Journal of Applied Biological Sciences
E-ISSN: 2146-0108 16(3): 563-574, 2022
DOI: 10.5281/zenodo.7114334
Research Article

MONOFLORAL *BRASSICA NIGRA* POLLEN IMPROVES OXIDATIVE STRESS AND METABOLIC PARAMETERS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Sibel Taş[1*], Emre Sarandöl[2], Cansu Nur Tekin[1], Aycan Tosunoğlu[1], Özgür Vatan[1], Huzeyfe Hürriyet[1], Cengiz Bozyiğit[2], Nilüfer Çinkılıç[1], Tolga Çavaş[1], Melahat Dirican[2]

[1]*University of Bursa Uludağ, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkiye*
[2]*University of Bursa Uludağ, Faculty of Medicine, Department of Biochemistry, Bursa, Turkiye*

**Corresponding Author:*
E-mail: smeral@uludag.edu.tr

(Received 14th February 2022; accepted 21th March 2022)

ABSTRACT. Diabetes mellitus is accompanied by metabolic and oxidative stress-related complications and supportive treatment with natural products might prevent or delay the progression of these complications. The present study hypothesized that *Brassica nigra* pollen (BNP), shown to have metabolic and antioxidative properties, can reduce oxidative stress and genotoxicity and improve metabolic parameters in diabetes mellitus. Diabetes was induced by intraperitoneal injection of STZ (65 mg/kg). The rats were divided into four groups; control rats; control group rats given BNP; diabetic rats; diabetic group rats given BNP. Rats were given with BNP for thirty days (350 mg/kg/day). Serum insulin, blood glucose, triglyceride, total cholesterol, high-density lipoprotein-cholesterol, alanine aminotransferase, aspartate aminotransferase levels were evaluated using an auto-analyzer. Plasma and tissue malondialdehyde (MDA) levels were measured with spectrophotometric methods. Serum paraoxonase (PON), arylesterase (ARE), superoxide dismutase, glutathione peroxidase activities were determined using commercial kits. Genotoxicity was determined by the bone marrow micronucleus (MN) method. BNP recovered the increased plasma and heart, muscle, liver, and kidney tissue MDA and serum glucose and total cholesterol levels. BNP increased serum PON, ARE activities and showed antigenotoxic activity by decreasing MN frequency in the BNP-treated diabetic rats. BNP has antihyperglycemic, antihyperlipidemic, antioxidant, and antigenotoxic properties and can be a promising supportive therapeutic agent in diabetes mellitus for improving treatment outcomes and reducing treatment-related and/or diabetes-related complications.

Keywords: *Diabetes mellitus, oxidative stress, black mustard, bee pollen, micronucleus.*

INTRODUCTION

In the human biological system, there is a struggle between the oxidant and antioxidant forces, and oxidative stress is the imbalance of these systems in favor of the former. Oxidative stress has been accepted to be related to the pathogenesis of various diseases together with, including diabetes mellitus and its complications [1, 2]. The fight against oxidative stress is provided by various antioxidant enzymes [Superoxide dismutase (SOD), glutathione peroxidase (GPx) catalase (CAT), and paraoxonase (PON)] and many antioxidant molecules (vitamin A, C, and E and uric acid, bilirubin, etc.) [3].

Diabetes mellitus (DM) is characterized by hyperglycemia, hyperlipidemia, and oxidative stress, and these situations affect each other in a vicious circle. Hyperglycemia causes glycosylation of proteins, enzymes, nucleic acid material, and lipoproteins, resulting in deactivation and deformation of these molecules [1, 4, 5]. The principal approach for attaining glycemic control in diabetes mellitus is lifestyle modification, insulin, or oral hypoglycemic agents. However, there are reports about the side effects of insulin and oral hypoglycemic agents [6, 7].

Bee products have been suggested to be used in the treatment of various disorders such as diabetes mellitus, cardiovascular diseases, and cancer [8], and the German Federal Board of Health acknowledges bee pollen as an official medicine [9]. Bee pollen is rich in amino acids, proteins, carbohydrates, fats, minerals, vitamins, phenolic substances, phytochemicals [10, 11]. *Brassica nigra* (L.) K. Koch, known as Black Mustard (*Brassicaceae* family), is an annual herbaceous wild plant that has a very wide natural distribution in Africa, Asia, and Europe, also widely cultivated crop. On the other hand, *Brassica* pollen is mainly preferred as a major source by honey bees. Since ancient times, mustard has been used by mankind for medicinal properties. Antihyperglycemic, antihyperlipidemic and antioxidant effects of *B. nigra* seeds, roots, and leaves were shown in experimental and clinical studies [12, 13, 14, 15, 16, 17].

Due to the specific chemical properties of herbal species, monofloral bee pollen is a safe alternative for medicinal practice [18] and according to our, this is the first study of the effects of monofloral *B. nigra* pollen on oxidative stress and metabolic changes in STZ-induced diabetic rats.

We hypothesized that BNP, can reduce oxidative stress and genotoxicity and improve metabolic disturbances in diabetes mellitus. For this reason, PON, ARE, SOD, and GPx were studied for antioxidant status. Plasma and tissue malondialdehyde (MDA) levels were determined to research the oxidative stress parameters. Total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C) and triglyceride (TG) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, and blood glucose, serum insulin levels were measured to investigate the metabolic status of the groups. Moreover, bone marrow micronucleus (MN) frequencies were also investigated as a marker of genotoxic damage.

MATERIALS AND METHODS

Pollen collection and analyses

From the colonies of Anatolian honey bee (*Apis mellifera Anatolia*) which were kept in Langstroth-type of hives in the Bursa lowland area (40º 13.8’ N, 28 º 49.8’ E), the pollen grains of *B. nigra* were collected in May 2019. The pollen loads which were accumulated in the bottom of drawers of each hive were removed twice a day and stored at -20°C until analysis. Pollen loads from different botanical origins were classified according to their color on the cold pack by using a stereomicroscope (Zeiss Stemi DV4, China). However, from each color, a small portion was mixed with glycerin jelly and stained utilizing basic fuchsine, and the uncatalyzed pollen grains were examined under the light microscope (Olympus BX 41, USA). The identification of pollen grains was carried out using a light microscope. The images of pollen grains were compared concerning slides of pollen collection of the Uludağ University, Palynology Laboratory (Bursa, Turkey). The pollen pellets which were identified as *B. nigra* pollen grains were

kept under -20 ℃ for further examination. These pellets were given to rats as frozen pollen. In addition, a purity test of the pellets was conducted to assess the level of contamination as follows: one g of pollen pellets was homogenized with 70% ethanol with three parallel uncatalyzed slides. A thousand pollen grains were counted and identified on each slide by using the light microscope. Then the total percentage of their purity was calculated. The results of this assay showed that the pollen grains purity was 98.9%.

Animals and induction of diabetes

Twenty-eight healthy male Wistar rats with an average weight of 350 g were used. The rats were maintained in the animal house at the Uludağ University in Bursa and were fed with standard laboratory chow produced by a commercial firm (Korkutelim Yem A.Ş. Antalya, Turkey) according to Turkish Standards Institution and tap water for a week before the experiment. Four rats were housed in each cage. All rats were kept at standard conditions of humidity (55±5%), temperature (25±2 °C), and light/dark cycle (12/12 h). Diabetes in rats was induced by preparing STZ (Sigma-Aldrich Chemicals, USA) in sodium citrate buffer (pH 4.5) and administering 65 mg/kg intraperitoneally to rats. At the same time, citrate buffer solution was injected into the control group. Blood glucose was detected after 48 h of STZ or buffer injections. When rats had blood glucose levels higher than 200 mg/dL, they were considered diabetes and used for further experiments.

Experimental design and sample collection

The rats were divided into four groups (n:7); control rats (C), control rats given *B. nigra* pollen; (C+BNP), diabetic rats (D); diabetic rats given *B. nigra* pollen (D+BNP). Seven days after injection of STZ, *B. nigra* pollens (350 mg/kg/day) were added to the drinking water of the groups C+BNP and D+BNP for thirty days. The drinking water containing BNP was prepared daily.

Weekly body weight and blood glucose levels, and daily food and fluid intake were recorded for all groups throughout the experimental procedure. Thirty days later, blood was collected by cardiac puncture under light ether anesthesia. The lipid profile, liver enzymes, insulin, and antioxidant enzymes were studied from the blood supernatant centrifuged at 3000 rpm for 15 minutes.

For MDA analysis, blood samples were taken into tubes including EDTA. Plasma and serum samples were stored at -20 °C. While the skeletal muscle (Musculus gastrocnemius), heart, kidney liver tissues were dissected straight away after the collection of blood from rats. In addition, all obtained tissues were rinsed with cold standard saline solution and then stored at -20 °C for the MDA analysis.

Estimation of biochemical parameters

Using glucometer (Abbott, USA) glucose levels were determined from blood samples taken from the tail of rats in all experimental groups each week. HDL-C, TG, TC, AST, ALT levels were determined in serum using an auto-analyzer (Architect C8000, Abbott Laboratories, IL, USA). Serum insulin, SOD, and GPx levels were measured by commercial (ELISA) kits (Bioassay Technology Laboratory, China). Serum PON and ARE enzyme activities were measured with Rel Assay Diagnostics kits (Rel Assay Diagnostics, Turkey) on an Architect C8000 device (Abbott Laboratories, IL, USA).

Tissue MDA (kidney, heart, liver, and muscle) levels were studied according to the method described by Ohkawa et al. [19] and the results were given as nmol/mg tissue. Plasma MDA concentrations were determined by the TBA method [20].

Bone marrow micronucleus assay

The methods used in previous studies have been applied with various modifications. Briefly, bone marrow was washed with 2 mL of fetal bovine serum from the femur bones removed by dissection and transferred to a sterile centrifuge tube via syringe. Bone marrow cells in fetal bovine serum were centrifuged at 1000 rpm for 10 minutes. After centrifugation, part of the supernatant was removed, and cells were suspended in the remaining supernatant. The cell suspension was spread on slides, and fixation was achieved by air drying for 24 hours. After that time, slides were first stained with 100% May Grünwald Giemsa stain for 3 minutes. It was then stained with 50% May Grünwald Giemsa for 3 minutes. After washing the slides, they were painted with 5% Giemsa dye prepared with pH 6.8 phosphate buffer for 20 min.

Microscopic evaluations of the MN slides were performed by light microscopy (Nikon Eclipse E100) with a 100X objective. Younger polychromatic erythrocytes (PCE) and older Normachromatic erythrocytes (NCE) were evaluated after staining. During the evaluation, 1000 PCE cells were counted for each rat, and MNs in 1000 PCE cells were recorded. PCE / NCE ratios were determined in 2000 cells for each rat as a cytotoxicity marker. Results are given as mean MN values in 100 PCE cells (MNPCE%) ± SEM (standard error of the mean) value and mean PCE / NCE value ± standard deviation value. The percent reduction in the frequency of MNPCEs between group D and group D+BNP was calculated according to Waters et al. [21] using the following formula:

$$\text{Reduction \%} = \frac{\text{frequency MNPCEs in group D} - \text{frequency of MNPCEs in group D+BNP}}{\text{frequency MNPCEs in group D} - \text{frequency MNPCEs in group C}} \text{ x } 100$$

Similarly, the percent increment of PCE/NCE ratios as a marker to cytotoxicity was calculated using the following formula;

$$\text{Increment PCE/NCE ratio \%} = \frac{\frac{\text{PCE}}{\text{NCE}}\text{ the ratio in group D+BNP} - \frac{\text{PCE}}{\text{NCE}}\text{ the ratio in group D}}{\frac{\text{PCE}}{\text{NCE}}\text{ the ratio in group C} - \frac{\text{PCE}}{\text{NCE}}\text{ the ratio in group D}} \text{ x } 100$$

Statistical analyses

Data were given as the mean ± standard error (SEM). Statistical analyses were taken using Statistical Package for the Social Sciences (SPSS) software (Version 13.0 for Window) and Kruskal Wallis (K-independent samples test). While the subsequently significant differences were determined using Mann-Whitney *U*- test. Values are significant at the $p<0.05$ level.

RESULTS AND DISCUSSION

We observed metabolic disturbances and oxidative stress in the diabetic rat model in this study as in our previous studies [22, 23, 24]. Oxidative stress was evident by the increased plasma and tissue MDA levels found in diabetic rats. We observed that SOD and GPx activities increased in the D group; however, they were not sufficient to compensate for the oxidative stress.

In this study, while the increase in food, water intake, serum glucose, TC, TG, AST, and ALT levels was found in the D group, the decrease in body weight and insulin levels were found to be significant compared to the control group (Table 1). In the D+BNP group compared to the D group, an increase in insulin levels and a diminish in blood sugar and TC levels were found. The positive changes detected in these parameters may have developed due to the multifaceted effect of pollen. Bee pollen has a very rich bioactive structure such as vitamins, minerals, proteins, amino acids, lipids, carbohydrates, and as well as polyphenols [8, 9]. In many different human and animal studies, it has been stated that polyphenols and flavonoids modulate lipid metabolisms [25, 26] as well as carbohydrate metabolism in diabetes (such as improvement of peripheral sensitivity to insulin, inhibition of intestinal disaccharidase activities such as alpha-amylase, competitive inhibition of sodium-dependent glucose transporter-1), and further, they have been shown to have the feature of optimizing oxidative stress as an antioxidant [27, 28, 29, 30].

Table 1. *Body weight, food, water consumption, and laboratory parameters of the study groups.*

Group	C	C+BNP	D	D+BNP
Food intake (g/24 h)	22±1	20±1	37±2^{a***}	32±1
Water intake (mL/24 h)	40±1	49±1	107±1^{a***}	107±1
Final body weight (g)	416±8	420±4	286±28^{a***}	321±18
Glucose (mg/dL)	137±6	144±9	546±11^{a***}	449±28^{b**}
Insulin (mIU/L)	3.09±0,6	2.63±0.7	0.51±0.34^{a***}	2.10±0.42^{b*}
TC (mg/dL)	85±7	88±4	232±27^{a***}	112±11^{b***}
TG (mg/dL)	82±7	71±8	179±19^{a***}	172±15
HDL-C (mg/dL)	59±4	60±3	55±3	58±5
ALT(IU/L)	59±2	57±4	200±13^{a***}	198±13
AST(IU/L)	103±4	125±12	232±4^{a***}	249±38

Abbreviations: TC: Total cholesterol, **TG:** Triglyceride, **HDL-C:** High-density lipoprotein-cholesterol, **ALT:** Alanine aminotransferase, **AST:** Aspartate aminotransferase. **C:** Control rats, **C+BNP:** Control rats given *B. nigra* pollen, **D:** Streptozotocin-induced diabetic rats, **D+BNP:** Diabetic rats given *B. nigra* pollen. Values are given as mean ± SEM (standard error of the mean), Statistical comparison; ^{a}C vs D, ^{b}D vs D+BNP, Statistical significance, *p<0.05, *p<0.01,***p<0.001.

The heart, muscle, liver, kidney tissues, and plasma MDA levels had reduced at the D+BNP group compared with the D group (Fig. 1 and 2). The metabolic improvements we detected in carbohydrate and lipid metabolisms may be one of the factors underlying the low plasma and tissue MDA levels in the D+BNP group. MDA is the best sign of oxidative stress, giving us information about lipid peroxidation [31]. Studies have shown that the seeds and leaves of *B. nigra* and bee pollen have direct antioxidant properties [32, 33, 34, 35], which may be another factor contributing to the low MDA levels observed in our study. However, when we analyzed the MDA levels of the C+BNP group, a significant decrease was found only in the muscle tissue of this group, suggesting that the effect of *B. nigra* pollen on these parameters is more effective in diabetic conditions. The liver has an effective role in the regulation of glucose and lipid homeostasis and the metabolism of reactive oxygen species (ROS). In our study, the increase in liver tissue MDA levels, which we detected in diabetic rats, was accompanied by an increase in serum AST and ALT levels, which supported deterioration in the liver. Similar to our findings, Laaroussi et al. [36] found that ALT and AST levels were increased in D-glucose-induced type 2 diabetes in rats and they also showed that enzyme levels were restored with pollen treatment. However, we did not observe any improvement in ALT and AST levels in this study. This discrepancy might result from the usage of different plant pollens or the amount and duration of pollen application.

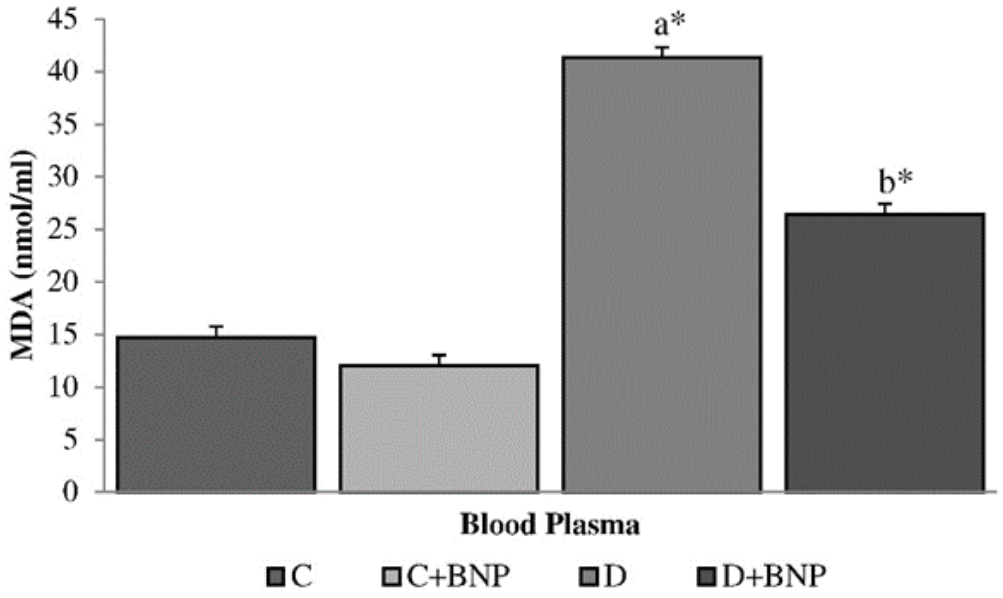

Fig. 1. *Plasma malondialdehyde (MDA). Values are given as mean±SEM (standard error of the mean) Statistical comparison: [a]C vs D, [b]D vs D+BNP, Statistical significance, *p<0.05 for abbreviations of study groups, see Table 1.*

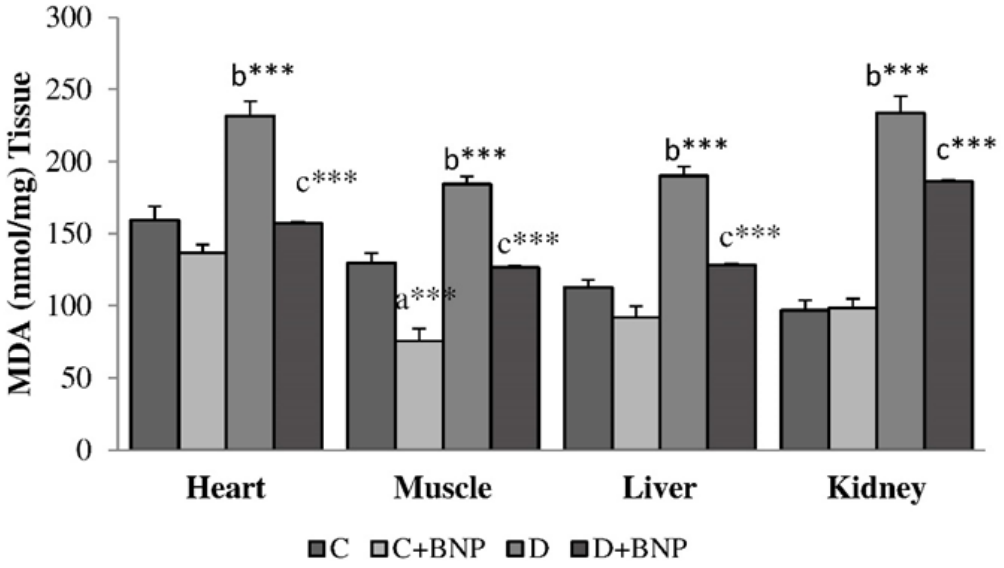

Fig. 2. *Tissue malondialdehyde (MDA) levels. Values are given as mean±SEM (standard error of the mean). Statistical comparison: [a]C vs C+BNP, [b]C vs D, [c]D vs D+BNP, Statistical significance, ***p<0.001, for abbreviations of study groups, see Table 1.*

There are different findings and opinions about antioxidant enzyme levels and activities in combating oxidative stress in diabetic conditions [37,38]. In this study, the increase in serum SOD and GPx activities in group D was significant compared to group C but the decrease in SOD and GPx activities in the D+BNP group was not statistically significant (Table 2). SOD and GPx are enzymes that protect cells from the toxic effects of endogenously produced free radicals. SOD dismutases superoxide radicals to hydrogen peroxide and oxygen [39]. Therefore, the increase observed in SOD and GPx activities in the D group can be a protective response against increased superoxide formation as observed in our previous studies [21, 22, 23, 24]. Furthermore, we may say that although statistically non-significant, there was a tendency to diminish in the activities of SOD and GPx in the D+BNP group (Table 2). Considering that plasma and tissue MDA levels in diabetic rats decreased with BNP treatment, we can speculate a decrease in superoxide formation.

PON1 is another antioxidant enzyme related to high-density lipoprotein (HDL). Many studies have reported PON1 and ARE enzyme activity and/or levels decrease due to ROS attack in high oxidative stress conditions such as coronary artery disease, dyslipidemia, and diabetes [40, 41, 42, 43]. While there was a significant diminish in serum PON and ARE activities in group D rats compared to group C, serum PON and ARE activities were significantly higher in the D+BNP group than in group D. The significant reduction in serum PON and ARE enzyme activities in diabetic rats might be related to glycation and/or oxidative modification of HDL and/or enzyme paraoxonase [42]. However, the increase in PON and ARE enzyme activity in the D+BNP group made us think that *B nigra* pollen may protect lipoproteins and tissues against oxidative modification in diabetic conditions and may have positive contributions to prevent atherosclerosis. In the C+BNP group, there was no change in PON and ARE activities as in SOD and GSH-Px

enzymes. This considered us the idea that BNP does not directly affect these enzymes, at least under normal physiological conditions.

Table 2. Serum superoxide dismutase, glutathione peroxidase paraoxonase, and arylesterase activities in the control and experimental groups.

Group	C	C+BNP	D	D+BNP
SOD (ng/ml)	1.96±0.16	2.05±1.42	2.60±0.22^{a***}	2.07±0.32
GPx(U/mL)	64.10±9.20	64.30±54.00	102.40±2.10^{a***}	78.20±22.30
PON (U/L)	56.70±6.90	59.70±8.50	36.40±1.40^{a***}	54.50±61.40^{b*}
ARE (U/L)	207.1±23.60	213.80±14.90	197.20±17.50	228.20±14.40^{b*}

Abbreviations: SOD: Superoxide dismutase, **GPx:** Glutathione peroxidase, **PON:** Paraoxonase, **ARE:** Arylesterase. Values are given as mean ± SEM (standard error of the mean), Statistical comparison: ^{a}C vs D, ^{b}D vs D+BNP, Statistical significance, *p< 0.05, ***p< 0.001, for abbreviations of study groups, see Table 1.

In this study, a bone marrow micronucleus test was performed to evaluate genotoxicity and anti-genotoxic effects of BNP pollen in STZ-induced diabetic rats in vivo. Table 3 and 4. and Figure. 3 show the results of the bone marrow MN assay. There was no difference between group C and group C+BNP in terms of both MNPCE % and PCE / NCE values.

Table 3. Frequency of micronucleus in polychromatic erythrocytes.

Groups	MNPCE %	p^a	p^b	Reduction MN % (between D and D+BNP)
C	0.500±0.043	-	0.0001	
C +BNP	0.500±0.030	1.000	0.0001	
D	4.342±0.188	0.0001	-	
D+ BNP	1.857±0.134	0.0001	0.0001	64.211

Abbreviations: MNPCE %: Percentage of Micronucleus in polychromatic erythrocytes, **PCE:** polychromatic erythrocytes. For abbreviations of study groups, see Table 1.
Values are given as mean ± SEM (standard error of the mean) p^a: Significance of MNPCE when compared with group C, p^b: Significance of MNPCE when compared with group D.

The MNPCE % values increased significantly in group D compared to group C. However, PCE / NCE values were significantly decreased in group D. While the MNPCE % value decreased significantly in the D+BNP group compared to the D group, the PCE / NCE values increased significantly.

Table 4. The ratio of polychromatic erythrocytes / Normachromatic erythrocytes.

Groups	PCE / NCE	p^a	p^b	Increment PCE/NCE ratio % (between D and D+BNP)
C	1.727±0.115	-	0.001	
C +BNP	1.721±0.066	1.000	0.0001	
D	0.711±0.022	0.001	-	
D+ BNP	1.013±0.028	0.004	0.0001	29.72

Values are expressed as mean ± SEM (standard error of the mean), PCE: polychromatic erythrocytes NCE: Normachromatic erythrocytes. For abbreviations of study groups, see Table 1. p^a: Significance of MNPCE when compared with group C, p^b: Significance of MNPCE when compared with group D.

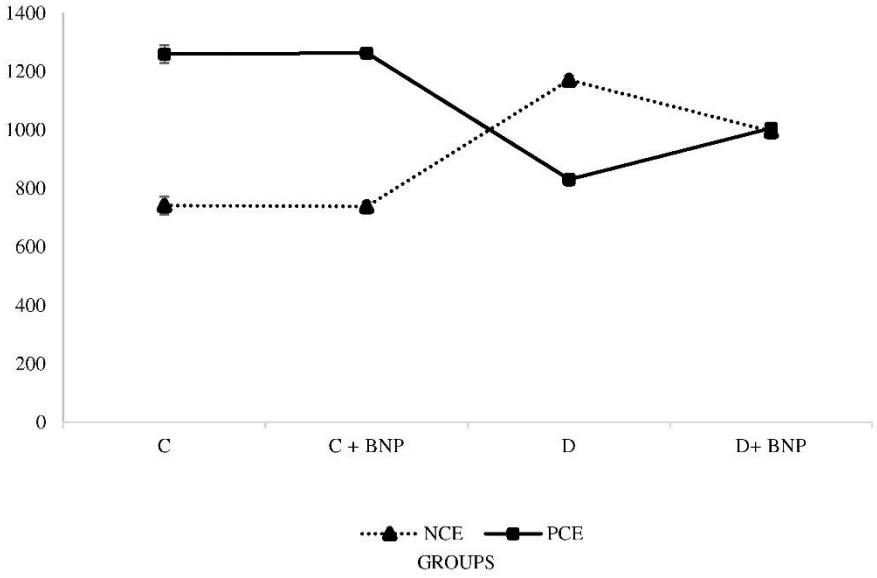

Fig. 3. *Numbers of PCE and NCE. Values are given as mean and SEM (standard error of the mean), PCE: polychromatic erythrocytes NCE: Normachromatic erythrocytes. For abbreviations of study, groups see Table 1.*

There are recent studies on the potential of STZ-induced diabetes to increase DNA damage and cytotoxicity [44, 45, 46, 47, 48], but, to our knowledge, there is not any study that investigates the bone marrow genotoxicity and anti-genotoxic effects of BNP pollen in diabetic conditions in vivo.

MNs are structures that are formed during cell division, do not join the main nucleus, and consist of whole chromosomes and/or chromosome fragments. Factors such as deficiency of some genes controlling the cell cycle, dysfunction of the mitotic spindle, or chromosomal damage can cause MN [47]. In this study, the increased frequency of MN (%MNPCE) and cytotoxicity (PCE/NCE) observed in group D are thought to be indicative of DNA damage caused by increased oxidative stress in diabetic conditions [44, 45, 46, 47]. Compared to the D group, the frequency of MNPCE decreased by approximately 65% and the PCE/NCE ratio increased by approximately 30% in the D+BNP group. These results suggest that *B. nigra* pollen does not have genotoxic and cytotoxic effects in healthy rat bone marrow further that *B. nigra* pollen may have protective/preventive effects against increased micronucleus formation as a result of DNA damage due to oxidative stress in diabetes.

CONCLUSION

The current study findings show that *B. nigra* pollen has antihyperglycemic, antihyperlipidemic, antioxidant, and antigenotoxic properties and may be a promising adjunctive therapeutic agent in curing diabetes and/or reducing diabetes-related complications. However, since there is no previous study of monofloral *B. nigra* pollen in diabetes, we suggest that further research is recommended to elucidate the mechanism behind its therapeutic/supportive effects in diabetes.

Ethics Committee Approval: This study was approved by Bursa Uludag University Animal Experiment Local Ethics Commission (Protocol number: 26. 2019. Mar). All experimental procedures on animals were carried out following ethical policies and procedures approved by the Animal Care and Use Committee of Uludağ University, Bursa, Turkey (Ethical approval number: 2019-04/08).

Conflict of Interest. "The authors declared that there is no conflict of interest."

Authorship Contributions. Concept: S.T., A.T., Design: S.T., E.S, Ö.V., M.D., Data collection: S.T., C.N.T., A.T., Ö.V., H.H., C.B., Analysis or Interpretation: S.T., E.S., M.D., Ö.V., Literature Search: S.T., E.S., Ö.V., N.Ç., T.Ç., Writing: S.T., E.S., A.T, Ö.V.

Financial Disclosure. This research received no grant from any funding agency/sector.

REFERENCES

[1] Giacco, F., Brownlee, M. (2010): Oxidative stress and diabetic complications. Circulation Research 107:1058–1070.

[2] Volpe, C.M.O., Villar-Delfino, P.H., Dos Anjos, P.M.F., Nogueira-Machado. J.A. (2018): Cellular death, reactive oxygen species (ROS), and diabetic complications. Cell Death & Disease 9:119.

[3] Forman, H.J., Zhang, H. (2021): Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nature Reviews Drug Discovery 30:1–21.

[4] Chung, S.S., Ho, E.C., Lam, K.S., Chung, S.K. (2003): Contribution of polyol pathway to diabetes-induced oxidative stress. Journal of the American Society of Nephrology 14:S233–236.

[5] Singh, R., Devi, S., Gollen, R. (2015): Role of free radical in atherosclerosis, diabetes, and dyslipidemia: larger-than-life. Diabetes/Metabolism Research and Reviews 31:113–126.

[6] Dave, H.D., Preuss C.V. (2021): Human Insulin. In: StatPearls Publishing; Treasure Island (FL).

[7] Stein, S.A., Lamos, E.M., Davis, S.N. (2013): A review of the efficacy and safety of the oral antidiabetic drug. Expert Opinion on Drug Safety 12:153–175.

[8] Denisow. B., Denisow-Pietrzyk, M. **(**2016): Biological and therapeutic properties of bee pollen: A review. Journal of the Science of Food and Agriculture 96:4303–4309.

[9] Linskens, H.F., Jorde, W. Pollen as food and medicine—A review. (1997): Economic Botany 51:78–86.

[10] Komosińska-Vassev, K., Olczyk, P., Kázmierczak, J., Mencner, L., Olczyk, K. (2015): Bee pollen: Chemical composition and therapeutic application. Evidence-Based Complementary and Alternative Medicine. Med ID 297425, 1-6. http://dx.doi.org/10.1155/2015/297425.

[11] Almaraz-Abarca, N., Campos, M.G., Ávila-Reyes, J.A., Naranjo-Jiménez, N., Corral, J.H., González-Valdez, L.S. (2007): Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (Prosopis juliflora, Leguminosae). Journal of Food Composition and Analysis 20:119–124.

[12] Anand, P., Murali, Y.K., Tandon, V., Chandra, R., Murthy, P.S. (2007): Preliminary studies on the antihyperglycemic effect of aqueous extract of Brassica nigra (L.) Koch in streptozotocin-induced diabetic rats. Indian Journal of Experimental Biology 45:696-701.

[13] Anand, P., Murali, Y.K., Tandon, V., Murthy, P.S., Chandra, R. (2009): Insulinotropic Effect of Aqueous Extract of Brassica nigra Improves Glucose Homeostasis in Streptozotocin-Induced Diabetic Rats. Experimental and Clinical Endocrinology and Diabetes Latest 117:251-256.

[14] Singhal, P., Singla, N., Sakhare, D., Sharma, A.K. (2017): A Comparative Evaluation of In-Vitro Antioxidant Activity of Some Commonly Used Spices of Northern India. The Natural Products Journal 7: 1-6.

[15] Biljana, S.Đ., Dragan, Z.T., Zoran, B.T., Ivica, G.Đ., Ljiljana, P.S., Mitrović, P.M, Veljković, V.B. (2020): The effect of the triethanolamine: glycerol deep eutectic solvent on the yield, fatty acid composition, antioxidant activity and physicochemical properties of black mustard (Brassica nigra L.) seed oil. Journal of Food Measurement and Characterization 14:2570–2577.

[16] Spulber, R., Doğaroğlu, M., Babeanu, N., Popa, O. (2018): Physicochemical characteristics of fresh bee pollen from different botanical origins. Romanian Biotechnological Letters 23:13357-13365.

[17] Ketkar, S.S., Rathore, A.S., Lohidasan, S., Rao, L., Paradkar, A.R., Mahadik, K.R. (2014): Investigation of the nutraceutical potential of monofloral Indian mustard bee pollen. Journal of Complementary and Integrative Medicine 12:379-389.

[18] Kalaycıoğlu, Z., Kaygusuz, H., Döker, S., Kolaylı, S., Erim, F.B. (2017): Characterization of Turkish honeybee pollens by principal component analysis based on their individual organic acids, sugars, minerals, and antioxidant activities. LWT– Food Science and Technology 84:402-408.

[19] Ohkawa, H., Ohishi, N., Yagi, K. (1979): Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. Analytical Biochemistry 95: 351–358.

[20] Young, I.S., Trimble, E.R. (1991): Measurement of malondialdehyde in plasma by high-performance liquid chromatography with fluorimetric detection. Annals of Clinical Biochemistry 28: 504–508.

[21] Waters, M.D., Brady, A.L., Stack, H.F., Brockman, H.E. (1990): Antimutagenicity profiles for some model compounds. Mutation Research 238:57–85.

[22] Taş, S., Sarandöl, E., Dirican, M. (2014): Vitamin B6 supplementation improves oxidative stress and enhances serum paraoxonase/arylesterase activities in streptozotocin-induced diabetic rats. The Scientific World Journal 351598. DOI: 10.1155/2014/351598.

[23] Sarandol, E., Tas, S., Serdar, Z., Dirican, M. (2020): Effects of thiamine treatment on oxidative stress in experimental diabetes. Bratislava Medical Journal 121:235–241.

[24] Bassalat, N., Taş, S., Jaradat, N. (2020): Teucrium leucocladum: An Effective Tool for the Treatment of Hyperglycemia, Hyperlipidemia, and Oxidative Stress in Streptozotocin-Induced Diabetic Rats. Evidence-based Complementary and Alternative Medicine 8. https://doi.org/10.1155/2020/3272103.

[25] Amal, D., Drira, M., Sana, B., Najla, H., Kais, M., Adel, K., Néji, GH. (2015): Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. Arabian Journal of Chemistry http://doi.org/10.1016/j.arabjc.

[26] Dragan, S., Andrica M.F., Maria-Corina, S., Timar, R. (2015): Polyphenols-rich natural products for the treatment of diabetes. Current Medicinal Chemistry 21:14-22.

[27] Hamden, K., Keskes, H., Belhaj, S., Mnafgui, K., Feki, A., Allouche, N. (2011): Inhibitory potential of omega-3 fatty and fenugreek essential oil on key enzymes of carbohydrate-digestion and hypertension in diabetes rats. Lipids in Health and Disease 10:226. DOI: 10.1186/1476-511X-10-226.

[28] Hogan, S., Zhang, L., Li, J., Sun, S., Canning, C., Zhou, K. (2010): Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase. Nutrition & Metabolism 7:71. DOI: 10.1186/1743-7075-7-71.

[29] Pereira, D.F., Cazarolli, L.H., Lavado, C., Mengatto, V., Santos, Figueiredo, M.R.B., Guedes, A., Pizzolatti, M.G., Silva, F.R.M.B. (2011): Effects of flavonoids on α-glucosidase activity: potential targets for glucose homeostasis. Nutrition 27:1161–1167.

[30] Sireesha, Y., Kasetti, R.B., Nabi, S.A., Swapna, S., Apparao, C. (2011): Antihyperglycemic and hypolipidemic activities of Setaria italica seeds in STZ diabetic rats. Pathophysiology18:159–164.

[31] Gaweł, S., Wardas, M., Niedworok, E., Wardas, P. (2004): Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci lekarskie 57:453-455.

[32] Karadal, F., Onmaz, N.E., Abay, S., Yildirim, Y., Al, S., Tatyuz, I., Akcay, Aytac. (2018): A Study of Antibacterial and Antioxidant Activities of Bee Products: Propolis, Pollen and Honey Samples. The Ethiopian Journal of Health Development 32:116-122.

[33] Saral, Ö., Yıldız, O., Yazıcıoğlu, RA., Yuluğ, E., Canpolat, S., Öztürk, F., Kolaylı S. **(**2016): Apitherapy products enhance the recovery of CCL4-induced hepatic damages in rats. Turkish Journal Of Medical Sciences 46:194–202.

[34] Ulusoy, E., Kolayli, S. (2013): Phenolic composition and antioxidant properties of Anzer bee pollen. Journal of Food Biochemistry 38:73–82.

[35] Rajamurugan, R., Selvaganabathy, N., Kumaravel, S., Ramamurthy, C.H., Sujatha, V., Thirunavukkarasu, C. (2012): Polyphenol contents and antioxidant activity of Brassica nigra (L.) Koch. leaf extract. Natural Product Research 26:2208-2210.

[36] Laaroussi, H., Bakour, M., Ousaaid, D., Aboulghazi, A., Ferreira-Santos P., Genisheva, Z., Teixeira, J.A., Lyoussia, B. (2020): Effect of antioxidant-rich propolis and bee pollen extracts against D-glucose induced type 2 diabetes in rats. Food Research International 138:109802 https://doi.org/10.1016/j.foodres.2020.109802.

[37] Lewandowski, L., Kepinska, M., Milnerowicz, H. (2019): The copper-zinc superoxide dismutase activity in selected diseases. European Journal of Clinical Investigation 49:e13036 DOI: 10.1111/eci.13036.

[38] Huanga, J.Q., Zhouc, J.C., Wua, Y.Y., Rena, F.Z., Le, X.G. (2018): Role of glutathione peroxidase 1 in glucose and lipid metabolism-related diseases. Free Radical Biology and Medicine 127:108–115. doi:10.1016/j.freeradbiomed.2018.05.077

[39] Ighodaro, O.M.,–Akinloye, O.A. (2018): First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defense grid. Alexandria Journal of Medicine 54:287-293.

[40] Meneses, M.J., Silvestre, R., Sousa-Lima, I., Macedo, M.P**.** (2019): Paraoxonase-1 as a Regulator of Glucose and Lipid Homeostasis: Impact on the Onset and Progression of Metabolic Disorders. International Journal of Molecular Sciences 20:4049. DOI: 10.3390/ijms20164049.

[41] Aviram, M., Fuhrman, B. (1998): LDL oxidation by arterial wall macrophages depends on the oxidative status in the lipoprotein and the cells: role of prooxidants vs. antioxidants. Molecular and Cellular Biochemistry 188: 149–159.

[42] Tsuzura, S., Ikeda, Y., Suehiro, T., Ota, K., Osaki, F., Arii, K., Kumon, Y., Hashimoto, K. (2004): Correlation of plasma oxidized low-density lipoprotein levels to vascular complications and human serum paraoxonase in patients with type 2 diabetes. Metabolism: Clinical and Experimental 53: 297–302.

[43] Karakaya, P., Ozdemir, B., Mert, M., Okuturlar, Y. (2018): Relation of Paraoxonase 1 Activity with Biochemical Variables, Brachial Artery Intima-Media Thickness in Patients with Diabetes with or without Obesity. Obesity Facts 11:56–66.

[44] Vikram, A., Tripathi, D.N., Ramarao, P., Jena, G.B. (2007): Evaluation of streptozotocin genotoxicity in rats from different ages using the micronucleus assay. Regulatory Toxicology and Pharmacology 49:238–244.

[45] Kita-Tomihara, T., Sato, S., Yamasaki, S., Ueno, Y., Kimura, G., Ketema, R.M., Kawahara, T., Kurasaki, M., Sait, T. (2019): Polyphenol-enriched azuki bean (Vina angularis) extract reduces the oxidative stress and prevents DNA oxidation in the hearts of streptozotocin-induced early diabetic rats. International Journal of Food Science 70: 845-855.

[46] Bolzán, A.D., Bianchi, M.S. (2002): Genotoxicity of streptozotocin. Mutation Research 512:121–134.

[47] Stopper, H., Müller, S.O. (1997): Micronuclei as a biological endpoint for genotoxicity: A minireview. Toxicology in Vitro 11:661–667.

[48] Lee, S.C., Chan, J.C.N. (2015): Evidence for DNA Damage as a Biological Link Between Diabetes and Cancer. Chinese Medical Journal 128:1543-1548. DOI: 10.4103/0366-6999.157693.