Effects of honeybee (apis mellifera) venom on redox balance, biochemical and hematological profile in diabetic rats: A preliminary study

Barış DENK*, Abdurrahman Fatih FİDAN
Department of Biochemistry, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

1. Introduction
Diabetes mellitus (DM) is a series of metabolic disorders that is common among endocrine system diseases [1]. Free radicals prevent the preservation of homeostasis in the redox system of the organism, causing oxidative stress. Thus, oxidative stress damages cellular structures and functions, disrupting glucose, protein, and lipid structures. These irregularities cause disorders in insulin secretion or interaction [2,3]. This chain of oxidative events that trigger each other prepares the ground for the development of diabetic complications [2].

Nowadays, we use a variety of medications to treat diabetes, but these are not always effective. Therefore, the quest for alternative treatment methods is paramount at this stage. However, scientific evidence for recognized alternative treatment methods is either limited or absent [4]. One of these alternative methods is apitherapy, and there is some scientific research on therapy with honeybee venom (apitoxin). Researchers have discovered that apitoxin interferes with inflammatory processes. Bioactive peptide components in apitoxin are responsible for this effect of apitoxin [5].

Some studies have emphasized that apitoxin causes in vitro insulin release [6] and the enhancement of in vivo insulin release [7]. One study also finds that apitoxin causes improvements in glucose metabolism and lipid profile [8].

The present study thus has been designed with an intention to assess the possible antidiabetic effects of apitoxin in the rat DM model induced by streptozotocin (STZ) and its effects on the redox system by supporting it with some biochemical and hematological parameters, which are all parameters related to diabetes.

2. Materials and methods
2.1. Animals
Three-month-old male Wistar Albino rats (200–320 g) were purchased from Afyon Kocatepe University Experimental Animal Research and Application Center (Afyonkarahisar, Turkey). All rats were acclimated 10 days before the study and were housed up to four per cage in polycarbonate cages and maintained in 12 h dark/light cycles and 65% humidity at 20 ± 1°C. Rats were randomly divided into four groups (n = 10 in each group).
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The biochemical profile was determined with an autoanalyzer (Cobas Integra 400), and the hematological parameters were determined using a fully automated instrument (Mindray BC-2800vet).

2.5. Biochemical and hematologic parameters
Blood samples (5-8 mL) were taken into tubes with K$_2$EDTA. The biochemical profile was determined with an autoanalyzer (Cobas Integra 400), and the hematological profile was determined with a fully automated instrument (Mindray BC-2800vet).

2.6. Statistical analysis
Data of the results are expressed as means ± S.D. The normality test was performed with the Kolmogorov–Smirnov test. Statistical differences among groups were determined using ANOVA followed by Bonferroni posthoc test. The data were evaluated with SPSS software (SPSS v.20; Chicago, IL, USA). Differences were considered significant at P < 0.05.

3. Results
3.1. Alterations in redox balance
The results showed that experimental DM led to lipid peroxidation, leading to an increase in MDA. Bee venom caused an increase in MDA in group A (Figure 1A). The levels of NOx increased significantly in the DA group (Figure 1B). PCO levels have increased in all groups compared with the control group (Figure 1C). No significant effect of apitoxin alone on the levels of 8-OHdG.
was detected, although an increase in those of the D and DA groups was observed (Figure 1D). The levels of TOS were increased in the D and DA groups compared with those of the C group, whereas the levels of TAS were decreased (Figure 1E, 1F). The levels of GSH decreased in all groups compared with those of the C group (Figure 1G).

3.2. Alterations in insulin levels
The levels of insulin decreased in group D compared with group C. No significant effects of apitoxin alone on the insulin levels were observed. There was a slight, insignificant increase in insulin levels in the DA group (Figure 1H).

3.3. Bee venom decreased FBG
All STZ-injected rats had developed hyperglycemia (200 mg/dl); FBG concentration of group D reached its highest level (P < 0.05) in the 4th week compared with STZ induction day. However, DA rats showed a significant (P < 0.05) decrease in FBG in the first week of the study. FBG concentration of the apitoxin-treated rats in group A shows a significant decrease (P < 0.05) in the 2nd week compared to day 0, and in the 2nd, 3rd, and 4th weeks compared to STZ-injection day (Figure 2).

3.4. Alterations in biochemical parameters
We observed an increase in AST in group A. AST also increased in diabetic rats. Alanine aminotransferase (ALT) did not show a significant increase in group A; however, the increase in D and DA groups is significant. Apitoxin caused an insignificant increase in TC and low-density lipoprotein cholesterol (LDL) in group A, while a significant increase was observed in group D and DA. While no significant change in very low-density lipoprotein cholesterol (VLDL) was observed in the A and D group, a significant decrease was observed in the DA group. The changes observed in triglyceride and high-density lipoprotein cholesterol (HDL) parameters are insignificant. It was observed that VLDL decreased when rats of group DA and rats of both A and D were compared (Table 1).

3.5. Alterations in hematologic parameters
No significant changes were observed in HCT, RBC, and HGB levels. An increase in MCV and a decrease in MCHC were observed in the DA group. The decrease in MCHC in the DA group was significant. Leucocytes (WBC) in group A did not show a significant change. The increase in WBC in the D and DA groups was also significant. There was a significant increase in lymphocyte (LYM) count in D and DA. The count of GRAN increased in DA. Apitoxin caused a decrease in monocyte (MON) percentage. DM caused a decrease in MON percentage. It was observed that the percentage of MON in the DA group approached that of the C group. The effect of apitoxin to increase the number of PLT has been observed. DM caused a decrease in the number of PLT. MPV increased in the D and DA groups (Table 2).

3.6. Changes in bodyweight, food, and water intake
Bodyweight changes of rats in groups C, A, D, and DA were observed as gain, no change, loss between 0 and 4 weeks and loss between 0 and 3 and 0 and 4 weeks during the study time, respectively (P < 0.05). A graph showing the change of bodyweight in the weekly time unit according to the in-group comparison of the differences (Figure 3A) is shown. We observed a decrease in food intake in the C group in the 1st and 2nd weeks, and in the A group in the 1st, 2nd, 3rd, and 4th weeks. There was a gradual increase in the food intake of D group. We did not observe in-group statistical differences in the DA group at the 2nd, 3rd, and 4th weeks (P < 0.05), (Figure 3B).

We did not observe any change in water intake in group C. We observed that in group A, water intake decreased in the 3rd and 4th weeks compared to the initiation day of the study and the 1st week. We observed that water intake increased in D and DA groups. We observed that in all weeks except week 2, water and food intake was significantly lower in group DA than group D (P < 0.05), (Figure 3C).

3.7. Behavioral and physical changes
Aggression, convulsion, swelling around the eyes, nose, mouth, and neck and difficulty in breathing were observed in rats treated with bee venom.

4. Discussion
As we have observed in our study, free radical production in DM [3] increases the susceptibility to lipid peroxidation [14,15]. In some studies, it was reported that blood MDA levels decreased in diabetic rats due to the effect of apitoxin [16]. Considering other studies, findings of elevated MDA levels have been reported in vitro, human whole blood samples, depending on the dose of apitoxin and the duration of exposure [17]. Similar findings have been reported in rabbit renal proximal tube cells [18], mouse serum [19], and liver tissue [20]. Apitoxin may cause lipid peroxidation. PLA₂ may disrupt membrane integrity by hydrolyzing the cell membrane [21], thus causing an increase in MDA.

The increase in NOx levels in diabetic rats with apitoxin injection is probably due to oxidative stress [22].

The production of modified proteins in diabetic rats with apitoxin injection increased, due to diabetic oxidative stress and glucose accumulation, or the effects of both [23]. In this study, apitoxin increased the production of oxidants, causing an increase in PCO levels that triggered uncompensated reactive oxygen species (ROS) production, as previously described [24].

In diabetic cases, 8-OHdG excretion increased in urine [25]. The increase in 8-OHdG in rats with DM was consistent with reports from others.
Figure 1. Redox parameters and insulin measurement graphs. Bar graphs showing the quantification, in various units and ±S.D., of malondialdehyde (MDA), nitric oxide (NOx), protein carbonyl (PCO), DNA damage marker (8-OHdG), total oxidant status (TOS), total antioxidant status (TAS), reduced glutathione (GSH), and insulin parameters measured in study groups (ANOVA, posthoc Bonferroni test, *P < 0.05; **P < 0.0001). C, control group; A, apitoxin treated group; D, diabetic group; DA, apitoxin treated diabetic group.
The increase in diabetic oxidative stress caused TOS levels to rise and TAS levels to decrease. The effect of apitoxin is insignificant as statistical. GSH reserve decreases due to elimination of oxidative molecules [21]. The toxic effects of apitoxin on healthy cells have been associated with an increase in ROS production which is in agreement with the mechanisms reported by others [17].

Due to the effect of STZ [26], plasma insulin concentration in diabetic rats has decreased. An insignificant increase in plasma insulin levels observed in the DA group does not comply with apitoxin's effect of increasing insulin plasma concentration, as discussed in previous studies [6,8].

The increase of ALT and AST observed in diabetic rats treated with apitoxin may be a sign of hepatotoxicity. Therefore, hepatic glycogen stores may have decreased, and/or hepatic glyconeogenesis capacity may have decreased. Perhaps this may be the reason for the decline in FBG level, especially in the 1st and 2nd weeks, in the DA group. To interpret this condition more clearly, it is obvious that, in future studies, measurement of hepatic...
glycogen stores and histopathological examination of the liver is required.

Increased AST and ALT parameters due to experimental DM have been reported previously [27]. This finding is consistent with our research findings. Apitoxin causes AST and ALT release from tissue and organ cells [19,28]. In this study, similar results were obtained as mentioned in previous studies on AST, but the results do not match in terms of ALT [16]. It is thought that this situation may be caused by apitoxin-induced tissue and organ damage and the combined effect of both apitoxin and experimental diabetes. The increase in ALT level observed in diabetics may be caused by hepatocellular damage similar to the literature [19,29]. However, the fact that there was no significant increase in ALT level suggests that apitoxin alone did not cause liver damage. Besides, the collection of blood samples was not divided by time intervals, but only at the end of the study were AST and ALT parameters examined. The method, frequency and dose of apitoxin administration may have caused this condition.

In experimental STZ-induced DM, an increase in total cholesterol (TC) levels has been reported in Wistar albino rats [30]. As we observed in our study, an increase in TC levels was reported in experimental DM, which is due to an increase in LDL. The TC levels of the diabetic rats injected with apitoxin were increased. Considering that apitoxin application has no effect on TC levels, it can be said that the reason for the increase in TC levels is caused by diabetes. The absence of changes in triglyceride and HDL cholesterol levels in the experimental DM model is in agreement with a previous study [31]. The increase of TC and LDL in the DA group can be discussed by speculating on the findings. The inability of apitoxin to fully normalize the levels of FBG, insulin, and oxidants may be responsible for this situation.

DM-induced oxidative stress, as well as changes in FBG and insulin levels, may have caused increases in WBC, GRAN, and LYM numbers [32–34], and a decrease in PLT count [35,36].

As expected, the food intake of diabetic animals in group D increased, and bodyweight decreased. Apparently, the saline injection did not adversely affect food intake. The trend of decreasing bodyweight observed in the DA group may indicate that apitoxin as a stressor reduced the appetite of rats and hindered bodyweight gain.

It is known that water intake shows a circadian rhythm parallel to food intake, which was observed in groups

| Table 2. Hematologic parameters measured in groups. |
|-----------------------------------------------|
|                  | C (n = 10) | A (n = 10) | D (n = 10) | DA (n = 10) | P  |
|------------------|-----------|-----------|-----------|------------|----|
| HCT (%)          | 42.33 ± 1.75 | 41.20 ± 2.14 | 40.15 ± 3.49 | 43.87 ± 1.81 | 0.07 |
| RBC (x10^{12}/L) | 8.23 ± 0.17 | 8 ± 0.37  | 7.79 ± 0.12 | 8.10 ± 0.22 | 0.14 |
| HGB (g/dL)       | 14.95 ± 0.76 | 14.37 ± 0.83 | 13.83 ± 1.15 | 14.88 ± 0.68 | 0.12 |
| MCV (IL)         | 51.50 ± 1.39<sup>a</sup> | 52.65 ± 1.28<sup>b</sup> | 53.24 ± 1.49<sup>b</sup> | 54.23 ± 1.32<sup>b</sup> | 0.02 |
| MCHC (g/dL)      | 35.27 ± 0.56<sup>a</sup> | 34.78 ± 0.29<sup>b</sup> | 34.42 ± 0.62<sup>b</sup> | 33.87 ± 0.54<sup>b</sup> | 0.00 |
| WBC (x10^{9}/L)  | 6.78 ± 3.32<sup>b</sup> | 5.12 ± 1.45<sup>a</sup> | 8.95 ± 1.46<sup>b</sup> | 11.17 ± 2.40<sup>a</sup> | 0.00 |
| LYM (x10^{9}/L)  | 4.07 ± 1.62<sup>b</sup> | 3.15 ± 1.44<sup>a</sup> | 6.07 ± 1.84<sup>b</sup> | 5.98 ± 2.16<sup>a</sup> | 0.02 |
| MON (x10^{9}/L)  | 0.48 ± 0.39 | 0.37 ± 0.56  | 0.33 ± 0.08  | 1.47 ± 1.41  | 0.06 |
| GRAN (x10^{9}/L) | 2.40 ± 1.47<sup>a</sup> | 1.84 ± 0.98<sup>a</sup> | 2.55 ± 0.61<sup>b</sup> | 4.78 ± 1.27<sup>b</sup> | 0.00 |
| LYM (%)          | 62.5 ± 14.57 | 61.78 ± 21.34 | 66.57 ± 9.89 | 54.1 ± 17.35 | 0.61 |
| MON (%)          | 9.37 ± 5.12<sup>a</sup> | 2.95 ± 1.29<sup>b</sup> | 4.05 ± 1.17<sup>b</sup> | 8.12 ± 4.74<sup>c</sup> | 0.01 |
| GRAN (%)         | 30.17 ± 9.58 | 33.22 ± 18.97 | 29.38 ± 8.89 | 37.78 ± 14.20 | 0.70 |
| PLT (x10^{9}/L)  | 589.17 ± 186.68<sup>b</sup> | 918.33 ± 118.96<sup>c</sup> | 116.83 ± 93.61<sup>d</sup> | 442 ± 188.75<sup>b</sup> | 0.00 |
| MPV (fL)         | 5.80 ± 0.46<sup>a</sup> | 5.58 ± 0.23<sup>a</sup> | 6.48 ± 0.28<sup>b</sup> | 6.70 ± 0.32<sup>b</sup> | 0.00 |
| PDW              | 16.43 ± 0.48<sup>b</sup> | 16.13 ± 0.29<sup>b</sup> | 17.48 ± 0.55<sup>a</sup> | 16.87 ± 0.33<sup>b</sup> | 0.00 |

Statistically significant differences were found as indicated (data were expressed as mean ± S.D., n = 10; ANOVA, posthoc Bonferroni test, *P < 0.05; a,b, c: different letters on the same line indicate the difference between groups). Abbreviations: C, control group; A, apitoxin treated group; D, diabetic group; DA, apitoxin treated diabetic group; HCT, hematocrit; RBC, red blood cells; HGB, hemoglobin; MCV, mean cell volume; MCHC, mean hemoglobin concentration per RBC; WBC, white blood cells; LYM, lymphocytes; MON, monocytes; GRAN, granulocytes; PLT, platelets; MPV, mean platelet volume; PDW, platelet distribution width.
C, A, and D [37]. However, lower water and food intake observation in the DA group than in the D group may be related to the FBG's tendency to decrease in the DA group especially in the 1st and 2nd weeks.

As stated in previous studies, aggression, hyperactivity, and convulsions that we observed in our study are likely caused by apitoxin [38]. Apparently, edema was observed in the rats due to histamine, which was both introduced into the injection medium as an apitoxin component, and released by mast cells during an allergic reaction. Breathing difficulties observed may have been due to swelling observed around the nose and neck.

In the present study, the effects of apitoxin therapy (which is becoming more widely-touted for use as an antiinflammatory) were evaluated on the oxidant-antioxidant status and some biochemical parameters in rats with experimental DM. In our study, we observed the negative effects of apitoxin on oxidative stress.

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**Figure 3.** Bodyweight, food, and water intake changes during the study period. Bar graph A showing the means, in g and +S.D., of bodyweight in-group comparison parameters measured weekly in study groups (ANOVA, post-hoc Bonferroni test, *P < 0.05, comparison of weeks amongst themselves). Bar graph B showing the means, in g and +S.D., of food intake in-group comparison parameters measured weekly in study groups, and bar graph C showing the means, in mL and +S.D., of water intake in-group comparison parameters measured weekly in study groups (ANOVA, post-hoc Bonferroni test, *P < 0.05, compared with day 0; **P<0.05, compared with 1st week; † P<0.05, compared with 2nd week; † † P<0.05, compared with 3rd week). C, control group; A, apitoxin treated group; D, diabetic group; DA, apitoxin treated diabetic group. 0, first day of FBG measurement; 1, 2, 3, 4, weeks after STZ injection day.
parameters in general, and positive effects on FBG. Results reported herein should be considered in the light of some limitations. The relationship between food intake and plasma insulin concentration should be supported by an oral glucose tolerance test. In the framework of the DM-apitoxin relationship, we think that the pathophysiology of changes in macroscopic and microscopic inflammation findings, inflammation mediators, and biochemical and hematological parameters in various tissues and organs, especially the pancreas, should be measured and evaluated at a molecular level. A study evaluating the histopathological measurements of apitoxin in the pancreas [16] and liver [39] in a similar experimental model by future researchers would be useful in this regard. In addition, it is proposed that the dose-response associations for various dosage-per-administration studies should be performed to illuminate the apitoxin effect in vivo, and the samples should be taken intermittently during the study and the results evaluated at peracute, acute, subacute, and chronic levels. Also, comparative analysis of a positive control agent known to be antidiabetic with apitoxin [16] may be useful in elucidating the mechanism of action of apitoxin, so that the possible effects of apitoxin on diabetes can be discussed less speculatively. In the current form of this research, the available findings for the use of apitoxin as an antidiabetic or as a regulator for diabetic complications are preliminary and a full investigation of the aforementioned conditions is envisaged in future studies.

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We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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