19 **study aimed to** evaluate the potential of topical resveratrol formulation on histological, immunohistochemical and anti-  
20 oxidative findings in experimentally induced full-thickness wound model in induced diabetic rats. Seventy - two albino  
21 rats were divided into two main groups: normal control group (18 rats) and diabetic group (54 rats) which divided  
22 equally into three sub groups: sub group (I) received plain ointment base and considered as control diabetic positive; sub  
23 group (II) received topical standard marketed product for wound healing containing  $\beta$ -Sitosterol (MEBO) (0.25% w/w)  
24 and considered as reference group, while sub group (III) received topical Resveratrol 0.5% ointment, and considered as  
25 test group. Wound surface area (W.S.A) and oxidative enzymes, Histological and Immunohistochemical studies were  
26 carried out for each group throughout treatment period (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> weeks). The obtained results showed that topical  
27 resveratrol ointment showed more potential healing effect on diabetic wound than the reference product. Resveratrol has  
28 percent wound contraction rate high than the wound closure rate than MEBO. Wound treated with resveratrol (group II)  
29 showed re-epithelialization and increased epidermal thickness compared with rats in control diabetic (group I). So,  
30 topical application of resveratrol ointment has potential effect on enhancing wound healing process in diabetic  
31 conditions, and this activity may be attributed – mainly – to its free-radical scavenging activity.

32 [Wardah Abdullah Alasmari, Naser A. ElSawy, Mohammed A.S. Abourehab, Eman Mohamed Faruk, Ashwaq Abdullah  
33 Alasmari. **Effect of Topical Resveratrol Formulation on Healing of Experimental Full Thickness Wound in**  
34 **Diabetic Male Albino Rats: (Histological and Immunohistochemical study).** *Life Sci J* 2026;23(1):26-43]. ISSN 1097-  
35 8135 (print); ISSN 2372-613X (online). <http://www.lifesciencesite.com>. 03. doi:[10.7537/marslsj230126.03](https://doi.org/10.7537/marslsj230126.03)

36  
37 **Keywords:** Resveratrol; Wound Healing; Diabetic Rats; topical formulation

38  
39 **1. Introduction:**

40 The prevalence of diabetes is increasing globally,  
41 where, it had been estimated that world prevalence of  
42 diabetes in 2013 was 8.3% (Guariguata et al. 2014).  
43 And chronic non healing foot ulcers was developed in  
44 15% of diabetic patients (Andrews et al.2015)  
45 Skin-wound healing process is sequential overlapping  
46 phases: hemostasis, inflammation, new tissue  
47 formation (proliferation) and remodeling (Janis et  
48 al.2010). An aberrant course of inflammation has been  
49 demonstrated in wounds of diabetic patients  
50 (Gallagher et al. 2015). Prolonged hyperglycemia has  
51 been estimated to decrease the healing rate of foot  
52 ulcers (Christman et al. 2011). Therefore, delayed  
53 healing rate of wound is one of the long-term  
54 complications of diabetes (Alavi et al.2014 &  
55 Yamagishi et al .2015). Which is mainly mediated by

56 methylglyoxal (Duscher et al.2015). In a study on  
57 diabetic animal models of impaired wound healing, it  
58 was found that the wound healing process was  
59 accelerated by suppressing reactive oxygen stress  
60 (Thiem et al. 2003). One of the pathogenic factor in  
61 delayed diabetic wound complications is Oxidative  
62 stress (Naito et al.2014). Delayed wound healing in  
63 diabetes was demonstrated to be mainly, associated  
64 with hyperglycemia, over-expression of inflammatory  
65 mediators (cytokines), oxidative stress, decreased  
66 synthesis of collagen, and reduced angiogenesis in  
67 addition to the infections of the wounds (Gautam and  
68 Goel .2013), where, different studies proved that ROS  
69 may promote the secretion of different factors as  
70 epidermal growth factor (EGF), fibroblast basic  
71 growth factors, and G protein-coupled receptors (Arul  
72 et al .2012) But, at higher concentrations of ROS,

73 severe tissue damage may be induced and in some  
74 cases it may lead to malignant transformation (Lee et  
75 al .1998& Roche et al.2009). Cells develop cellular  
76 defensive mechanisms which maintain balanced redox  
77 state, these mechanisms include the antioxidant  
78 enzymes superoxide dismutase (SOD), catalase (CAT),  
79 glutathione peroxidase, glutathione reductase, in  
80 addition to the other endogenous free radical  
81 scavengers as GSH (Sarsour et al. 2009& Sen .2009  
82 and Paul et al.2009).

83 Resveratrol (3,5,4'-trihydroxystilbene), a natural  
84 polyphenolic flavonoid compound found in many  
85 plants, is widely distributed in edible fruits and  
86 vegetables, including red grapes. Resveratrol showed  
87 antioxidant, anti-inflammatory, anti-hyperlipidemic,  
88 and antitumor activities (Amri et al.2012). Resveratrol  
89 has been demonstrated to prevent cell damage caused  
90 by free radicals by virtue of its strong antioxidant  
91 properties, as well as by inhibiting cell death (Møller  
92 Poulsen et al.2015 &Kapetanovic et al. 2011).  
93 Moreover, resveratrol appears to protect against  
94 diabetes (Balata et al.2016). As it has been reported  
95 that resveratrol reduces hyperglycemia in humans  
96 through the mechanism of increasing insulin  
97 sensitivity. Resveratrol was demonstrated to have the  
98 ability to enhance stimulation of glucose uptake in the  
99 absence of insulin (Hausenblas et al.2015).

100 Recent study has demonstrated that oral resveratrol  
101 supplementation enhances the healing of the foot ulcer  
102 and decreases plasma fibrinogen level in type 2  
103 diabetic patients (Bashmakov et al.2014).

104 Due to its hydrophobicity, resveratrol is poorly  
105 absorbed following oral administration, Because of its  
106 low bioavailability and extensive rapid first-pass  
107 metabolism (Kristl et al.2009), resveratrol is a suitable  
108 candidate for topical applications. No previous studies  
109 had been carried out to evaluate the potential effects  
110 of topical formulations of resveratrol on wound in  
111 streptozotocin-induced diabetic rats, therefore, so the  
112 present work designed to evaluate the wound healing  
113 and antioxidant potential of topical resveratrol  
114 formulation in diabetic rats compared with a standard  
115 marketed reference formulation.

## 116 **2. Materials and Methods:**

### 117 **2.1. Materials**

118 Resveratrol was purchased from Xian Lukee Bio-Tech  
119 Co., Ltd., (Xi ' an, Shaanxi Sheng, China),  
120 Streptozotocin (STZ) was purchased from Sigma-  
121 Aldrich Chemical Co. (St. Louis, MO, USA). White  
122 soft paraffin, hard paraffin, liquid paraffin, Cetostearyl  
123 alcohol and bees wax were purchased from (BDF,  
124 UK), MEBO (Batch No. 0202, Gulf Pharmaceutical  
125 Industries, Ras Al Khaimah, U.A.E.), other solvents  
126 and materials were of analytical grades and used  
127 without further purification.

### 128 **2.2. Animals**

129 Ninety-four adult male Albino rats (150-180 gm).  
130 Animals were obtained from the animal house of Faculty  
131 of Medicine - Umm Al Qura University. Our experiment  
132 was done according to the International Principles of  
133 Laboratory Animal Research. Animals were individually  
134 housed in well-ventilated stainless steel cages in a  
135 controlled temperature (23-25°C) and relative humidity of  
136 45-50 % with a light dark cycles of 10 and 14 h,  
137 respectively. Animals were kept in this standard conditions  
138 as an acclimatization period to the laboratory environment  
139 for one week prior to the study. During the entire period of  
140 the study, animals were provided with standard rodent  
141 pellet diet and tap water *ad libitum*.

142 Rats were divided into two groups: normal control group  
143 (18 rats) and diabetic group (54 rats) which divided  
144 equally (18 rats each) into three sub groups sub group (I)  
145 received simple ointment base and considered as control  
146 positive; sub group (II) received topical standard marketed  
147 product for wound healing containing  $\beta$ -Sitosterol (**MEBO**)  
148 0.25% and considered as reference group, while sub group  
149 (III) received topical Resveratrol 0.5% ointment, and  
150 considered as test group.

### 151 **2.3. Experimental**

#### 152 **2.3.1. Formulation of Resveratrol Ointment:**

153 Resveratrol was prepared with simple ointment base  
154 B.P. in a concentration of (0.5 % w/w) by fusion  
155 method. The prepared ointment formulation was kept  
156 in a tightly closed glass container used for topical  
157 application and stored at 4 °C.

158 High reputation marketed ointment formulation for  
159 wound healing, 0.25% w/w  $\beta$ -Sitosterol Ointment  
160 USP was selected as a reference for comparison.

#### 161 **2.3.2. Dermal Irritation Study:**

162 A primary skin irritation test was done on animals to  
163 show any skin allergy that happen from topical  
164 application of ointment. Animals that showed  
165 preexisting skin irritation or abnormalities was  
166 excluded from the study. A suitable amount of the  
167 formula was applied to 6 cm<sup>2</sup> intact area on each  
168 animal which was then caged separately. After 4 h of  
169 ointment application, the amount of ointment was  
170 removed and the sites of ointment application  
171 (Rogiers and Pauwels. 2008).

#### 172 **2.3.3. Induction of Diabetes:**

173 Experimental diabetes was induced in animals by  
174 a single intraperitoneal injection of streptozotocin (80  
175 mg/kg body weight) freshly prepared in Citrate Buffer  
176 (0.1 M, pH 4.5) after overnight fasting. All the  
177 animals were given 5% glucose solution to avoid  
178 sudden post-injection hypoglycemia- first phase  
179 hypoglycemic condition (Kumar et al .2017). Blood  
180 samples were obtained from the rat tail, 72 h after the  
181 injection for estimation of blood glucose level using  
182 Glucometer. The animals showing stable blood

183 glucose level >250 mg/dL were considered diabetic  
184 then full-thickness excision wound model was  
185 persistent. Blood glucose level was estimate two hours  
186 after creation of the wounds and after treatment every  
187 1,2, 3 weeks (Prasad et al.2010).

#### 188 **2.3.4. Wound Creation:**

189 The upper back of rats was shaved by electrical  
190 small animal clipper, and the animals were examined  
191 for any skin abnormalities. The shaved areas were  
192 disinfected with 70% alcohol, then, rats were  
193 anesthetized by the open mask method with  
194 anaesthetic ether, then 2x2.5 cm wound area was  
195 created (a full-thickness excisional wound of circular  
196 area was performed, as (500 mm<sup>2</sup>) wound area was  
197 excised from the back of all rats by surgical blade  
198 (Pandian et al.2013). The same researcher performed  
199 all surgical procedures. Animals were strictly  
200 observed for any signs of infection, and those arise  
201 any signs of infection were excluded from the study  
202 protocol.

#### 203 **2.3.5. Experiment Design (Protocol):**

204 After creation of wound, rats of diabetic group were  
205 randomly divided into three groups (eighteen rats each)  
206 (Harish et al.2008):

207 Group (IIa) received plain ointment base and  
208 considered as control positive;

209 Group (IIb) received topical standard marketed  
210 product for wound healing containing  $\beta$ -Sitosterol  
211 (MEBO) and considered as reference group.

212 Group (IIb) received topical Resveratrol 0.5%  
213 ointment and considered as test group.

#### 214 **2.3.5.1. Treatment:**

215 As wound dimensions were approximately (20 mm x  
216 25 mm x 2 mm) so amount of different ointment  
217 treatments about (1.5 cm<sup>3</sup>) used to cover wound cavity  
218 and boundaries of wound through using a sterile  
219 syringe; this amount was fixed for all treatments  
220 throughout all time of experiment. The treatment  
221 started in each group just 2 hours after wound creation.  
222 For all animals, the treatments were applied once daily  
223 with topical application for all treatments. Periodically,  
224 we follow the wound healing by the biochemical and  
225 histological parameters in which the tissue was  
226 obtained at 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> day of wound creation.

#### 227 **2.3.5.2. Wound Surface area, percent wound 228 contraction and epithelialization time measurements:**

229 By placing a transparent tracing paper over the wound  
230 we measure the wound area then counted the squares  
231 and the area was recorded (Al Bayaty et al.2010).

232 The percentage of wound contraction was calculated  
233 using the following formula:

$$234 \text{ Wound Area Contraction (\%)} = [(A_0 - A_t)/A_0] * 100$$

235 Where: A<sub>0</sub>: the initial wound surface area

236 A<sub>t</sub>: the wound surface area at time t

237 For each rat, measurement of wound area was  
238 repeated three times at the end of (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup>  
239 weeks) time intervals of treatment. Additionally,  
240 wounds were photographed by digital camera at these  
241 intervals. At the end of the specified intervals (1<sup>st</sup>, 2<sup>nd</sup>,  
242 and 3<sup>rd</sup> weeks), 8 rats from each group were randomly  
243 selected and sacrificed then the skins of wound areas  
244 were dissected and isolated for histological and  
245 immunohistochemical examination.

#### 246 **2.3.5.3. Enzymatic and non-enzymatic antioxidant assay**

247 From the wound area, the small part of granuloma  
248 tissue was used for antioxidant assay. The granuloma  
249 tissues were homogenized in phosphate buffer (pH 7.0)  
250 and centrifuged under cold condition. The clear  
251 supernatant was taken to assay of antioxidants level.  
252 Catalase activity was estimated according to the  
253 method described by (Woessner et al.1961).  
254 Superoxide dismutase (SOD) activity was measured,  
255 according to the method of (Beers et al.1952). Total  
256 reduced glutathione (GSH) level was determined in  
257 tissues homogenates, according to the method  
258 described by (Misra et al.1972). For Lipid  
259 peroxidation analysis, we used the method described  
260 by (Moron et al.1972).

#### 261 **2.3.5.4. Histological study:**

262 The animals were anaesthetized by inhalation using  
263 diethyl ether and sacrificed, skin specimens were  
264 taken from the wound area with the surrounding  
265 normal skin. Skin samples were collected and cleaned  
266 properly from connective tissue and stored in 10%  
267 neutral buffered formaldehyde. Then tissues were  
268 dehydrated, cleared in xylol, and then embedded in  
269 paraffin blocks and sectioned (4~5  $\mu$ m thickness)  
270 were prepared using microtome. Finally, skin sections  
271 were stained with haematoxylin and eosin (H&E)  
272 stain and Masson's trichrome. Then examined under  
273 light microscope. Sections were assessed for, collagen  
274 maturation, angiogenesis and epithelialization  
275 (Bancroft and Layton,2013).

#### 276 **2.3.5.5. Immunohistochemically study**

277 For detection of the cytokeratin intermediate filaments  
278 on keratinocytes. The deparaffinized and rehydrated  
279 Sections were treated with 0.01m citrate buffer (pH  
280 6.0) for 10min and heated twice in a microwave oven  
281 to unmask antigens. To abolish endogenous  
282 peroxidase activity sections were incubated in 0.3%  
283 hydrogen peroxide for 30min Slides were incubated  
284 with the primary antibody (1: 1500 monoclonal mouse  
285 anticytokeratin) (Dako Biotechnology, Denmark) for  
286 2h, then washing, then incubated with biotinylated  
287 secondary antibodies (ABC kit, 1: 1200) and then with  
288 the avidin-biotin complex. Slides were counterstained  
289 for 1 min with Mayer's hematoxylin, and dehydration,  
290 clearing, and mounting were carried out to be  
291 examined under light microscope. Keratinocytes

292 containing cytokeratin appeared brown, whereas  
293 nuclei appeared blue in color ( Shivananda et al.2006).

#### 294 **2.3.5.6. Morphometric study**

295 a. Epidermal total thickness and the number of  
296 fibroblasts were estimated in H&E-stained sections. b.  
297 The area percentage of the collagen fibers in the  
298 dermis was estimated in Masson's trichrome-stained  
299 sections. c. cytokeratin intermediate filaments  
300 expression. All above were quantified in in five non  
301 overlapping fields from ten different sections for each  
302 group at  $\times 400$ . by using Image-Pro Plus program  
303 version 6.0 (Media Cybernetics Inc., Bethesda,  
304 Maryland, USA) in the Histology Department, Faculty  
305 of Medicine, Benha University which consisting of a  
306 microscope equipped with a high resolution video  
307 camera.

#### 308 **2.3.5.7 Statistical Analysis**

309 Data were collected and analyzed as means  $\pm$  SD for  
310 eight rats in each group. Statistical comparison was  
311 performed using one-way analysis of variance  
312 (ANOVA) followed by Tukey's Multiple Comparison  
313 test using SPSS statistical version 16.0 software  
314 package (SPSS® Inc., USA). Values were considered  
315 statistically significant when  $p < 0.05$ .

### 316 **RESULTS**

#### 317 **Dermal Irritation Study:**

318 Showed negative signs of dermal reaction towards the  
319 applied formulation, as, very slight erythema was  
320 observed only after 24 hrs. of application, and this  
321 erythema was disappeared after 48 hrs. No edema was  
322 observed in any treated rats

#### 323 **Wound Surface Area (WSA):**

324 The representative photographs of the wound for  
325 treated animals of each group taken at different time  
326 intervals are given in Figure (1).

327 The effect of different treatments on excision wound  
328 model in diabetic rats are given in Table (1) and  
329 Figure (2). The obtained results revealed a significant  
330 reduction in wound surface area during treatment  
331 period (21 days) within all three groups. The topical  
332 application of resveratrol ointment significantly  
333 ( $p < 0.05$ ) decreased the wound surface area compared  
334 to the control diabetic group. After the first week of  
335 treatment, the mean value of WSA in test group was  
336 about ( $374.81 \pm 4.13$ ) and it was ( $259.21 \pm 5.78$ ) for  
337 reference group while it was ( $244.18 \pm 4.67$ ) for  
338 control group. While, at the end of the experiment,  
339 after 21 days of treatment, the mean value of WSA in  
340 test group was about ( $00.00 \pm 0.00$ ) and it was ( $05.39$   
341  $\pm 1.61$ ) for reference group while it was ( $152.60 \pm$   
342  $7.11$ ) for control group. From the results, it is clear  
343 that only rats treated with topical resveratrol  
344 formulation showed complete healing of the wound  
345 (WSA =  $00.00$ ) at the end of experiment.

#### 346 **Rate of healing studies:**

347 The results of wound healing are shown in Table (2)  
348 and Figure (3). The results demonstrate that, the  
349 percentage of healing in diabetic group was  
350 significantly lower than those of treated groups.

351 After the first week of treatment, the mean value of  
352 healing rate in test group was about ( $52.22 \pm 0.91$ ), and  
353 it was ( $52.52 \pm 2.07$ ) for reference group while it was  
354 ( $26.05 \pm 0.81$ ) for control group. Analysis of results by  
355 one-way ANOVA revealed that there was statistically  
356 significant difference between group the test and  
357 reference groups compared to the control group as ( $P$   
358 values  $< 0.05$ , but the difference was not significant  
359 between the test and the reference group ( $p > 0.05$ ).

360 At the end of the experiment, after 21 days, rats  
361 treated either with topical resveratrol or reference  
362 topical formulation showed nearly complete healing  
363 of the wound with healing rate about ( $100.0\% \pm 0.00$ )  
364 and ( $98.94 \pm 0.32$ ) respectively, while the healing rate  
365 mean for animals in the control group was  
366 ( $69.89 \pm 1.40$ ). These results showed that wound  
367 closure and healing were accelerated in diabetic rats  
368 treated topically with resveratrol and the reference  
369 formulation compared with control diabetic rats.

370 The mean period of epithelialization in control  
371 diabetic rats cannot be calculated as the healing was  
372 not complete, where it was ( $19.53 \pm 0.61$ ) and  
373 ( $18.63 \pm 0.54$ ) days for animals in reference and test  
374 groups respectively. As results showed in Table (2),  
375 the difference was not significant between the  
376 reference and the test group ( $p > 0.05$ ).

#### 377 **Biochemical analysis**

##### 378 **Blood Glucose Level:**

379 Blood glucose level was determined at the time of  
380 creation of the wounds and after treatment every 1,2  
381 and 3 weeks. The obtained results for blood glucose  
382 level throughout the experiment period are shown in  
383 Table (3). The results revealed that the diabetic rats in  
384 the three groups exhibited significant elevation  
385 ( $p < 0.001$ ) in the glucose levels compared to the  
386 normal non-diabetic rats. Results also revealed that,  
387 blood glucose levels were not significantly ( $p > 0.05$ )  
388 decreased upon treatment with either topical  
389 resveratrol or the reference drug compared to control  
390 group.

##### 391 **-Anti-oxidant activity:**

392 The enzymatic assays for antioxidants during wound  
393 healing processes in skin tissues are shown in table (4)  
394 and figures (4-7).

395 The wound tissue from diabetic rats showed  
396 decreased extracellular SOD activity as compared to  
397 non-diabetic rats. Treatment of diabetic rats with  
398 resveratrol or the reference drug showed significant  
399 ( $p < 0.05$ ) increase in the SOD levels. Similar results  
400 were obtained for both CAT and GSH parameters.  
401 On the other hand, diabetic non-treated rats, showed

402 elevated levels of LPO, which is a marker for lipid  
403 peroxidation, compared to normal control non-  
404 diabetic rats. Treatment of diabetic rats with  
405 resveratrol or the reference drug showed significant  
406 ( $p < 0.05$ ) decrease in the LPO levels. The results for  
407 antioxidant activity of resveratrol indicate potent  
408 antioxidant activity through decreasing lipid  
409 peroxidation, and increasing the levels of reduced  
410 glutathione (GSH), SOD and CAT activities. This  
411 validates the potent wound healing activity of  
412 resveratrol.

#### 413 **Histological results:**

##### 414 **Hematoxylin and eosin stain:**

415 Group I: Sections of control un-wounded skin (-ve  
416 control G) revealed a normal histological structure of  
417 the skin of two basic layers: the epidermis and the  
418 dermis. The epidermis appeared stratified squamous  
419 keratinized epithelium resting on a wavy basement  
420 membrane with four distinct cell layers: stratum  
421 basale (columnar cells), stratum spinosum (polyhedral  
422 cells), stratum granulosum, plus and on the top there is  
423 superficial noncellular acidophilic horny layer  
424 (stratum corneum). The epidermal-dermal junction  
425 showed many dermal papillae. The underlying dermal  
426 layer contained thin superficial papillary layer with  
427 connective tissue and thick deep reticular layer with  
428 dense connective tissues and collagen fibers, the  
429 epidermal appendages (hair follicles & sebaceous  
430 gland) were seen in dermis (Fig.8 A,B). While in  
431 diabetic group without any treatment (control  
432 positive), skin specimens showed loss of covering  
433 epidermal layer (wound area after 7 days) (Fig.9A).  
434 There is granulation tissue entangling mononuclear  
435 inflammatory cells and congested blood vessels filling  
436 the wound gap in the untreated diabetic group after  
437 2 weeks of wound (Fig.9B). While in three weeks'  
438 post wounding the wound showing loss of the  
439 epidermal bridges and presence of inflammatory cells  
440 and keratocytes migration (Fig.9C). In the test group  
441 (treated with resveratrol) there is an epidermal tongue  
442 seen growing from the wound with scab and decrease  
443 of granulation tissue in 1<sup>st</sup> and 2<sup>nd</sup> week and was  
444 appeared more vascular with deposition of collagen  
445 fibers and presence of hair follicles (Fig. 10 A, B) the  
446 wound totally covered with epidermis and apparent  
447 normal after 3 weeks of wound (F.10 C). While in  
448 reference drug treated group the wound totally  
449 uncovered with epidermis in 1<sup>st</sup> week (Fig.11A) and  
450 after 2<sup>nd</sup> and 3<sup>rd</sup> weeks the wound covered with very  
451 thin epidermis that showed vacuolated and  
452 degenerated keratocytes cells with little deposition of  
453 collagen fibers in dermal layer in contrast to the  
454 control normal skin (Fig.11B, C).

##### 455 **-Masson's trichrome stain:**

456 In the specimen of the normal skin of control group  
457 revealed in the papillary layer of the dermis thin  
458 interlacing bundles of collagen fibers, while coarse  
459 collagen fibers in the reticular layer (Fig. 12A). In  
460 diabetic untreated group, the collagen fibers in the  
461 dermis were disorganized at the wound edge. (Fig.  
462 12B). In the test resveratrol group an apparent  
463 increase in collagen content in the papillary dermis  
464 was seen (Fig. 12C), while in the reference group,  
465 there are less collagen fiber in the dermis compared  
466 with the control skin (Fig. 12D). There was a  
467 significant increase in the collagen content of the  
468 papillary dermis in the reference group wound after 7  
469 days compared with the untreated diabetic wound at  
470 the same periods (Table 5).

##### 471 **-Immunohistochemically staining:**

472 There were normal apparent of  
473 immunohistochemically staining of cytokeratin  
474 intermediate filaments in the keratocytes cells of the  
475 epidermis (Fig.13A). In diabetic control group without  
476 any treatment, epidermal cells showed loss of  
477 immunohistochemically staining of cytokeratin  
478 intermediate filaments (Fig.13B), whereas in the test  
479 group the immunohistochemically staining of  
480 cytokeratin intermediate filaments appeared increase  
481 as in normal skin (Fig. 13C). There is less the  
482 immunohistochemically staining of cytokeratin  
483 intermediate filaments in the epidermal cells  
484 compared with the control skin (Fig. 13D).

##### 485 **-Morphometric results:**

486 a. The thickness of the epidermis showed a significant  
487 decrease in the un treated diabetic group ( $P < 0.01$ )  
488 whereas that of the test group showed a significant  
489 increase in the untreated diabetic group ( $P < 0.01$ )  
490 with a non-significant change ( $P > 0.05$ ) in reference  
491 group (Table 5).

492 b. The area percentage of collagen fibers was  
493 significantly decreased ( $P < 0.01$ ) in the untreated  
494 diabetic group (group IIA) and not significantly  
495 changed ( $P > 0.05$ ) in the test group (group IIB) as  
496 compared with that of the control group (Table 4).  
497 The mean area percentage of collagen fibers was  
498  $43.32 \pm 1.12\%$  in the control group compared with  
499  $40.23 \pm 1.15\%$  in the test group, which was  
500 statistically significant ( $P=0.02$ ). (Table 6).

501 c. The area percentages of cytokeratin intermediate  
502 filaments expression was  $18.72 \pm 0.3\%$  in the  
503 resveratrol -treated group compared with  $20.77 \pm 0.$   
504  $33\%$  in the control-ve group. The difference in area  
505 percentages of cytokeratin intermediate filaments  
506 expression between the two groups was statistically  
507 significant ( $P < 0.001$ ). The area percentage of  
508 cytokeratin intermediate filaments expression was  $16.$   
509  $13 \pm 0.56\%$  in the Mebo-treated group compared with  
510  $20.77 \pm 0. 33\%$  control -ve group. Expression of

511 cytokeratin intermediate filaments was statistically  
512 significant in the control +ve group compared with the  
513 control- ve group ( $P < 0.001$ ) (Table 7).

#### **514 Discussion**

515 Wound healing is the process in which a  
516 affected tissue is restored its normal structure and it  
517 depends mainly on both; the repairing ability and the  
518 general health state of the tissue (Kasuya et al.2014).  
519 In diabetic wound conditions, impairment of healing  
520 occurs as a result of ischemia, excessive production  
521 of reactive oxygen species (ROS), and inflammatory  
522 mediators (Santram and Abhay.2013). High blood  
523 glucose levels may lead to occlusion of capillary  
524 vessels as a consequence for endothelial damage as  
525 well as hyperglycemia-induced leukocyte dysfunction  
526 and phagocytosis (Gowda et al .2013). In  
527 experimental diabetic wound, healing process is  
528 characterized by delayed cellular infiltration,  
529 impaired granulation tissue formation, decreased  
530 collagen deposition, and increased epithelialization  
531 time (Vidinskýet al. 2006).

532 In our study an excision a full-thickness  
533 wound model was employed for the assessment of  
534 wound healing effect of topical formulation of  
535 resveratrol. In this study, the healing of skin wounds  
536 was significantly delayed in diabetic rats, and this  
537 may be attributed to the high blood glucose levels,  
538 where, it delayed cellular infiltration through  
539 abnormal physiological response. In the present study,  
540 there is delayed wound healing in untreated diabetic  
541 rats when compared with treated animals as naked  
542 eye examination of the wound surface area after 7  
543 and 14 days revealed that incomplete closure of the  
544 wound. The epithelization time is an important factor  
545 that can be used to assess the wound healing process.  
546 In the diabetic control animals, epithelial  
547 reorganization process was very slow compared to  
548 that of the treated animals. The obtained results show  
549 that topical application of resveratrol clearly  
550 enhanced wound healing from the first stage, and this  
551 effect can be attributed to its known angiogenic and  
552 mitogenic potential.

553 Also, histological examination of tissue samples  
554 showed loss of the epidermis and dermis which  
555 suggested a delayed wound healing stated by some  
556 authors (Tse et al.2003) and the wounded areas of the  
557 untreated group (group I) did not show complete  
558 closure till the end of experiment with presence of  
559 wound scab and keratocytes migration. These above  
560 data were observed by several studies that explained  
561 the presence of scab filled with inflammatory cells  
562 and keratinocyte migration beneath the scab from  
563 thickened epidermis on the second day, but the whole  
564 wound was covered only after 14 days (Vidinskýet al.  
565 2006).

566 The percentage of collagen fibers of the same group  
567 increased progressively in an irregular manner and  
568 this results are in agreement with results obtained by  
569 Gal et al. 2006, who noticed an increase in  
570 proteoglycans, glycoproteins, and collagen  
571 synthesized by fibroblasts one week after wounding  
572 (Medina et al.2005). In diabetic patient the delayed  
573 healing occurs as result of poor neovascularization,  
574 decreased growth factors, with failure of migration of  
575 keratocytes and so failure of reepithelialization and  
576 wound closure (Asai et al.2006). While other study  
577 revealed decreased cellular infiltration and delayed  
578 collagen fiber formation and reorganization  
579 (Pattanayaka et al.2008).

580 Our study, showed that the wound of resveratrol  
581 treated rats had highly improvement effects in wound  
582 healing as increase granulation tissue formation,  
583 angiogenesis, epithelialization, keratinization,  
584 restoration of hair follicles, and collagen fibers  
585 deposition with prominent increase in cytokeratin  
586 immunohistochemical staining. This results are in  
587 agreement with results obtained in a previous study  
588 which demonstrated that the thickness of the newly  
589 formed epidermis was not similar to intact epidermis  
590 (Alfars,2009). In our study there are regularly  
591 appearing of collagen fibers in the dermis and this as  
592 the results of other experimental studies (Rastogi et  
593 al.2006). Our study revealed that there is mild  
594 positive reaction for cytokeratin  
595 immunohistochemical staining in the skin of the  
596 untreated diabetic group and this is due decrease  
597 kertaocytes formation as they produce cytokeratins  
598 for protection and providing mechanical support to  
599 the cells (Maruyama et al.2007). The mechanical  
600 support of keratins is the ability to flexible easily to  
601 resist as well as softness, strength, self-repair  
602 (Wagner et al.2007).

603 Flavonoids have astringent and antimicrobial  
604 property which lead to accelerate the wound healing  
605 process and so wound closure (Hesham et al.2014).  
606 Oxidative stress, originated from over production of  
607 reactive oxygen species (ROS), may lead to  
608 cytotoxicity and delayed wound healing, therefore,  
609 elimination of ROS may be a key strategy for chronic  
610 wounds healing (Mikhailchik et al.2006). The  
611 antioxidants have been reported to hasten wound  
612 healing by decreasing the free radicals (Weindl et  
613 al.2004). Results in this study on the antioxidants  
614 revealed that topical resveratrol had significant  
615 antioxidant activity which is directly expressed as  
616 decrease in inflammation and oxidative damage and  
617 enhancing the healing process.

618 Flavonoids have been estimated to exert several  
619 biological effects on collagen synthesis process, as  
620 enhancing collagen synthesis, promoting the collagen

621 fibers cross-linking, decreasing soluble collagen  
622 degradation in addition to acceleration of the  
623 conversion of soluble collagen into insoluble collagen  
624 (Inan et al.2006). Collagen- major protein of the  
625 extracellular matrix- imparts strength to wound.  
626 Breakdown of collagen results in free hydroxyproline  
627 and its peptides, therefore, hydroxyproline content  
628 has been used as an index for collagen turnover. High  
629 content of collagen in the excision wound is a clear  
630 indicator for faster collagen turnover which  
631 accelerates wound healing process (Medina et  
632 al.2005).

633 Resveratrol has been demonstrated to have a  
634 significant enhancing effect on skin fibroblast  
635 proliferation in addition to its anti-collagenase  
636 activity. It also promotes maturation of mesenchymal  
637 stem cells in adipose tissue in a dose-dependent  
638 scheme (Giardina et al. 2010). Furthermore,  
639 flavonoids are known to decrease lipid peroxidation  
640 through improving vascularity and decreasing the  
641 onset of cell necrosis. Therefore, drugs which reduce  
642 lipid peroxidation are thought to enhance the collagen  
643 fibrils viability by increasing the collagen fibers  
644 strength, increasing the circulation and preventing the  
645 cell damage (Das and Kuntal.2013).

646 The main valuable pharmacological effect of  
647 resveratrol which may be directly affect wound  
648 healing is attributed to its inhibitory activity on  
649 matrix metalloproteinases (MMPs) which play the  
650 key role in diabetic wound healing, where, these  
651 enzymes promote collagen and other extracellular  
652 matrix of the skin degradation (Lobmannet al.2002).  
653 High level of MMP-8 and MMP-9 in diabetic wounds  
654 revealed a negative sign of wound healing process  
655 (Muller et al.2008).

656 The obtained data from biochemical,  
657 histopathological and immunohistochemical studies  
658 in the present study are in line with the regular  
659 findings of normal wound healing phases, and this  
660 results are similar to that obtained from other studies  
661 using different natural constituents for treatment of  
662 excision wound in diabetic rats (Pillai et al.2010)

### 663 Conclusion

664 In conclusion, the observation and results obtained  
665 from the present study indicated that topical  
666 resveratrol possesses a powerful modulating effects  
667 on wound healing, as it promotes wound healing by  
668 reepithelization and regular collagen fibers synthesis.  
669 This study confirms the promising wound healing  
670 activity of topical resveratrol in diabetic animals, and  
671 deserve for more investigations on the scale of  
672 cellular level in future and further studies may be  
673 required to determine the underlying mechanisms  
674 through which resveratrol affects wound healing and

675 other body system to establish resveratrol as a  
676 potential candidate for clinical settings.

### 677 Acknowledgements

678 The authors would like to thank Institute of Scientific  
679 Research and Revival of Islamic Heritage at Umm  
680 Al-Qura University, Makkah, KSA (Project No:  
681 43509022) for the financial support.

### 683 Disclosure and Conflicts Statement

684 The author declares no conflicts of interest.

685

### 686 References

687

- 688 - Al Bayaty, M. Abdulla, M. I. Abu Hassan M.  
689 Masaud. Wound healing potential by hyaluronate gel  
690 in streptozotocin-induced diabetic rats. Scientific  
691 Research and Essays, 2010; 5(18): 2756-2760.  
692 - Alavi A, Sibbald RG, Mayer D, Goodman L, Botros  
693 M, Armstrong DG, Woo K, Boeni T, Ayello EA and  
694 Kirsner RS. Diabetic foot ulcers: Part I.  
695 Pathophysiology and prevention. J Am Acad  
696 Dermatol 2014; 70(1):1.e1-1.e18.  
697 - Alfars, A. Histological study of early events during  
698 wound healing in rabbits. Bas.J.Vet.Res . (2009); 8  
699 (1):130-137.  
700 - Amri A, Chaumeil JC, Sfar S, Charrueau C.  
701 Administration of resveratrol: What formulation  
702 solutions to bioavailability limitations? J Control  
703 Release. 2012; 158:182–193.  
704 - Andrews K.L., Houdek M.T. and Kiemele L.J.  
705 Wound management of chronic diabetic foot ulcers:  
706 From the basics to regenerative medicine. Prosthetics  
707 and Orthotics International 2015; 39(1) 29–39.  
708 - Arul, V ., Masilamouni, J.K., Jesudason, E.P., Jaji, P.J.,  
709 Inayathuliah, M., Dicky, D.G., Vignesh, I.S.,  
710 Jayakumar, R. Glucose Oxidase Incorporated Collagen  
711 Matrices for Dermal Wound Repair in Diabetic Rat  
712 Models: A Biochemical Study. Journal of biomaterials  
713 Volume 26 —2012: 917-938  
714 - Asai J, Takenaka H, Katoh N and Kishimoto S.  
715 Dibutyryl cAMP influences endothelial progenitor cell  
716 recruitment during wound neovascularisation. J  
717 Invest Dermatol 2006; 126:1159–1167.  
718 - Balata G.F., Essa A.E., AShamardl H, Zaidan S.H.,  
719 Abourehab M.A.S. Self-emulsifying drug delivery  
720 systems as a tool to improve solubility and  
721 bioavailability of resveratrol. Drug Design,  
722 Development and Therapy 2016; 10: 117–128.  
723 - Bancroft JD and Layton C: The Hematoxylin and  
724 eosin. In: Suvarna SK, Layton C and Bancroft JD  
725 editors. Theory & Practice of histological  
726 techniques. 7th ed., Churchill Livingstone of El Sevier.  
727 Philadelphia. Ch. 10 and 11. 2013; 172 – 214  
728 - Bashmakov Y.K., Assaad-Khalil S.H., Abou Seif  
729 M., Udumyan R., Megallaa M., H. Rohoma

- 730 K.H., Zeitoun M., and Petyaev I.M. Clinical Study: 785  
 731 Resveratrol Promotes Foot Ulcer Size Reduction in 786  
 732 Type 2 Diabetes Patients. Volume 2014 (2014), 787  
 733 Article ID 816307, 8 pages 788  
 734 - Beers, Jr. R.F., and Sizer, I.W. A 789  
 735 Spectrophotometric Method for Measuring the 790  
 736 Breakdown of Hydrogen Peroxide by Catalase, *J. Biol.* 791  
 737 *Chem.*, 1952; 195: 133–140. 792  
 738 - Christman AL, Selvin E, Margolis DJ, Lazarus GS 793  
 739 and Garza LA. Hemoglobin A1c Predicts Healing 794  
 740 Rate in Diabetic Wounds. *J Invest Dermatol* 2011; 795  
 741 45(2):1870-90. 796  
 742 - Das K and Kuntal D. Wound healing potential of 797  
 743 aqueous crude extract of *Stevia rebaudiana* in mice. 798  
 744 *Brazilian Journal of Pharmacognosy.* 2013; 23(2): 799  
 745 351-357. 800  
 746 - Duscher D, Neofytou E, Wong VW, Maan ZN, 801  
 747 Rennert RC, Inayathullah M, Januszyk M, Rodrigues 802  
 748 M, Malkovskiy AV, Whitmore AJ, Walmsley GG, 803  
 749 Galvez MG, Whittam AJ, Brownlee M, Rajadas J, 804  
 750 Gurtner GC. Transdermal deferoxamine prevents 805  
 751 pressure induced diabetic ulcers. *Proc Natl Acad Sci* 806  
 752 *U S A* 2015; 112(1):94-9. 807  
 753 - Gal P, Toporcer T, Vidinsky B, Mokry M, Novotny 808  
 754 M and Kilik R. Early changes in the tensile strength 809  
 755 and morphology of primary sutured skin wounds in 810  
 756 rats (wound healing/tensile strength 811  
 757 measurement/tissue repair and regeneration). *Folia* 812  
 758 *Biologica Praha* 2006; 52:109–115. 813  
 759 - Gallagher KA, Joshi A, Carson WF, Schaller M, 814  
 760 Allen R, Mukerjee S, Kittan N, Feldman EL, Henke 815  
 761 PK, Hogaboam C, Burant CF and Kunkel SL. 816  
 762 Epigenetic Changes in Bone Marrow Progenitor Cells 817  
 763 Influence the Inflammatory Phenotype and Alter 818  
 764 Wound Healing in Type 2 Diabetes. *Diabetes* 2015; 819  
 765 64(4):1420-30. 820  
 766 - Gautam M.K. and Goel R.K.. Wound Healing Effect 821  
 767 of *Ocimum sanctum* Leaves Extract in Diabetic Rats. 822  
 768 *International Journal of Pharmacological and* 823  
 769 *Pharmaceutical Sciences.* 2013; 7 (9): 538-541. 824  
 770 - Giardina S, Michelotti A, Zavattini G, Finzi S, 825  
 771 Ghisalbetti C, Marzatico F and Minerva G. Efficacy 826  
 772 study in vitro: assessment of the properties of 827  
 773 resveratrol and resveratrol + N-acetyl-cysteine on 828  
 774 proliferation and inhibition of collagen activity. 829  
 775 *Minerva Ginecol* 2010; 62(3): 195–201. 830  
 776 - Gowda A., Shanbhag V, Shenoy S, Bangalore E.R., 831  
 777 Prabhu K., Murthy R., Venumadhav N., Goudapalla 832  
 778 P.K., Narayanareddy M and Shanbhag T. Wound 833  
 779 healing property of topical application of ethanolic 834  
 780 extract of *Michelia champaca* flowers in diabetic rats. 835  
 781 *International Journal of Pharmacology and Clinical* 836  
 782 *Sciences.* 2013; 2 (3): 67-74. 837  
 783 - Guariguata L, Whiting DR, Hambleton I, Beagley J, 838  
 784 Linnenkamp U and Shaw JE. Global estimates of  
 diabetes prevalence for 2013 and projections for 2035.  
*Diabetes Res Clin Pract* 2014; 103(2):137-49.  
 - Harish BG, Krishna V, Santosh Kumar HS, Khadeer  
 Ahamed BM, Sharath R, Kumara Swamy HM:  
 Wound healing activity and docking of glycogen-  
 synthase-kinase-3-beta-protein with isolated  
 triterpenoid lupeol in rats. *Phytomedicine.* 2008; 15:  
 763–767.  
 - Hausenblas HA, Schoulda JA, Smoliga JM.  
 Resveratrol treatment as an adjunct to  
 pharmacological management in type 2 diabetes  
 mellitus – systematic review and meta-analysis. *Mol*  
*Nutr Food Res.* 2015;59(1):147–159.  
 - Hesham G. M., Mohamed A.S and Aborehab.A.  
 Phonopheresis Versus Topical *Salvadora Persica* in  
 Healing of Full-Thickness Wound: An experimental  
 Study. *International Journal of Advanced Research*  
 (2014), Volume 2, Issue 5, 919-928.  
 - Inan A, Sen M, Koca C, Akpınar A and Dener C.  
 The effect of purified micronized flavonoid fraction  
 on the healing of anastomoses in the colon in rats.  
*Surg Today* 2006; 36: 818-822.  
 - Janis JE, Kwon RK and Lalonde DH. A Practical  
 Guide to Wound Healing. *Plast. Reconstr. Surg.* 2010;  
 125: 230-244.  
 - Kapetanovic I.M., Muzzio M., Huang Z., Thompson  
 T.N., McCormick D.L. Pharmacokinetics, oral  
 bioavailability, and metabolic profile of resveratrol  
 and its dimethylether analog, pterostilbene, in rats.  
*Cancer Chemother Pharmacol.* 2011; 68(3): 593–601.  
 - Kasuya A and Tokura Y. Attempts to accelerate  
 wound healing. *Journal of Dermatological Science.*  
 2014; 76: 169–172.  
 - Kristl J, Teskac K, Caddeo C, Abramović Z,  
 Sentjurc M. Improvement of cellular stress response  
 on Resveratrol in liposomes. *Eur J Pharm Biopharm.*  
 2009;73(2):253–259.  
 - Kumar R.S., Rajkiran, K., Patnaik S.K. Evaluation  
 of Incisional Diabetic Wound Healing Activity of  
 Ethanolic Leave Extract of *Mimosa pudica* L. in Rats.  
*International Journal of Pharmacognosy and*  
*Phytochemical Research* 2017; 9(8); 1143-1147.  
 - Lee, Z.W., Kweon, S.M., Kim, B.C. Phosphatidic  
 Acid-Induced Elevation of Intracellular Ca<sup>2+</sup> is  
 Mediated by RhoA and H<sub>2</sub>O<sub>2</sub> in Rat-2 Fibroblasts, *J.*  
*Biol. Chem.*, 1998: 273: 12710–12715.  
 - Lobmann R, Ambrosch A, Schultz G, Waldmann K,  
 Schiweck S and Lehnert H. Expression of matrix-  
 metalloproteinases and their inhibitors in the wounds  
 of diabetic and non-diabetic patients. *Diabetologia*  
 2002; 45(7): 1011–6.  
 - Maruyama K, Asai J, Ii M, Thorne T, Losordo DW  
 and D'Amore PA. Decreased macrophage number and  
 activation lead to reduced lymphatic vessel formation

- 839 and contribute to impair diabetic wound healing. *Am J*  
840 *Pathol* 2007; 170(4):1178-91.
- 841 - Medina A, Scott PG and Ghahary A.  
842 Pathophysiology of chronic non healing wounds. *J*  
843 *Burn Care Rehabil* 2005; 26:306-319.
- 844 - Mikhailchik E.V., Anurov M.V., Titkova S.M.,  
845 Miroshnikova E.A., Lukasheva E.V., Deeva I.B.,  
846 Ibragimova G.A and Korkina L.G. Activity of  
847 antioxidant enzymes in the skin during surgical  
848 wounds. *Bul. Exp. Biol. Med.* 2006; 142:667-669.
- 849 - Misra, H.P. and Fridovich, I. The Role of  
850 Superoxide Anion in the Autoxidation of Epinephrine  
851 and a Simple Assay for Superoxide Dismutase, *J. Biol.*  
852 *Chem.*, 1972; 247: 3170-3175.
- 853 - Møller Poulsen, M., Fjeldborg, K., Juul Ornstrup, M.,  
854 Nordstrøm Kjær, T., Klitgaard Nøhr, M., Bønløkke  
855 Pedersen, S. Resveratrol and inflammation: Challenges  
856 in translating pre-clinical findings to improved patient  
857 outcomes. *Biochimica et Biophysica Acta* 1852 (2015)  
858 1124-1136.
- 859 - Moron, M.S., Depierre, J.W. and Mannervik, B.  
860 Levels of Glutathione, Glutathione Reductase and  
861 Glutathione S-Transferase Activities in Rat Lung and  
862 Liver, *Biochim. Biophys. Acta*, 1979; 582: 67-78.
- 863 - Muller M, Trocme C, Lardy B, Morel F, Halimi S  
864 and Benhamou PY. Matrix metalloproteinases and  
865 diabetic foot ulcers: the ratio of MMP-1 to TIMP-1 is  
866 a predictor of wound healing. *Diabet Med* 2008; 25(4):  
867 419-26.
- 868 - Naito R, Nishinakamura H, Watanabe T, Nakayama  
869 J, Kodama S. Edaravone, a free radical scavenger,  
870 accelerates wound healing in diabetic mice. *Wounds.*  
871 2014; 26(6):163-71.
- 872 - Pandian C., Srinivasan A. and Pelapolu I. C.  
873 Evaluation of wound healing activity of hydro  
874 alcoholic extract of leaves of *Stachytarpheta*  
875 *jamaicensis* in streptozotocin induced diabetic rats.  
876 *Der Pharmacia Lettre.* 2013; 5 (2):193-200.
- 877 - Pattanayaka S.P and Sunitab P. Wound healing, anti-  
878 microbial and antioxidant potential of  
879 *Dendrophthoe falcata* (L.f) Ettingsh. *Journal of*  
880 *Ethnopharmacology.* 2008; 120: 241-247.
- 881 - Paul, A.G., Ahmad, N.W., Lee, H.L. Maggot  
882 Debridement Therapy with *Lucilia Cuprina*: A  
883 Comparison with Conventional Debridement in  
884 Diabetic Foot Ulcers, *Int. Wound J.*, 2009; 6: 39-46.
- 885 - Pillai S.I., Palsamy P., Subramanian S. and  
886 Kandaswamy M. Wound healing properties of Indian  
887 propolis studied on excision wound-induced rats.  
888 *Pharmaceutical Biology*, 2010; 48(11): 1.
- 889 - Prasad SK, Kumar R, Patel DK, Hemalatha S.  
890 Wound healing activity of *Withania coagulans* in  
891 streptozotocin- induced diabetic rats. *Pharm Biol.*  
892 2010; 48(12): 1397-1404.
- 893 - Rastogi S, Goyal P, Mangla K, Bhavsar N, Patel H  
894 and Rawal RC. Study of transplantation of melanocyte  
895 keratinocyte suspension in treatment of vitiligo. *Indian*  
896 *J Dermatol* 2006; 51:142-144.
- 897 - Roche, M., Tarnus, E., Rondeau, P. and Bourdon, E.  
898 Effects of Nutritional Antioxidants on AAPH- or  
899 AGEs-Induced Oxidative Stress in Human SW872  
900 Liposarcoma Cells, *Cell. Biol. Toxicol.*, 2009: 25:  
901 635-644.
- 902 - Rogiers V, Pauwels M. Safety Assessment of  
903 cosmetics in europe. *Curr Prob Dermatol* 2008; 36:  
904 134.
- 905 - Santram L and Abhay K. Wound healing effect of  
906 flavonoid rich fraction and luteolin isolated from  
907 *Martynia annua* Linn. on streptozotocin induced  
908 diabetic rats. *Asian Pacific Journal of Tropical*  
909 *Medicine.* (2013)253-259.
- 910 - Sarsour, E.H., Kumar, M.G., Chaudhuri, L., Kalen,  
911 A.L. and Goswami, P.C. Redox Control of the Cell  
912 Cycle in Health and Disease, *Antioxid. Redox Signal.*,  
913 2009; 11: 2985-3011.
- 914 - Sen, C.K. Wound Healing Essentials: Let There be  
915 Oxygen, *Wound Repair Regen.*, 2009; 17: 1-18.
- 916 - Shivananda N, Poorna N, Steve Vidyasagar B and  
917 Andrew A. Evaluation of wound healing activity of  
918 *Allamanda cathartica*. L. and *Laurus nobilis*. L.  
919 extracts on rats. *BMC Complementary and Alternative*  
920 *Medicine* 2006, 6: 12.
- 921 - Thiem B., Grosslinka O. Antimicrobial activity of  
922 *Rubushamaemorus* leaves. *Fitoterapia.* 2003; 75: 93-  
923 95.
- 924 - Tse WT, Pendleton JD, Beyer WM, Egalka MC and  
925 Guinan EC. Suppression of allogeneic T-cell  
926 proliferation by human marrow stromal cells:  
927 implications in transplantation. *Transplantation* 2003;  
928 75:389-397.
- 929 - Vidinský B, Gál P, Toporcer T, Longauer F,  
930 Lenhardt L, Bobrov N and Sabo J. Histological study  
931 of the first seven days of skin wound healing in rats.  
932 *Acta Vet Brno* 2006; 75:197-202.
- 933 - Wagner OI, Rammensee S, Korde N, Wen Q,  
934 Leterrier JF and Janmey PA. Softness, strength and  
935 self-repair in intermediate filament networks. *Exp Cell*  
936 *Res* 2007; 313:2228-2235.
- 937 - Weindl G., Schaller M and Korting H.C. Hyaluronic  
938 acid in the treatment and prevention of skin diseases:  
939 molecular biological, pharmaceutical and clinical  
940 aspects. *Skin Pharmacol. Physiol.* 2004; 17:207-213.
- 941 - Woessner JF. The determination of hydroxyproline  
942 in tissue and protein samples containing small portion  
943 of this Imino acid. *Arch Biochem Biophys* 1961; 193:  
944 440-447.
- 945 - Yamagishi S, Nakamura N, Suematsu M, Kaseda K  
946 and Matsui T. Advanced Glycation End Products: A

947 Molecular Target for Vascular Complications in 948 Diabetes. Mol Med 2015; 21 Suppl 1:S32-S40.

949  
950  
951  
952  
953  
954  
955  
---

**Control Group (IIB)                      Reference Group                      Test Group**

**2 Hours  
pre-treatment**

961  
962  
963  
964  
**1 Week  
post-treatment**

965  
966  
967  
968  
969  
970  
971  
972  
**2 Weeks  
post-treatment**

973  
974  
975  
976  
977  
978  
979  
980  
**3 Weeks  
post-treatment**

981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992

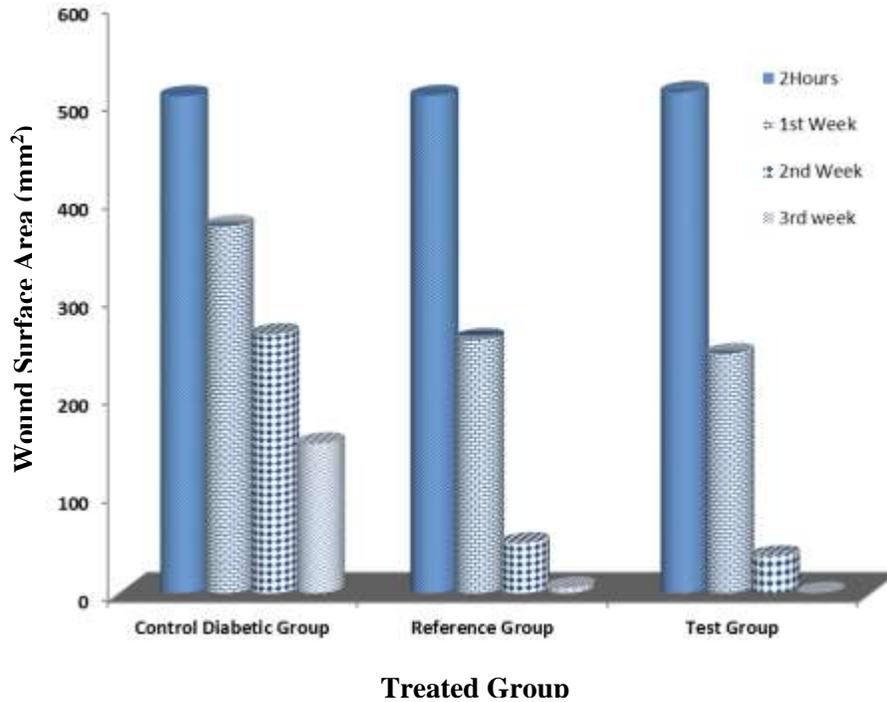


Figure (1): Examples of captured photographs by digital camera providing comparative images for wounds in different groups pretreatment and after

**Table 1: Effect of different treatments on Wound Surface Area by excision wound model.**

Post-wounding (days)	Wound Surface Area (mm <sup>2</sup> ) (mean±SD)		
	Control positive (diabetic group )	Reference Mebo ointment (0.25%, w/w)	Resveratrol ointment (0.5%, w/w)
0	506.86 ± 4.82	507.36 ± 9.59	511.06 ± 9.28
7	374.81 ± 4.13	259.21 ± 5.78**	244.18 ± 4.67**
14	264.06 ± 7.76	50.82 ± 2.88**	37.60 ± 3.08**
21	152.60 ± 7.11	05.39 ± 1.61**	00.00 ± 0.00**

993 Results are expressed as mean±SD.  
 994 \*\*significantly different compared to control group (p<0.05)  
 995 <sup>ns</sup> not significantly different compared to reference group (p>0.05)  
 996  
 997  
 998  
 999



1021  
 1022 **Treated Group**  
 1023 Figure (2): Effect of different treatments on Wound  
 1024 Surface Area within each group at different treatment  
 1025  
 1026  
 1027  
 1028

1029 **Table (2): Comparison of wound contraction (healing rate) and epithelialization period after different**  
 1030 **treatments.**

Post-wounding (days)	Percent wound contraction % (Healing Rate %) (mean±S.E.)		
	control positive (Diabetic G)	Reference Mebo ointment (0.25%, w/w)	Resveratrol ointment (0.5%, w/w)
0	0	0	0
6	26.05±0.81	52.52±2.07**	52.22±0.91**
14	47.90±1.53	89.98±0.57**	92.64±0.60**, <sup>ns</sup>
21	69.89±1.40	98.94±0.32**	100.00±0.00**, <sup>ns</sup>
Epithelialization period (days)	Incomplete	19.53 ± 0.61	18.63 ± 0.54

1031 Results are expressed as mean±SD (n=8).  
 1032 \*\*significantly different compared to control group (p<0.05)  
 1033 <sup>ns</sup> not significantly different compared to reference group (p>0.05)  
 1034  
 1035  
 1036

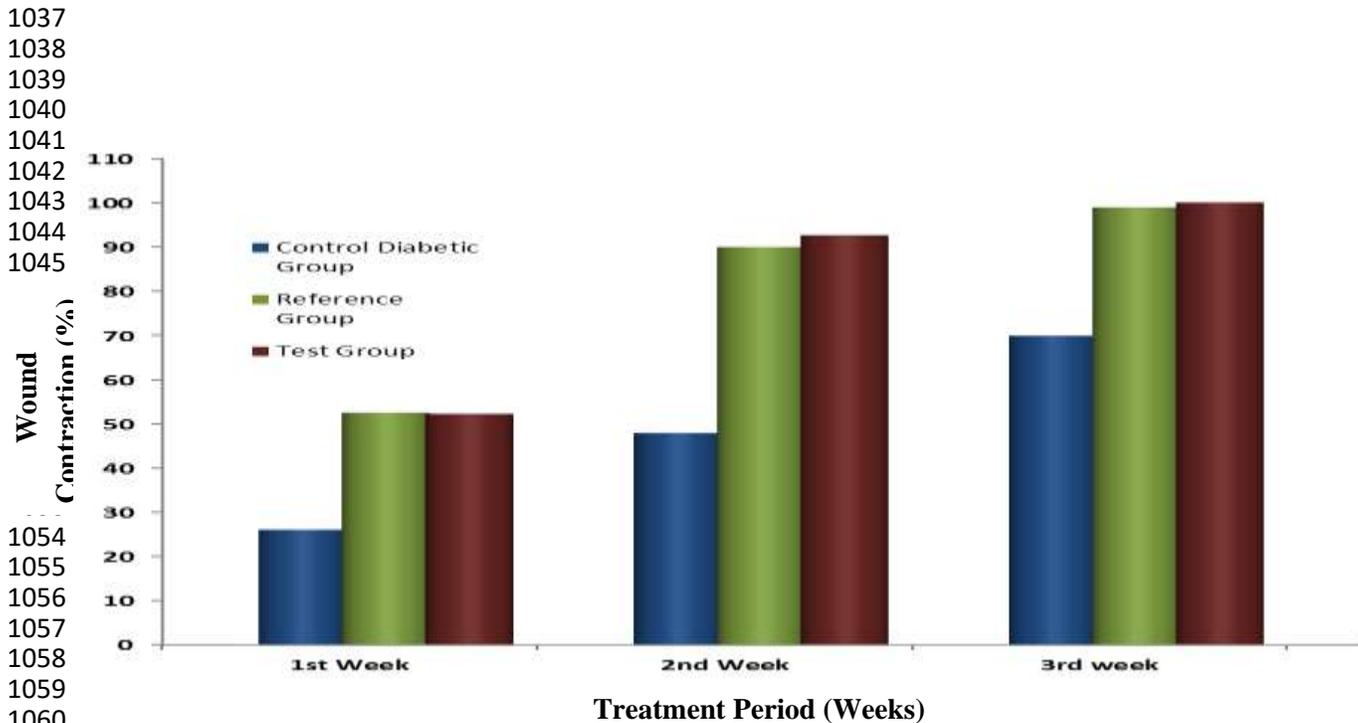


Figure (3): Effect of different treatments on Wound Healing (%).

1064  
1065  
1066  
1067  
1068  
1069  
1070  
1071  
1072  
1073  
1074  
1075  
1076  
1077  
1078  
1079  
1080  
1081  
1082  
1083  
1084  
1085

Table (3): Comparison of fasting serum glucose (mg/dl) mean values after (1st, 2nd and 3rd weeks) between groups.

Mean $\pm$ SD	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week
Normal Control group (NC)	96.64 $\pm$ 2.72	95.68 $\pm$ 5.40	91.13 $\pm$ 2.96
Control Diabetic group (group IIA)	395.35 $\pm$ 5.21 <sup>***</sup>	388.46 $\pm$ 5.05 <sup>ns,***</sup>	367.99 $\pm$ 5.93 <sup>ns,***</sup>
Mebo ointment (0.25%, w/w) (Reference group IIB)	392.11 $\pm$ 3.24 <sup>***</sup>	381.20 $\pm$ 6.88 <sup>ns,***</sup>	360.13 $\pm$ 7.31 <sup>ns,***</sup>
Resveratrol ointment (0.5%, w/w) (Test group IIC)	394.07 $\pm$ 4.21 <sup>***</sup>	377.85 $\pm$ 6.79 <sup>ns,***</sup>	356.83 $\pm$ 7.45 <sup>ns,***</sup>
P value	< 0.001	< 0.001	< 0.001

Results are expressed as mean $\pm$ SD.

. \*\*\* Significant difference compared to normal control group (p<0.001).

<sup>ns</sup> not significantly different compared to diabetic control group (p>0.05)

Table (4): Effect of different treatments on different biochemical parameters of tissues from diabetic wound.

Parameter	LPO (nmoles/mg protein)	GSH ( $\mu$ mol/50mg tissue)	Catalase ( $\mu$ mol/50mg tissue)	SOD ( $\mu$ g/50 mg tissue)
Normal Control group (NC)	0.53 $\pm$ 0.02	26.65 $\pm$ 0.55	37.51 $\pm$ 1.82	34.95 $\pm$ 0.78
Control Diabetic	6.66 $\pm$ 0.25	10.72 $\pm$ 0.42	16.83 $\pm$ 0.31	10.89 $\pm$ 0.38

group (IIA )				
<b>Mebo ointment (0.25%, w/w) (Reference group IIB)</b>	<b>1.75 ± 0.13<sup>**</sup></b>	<b>20.40±0.63<sup>**</sup></b>	<b>24.79±0.90<sup>**</sup></b>	<b>22.28±0.76<sup>**</sup></b>
<b>Resveratrol ointment (0.5%, w/w) (Test group IIC)</b>	<b>0.78 ± 0.05<sup>*,***</sup></b>	<b>26.48±0.51<sup>*,***</sup></b>	<b>34.35±0.65<sup>*,***</sup></b>	<b>28.47±0.91<sup>*,***</sup></b>

1086

1087

Results are expressed as mean±SD (n=8).

1088

<sup>\*\*\*</sup> significantly different compared to control group (p<0.001)

1089

<sup>\*\*</sup> significantly different compared to reference group (p<0.05)

1090

<sup>ns</sup> not significantly different compared to reference group (p>0.05)

1091

1092

1093

1094

1095

1096

1097

1098

1099

1100

1101

1102

1103

1104

1105

1106

1107

1108

1109

1110

1111

1112

1113

1114

1115

1116

1117

1118

1119

1120

1121

1122

1123

1124

1125

1126

1127

1128

1129

1130

1131

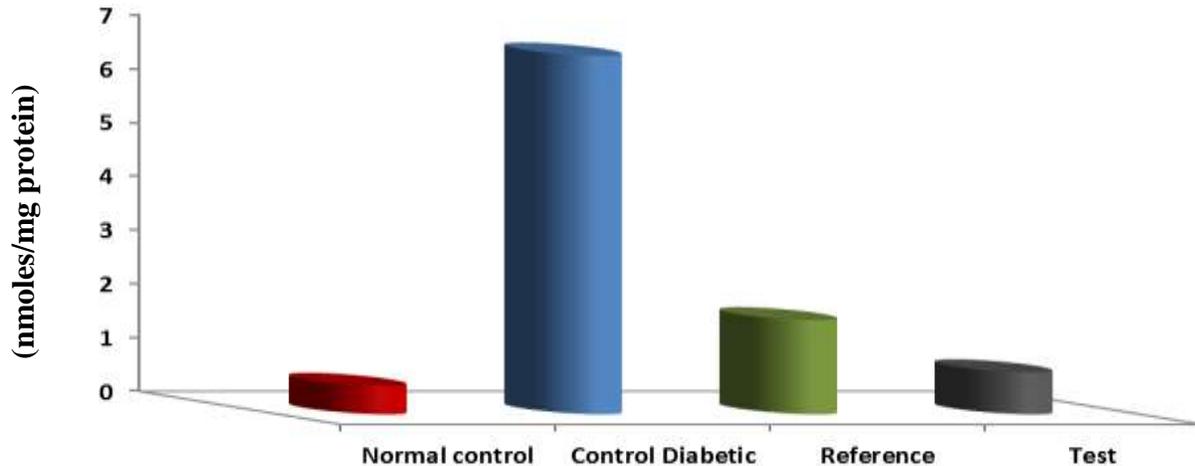


Figure (4): Effect of different treatments on LPO parameter.

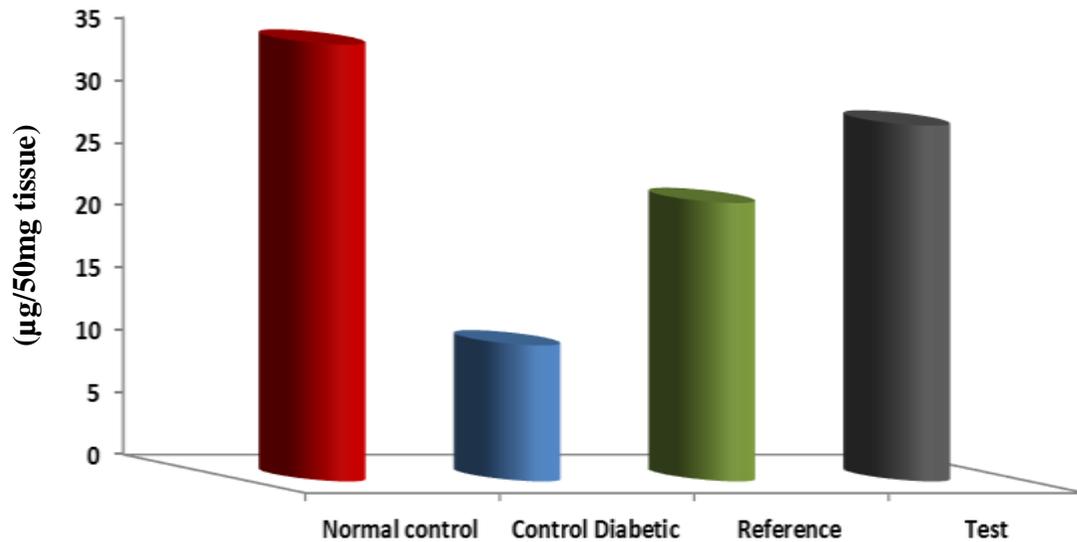


Figure (7): Effect of different treatments on SOD parameter.

Table (5): showing the mean epidermal thickness (mean  $\pm$  SD) in the different groups compared with control-ve group.

Groups	Control (-ve) group	Diabetic group	Resveratrol ointment (0.5%, w/w)	Mebo ointment (0.25%, w/w)
1 <sup>st</sup> week post wound	39.79 $\pm$ 9.11	11.21 $\pm$ 1.21	24.23 $\pm$ 0.12	19.96 $\pm$ 0.18
2 <sup>nd</sup> week post wound		15.94 $\pm$ 0.21	31.12 $\pm$ 0.82	28.25 $\pm$ 1.51
3 <sup>rd</sup> week post week		20.89 $\pm$ 0.34	38.19 $\pm$ 0.15	32.73 $\pm$ 1.82
Significance		S	S	NS

SD = standard deviation      S = Significant      NS = Significant

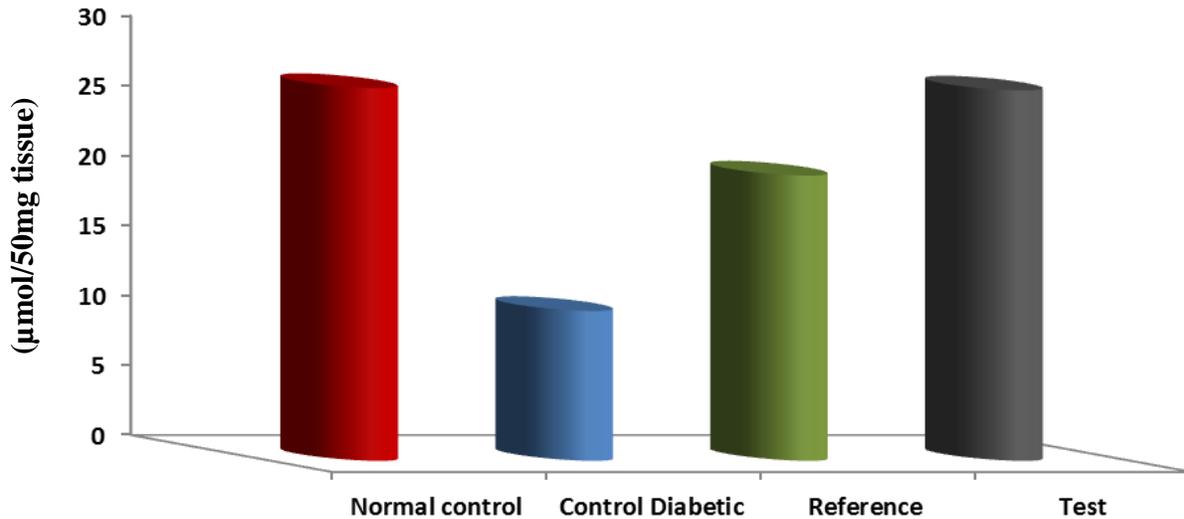


Figure (5): Effect of different treatments on GSH parameter.

Table (6): The mean area percentage of collagen fibers in the different groups studied.

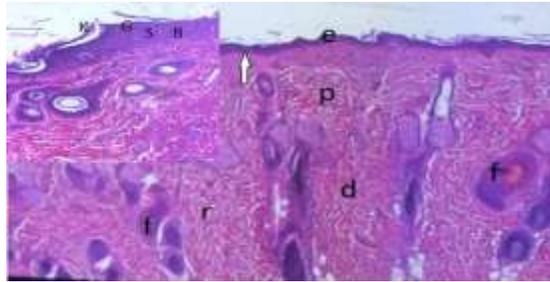
Groups	Control (-ve) group	Diabetic group	Resveratrol ointment (0.5%, w/w)	Mebo ointment (0.25%, w/w)
1 <sup>st</sup> week post wound	43.32 ± 1.12	10.11 ± 0.21	37.23 ± 2.05	29.34 ± 0.19
2 <sup>nd</sup> week post wound		18.14 ± 0.41	39.19 ± 0.45	34.28 ± 0.13
3 <sup>rd</sup> week post week		26.79 ± 0.48	40.23 ± 1.15	37.31 ± 0.62
Significance		S	S	NS

SD = standard deviation S = Significant NS = Significant

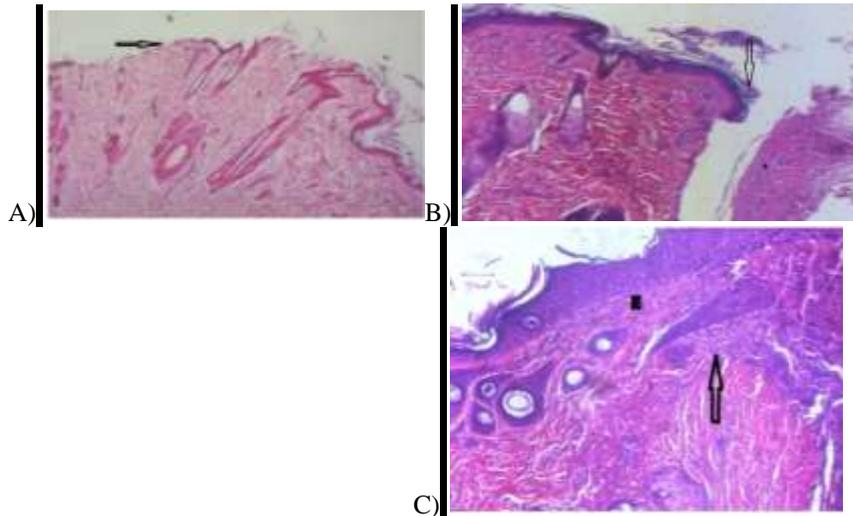
Table (7): Showing The area percentage of cytokeratin intermediate filaments expression in-positive cells, SD e compared with control-ve group.

Groups	Control (-ve) group	Diabetic group	Resveratrol ointment (0.5%, w/w)	Mebo ointment (0.25%, w/w)
1 <sup>st</sup> week post wound	20.77 ± 0.33	9.12 ± 1.09	15.03 ± 0.15	14.39 ± 2.84
2 <sup>nd</sup> week post wound		11.04 ± 2.91	16.18 ± 0.98	15.26 ± 1.19
3 <sup>rd</sup> week post week		12.39 ± 2.08	18.72 ± 0.30	16.13 ± 0.56
Significance		S	s	NS

SD = standard deviation S = Significant NS = Significant

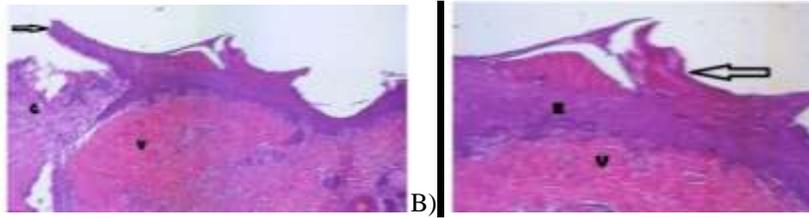


1214 Figure (8). A keratinized stratified squamous epithelium of skin epidermis (e) in skin from a control rat (group I).  
 1215 The dermis (d) with upper thin papillary layer (p) and basal thick reticular layer (r). Apparent epidermal-dermal  
 1216 junction (arrow) with hair follicles (f). Above magnify photo showing the layers of the epidermis: stratum basale (B),  
 1217 stratum spinosum (S), stratum granulosum (G), and stratum corneum with keratin (K). H&E  $\times$  200. (B) H&E  $\times$  400.  
 1218  
 1219



1220 A) B) C)  
 1221 Figure (9). (A) Photomicrographs of skin wounds from a control +ve rats (IIA) showing loss of the epidermis at the  
 1222 site of the wound defect (arrow). (B) Two weeks' post wounding the wound showing granulation tissue filled the  
 1223 wound gap and creeping epithelium filling the defect (arrow). Note that granulation tissue is formed of fine fibrous  
 1224 material entangling mononuclear inflammatory cells and containing congested blood vessels (G). three weeks' post  
 1225 wounding the wound showing loss of the epidermal bridges(E) and presence of keratocytes migration with  
 1226 inflammatory cells (arrow). H&E  $\times$  200.  
 1227  
 1228  
 1229  
 1230  
 1231  
 1232  
 1233  
 1234  
 1235  
 1236  
 1237  
 1238  
 1239  
 1240  
 1241  
 1242  
 1243  
 1244  
 1245

1246



1247

1248

A)

B)

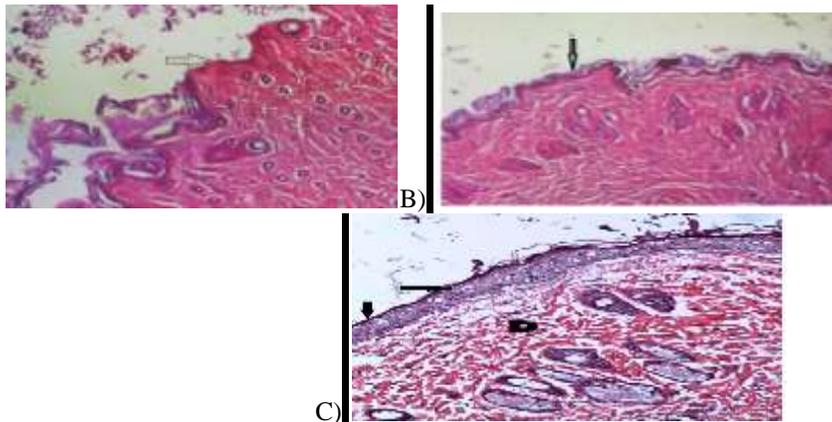
C)

1249

1250 Figure (10). (A) Photomicrographs of skin wounds from group IIB (topical resveratrol formulation treated rats)  
 1251 showing Discontinuity in the epidermis and it partially covering the wound with presence of skin tongue (arrow)  
 1252 with underlying granulation tissue (G) (one-week post wound). Note that the granulation tissue appears more  
 1253 vascular and more fibrous (V). (B) two -weeks post wound apparent increase in the epidermal epithelium (E) with  
 1254 presence of wound scab (arrow). Note increase vascularization in dermis (V) (C) three -weeks post wound the  
 1255 epidermis is totally covers most of the wound and an apparent increase in epidermal thickness (E) with presence of  
 1256 epidermal ridges (arrow). Note the hair follicle (F). (H&E  $\times$  200.)

1257

1258



1259

A)

B)

C)

1260

1261 Figure (11). (A) Photomicrographs of skin wounds from group IIC (topical mebo treated rats) showing loss in the  
 1262 epidermis with scab (arrow) with no underlying granulation tissue (one-week post wound). (B) two weeks' post  
 1263 wound an apparent thinning out of the epidermis (arrow), loss of epidermal ridges, and dermal papillae as compared  
 1264 with that of the control rats. The dermis shows less dense connective tissue fibers. (C) three weeks post wound the  
 1265 epidermis completely cover the wound with loss of its ridges and vacuolation of keratocytes (arrow). Note increase  
 1266 connective tissue in dermal layer (D) (H&E  $\times$  200.)

1267

1268

1269

1270

1271

1272

1273

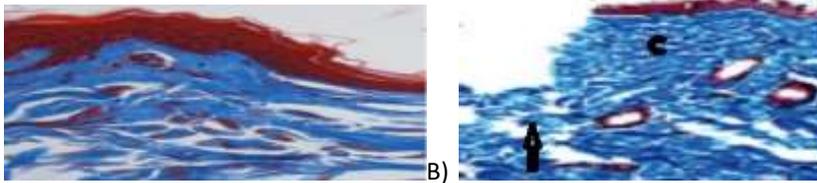
1274

1275

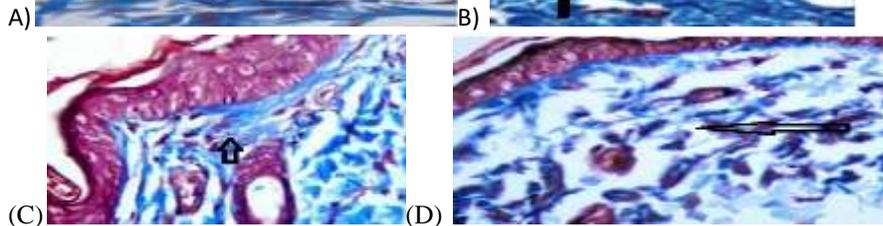
1276

1277

1278



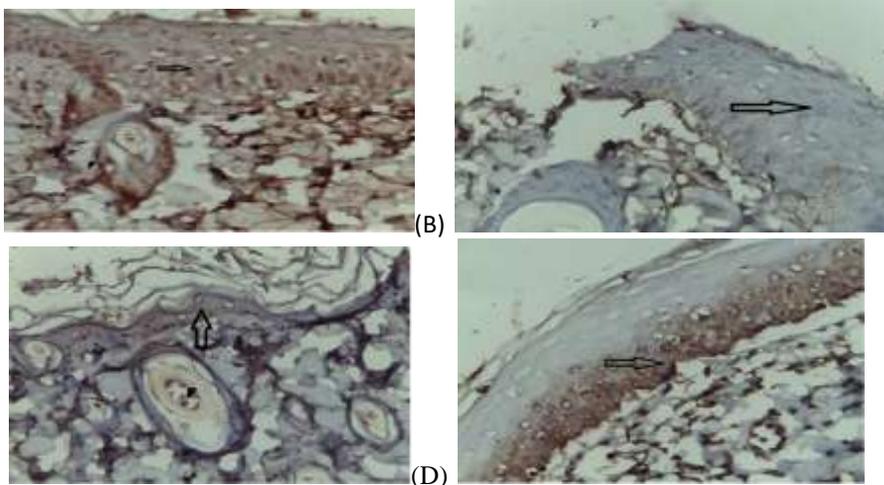
1279



1280

1281 Figure (12). A) A photomicrograph of a section of rat skin of the diabetic control group (group I) showing the  
 1282 collagen fiber content in dermis. Thin collagen bundles are present in the papillary layer of the dermis appear (P),  
 1283 Notice that collagen fibers in the reticular dermis appear as coarse, wavy bundles (R). (B) A photomicrograph of a  
 1284 section of rat skin of the diabetic group (group IIA) showing more collagen fiber content (c) and irregular  
 1285 arrangement of collagen fibers at the wound area (arrow). (C) A photomicrograph at the skin of group IIB showing  
 1286 regular arrangement of collagen fibers in dermis (arrow). (D) A photomicrograph at the skin of subgroup IIC  
 1287 showing less collagen fiber content with irregular arrangement of them in dermis compared with control skin. (arrow).  
 1288 Masson's trichrome stain,  $\times 400$ .

1289



1290

1291

1292 Figure (13). (A) A photomicrograph of a section of rat skin of the -ve control group showing the positive cytokeratin  
 1293 intermediate filaments (brown color) in the epidermal cells (arrow) and around hair follicles (F). (B) A  
 1294 photomicrograph of a section of rat skin of the diabetic group (group IIA) showing an apparent decrease in the  
 1295 cytokeratin intermediate filaments in the epidermis (arrow) as compared with that of the control group. (C) A  
 1296 photomicrograph at the skin of group IIB showing apparent increase in the cytokeratin intermediate filaments in the  
 1297 epidermis (arrow) as compared with that of the diabetic untreated group. Note positive staining in hair follicle (F).  
 1298 (D). A photomicrograph at the skin of group IIC showing apparent moderate increase in the cytokeratin intermediate  
 1299 filaments in the epidermis (arrow) as compared with that of the diabetic untreated group. Cytokeratin  
 1300 Immunohistochemical staining  $\times 400$

1301

1302