

**Physiological studies on the effect of a bradykinin potentiating factor (BPF) isolated from scorpion venom on the burnt skin of alloxan-induced diabetic Guinea pigs.**

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**ABSTRACT**

It is well known that, Wound healing in the diabetes is enormous, growing problem and having cost Medicaid. Therefore, this study produces an exciting prospect, to improve diabetic burn healing, and evaluates the ability of a bradykinin potentiating factor (BPF) isolated from scorpion venom (*Buthus occitanus*) in treatment of burns in diabetic male Guinea pigs. Male Guinea pigs of approximate (550 g body weight each) were divided into five groups. In the normal group; Guinea pigs were interaperitoneally (i.p) injected with 100 µL saline solution. The second, served as control group which were injected with 100 µL saline solution then standard burns were obtained on the dorsal skin. The 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> groups were i.p. injected with (750 mg /kg, body weight) with a single dose of Alloxan, then standard burns were obtained, after that the 4<sup>th</sup> group was treated by BPF in 100 µL saline solutions (1µg/gm. b. w.) topically and the 5<sup>th</sup> group treated with BPF (1µg/gm. b. w.) topically and interperitonially (1µg/gm. b. w. twice a week). Every group contains 20 animals and sacrificed at 15 and 30 days post-treatment by BPF (10 animals per each). The results are recorded after monitoring the CBC including (RBCs, WBCs, platelets, Hb content and HCT percentage), lipid profile including (total cholesterol (TC), triglyceride (TGs), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL)) and the skin antioxidant status (catalase activity (CAT) and superoxide dismutase (SOD) and serum nitric oxide (NO) beside malondialdehyde (MDA) concentration were also monitored during the study compared to normal animals. Also serum glucose level was monitored. The recorded results declared that, the treatment with BPF has shown an ameliorative effect on burn healing in diabetic animals. These observations and investigations were the pacemaker for the hypothesized ameliorating activity of BPF in the present study.

**Keywords:** scorpion venom, *Buthus occitanus*, BPF, burnt wound healing, diabetes and Guinea pigs.

**INTRODUCTION:**

Diabetes mellitus DM is a group of metabolic disorders with one common manifestation: hyperglycemia associated with defects in insulin secretion, action or both. It is well known that diabetes mellitus results in many complications to the individual, foot ulceration taking the greatest toll, which will be focused in this study. Several laboratory rodent models (Buschard, 1996) as well as non-rodent species (Sun *et al.*, 1996) are available for studying both types of diabetes. The

development of the syndrome in these models is either spontaneous (Weiss *et al.*, 2005), induced by chemical agents such as alloxan or streptozotocin (Portha *et al.*, 1989), or caused by a virus infection (Filippi and Von Herrath, 2005).

A variety of factors including tissue damage, allergic reactions, viral infection and other inflammatory events activate a series of proteolytic reactions that generate bradykinin and kallidin in the tissues (Wachtfogel *et al.*, 1998).

The nonapeptide bradykinin has a wide range of biological effects on a number of different tissues and organs. Bradykinin is most known for its potent vasodilatory effect. It is also involved in smooth muscle contraction, regulation of vascular permeability, pain induction, cell proliferation and development of pathological states such as inflammation (Hall, 1992). The bradykinin potentiating factor BPF extracted from *Leiurus quinquestriatus* venom was shown to enhance the cellular growth of the uterus and development of the ovarian follicle in female mice (Abdel Raheim *et al.*, 1995). Similarly, injection of this BPF enhanced spermatogenesis. Moreover, Salman (2002) declared that injection of BPF in sublethally-irradiated and non-irradiated Guinea pigs accelerated the generation of thymus and spleen cellularity without noticeable toxic effects in non-irradiated control animals. Nassar *et al.*, 1990 and Abd-El-Rahim, 1990 stated that, BPF of *Buthus occitanus* venom showed growth factor-like activity.

It is worth to mention that, the recent researches recorded that there is a relation between bradykinin and oxidative stress, kinin may attenuate inflammatory responses and renal fibrosis by inhibiting oxidative stress and mitogen-activated protein kinase (MAPK) activation (Chao *et al.*, 2007), therefore, oxidative stress parameters (MDA, CAT, SOD and Nitric oxide) were monitored during our study. In addition this study monitors lipid profile (cholesterol, HDL, LDL, vLDL and triglycerides) and serum glucose because alloxan causes diabetes which leads to a massive reduction in insulin release by the destruction of  $\beta$ -cells of the islets of langerhans, thereby, inducing hyperglycaemia and hyperlipidemia as consequent (Grover *et al.*, 2000 and Sharma *et al.*, 2010).

## MATERIALS AND METHODS

**Chemicals:** Alloxan monohydrate was obtained from Sigma Chemicals Company, Cairo, Egypt. Kits of glucose, cholesterol, triglycerides, HDL, LDL, MDA, SOD, NO and catalase were obtained from Biodiagnostic Company, Cairo, Egypt.

### **Isolation of Bradykinin Potentiating factor (BPF):**

Firstly the crude venom was collected from scorpion, *Buthus occitanus* gathered from the Aswan area. The scorpions were milked in the physiology laboratory at the Faculty of Science in Qena, South Valley University with a specific device using electrical shocks (6 volts) at the articular membrane of the telson. The collected droplets were received into a clean dry glass container. The venom was lyophilized and freeze dried after which it was kept at -10 °C (in the dark) until it was used. The venom fraction (BPF) separated from *Buthus occitanus* was isolated, purified and detected according to the method of (Ferreira, 1965).

**Animals:** Adult Guinea pigs of approximate (550 g body weight each) were selected. The animals were housed in the Animal House of the Faculty of science, South Valley University, Qena, Egypt, for two weeks under natural day and night periods and supplied with a balanced diet and water *ad libitum*. Animals were divided into four groups each was sacrificed every 15 and/or 30 days as the following:

**Group 1** (normal group): Each of 20 animals injected with (100 ml/kg body weight) of saline solution intraperitoneally. This group served as normal group.

**Group 2** (normal burned group): Each of 20 animals injected i.p. with (100 ml/kg body weight) saline solution then standard burns were obtained on the dorsal skin without treatment. Then they were sacrificed after 15 and 30 days.

**Group 3** (diabetic burned group): Each of 20 animals injected i.p. with (750

mg /kg), alloxan to induce diabetes then standard bruns of skin were obtained without treatment. Then they were sacrificed after 15 and 30 days.

**Group4** (BPF topically treated group): Each of 20 animals injected i.p. with alloxan to induce diabetes then standard bruns of skin are obtained. This group was treated topically with BPF daily. Then 10 animals were sacrificed after 15 (i.e., after 15 doses of topical treatment). The other 10 animals were sacrificed after 30 days (i.e., after 30 doses of topical treatment).

**Group 5** (BPF top. and i.p. treated group): Each of 20 animals injected i.p. with alloxan to induce diabetes then standard bruns of skin are obtained. They were treated topically with BPF daily and i.p. with BPF also twice a week. After this 10 animals were sacrificed after 15 (i.e., after having 15 doses of topical treatment and 4 i.p. doses of BPF). The other 10 animals were sacrificed after 30 days (i.e., after having 30 doses of topical treatment and 8 i.p. doses of BPF)

#### **Collection of samples:**

12 hrs Fasting animals were sacrificed. Peripheral blood was divided into two portions (the first portion was used as whole blood for complete blood count (CBC), the second was used for separation of serum) also a part of the skin tissue was taken to prepare tissue homogenate as the following:

#### **I. Whole blood collection:**

Whole blood samples were received in clean EDTA tubes. These fresh samples were directly used in RBCs. count, WBCs count, platelets count, hematocrit and HB content.

#### **II. Serum preparation:**

Blood samples in this case were collected in clean and dry tubes. Then the blood was let to clot at room temperature. After an hour, serum was separated by centrifugation for 30 minutes at 3000 rpm. Then collected in labeled

Epindroff's tubes and stored at -20 C for biochemical analysis.

#### **III. Skin Tissue Homogenate preparation:**

Skin tissue was perfused with PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. Tissue then was homogenized in 5\_10 ml cold buffer (i.e. 50 Mm potassium phosphate, pH 7.4, 1 mM EDTA and 1 ml/L Triton X-100) per gram tissue. After this the sample was centrifuged at 4,000 rpm for 15 minutes at 4 C. Finally the supernatant was removed for assay and freeze at -80C (Nishikimi *et al.*, 1972).

#### **Biochemical parameter of whole blood, serum and skin tissue:**

Biochemical parameters; Serum glucose was determined with an enzymatic colorimetric method. Lipid profile was estimated (Trivedi *et al.*, 2004) by enzymatic method using reagent kit obtained from (Bio-diagnostic Company, Cairo, Egypt) and was analyzed according to the reported methods. Malondialdehyde MDA (Ohkawa, *et al.*, 1979), Catalase CAT (Fossati *et al.*, 1980) and super oxide dismutase SOD (Nishikimi *et al.*, 1972), were analyzed also using available kits from (Bio-diagnostic Company, Cairo, Egypt) according the reported methods. Blood cell counts, hematocrit or packed cell volume (PCV) and hemoglobin (Hb) content were determined using Celltac  $\alpha$  apparatus from NIHON KOHDEN (MEK-6410K).

#### **Statistical analysis:**

The variability degree of results was expressed as means  $\pm$  standard deviation of means (Mean  $\pm$  S.D). The significance of the difference between samples was determined using Graph Pad Prism 03n software, where appropriate. The difference was regarded significant at  $P < 0.05$ .

## RESULTS

### Effect of BPF on serum glucose level of the diabetic burned *Guinea pigs*:

The recorded results in tables (1) showed that glucose level in diabetic burned male *Guinea Pigs* after 15 days was significantly increased ( $p < 0.05$ ) when compared with normal and normal burned groups.

With time proceeding (i.e. after 30 days) a highly significant increase ( $p <$

0.01) occurred. With topical treatment there was a non-significant decrease in glucose level after 15 or 30 days when compared with those of non treated groups under normal conditions. In the case of both 15 and 30 days of topical and i.p. BPF treatment a significant decrease ( $P < 0.05$ ) in glucose level was observed in comparing with diabetic burned group.

Table 1: Effect of interperitoneally (i.p.) and/or topically treatment with bradykinin potentiating factor (BPF) 1 $\mu$ g/gm isolated from scorpion venom *Buthus occitanus* within 15 and 30 days on serum glucose of burned skin of alloxan-induced diabetic Guinea Pigs:

Groups	Glucose level after 15 days	Glucose level after 30 days
	Mean $\pm$ S. D.	Mean $\pm$ S. D.
Normal	90.0 $\pm$ 5.2	95.0 $\pm$ 6.0
Normal Burned	98.0 $\pm$ 6.0	88.0 $\pm$ 7.0
Diabetic burned (control)	260.0 <sup>+a</sup> $\pm$ 9.0	337.0 <sup>++a</sup> $\pm$ 8.0
Top. BPF (Treatment)	236.0 $\pm$ 9.0	248.0 $\pm$ 5.0
Top. + i.p. BPF (Treatment)	220.0 <sup>-b</sup> $\pm$ 9.0	218.0 <sup>--b</sup> $\pm$ 10.0

Results are expressed as mean  $\pm$  S.D. of 10 animals.

+a = significantly increased from the normal at  $p < 0.05$ .

++a = highly significantly increased from the normal at  $p < 0.01$ .

-b = significantly decreased from diabetic burned group at  $p < 0.05$ .

--b = highly significant decreased from the normal at  $p < 0.01$

### Effect of BPF on complete blood count (CBC) of the diabetic burned *Guinea pigs*:

As recorded in Table (2), diabetic burned group showed a significant decrease in RBCs, Hb, HCT and platelets after 15 days and a highly significant decrease after 30 days of the same parameters. In contrast, WBCs count was significantly increased after 15 days and highly significant increased after 30 days. After 15 days of topical treatment, there was neither a significant increase in RBCs, Hb, HCT and Platelets nor a significant decrease in WBCs when compared with control group. While in

the case of topical and i.p. treatment (after 15 days) there was a significant increase in RBCs, Hb, HCT and Platelets while WBCs recorded a significant decrease.

Also in comparing animals with diabetic burned group the RBCs, Hb, HCT and Platelets showed a significant increase after 30 days of topical treatment with BPF in and a corresponding significant decrease in WBCs. Similarly a highly significant increase was observed in RBCs, Hb, HCT and Platelets and a corresponding highly significant decrease in WBCs.

Table 2: Complete blood count (RBCs, Hb, WBCs, and HCT) after 15 and 30 days in topically treatment with BPF and intraperitoneally + topically treatment with BPF groups in comparison with normal and control groups

Groups	RBCs X 10 <sup>12</sup> /l	Hb% g/dl	WBCs x10 <sup>9</sup> /l	Platelets x 10 <sup>9</sup> /l	HCT %
	Mean ± S. D.	Mean ± S. D.	Mean ± S. D.	Mean ± S. D.	Mean ± S. D.
<i>After 15 days</i>					
Normal	6.52 ± 0.09	13.1 ± 0.36	7.7 ± 0.24	392 ± 9.0	39.5 ± 1.18
Normal burned	5.90 ± 0.12	11.1 ± 0.29	10.7 <sup>++a</sup> ± 0.37	388 ± 9.0	37.3 ± 1.11
Diabetic burned (control)	4.22 <sup>-a</sup> ± 0.14	8.3 <sup>-a</sup> ± 0.23	19.3 <sup>-a</sup> ± 0.46	301 <sup>-a</sup> ± 7.0	28.3 <sup>-a</sup> ± 0.91
Topical BPF (Treatment)	4.94 ± 0.18	9.2 ± 0.34	14.5 ± 0.31	327 ± 7.0	34.4 ± 0.76
Topical + i.p. BPF (Treatment)	5.91 <sup>++b</sup> ± 0.12	10.7 <sup>++b</sup> ± 0.32	11.5 <sup>-b</sup> ± 0.36	331 <sup>++b</sup> ± 10.0	36.5 <sup>++b</sup> ± 1.13
<i>After 30 days</i>					
Normal	6.55 ± 0.11	14.2 ± 0.34	7.9 ± 0.26	382 ± 7.0	42.1 ± 1.16
Normal burned	5.76 ± 0.17	11.0 ± 0.31	12.6 <sup>++a</sup> ± 0.34	391 ± 8.0	38.0 ± 1.04
Diabetic burns (control)	3.75 <sup>-a</sup> ± 0.24	7.0 <sup>-a</sup> ± 0.23	21.3 <sup>-a</sup> ± 0.76	283 <sup>-a</sup> ± 9.0	22.2 <sup>-a</sup> ± 1.08
Topical BPF (Treatment)	5.28 <sup>++b</sup> ± 0.12	10.6 <sup>++b</sup> ± 0.28	13.5 <sup>++b</sup> ± 0.33	340 <sup>++b</sup> ± 10.0	36.2 <sup>++b</sup> ± 1.06
Topical + i.p. BPF (Treatment)	6.34 <sup>+++b</sup> ± 0.11	13.3 <sup>+++b</sup> ± 0.34	9.5 <sup>-b</sup> ± 0.33	387 <sup>+++b</sup> ± 11.0	38.2 <sup>+++b</sup> ± 0.97

Results are expressed as mean ± S.D. of 10 animals.

+a = significantly increased from normal at p<0.05

++a = highly significant increased from normal at p<0.01

-a = significantly decreased from normal at p<0.05

--a = highly significant decreased from normal at p<0.01

+b = significantly increased from diabetic burned (control) at p<0.05

++b = highly significant increased from diabetic burned (control) at p<0.01

-b = significantly decreased from diabetic burned (control) at p<0.05

--b = highly significant decreased from diabetic burned (control) at p<0.01

### Effect of BPF isolated from scorpion venom on antioxidant status and MDA concentration in burnt skin tissue of diabetic Guinea pigs:

#### Effect of BPF on antioxidant parameters catalase (CAT), superoxide dismutase (SOD) and nitric oxide (NO):

The activity of CAT and SOD in skin tissue of diabetic burned group after 15 days were significantly decreased (P<0.05) as recorded in table 3 and highly significant decreased (p < 0.01) after 30 days. In addition the concentration of serum NO in burnt diabetic group was significantly decreased (P<0.05) and a highly significant decreased (p < 0.01) as shown in Table 3.

There was a significant increase (P<0.05) in CAT, SOD and serum NO of diabetic burnt group after 30 days of treatment with BPF topically. The treatment with BPF topically and interperitonially showed a significant increase (P<0.05) in NO, SOD activity and CAT activity after 15 days as recorded in table 3 and highly significant increase (p < 0.01) after 30 days, as shown in Table 3.

#### Effect of BPF malondialdehyde level (MDA):

The level of MDA in skin tissue of diabetic burned group was significantly increased (P<0.05) as recorded in table 3 (after 15 days) and a highly significant increased (p<0.01) after 30 days.

In contrast, the treatment with BPF topically recorded a significant decrease ( $P < 0.05$ ) after 30 days as shown in table 3. While treatment with BPF topically and interperitonally of burnt diabetic animals, showed a decrease ( $P < 0.05$ ) and highly significant decrease ( $p < 0.01$ ) after 30 days, as recorded in Table 3.

Table 3: Effect of interperitonally (i.p.) and/or topically treatment with bradykinin potentiating factor (BPF) 1 $\mu$ g/gm isolated from scorpion venom *Buthus occitanus* within 15 and 30 days on MDA, CAT, SOD and NO of burned skin of alloxan-induced diabetic *Guinea Pigs*.

Groups	MDA	CAT	SOD	NO
	Nmol/g	U/ g protein	u/g protein	$\mu$ mol / L
	Mean $\pm$ S.D.	Mean $\pm$ S. D.	Mean $\pm$ S. D.	Mean $\pm$ S. D
<i>After 15 days</i>				
Normal	179 $\pm$ 4.99	3.19 $\pm$ 0.13	79.19 $\pm$ 1.67	44.8 $\pm$ 1.08
Normal burned	200.5 $\pm$ 7.24	2.99 $\pm$ 0.06	70.13 $\pm$ 1.24	37.97 $\pm$ 0.87
Diabetic burned (control)	262.5 <sup>+a</sup> $\pm$ 4.80	1.92 <sup>-a</sup> $\pm$ 0.07	33.49 <sup>-a</sup> $\pm$ 1.76	18.47 <sup>-a</sup> $\pm$ 0.95
Topical BPF (Treatment)	227.4 $\pm$ 6.11	2.01 $\pm$ 0.09	41.89 $\pm$ 1.50	24.89 $\pm$ 1.00
Topical + i.p. BPF (Treatment)	209.2 <sup>-b</sup> $\pm$ 8.79	2.35 <sup>+b</sup> $\pm$ 0.12	51.75 <sup>+b</sup> $\pm$ 2.12	32.16 <sup>+b</sup> $\pm$ 1.01
<i>After 30 days</i>				
Normal	184.0 $\pm$ 6.01	3.39 $\pm$ 0.10	84.02 $\pm$ 2.66	41.08 $\pm$ 0.89
Normal burned	207.6 $\pm$ 11.55	2.77 $\pm$ 0.08	69.46 $\pm$ 1.10	37.12 $\pm$ 1.01
Diabetic burns (control)	400.9 <sup>++a</sup> $\pm$ 7.54	0.91 <sup>-a</sup> $\pm$ 0.11	18.59 <sup>-a</sup> $\pm$ 1.85	12.02 <sup>-a</sup> $\pm$ 1.00
Topical BPF (Treatment)	203.7 <sup>-b</sup> $\pm$ 6.94	2.43 <sup>+b</sup> $\pm$ 0.08	56.31 <sup>+b</sup> $\pm$ 1.80	31.10 <sup>+b</sup> $\pm$ 1.04
Topical + i.p. BPF (Treatment)	191.0 <sup>-b</sup> $\pm$ 9.88	3.17 <sup>++b</sup> $\pm$ 0.10	70.61 <sup>++b</sup> $\pm$ 1.92	37.92 <sup>++b</sup> $\pm$ 1.03

Results are expressed as mean  $\pm$  S.D. of 10 animals.

+a = significantly increased from normal at  $p < 0.05$

++a = highly significant increased from normal at  $p < 0.01$

-a = significantly decreased from normal at  $p < 0.05$

--a = highly significant decreased from normal at  $p < 0.01$

+b = significantly increased from diabetic burned (control) at  $p < 0.05$

++b = highly significant increased from diabetic burned (control) at  $p < 0.01$

-b = significantly decreased from diabetic burned (control) at  $p < 0.05$

--b = highly significant decreased from diabetic burned (control) at  $p < 0.01$

#### Effect of BPF isolated from scorpion venom on serum lipid profile diabetic burned *Guinea Pigs*:

The recorded results in Tables (4) showed that TGs, T. Cholesterol, LDL and vLDL level in diabetic burned male *Guinea pigs* after 15 days increased significantly ( $p < 0.05$ ) when compared with normal and normal burned group. While after 30 days a highly significant ( $p < 0.01$ ) increase in their levels were

recorded. In contrast, HDL level was significantly decreased after 15 days and was highly significant ( $p < 0.01$ ) decreased after 30 days.

Daily topical treatment with BPF for 15 days had a non significant decrease in serum TGs, T. Cholesterol, LDL and vLDL levels in comparing with the diabetic burned group. While after 30 days, the decrease became significant ( $P <$

0.05) in comparing with the corresponding diabetic burned group.

Also the increase of HDL level was non significant while the increase became significant ( $p < 0.05$ ) after 30 days.

Treatment topically and i.p. with BPF for 15 days had a significant decrease ( $p < 0.05$ ) in serum TGs, T. Cholesterol, LDL and vLDL levels in

comparing with the diabetic burned group. While after 30 days, the decrease became highly significant ( $P < 0.01$ ) in comparing also with the corresponding diabetic burned group. Also the increase of HDL level after 15 days was significant ( $p < 0.05$ ) while, the increase became highly significant ( $p < 0.01$ ) after 30 days.

Table 4: Effect of interperitoneally (i.p.) and/or topically treatment with bradykinin potentiating factor (BPF)  $1\mu\text{g/gm}$  isolated from scorpion venom *Buthus occitanus* within 15 and 30 days on (Chol., TGs, HDL-C, LDL-C and vLDL) of burned skin of alloxan-induced diabetic *Guinea pigs*.

Groups	Cholesterol (TC) mg/dl	HDL mg/dl	LDL mg/dl	Vldl mg/dl	Triglycerides (TGs) mg/dl
	Mean $\pm$ S. D.	Mean $\pm$ S.D.	Mean $\pm$ S. D.	Mean $\pm$ S. D	Mean $\pm$ S. D
<i>After 15 days</i>					
Normal	102.0 $\pm$ 5.16	44.33 $\pm$ 0.77	36.8 $\pm$ 1.84	19.5 $\pm$ 1.10	97.7 $\pm$ 6.20
Normal diabetic	93.7 $\pm$ 4.7	72.7 $\pm$ 1.06	34.0 $\pm$ 2.37	19.33 $\pm$ 1.03	95.5 $\pm$ 4.07
Diabetic burns (control)	156.0 <sup>+a</sup> $\pm$ 4.75	24.0 <sup>-a</sup> $\pm$ 0.67	101.6 <sup>+a</sup> $\pm$ 3.06	30.5 <sup>+a</sup> $\pm$ 1.08	152.0 <sup>+a</sup> $\pm$ 4.7
Topical BPF (Treatment)	150.5 $\pm$ 3.76	25.8 $\pm$ 0.66	95.8 $\pm$ 4.00	29.2 $\pm$ 0.72	146.0 $\pm$ 4.5
Topical + i.p. BPF (Treatment)	133.0 <sup>b</sup> $\pm$ 3.31	33.6 <sup>b</sup> $\pm$ 0.82	73.2 <sup>b</sup> $\pm$ 2.79	26.1 <sup>b</sup> $\pm$ 0.71	130.7 <sup>b</sup> $\pm$ 5.9
<i>After 30 days</i>					
Normal	92.3 $\pm$ 4.23	46.5 $\pm$ 1.14	40.0 $\pm$ 3.21	20.5 $\pm$ 0.99	102.5 $\pm$ 5.11
Normal burned	100.0 $\pm$ 6.24	44.9 $\pm$ 1.04	36.7 $\pm$ 3.18	21.0 $\pm$ 0.67	106.0 $\pm$ 4.34
Diabetic burns (control)	178.0 <sup>+++</sup> $\pm$ 3.82	18.7 <sup>-a</sup> $\pm$ 0.59	191.8 <sup>+++</sup> $\pm$ 4.36	34.2 <sup>+++</sup> $\pm$ 1.14	170.0 <sup>+++</sup> $\pm$ 5.13
Topical BPF (Treatment)	140.0 <sup>-b</sup> $\pm$ 3.31	27.0 <sup>+b</sup> $\pm$ 0.78	85.4 <sup>-b</sup> $\pm$ 2.17	27.6 <sup>-b</sup> $\pm$ 0.77	138.0 <sup>-b</sup> $\pm$ 3.48
Topical + i.p. BPF (Treatment)	116.7 <sup>-b</sup> $\pm$ 4.03	38.0 <sup>+++</sup> $\pm$ 0.91	54.7 <sup>-b</sup> $\pm$ 3.45	24.0 <sup>-b</sup> $\pm$ 1.28	120.0 <sup>-b</sup> $\pm$ 4.09

Results are expressed as mean  $\pm$  S.D. of 10 animals.

+a = significantly increased from normal at  $p < 0.05$

+++ = highly significant increased from normal at  $p < 0.01$

-a = significantly decreased from normal at  $p < 0.05$

--a = highly significant decreased from normal at  $p < 0.01$

+b = significantly increased from diabetic burned (control) at  $p < 0.05$

+++b = highly significant increased from diabetic burned (control) at  $p < 0.01$

-b = significantly decreased from diabetic burned (control) at  $p < 0.05$

--b = highly significant decreased from diabetic burned (control) at  $p < 0.01$

## DISCUSSION

The red blood cells count can be considered as a biological index for the determination of the effect of diabetic anemia. The present study demonstrated that alloxan induced diabetes which resulted in a significant decrease in red blood cells count, hemoglobin and hematocrit when compared with the normal group. The significant decrease of erythrocytic count and blood hemoglobin content in the present study is in the accordance with the finding of (Salman, 2008).

The improvement of RBCs, HB, PCV and platelets count in the present study after treatment with BPF, are in accordance with finding of (Salman, 2002 and 2009), who showed that injection of BPF in sub-lethally-irradiated and non-irradiated growing guinea pig accelerated the generation of thymus and spleen cellularity and completely recovered.

These observations and investigations were the base for the hypothesized ameliorating activity of BPF in the present study.

Since oxidative damage of tissues is a major pathogenesis of many human diseases including diabetes, this study will summarize the available data which indicated the role of the preventive effect of BPF on the development of burnt wound healing in diabetic animal by mechanisms may include the role of BPF of endogenous antioxidant enzymes of burned animals (Mikrut *et al.*, 2001).

There is the fact that, glucose auto-oxidation is considered to be a source of free radicals and hydrogen peroxide (Ceriello *et al.*, 1992), These processes could contribute to the elevated levels of plasma peroxide and MDA (Olczyk *et al.*, 1994), and hence these free radical may delay the healing of burned skin. However, BPF injection to the diabetic animals, for 4 weeks, controlled the glucose levels and improved the antioxidant status, which hastened the

dermal wound healing process. The mechanisms by which BPF hastened the healing process was Activation of bradykinin and consequently activation of B2R, which results in the formation of nitric oxide (NO), a potent scavenger molecule, which could reduce the accumulation of reactive oxygen species (ROS) (Allard *et al.*, 2007). The other mechanism is the role of BPF in enhancing connective tissue synthesis, cell division and fiber secretion, re-epithelization, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), NO, serotonin, prostacyclin I<sub>2</sub> (PGI<sub>2</sub>) production and stimulating chemotaxis as well as healing-inducing inflammation (Salman, 1995; Özotürk, 2001 and Qiu *et al.*, 2002).

It is worth to mention that, in this study, the treatment with BPF was effective in the dermal healing in both cases topical and interperitoneal, and that may be sustain, the BPF may be considered potentially in the treatment of severe skin burns.

While diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities and raised basal metabolic rate (Owu *et al.*, 2006), the present study will summarize the beneficial effects of BPF on the lipid profile of the diabetic burned guinea pigs.

According to (Grover *et al.*, 2000), alloxan causes a massive reduction in insulin release by the destruction of  $\beta$ -cells of the islets of langerhans, thereby inducing hyperglycemia. Consequently, insulin deficiency leads to various metabolic alterations in the animals as increased blood glucose and increased cholesterol (Shanmugasundaram *et al.*, 1983).

According to (Salman *et al.*, 2002) the nonapeptide bradykinin has a wide range of biological effects on a number of different tissues and organs.

The present study has revealed decreases in serum cholesterol and triglycerides levels in diabetic burned

guinea pigs following BPF treatment. These findings are in agreement with other investigators who recorded the decrease in serum cholesterol and triglycerides levels evenenomated rats was in laboratory animals injected with viper snake venoms. They suggested that, the snake venom might have mobilized the lipids from adipose and other tissues by activation the lipolytic enzymes which could have split tissue lipid with the liberation of free fatty acids (Al-Jammaz, 2002 and Salman, 2010)

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## ARABIC SUMMARY

دراسات فسيولوجية على - مستخلص من سم العقرب - كعامل منشط للبراديكينين على الحروق الجلدية لخنازير غينيا المصابة بسكر الدم المستحدث بواسطة الألوكران.

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رغم ما هو معروف من التأثيرات السمية لسُموم العقارب والثعابين السامة، فإن هناك كثير من الأبحاث الحديثة تعمل على استخلاص مكونات مفيدة من هذه السموم والتي من بينها عامل منشط للبراديكينين الذي تم فصله وتعريفه في دراسات سابقة من سموم العقارب والثعابين (*Bradykinin potentiating factor, BPF*) وقد أشارت الدراسات السابقة إلي أن هذا المستخلص يزيد من سرعة النشاط الانقباضي في الخلايا العادية وخلايا نخاع العظام و كان لاستخدام هذا المستخلص دور حاسم وفعال في علاج الحيوانات المشععة من ذلك التأثير المدمر للإشعاع. كما ثبتت فعاليته في علاج الحروق. ومن المعروف أن الجروح أو الحروق السكرية تستغرق وقتاً طويلاً حتى تلتئم تماماً. لذلك يهدف هذا البحث إلى دراسة تأثير هذا المستخلص (*BPF*) في علاج حروق الجلد لذكور خنازير غينيا المصابة بداء السكري المستحدث بواسطة الألوكران.

ومن ثم فقد تم تقسيم الحيوانات (خنازير غينيا) إلى أربع مجموعات :  
**المجموعة الأولى:** حقنت بمحلول فسيولوجي (كلوريد الصوديوم) (0.9% NaCl) وكانت تلك المجموعة العادية.

**أما المجموعة الثانية:** حقنت بمحلول فسيولوجي وتم حرق الجلد بحروق متساوية (قياسية) على الظهر ثم تركت بدون علاج. وعرفت بالمجموعة المصابة بحروق جلدية.

**المجموعة الثالثة:** حقنت ب ٧٥٠ ملجم لكل كيلوجرام من وزن الجسم لاستحداث مرض السكر ثم حرق الجلد بحروق متساوية (قياسية) ثم تركت بدون علاج.

**المجموعة الرابعة:** حقنت ب ٧٥٠ ملجم لكل كيلوجرام من وزن الجسم لاستحداث مرض السكر ثم حرق الجلد بحروق متساوية (قياسية) ثم عولجت سطحياً فقط باستخدام المستخلص.

**المجموعة الخامسة:** حقنت ب ٧٥٠ ملجم لكل كيلوجرام من وزن الجسم لاستحداث مرض السكر ثم حرق الجلد بحروق متساوية (قياسية) ثم عولجت باستخدام المستخلص سطحياً وبالحقن ب(١) ميكروجرام لكل جرام من وزن الجسم من المستخلص السمي (*BPF*).

ولمعرفة مدى التغييرات الدموية و البيوكيميائية ومتابعة عملية الشفاء والالتئام خلال مدة التجارب. فقد ذبحت الحيوانات بعد ١٥ و ٣٠ يوماً من العلاج و تم جمع جزء من عينات الدم في أنابيب ايدتا وذلك لعد كريات الدم الحمراء و خلايا الدم البيضاء والصفائح الدموية وتقدير محتوى الهيموجلوبين ونسبة الهيماتوكريت. وكذلك تم فصل المصل لتقدير كلا من مستوى الدهون الثلاثية والكوليسترول الكلي والكوليسترول عالي الكثافة (*HDL*) وكذلك الكوليسترول منخفض الكثافة (*LDL*) والنيتريك اوكسيد (*nitric oxide*) مستوى السكر. وتم أيضاً نزع عينات متساوية في الوزن من الجلد لقياس مستوى مالون داي أدهيد (*MDA*) ونشاط انزيمي الكاتاليز (*CAT*) وسوبر أكسيد دسميوتيز (*SOD*) في أنسجة الجلد لخنازير غينيا.

وقد كان عد كريات الدم الحمراء والصفائح الدموية ومحتوى الهيموجلوبين ونسبة الهيماتوكريت منخفض جداً في المجموعة الثالثة (الغير معالجة) يقابله زيادة كبيرة في عد كريات الدم البيضاء. بينما انخفض نشاط كلا من إنزيم الكاتاليز، السوبر أكسيد ديسموتيز و النيتريك اوكسيد وارتفعت كثافة المالون داي أدهيد مما يدل على وجود إجهاد تأكسدي في أنسجة الحيوانات الغير معالجة. وكان مستوى الكوليسترول، الدهون الثلاثية و الكوليسترول منخفض الكثافة مرتفع جداً نتيجة الحقن بالالوكزان المسبب للسكري وترتب على ذلك انخفاضاً ملحوظاً أيضاً في مستوى الكوليسترول عالي الكثافة. ومما جذب الانتباه أن المجموعات المعالجة بمستخلص سموم العقارب (*BPF*) سطحياً فقط أو سطحياً وبالحقن أظهرت تحسناً ملحوظاً في عد كريات الدم الحمراء والبيضاء والصفائح الدموية و محتوى الهيموجلوبين ونسبة الهيماتوكريت مقارنة بالمجموعة الغير معالجة. وإن كان التحسن في المجموعة المعالجة سطحياً بطيء نسبياً عن المجموعة المعالجة سطحياً بالإضافة إلى الحقن بالمستخلص.

أما بالنسبة للإجهاد التأكسدي فقد كان للمستخلص السمي (*BPF*) تأثيراً فعالاً في خفض الزيادة الملحوظة في تركيز المالون داي أدهيد ورفع نسبة نشاط انزيمي الكاتاليز وسوبر أكسيد دسميوتيز في أنسجة الجلد لخنازير غينيا بالإضافة إلى رفع مستوى النيتريك اوكسيد بمصل الدم.

وكان التأثير العلاجي للمستخلص واضحاً أيضاً في تحسين مستوى الدهون بالدم للمجموعات المعالجة مقارنة بالمجموعة الثالثة (الغير معالجة).

وم سبق يتبين أن هذا المستخلص السمي (*BPF*) - وهو ببينيد - له تأثير منشط للأداء الفارماكولوجي لمركب البراديكينين والذي ينطلق أثناء الحروق من الأنسجة ومن ثم يمكن الاعتقاد بأن هذا المستخلص السمي بطريقة مباشرة أو غير مباشرة ينشط عوامل النمو الخلوي في الجلد المحروق للخنازير المصابة بداء السكري ويعجل عملية الشفاء والالتئام.