Sohag Journal of Sciences
*An International Journal*

# Protective effect of bradykinin potentiating factor on haematological parameters of diabetic male albino rats

*El-Sabry Abu Amra[1], Soheir A. Abd El Raheem[1], Tito N. Habib, Hossam A. AboElkhair[1]*

[1]Zoology Department, Faculty of Science, Sohag University, Sohag 82524, Egypt

Received: 11 Apr. 2022, Revised: 27 Apr. 2022, Accepted: 28 Apr. 2022.
Published online: 1 May. 2022

**Abstract:** The aim of the study was to evaluate the protective effect of bradykinin potentiating factor extracted from honey bee venom, *Apis mellifera* on hematological parameters in diabetic rats. Forty adult male albino rats classified into five groups, control group (G1), STZ diabetic group (G2), BPF group (G3), pre-treatment group BPF+STZ (G4) and post-treatment groupSTZ+BPF (G5). After 30 days from treatment, rats were sacrificed and dissected and the blood were collected from heart. In blood samples, RBCs, HGB, HCT, PLTs, MCV, MCH, WBCs and differential leucocyte count were examined. In the study, STZ diabetic group (G2) showed a significant ($P<0.05$) and highly significant ($p<0.001$) decrease in all tested haematological parameters except MCV and MCH, which showed non-significant change ($P>0.05$) compared to control value. These parameters showed improvement in pre- and post- treated groups as compared to diabetic group. In conclusion our results revealed a Protective effect of bradykinin potentiating factor on haematological parameters of diabetic male rats

**Keywords**: Diabetes Mellitus – Bradykinin Potentiating Factor – Haematological parameters – Streptozotocin

## 1 Introduction

Diabetes mellitus (DM) is a metabolic disorder of the endocrine system. It has been recognized as a clinical syndrome since ancient times and remains a crippling global health problem today. The disease is found in all parts of the world. It is a common chronic disease affecting up to 6 % of the world population. According to World Health Organization (WHO) projections, the number of people with DM is rapidly increasing worldwide and has become of major public health concern[1] .

Since ancient times, many natural products have played and continue to play a valuable role in the treatment of various diseases and in drug discovery processes. It has remained a source of new compounds with diversified structural arrangement possessing interesting biological activities for various disease treatments[2]**.** The using of natural products has been focused in recent years as honey bee venom for treatment of different diseases in traditional medicine[3], it contains several biochemical or pharmacologically active substances including polypeptides, enzymes, amines, lipids and amino acids [4-6] that express its potency and medical efficacy. Bee venom was revealed to be effective in treat a variety of diseases and complications such as arthritis, rheumatism, back pain, cancerous tumors and skin disease [7-9]. Also, bee venom has a curative effect on oxidative stress induced by γ – irradiation [10] and protective effect against acute pancreatitis[11], hepatoprotective characteristics [12] and anticancer and radioprotective characteristics[13,14].Moreover it increases coronary and peripheral blood circulation regulates pro-inflammatory cytokines in hepatocyte , liver fibrosis and renal tubular injury and decrease in the complications of diabetes and multiple sclerosis [4,6,14,15].

Animal venom mostly contains characteristically small bioactive peptides which referred to as bradykinin potentiating factors (BPFs). These factors have been implicated in multiple physiological processes such as potentiate the pharmacological effect of bradykinin both in *vivo* and in *vitro* [16] , promote vasodilation , reduce blood pressures and increase vascular permeability and vascular exchange [17-19], induce cell division and cell differentiation [20,21] , induce prostaglandin biosynthesis and act as a cytoprotective regulatory substances[22].

Diabetes mellitus (DM) characterized by hyperglycemia [23] .Sustained hyperglycemia has been shown to progress the pathology of cardiac damage through haematological changes which include haemolysis of erythrocytes consequent decrease in red blood cell (RBC) count and haemoglobin (Hb) concentrations which is associated with the development of diabetic anaemia and

* Corresponding author E-mail: aboelkhair.h.a@science.sohag.edu.eg

increase the risk of cardiovascular complications if left untreated [24]. The erythrocyte membrane plays as essential role in the regulation of surface deformability and flexibility [25,26]. Hyperglycemia has been shown to induce modifications and impairment of the erythrocyte membrane via increased production of reactive oxygen species (ROS). ROS causes nonenzymatic glycosylation of the RBC membrane proteins resulting in the non-specific aggregation of protein molecules and alters the protein-protein and protein-lipid interaction leading to the modification and disrupts the integrity /and symmetry of the erythrocyte membrane[27,28]. A loss of membrane symmetry increases erythrocyte aggregation, decreases mobility of the RBCs and increases blood viscosity , which consequently elevates arterial pressure, thus increasing cardiovascular complications [29,30]. Furthermore, chronic hyperglycemia induced ROS production such as hydrogen peroxide ($H_2O_2$) crosses the erythrocyte membrane and oxidizes heme proteins , which have been shown to lead to the progressive loss of deformability and increased osmotic fragility of RBCs [31]. Consequently, diabetic patients with cardiovascular complications present with a decrease in Hb concentrations[32]. In addition , these changes induce the rapid initiation of apoptosis in damaged erythrocytes, decreasing the oxygen-carrying capacity of erythrocyte due to the increased haemolysis rate thereby significantly decreasing their lifespan [33]. Moreover, diabetic nephropathy caused by ongoing hyperglycaemia results in the destruction of the renal interstitium which is composed of interstitial peritubular fibroblasts, further causing the impairment in erythropoietin (EPO) production [34,35] which associated with a decrease in the production of RBC by the bone marrow and consequently diabetic anaemia [36].

Streptozotocin is a well known chemical that suppresses the immune system by damaging WBC and certain organs in the body[37]. The intraperitoneal injection of STZ in to rats significantly reduced the WBC count and its differentials such as basophils, eosinophils, neutrophils, monocytes and lymphocytes. This reduction could be linked to suppression of leucoytosis from the bone marrow which may account for poor defensive mechanism against infection[38]. Consequently, they might have effects on the immune system and phagocytic activity of the animals[39].

Platelets aggregation ability has been shown in diabetic patient with long term poor glycaemic control due to lack or deficiency of insulin [40].

Consequently, the study aims to investigate the protective effect of bradykinin potentiating factor on the induced complications of haematological parameters of diabetic animals.

## 2 Results and Discussion

The results indicated that, STZ diabetic group (G2) showed a significant ($P<0.05$) and highly significant ($p<0.001$) decrease in all tested haematological parameters except MCV and MCH, which showed non-significant change ($P>0.05$) compared to control value. In contrast to that, BPF treated group (G3) showed a significant and highly significant increase in WBCs, lymphocytes, granulocytes, monocytes and platelets and a non-significant change in all other tested hematological parameters (RBCs, Hgb, HCT, MCV and MCH) compared to control group. while the results showed that, pre-treatment group BPF plus STZ (G4) induced a significant increase in WBCs and PLT and highly significant increase in lymphocytes, but a non-significant change were detected in other tested haematological parameters compared to control group. Also, the post-treatment group STZ plus BPF (G5), showed a significant increase in WBCs, granulocytes, MCH and a highly significant increase in monocytes, and a non-significant change were observed in all of the other haematological parameters compared to control value. In addition, the result revealed that, the treated and co-treated (G3, G4 and G5) showed a significant and highly significant increase in tested haematological parameters except MCV and MCH which decreased significantly compared to STZ diabetic group (G2). These data revealed that, a highly significant improvement in all tested parameters in treated and co-treated groups compared to STZ diabetic group. These data observed in tables (1, 2, 3 and 4) and figures (1, 2, 3, 4, 5, 6, 7, 8, 9 and10).

**Table 1:** Effect of BPF (2.314 mg/kg b.w) on WBCs, Lymphocytes and granulocytes count of male albino rats induced by Streptozotocin (45 mg/kg b.w) for 30 days in different groups.

| parameters | | Control | STZ | BPF | BPF+STZ | STZ+BPF |
|---|---|---|---|---|---|---|
| WBCs $\times 10^3/\mu L$ | Mean ±SE | 7.275±0.78 | $3.1^{a**}$ ± 0.37 | $11.725^{ab**}$ ± 2.45 | $9.375^{a*b**}$ ±1.7 | $9.35^{a*b**}$ ±1.19 |
| | % of change (1) | | -57.38 | +61.16 | +28.86 | +28.52 |
| | % of change (2) | | | +278.22 | 202.4 | +201.6 |
| Lymphocytes $\times 10^3/\mu L$ | Mean ±SE | 3.2±0.08 | $1.825^{a**}$ ±0.34 | $5.775^{ab**}$ ±1.03 | $4.6^{ab**}$ ±0.9 | $3.75^{b**}$ ±0.37 |
| | % of change (1) | | -42.96 | +80.46 | +43.75 | +17.18 |
| | % of change (2) | | | +216.43 | +152.05 | +105.47 |
| Granulocytes $\times 10^3/\mu L$ | Mean ±SE | 1.9±0.37 | $0.475^{a**}$ ±0.04 | $2.525^{a*b**}$ ±0.32 | $2.275^{b**}$ ±0.26 | $2.425^{a*b**}$ ±0.55 |
| | % of change (1) | | -75 | +32.89 | +19.73 | +27.63 |
| | % of change (2) | | | +431.57 | +378.94 | +410.52 |

Significant difference between control and different groups. N=8.

* = $P<0.05$ Significant. ** = $P<0.001$ Highly Significant. Non-Significant$P>0.05$

% of change (1) different from normal control group G1. % of change (2) different from Streptozotocin group G2. a=significant different from control group. b= significant different from Streptozotocin group.

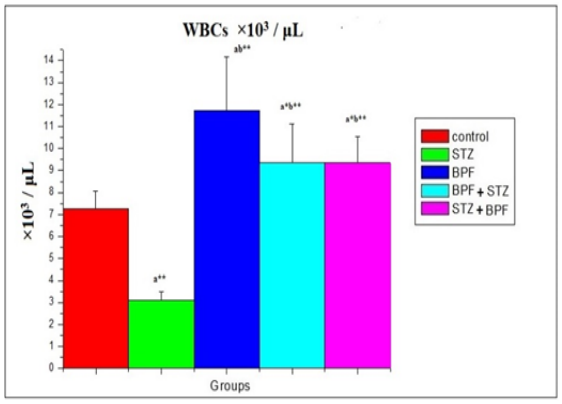


**Figure 1:** Effect of BPF (2.314 mg/kg b.w) on WBCs count of male albino rats induced by Streptozotocin (45 mg/kg b.w) for 30 days in different groups.

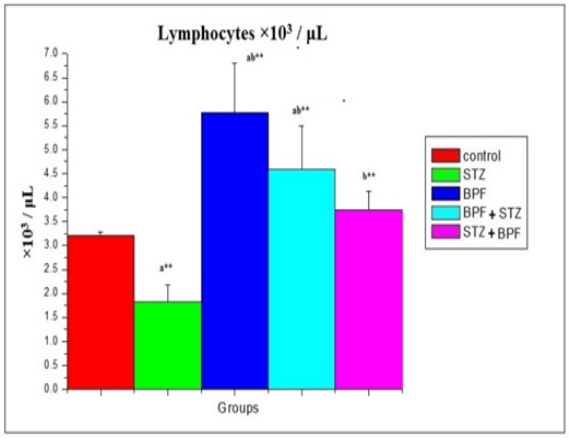


**Figure 2:** Effect of BPF (2.314 mg/kg b.w) on lymphocytes count of male albino rats induced by Streptozotocin (45 mg/kg b.w) for 30 days in different groups.

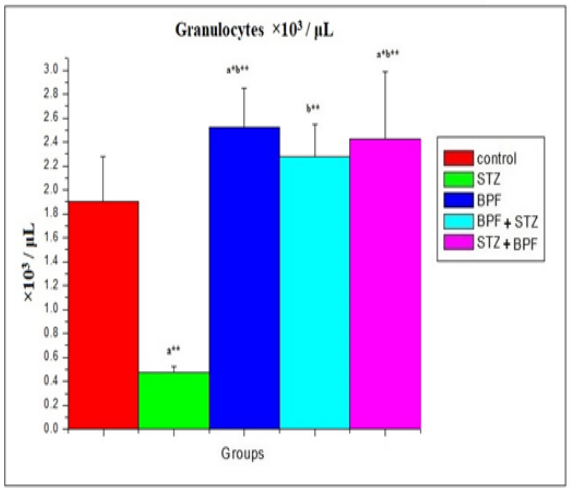


**Figure3:** Effect of BPF (2.314 mg/kg b.w) on granulocytes count of male albino rats induced by Streptozotocin (45 mg/kg b.w) for 30 days in different groups.

**Table 2:** Effect of BPF (2.314 mg/kg b.w) on Monocytes, RBCs count and Hgb level of male albino rats induced by Streptozotocin (45 mg/kg b.w) for 30 days in different groups.

| parameters | | Control | STZ | BPF | BPF+STZ | STZ+ BPF |
|---|---|---|---|---|---|---|
| Monocytes ×10³ / µL | Mean ±SE | 2.175±0.70 | $0.8^{a**}$ ±0.09 | $3.425^{ab**}$ ±0.58 | $2.5^{b**}$ ±0.54 | $3.125^{ab**}$ ±0.56 |
| | % of change (1) | | -63.21 | +57.47 | +14.94 | +43.67 |
| | % of change (2) | | | +328.12 | +212.5 | +290.62 |
| RBCs ×10⁶/ µL | Mean ±SE | 5.825±0.8 | $3.64^{a*}$ ±0.2 | $6.85^{b**}$ ±0.7 | $5.925^{b**}$ ±0.8 | $6.325^{b**}$ ±0.4 |
| | % of change (1) | | -37.5 | +17.59 | +1.716 | +8.58 |
| | % of change (2) | | | +88.18 | +62.77 | +73.76 |
| Hgb g / dL | Mean ±SE | 13.05 ±1.5 | $8.55^{a*}$ ±0.4 | $13.4^{b**}$ ±1.06 | $11.53^{b*}$ ±0.9 | $10.925^{b*}$ ±0.5 |
| | % of change (1) | | -34.48 | +2.68 | -11.6 | -16.28 |
| | % of change (2) | | | +56.72 | +34.85 | +27.77 |

Significant difference between control and different groups. N=8.
* = P<0.05 Significant. ** = P<0.001 Highly Significant. Non-SignificantP>0.05
% of change (1) different from normal control group G1. % of change (2) different from Streptozotocin group G2. a=significant different from control group. b= significant different from Streptozotocin group.

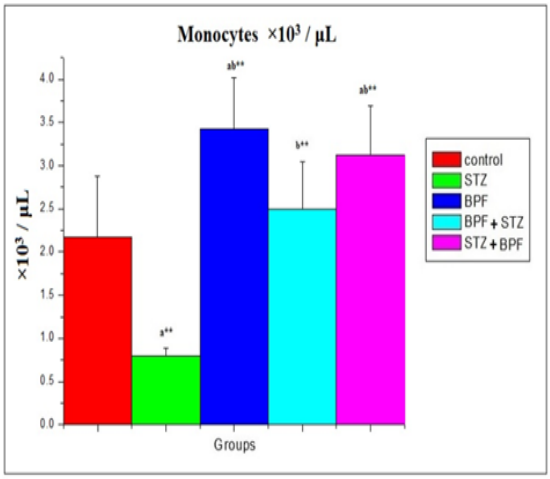

**Figure 4 :** Effect of BPF (2.314 mg/kg b.w) on monocytes count of male albino rats induced by Streptozotocin (45 mg/kg b.w) for 30 days in different groups.

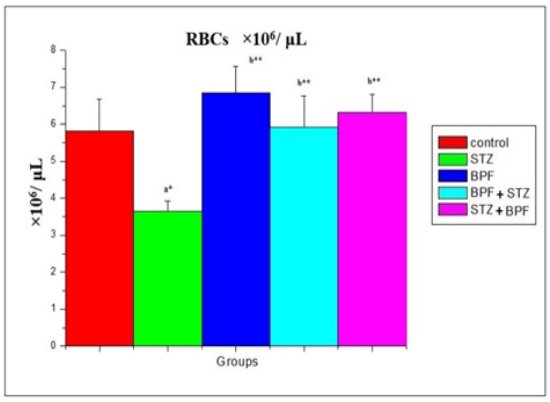


**Figure 5:** Effect of BPF (2.314 mg/kg b.w) on RBCs count of male albino rats induced by Streptozotocin (45 mg/kg b.w) for 30 days in different groups.

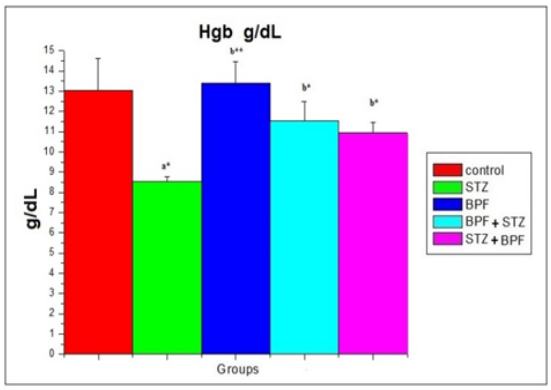


**Figure 6 :** Effect of BPF (2.314 mg/kg b.w) on Hgb level of male albino rats induced by Streptozotocin (45 mg/kg b.w) for 30 days in different groups.

**Table 3** : Effect of BPF (2.314 mg/kg b.w) on HCT and MCV values of male albino rats induced by Streptozotocin (45 mg/kg.bw) for 30 days in different groups.

| Parameters | | Control | STZ | BPF | BPF+STZ | STZ+BPF |
|---|---|---|---|---|---|---|
| HCT % | Mean ±SE | 39.85±4.28 | $27.5^{a*}$ ±1.17 | $40.825^{b**}$ ±3.08 | $38.36^{b**}$ ±2.89 | $32.5^{b*}$ ±1.54 |
| | % of change (1) | | -30.99 | +2.44 | -3.73 | -18.44 |
| | % of change (2) | | | +48.45 | +39.49 | +18.18 |
| MCV Fl | Mean ±SE | 64.5±6.19 | 70.457.94 | $57.15^{b*}$±7.66 | $51^{b*}$±6.69 | $50.52^{b*}$±5.58 |
| | % of change (1) | | +9.22 | -11.39 | -20.9 | -21.67 |
| | % of change (2) | | | -18.87 | -27.60 | -28.28 |

Significant difference between control and different groups. N=8.

* = P<0.05 Significant. ** = P<0.001 Highly Significant. Non-SignificantP>0.05

% of change (1) different from normal control group G1.

% of change (2) different from Streptozotocin group G2.

a=significant different from control group.

b= significant different from Streptozotocin group.

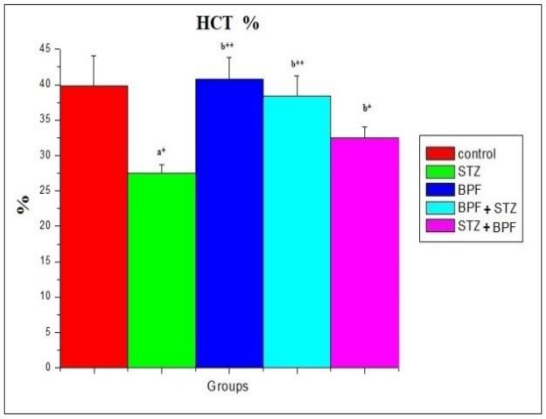


**Figure 7:** Effect of BPF (2.314 mg/kg b.w) on HCT value of male albino rats induced by Streptozotocin (45 mg/kg.bw) for 30 days in different groups.

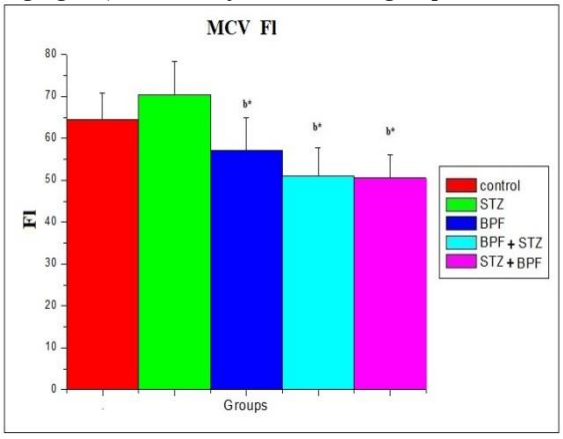


**Figure 8:** Effect of BPF (2.314 mg/kg b.w) on MCV value of male albino rats induced by Streptozotocin (45 mg/kg.bw) for 30 days in different groups.

**Table 4 :** Effect of BPF (2.314 mg/kg b.w) on MCH value and PLTs count of male albino rats induced by Streptozotocin (45 mg/kg.bw)for 30 days in different groups.

| Parameters | | Control | STZ | BPF | BPF+STZ | STZ+BPF |
|---|---|---|---|---|---|---|
| MCH Pg | Mean ±SE | 23.5±2.62 | 25.97±2.73 | $20^{b*}$±3.30 | $20.54^{b*}$±5.57 | $17.8^{ab*}$±1.73 |
| | % of change (1) | | +10.51 | -14.89 | -12.57 | -24.25 |
| | % of change (2) | | | -22.98 | -20.88 | -31.45 |
| PLT×$10^3$/µL | Mean ±SE | 246.75±42.17 | $172.5^{a*}$±15.5 | $318.75^{a*b**}$±19.37 | $302^{a*b**}$±1.95 | $245.75^{b**}$±14.4 |
| | % of change (1) | | -30.09 | +29.17 | +22.39 | -0.4 |
| | % of change (2) | | | +84.78 | +75.07 | +42.46 |

Significant difference between control and different groups. N=8.
* = P<0.05 Significant. ** = P<0.001 Highly Significant. Non-Significant P>0.05
% of change (1) different from normal control group G1.
% of change (2) different from Streptozotocin group G2.
a=significant different from control group.
b= significant different from Streptozotocin group.

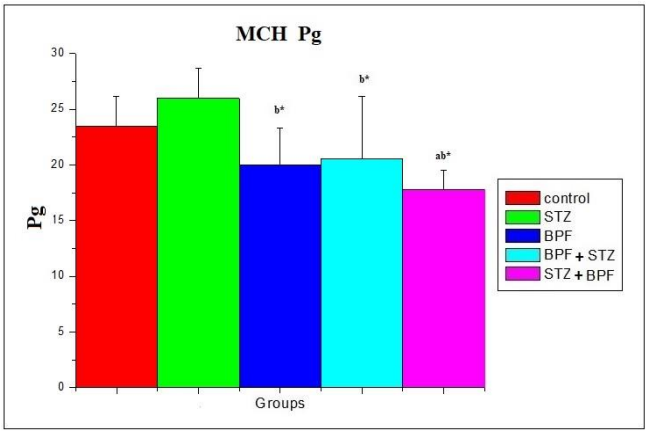


**Figure 9 :** Effect of BPF (2.314 mg/kg b.w) on MCH value of male albino rats induced by Streptozotocin (45 mg/kg.bw)for 30 days in different groups**.**

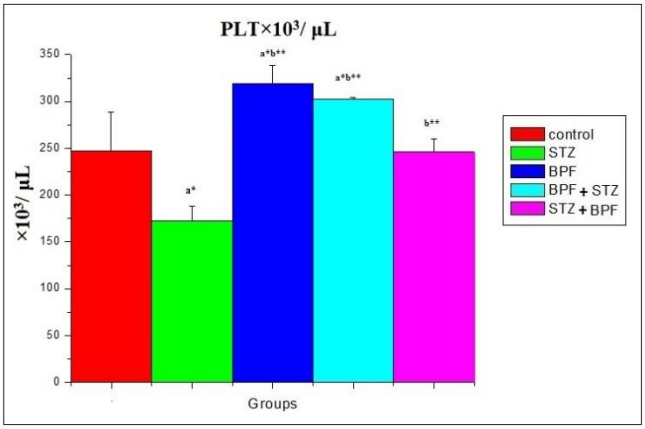


**Figure 10 :** Effect of BPF (2.314 mg/kg b.w) on PLTs count of male albino rats induced by Streptozotocin (45 mg/kg.bw)for 30 days in different groups.

The data obtained from the present investigation revealed a significant decrease in red blood cells (RBCs) count, hemoglobin (Hb) and hematocrit (HCT) values, white blood cells (WBCs) count and its differentials (basophils, eosinophils, neutrophils, monocytes and lymphocytes) and platelets count in the positive control group as compared to the negative control group.

The significant decrease in RBCs count and its related indices in the STZ induced diabetic group is similar to the result observed by many investigators which reported that, hyperglycaemia through the increased formation of ROS has been shown to impaire RBCs deformability, further causing an increase in RBCs haemolysis [41,42]. The impairment in RBCs deformability is correlated with haematological changes which include reduced RBCs count, Hb concentration, HCT levels, MCV, MCHC and RDW concentration as demonstrated by the hyperglycaemic STZ -induced diabetic animals in our study [43,44]. Increased ROS formation causes nonenzymatic glycosylation of proteins on the erythrocyte membrane , cross-linking of membrane lipids and inactivation of RBCs antioxidant enzymes [44]. Furthermore , hyperglycemia results in the development of diabetic anaemia [35] which causes a reduction in RBCs viscosity and increases aggregation and agglutination within the blood vessels, which consequently increases the formation of atherosclerosis plaque[34]. In addition, diabetic nephropathy caused by ongoing hyperglycemia results in the destruction of the renal interstitium causing the impairment in the erythropoietin (EPO) production [34,35], this is associated with a decrease in the production of RBCs by the bone marrow and consequently diabetic anaemia [36]. In addition , this effect may be related to the inhibition of mitochondrial protein synthesis or DNA damage that occurs in the haemopoietic stem cells leading to a severe reduction in proliferation which in turn inhibits erythropoiesis[45,46]**.**

The significant decrease in the WBCs count and its differentials such as basophils, eosinophils, neutrophils, monocytes and lymphocytes in the STZ- induced diabetic groups may be attributed to the effect of streptozotocin which is well known chemical that suppresses the immune system by damaging WBCs and certain organs in the body [47] . This is in agreement with Oyedemi *et al*.,[38] who reported that the reduction of these parameters could be linked to suppression of leukocytosis from the bone marrow which may account for poor defensive mechanisms against infection. Consequently, they might have effects on the immune system and phagocytic activity of the animals[39].

In this study platelets count significantly decreased in the diabetic group. This reduction indicates suppression of

haemopoiesis as a result of STZ application and hyperglycemia[48].

In contrast, the results illustrated that the post or pretreatment of positive control group with BPF extracted from the bee venom showed a significant alteration in all the above haematological parameters. This improvement in haematological parameters may be due to the effect of this factor which has the potential of ameliorating the toxic effects of STZ possibly by acting as an anti-inflammatory agent. In support of this, several compounds or natural products extracted from marine organisms were recognized to exert an activity against human pathologies[49,50] . Also, it has influenced on some diseases and to be anti-inflammatory, antiplatelet and antitumor as reported [51-53].

Moreover, the improvement of the erythrocyte count accompanied with an improvement in the Hb and HCT may be related to the ability of this factor to potentiate the effects of bradykinin (BK) in the mammalian cells. Because BK is considered as an important mediator of the inflammatory response in many organs and it can activate the kinin B1 receptor (bradykinin receptor B1) which expressed only as a result of tissue injury. The B1 receptor plays an important role in chronic pain and inflammation [54].In addition, Chihara *et al*., ; Enjalbert *et al*., ; Frawley and Neill, and Vijayan *et al*., [55-58] demonstrated that BK and classical hormonal transmitters stimulate the synthesis or release of prolactin and growth hormones, which in turn increase protein synthesis [59,60], stimulate the proliferation of mammalian cells [61] and differentiation of bone marrow hematopoietic cells [62] leading to the erythrocyte formation and improvement. Furthermore, BK stimulates prostaglandin release and synthesis [22]which may be promote the observed effects through the stimulation of kidney erythropoietin leading to enhanced erythropoiesis [63] and consequently improving Hb and HCT values [64].

Furthermore, the increase and improvement in the leucocytes and its different cells may be due to a direct effect of factor extracted from bee venom which stimulates and enhanced the lymphoid organs leading to an increase of lymphocytes count[65] . On the other hand , the endogenous BK which potentiating by this factor enhances prostaglandin synthesis [66] which have a regulatory role in myelopoiesis[67] leading to an increase and improvement in the granulocytes and monocytes counts as observed in the present study[68].

The improvement of platelet count may be due to the effect of prostaglandins which are intimately involved in the control of platelet aggregation [69,70]. This control seems to be exercised via modulation of the levels of cyclic adenosine monophosphate within the platelet[71].

## 3 Experimental

### Chemicals

Streptozotocin≥75% α-anomer basis, ≥98% (HPLC) powder (Sigma-Aldrich ), Bee venom of *Apis mellifera* was obtained from commercial wild bee culture (Tanta government), north Egypt. Bradykinin potentiating factor (BPF) was isolated from bee venom according to the chemical methods of Ferreira,[72].

### Animals groups

Forty adult male albino rats (*Rattus rattus*), weighing approximately 180–200 gm. Animals were kept in the laboratory under normal conditions of light, temperature, and humidity with the access of food and water. Animals were classified into five groups (G1-G5) with eight animals for each group. A control represented (G1) was injected with citrate buffer. The animals were made diabetic (G2, G4 and G5) by an i. p injection of STZ (45 mg/Kg b.w) dissolved in citrate buffer (pH 4.5) to overnight fasted animals [73]. Animals with glucose level 200 mg/dL or more were considered as diabetic animals. STZ diabetic group (G2) injected i.p one time (45 mg/Kg b.w) to induce hyperglycemia according to Festa et al., [73] ,this group was used as a positive control group. BPF group (G3) was represented by animals treated with BPF (i.p 2.314 mg/ Kg b.w/ day after the other).The fourth group (G4) was treated with BPF for 15 days and injected with STZ as the same as G3 and G2 respectively, while, the fifth group (G5) was injected with BPF for15th day, after the dose of STZ as the same as G2 and G3 respectively. G5 G3, G4 and G5 continued with BPF injection for a period of the experiment. After 30 days from treatment, the animals were sacrificed and dissected. The blood samples were collected from the heart. They were taken in heparinized tubes as an anticoagulant for haematological parameters.

### Haematological assays

Haematological investigations included white blood cells count (WBCs), lymphocytes count (Ly), granulocytes count (Gr), monocytes count (Mon), RBCs count, haemoglobin concentration (Hb), HCT%, MCV, MCH and PLTs. These parameters were measured using cell counter HA-Vet Automatic Hematology Analyzer, Belgium; S/N HA3DM004.

### Statistical analysis

Results are presented as means ± SE for comparison of different experimental animal groups and control ones. Student's t-test was used and the results were calculated by using origin program (version 6). Significance difference between control and treated groups of the studied parameters n= 8.

## 4 Conclusion

The results showed an observable protective effect of BPF on the haematological parameters of diabetic male rats due to its antioxidant properties.

## 5 References


[1]Akbarzadeh, A.; Norouzian, D.; Mehrabi, M.; Jamshidi, S.; Farhangi, A.; Verdi, A. A.; Mofidian, S.; Rad, B. Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry, 2007, 22, 60-64.

[2]Osadebe, P. O.; Odoh, E. U.; Uzor, P. F. Natural products as potential sources of antidiabetic drugs. Journal of Pharmaceutical Research International, 2014, 2075-2095.

[3]Abdela, N.; Jilo, K. Bee venom and its therapeutic values: a review. Adv Life Sci Technol , 2016, 44, 18-22.

[4]Chen, J.; Lariviere, W. R. The nociceptive and anti-nociceptive effects of bee venom injection and therapy: a double-edged sword. Progress in neurobiology, 2010, 92, 151-183.

[5]Danneels, E. L.; Van Vaerenbergh, M.; Debyser, G.; Devreese, B.; De Graaf, D. C. Honeybee venom proteome profile of queens and winter bees as determined by a mass spectrometric approach. Toxins, 2015, 7, 4468-4483.

[6]Son, D. J.; Lee, J. W.; Lee, Y. H.; Song, H. S.; Lee, C. K.; Hong, J. T. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacology & therapeutics, 2007, 115, 246-270.

[7]Tu, W.C.; Wu, C.C.; Hsieh, H.-L.; Chen, C.Y.; Hsu, S.L. Honeybee venom induces calcium-dependent but caspase-independent apoptotic cell death in human melanoma A2058 cells. Toxicon, 2008, 52, 318-329.

[8]Jang, H.-S.; Chung, H.S.; Ko, E.; Shin, J.S.; Shin, M.-K.; Hong, M.C.; Kim, Y.; Min, B.-I.; Bae, H. Microarray analysis of gene expression profiles in response to treatment with bee venom in lipopolysaccharide activated RAW 264.7 cells. Journal of ethnopharmacology, 2009, 121, 213-220.

[9]Lee, K.-G.; Cho, H.-J.; Bae, Y.-S.; Park, K.-K.; Choe, J.-Y.; Chung, I.-K.; Kim, M.; Yeo, J.-H.; Park, K.-H.; Lee, Y.-S. Bee venom suppresses LPS-mediated NO/iNOS induction through inhibition of PKC-α expression. Journal of ethnopharmacology, 2009, 123, 15-21.

[10]Elshater, A.-E. A.; Mohi Eldin, M. M.; Salman, M.; Kasem, N. R. The curative effect of Bee Venom and Propolis on oxidative stress induced by γ-irradiation on blood and tissues of rats. Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology, 2014, 6, 53-69.

[11]Seo, S.-W.; Jung, W.-S.; Lee, S.-E.; Choi, C.-M.; Shin, B.-C.; Kim, E.-K.; Kwon, K.-B.; Hong, S.-H.; Yun, K.-J.; Park, R.-K. Effects of bee venom on cholecystokinin octapeptide-induced acute pancreatitis in rats. Pancreas ,2008, 36, e22-e29.

[12]Darwish, S. F.; El-Bakly, W. M.; Arafa, H. M.; El-Demerdash, E. Targeting TNF-α and NF-κB activation by bee venom: role in suppressing adjuvant induced arthritis and methotrexate hepatotoxicity in rats. PLoS One ,2013, 8, e79284.

[13]Gajski, G.; Garaj-Vrhovac, V. Radioprotective effects of honeybee venom (Apis mellifera) against 915-mhz microwave radiation–induced DNA damage in wistar rat lymphocytes: In vitro study. International journal of toxicology ,2009, 28, 88-98.

[14]Oršolić, N. Bee venom in cancer therapy. Cancer and metastasis reviews ,2012, 31, 173-194.

[15]Behroozi, J.; Divsalar, A.; Saboury, A. A. Honey bee venom decreases the complications of diabetes by preventing hemoglobin glycation. Journal of Molecular Liquids, 2014, 199, 371-375.

[16]Ferreira, S.; Greene, L.; Alabaster, V. A.; Bakhle, Y.; Vane, J. Activity of various fractions of bradykinin potentiating factor against angiotensin I converting enzyme. Nature ,1970, 225, 379-380.

[17]Camargo, A. C.; Ianzer, D.; Guerreiro, J. R.; Serrano, S. M. Bradykinin-potentiating peptides: beyond captopril. Toxicon, 2012, 59, 516-523.

[18]Jain, R. K. Molecular regulation of vessel maturation. Nature medicine, 2003, 9, 685-693.

[19]Sonveaux, P. Provascular strategy: targeting functional adaptations of mature blood vessels in tumors to selectively influence the tumor vascular reactivity and improve cancer treatment. Radiotherapy and Oncology ,2008, 86, 300-313.

[20]Abd-El-Raheim, S. Physiological studies of a separated fraction from, the venom of Egyptian scorpion on the living cells of some organs. Ph. D. Thesis Assuit Univ.(Sohag Campus), 1990.

[21]Nassar, A. Y.; Abu-Sinna, G.; Rahim, S. A. Effect of a bradykinin potentiating fraction, from venom of the Egyptian scorpion, Buthus occitanus, on the ovaries and endometrium of mice. Toxicon ,1990, 28, 525-534.

[22]El-Saadani, M. A. A scorpion venom peptide fraction induced prostaglandin biosynthesis in guinea pig kidneys: incorporation of 14C-linoleic acid. Journal of biochemistry, 2004, 135, 109-116.

[23]Mellitus, D. Diagnosis and classification of diabetes mellitus. Diabetes care, 2005, 28, S5-S10.

[24]Thomas, M. C.; MacIsaac, R. J.; Tsalamandris, C.; Power, D.; Jerums, G. Unrecognized anemia in patients with diabetes: a cross-sectional survey. Diabetes care, 2003, 26, 1164-1169.

[25]Simchon, S.; Jan, K.-M.; Chien, S. Influence of reduced

red cell deformability on regional blood flow. American Journal of Physiology-Heart and Circulatory Physiology, 1987, 253, H898-H903.

[26]Brown, C. D.; Ghali, H. S.; Zhao, Z.; Thomas, L. L.; Friedman, E. Association of reduced red blood cell deformability and diabetic nephropathy. Kidney international ,2005, 67, 295-300.

[27]Awasthi, S.; Gayathiri, S.; Ramya, R.; Duraichelvan, R.; Dhason, A.; Saraswathi, N. Advanced glycation-modified human serum albumin evokes alterations in membrane and eryptosis in erythrocytes. Applied biochemistry and biotechnology, 2015, 177, 1013-1024.

[28]Chen, P.-M.; Gregersen, H.; Zhao, J.-B. Advanced glycation end-product expression is upregulated in the gastrointestinal tract of type 2 diabetic rats. World journal of diabetes ,2015, 6, 662.

[29]Sila, A.; Kamoun, Z.; Ghlissi, Z.; Makni, M.; Nasri, M.; Sahnoun, Z.; Nedjar-Arroume, N.; Bougatef, A. Ability of natural astaxanthin from shrimp by-products to attenuate liver oxidative stress in diabetic rats. Pharmacological reports, 2015, 67, 310-316.

[30]Diederich, L.; Suvorava, T.; Sansone, R.; Keller IV, T.; Barbarino, F.; Sutton, T. R.; Kramer, C. M.; Lückstädt, W.; Isakson, B. E.; Gohlke, H. On the effects of reactive oxygen species and nitric oxide on red blood cell deformability. Frontiers in physiology, 2018, 9, 332.

[31]Shilov, A.; Avshalumov, A. S.; Markovsky, V.; Sinitsyna, E.; Poleshchuk, O. Changes of blood rheological properties in patients with metabolic syndrome. Russ. Med. J, 2008, 4, 200-204.

[32]Miikue-Yobe, T. F. B. Effect of aqueous leaf extract of Heinsia crinata on haematological and some biochemical indices of toxicity in streptozotocin induced diabetic rats. International Journal for Innovative Research in Science & Technology ,2015, 2, 116-126.

[33]Wesseling, M. C.; Wagner-Britz, L.; Huppert, H.; Hanf, B.; Hertz, L.; Nguyen, D. B.; Bernhardt, I. Phosphatidylserine exposure in human red blood cells depending on cell age. Cellular physiology and biochemistry, 2016, 38, 1376-1390.

[34]Vercaemst, L. Hemolysis in cardiac surgery patients undergoing cardiopulmonary bypass: a review in search of a treatment algorithm. The Journal of extra-corporeal Technology ,2008, 40, 257.

[35]Bashiru Shola, O.; Olatunde Olugbenga, F. Hyperglycaemic environment: contribution to the anaemia associated with diabetes mellitus in rats experimentally induced with alloxan. Anemia, 2015, 2015.

[36]de Jong, K.; Emerson, R. K.; Butler, J.; Bastacky, J.; Mohandas, N.; Kuypers, F. A. Short survival of phosphatidylserine-exposing red blood cells in murine sickle cell anemia. Blood, The Journal of the American Society of Hematology ,2001, 98, 1577-1584.

[37]Oyedemi, S.; Adewusi, E.; Aiyegoro, O.; Akinpelu, D. Antidiabetic and haematological effect of aqueous extract of stem bark of Afzelia africana (Smith) on streptozotocin–induced diabetic Wistar rats. Asian Pacific journal of tropical biomedicine ,2011, 1, 353-358.

[38]Oyedemi, S.; Yakubu, M.; Afolayan, A. Effect of aqueous extract of Leonotis leonurus (L.) R. Br. leaves in male Wistar rats. Human & experimental toxicology ,2010, 29, 377-384.

[39]Torel, J.; Cillard, J.; Cillard, P. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry, 1986, 25, 383-385.

[40]Jarald, E.; Joshi, S. B.; Jain, D. Diabetes and herbal medicines. 2008.

[41]Fırat, U.; Kaya, S.; Çim, A.; Büyükbayram, H.; Gökalp, O.; Dal, M. S.; Tamer, M. N. Increased caspase-3 immunoreactivity of erythrocytes in STZ diabetic rats. Experimental Diabetes Research ,2012.

[42]Odoh, U.; Onugha, V.; Chukwube, V. Evaluation of antidiabetic effect and hematotological profile of methanol extract of Ceiba pentandra G (Malvaceae) stem bark on alloxan-induced diabetic rats. African Journal of Pharmacy and Pharmacology, 2016, 10, 584-590.

[43]Gamede, M.; Mabuza, L.; Ngubane, P.; Khathi, A. The effects of plant-derived oleanolic acid on selected parameters of glucose homeostasis in a diet-induced pre-diabetic rat model. Molecules ,2018, 23, 794.

[44]Lippi, G.; Targher, G.; Salvagno, G. L.; Guidi, G. C. Increased red blood cell distribution width (RDW) is associated with higher glycosylated hemoglobin (HbA1c) in the elderly. Clin Lab ,2014, 60, 2095-2098.

[45]Basivireddy, J.; Vasudevan, A.; Jacob, M.; Balasubramanian, K. A. Indomethacin-induced mitochondrial dysfunction and oxidative stress in villus enterocytes. Biochemical pharmacology ,2002, 64, 339-349.

[46]Strom, B. L.; Carson, J. L.; Schinnar, R.; Snyder, E. S.; Shaw, M.; Lundin, F. E. Nonsteroidal anti-inflammatory drugs and neutropenia. Archives of internal medicine, 1993, 153, 2119-2124.

[47]Muller, Y. D.; Golshayan, D.; Ehirchiou, D.; Wyss, J. C.; Giovannoni, L.; Meier, R.; Serre-Beinier, V.; Puga Yung, G.; Morel, P.; Bühler, L. H. Immunosuppressive effects of streptozotocin-induced diabetes result in

absolute lymphopenia and a relative increase of T regulatory cells. Diabetes ,2011, 60, 2331-2340.

[48]Keskin, E.; Dönmez, N.; Kılıçarslan, G.; Kandır, S. Beneficial effect of quercetin on some haematological parameters in streptozotocin-induced diabetic rats. Bull Environ Pharmacol Life Sci ,2016, 5, 65-68.

[49]Jimeno, J.; Faircloth, G.; Sousa-Faro, J.; Scheuer, P.; Rinehart, K. New marine derived anticancer therapeutics─ a journey from the sea to clinical trials. Marine Drugs, 2004, 2, 14-29.

[50]Proksch, P.; Edrada-Ebel, R.; Ebel, R. Drugs from the sea-opportunities and obstacles. Marine Drugs ,2003, 1, 5-17.

[51]Cragg, G. M.; Newman, D. J. Discovery and development of antineoplastic agents from natural sources. Cancer investigation ,1999, 17, 153-163.

[52]Mayer, A. M.; Hamann, M. T. Marine pharmacology in 1999: compounds with antibacterial, anticoagulant, antifungal, anthelmintic, anti-inflammatory, antiplatelet, antiprotozoal and antiviral activities affecting the cardiovascular, endocrine, immune and nervous systems, and other miscellaneous mechanisms of action. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 2002, 132, 315-339.

[53]Mayer, A. M.; Rodríguez, A. D.; Berlinck, R. G.; Hamann, M. T. Marine pharmacology in 2005–6: Marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Biochimica et Biophysica Acta (BBA)-General Subjects, 2009, 1790, 283-308.

[54]McLean, P. G.; Ahluwalia, A.; Perretti, M. Association between kinin B1 receptor expression and leukocyte trafficking across mouse mesenteric postcapillary venules. The Journal of experimental medicine ,2000, 192, 367-380.

[55]Chihara, K.; Iwasaki, J.; Minamitani, N.; Kaji, H.; Matsukura, S.; Tamaki, N.; Matsumoto, S. Effect of vasoactive intestinal polypeptide on growth hormone secretion in perifused acromegalic pituitary adenoma tissues. The Journal of Clinical Endocrinology & Metabolism, 1982, 54, 773-779.

[56]Enjalbert, A.; Arancibia, S.; Ruberg, M.; Priam, M.; Bluet-Pajot, M.; Rotsztejn, W.; Kordon, C. Stimulation of in vitro prolactin release by vasoactive intestinal peptide. Neuroendocrinology ,1980, 31, 200-204.

[57]Frawley, L.; Neill, J. Stimulation of prolactin secretion in rhesus monkeys by vasoactive intestinal polypeptide. Neuroendocrinology ,1981, 33, 79-83.

[58]Vijayan, E.; Samson, W.; Said, S.; McCann, S. Vasoactive intestinal peptide: evidence for a hypothalamic site of action to release growth hormone, luteinizing hormone, and prolactin in conscious ovariectomized rats. Endocrinology ,1979, 104, 53-57.

[59]Li, N.; Zhou, L.; Zhang, B.; Dong, P.; Lin, W.; Wang, H.; Xu, R.; Ding, H. Recombinant human growth hormone increases albumin and prolongs survival in patients with chronic liver failure: a pilot open, randomized, and controlled clinical trial. Digestive and Liver Disease ,2008, 40, 554-559.

[60]Moller, N.; Vendelbo, M. H.; Kampmann, U.; Christensen, B.; Madsen, M.; Norrelund, H.; Jorgensen, J. O. Growth hormone and protein metabolism. Clinical Nutrition, 2009, 28, 597-603.

[61]Montgomery, R.; Conway, T. W.; Spector, A. A.; Chappell, D.: Biochemistry: a case-oriented approach; Mosby Incorporated, 1996; Vol. 10.

[62]Peng, M.-Y.; Zhao, X.-L.; Gao, X.; Lei, H.-Y. Renin angiotensin system in bone marrow of patients with aplastic anemia. Zhongguo shi yan xue ye xue za zhi ,2006, 14, 512-515.

[63]Ganong, W. F. Review of medical physiology. Dynamics of blood and lymph flow ,1995, 30, 525-541.

[64]Piron, M.; Loo, M.; Gothot, A.; Tassin, F.; Fillet, G.; Beguin, Y. Cessation of intensive treatment with recombinant human erythropoietin is followed by secondary anemia. Blood, The Journal of the American Society of Hematology, 2001, 97, 442-448.

[65]Abu Amra, E.; El Rehim, S. A. A.; Lashein, F. M.; Shoaeb, H. S. Effect of a bradykinin potentiating factor separated from honey bee venom on thyroid gland and testis in hypothyroid white rats. The Journal of Basic and Applied Zoology ,2022, 83, 1-10.

[66]Levant, A.; Levy, E.; Argaman, M.; Fleisher-Berkovich, S. Kinins and neuroinflammation: dual effect on prostaglandin synthesis. European journal of pharmacology ,2006, 546, 197-200.

[67]Kornberg, A.; Rachmilewitz, E. Aplastic anemia after prolonged ingestion of indomethacin. Acta Haematologica ,1982, 67, 136-138.

[68]Hoffbrand, A. V.; Steensma, D. P.: Hoffbrand's essential haematology; John Wiley & Sons, 2019.

[69]Allen, J. E.; Valeri, C. R. Prostaglandins in hematology. Archives of Internal Medicine, 1974, 133, 86-96.

[70]Ribardo, D. A.; Crowe, S. E.; Kuhl, K. R.; Peterson, J. W.; Chopra, A. K. Prostaglandin levels in stimulated macrophages are controlled by phospholipase A2-activating protein and by activation of phospholipase C and D. Journal of Biological Chemistry ,2001, 276, 5467-5475.

[71]Salzman, E.; Weisenberger, H. Role of cyclic AMP in

platelet function. Advances in cyclic nucleotide research ,1972, 1, 231-247.

[72]Ferreira, S. A bradykinin-potentiating factor (BPF) present in the venom of Bothrops jararaca. British journal of pharmacology and chemotherapy ,1965, 24, 163.

[73]Festa, A.; D'Agostino Jr, R.; Tracy, R. P.; Haffner, S. M. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes ,2002, 51, 1131-1137.