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Scientific paper

Glycoprotein Levels and Oxidative Stomach Damage in Diabetes and Prostate Cancer Model: Protective Effect of Metformin

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Abstract

People with diabetes have a higher risk of prostate cancer and people with prostate cancer are prone to stomach metastases. Therefore, researchers continue to search for new approaches in the treatment of individuals with all the above diseases at the same time. The protective effect of metformin (which is used in the treatment of diabetes) on cancer continues to be supported by studies. In this study, it was determined that the biochemical parameters showed a protective effect on stomach tissues with the administration of metformin to cancer and group with both cancer and diabetes groups. With the principal component analysis, it was determined that the biochemical parameters studied in the stomach tissue showed a correlation.

Keywords: Dunning Prostate Cancer, Diabetes, Metformin, Stomach Damage, Oxidative Stress.

1. Introduction

Diabetes mellitus (DM) is induced by many factors such as genetic, dietary and environmental factors. It is mainly divided into two groups; while type 1 diabetes occurs due to insufficient secretion of the hormone insulin, type 2 diabetes occurs due to insulin resistance, with high blood glucose levels observed in both cases.[1] Its incidence is increasing daily depending on the factors. Cancer, a disease that occurs due to the abnormal proliferation of cells requires intensive treatment. It can result in death unless diagnosed at an early stage.[2] The increasing incidence of cancer, high mortality rates, and the fact that a treatment has not been found yet, and for that reason, cancer research remains important scientific.

The incidence of diabetes and many types of cancer has risen especially in recent years owing to changing dietary habits, and external and genetic factors. It is predicted that the incidence of these diseases will increase in the coming years. The prostate cancer is shown common type of cancer in men, having important social and economic consequences. It accounts for nearly 25 per cent of all new male cancer diagnoses in the UK.[3] Prostate cancer incidence varies regionally and it is known that the highest rates are in Western and the lowest rates are in Asia countries.[4] In addition to this, epidemiological evidence suggests that people with diabetes are at higher risk for cancer[5] and hence, it is essential to find new approaches to the treatment of people with both diseases using existing or newly discovered drugs.

Investigation of the effects of known and currently used drugs on different diseases provides importance for the discovery of more than one targeted drug. Knowing the side effects of these drugs, which will be investigated, and proven by scientific research accelerates their use in the treatment of different diseases. One of them, metformin (1,1-Dimethylbiguanide) is a lipophilic biguanide drug that inhibits hepatic gluconeogenesis and improves peripheral utilisation of glucose. However, in recent studies,

the approach to this molecule has changed, considering that it has anti-tumour properties directly and indirectly, in addition to type 2 diabetes. A potential anti-tumorigenic effect of metformin directly is thought to be exerted by activating AMP-kinase, which inhibits the mammalian target of rapamycin (mTOR).[6] The indirect antitumor effect of metformin is presumed to be by inhibiting hepatic gluconeogenesis. AMPK activation in the liver causes hepatic gluconeogenesis inhibition by acting on gluconeogenesis genes. Inhibited gluconeogenesis genes stimulate the entrance of glucose into the muscles. As a result of this stimulation, blood glucose and insulin levels decrease.[7] Tumour cells have been found to express high levels of insulin receptors. Therefore, this is accepted as an unfavourable prognostic factor for prostate, breast, and colon cancer.[8] In one study, metformin was found to reduce the risk of prostate cancer as well as diabetes in a dose-dependent manner.[9] Considering the effects of metformin on cancer, it is very important to examine the effects of metformin on many tissues, especially in animal models of prostate cancer and diabetes.

The presented study aimed to examine the effects of prostate cancer and diabetic rats on stomach tissue through biochemical parameters.

2. Experimental

2. 1. Prostate Cancer Cell Protocol

Mat-LyLu cells were used according to instructions in our prior investigation.[10] Cell culture and functional assays. Mat-LyLu were grown in a 37 °C/5% CO_2 incubator in RPMI (RPMI-1640; Gibco;Life Technologies, Waltham, MA, USA) culture medium supplemented with 1% fetal bovine serum (FBS) (Gibco; LifeTechnologies).

2. 2. Experimental Protocol

In this research, Copenhagen rats were employed. Tubitak MAM Genetic Engineering and Biotechnology Institute produced the rats. The present study was carried out within the framework of the rules determined by the Istanbul University Animal Care and Use Committee (Protocol no: 2014/28- 27.02.2014 the ethics committee decision number and date).

2. 3. Pharmacological Application and Experimental Groups

Rats, 150–220 g, were housed individually in a light and temperature-controlled room on a 12 h/12 h light-dark cycle and fed a standard pellet lab chow. Streptozotocin (STZ) was given intraperitoneally (i.p.) to the diabetes groups to induce diabetes. At the end of the 72nd hour of the experimental process, blood glucose levels were measured and the rats were considered diabetic if the values were above 200 mg/dL. Rats were given 250 mg/kg of metformin (Sigma, D150959) orally and Table 1 shows the applications to the experimental groups.

At the end of the experimental process, all animals were dissected under ketamine hydrochloride (Ketalar®, Eczacıbaşı) and xylazine HCl (Alfazyne®, Holland) anaesthesia. After that, stomach tissues were taken for biochemical analyses. Stomach tissue was not examined histologically and only biochemical parameters were determined in this study. Also, no other organs, apart from the stomach, were not included in the study.

2. 4. Biochemical Analyses

2.4.1. Preparation of Stomach Tissues for Biochemical Analyses

Stomach tissues taken for use in biochemical parameters were first washed in cold physiological saline (0.9% NaCl) and then 1 g of stomach tissue was homogenized in 10 mL of saline solution using a glass homogenizer (Tenbroeck glass tissue homogenizer). After homogenization, it was centrifuged and the supernatants were stored at –20 °C to be used for experiments. All chemicals used in the experiments were obtained from Sigma-Aldrich.

2.4.2. Determination of Lipid Peroxidation (LPO) Levels and Myeloperoxidase (MPO) Activities

Lipid peroxidation (LPO) levels of stomach tissues were determined spectrophotometrically by measurement of the LPO products reacted with thiobarbituric (TBA) acid at high temperature and low pH.[11] 0.25 mL of homogenized tissue was mixed with 1.22 M trichloroacetic acid (TCA) and left at room temperature for 15 minutes. Then,

Table 1. Application for the experimental groups.

Groups	*n*	Application
The control	5	0.9% PS was given for 14 days.
The diabetic	7	65 mg/kg STZ was given to the group with a single injection.
The cancer	8	2.10^4 MATLyLu cells were given subcutaneously (s.c) inoculated with only one injection.
The cancer+metformin (CM)	8	250 mg/kg metformin was given to the group for 14 days after Mat-LyLu cells inoculation.
The diabetic+cancer (DC)	8	2.10^4 MAT-LyLu cells and STZ were injected.
The diabetic+cancer+metformin (DCM)	8	Metformin was given for 14 days to treat of STZ and Mat-LyLu cells.

0.375 mL TBA (0.047 M) was added and kept in a boiling water bath for 30 minutes. After cooling, 1 mL n-butanol was added to each tube and centrifuged at 4000 rpm for 10 minutes. Absorbance values of the organic phase were read against the blank at 532 nm. Results were reported as nmol MDA/mg protein.

Myeloperoxidase (MPO) activities were determined by the reaction of 0.13 mL 4-aminoantipyrine (25 mM), 0.13 mL phenol (2%) and 0.26 mL H_2O_2 (1.7 mM) with 0.4 mL homogenized. The resulting colour change was read at 510 nm in the spectrophotometer.12 The results were defined as mU/g protein.[12]

2.4.3. Determination of Superoxide Dismutase (SOD) and Catalase (CAT) Activities

130 µL phosphate buffer (50 Mm, pH:7.8, 0.1 Mm EDTA), 5 µL o-dianisidine (0.19%), 5 µL sample and 10 µL riboflavin (0.2 mM) were placed in a tube and the absorbance values at 0 and 8 minutes were read at 460 nm for the determination of superoxide dismutase (SOD) activities.[13] The results were expressed as U/mg protein.

The activity of the catalase (CAT) of stomach tissues was determined by converting H_2O_2 to H_2O and measuring the decreasing absorbance value due to H_2O_2 consumption at 240 nm in the spectrophotometer.[14] 0.1 mL sample and 0.4 mL H_2O_2 (30 mM) were added to the same tube and the absorbance values were read at 240 nm. The results were defined as U/mg protein.

2.4.4. Determination of Glutathione Reductase (GR), Glutathione Peroxidase (GPx), and Glutathione-S-Transferase (GST) Activities

NADPH and oxidized glutathione (GSSG) with glutathione reductase (GR) cause a decrease in absorbance due to the consumption of NADPH in the test tube.[15] 870 µL tris-HCl buffer (50 mM, pH:8.0 and 1 mM EDTA), 50 µL NADPH (2 mM) and 50 µL GSSG (20 mM) were added to the same tube. Then, 30 mL samples were placed in the same tube and the absorbance changes were determined at 340 nm. GR activity was expressed as U/g protein.

Glutathione peroxidase (GPx) provides GSSG by oxidation of GSH in the presence of H_2O_2. The resulting GSSG is converted to GSH by the oxidation of NADPH to NADP. 400 µL phosphate buffer (0.25 M, pH:7.0, 2.5 mM EDTA, 2.5 mM NaN_3), 100µL GSH (10 mM), 100µL NADPH (2.5 mM), 100 µL GR (6U/mL) and 100 µL H_2O_2 (12 mM) were added the tube and then sample 200 µL sample also added the same tube. Finally, the absorbance changes were read spectrophotometrically at 340 nm.[16] GPx activity was expressed as U/mg protein.

Glutathione-S-transferase (GST) activity was determined according to the spectrophotometric evaluation of the absorbance at 340 nm of the product formed by the conjugation of GSH and 1-chloro-2,4-dinitrobenzene (cDNB).[17] For this experiment, 400 µL phosphate buffer (0.2 M, pH: 6.6), 10 µL GSH (60 mM), 10 µL cDNB, 180 µL water and 100 µL sample were reacted in the same tube and absorbance changes were watched at 340 nm. GST activity was expressed as U/g protein.

2.4.5. Determination of Reactive Oxygen Species (ROS), Protein Carbonyl (PC), and Homocysteine (HCy) Levels

Reactive oxygen species (ROS) levels were determined by the reaction of 2000 µM 2',7'-dichlorofluorescein diacetate (DCF) compound dissolved in 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazinetansulfonic acid) buffer.[18] 5 µL sample, 55 µL HEPES buffer and 90 µL DCF were added in the same tube and the first read was observed fluorometrically at Ex. 480 nm/Em. 535 nm. The second read was recorded after incubation at 30 min and 37ºC.The results were given as ΔRFU/mg protein.

Protein carbonyl (PC) levels are determined with 2,4-dinitrophenylhydrazone, which is formed by the reaction of carbonyl groups in proteins with 2,4-dinitrophenylhydrazine (DNPH).[19] 0.5 mL sample, and 2 mL DNPH (10 mM, in 2.5 M HCl) were added same tube and the incubation was performed at room temperature. After 2.5 mL TCA (20%) was added to each tube, and the tubes were washed with ethyl alcohol and ethyl acetate mixture (1:1). In every washing, the tubes were centrifuged at 300 rpm and 10 min. Then, 1 mL guanidine-HCl (6 M) was put into each tube and the incubation was formed at 30 min and 37ºC. Finally, the absorbance values were taken by using a spectrophotometer at 370 nm. The results were given in nmol PC /mg protein.

Homocysteine (HCy) levels of the stomach tissues were measured according to the manufacturer's procedure via an ELISA kit. The homocysteine levels were given in nmol HCy/mg protein.

2.4.6. Determination of Xanthine Oxidase (XO) and Lactate Dehydrogenase (LDH) Activities

Xanthine oxidase (XO) is the enzyme that converts xanthine to uric acid. For this purpose, 870 µL phosphate buffer (50 mM, pH: 7.4), 33 µL EDTA (3 mM), 33 µL xanthine (2 mM) and 10 µL sample were kept in the same environment and the first reading was taken on the spectrophotometer. Second absorbance values were taken after 10 minutes of incubation at room temperature and at 286 nm in the spectrophotometer.[20] XO activity was expressed as U/mg protein.

Lactate dehydrogenase (LDH) catalyses the conversion of pyruvate to lactate in the presence of NADH. LDH activity was calculated by measuring the oxidation of NADH to NAD^+.[21] 2 mL NADH (170 µM) and 50 µL sample

was incubated at 5 min and 37 °C. After incubation, 250 µL pyruvate solution (14 mM) was added to each tube and the decreasing absorbances of each tube were recorded at 340 nm. LDH activity was defined as U/mg protein.

2.4.7. Determination of Sodium-Potassium ATPase (Na+/K+-ATPase) and Histone Deacetylase (HDAC) Activities

Ridderstap and Bonting methods were used for the determination of sodium-potassium ATPase (Na^+/K^+-ATPase) activity in stomach tissue with the help of the determination of Mg^{2+} ATPase in the presence of 20 mM ouabain and 11 mM ATP in acidic medium.[22] Na^+/K^+-ATPase activity was given in nmol Pi/mg protein/h.

Histone deacetylase (HDAC) activities of the stomach tissues were measured according to the manufacturer's procedure by using an ELISA kit. HDAC activity was given in U/mg protein.

2.4.8. Determination of Sialic Acid (SA), Hexose, Hexosamine and Fucose Levels

The method for determining the sialic acid (SA) levels of gastric tissues is based on reading the absorbance at 546 nm of the coloured compound formed by the reaction of 2-formyl pyruvic acid, which is formed as a result of the oxidation of periodic acid, with two moles of thiobarbituric acid.[23] 10 µL sample were added to the tube. Then, 100 µL NaCl (155 mM) and 300 µL H_2SO_4 (6.7 mM) were added to all tubes, respectively. It was incubated at 80 °C for one hour. After cooling, 100 µL sodium meta periodate (0.2 M) was added to all tubes and kept at room temperature for 20 minutes. Then, 400 µL sodium meta arsenite (1.54 M) was added and the tubes were shaken until the colour of the iodine disappeared. 1 mL of thiobarbituric acid (7.102%) was added to all tubes and kept at 90 °C for 15 minutes. After cooling, 2 mL of cyclohexanone was added and centrifuged for 10 minutes. SA was absorbed into the cyclohexanone phase and absorbance values were read on the spectrophotometer at 546 nm. The results were given as µmol SA/g protein.

In order to determine hexose compounds of the stomach tissues, spectrophotometrically, this method creates the colour reaction method of carbohydrates with orcinol in the presence of concentrated sulphuric acid.[24] 0.25 mL orcinol solution (1.6%) and 2 mL H_2SO_4 (60%) were added to 0.25 mL of the sample, respectively. After the mixture was boiled in a boiling water bath for 10 minutes and cooled, the absorbances were read on a spectrophotometer at 425 nm. The results were defined as µg hexose / mg protein.

The method used is based on measuring the absorbance of the pink colour formed as a result of the reaction of hexosamine compounds in the tissue with acetylacetone and p-dimethylaminobenzaldehyde in a spectrophotometer at 530 nm.[24] The results were defined as µg hexosamine /mg protein.

1 mL of sample was mixed with 1 mL of acetylacetone (0.5% in 0.5 Na_2CO_3) and then kept in a boiling water bath for 15 minutes. At the end of this period, 5 mL ethyl alcohol (96%) and 1 mL Ehrlich reagent were added to all tubes and the tubes were incubated at room temperature for 1 hour. At the end of this period, absorbance values were taken at 530 nm. The basis determination of fucose in stomach tissue is based on the colour reaction of carbohydrates with thiol groups in a sulfuric acid medium.[25] The results were expressed as µg fucose /mg protein.[25]

2.4.9. Determination of Protein Levels

The amount of protein in the stomach tissue is determined on the basis of the method of measuring the intensity of the blue-violet colour, which is formed as a result of the reduction of proteins reacted with copper ions in an alkaline medium with Folin reagent (phosphomolybdotungstic acid), spectrophotometrically at 500 nm.[26]

2. 5. Statistical Analysis

Graph-Pad Prism 3.0 (GraphPad Software, San Diego, CA, USA) program was used to interpret the experimental results statistically. Tukey's test was applied to determine the significance between groups and/or parameters, and the obtained data were expressed as mean ± standard deviation (SD). Tukey's test is ANOVA post hoc test, meaning that ANOVA was first performed. p values of less than 0.05 were accepted as a statistically significant difference. Principal component analysis (PCA) was also used to visualize the biomarker's responses for all exposure conditions. PCA was performed using GraphPad Prism Software, version 9 (San Diego, USA).

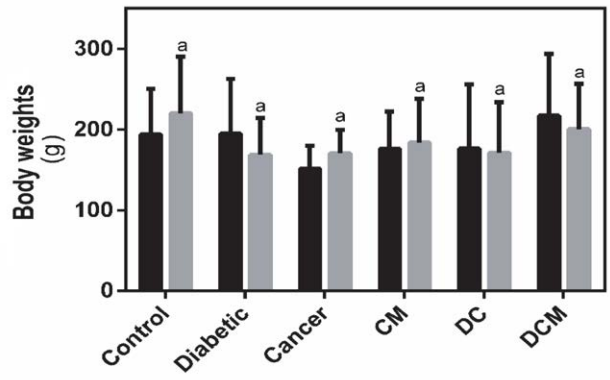

Figure 1. The body weights of all groups of rats. The black columns represent the first body weight of the groups. The grey columns represent the final body weight of the groups. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^a p < 0.05$ vs the control group.

3. Results

3. 1. Body Weights and Blood Glucose Levels

The body weights of all groups are shown in Figure 1. The first and last body weights of all groups were measured. It was observed that the first and last body weights of all groups except the DC group changed significantly ($^{a}p < 0.05$) and the significance of the body weights and blood glucose levels were determined by using Tukey's test.

The levels of blood glucose of all groups were measured during the experiment and are shown in Figure 2. The blood glucose values measured after 72 hours in the groups given STZ to create diabetes showed an important increase and exceeded 200 mg/dL. All experiment groups were found to be significantly changed when compared with the control group at the end of the experiment ($^{a}p < 0.01$). In addition, blood glucose levels were measured again at the end of the experiment (14 days later) and an increase was observed in the diabetic, DC, and DCM groups when compared to the control group, but it was observed that the blood glucose level measured at the end of the experiment in the DCM group decreased when compared to the 72nd-hour blood glucose level ($^{b}p < 0.05$). When the blood glucose levels of the DC group and the DCM group were compared, a decrease was observed in the DCM group ($^{c}p < 0.05$).

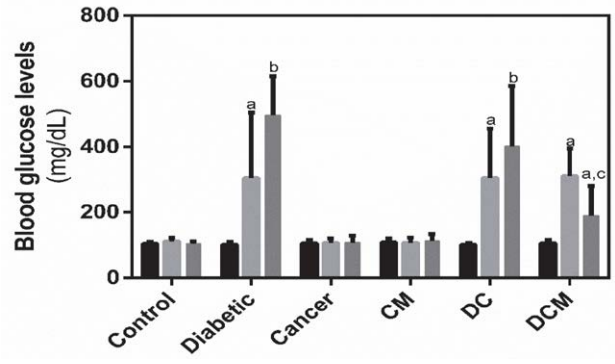

Figure 2. The blood glucose levels of all groups of rats. The black columns represent the blood glucose level at the beginning of the experiment of the groups. The light grey columns represent the 72nd h blood glucose level of the groups. The dark grey columns represent the blood glucose level at the end of the experiment of the groups. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^{a}p < 0.05$ vs control group; $^{b}p < 0.001$ vs control group; $^{c}p < 0.05$ vs DC group.

3. 2. Biochemical Results

3.2.1. Lipid Peroxidation (LPO) Levels and Myeloperoxidase (MPO) Activities

LPO levels and MPO activities of stomach tissues are presented in Figure 3(A-B) and it was found that the levels of LPO and activities of MPO incremented in the group of the diabetic ($p < 0.05$; $p < 0.0001$), cancer ($p < 0.05$; $p < 0.0001$) and DC ($p < 0.05$; $p < 0.0001$) when compared to the control group. Metformin reduced LPO levels and MPO activities in cancer and DC groups when compared to CM ($p < 0.05$; $p < 0.05$) and DCM ($p < 0.05$; $p < 0.0001$) groups respectively.

3.2.2. Superoxide Dismutase (SOD) and Catalase (CAT)) Activities

SOD and CAT activities presented in Figure 4(A-B) indicated that their activities decreased in the diabetic ($p < 0.01$; $p < 0.0001$), cancer ($p < 0.0001$; $p < 0.0001$) and DC ($p < 0.001$; $p < 0.0001$) groups. At the end of the treatment with metformin, SOD and CAT activities were advanced in CM ($p < 0.001$; $p < 0.01$) and DCM ($p < 0.0001$; $p < 0.0001$) groups.

3.2.3. Glutathione Reductase (GR), Glutathione Peroxidase (GPx), and Glutathione-S-Transferase (GST) Activities

GR, GPx and GST activities of all groups were given in Figure 5(A-C) and it was determined that the GR, GPx

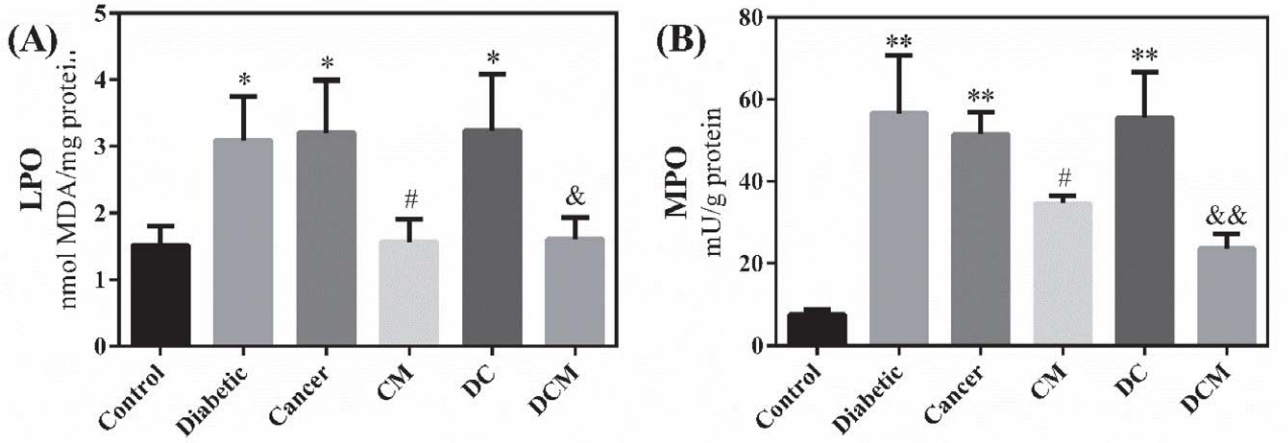

Figure 3. (A) Lipid peroxidation (LPO) levels and (B) myeloperoxidase (MPO) activities of all groups of rats. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^{*}p < 0.05$ vs control group; $^{**}p < 0.0001$ vs control group; $^{\#}p < 0.05$ vs cancer group; $^{\&}p < 0.05$ vs DC group; $^{\&\&}p < 0.0001$ vs DC group.

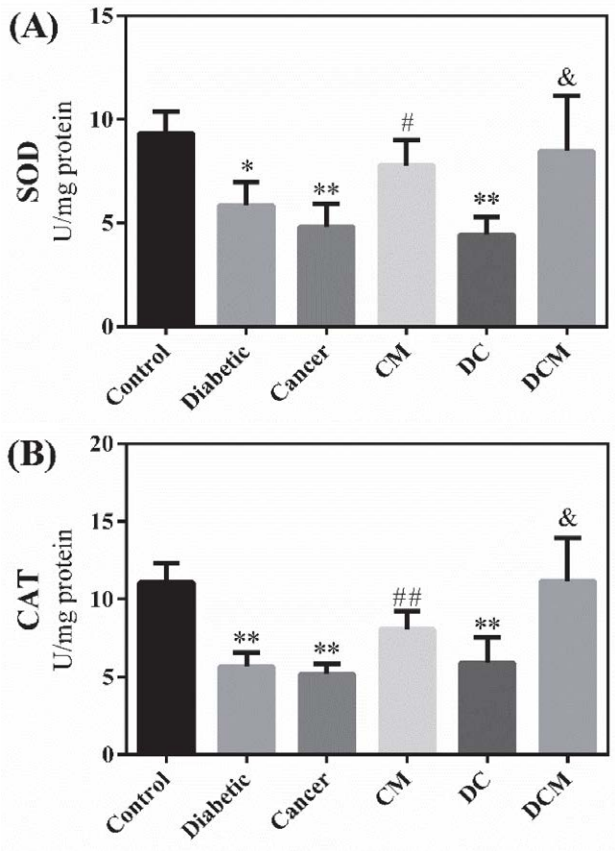

Figure 4. (A) Superoxide dismutase (SOD) and (B) catalase (CAT) activities of all groups of rats. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^{*}p < 0.01$ vs control group; $^{**}p < 0.0001$ vs control group; $^{\#}p < 0.001$ vs cancer group; $^{\#\#}p < 0.01$ vs cancer group; $^{\&}p < 0.0001$ vs DC group.

and GST activities of the diabetic ($p < 0.05$; $p < 0.0001$; $p < 0.0001$), cancer ($p < 0.001$; $p < 0.001$; $p < 0.0001$) and DC ($p < 0.05$; $p < 0.0001$; $p < 0.0001$) groups were decreased meaningfully when compared to the control group. Administration of metformin reversed GR, GPx and GST activities of CM ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$) and DCM ($p < 0.01$; $p < 0.0001$; $p < 0.01$) groups.

3.2.4. Reactive Oxygen Species (ROS), Protein Carbonyl (PC), and Homocysteine (HCy) Levels

ROS, PC, and HCy levels of stomach tissues are presented in Figure 6(A-C) and it was found that ROS, PC and HCys levels of diabetic ($p < 0.001$; $p < 0.0001$; $p < 0.0001$), cancer ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$) and DC ($p < 0.0001$; $p < 0.05$; $p < 0.0001$) increased when compared to the control groups. Metformin changed the levels of ROS, PC, and HCy. The CM ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$) and DCM ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$) groups showed decreasing ROS, PC and HCy levels when compared to cancer and DC groups respectively.

3.2.5. Xanthine Oxidase (XO) and Lactate Dehydrogenase (LDH) Activities

XO and LDH activities of stomach tissues were presented in Figure 7(A-B). It was found that the activities of XO and LDH were meaningfully increased in the diabetic ($p < 0.0001$; $p < 0.05$), cancer ($p < 0.0001$; $p < 0.0001$) and DC ($p < 0.0001$; $p < 0.0001$) groups. These increases in activities were reversed by metformin administration, by de-

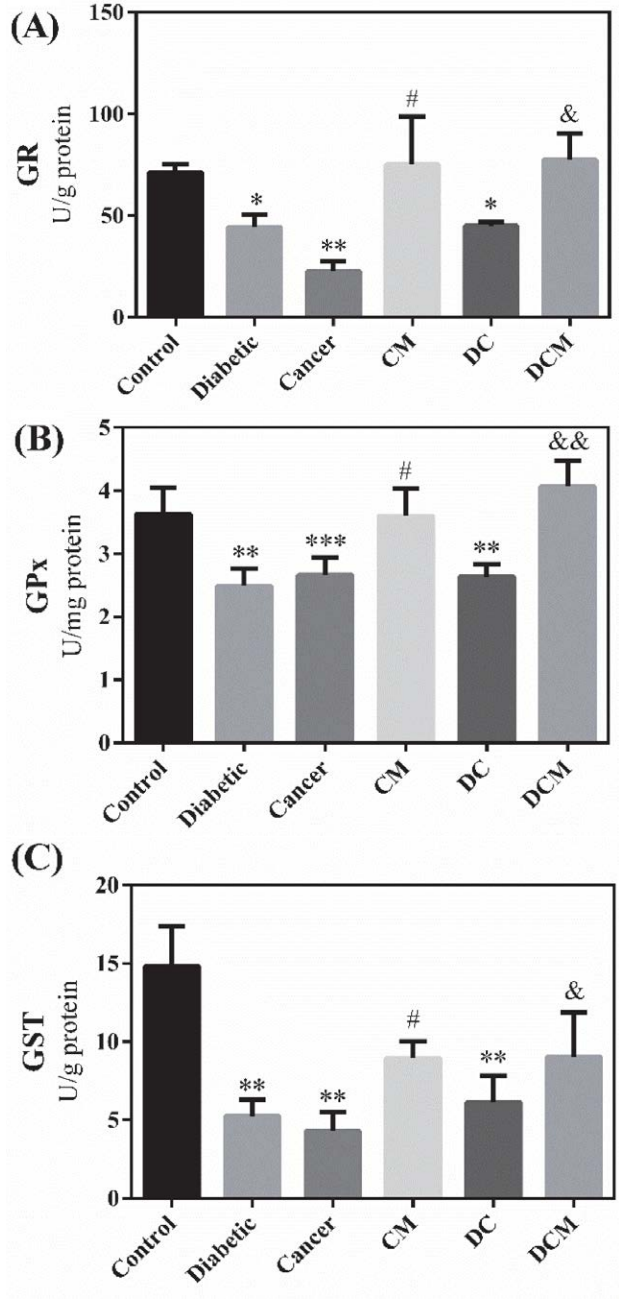

Figure 5. (A) Glutathione reductase (GR), (B) glutathione peroxidase (GPx) and (C) glutathione-S-transferase (GST) activities of all groups of rats. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^{*}p < 0.05$ vs control group; $^{**}p < 0.0001$ vs control group; $^{***}p < 0.001$ vs control group; $^{\#}p < 0.0001$ vs cancer group; $^{\&}p < 0.01$ vs DC group; $^{\&\&}p < 0.0001$ vs DC group.

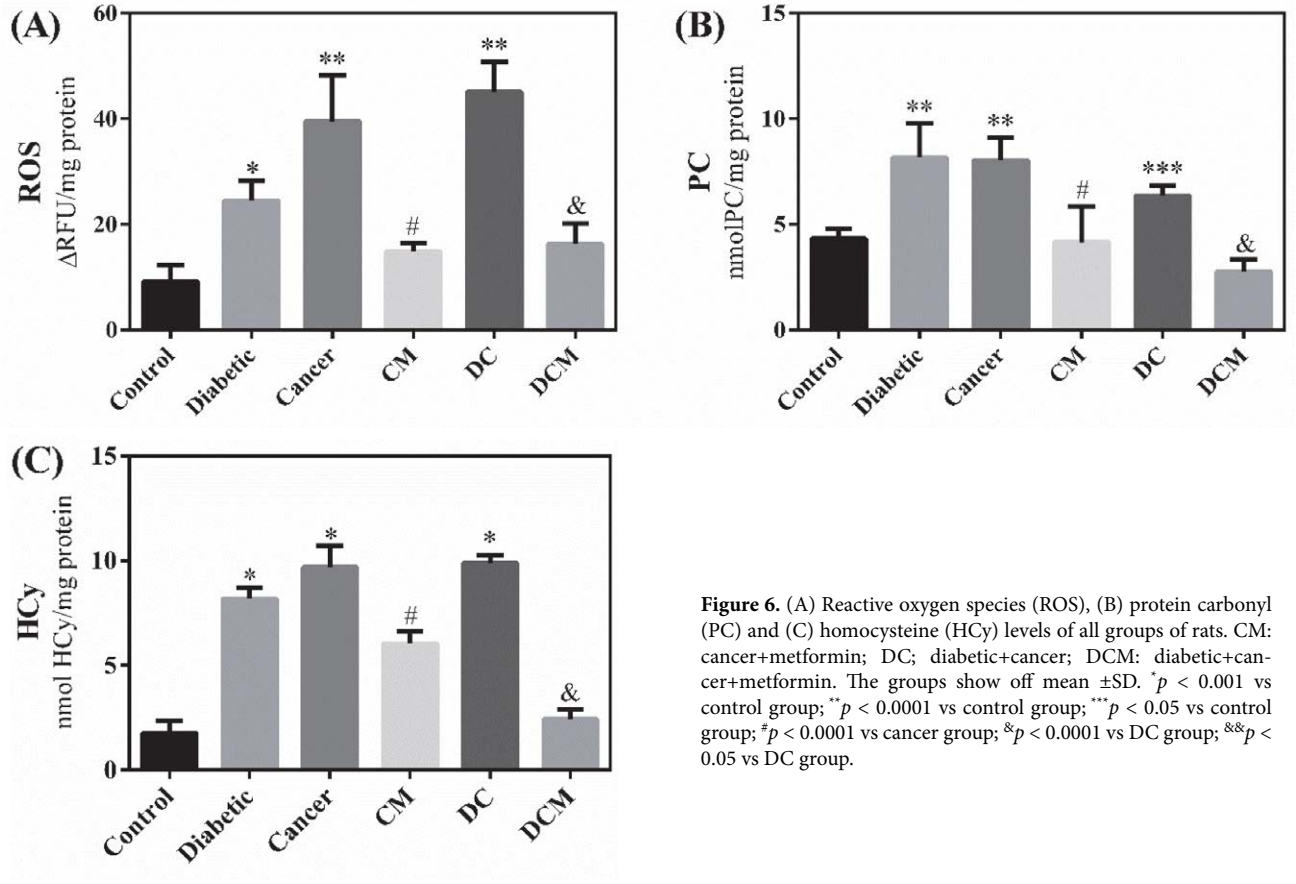

Figure 6. (A) Reactive oxygen species (ROS), (B) protein carbonyl (PC) and (C) homocysteine (HCy) levels of all groups of rats. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^{*}p < 0.001$ vs control group; $^{**}p < 0.0001$ vs control group; $^{***}p < 0.05$ vs control group; $^{\#}p < 0.0001$ vs cancer group; $^{\&}p < 0.0001$ vs DC group; $^{\&\&}p < 0.05$ vs DC group.

creasing the activities of XO and LDH in CM ($p < 0.0001$; $p < 0.05$) and DCM ($p < 0.0001$; $p < 0.0001$) groups.

3.2.6. Sodium-Potassium ATPase (Na+/K+-ATPase) and Histone Deacetylase (HDAC) Activities

Na^+/K^+-ATPase and HDAC activities of stomach tissues were shown in Figure 8 and the results showed that the activity of Na^+/K^+-ATPase diminished in diabetic, cancer and DC ($p < 0.01$; $p < 0.0001$; $p < 0.0001$ respectively) groups, while HDAC activities were raised in diabetic, cancer and DC ($p < 0.05$; $p < 0.0001$; $p < 0.001$) groups. Metformin supplementation resulted in significantly raised Na^+/K^+-ATPase activity in CM and DCM ($p < 0.0001$), while HDAC activity significantly diminished in CM and DCM ($p < 0.0001$; $p < 0.001$).

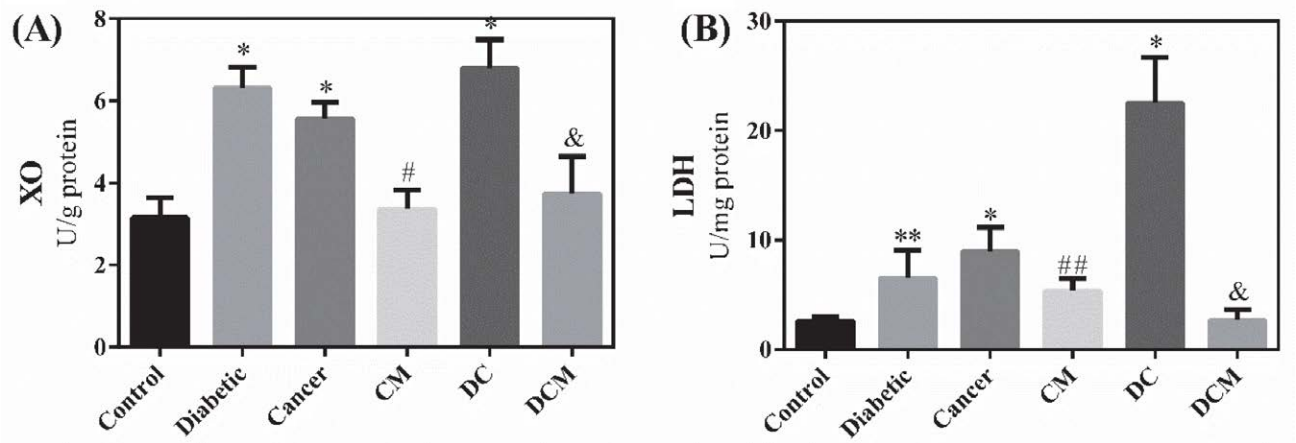

Figure 7. (A) Xanthine oxidase (XO) and (B) lactate dehydrogenase (LDH) activities activities of all groups of rats. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^{*}p < 0.0001$ vs control group; $^{**}p < 0.05$ vs control group; $^{\#}p < 0.0001$ vs cancer group; $^{\#\#}p < 0.05$ vs cancer group; $^{\&}p < 0.0001$ vs DC group.

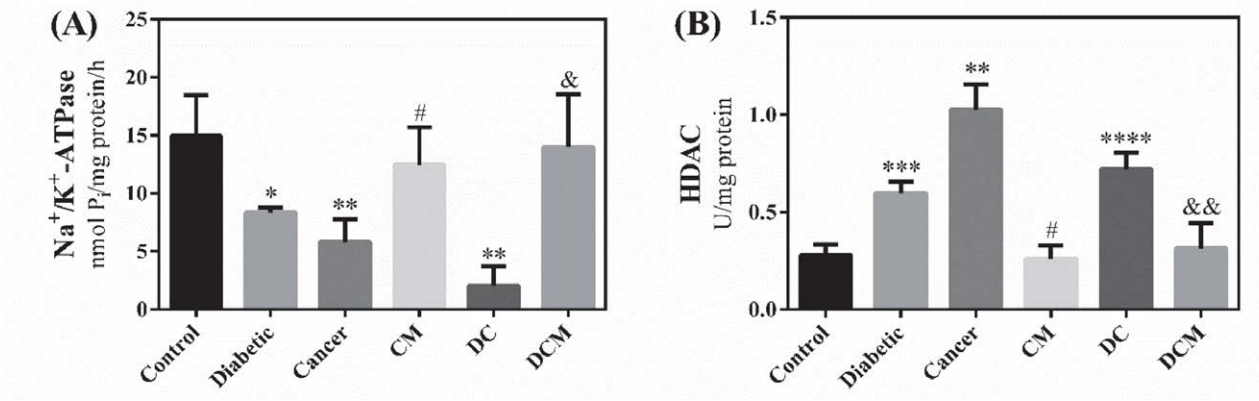

Figure 8. (A) Sodium/potassium ATPase (Na^+/K^+-ATPase) and (B) histone deacetylase (HDAC) activities of all groups of rats. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^{*}p < 0.01$ vs control group; $^{**}p < 0.0001$ vs control group; $^{***}p < 0.05$ vs control group; $^{****}p < 0.001$ vs control group; $^{\#}p < 0.0001$ vs cancer group; $^{\&}p < 0.0001$ vs DC group; $^{\&}p < 0.001$ vs DC group.

3.2.7. Sialic Acid (SA), Hexose, Hexosamine and Fucose Levels

Glycoprotein parameters which are SA, hexose, hexosamine and fucose levels are given in Figure 9. Determining glycosylation patterns in diabetes and cancer is of significant importance in both fields of research and clinical applications. Glycosylation refers to the process by which carbohydrates are added to proteins and lipids, and it plays a crucial role in various biological processes. They can help predict the risk of complications, such as diabetic nephropathy in diabetes. Also, altered glycosylation can differentiate between different cancer subtypes, helping to tailor treatment strategies. Therefore, the determination of glycosylation provides biochemical information about the status of both diseases. Determination of the results

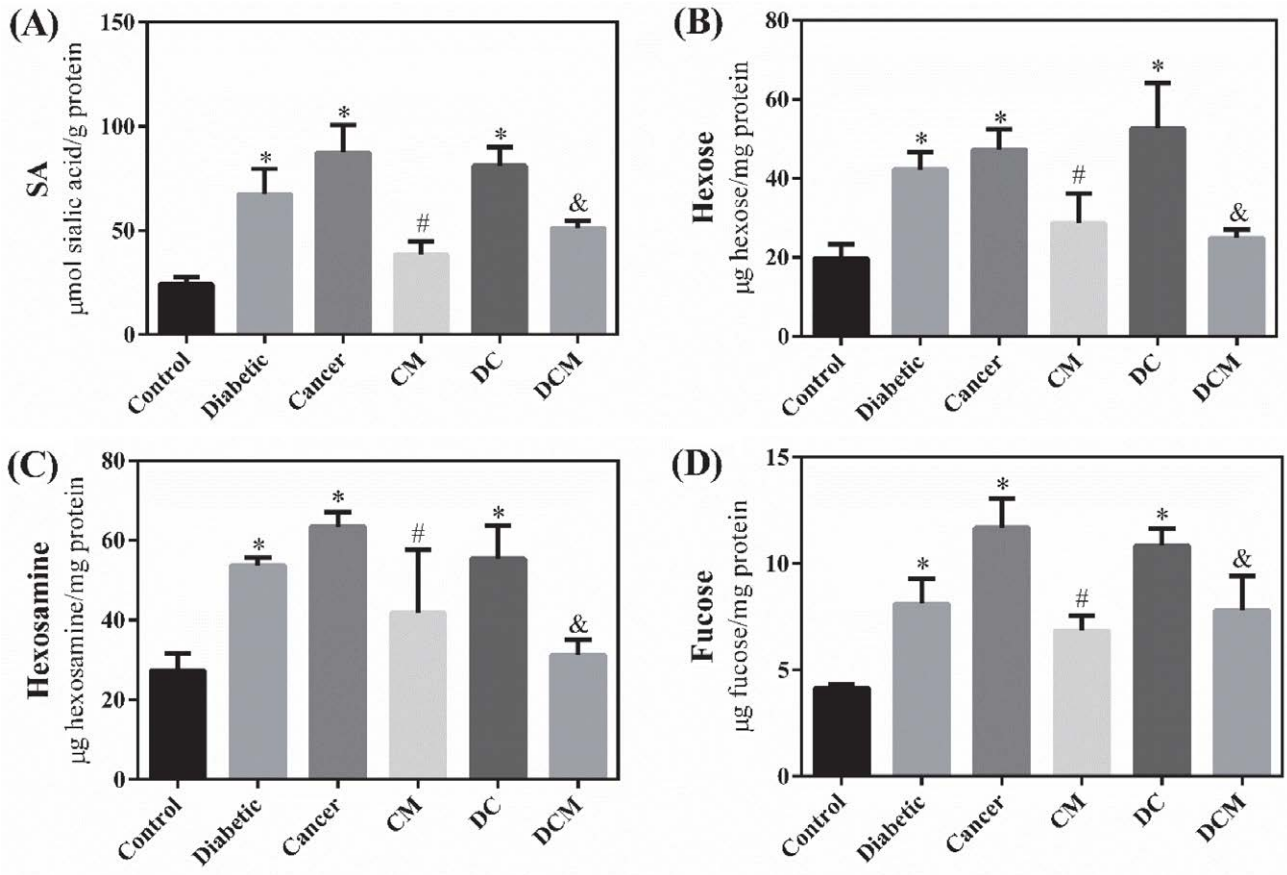

Figure 9. (A) Sialic acid (SA), (B) hexose, (C) hexosamine and (D) fucose levels of all groups of rats. CM: cancer+metformin; DC; diabetic+cancer; DCM: diabetic+cancer+metformin. The groups show off mean ±SD. $^{*}p < 0.0001$ vs control group; $^{\#}p < 0.0001$ vs cancer group; $^{\&}p < 0.0001$ vs DC group.

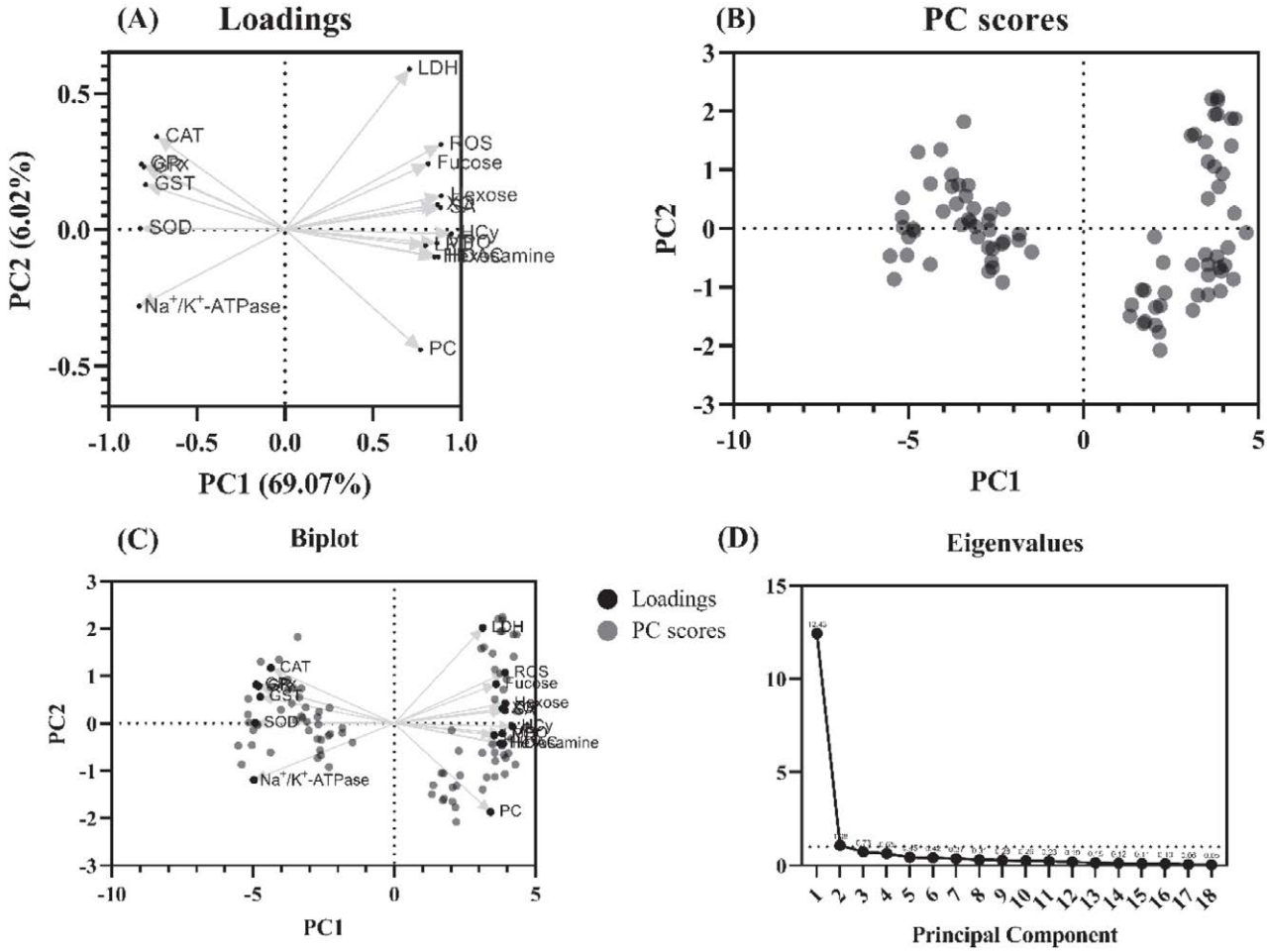

Figure 10. Principal component analysis of stomach biochemical parameters plots. (A) Loadings plot, (B) PC Scores, (C) Biplot and (D) Eigenvalues plots.

showed that SA, hexose, hexosamine and fucose levels were raised in the diabetic ($p < 0.0001$), cancer ($p < 0.0001$) and DC ($p < 0.0001$) groups. Treatment with metformin to cancer and DC groups resulted in significantly diminished SA, hexose, hexosamine and fucose levels in CM ($p < 0.0001$) and DCM ($p < 0.0001$) groups.

3. 3. Principal Component Analysis

Loadings, PC Scores, Biplot and Eigenvalues graphs of principal component analysis (PCA) of stomach tissues are presented in Figure 10(A-D), respectively. The purpose of the PCA method is to assist in the general interpretation of data and to simplify the complexity of high-dimensional data. It does this by converting the data into fewer dimensions that act like a summary of the properties. In the PCA method, it combines highly correlated variables to create a smaller set of artificial variables called principal components. That's why analysts use PCA as a tool for data analysis and building predictive models. Each PC is a linear combination of the variables that went into it and principal component 1 (PC1) is the one that extracts the maximum variance, and principal component 2 (PC2) is the one that extracts the maximum variance from what is left. It aims to show that there is a correlation between the results obtained and the parameters by performing PCA analysis for biochemical experiments in stomach tissue, and the obtained analysis results prove this accuracy.

PCA was used to prove the relationship between the biochemical results of gastric tissues and the analysis results showed that it details approximately 75.09% of the total variation (PC1: 69.07%, PC2: 6.02%). CAT, GR, GPx, GST, SOD, ROS, fucose, hexose, XO and SA are clustered together in the first component and these are negatively correlated with HCy, LPO, MPO, HDAC, hexosamine, PC and Na^+/K^+-ATPase (Figure 10A and 10C).

4. Discussion

Patients with diabetes have a very high risk of developing prostate cancer depending on age. Prostate cancer is usually associated with bones and lymph nodes metastasizing, but it has been reported that it metastasizes to the

stomach, albeit rarely.[27–30] Therefore, examining the effects of prostate cancer on stomach tissue is important because the subject is still controversial.

Although metformin is used in treating type 2 diabetes, its limited side effects make it easier to use. The most important of its side effects is that it increases the amount of lactic acid in the blood. Metformin exerts its antioxidant and anti-inflammatory effects with the activation of adenosine monophosphate protein kinase (AMPK). This activation by metformin causes inhibition of nuclear factor kappa light-chain-enhancer of activated B-cells (NF-κB) transcription. In addition, metformin inhibits Poly [ADP ribose] polymerase 1 (PARP-1), which acts as a cofactor of NF-kB. These inhibitions reduce reactive oxygen species (ROS) production, inflammatory pathways and proinflammatory cytokines. In addition, metformin increases the amount of NO, which antagonizes inflammation and ROS production, and this increase is due to its activation effect on AMPK.[31,32] On the other hand, metformin inhibits the complex I (NADH: ubiquinone oxidoreductase) of the electron transport chain, thereby helping decrease mitochondrial reactive oxygen species.[33]

Oxidative stress is a condition that arises due to the insufficiency of the organism's own defence system and insufficient antioxidant molecule intake, due to metabolic diseases such as diabetes and cancer. Therefore, studying antioxidant systems gives information about the status of oxidative stress in these diseases. Organism fights against free radicals in two ways. Via antioxidant enzyme systems and antioxidant molecules.[34] Cell defence mechanisms under oxidative stress work to correct this condition and minimise its effects. The enzymatic antioxidant system consists of SOD, CAT, GR, GPx, and GST enzymes, while nonenzymatic antioxidants consist of vitamin E, beta carotene, vitamin C, and GSH molecules. In addition, to understand the oxidative state, not only antioxidant systems, but also some other enzyme activities and biomarkers (LPO, MPO, ROS, PC etc.) are investigated.[35]

The reactive oxygen species levels increase in the case of oxidative stress. This increase affects the functionality of antioxidant systems of organisms. The enzymatic antioxidant system is involved in the removal of reactive oxygen species formed during oxidative stress. However, the decrease in the activity of this enzyme system contributes to the formation of oxidative stress. The superoxide radical is responsible for converting to H_2O_2 by SOD catalysis, and the CAT enzyme converts H_2O_2 to H_2O, forming a defence system against the harmful effects of the superoxide radical. During these reactions, the enzyme GPx reduces H_2O_2 to H_2O with the natural antioxidant molecule GSH. The organism reduces GSSG to GSH with the GR enzyme to provide a concentration of GSH for this reaction, and thus the continuity of the antioxidant enzyme system is ensured. Due to the decrease in the activity of this enzyme system, an increase in the amount of ROS is likely to be observed due to the effects of the antioxidant enzyme system, as well as Fenton reactions.[36] In addition, oxidative stress causes an increase in some biomarkers which are directly related to oxidative stress such as LPO and MPO. Increasing LPO levels in tissues can affect membrane fluidity and decrease the activity of membrane-bound enzymes. Due to the increased activity of MPO, the amount of hypochlorous acid (HOCl) and other strong oxidant substances increases.[37] The increase in LPO levels and MPO activities are biochemical parameters that are often used to provide information about the oxidative states of metabolic diseases, as they are parameters that prove the presence of oxidative stress.

In diabetes and cancer diseases, the antioxidant/oxidant balance of the organism is disrupted and oxidative stress occurs due to the increase in oxidant molecules. In a study by Chukwunonso Obi et al., it was reported that diabetic rats were given the diabetes drugs metformin, glibenclamide (GLI), and repaglinide (REP). They found that metformin increased serum SOD, CAT activity, and GSH amount compared to the diabetes group.[38] Ahmed Amar et al. investigated the activity of antioxidant enzymes and LPO levels in patients with prostate cancer. SOD, CAT activity, and GSH levels decreased in prostate cancer patients, while LPO levels increased.[39] Ozel et al. found that MPO activity increased in diabetes, cancer and diabetes+-cancer groups and decreased with metformin administration.[40] In our study, it was found that the activities of antioxidant enzymes SOD, CAT, GR, GPx, and GST decreased in diabetic, cancer, and diabetes+cancer groups, while the levels of LPO and MPO, which are biomarkers of oxidative stress, increased. It was observed that these parameters were reversed upon treatment of these groups with metformin. It can be suggested that these effects occur due to the fact that metformin acts in the direct reduction of ROS concentrations in organisms.

Oxidative stress causes the amount of ROS to increase. Increased amount of ROS has many dangerous effects, such as disruption of the cell membrane structure and DNA damage.[41] The mitochondrial effects of metformin include decreased endogenous ROS production, oxidative stress, decreased DNA damage, and decreased mutagenesis in normal somatic cells.[42] Metformin also inhibits Ras-induced ROS production and DNA damage. PC, another oxidative stress parameter, is a very important early marker of oxidative stress due to its high stability. The high levels of protein carbonyl (CO) groups have been observed in some metabolic diseases such as diabetes, and Alzheimer's.[43] In addition, ROS activates p38 MAPK phosphorylation and inflammation which enhances protein modification by carbonylation.[44] In the present study, it was found that the amount of ROS and PC increased in the damage groups (diabetes, cancer and DC), and the levels of ROS and PC decreased with the administration of metformin. These indicate that metformin might have reduced the formation of ROS in mitochondria.

Homocysteine (HCy) derived from the metabolism of methionine is a sulphur-containing amino acid. Its uncontrolled level in patients is associated with the incidence of stroke. Additionally, HCy level in plasma is a biomarker for metabolic diseases such as diabetes, neural tube defects, Down syndrome, megaloblastic and neurodegeneration. Also, the HCy level is a biomarker of cancer. Hence, the determination of HCy levels in plasma and tissues is correlated to the status of diseases biochemically. Methionine metabolites homocysteine, cystathionine and cysteine are accepted as metastatic risk factors for prostate cancer. The high serum levels of these methionine metabolites have been used to predict the risk of early biochemical relapse and the aggressiveness of the disease.[45] Sannigrahi et al. showed that HCy levels of men with prostate cancer increased significantly when compared to healthy men.[46] The effect of metformin on serum HCy level is upward, but studies show that this effect occurs in the absence of B group vitamins or folic acid supplementation.[47] This may be the reason why the CM and DCM groups showed an increase of HCy levels compared to the control group. In our previous study, it was shown that HCy levels in heart tissue increased in diabetes, cancer, and DC groups[48], and similar results were seen in the present study.

XO is a purine metabolism enzyme that converts xanthine and hypoxanthine to uric acid. The reaction of XO may cause oxidative stress due to the formation of H_2O_2. Hence, the activity of XO in tissues is important in determining tissue damage. The activity of XO might increase in various diseases, especially cancer and diabetes.[49] The changes in oxidative stress may alter p53 protein's function and affect many cellular pathways such as; DNA repair. In addition to being a genome protector, p53 protein is involved in the regulation of DNA repair, apoptosis, and cellular responses to oxidative stresses. Due to the antioxidant property of metformin, a decrease in ROS levels is observed. This effect of metformin prevents p53 from showing antioxidant properties and prevents damage to cells by preventing DNA damage.[42] It has been reported that p53 protein also decreases due to the decrease in oxidative stress, and this decrease is thought to be due to the antioxidant property of metformin.[50] Depending on the increase in DNA damage, it is possible to see an increase in the activity of enzymes in purine catabolism. XO is an enzyme involved in both purine metabolism and oxidative stress formation. It has been reported that metformin prevents oxidative stress by reducing ROS levels in addition to its protective effect on DNA.[51] It was observed that XO activity increased in diabetic, cancer and DC groups, but decreased with metformin administration to cancer and DC groups in the study. It can be argued that this decrease is due to the effect of metformin on both DNA repair and the prevention of oxidative stress formation.

LDH is located in cytoplasmic and catalyses the reversible conversion of lactate to pyruvate by reduction of NAD^+ to NADH. Increased LDH activity is seen in many diseases, but especially pernicious anaemia and haemolytic disorders, liver disorders, skeletal muscle disorders, and some leukaemias.[52] In addition, patients with cancer and/or diabetes have increasing LDH activity and lactate amounts due to anaerobic glycolysis.[53] Bayrak et al. found that increased LDH activity in heart tissues of diabetes, cancer and diabetes+cancer group when compared to the control group. Metformin reversed LDH activities.[48] Similarly, the present study found that LDH activity increased in diabetes, cancer, and diabetes+cancer groups, while metformin treatment reduced LDH activity in all these groups. It can be said that metformin may cause an effect on the protective LDH activity against oxidative stress.

Na^+/K^+-ATPase is an enzyme located on the surface of the cell membrane. It has an effect on energy metabolism and helps maintain osmotic balance and membrane potential. Changes in its activity are quite significant, as they have many effects.[54] In a study conducted on diabetic rats, it was found that metformin increased Na^+/K^+-ATPase activity.[55] In the present study, it was found that Na^+/K^+-ATPase activity decreased in diabetes, cancer and diabetes+cancer groups. Metformin increased the activity of Na^+/K^+-ATPase when given compared to the experimental groups. It can be suggested that these changes may be due to both the antioxidant properties of metformin and the fact that AMPK activation increases Na^+/K^+-ATPase activity.[56]

Histone deacetylases (HDACs) are a parameter used in the development of inhibitors for use in the treatment of cancer. The purpose of the development and administration of HDAC inhibitors is to increase histone acetylation and transcription of tumour suppressor genes. In addition, HDAC inhibitors induce apoptosis and do so by increasing histone acetylation, expression of p21 and proapoptotic genes. Also, AMPK activation is known to increase histone acetylation. The fact that metformin stops ROS production allows it to be evaluated as a potential inhibitor of HDAC, since it performs it through this pathway.[57] In addition, it has been established that metformin increases histone acetylation by activation of AMPK in prostate and ovarian cancer cells.[58] Interleukin-1β is involved in the formation of insulin resistance and β-cell insufficiency in diabetes, and the use of HDAC inhibitors is effective in the development of β-cells. These two connections mean that histone acetylation decreases in diabetes, and HDAC activity decreases. Considering that metformin increases histone acetylation by HDAC inhibition, it is thought that it may be useful in the treatment approach.[59] In the present study, it was found that HDAC activities decreased in diabetes, cancer, and diabetes+cancer groups. The treatment of these groups with metformin increased HDAC activities. It can be suggested that metformin carries out this change in HDAC activities by promoting the activation of the AMPK pathway.

Glycoproteins are important macromolecules with many metabolic effects, their levels can change in many

diseases. Glycoproteins have many functions such as cell differentiation and recognition, membrane transport, structural components of enzymes, hormones, and act as blood group substances. Alterations in glycoprotein levels have been shown to correlate with the development and/or progression of cancer, diabetes and other disease states.[60] Since they have many metabolic effects, it is very important to determine glycoprotein levels, and determine their connections with diseases. In diabetic individuals, increased glycation can be seen due to increased blood glucose levels. Similarly, changes in glycoprotein levels can be observed in cancer patients due to the deterioration of energy metabolism depending on the type of cancer. Similar to the findings of the present study, Chinnannavar et al. found that patients with oral squamous cell carcinoma had increased SA and fucose levels.[61] The outcome of the present investigation indicates that SA, hexose, hexosamine and fucose levels increased in diabetic, cancer and DC groups. All the glycoprotein parameters were reversed in metformin-treated groups. This indicates that metformin both has a protective effect against oxidative stress and lowers blood sugar levels, thereby resulting in a decrease in glycoprotein parameters.

Other publications of our study have been made on the heart, brain, kidney, testicular, and liver.[40,48,62–64] In all studies, it was determined that metformin had a protective effect on the damage groups diabetic, cancer, and group with both cancer and diabetes. Both studies determined that the damage caused by oxidative stress resulting from diabetes was reduced by metformin treatment, based on the relevant parameters. The data obtained in this study showed parallelism with other related studies and It has been determined that oxidative stress caused by diabetes and cancer is reduced by metformin treatment.

PCA is a method of size reduction often used to reduce the dimensionality of large datasets by converting a large set of variables into a smaller variable that still contains most of the information in the large set. PCA analysis is important in terms of making the results more understandable due to the multiplicity of biochemical parameters studied. The correlation between the obtained data and the PCA results reflects the consistency of the results. The PCA analysis applied as a result of the biochemical parameters in the stomach tissue showed a correlation between the biochemical parameters studied.

5. Conclusion

Men with diabetes have a higher risk of prostate cancer than healthy individuals, and it is a type of cancer that occurs especially at later ages. Prostate cancer especially metastasizes to the lymph nodes and bone, but rarely metastasizes to the stomach. Although metformin is an old drug, its popularity has increased as a result of research in recent years and it is preferred in research especially because of its effect on oxidative stress and cancer. In this study, the protective effect of metformin on the gastric tissues of diabetic rats with prostate cancer was investigated within the framework of biochemical parameters. In rats with cancer and/or diabetes, the decrease in oxidative damage after metformin treatment was determined through the studied biochemical parameters. The findings show that oxidative stress as well as alteration of glycoprotein contents are stopped by metformin treatment. Therefore, it can be said that metformin has a protective effect on the gastric tissue of diabetic and prostate cancer rats.

Authors' Contributions

Onur Ertik: formal analysis; investigation; data curation; writing – original draft. Pinar Koroglu Aydin: formal analysis; investigation; data curation; writing – original draft. Omur Karabulut Bulan: methodology; project administration; resources; supervision; writing – review & editing. Refiye Yanardag: Conceptualisation; project administration; resources; supervision; writing – review & editing.

Conflict of Interest

The authors declare no conflict of interest

6. References

1. M. Blair. *Urol. Nurs*. **2016**, *36*, 27–36. **DOI:**10.7257/1053-816X.2016.36.1.27
2. G. Mathur, S. Nain, P. K. Sharma. *Academic J. Cancer Res.* **2022**, *8*, 1–9. **DOI:** 10.5829/idosi.ajcr.2015.8.1.9336
3. B. Turner, L. Drudge-Coates. *Cancer Nurs. Pract.* **2010**, *9*, 29–36. **DOI:**10.7748/cnp2010.12.9.10.29.c8126
4. D. M. Parkin, F. Bray, J. Ferlay, P. Pisani. *CA Cancer J. Clin.* **2005**, *55*, 77–108. **DOI:**10.3322/canjclin.55.2.74
5. E. Giovannucci, D. M. Harlan, M. C. Archer, R. M. Bergenstal, S. M. Gapstur, L. A. Habel, M. Pollak, J. G. Regensteiner, D. Yee. *Diabetes Care.* **2010**, *33*, 1674–85. **DOI:**10.2337/dc10-0666
6. R. Mallik, T. A. Chowdhury. *Diabetes Res. Clin. Pract*. **2018**, *143*, 409–19. **DOI:**10.1016/j.diabres.2018.05.023
7. R. J. Shaw, K. A. Lamia, D. Vasquez, S. H. Koo, N. Bardeesy, R. A. Depinho, M. Montminy, L. C. Cantley. *Science*. **2005**, *310*, 1642–46. **DOI:**10.1126/science.1120781
8. J. Whitburn, C. M. Edwards, P. Sooriakumaran. *Curr. Urol. Rep*. **2017**, *18*, 46. **DOI:**10.1007/s11934-017-0693-8
9. A. Haring, T. J. Murtola, K. Talala, K. Taari, T. L. J. Tammela, A. Auvinen. *Scand. J. Urol.* **2017**, *51*, 5–12. **DOI:**10.1080/21681805.2016.1271353
10. I. Bugan, Z. Karagoz, S. Altun, M. B. Djamgoz. *Basic Clin. Pharmacol. Toxicol.* **2016**, *118*, 200–07. **DOI:**10.1111/bcpt.12484
11. A. Ledwozyw, J. Michalak, A. Stępień, A. Kałdziołka. *Clin.*

Chim. Acta. **1986**, *155*, 275–83.
DOI:10.1016/0009-8981(86)90247-0
12. H. Wei, K. Frenkel. *Cancer Res*. **1991**, *51*, 4443–49.
13. A. A. Mylroie, H. Collins, C. Umbles, J. Kyle. *Toxicol. Appl. Pharmacol*. **1986**, *82*, 512–20.
DOI:10.1016/0041-008X(86)90286-3
14. H. Aebi. *Methods Enzymol*. **1984**, *105*, 121–26.
DOI:10.1016/S0076-6879(84)05016-3
15. E. Beutler. Red cell metabolism: A manual of biochemical methods, Vol. 12, Grune & Stratton, **1971**.
16. D. E. Paglia, W. N. Valentine. *J. Lab. Clin. Med*. **1967**, *70*, 158–69.
17. W. H. Habig, W. B. Jakoby. *Methods Enzymol*. **1981**, *77*, 398–405. **DOI:**10.1016/S0076-6879(81)77053-8
18. Y. Zhang, J. Chen, H. Ji, Z. G. Xiao, P. Shen, L. H. Xu. *BMC Complement. Altern. Med*. **2018**, *18*, 343.
DOI:10.1186/s12906-018-2414-3
19. R. L. Levine, D. Garland, C. N. Oliver, A. Amici, I. Climent, A. G. Lenz, B. W. Ahn, S. Shaltiel, E.R. Stadtman. *Methods Enzymol*. **1990**, *186*, 464–78.
DOI:10.1016/0076-6879(90)86141-H
20. E. D. Corte, F. Stirpe. *Biochem. J*. **1968**, *108*, 349–51.
DOI:10.1042/bj1080349
21. R. Bais, M. Philcox. *Eur. J. Clin. Chem. Clin. Biochem*. **1994**, *32*, 639–55.
22. A. Ridderstap, S. Bonting. *Am. J. Physiol*. **1969**, *217*, 1721–27.
DOI:10.1152/ajplegacy.1969.217.6.1721
23. K. Lorentz, T. Weiß, E. Kraas. *J. Clin. Chem. Clin. Biochem*. **1986**, *24*, 189–98. **DOI:**10.1515/cclm.1986.24.3.189
24. R. J. Winzler. *Methods Biochem. Anal*. **1955**, *2*, 279–311.
DOI:10.1002/9780470110188.ch10
25. Z. Dische, L. B. Shettles. *J. Biol. Chem*. **1948**, *175*, 595–603.
DOI:10.1016/S0021-9258(18)57178-7
26. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall. *J. Biol. Chem*. **1951**, *193*, 265–75.
DOI:10.1016/S0021-9258(19)52451-6
27. F. Christoph, M. Grünbaum, F. Wolkers, M. Müller, K. Miller. *Urology.* **2004**, *63*, 778–79.
DOI:10.1016/j.urology.2003.12.009
28. W. H. Holderman, J. M. Jacques, M. O. Blackstone, T. A. Brasitus. *J. Clin. Gastroenterol*. **1992**, *14*, 251–54.
DOI:10.1097/00004836-199204000-00012
29. K. P. Hong, S. J. Lee, G. S. Hong, H. Yoon, B. S. Shim. *Korean J. Urol*. **2010**, *51*, 431–33. **DOI:**10.4111/kju.2010.51.6.431
30. A. A. Onitilo, J. M. Engel, J. M. Resnick. *Clin. Med. Res*. **2010**, *8*, 18–21. **DOI:**10.3121/cmr.2010.855
31. A. Chakraborty, S. Chowdhury, M. Bhattacharyya. *Diabetes Res. Clin. Pract*. **2011**, *93*, 56–62.
DOI:10.1016/j.diabres.2010.11.030
32. C. Wang, C. Liu, K. Gao, H. Zhao, Z. Zhou, Z. Shen, Y. Guo, Z. Li, T. Yao, X. Mei. *Biochem. Biophys. Res. Commun.* **2016**, *477*, 534–540. **DOI:**10.1016/j.bbrc.2016.05.148
33. D. Diniz Vilela, L. Gomes Peixoto, R. R. Teixeira, N. Belele Baptista, D. Carvalho Caixeta, A. Vieira de Souza, H. L. Machado, M. N. Pereira, R. Sabino-Silva, F. S. Espindola. *Oxid. Med. Cell Longev*. **2016**, *2016*, 1–9. **DOI:**10.1155/2016/6978625
34. C. K. Roberts, K. K. Sindhu. *Life Sci*. **2009**, *84*, 705–12.
DOI:10.1016/j.lfs.2009.02.026
35. E. Birben, U. M. Sahiner, C. Sackesen, S. Erzurum, O. Kalayci. *World Allergy Organ. J*. **2012**, *5*, 9–19.
DOI:10.1097/WOX.0b013e3182439613
36. Z. Haida, M. Hakiman. *Food Sci. Nutr*. **2019**, *7*, 1555–63.
DOI:10.1002/fsn3.1012
37. M. J. Davies, C. L. Hawkins. *Antioxid. Redox Signal.* **2020**, *32*, 957–81. **DOI:**10.1089/ars.2020.8030
38. B. Chukwunonso Obi, T. Chinwuba Okoye, V. E. Okpashi, C. Nonye Igwe, E. Olisah Alumanah. *J Diabetes Res*. **2016**, *2016*, 1635361. **DOI:**10.1155/2016/1635361
39. S. A. Ahmed Amar, R. Eryilmaz, H. Demir, S. Aykan. *Aging Male.* **2019**, *22*, 198–206. **DOI:**10.1080/13685538.2018.1488955
40. A. B. Ozel, E. Dagsuyu, P. K. Aydın, I. Bugan, O. K. Bulan, R. Yanardag, A. Yarat. *Biol. Trace Elem. Res*. **2022**, *200*, 1164–70.
DOI:10.1007/s12011-021-02708-z
41. S. Hebbar, E. Knust. *Bioessays.* **2021**, *43*, e2100096.
DOI:10.1002/bies.202100096
42. C. Algire, O. Moiseeva, X. Deschênes-Simard, L. Amrein, L. Petruccelli, E. Birman, B. Viollet, G. Ferbeyre, M. N. Pollak. *Cancer Prev. Res*. **2012**, *5*, 536–43.
DOI:10.1158/1940-6207.CAPR-11-0536
43. N. Fernando, S. Wickremesinghe, R. Niloofa, C. Rodrigo, L. Karunanayake, H. J. de Silva, A. R. Wickremesinghe, S. Premawansa, S. Rajapakse, S. M. Handunnetti. *PloS One.* **2016**, *11*, e0156085. **DOI:**10.1371/journal.pone.0156085
44. N. Nokkaew, P. Mongkolpathumrat, R. Junsiri, S. Jindaluang, N. Tualamun, N. Manphatthanakan, N. Saleesee, M. Intasang, J. Sanit, P. Adulyaritthikul, K. Kongpol, S. Kumphune, N. Nernpermpisooth. *Indian J. Clin. Biochem*. **2021**, *36*, 228–34.
DOI:10.1007/s12291-019-0815-9
45. S. Stabler, T. Koyama, Z. Zhao, M. Martinez-Ferrer, R. H. Allen, Z. Luka, L. Loukachevitch, P. E. Clark, C. Wagner, N. A. Bhowmick. *Plos One.* **2011**, *6*, e22486.
DOI:10.1371/journal.pone.0022486
46. S. Sannigrahi, D. K. Pal, M. Mukhopadhyay. *JCDR*. **2020**, *14*, XCO1–XCO4. **DOI:**10.7860/JCDR/2020/42790.13541
47. Q. Zhang, S. Li, L. Li, Q. Li, K. Ren, X. Sun, J. Li. *Nutrients.* **2016**, *8*, 798. **DOI:**10.3390/nu8120798
48. B. Bayrak, P. Koroglu, O. Karabulut Bulan, R. Yanardag. *Hum. Exp. Toxicol*. **2021**, *40*, 297–309.
DOI:10.1177/0960327120947452
49. M. G. Battelli, L. Polito, M. Bortolotti, A. Bolognesi. *Cancer Med*. **2016**, *5*, 546–557. **DOI:**10.1002/cam4.601
50. L. E. Nelson, R. J. Valentine, J. M. Cacicedo, M. S. Gauthier, Y. Ido, N. B. Ruderman. *Am. J. Physiol. Cell Physiol*. **2012**, *303*, C4–C13. **DOI:**10.1152/ajpcell.00296.2011
51. I. Dogan Turacli, T. Candar, E. B. Yuksel, S. Kalay, A. K. Oguz, S. Demirtas. *Biochimie.* **2018**, *154*, 62–68.
DOI:10.1016/j.biochi.2018.08.002
52. R. Klein, O. Nagy, C. Tóthová, F. Chovanová. *Vet. Med. Int*. **2020**, *2020*, 5346483. **DOI:**10.1155/2020/5346483
53. Y. Wu, Y. Dong, M. Atefi, Y. Liu, Y. Elshimali, J. V. Vadgama. *Mediat. Inflamm*. **2016**, *2016*, 1–12.
DOI:10.1155/2016/6456018

54. W. Kopec, B. Loubet, H. Poulsen, H. Khandelia. *Biochem.* **2014**, *53*, 746–54. **DOI:**10.1021/bi401425g
55. A. A. Safiriyu, I. Semuyaba, S. K. Lawal, M. O. Buhari, E. A. Tiyo, M. Kalange, A. O. Okpanachi, M. Nansunga. *J. Biomed. Eng*. **2018**, *11*, 254–62. **DOI:**10.4236/jbise.2018.119021
56. B. Benziane, M. Björnholm, S. Pirkmajer, R. L. Austin, O. Kotova, B. Viollet, J. R. Zierath, A. V. Chibalin. *J. Biol. Chem.* **2012**, *287*, 23451–63. **DOI:**10.1074/jbc.M111.331926
57. L. Galdieri, H. Gatla, I. Vancurova, A. Vancura. *J. Biol. Chem.* **2016**, *291*, 25154–66. **DOI:**10.1074/jbc.M116.742247
58. A. Vancura, I. Vancurova. *Oncotarget.* **2017**, *8*, 39939. **DOI:**10.18632/oncotarget.17829
59. D. P. Christensen, M. Dahllöf, M. Lundh, D. N. Rasmussen, M. D. Nielsen, N. Billestrup, L. G. Grunnet, T. Mandrup-Poulsen. *Mol. Med*. **2011**, *17*, 378–90. **DOI:**10.2119/molmed.2011.00021
60. J. Zhao, T. H. Patwa, D. M. Lubman, D. M. Simeone. *Curr. Opin. Mol Ther*. **2008**, *10*, 602–10.
61. S. Chinnannavar, L. Ashok, K. Vidya, S. K. Setty, G. Narasimha, R. Garg. *J. Int. Soc. Prev. Community Dent*. **2015**, *5*, 446–50. **DOI:**10.4103/2231-0762.169211
62. P. Koroglu Aydın, B. B. Bayrak, O. Karabulut Bulan, R. Yanardag. *Toxicol. Mech. Methods.* **2021**, *31*, 489–500. **DOI:**10.1080/15376516.2021.1919810
63. P. Koroglu Aydın, O. Karabulut Bulan, I. Bugan, I. B. Turkyilmaz, S. Altun, R. Yanardag. *Cell Biochem. Funct*. **2022**, *40*, 60–70. **DOI:**10.1002/cbf.3674
64. P. Koroglu Aydın, I. B. Turkyilmaz, I. Bugan Gul, O. Karabulut Bulan, R. Yanardag. *J. Diabetes Metab. Disord.* **2023**, *22*, 225–236. **DOI:**10.1007/s40200-022-01109-w

Povzetek

Ljudje s sladkorno boleznijo imajo večje tveganje za nastanek raka prostate, ljudje z rakom prostate pa so nagnjeni k metastazam na želodcu. Zato raziskovalci še naprej iščejo nove pristope k zdravljenju posameznikov z vsemi navedenimi boleznimi hkrati. Zaščitni učinek metformina (ki se uporablja pri zdravljenju sladkorne bolezni) pri raku še naprej potrjujejo številne raziskave. V tej raziskavi je bilo ugotovljeno, da biokemični parametri kažejo zaščitni učinek na tkiva želodca ob dajanju metformina pri skupini z rakom in skupini z rakom ter sladkorno boleznijo. Z analizo glavnih komponent je bilo ugotovljeno, da proučevani biokemični parametri v tkivu želodca kažejo korelacijo.