The Hepatoprotective Effect of Bradykinin Potentiating Factoron Streptozotocin-induced Diabetic Rats

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Research Article

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Abstract

Background

Natural products are considered the most successful source of potential drug leads. Accordingly, the present study investigated the potential antidiabetic effect of the Egyptian honey bee venom fraction known as bradykinin potentiating factor (BPF) in streptozotocin-induced (STZ) diabetic rats.

Materials & Methods

An *in vivo* study was performed on fifty albino male rats that were divided into five groups. (G1): vehicle control animals, (G2): diabetic STZ-induced group, (G3): nondiabetic BPF-treated group, (G4): BPF-injected animals and post-treated with STZ, (G5): STZ-injected animals and post-treated with BPF. Plasma glucose levels and ALT, AST, C reactive protein (C-RP), apelin, and resistin gene expression in BPF-treated rats were evaluated and compared to STZ-treated diabetic rats and vehicle control rats. The plasma protein profile of the five animal groups was investigated by sodium dodecyl sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE).

Results

The data indicated that the STZ-treated (G2) group showed a highly significant increase in the levels of plasma glucose, ALT, and AST compared to the BPF-treated (G3, G4, G5), and nondiabetic control (G1) groups. Quantitative reverse transcription PCR (RT-qPCR) was carried out to amplify the apelin and resistin genes with an internal reference gene (18S *rRNA*) using a real-time PCR system. The concentrations of C-RP (28.3 kDa) and apelin (16 kDa) proteins observed by SDS-PAGE were higher than those of apelin, and resistin gene expression was revealed by RT-qPCR in STZ-treated (G2) rats compared with BPF-treated (G4, G5) and negative control (G1) rats.

Conclusion

The study concluded the importance of BPF, which has therapeutic and protective effects against STZ-induced diabetes complications. The hypoglycemic effect is revealed by the improvement of the biochemical and genetic markers, which may be attributed to BPF against diabetes complications.

Introduction

Diabetes mellitus (DM) is a common health problem with multiple etiologies mainly characterized by hyperglycemia resulting from either a decrease in insulin production, insulin resistance, or both [1]. Diabetes is also associated with major defects in lipid and protein metabolism, thereby leading to many severe and life-threatening complications, including neuropathy, nephropathy, retinopathy,

vasculopathy, and hepatopathy [2,3]. Several studies reported that the most important cause of liver damage in diabetic patients is hyperglycemia-induced oxidative stress and subsequent disturbance in carbohydrate, protein, and lipid metabolism [4], increased gluconeogenesis and ketogenesis [5], and elevated plasma alanine transaminase (ALT), and aspartate transaminase (AST) [4].

STZ-induced diabetic animal models have proven extremely beneficial in understanding diabetic pathogenesis and screening artificial chemicals, natural products, and pharmaceutical agents that have the potential to lower blood glucose levels [6, 7]. STZ has been used to induce type 1 or type 2 diabetes (DM2) [8, 9] since its diabetogenic effects were first reported in 1963 [10]. It has been administered alone, in combination with other chemicals, or with dietary manipulations. A single STZ injection can induce type 1 diabetes (DM1) in rodents [11, 12], while DM2 can be induced by three methods, which include STZ injection after nicotinamide administration [13, 14], high-fat diet (HFD) feeding followed by a low-dose STZ injection [15], and STZ injection during the neonatal period [16, 17].

Several anti-diabetic drugs are used to treat DM. These drugs, however, are not without negative effects and cost money to the patient. When compared to synthetically engineered compounds, the success of venom-derived compounds could be linked to their increased bioactivity, specificity, and stability. Bee venom (BV) contains peptides, enzymes, active amines, and many other substances [18]. It has previously been shown that BV has therapeutic benefits in diabetic rats [19], improves β-cell insulin secretion, and reduces the levels of glucose and its lipolysis [20]. Hassan *et al* [21] revealed the antidiabetic effect of the Egyptian honeybee (*Apis mellifera*) venom in alloxan-induced diabetic rats. Ferreira *et al* [22] purified and characterized bradykinin-potentiating factors (BPFs) from the Brazilian viper snake (*Bothrops jararaca*) venom (Fig. 1), which potentiated the actions of bradykinin on various isolated organs and the blood pressure of the cat. This potentiating action was later correlated with an inhibition of kinin-destroying enzymes [23-26]. The present study was developed based on previous studies carried out by our colleagues on bee venom and its extracted BPF [27-29].

Apelin regulates adipogenesis, fatty acid oxidation, and insulin secretion in DM, and apelin-activated mechanisms play a role in the pathogenesis of DM complications. Apelin improves insulin sensitivity, stimulates glucose use in different tissues linked to DM, and improves brown adipogenesis. Additionally, it was shown that resistin in cell-cultured media decreased following the addition of (BPF) into adipocytes [30]. Therefore, we can conclude that BPF could strongly have an effect on hypoglycemic agent sensitivity (insulin) [31] and consequently have an effect on resistin expression since the expression of resistin in fatty tissues is strongly regulated by insulin. It is particularly important, as resistin plasma concentration is high in weighty and hyperinsulinemic humans and mice [32].

Because apelin and resistin may have a role in reducing diabetes symptoms, especially insulin resistance, and increasing blood glucose, the effects of substances that influence their expression may well be effective in preventing DM or treating it using therapeutic strategies. Thus, the present study aimed to investigate the effect of BPF on biochemical (plasma glucose, ALT, AST), and plasma protein profile and the expression of resistin and apelin which are adipocytokines, in an animal model of DM1.

Experimental Procedures

Chemicals:

STZ ≥75% α-anomer basis, ≥98% (HPLC) powder (Sigma-Aldrich, US), and *A. mellifera* venom were obtained from a commercial wild bee culture (Tanta Government), North Egypt. BPF was isolated from bee venom [29] according to the chemical methods of Ferreira [22].

Animal groups:

A total of 50 healthy adult male albino rats, 12 to 15 weeks old, and weighing 180-200 gm were used and kept in the laboratory under normal conditions of light, temperature, and humidity with access to food and water formulated according to AIN [33]. Animals were classified into five main subgroups. The first main group (G1) (n=10) served as the vehicle control group and was only injected with citrate buffer. The second main group was a model of type I diabetes (n=20), induced by intraperitoneal injection (IP) of STZ [N-(methyl nitroso carbamoyl)-α-D-glucosamine, Sigma, St. Louis, MO, USA] dissolved in citrate buffer (pH 4.5) at a dose of (45 mg/kg b.w.) to overnight fasted animals [34]. The animals with glucose levels over 250 mg/dL were considered diabetic [35]. Because STZ can produce deadly hypoglycemia as a result of the increased pancreatic insulin release, the animals were provided with a 10% glucose solution for the next 48 hours after 6 hours of STZ injection to prevent hypoglycemia. Furthermore, the second main group was divided into 2 equal subgroups: (**G_2**) (n=10) served as the positive control and was injected (*i.p.*) with saline (0.4 ml), and (**G_5**) (n=10) was used as the post-treated BPF group after subcutaneous injection (sub-Q) of BPF (2.314 mg/kg b.w.) The third main group served as the BPF-treated group (n=20), and was divided into 2 equal subgroups: (**G3**) (n=10) was considered the BPF-treated control and only injected (*i.p.*) with saline (0.4 ml), while (**G_4**) served as the pre-treated BPF group and was injected (*i.p.*) 15 days after STZ with the same dose of STZ. After 30 days of treatment, all animals were sacrificed and dissected. Blood samples were collected from the heart. The blood sample was centrifuged for 20 minutes at 3000 RPM, at 4°C to obtain clear plasma for biochemical analysis, apelin, resistin gene expression, and plasma protein profiling. Until the time of measurement, the separated plasma was kept at -20°C. Repeated freezing and thawing were avoided according to Nishizawa *et al.* [36].

Biochemical analysis

The biochemical parameters were assayed spectrophotometrically using commercially available specific kits purchased from the Biodiagnostic company, Giza, Egypt. The plasma levels of glucose, AST, and ALT were measured according to the methods of Trinder [37] and Young [38], respectively.

Real-time reverse transcription-quantitative PCR (RT-qPCR):

Total RNA was purified from blood plasma using NucleoSpin RNA purification kits (catalog no. 740955-250; D-MARK Biosciences) following the manufacturer's instructions. For cDNA synthesis, we used 500 ng of total RNA and a qScript cDNA Synthesis kit (catalog no. CA101414-098; Quanta Biosciences). RT-

PCR was performed using Fast SYBR Green master mix (catalog no. 4385618; Life Technologies) and a StepOne Plus thermal cycler (Applied Biosystems, Foster City, CA). The PCR program parameters were 95°C for 20 seconds followed by forty cycles of 95°C for 3 seconds and 60°C for 30 seconds. The primer sequences used were apelin (forward 5' TCC TCT TGA TGA TGT CCT TTC 3', reverse 5´ TCC TCT TGA TGA TGT CCT TTC 3'), and resistin (forward 5' GAC GGT TGA TTG AGA ACT GA 3', reverse 5' TTG TGT ATT TCC AGA CCC TC 3'). 18S rRNA (forward 5' GTA ACG CGT TGA ACC CCA TT 3', reverse 5' CCA TCC AAT CGG TAG TAG CG 3')

Relative quantitative analysis of apelin and resistin genes was carried out with a reference gene (*18S rRNA*) using a real-time PCR system. Reactions were performed in triplicate. All runs included one negative-template control consisting of PCR-grade water instead of cDNA. The cycle threshold (Ct) was determined for the studied genes in all five animal groups. Relative quantification ΔΔCt and $2^{-\Delta\Delta Ct}$ were calculated using Lightcycler 96 system software. Validation of the assay was performed to ensure that the primers used for the target and internal reference genes had similar amplification efficiencies. All qPCR analyses were performed according to the Minimum Information for Publication RT-PCR (qPCR) experiments (MIQE) guidelines [39].

Plasma protein profile analysis

The plasma protein profile was determined by SDS-PAGE, which was performed by preparation of 17% polyacrylamide gel, according to the method of Laemmli [40].

Statistical analysis

The results are presented as the means ± SE for comparison of different experimental animal groups and control groups. The student's t-test was used, and the results were calculated by using the Origin program (version 6). The potential effects of BPF were initially evaluated using a one-way analysis of variance (ANOVA) test followed by Tukey's test in SPSS (v. 16).

Results

Effects of BPF on biochemical parameters:

Plasma glucose level:

The present data indicated that the STZ diabetic group (G2) showed a highly significant increase ($P<0.001$) in plasma glucose levels compared to the normal value. No significant difference in plasma glucose levels was observed in the BPF-treated group (G3), while, the cotreated groups (G4 and G5) showed a highly significant increase in glucose levels compared to the control group (G1). In comparison with the STZ diabetic group (G2), the results indicated that the cotreated groups (G4) and (G5) showed a highly significant improvement in the level of glucose. These data are presented in Table 1 and Fig. 2.

Plasma ALT and AST levels:

The results are presented in Table 2 and Figures 3A & B demonstrated that the STZ diabetic group (G2) showed a highly significant increase (P<0.001) in plasma ALT and AST levels compared to the normal control group (G1). In the BPF group (G3) and cotreated groups (G4 and G5), the results revealed a highly significant increase and decrease (P<0.001) in ALT and AST levels compared to the (G1) and (G2) groups, respectively. The cotreated groups (G4, G5) exhibited highly significant improvements in both ALT and AST compared to STZ-injected animals (G2).

The present data demonstrated that there was no significant change in plasma glucose level in the BPF-treated group (G3), while, both the pretreatment and post-treated (G4, G5) groups had a highly significant decrease in the level of serum glucose compared to the STZ-injected rats (G2). The results showed that the activities of ALT and AST were significantly elevated in the positive control (G2) compared to the (G1) group (P<0.001). AST levels were higher than ALT levels for all animal groups.

Apelin and resistin gene expression:

Resistin gene expression showed significant changes in all studied groups when normalized Ct values were compared to the 18S rRNA (a ribosomal protein), whereas apelin gene expression showed no significant changes (Table 3 & Fig. 4). An increase in resistin gene expression was detected in the diabetic STZ-treated (G2) group compared to the non-diabetic control (G1) group (ΔCt, P < 0.01). Resistin expression was decreased in all BPF-injected groups, (G3), pre-treated (G4), and post-treated (G5) compared with the untreated diabetic group (ΔCt, P < 0.01) (Table 3 & Fig. 4). No significant changes were observed in the expression of apelin in the diabetic STZ-treated (G2) group, compared with all BPF-treated (G3, G4, G5) groups, and the vehicle control (G1) group, (Ct, P < 0.01).

Table 1. Effect of BPF (2.314 mg/kg b.w.) on the serum glucose level of male albino rats induced by STZ (45 mg/kg b.w.) for 30 days in different groups.

Parameters		G1	G2	G3	G4	G5
Glucose mg/dL	Mean ±SE	110 ± 5.87	549.5 ^{a**} ± 46.08	129.75 ^{b**} ± 9.62	248.25 ^{ab**} ± 21.61	253.5 ^{ab**} ± 25.15
	% of change (1)	--	+399.5	+17.95	+125.68	+130.45
	% of change (2)	--	--	-76.38	-54.82	-53.86

There was a significant difference between G1 and the different rat groups.
*: P<0.05 Significant.
**: P<0.001 Highly Significant.
Nonsignificant (NS) P> 0.05
% of change (1) different from the normal control group (G1).
% of change (2) different from the STZ (G2) group.
a: significant difference from the control group.
b: significant difference from the STZ (G2) group.

Table 2. Effect of BPF (2.314 mg/kg b.w.) on plasma, ALT, and AST concentrations induced by STZ (45 mg/kg b.w.) for 30 days in different rat groups.

Parameters		G1	G2	G3	G4	G5
ALT mg/dl	Mean ±SE	30.75 ±3.42	125.5^{a**} ±6.61	72.25^{ab**} ±7.25	57.25^{ab**} ±4.51	70 ^{ab**} ±5.5
	% of change (1)	--	+308.1	+134.9	+86.17	+127.64
	% of change (2)	--	--	-42.4	-54.38	-44.22
AST mg/dl	Mean ±SE	54 ±5.81	162.5 ^{a**} ±7.48	94 ^{ab**} ±9.95	80.25 ^{ab**} ±4.02	102 ^{ab**} ±6.09
	% of change (1)	--	+200.9	+74.04	+48.6	+88.8
	% of change (2)	--	--	-42.1	-50.61	-37.2

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

Table 3. RT-qPCR data analysis of BPF on the gene expression in rats was normalized to those of 18S rRNA and relative to the negative control (G1).

Groups	G1	G2	G3	G4	G5
Apelin	0.35 ± 0.017	0.41 ± 0.013	0.33 ± 0.015	0.36 ± 0.021	0.38 ± 0.017
Resistin	0.37 ±0.019	0.48^{a*} ±0.014	0.35^{b*} ±0.016	0.40^{b*} ±0.026	0.42^{b*} ±0.020

Data are the means of three independent experiments, *p ≤ 0.05 considered significant. a= P < 0.05 compared with the control group (G1), b: P < 0.05 compared with the STZ-treated diabetic group (G2).

Plasma protein profile by SDS-PAGE:

The plasma protein profile by SDS-PAGE analysis followed a similar pattern of mRNA levels by RT-qPCR indicating that BPF has a broad impact and differentially modulates not only mRNA messages but also the levels of their functional proteins. The results showed that all plasma samples, whether control (G1), STZ-injected (G2), BPF (G3), or pre-and post-treated (G4, G5) had a similar protein composition (Fig. 5), namely, ankyrin (200 kDa), IgG (150 kDa), nephrine (136 kDa), IDE (112 kDa), albumin (65 kDa), prealbumin (55 kDa), ClCP (43 kDa), ApoA-V (39 kDa), GAPDH (35 kDa), C-RP (28.3 kDa), leptin (17 kDa) and apelin (16 kDa).

Based on Figure 5, the intensity of the C-RP and apelin proteins in the STZ-treated (G2) group was greater than that in the nondiabetic control (G1). There was a distinct absence of ankyrin, Ig-G, GAPDH, and leptin in STZ-treated animals (G2), but they have recovered in BPF-treated (G3, G4, G5) rats with almost the same profile as the nondiabetic (G1) group. The BPF-treated group had the best plasma protein profile (G4). The albumin was not changed in either STZ-treated (G2, G4) or BPF-treated (G3, G5) rats compared to the vehicle control (G1).

Discussion

Effects of BPF on biochemical parameters

DM is a life-threatening metabolic disease characterized by chronic hyperglycemia. It develops when insulin secretion and/or action are impaired, resulting in micro-and macrovascular consequences [41]. Most diabetic drugs have adverse side effects, requiring the introduction of other modalities, such as natural products, to replace those [42]. Recently, venomous products of some animals have received great attention in the development of treatments for many diseases, one of them being DM [43].

One of the most widely used diabetogenic agents is STZ. STZ causes selective damage to pancreatic B-cells and is transported through glucose transporter-2 (GLUT2) into B-cells and causes changes in DNA fragmentation, which leads to the cessation of insulin production [44-47] and has been proven to induce DM1.

Previous studies by Asokan *et al* [48] and Mohammed *et al* [49] reported a significant increase in glucose levels and a significant decrease in insulin levels in diabetic rats because of the cytotoxic influence of STZ, which leads to b-cell degradation and reduced insulin secretion. The current results revealed that the STZ-injected animals had a highly significant increase in plasma glucose levels compared to the non-diabetic control group. On the other hand, plasma glucose was significantly decreased in BPF-treated animal groups. This decrease and the improvement in plasma glucose may be attributed to endogenous bradykinin, which was potentiated by BPF. In support of this, bradykinin can upregulate insulin receptor tyrosine kinase activity and increase insulin-stimulated glucose uptake through the translocation of glucose transporter 4 (GLUT4). Bradykinin can improve glucose metabolism by increasing insulin sensitivity in diabetic rats [50-53]. Moreover, bradykinin can directly stimulate glycolytic flux by glucose away from incorporation into glycogen or activation of key glycolytic enzymes such as phosphofructokinase [54], and pyruvate dehydrogenase [55]. Furthermore, bradykinin can inhibit gluconeogenesis [56]. Most physiological effects of bradykinin are mediated *via* local stimulation of prostaglandin synthesis [57], which stimulates the production of insulin-like growth factors[58,59].

The damaging action of STZ on pancreatic β-cells in diabetic rats may result in a decrease in insulin production, hyperglycemia, and hyperlipidemia [60]. These findings have been further confirmed by Al-Rawi *et al.* [61], who found that the elevation in the enzymatic activities of the liver was attributed to their greater need for gluconeogenic substrates.

Liver enzymes such as aminotransferase (ALT and AST) are biomarkers for liver integrity and function [62]. The present study showed that a significant increase in ALT and AST activities was detected in the STZ-treated diabetic rats in comparison to the non-diabetic control, and BPF-treated groups. This result may be due to endogenous potentiation by the venom fraction (BPF) on bradykinin, which stimulates growth factors and increases protein synthesis, causing preservation of liver function, which was demonstrated by the significant decrease in ALT and AST levels, along with protection against liver tissue damage [63]. Furthermore, bradykinin triggers the release of many cytokines that are key molecules in cell proliferation [64] and inhibits aminotransferase enzymes [65].

Effects of BPF on Apelin and Resistin gene expression:

Apelin is a bioactive peptide identified in 1998 as the endogenous ligand of the orphan G-protein coupled receptor APJ [66]. Apelin has recently been found to be an adipokine released from adipocytes [67,68]. Food intake control, glucose homeostasis, and obesity are all thought to be regulated by this peptide [69]. Resistin is considered to represent a link between obesity and diabetes and to play a role in the pathogenesis of insulin resistance [70]. The present results demonstrated nonsignificantly increased circulating apelin levels by RT-qPCR and SDS-PAGE in diabetic STZ-treated rats compared to BPF-treated, and non-diabetic vehicle controls. The study of Meral *et al.* [71] indicated that children with type I diabetes have significantly higher circulating apelin levels than healthy controls, and there is no significant relationship between apelin and BMI, glucose, lipids, adiponectin levels, or insulin sensitivity. In contrast, Dray *et al* [72] found that apelin and insulin had a synergistic effect in this regard. This apparent contradiction can be explained by the fact that Dray *et al* carried out their experiment on type II (T_2D) diabetes with an *ex vivo* model, whereas Meral *et al*. [71] and our study were conducted on type I (T_1D) diabetes with an *in vivo* model. Drougard *et al* [73] reported that chronic intracerebroventricular (*icv*) apelin increases hepatic neo-glucogenesis and hence contributes to the development of the diabetic phenotype in mice. Along the same line, Duparc *et al* [74] demonstrated that chronic *icv* apelin infusion triggered the onset of glucose homeostasis disorders, *e.g.,* glucose intolerance and insulin resistance, and considered apelin/APJ to be a novel mechanistic candidate for the transition from normal to diabetic states. This last result fits with the previous study of Drougard *et al*. [73], who highlighted that overexpression of apelin in the brain participates in the establishment of DM2.

Resistin was initially identified in a screen for adipocyte genes that are suppressed by insulin-sensitizing drugs in rodents [75]. In this study, we found a significant increase in resistin gene expression by RT-qPCR in the diabetic STZ-treated group compared with the control group and in all BPF-treated groups. It can be inferred that the down-regulation in resistin gene expression by BPF can be a mechanism due to the antidiabetic effect of the BPF compound. Resistin mRNA levels were increased in response to acute hyperglycemia and decreased in response to hyperinsulinemia in mice [76]. In contrast, insulin-stimulated resistin gene expression in STZ-treated mice [77]. Our finding is inconsistent with prior studies that have indicated no difference or elevation in plasma resistin levels in patients with DM2 and controls [78,79].

Effects of BPF on plasma protein profile by SDS-PAGE:

According to Beck *et al.* [80], and Gupta [81], protein functions as a diagnostic and prognostic marker, and the expression variety of proteins can be used as markers to analyse the normal or destruction of tissue. Blood circulation is the main source of samples used for proteomics analysis.

The protein composition obtained in this study is the types of proteins found in normal blood plasma. The largest component of blood plasma of normal rats is generally derived from albumin and globulin (α1, α2, β, and γ) [82]. Alberts *et al* [83], and East & Dehnhard [84] reported that proteins of higher intensity showed a higher protein concentration. The present data found that the plasma protein profile was well protected in the BPF-treated groups (G4, G5). Perhaps prolonging the time of cell exposure to the daily dose of BPF may be important in eliciting the insulinotropic effects.

The present study demonstrated that a higher C-RP is significantly associated with a greater likelihood of higher apelin and resistin among induced hyperglycemic STZ-treated animals compared to the normal C-RP level in control and BPF-treated groups. Previous research has also established that C-RP levels are higher in people with diabetes [85]. These changes are in line with Nam *et al.* [86], who mentioned that the anti-inflammatory activity of BV is mediated through suppression of the NF-κB signaling cascade. Therefore, increased C-RP levels can be reduced by treating rats with honey BV for 14 days.

Thus, we can conclude that the inflammatory and metabolic variables associated with diabetes, such as high levels of blood glucose, AST, ALT, apelin, and resistin, stimulate CRP production by endothelial cells, smooth muscle cells, and monocytes and/or macrophages. The albumin level was not changed in either STZ-or BPF-treated animals compared to the vehicle control.

Finally, it should be noted that, despite the results found in this and other studies, further studies in diabetic patients are required to reveal more details regarding the discussed issues.

Conclusion

Our results suggest that BPF has therapeutic and protective effects against STZ-induced diabetes complications. The hypoglycemic effect is revealed by decreased serum glucose levels and ALT, and AST, levels, and improved apelin, resistin, and C-RP profiles, which may be attributed to some potent bioactive constituents of honey bees against diabetes complications.

Abbreviations

Bradykinin potentiating factor: BPF

STZ Streptozotocin

DM Diabetes mellitus

DM1 Type 1 diabetes

DM2 Type 2 diabetes

ALT Plasma alanine transaminase

AST Aspartate transaminase

RT-qPCR Quantitative reverse transcription PCR

SDS-PAGE Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

C-RP C reactive protein

Declarations

Acknowledgment

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Author Contributions

T.N. Habib, S.A. Abd El Raheem, S.A. Hassanain, supervision, study design, data analysis, and data discussion.

H.A. AboElkhair, Methodology, data collection, writing -review & editing.

T.N. Habib, H.A. AboElkhair, Formal analysis, Methodology.

All authors reviewed the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethical approval: All procedures performed in studies involving animals were approved with the ethical standards of the experimental animal protocol (92-06, Sohag, Egypt) by the regulation of Animal Care and Use of Sohag University, Faculty of Medicine.

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Figures

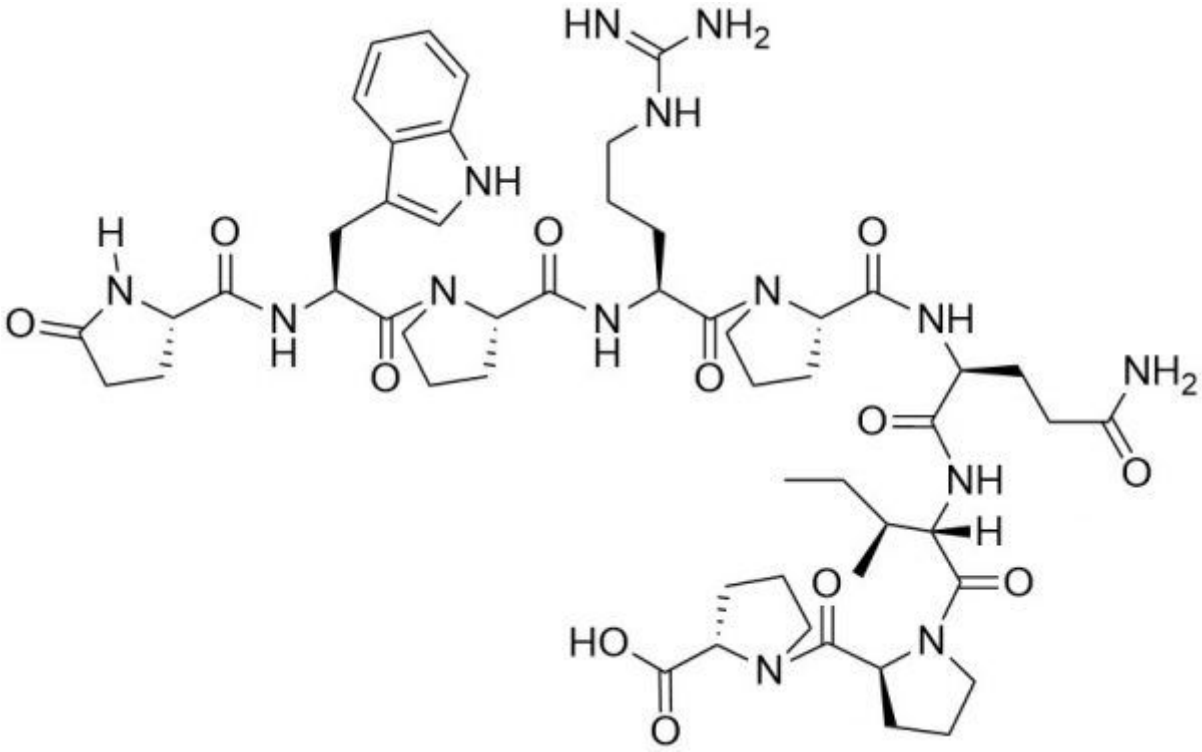

Figure 1

Bradykinin Potentiating Factor BPF (Angiotensin Converting Enzyme Inhibitor).

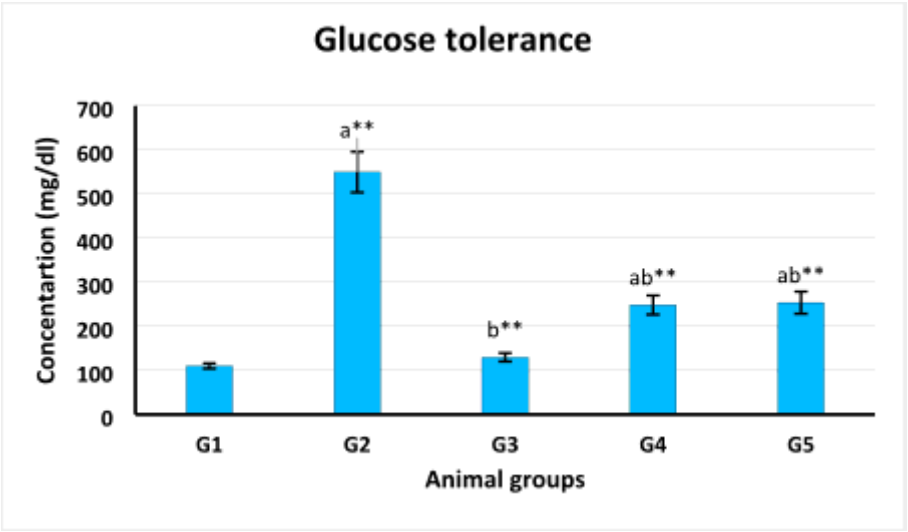

Figure 2

Effect of BPF (2.314 mg/kg b.w.) on the serum glucose level of male rats induced by STZ (45 mg/kg b.w.) for 30 days in different groups.

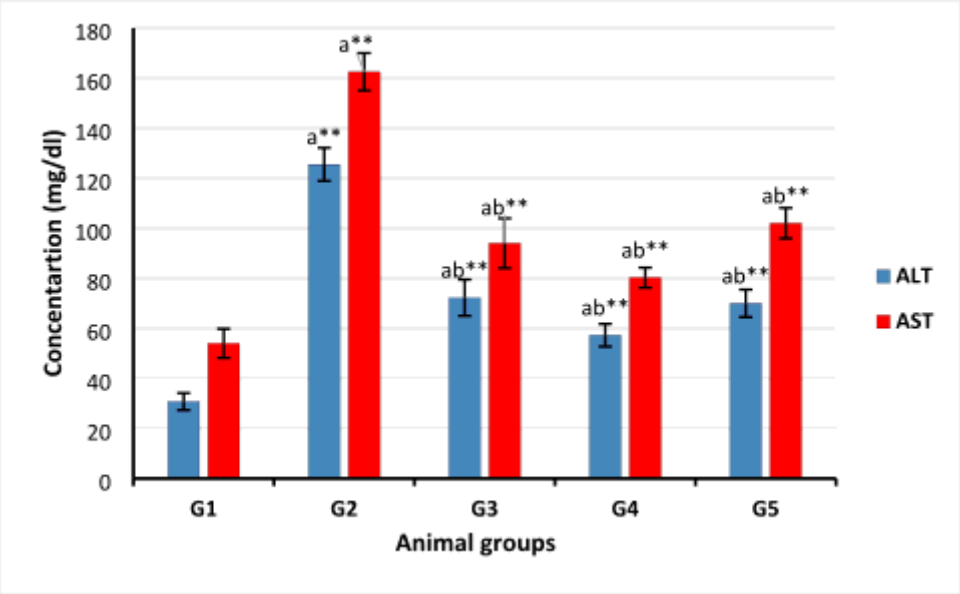

Figure 3

Effect of BPF (2.314 mg/kg b.w) on plasma ALT and AST concentrations by STZ (45 mg/kg b.w.) for 30 days in different rat groups.

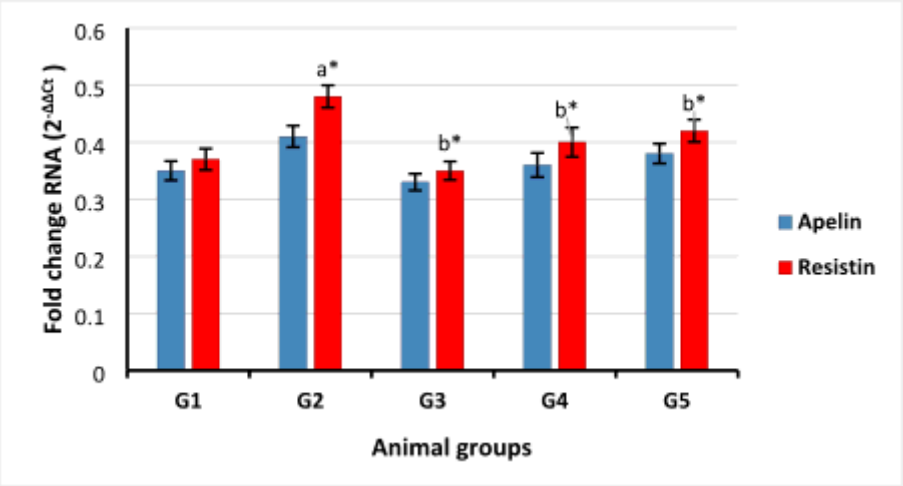

Figure 4

A histogram showing the concentration of RNA fold yields from the different animal groups.

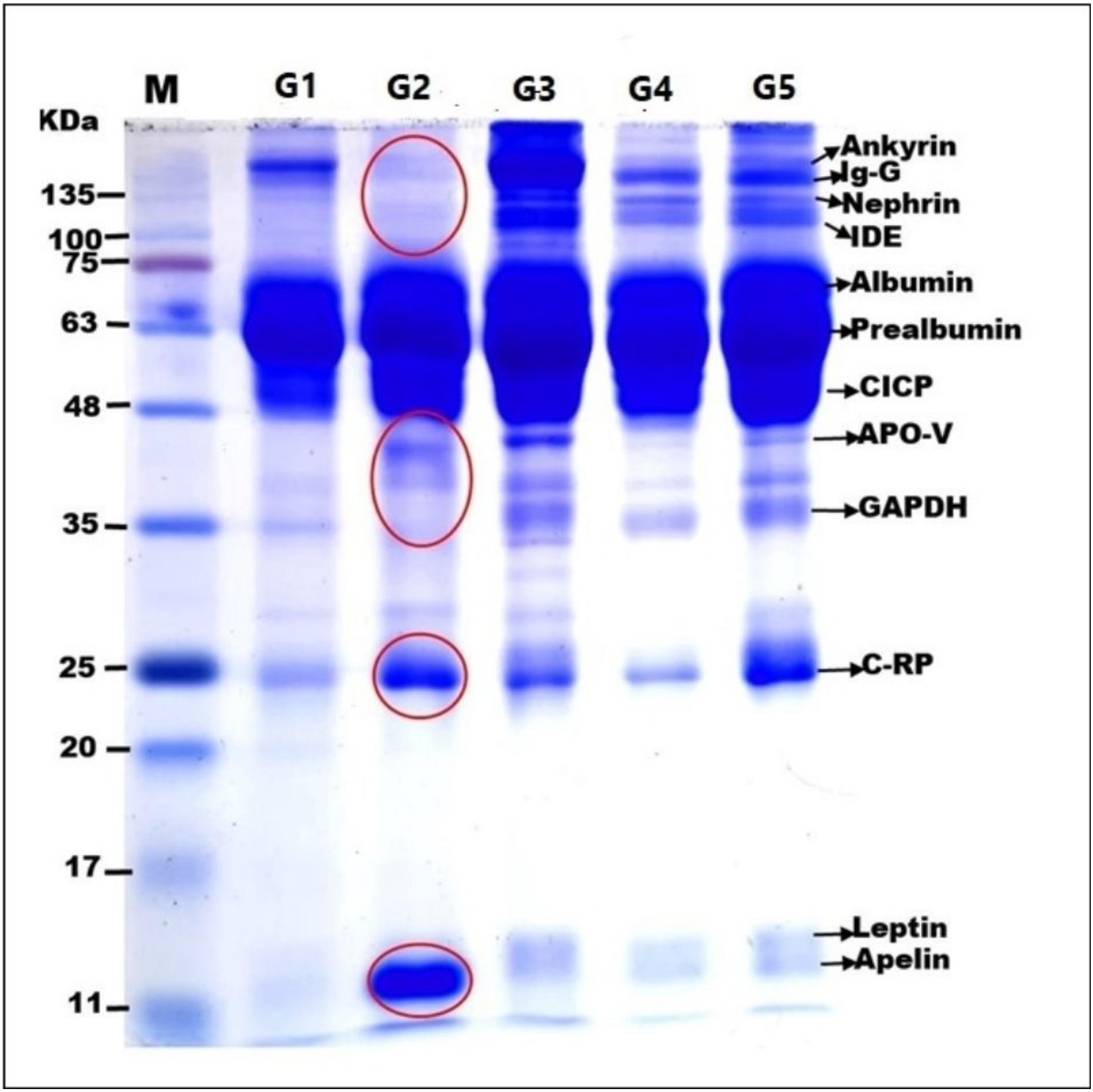

Figure 5

A photomicrograph showing SDS-gel electrophoresis of the migration pattern bands of rat plasma proteins. M= protein marker, G1= negative control, G2= STZ-induced diabetic, G3= BPF treated, G4= BPF-pretreated G5= BPF post-treated.

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