Original Article

Effects of Total Suspended Particulates on the Chronotropic, Inotropic, and Dromotropic Parameters of the Heart, Blood Pressure, and Oxidative Stress in Diabetic Rats and the Protective Effects of Crocin and Insulin

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ABSTRACT

Background: Diabetes mellitus is a metabolic disease characterized by hyperglycemia that results from inadequacies in secreting insulin and/or the action of insulin. Increased exposure to particulate matter at high concentrations is associated with increased mortality in heart diseases. This study aimed to evaluate the effects of insulin and crocin on cardiac electrophysiological parameters, blood pressure, and oxidative stress in alloxan-induced diabetic rats exposed to the total suspended particulate (TSP).

Methods: Adult male Wistar rats (n=60) with bodyweight between 200 and 250 g were divided into 10 experimental groups (6 animals per group): control, crocin, diabetic, TSP (5 mg/kg TSP, intratracheal instillation), diabetic-crocin, diabetic-insulin, diabetic-TSP, crocin-TSP, diabetic-TSP-insulin, and diabetic-TSP-crocin. The effects of chronotropic (heart rate), inotropic (QRS voltage), and dromotropic (P-R intervals and QTc intervals) were evaluated with standard bipolar limb lead II. Systolic and diastolic blood pressure measurements were recorded with the tail cuff. Antioxidant and lactate dehydrogenase enzymes were also measured.

Results: The diabetic groups and groups exposed to TSP experienced a deleterious effect on cardiac electrophysiological parameters and blood pressure, with a significant decrease in the activity of antioxidant enzymes. These changes were improved with crocin and insulin.

Conclusions: In this work, the protective role of crocin and insulin alone was observed in diabetic groups and groups exposed to TSP by improving the electrophysiological parameters of the heart, blood pressure, and oxidative stress. ***(Iranian Heart Journal 2022; 23(3): 6-23)***

KEYWORDS: Diabetes, Cardiac electrophysiological, TSP, Crocin, Insulin

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Diabetes mellitus (DM) is characterized by hyperglycemia due to deficiencies in insulin secretion, insulin action, or both.[1] Some of the common complications of DM are peripheral neuropathy, chronic kidney disease, albuminuria, coronary artery disease, heart failure, and stroke.[2] In addition, DM increases the risk of developing peripheral vascular disease, dyslipidemia, hypertension, and obesity.[3]

In DM, the risk of developing myocardial infarction or stroke is increased by 2 to 3 folds, while the risk of death is increased by 2 folds.[4] The prevalence of heart failure and left ventricular dysfunction is greater in patients with DM,[5] and hypertension is diagnosed in about one-third of diabetic patients.[6] The hyperglycemic state can lower the activity of antioxidant enzymes and increase the levels of oxidative stress-induced DNA damage markers.[7]

Oxidative stress is considered the reason for type II DM, β-cell dysfunction, and the development of insulin resistance.[8] Lipid peroxidation increases free radical production and diminishes antioxidant status, and it is a biomarker of oxidative stress in diabetic patients.[9] Increased particulate matter (PM) (2.5–10) is linked to increased mortality due to respiratory, cardiac, and cerebrovascular conditions.[10]

Oxidative stress plays an important role in the cardiac and vascular abnormalities of cardiovascular diseases (CVDs). Inhaling air pollutants causes inflammation and major reactive oxygen species (ROS), indicative of pulmonary oxidative stress.[11] Oxidative stress can occur in the body following exposure to pro-oxidant air pollutants.[12] Air pollution exposure-related endothelial dysfunction is considered an activating factor for CVDs.[13]

Total suspended particulates (TSPs) are fine and ultrafine particles dispersed into the air from combustion processes, traffic-related particles, industrial activities, and natural sources, as well as particles with a diameter of 10 μm or less.[14] Studies in Japan have shown a positive association between suspended PM concentrations and daily mortality[15] and an association between increased PM (2.5 concentration) with increased mortality from heart diseases.[16] Research has also indicated an association between oxidative stress and depressed antioxidant capacity and an increased risk of CVDs.[11] Following exposure to air pollution, the response of oxidative stress to ROS and subsequent inflammation may be an important mechanism.[17]

Antioxidants may defend against the adverse effects of oxidative stress.[18] Antioxidant crocin, an active component of *Crocus sativus* (saffron), decreases fasting blood glucose and HbA1 levels, increases serum insulin levels, enhances antihyperlipidemic effects,[19] and stimulates pharmacological events such as glucose uptake by peripheral tissues.[20] Crocin reduces the incidence of oxidative stress and improves antioxidant defenses.[21] Insulin produced in β-cells in the islet of Langerhans lowers elevated blood glucose; hence, the loss of insulin is related to DM.[22]

In the current study, we sought to evaluate the effects of insulin and crocin on cardiac electrophysiological parameters, blood pressure, and oxidative stress in alloxan-induced diabetic rats exposed to TSPs.

METHODS

Study Area

Abadan is a city in Khuzestan Province located in southwest Iran at 30°20′21″N 48°18′15″E. It is one of the hottest places on the earth and experiences frequent sand and dust storms. A Moderate Resolution Imaging Spectroradiometer (MODIS) image and a backward trajectory of the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) previously showed that the sources of a dust storm were generally western countries in the vicinity of Iran, with the red line backward trajectory on 500 m above the ground level and the wind direction (~160˚)

suggesting that the source of the dust storm was the southwestern plain in Khuzestan province (Fig. 1). In that instance, air pressure was at the lowest level 2 days before the occurrence of the dust storm. In another study in Ahvaz, PM10 peaks during local storms, dust from Iraq, dust from both sources, and 1- and 2-peak dust events decreased the dewpoint and relative humidity.[23]

Chemicals

Alloxan monohydrate (Sigma Chemical Company Inc, USA), ketamine HCl (10%), and xylazine (2%) were obtained from Alfasan Company (the Netherlands). Crocin sodium was purchased from Sigma-Aldrich Company (USA), and insulin (LANSULIN) was obtained from Exir Pharmaceutical Company (Iran). SOD and CAT kits were purchased from Zell Bio GmbH Company (Germany). A lactate dehydrogenase (LDH) kit was purchased from Pars Azmoun (Iran).

Ethical Statement

The animals in the present study were treated according to the guidelines of the Laboratory Animal Care and Use Ethics Committee, Abadan Faculty of Medical Sciences. They were maintained in standard conditions (50% humidity, 12-h dark-light cycle, and 22 ± 2 °C temperature), with free access to tap water and a standard rat chow diet. Ethical approval was obtained (reference number: IR.ABADANUMS.REC.1395.93). This research was a funded project (95U-1065).

Instruments, Reagents, and Sample Preparations

All the standard stock solutions of nickel (Ni), mercury (Hg), chromium (Cr), arsenic (As), lead (Pb), and cadmium (Cd) were of the analytical grade and purchased from Merck (purity >99%). High-purity concentrated nitric acid (HNO_3, 65%) and perchloric acid ($HClO_4$, 70%) were purchased from Merck. Ultrapure water was prepared using the Milli-Q System from Millipore (USA). The calibration curve was prepared by diluting a mixed standard stock solution to obtain quantitative concentrations. Inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500, and American) was applied to determine the concentration of heavy metals. The instrumental conditions of the ICP MS are summarized in Table 1. ICP-MS analyses were done by using the following wavelengths: As 188.98 nm, Cd 214.43 nm, Cr 267.71nm, Hg 184.88 nm, Ni 231.64 nm, and Pb 220.35 nm.

The concentrations of heavy metals such as Ni, Hg, Cr, As, Pb, and Cd in Abadan were investigated on April 29, 2017, in Iran. A mixture of nitric acid and $HClO_4$ was used for acid digestion. A dried soil sample (1 g) was poured into an Erlenmeyer; then, 10 mL of nitric acid 65% was added and kept at the ambient temperature for 24 hours. The next day, 5 mL of 70% $HClO_4$ was added to the samples. The samples were digested in a Bain Marie water bath at 80 °C for 5 hours and proceeded to a liquid solution. After digestion, the samples were placed at room temperature to cool down, and the digested samples were filtered through a 0.45 µm filter and diluted to 25 ultra-pure water.[24] After preparation, heavy metal concentrations were determined using an ICP MS.

Animals and Treatments

The animals were maintained under standard conditions (50% humidity, 12-h dark-light cycle, and temperature 22 ± 2 °C). They had free access to tap water and a standard rat chow diet. The animals were treated according to the guidelines of the Laboratory Animals Care and Use Ethics Committee, Abadan Faculty of Medical Sciences.

Sixty adult male Wistar rats with bodyweight between 200 and 250 g were divided into 10 experimental groups (6

animals per group: control, crocin (receiving crocin, 50 mg/kg[25] [Crocin was suspended in 0.1 mL of normal saline and administered to the rats, intraperitoneally (IP).], diabetic (treatment with alloxan; a single dose of 120 mg/kg of alloxan monohydrate, IP),[26] TSP (5 mg/kg of TSP, intratracheal instillation), diabetic-crocin (treatment with alloxan; a single dose of 120 mg/kg of alloxan monohydrate, IP, and crocin, 50 mg/kg, IP), diabetic-insulin (treatment with alloxan; a single dose of 120 mg/kg of alloxan monohydrate, IP, and regular insulin (5 U/kg) through the subcutaneous injection of a single dose),[26,27] diabetic-TSP (treatment with alloxan; a single dose of 120 mg/kg of alloxan monohydrate, IP, and 5 mg/kg of TSP, intratracheal instillation), crocin-TSP (crocin, 50 mg/kg, IP, and 5 mg/kg of TSP, intratracheal instillation), diabetic-TSP-insulin (treatment with alloxan; a single dose of 120 mg/kg of alloxan monohydrate, IP, 5 mg/kg of TSP, intratracheal instillation, and regular insulin (5 U/kg) through a subcutaneous injection of a single dose), and diabetic-TSP-crocin (treatment with alloxan; a single dose of 120 mg/kg of alloxan monohydrate, IP, 5 mg/kg of TSP, intratracheal instillation, and crocin, 50 mg/kg, IP).

The basal glucose level of the rats was measured before inducing diabetes. Alloxan monohydrate dissolved in sterile normal saline at a dose of 120 mg/kg was injected into the 6 groups of rats IP to induce DM. Following 3 days of alloxan injection, DM with hyperglycemia (ie, with blood glucose levels >50 mg /dL) was confirmed.[26,28]

TSP Intratracheal Instillation

The TSP sample was suspended in normal saline and mixed; then, it was introduced into the trachea through an intubation tube after anesthetizing the animals with IP injections of ketamine-xylazine (xylazine (10 mg/kg), ketamine (50 mg/kg), and 0.1 mL of saline.

Electrocardiogram (ECG) Recording

Crocin was given 24 hours after anesthetizing the animals with an IP injection of ketamine-xylazine. For the evaluation of the effects of crocin, 15 minutes after the administration of crocin, ECG was done with a standard bipolar limb lead II to measure chronotropic (heart rate), inotropic (QRS voltage), and dromotropic (P-R intervals and QTc intervals) changes. Since QT interval changes with heart rate, the corrected QT (QTc) was calculated. The most frequently used Bazett formula was employed to correct the QT for the heart rate (QTc).

Bazett formula: QTc (QT corrected for HR) = QT/square root RR

Systolic and Diastolic Blood Pressure (SBP and DBP) Measurements

SBP was recorded before and after crocin administration by the tail-cuff method 15 minutes after anesthetizing the rats.

Antioxidant Enzymes and LDH

Blood samples were collected directly from the heart of the rats, transferred to EDTA-containing tubes, and centrifuged at 4000*g* for 10 minutes to obtain plasma. The plasma was then stored at −80 °C for biochemical analysis. While LDH was measured using a Pars Azmoun kit (Iran), antioxidant enzymes (CAT and SOD) were measured using Zell Bio GmbH kits (Germany).

Statistical Analysis

The results were analyzed using GraphPad Prism8 and expressed as the mean ± standard error of the mean (SEM).

The normality of the distribution of quantitative variables was assessed through the Kolmogorov–Smirnov test. One-way ANOVA (analysis of variance), followed by

the Fisher LSD (least significant difference) test, was utilized to compare the mean of 1 group with the mean of another. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Concentrations of Heavy Metals

The results of heavy metal concentrations using the proposed methods are shown in Table 2. In this research, 6 heavy metals, namely Ni, Hg, Cr, As, Pb, and Cd, were selected in a soil sample. The sample was investigated using ICP-MS. For the preparation of the heavy metals in the sample, acid digestion was used. After preparations, some toxic metals in the soil sample were analyzed by ICP-MS. As shown in Table 1, a lower concentration of heavy metals was observed in As. The mean concentration of As was 0.02 ppm. The mean concentrations of Cr and Ni were at the same level. The mean concentration of Pb was obtained as 0.74 ppm. The highest concentration of heavy metals was found in Pb. The mean concentration was 0.74 ppm. In this study, the Hg concentration was 0.05 ppm. The results showed that the concentration of Cd in the sample was 0.1 ppm, which was below the standard limit.

Table 1: Instrumental condition of the ICP MS

Parameters	Value / Type
RF generator power	1200 W
RF frequency	Resonance Frequency: 24 MHz
Plasma, auxiliary, and nebulizer gas	Argon
Plasma gas flow rate	12.2 (L/min)
Auxiliary gas flow rate	0.8 (L/min)
Nebulizer gas flow rate	0.8 (L/min)
Sample uptake time	260 total (S)
Measurement replicate	3
Type of detector solid-state	CCD
Type of spray chamber cyclonic	Modified Lichte

Table 2: Concentrations of the heavy metals in the sample

Heavy Metals	Concentration ppm
As	0.02
Cd	0.10
Cr	0.08
Hg	0.05
Ni	0.08
Pb	0.74

Heart Rate (the chronotropic parameter of the heart)

In comparison with the control group, heart rate increased significantly in the TSP group (P=0.03). Heart rate rose significantly in the diabetic group compared with the control group (P=0.03), but no significant change was observed in the crocin group (P=0.07). There was also a significant increase in heart rate in the diabetic–TSP group (P=0.02). In the diabetic-crocin group, a significant decrease was found in heart rate compared with the diabetic group (P<0.0001), but no significant change was found compared with the control group, indicating that crocin had a positive effect on heart rate in the diabetic group. In the diabetic-insulin groups, a significant decrease in heart rate was observed compared with the diabetic group (P<0.0001). In comparison with the decrease in heart rate among the rats treated with crocin, the rats treated with insulin showed a more marked decrease in heart rate, indicating that insulin significantly reduced heart rate in both healthy and diabetic groups. In the TSP group receiving crocin, there was a significant increase in heart rate compared with the control group (P=0.03), but no significant change was observed compared with the TSP group (P=0.29). In the diabetic TSP group receiving insulin, heart rate was significantly decreased compared with the TSP group (P=0.007). In the diabetic-TSP-insulin group, a significant decrease occurred in heart rate compared with the TSP-diabetic group (P=0.01) (Fig. 2).

PR Interval (the dromotropic parameter of the heart)

By comparison with the control group, the PR interval was increased in the TSP group and the diabetic group, but it was not significant. Compared with the diabetic group, the diabetic-TSP groups receiving insulin or crocin had shortened PR intervals, which was significant in the diabetic-TSP-insulin group (P=0.02). In the crocin group, a significant reduction in the PR interval was observed compared with the TSP group (P=0.02). In the group receiving TSP and crocin, a significant decrease in the PR interval was observed compared with the TSP group (P=0.01). In the diabetic groups receiving TSP with insulin or crocin, a significant reduction in the PR interval was observed compared with the TSP group (P=0.002 and P=0.03) (Fig. 3A).

QTc Interval (the dromotropic parameter of the heart)

In comparison with the control group, the QTc interval was significantly increased in the TSP group (P=0.004). The diabetic group showed a significant increase in the QTc interval compared with the control group (P=0.006). The QTc interval was significantly greater in the diabetic groups receiving TSP than in any stand-alone diabetic (P<0.0001) or TSP (P=0.0009) groups, indicating a synergistic effect for DM and TSP on the QTc interval. A significant decrease in the QTc interval was observed in the diabetic-insulin group when compared with the diabetic group (P=0.04). A significant fall in the QTc interval was observed in the diabetic-TSP-insulin group compared with the TSP group (P=0.02). A significant drop was observed in the crocin group compared with the diabetic group (P=0.007) and the TSP group (P=0.001) (Fig. 3B).

The Voltage of QRS (the inotropic parameter of the heart)

By comparison with the control group, the TSP group displayed a significant rise in the voltage of QRS (P=0.04). A similar increase was seen in the diabetic group when compared with the comparison group (P=0.04). However, no significant change was observed in the crocin group compared with the control group. When assessed against the control group, the diabetic groups receiving TSPs exhibited a significant increase in the voltage of QRS (P=0.04). A significant decline was observed in the crocin group compared with the diabetic group (P=0.04). An insignificant decrease in the QRS voltage was seen in the diabetic group receiving crocin compared with the diabetic group. Nonetheless, the decrease was significant in the diabetic group receiving insulin when compared with the diabetic group (P=0.04). Compared with the TSP group, the voltage of QRS was significantly reduced in the TSP groups receiving crocin (P=0.02) (Fig. 3C).

SBP

A significant rise in SBP was detected in the TSP groups compared with the control group (P<0.0001). Additionally, a substantial rise in SBP was noted in the diabetic groups compared with the control group (P=0.0002). Blood pressure was similar in both crocin and control groups. SBP was considerably greater in the diabetic groups receiving TSPs (P<0.0001). In the diabetic-crocin and diabetic-TSP-crocin groups, there was a significant decrease compared with the diabetic group (P=0.002 and P=0.0004), indicating the protective role of crocin in diabetics with blood pressure.

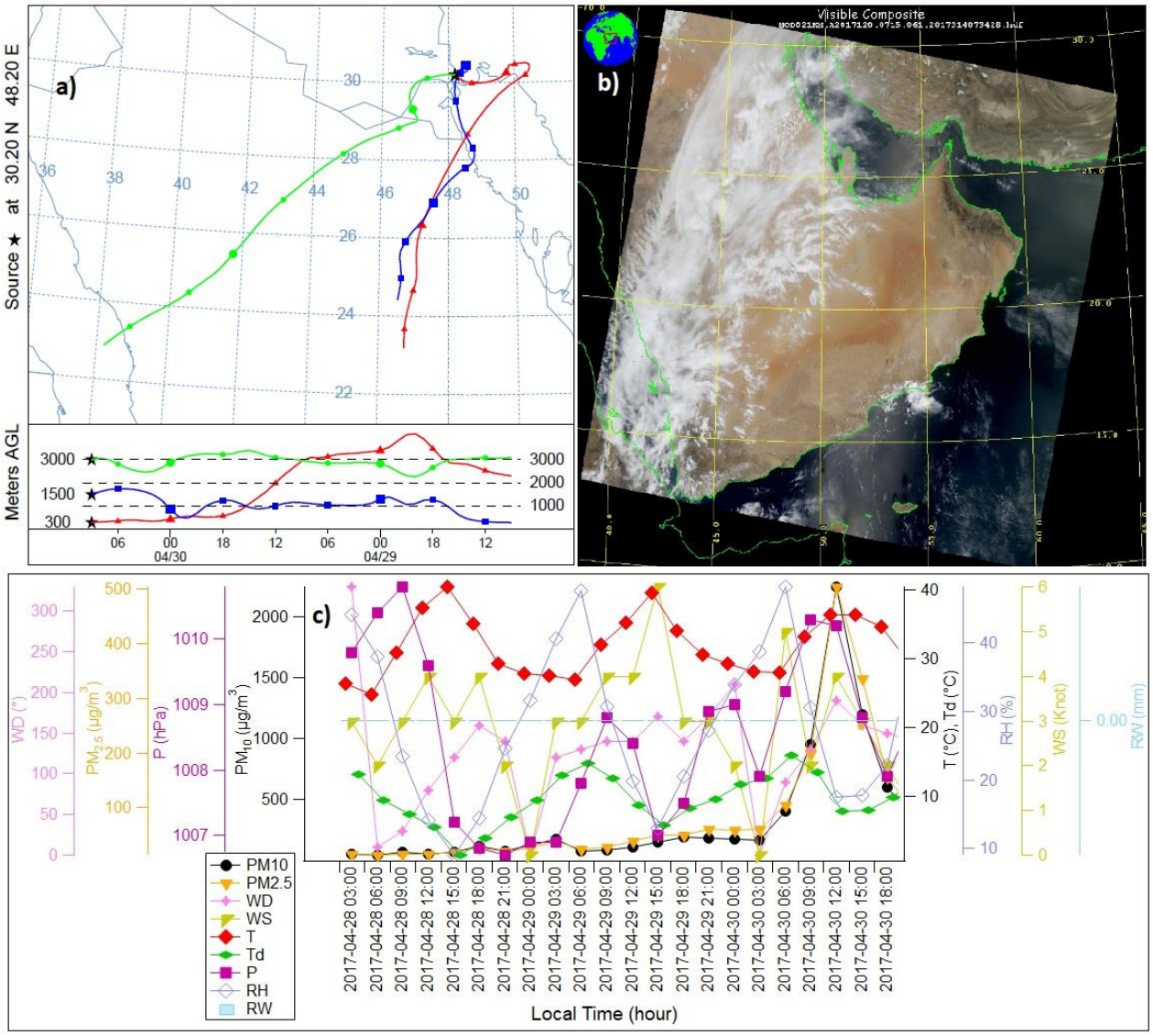

Figure 1: The properties of the dust storm that occurred on April 30, 2017, are (**a**) a 48-hour backward trajectory, (**b**) MODIS imagery from the airflow to the region, and (**c**) meteorological conditions during the dusty day and 2 preceding days.

P, Pressure; WD, Wind direction; T, Temperature; Td, Dewpoint; RH, Relative humidity; WS, Wind speed; RW, Rainwater

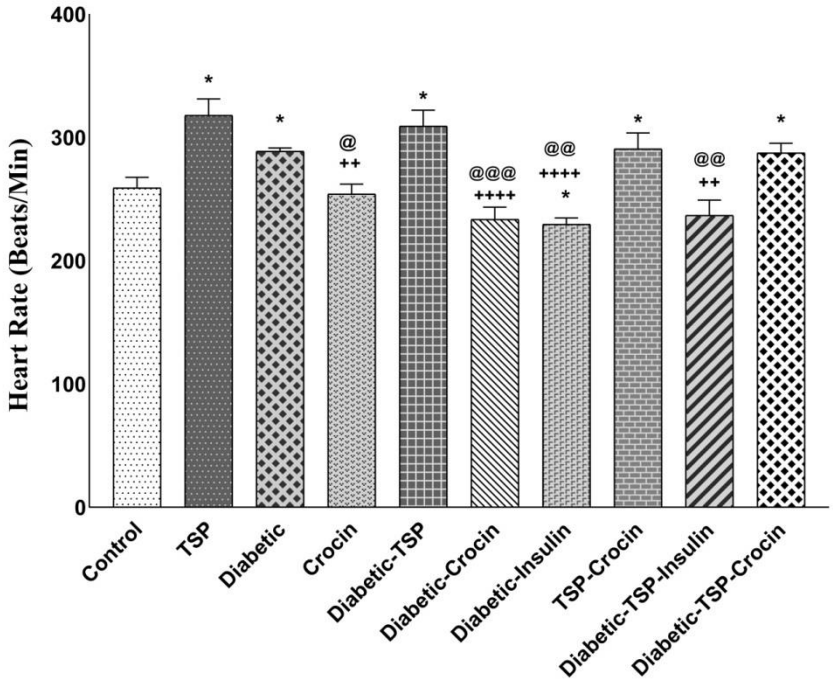

Figure 2: The effects of crocin, insulin, and TSPs on heart rate in diabetic rats are presented herein. For the total test, one-way ANOVA, followed by the Fisher LSD test, was used.
P*<0.05, ***P*<0.01, ****P*<0.001, ** *P*<0.0001 vs the control group. +*P*<0.05, ++*P*<0.01, +++*P*<0.001, ++++ *P*<0.0001 vs the diabetic group. @*P*<0.05, @@*P*<0.01, @@@*P*<0.001, @@@@ *P*<0.0001 vs the TSP group

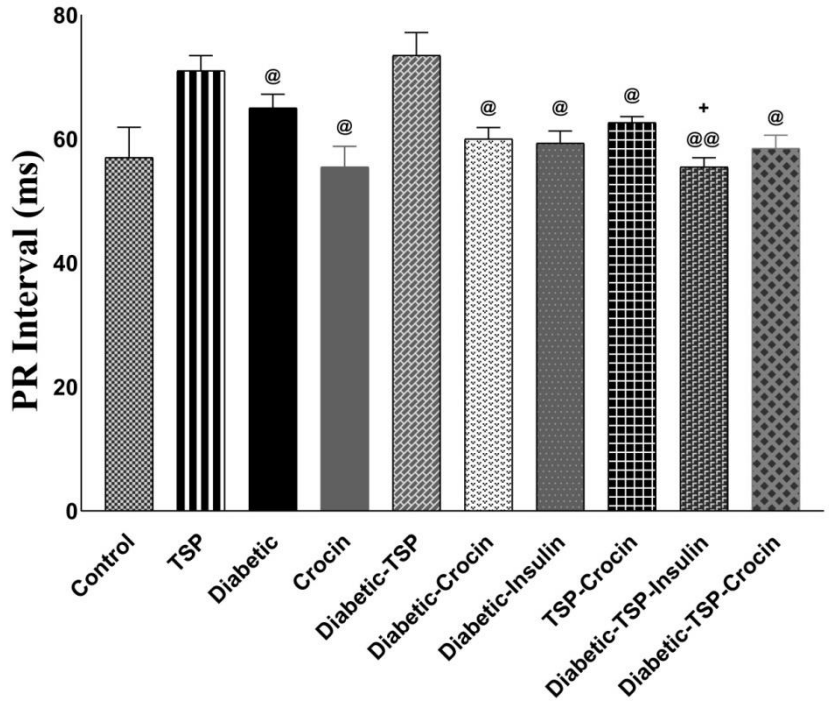

Figure 3.A

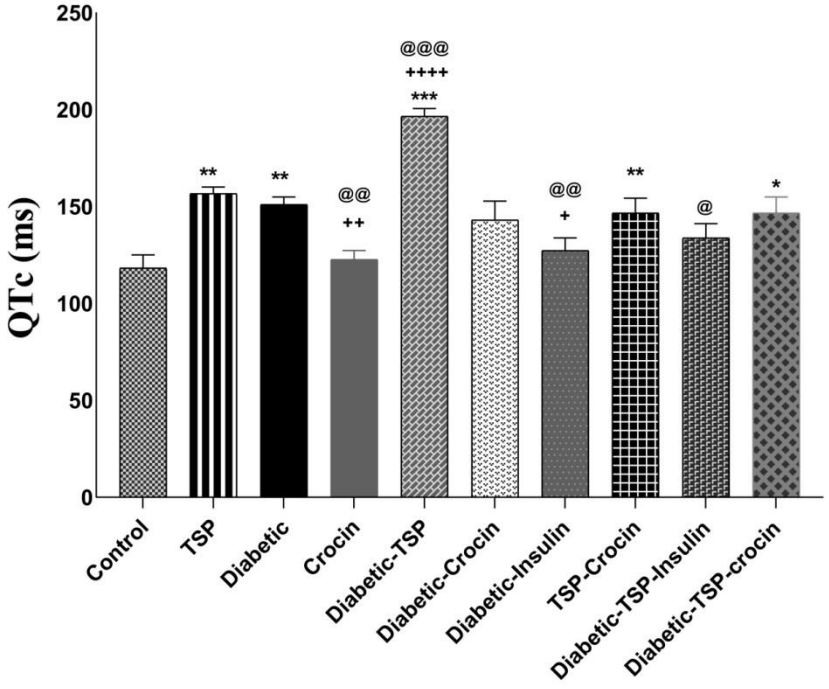

Figure 3.B

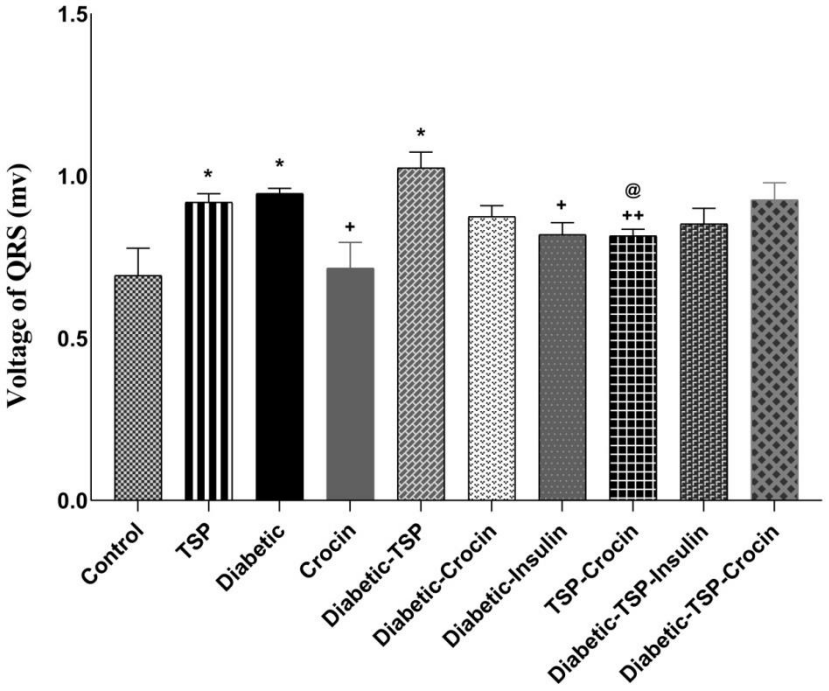

Figure 3.C

Figure 3: The effects of crocin, insulin, and TSPs on electrocardiographic parameters are illustrated herein. A, PR interval; B, QTc interval; C, Voltage of QRS) in diabetic rats
For the total test, one-way ANOVA, followed by the Fisher LSD test, was used.
P*<0.05, ***P*<0.01, ****P*<0.001, ** *P*<0.0001 vs the control group. +*P*<0.05, ++*P*<0.01, +++*P*<0.001, ++++ *P*<0.0001 vs the diabetic group. @*P*<0.05, @@*P*<0.01, @@@*P*<0.001, @@@@ *P*<0.0001 vs the TSP group

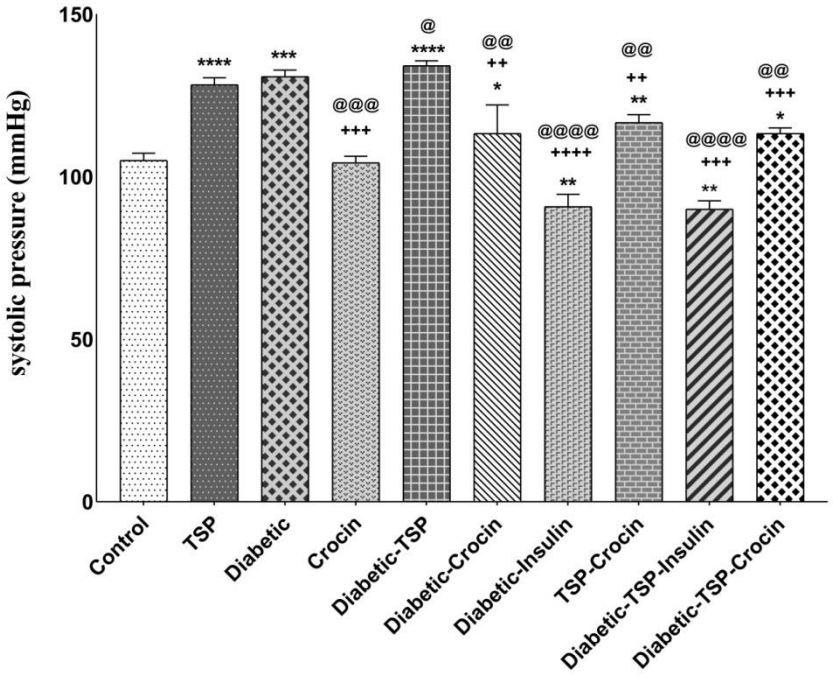

Figure 4.A

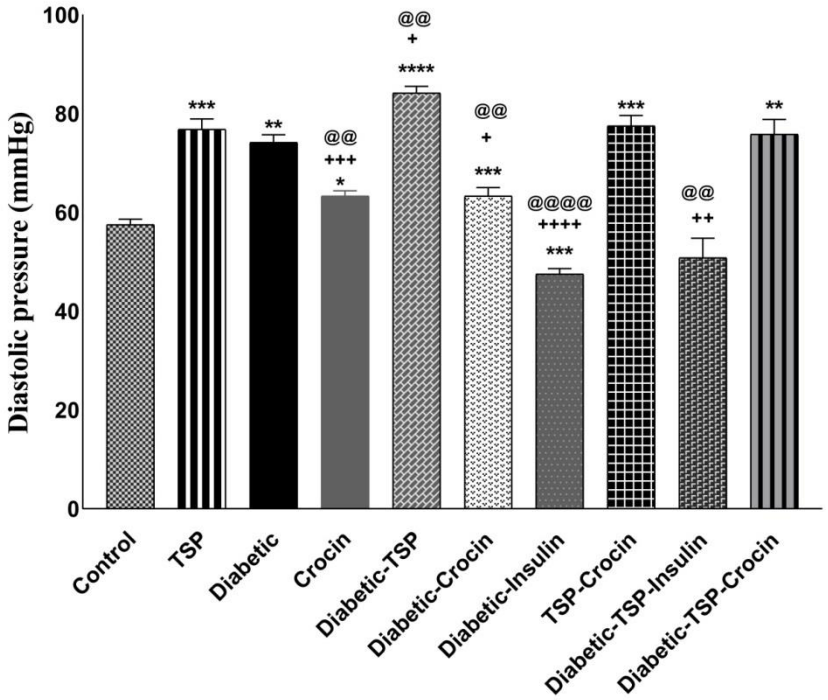

Figure 4.B

Figure 4: The image depicts the effects of crocin, insulin, and TSPs on blood pressure.
A, Systolic pressure; B, Diastolic pressure in diabetic rats
For the total test, one-way ANOVA, followed by the Fisher LSD test, was used.
**P*<0.05, ***P*<0.01, ***P<0.001, **** P<0.0001 vs the control group
\+ *P*<0.05, ++ *P*<0.01, +++ *P*<0.001, ++++ *P*<0.0001 vs the diabetic group
@ *P*<0.05, @@ *P*<0.01, @@@*P*<0.001, @@@@ *P*<0.0001 vs the TSP group

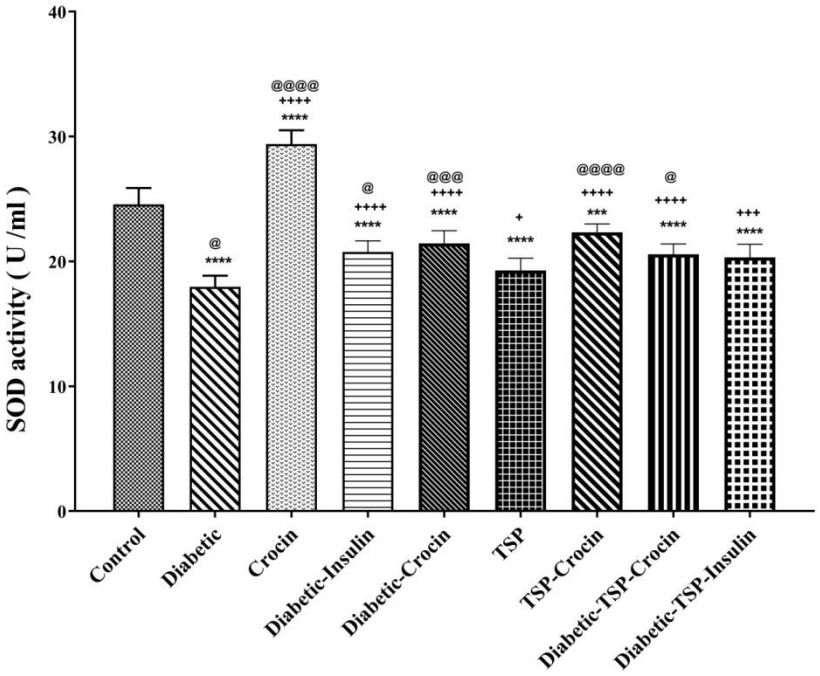

Figure 5.A

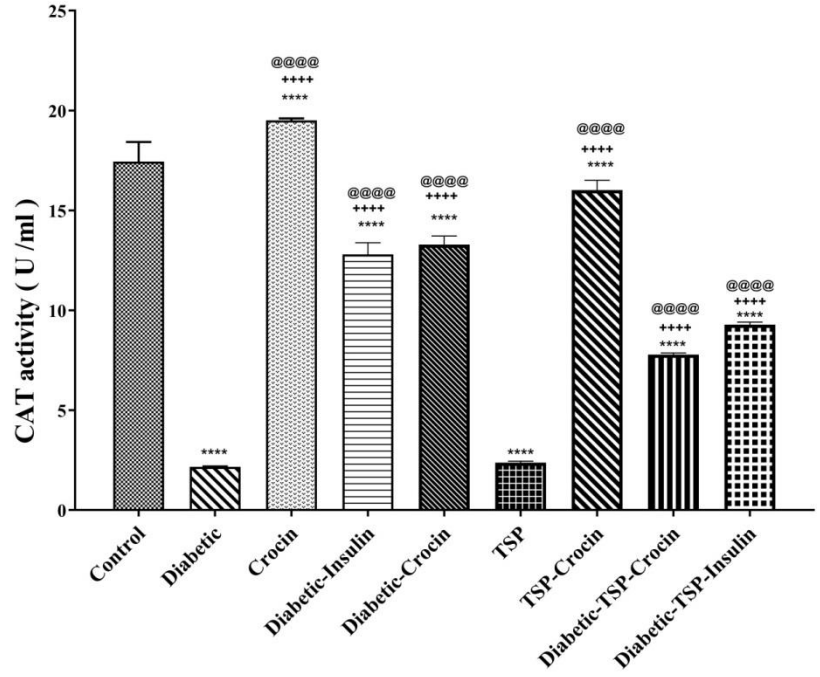

Figure 5.B

Figure 5: The image illustrates the effects of crocin, insulin, and TSPs on antioxidant enzymes. A, Activity of the SOD enzyme; B, Activity of the catalase enzyme in diabetic rats
For the total test, one-way ANOVA, followed by the Fisher LSD test, was used.
**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 vs the control group
+*P*<0.05, ++*P*<0.01, +++*P*<0.001, ++++*P*<0.0001 vs the diabetic group
@*P*<0.05, @@*P*<0.01, @@@*P*<0.001, @@@@*P*<0.0001 vs the TSP group

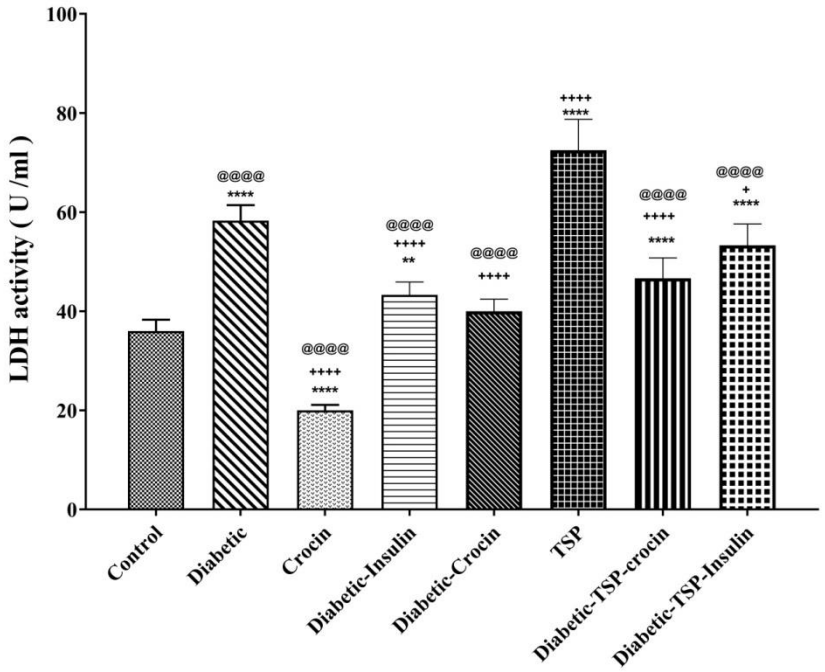

Figure 6: The effects of crocin, insulin, and TSPs on the activity of the LDH enzyme in diabetic rats are depicted herein. For the total test, one-way ANOVA, followed by the Fisher LSD test, was used.
**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 vs the control group
+*P*<0.05, ++*P*<0.01, +++*P*<0.001, ++++*P*<0.0001 vs the diabetic group
@*P*<0.05, @@*P*<0.01, @@@*P*<0.001, @@@@*P*<0.0001 vs the TSP group

Insulin was effective in decreasing SBP in the diabetic group compared with the control (*P*=0.007). The positive effect of insulin on SBP was also shown in the diabetic-TSP-insulin and diabetic-insulin groups compared with the diabetic groups (*P*=0.0002 and *P*<0.0001, respectively). A significant decrease in SBP in the crocin group (*P*=0.0005), the TSP-crocin group (*P*=0.007), and the diabetic-TSP-crocin group (*P*=0.0004) was seen compared with the TSP group (Fig. 4A).

DBP

In comparison with the control group, a significant increase in DBP was observed in the TSP group (*P*=0.0002). A similar increase was seen in the diabetic group compared with the control group (*P*=0.001). A significant decrease was observed in DBP in the crocin and control groups (*P*=0.03). The diabetic-TSP group displayed a significant increase in DBP compared with the control group (*P*<0.0001). Both diabetic groups receiving insulin or crocin showed a profound decrease in DBP compared with the diabetic group (*P* <0.0001 and *P*=0.01, respectively). The risk of developing hypotension was greater with insulin than with crocin. A significant increase in DBP was also observed in the diabetic-TSP group compared with the TSP group (*P*=0.006). Significant effects on DBP were seen in the diabetic-TSP-insulin group compared with the TSP group (*P*=0.001) (Fig. 4B).

Superoxide Dismutase (SOD) Enzyme

A significant increase was observed in SOD activity in the crocin-treated group compared with the control group (*P*<0.0001), indicating that crocin was a strong antioxidant. The TSP group showed a significant decrease in the SOD enzyme activity compared with the control group (*P*<0.0001). Furthermore, the

diabetic group treated with insulin or crocin showed a significant increase in SOD enzyme activity compared with the diabetic group (P <0.0001 and P<0.0001, respectively), representing the antioxidant mechanism of insulin and crocin. Still, the antioxidant effect was more profound in crocin than in insulin.
The TSP group treated with crocin displayed a significant increase in SOD enzyme activity compared with the similar group without crocin treatment (P<0.0001). The SOD enzyme activity decreased in the diabetic-TSP-crocin (P<0.0001) and diabetic-TSP-insulin (P=0.0001) groups compared with the diabetic group significantly (Fig. 5A).

Catalase Enzyme

Catalase activity decreased significantly in the diabetic group compared with the control group (P<0.0001). A significant increase in catalase activity was observed in the crocin receiving group compared with the control group (P<0.0001). In the insulin-treated diabetic group, there was a significant increase in the activity of catalase compared with the diabetic group (P<0.0001), indicating the antioxidant capacity of insulin. A significant increase in catalase activity was observed in the diabetic group receiving crocin compared with the diabetic group (P<0.0001), which was higher than that in the diabetic group receiving insulin and indicated the strong antioxidant capacity of crocin. A significant decrease in catalase activity was observed in the TSP groups compared with the control group (P<0.0001). A significant increase in catalase activity was observed in the TSP groups receiving crocin compared with TSP groups (P<0.0001). The diabetic-TSP-crocin group showed a significant increase in catalase activity compared with the TSP and diabetic groups (P<0.0001 and P<0.0001, respectively). A significant increase in catalase activity was observed in the diabetic-TSP-insulin group compared with the TSP (P<0.0001) and diabetic (P<0.0001) groups (Fig. 5B).

LDH

Compared with the control group, the diabetic group showed a significant increase in LDH activity (P<0.0001). LDH activity was also increased in the groups receiving TSPs compared with the control group (P<0.0001). The crocin group displayed a significant reduction in LDH activity compared with the control group (P<0.0001). A similar effect of crocin in the diabetic-TSP groups (P<0.0001) indicated the antioxidant effect of crocin in rats. Correspondingly, insulin, too, displayed significant antioxidant effects in the insulin-treated diabetic-TSP group by reducing LDH activity (P<0.0001). A decrease in LDH enzyme activity in the TSP-diabetic group treated with crocin or insulin further indicated the antioxidant effects of insulin and crocin (Fig. 6).

DISCUSSION

The results of the present study showed a significant difference in cardiac electrophysiological parameters, including heart rate, the PR interval, the QTc interval, and the QRS interval (Fig. 2, 3A, B, & C), as well as SBP and DBP, between diabetic and control groups (Fig. 4A & B).
Stricker–Krongrad et al[29] in 2018 reported that the mean PR interval and the mean QRS interval were increased in all diabetic animals compared with normal animals, but there were no pronounced QTc abnormalities when comparing diabetic to normal animals. The results of a study by Pour Moghadas et al[30] demonstrated that the prevalence of a prolonged QTc interval was significantly increased in the diabetic group in comparison with the control group. In our study, we observed a significant increase in the PR interval, the QTc interval, and the voltage of QRS in the diabetic group compared with the control group.

Recent studies have elucidated pathophysiological and genetic links between DM and hypertension.[31] Matteucci et al[32] reported that their diabetic patients achieved a higher maximal exercise SBP. In the present study, we observed a significant increase in DBP and SBP in the diabetic group. Optimal levels of blood pressure in diabetic patients are associated with a substantially lower risk of coronary health disease and CVDs.[33]

The difference was more prominent in diabetic groups receiving TSPs, indicating worsening effects on cardiovascular parameters by both DM and TSPs.

The results of this study showed that TSPs significantly increased cardiac electrophysiological parameters, including heart rate, the PR interval, the QTc interval, and the voltage of QRS (Fig. 2, 3A, B, & C), as well as blood pressure, compared with the control group (Fig. 4). This study showed a significant reduction in the activity of antioxidant enzymes (ie, SOD and CAT) by TSPs compared with the control group (Fig. 5A & B). The study results depicted the detrimental effects of TSPs on cardiovascular parameters and antioxidant enzymes.

An association was observed between daily cardiovascular mortality and short-term exposure to PM10 during dust days, specifically a 2.43% increase in daily cardiovascular mortality associated with each 10 mg/m^3 increase in PM10 concentrations, but no associations during non-dust days.[34] Exposure to desert dust aerosols and combustion aerosols probably resulted in different types of CVDs. An association has been noted between desert dust aerosols and heart failure. Exposure to increased dust concentrations is associated with in-hospital mortality among patients with heart failure.[35] Air pollution increases the risk of myocardial infarction, with worsening effects on the elderly.[36]

An increase in DBP or SBP is associated with exposure duration and concentration of respirable suspended PM.[37] In a study by Murakami and Ono,[38] myocardial infarction deaths increased a few hours after exposure to suspended PM.

The results regarding heavy metal concentrations using the proposed methods are shown in Table 2. In this research, we selected 6 heavy metals, namely Ni, Hg, Cr, As, Pb, and Cd, in a soil sample. The sample was investigated using ICP-MS. For the preparation of the heavy metals in the sample, acid digestion was used. After preparations, some toxic metals in the soil sample were analyzed by ICP-MS. In this study (Table 2), a lower concentration of heavy metals was observed in As. The mean concentration of As was 0.02 ppm. The mean concentrations of Cr and Ni were at the same level. There was a correlation concerning the concentration between Cr and Ni. In this work, the concentration of Cr and Ni were close to those obtained by Heidari-Farsani[39] in the air pollution of Ahvaz. The author reported that the higher PM concentration was due to the lack of precipitation and proximity to large arid deserts in the city.

In the present study, the highest concentration of heavy metals was found in Pb (Table 2). The results of Hg and Cd were the same as those determined by Rasmussen et al,[40] who investigated air pollution in Ottawa, Canada, and reported that the higher PM concentration was due to the lack of precipitation and street dust.

Cao et al[41] found that higher concentrations of heavy metals were observed during winter. Heavy metals were attributed to more vehicular activities, low temperature, and temperature inversion during the winter season.[27,42,43] According to the results, the concentrations of the 6 selected heavy metals were lower than the toxicity limits for heavy metals in natural soil.

ROS can play a role in endothelial dysfunction, vasoconstriction, and hypertrophy, ultimately leading to

hypertension.[44] One molecular pathway linking PM to blood pressure may be oxidative stress.[45] PM can induce oxidative stress and pro-inflammatory events in the vascular system, and ultrafine and fine components of PM are linked to developing atherosclerosis and cardiovascular ischemic events.[46]

In a prior investigation, within the initial hours of introducing PM into the pulmonary system, the release of myeloperoxidase from leukocytes into the vasculature and the vascular dysfunction into systemic micro-vessels was observed.[47]

One of the factors indicating oxidative stress is the LDH enzyme. In this study, the increased activity of the LDH enzyme in diabetic groups improved with insulin or crocin treatment, indicating the protective role of crocin and insulin on oxidative stress in diabetic rats. Furthermore, the activity of the LDH enzyme in TSP groups showed a significant increase compared with the control group (Fig. 6).

Improved access to antioxidant-rich foods in polluted urban areas may protect cardiovascular health.[18] Oxidative stress is the common pathogenic factor leading to β-cell dysfunction, insulin resistance, and impaired glucose tolerance, ultimately leading to DM. Reducing oxidative stress would benefit patients with DM and those at risk of developing DM.[8]

In a previous investigation, crocin improved blood glucose, bodyweight, whole heart weight, the whole heart weight/bodyweight ratio, ECG changes, serum LDH and CK-MB activities, heart tissue levels of malondialdehyde (MDA) and SOD, and histological outcomes of cardiomyopathy induced by *streptozotocin* (STZ).[48]

Diabetic rats receiving crocin showed a significant reduction in the levels of serum triglyceride, total cholesterol, glucose, advanced glycation end-products, and low-density lipoprotein, while they exhibited increased high-density lipoprotein. Crocin was also effective in decreasing microalbuminuria and HbA1c.[49]

Crocin is further effective in reducing the symptoms of obesity and diabetes, including hyperinsulinemia, hyperleptinemia, insulin resistance, weight gain, and reducing inflammation in diabetic rats.[50]

In the present study, the diabetic group receiving crocin showed a significant decrease in cardiac electrophysiological parameters (ie, heart rate, the PR interval, the QTc interval, and the voltage of QRS) (Fig. 2, 3A, B, & C), as well as blood pressure (Fig. 4), and a significant increase in the activity of antioxidant enzymes (Fig. 5), which were increased by developing diabetes. No significant changes were observed in cardiovascular parameters in the group receiving crocin alone. The activity of antioxidant enzymes in this group was significantly increased compared with the control and other groups, confirming the antioxidant role of crocin.

Cardiovascular safety has been very reassuring with insulin therapy.[51] In this study, insulin significantly improved cardiac electrophysiological parameters and blood pressure in the diabetic group (Fig. 2, 3A, B, C, & 4). It showed a more protective effect than crocin. In the diabetic group treated with insulin, an increase in the activity of the catalase antioxidant enzyme was significant, while the change in the activity of the SOD enzyme was insignificant compared with the diabetic group with no treatment (Fig. 5). In the diabetic groups receiving TSPs, the severity of complications in cardiac electrophysiology and blood pressure parameters were significant. Crocin and insulin were found to play protective roles in improving these parameters.

CONCLUSIONS

The results of the current study showed that diabetic groups experienced deleterious effects

on cardiac electrophysiological parameters (ie, heart rate, the PR interval, the QTc interval, and the voltage of QRS), as well as blood pressure and the activity of antioxidant enzymes. These markers improved in the groups of diabetics and TSP-exposed following treatment with insulin or crocin, demonstrating the protective effect of both insulin and crocin. All the groups exposed to TSPs showed destructive changes in cardiac electrophysiological parameters and blood pressure insofar as the activity of antioxidant enzymes decreased while the activity of LDH increased. The diabetic groups exposed to TSPs were worse. In this study, the protective roles of insulin and crocin were depicted through improving electrophysiological parameters, blood pressure, and the activity of antioxidant enzymes.

Conflict of Interest

None declared.

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