Neuro-Cell Molecular Research

Research Article

Neuro-Cell Mol Res 2024;1(3):60-73

Protective effects of curcumin on the structure of the adrenal gland in diabetic rat model: A stereological, immunohistochemical and electron microscopic study

Gamze Altun[1*], Mahmut Ulubay[2], Elfide Gizem Bakirhan[3], Arife Ahsen Kaplan[4], Kıymet Kübra Tüfekci[5], Ayşenur Kaya Mutlu[6], Mohamed K.E. Ahmed[1], Emrah Küçük[7], İlknur Keskin[4], Süleyman Kaplan[1]

[1]Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[2]Department of Urology, Faculty of Medicine, Samsun University, Samsun, Türkiye
[3]Department of Histology and Embryology, Faculty of Medicine, Adiyaman University, Adiyaman, Türkiye
[4]Department of Histology and Embryology, Faculty of Medicine, İstanbul Medipol University, İstanbul, Türkiye
[5]Department of Histology and Embryology, Faculty of Medicine, Kastamonu University, Kastamonu, Türkiye
[6]Department of Histology and Embryology, Faculty of Medicine, Karamanoğlu Mehmetbey University, Samsun, Türkiye
[7]Department of Urology, Samsun Training and Research Hospital, Samsun, Türkiye

***Corresponding Author:**
Gamze Altun,
Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
Mail: gamzeyayla.omu@gmail.com
Orcid ID: 0000-0003-3959-9893

DOI: 10.5281/zenodo.14552949

Received: 5 November 2024
Accepted: 16 December 2024
Published: 31 December 2024

The author(s) - Available online at **www.neurocellmolres.com.tr**

This open-access article is distributed under the terms of Creative Commons Attribution-Non Commercial 4.0 International License

Copyright © 2024

Abstract

This study investigated the ultrastructural changes in the adrenal gland in different stages of diabetes (onset, early, late) and the protective effects of curcumin. Seven groups of *Wistar albino* rats have six rats in each group. These were control, sham, curcumin, and four different Diabetes mellitus (DM) groups. For 14 days, the Sham group received corn oil, and the curcumin group received 30 mg/kg curcumin via intragastric gavage. DM group received a single dose of 50 mg/kg streptozotocin (STZ*) I.P*. for induction of diabetes. The DM-treated groups were given 30 mg/kg curcumin after seven days (DC1 group), after 21 days (DC2 group), and concurrently with STZ injection (DC3 group), respectively. Apoptotic processes in the adrenal gland were evaluated by immunohistochemistry.

Stereological analyses showed that the cortex volume of DM and DC2 groups was significantly increased compared to the Cont group. An increased medulla volume was also observed in the DM and DC2 groups. Histopathological analysis showed numerous lipid droplets and increased cytoplasmic density of cells in the zona fasciculata in the DM group. The adrenal gland showed a healthy structure in the early curcumin treatment group (DC1). Findings indicate that curcumin administration exhibits a protective effect on the structure of the diabetic adrenal gland. However, a late curcumin treatment did not provide a sufficient protective effect. Compared to other groups, a strong expression of anti-caspase-3 in the cortex and medulla of the adrenal gland belonging to the DM group was found. Our results showed that giving curcumin at the beginning of diabetes ameliorates the adrenal gland.

Keywords: Adrenal glands, Curcumin, Diabetes mellitus, Rat, Immunohistochemistry

Introduction

Diabetes mellitus (DM) causes complications in the brain, eyes, kidneys, adrenal gland, heart, liver, and central nervous system through mechanisms such as permanent hyperglycemia, inflammation, and oxidative stress [1]. The disease mainly affects the central nervous system by impairing the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol synthesis and adrenal gland volume increase as diabetes impairs the HPA axis [2]. Diabetes-related autonomic nervous system damage also disrupts the adrenal medulla responses [3]. Experimental animal studies have shown that uncontrolled diabetes damages the HPA axis even if it does not affect its basal functions [4, 5]. Similarly, studies examining the connection between diabetes and adrenal gland morphology have discovered that people with diabetes have larger adrenal glands [6, 7].

The advantages of curcumin, a natural compound of *Curcuma longa*, for health have been investigated recently. This substance consists of two aromatic O-methoxy phenolic groups, an a-dicarbonyl moiety, and two enon moieties with a seven-carbon binder. These structural modifications, formed by different functional groups, yield advanced physicochemical and bioactive curcumin [8]. It also contains a polyphenolic group and exhibits therapeutic effects, such as antioxidant, anti-inflammatory, antiviral, antitumoral, antidiabetic, neuroprotective, cardiovascular protective, and hepatoprotective activities [9]. Curcumin may have anti-diabetic properties because it increases insulin sensitivity, affects transcription factors and enzymes at the molecular level, reduces intracellular sorbitol, reduces the accumulation of glycation end products in the cell, and lowers polyol pathway activity [10, 11].

The aim of this study was to assess the quantitative effects of diabetes on the adrenal gland in different stages of the disease (onset, early, and late) and to examine curcumin's beneficial effects, whose hypoglycemic effects are already well known, on the diabetic adrenal gland.

Materials and Methods

Animals and Study Groups

A total of 42 female Wistar albino rats were divided into seven groups of six at random. Animals in the control (Cont) group were not subjected to any procedure. Corn oil was given to the Sham group for 14 days. Curcumin was given for 14 days in the curcumin (Curc) group. For diabetes creation, a single dose of 50 mg/kg of streptozotocin (STZ) was applied intraperitoneally in the diabetes mellitus group (DM) [12]. Thirty mg/kg of curcumin for 14 days was given to the DM-treated groups after seven days (DC1 group), after 21 days (DC2 group), and simultaneously with diabetes (DC3 group). The curcumin dose (30 mg/kg) was selected [13, 14]; it was dissolved in corn oil and given to all curcumin-treated groups via gastric gavage. The establishment of the experimental diabetic model of rats and blood glucose measurements was explained in detail in our previous study [15]. A power analysis test was performed using the G*Power 3.1 program to determine the number of animals for each group. When the sample value was 6 for each group, effect size (d)=0.77, power (1-β err prob) =0.95, α err prob=0.05 was found.

The tissue samples used in this study are cadaver samples of the study carried out with the permission numbered 2017-53 and dated 30.03.2018, approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (HADYEK) (with the approval numbered 100-E.25374 dated 05.03.2018). Ethical rules performed experimental procedures according to the guidelines of the Local Animal Experiments Ethics Committee of Ondokuz Mayıs University. Experimental procedures on animals were carried out in the Experimental Animal Application and Research Center.

Tissue Preparation

Ketamine and xylazine were administered intramuscularly to anaesthetise the rats (90 mg/kg and 10 mg/kg, respectively). Intracardiac perfusion was performed using 10% buffered formalin, and adrenal gland tissues were removed for histopathological analysis. For light microscopic examination, adrenal gland tissues were immersed in 10% formalin and fixed at 4°C for two weeks. Tissue samples were processed through gradual alcohol and xylene series and embedded in paraffin (Merck, Darmstadt, Germany).

Stereological Analysis

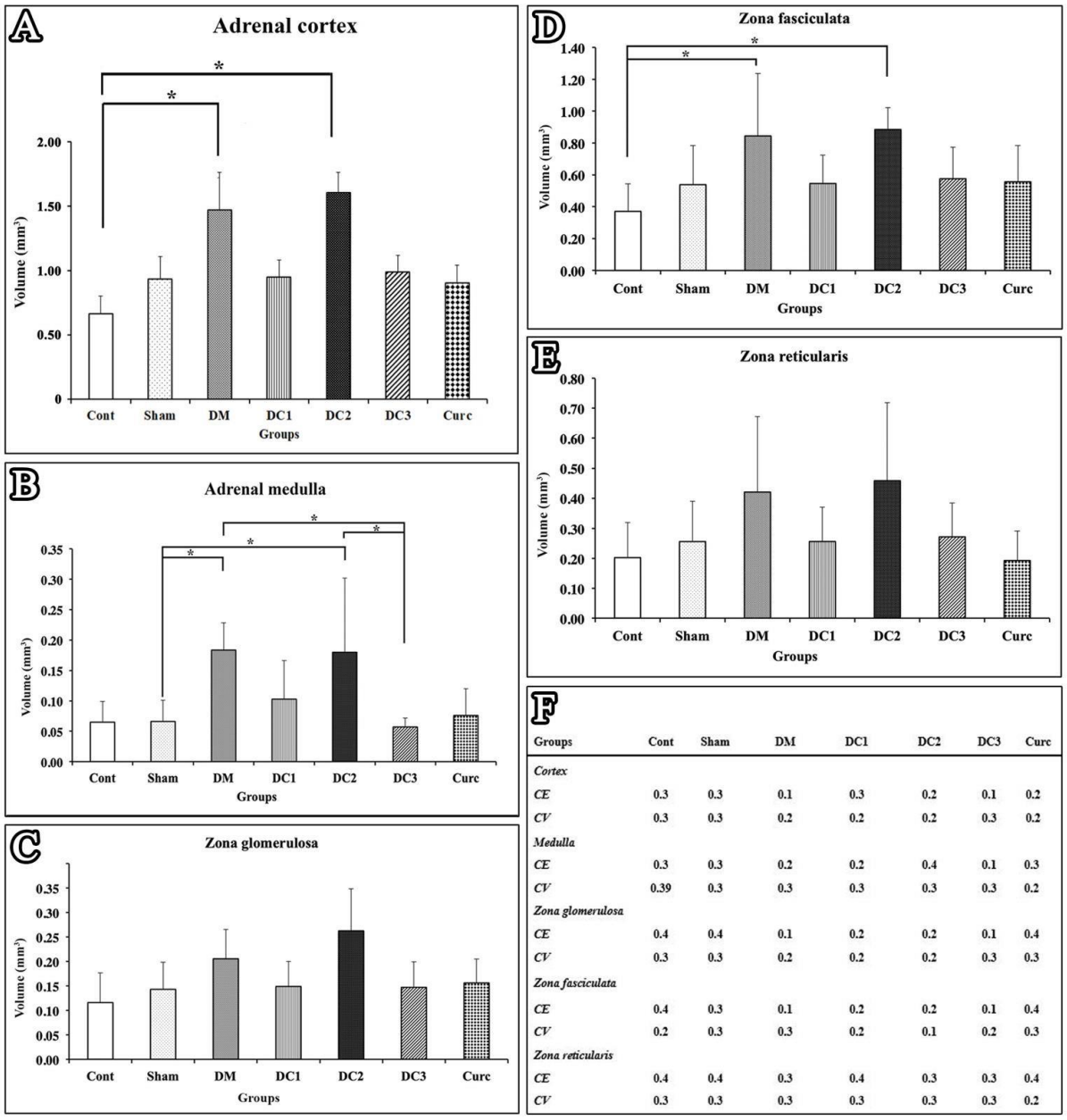
The study estimated the volumes of the adrenal cortex layers and medulla with Cavalieri's principle. According to the systematic random sample procedure, paraffin sections with a thickness of seven microns were cut at intervals of 1/24. Hematoxylin and eosin are used to stain the sections. For the study, the cortex and medulla images were captured at a 10X magnification under the camera-attached microscope. Using the point counting grid on the Image J program, the volumes of the structures were estimated. The distance between the points of the grid was 30 μm, and the area of the point was 900 μm^2 for the cortex, cortex layers, and medulla. These values were determined according to the pilot study. The volume of all parameters on the adrenal gland was calculated using the formula below:

$$V = (a/p) \times \sum P \times t$$

Where "P" is all the points in the adrenal tissue that hit the cut surface of the relevant area, "(a / p)" was the area associated with each point reflected on the adrenal and "t" section thickness [16].

Electron Microscopic Analysis

Adrenal gland tissue was embedded in resin blocks after electron microscopic tissue processing, and 70 nm thin sections were cut on copper grids. The sections' structures



Groups	Cont	Sham	DM	DC1	DC2	DC3	Curc
Cortex							
CE	0.3	0.3	0.1	0.3	0.2	0.1	0.2
CV	0.3	0.3	0.2	0.2	0.2	0.3	0.2
Medulla							
CE	0.3	0.3	0.2	0.2	0.4	0.1	0.3
CV	0.39	0.3	0.3	0.3	0.3	0.3	0.2
Zona glomerulosa							
CE	0.4	0.4	0.1	0.2	0.2	0.1	0.4
CV	0.3	0.3	0.2	0.2	0.2	0.3	0.3
Zona fasciculata							
CE	0.4	0.3	0.1	0.2	0.2	0.1	0.4
CV	0.2	0.3	0.3	0.2	0.1	0.2	0.3
Zona reticularis							
CE	0.4	0.4	0.3	0.4	0.3	0.3	0.4
CV	0.3	0.3	0.3	0.3	0.3	0.3	0.2

Figure 1. *Graph comparing the adrenal cortex, zona glomerulosa, fasciculata, reticularis, and medulla volumes in the study groups (mean ± SEM).* ***(A)*** *An increase in cortex volume can be seen in the DM and DC2 groups compared to the control group (p<0.05).* ***(B)*** *Medulla volume increases in the DM and DC2 groups compared to the sham group (p<0.05). The volume of the adrenal medulla in the DC3 group was also significantly lower than in the DM and DC2 groups (p<0.05).* ***(C, E)*** *No significant differences were determined in zona glomerulosa or zona reticularis.* ***(D)*** *An increase in volume can be seen in the DM and DC2 groups compared to the control group (p<0.05) (n=6 for all groups; (*) p<0.05).* ***(F)*** *The mean coefficient of error (CE) and coefficient of variation (CV) for the stereological analysis of adrenal gland volume estimation.*

were visualized with uranyl acetate and lead citrate to be examined under the electron microscope [17].

Immunohistochemical Analysis

In the sections of 4-µm thickness obtained from paraffin blocks, an anti-caspase-3 (1:500, Abcam, USA) antibody was used to detect apoptosis. Immunohistochemical staining was performed using the HRP/AEC chromogen kit (Abcam, USA). Positive staining areas observed in the cortex and medulla were evaluated under a camera-attached microscope (Olympus BX43F, Tokyo, Japan).

Statistical Analysis

IBM SPSS version 21.0 software (SPSS Inc., Chicago, IL, USA) was used for statistics. The data of each group was subjected to the normality test, and it was seen that the data were homogeneous. One-way ANOVA was used to examine all data, and the $p<0.05$ value was considered significant.

Results

Stereological Analysis

The volume of the adrenal cortex in the DM and DC2 groups was higher than in the control group ($p<0.05$). No significant difference was found between the control and the other groups ($p>0.05$) **(Figure 1A).** Adrenal medulla volumes were higher in the DM and DC2 groups compared to the sham group ($p<0.05$). The DC3 group exhibited a lower medulla volume than the DM and DC2 groups ($p <0.05$). No significant difference was found between the control and other groups in the medulla ($p>0.05$) **(Figure 1B).** The total volume of the zona fasciculata in the DM and DC2 groups increased compared to the control group ($p <0.05$). No significant difference was observed between the remaining groups **(Figure 1D).**

No statistically significant differences were seen between the groups in the volume of the zona reticularis and zona glomerulosa layers ($p>0.05$) **(Figure 1C, 1E).**

The coefficient of errors and coefficient of variation for stereological analysis belonging to all groups were given in **Figure 1F.**

Histopathological Analysis

Electron Microscopic Findings

The zona fasciculata of the adrenal gland from the control group was examined. The general structures of the cells in the gland were healthy, with lipid droplets, lysosomes, mitochondria, and sinusoids exhibiting a standard form. The cells in the zona fasciculata from the sham group contained numerous lipid droplets, mitochondria, and an intact nucleus and nucleoli. Numerous lipid droplets and increased cytoplasmic density were noted in most zona fasciculata cells in the DM group. The sinusoid lumens increased in size due to hyperglycemia and the accumulation of blood cells in the lumens. The nuclear boundaries of some parenchymal cells in this layer were disrupted. The number of lipid droplets increased significantly in the sections taken from the zona fasciculata of the adrenal gland of the DC1 group animals. The diameter of the sinusoids was partially reduced to normal size. Most of the cells in the zona fasciculata of the adrenal gland from the DC2 group were dark-stained, and the structure of the sinusoids in this region was normal. Images from the zona fasciculata of the glands from the DC3 group showed that the sinusoid lumen diameter and the density of the cytoplasm had increased significantly. In the curcumin group, cells and sinusoids in the zona fasciculata exhibited a normal structure. The borders of the cells in this zone can be easily distinguished **(Figure 2).**

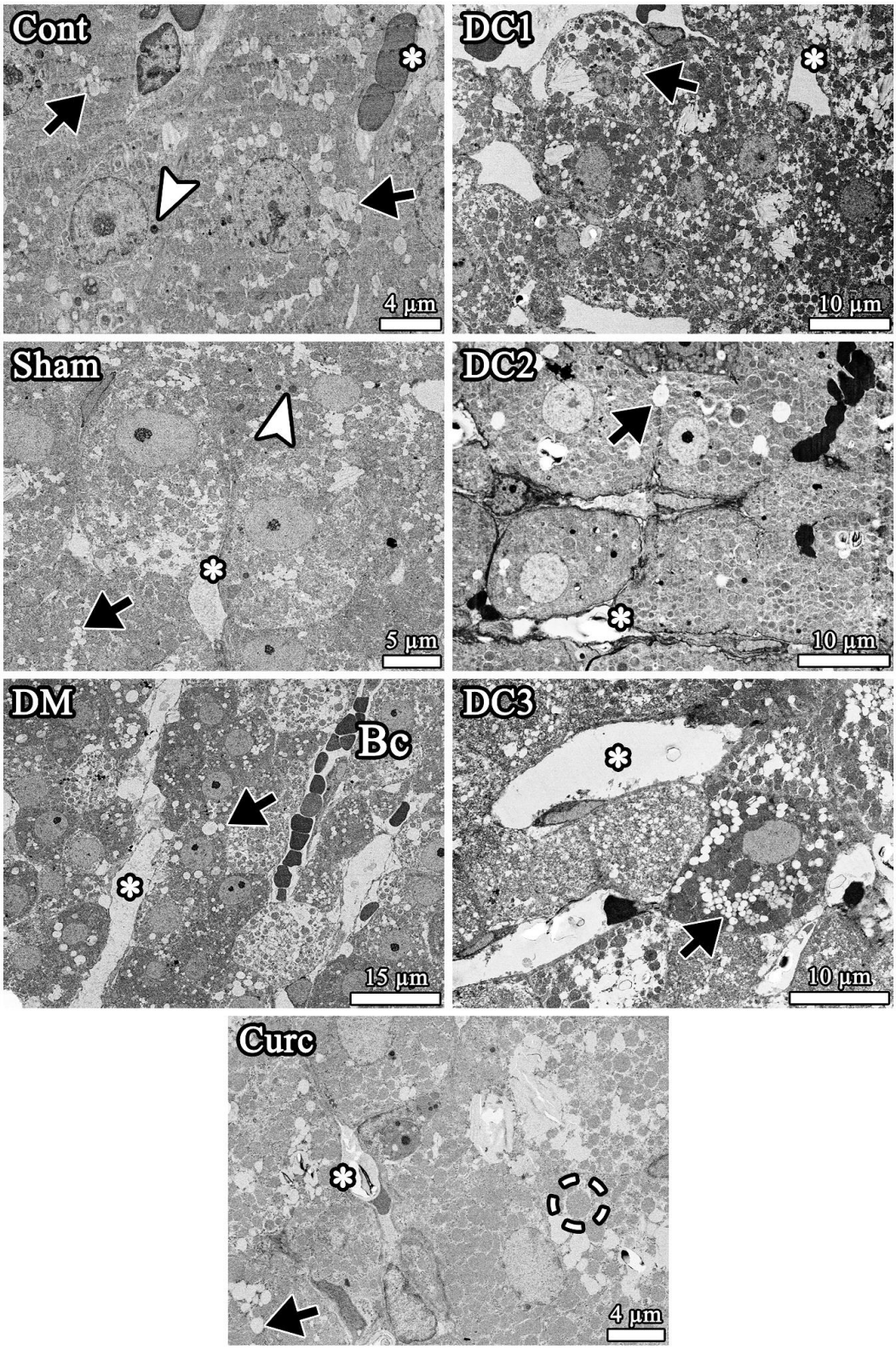


Figure 2. *Images from the zona fasciculate of the adrenal gland. The general structures of the cells in the Cont group are healthy in appearance. All cells in the Sham group have a normal structure. Most of the cells in the DM group contain a few lipid droplets, some of them large. The increased cytoplasmic density is also remarkable. Increased size of the sinusoid lumen can be seen. Some parenchymal cells have an impaired nuclear border. In the DC1 group, although the cell density is high, the number of lipid droplets has increased significantly. The diameter of the sinusoids has partly decreased to normal size. Increased numbers of mitochondria but fewer lipid droplets can be seen in the DC2 group. Sinusoids in this zone appear normal. DC3 group rats showed that the diameter of the sinusoid lumens and the cytoplasm density had increased substantially. In the Curc group, cells and sinusoids have a healthy structure. Lipid droplets (arrow), lysosomes (arrowhead), mitochondria (dashed circled), and sinusoids (*).*

The cells' morphology in the zona reticularis in the control group was healthy in appearance. The cytoplasmic density of the cells was higher than that in the cells in the zona fasciculata, as shown by fewer lipid droplets. Sinusoids also exhibited normal structure. Examination of the sham group revealed that all cells in this region had a typical structure. In addition to mitochondria, the zona reticularis cells also contained many lipid droplets. Enlarged sinusoids were particularly notable structures in the DM group. In addition to many lipid droplets in the cells in this layer, the nucleus shape was distorted, and the number of mitochondria had also decreased. Also, macrophages can be seen near the connective tissue fibers of the sinusoid in the DM group. This contains much phagocytic debris, and these phagosomes differ significantly in density. In addition, the mitochondria of cells in this layer, the zona reticularis, were intensely stained, and most cell structures were severely disrupted. Although the cells in the zona reticularis from the DC1 group were small, most had a well-conserved structure. The cells contained few lipid droplets, giant lysosomes, and intact mitochondria. The walls of the sinusoids were clearly visible. Cells in the zona reticularis of the DC2 group were healthy in appearance. Most of the cells were heavily stained as they contained many mitochondria and few lipid droplets. The structure of the sinusoids in this region also appeared normal. Cell division and a few lipid droplets were also observed in the zona reticularis of the DC3 group. Cells and sinusoids in this layer seemed to be healthy. In the Curc group, mitochondria and a few lipid droplets had been noticed in the cytoplasm of zona reticularis cells **(Figure 3).**

When the medulla was examined in detail in the Cont group, the general morphology of the cells in this region was also healthy. There were two types of cells in the medulla, light- and dark-stained. Light-stained medullary cells had a lower secreted granule density than dark-stained cells. These granules contained epinephrine or norepinephrine. The cells exhibited a healthy structure in the images taken from the sham group's medulla of the adrenal gland. The nuclei and nucleoli of these cells were intact. As seen in the previous groups, there were also two types of cells in the adrenal medulla, dark- and lightly stained, in the DM group. The nuclei of the dark-stained cells were partially deformed. In the DC1 group, although lightly stained cells had well-preserved nuclei, deformed nuclei were observed in most of the heavily stained cells. Densely and lightly stained cells were healthy on the medulla from the DC2 group. Some mega-sized macrophages with many phagosomes of different sizes in the connective tissue of the medulla were particularly noteworthy. Norepinephrine and epinephrine cells in the medulla of the DC3 group were also found to be healthy. The cells, lightly and darkly stained in the medulla of the adrenal gland from the curcumin group, had a healthy structure. The endothelial cells of the sinusoids were well preserved **(Figure 4).**

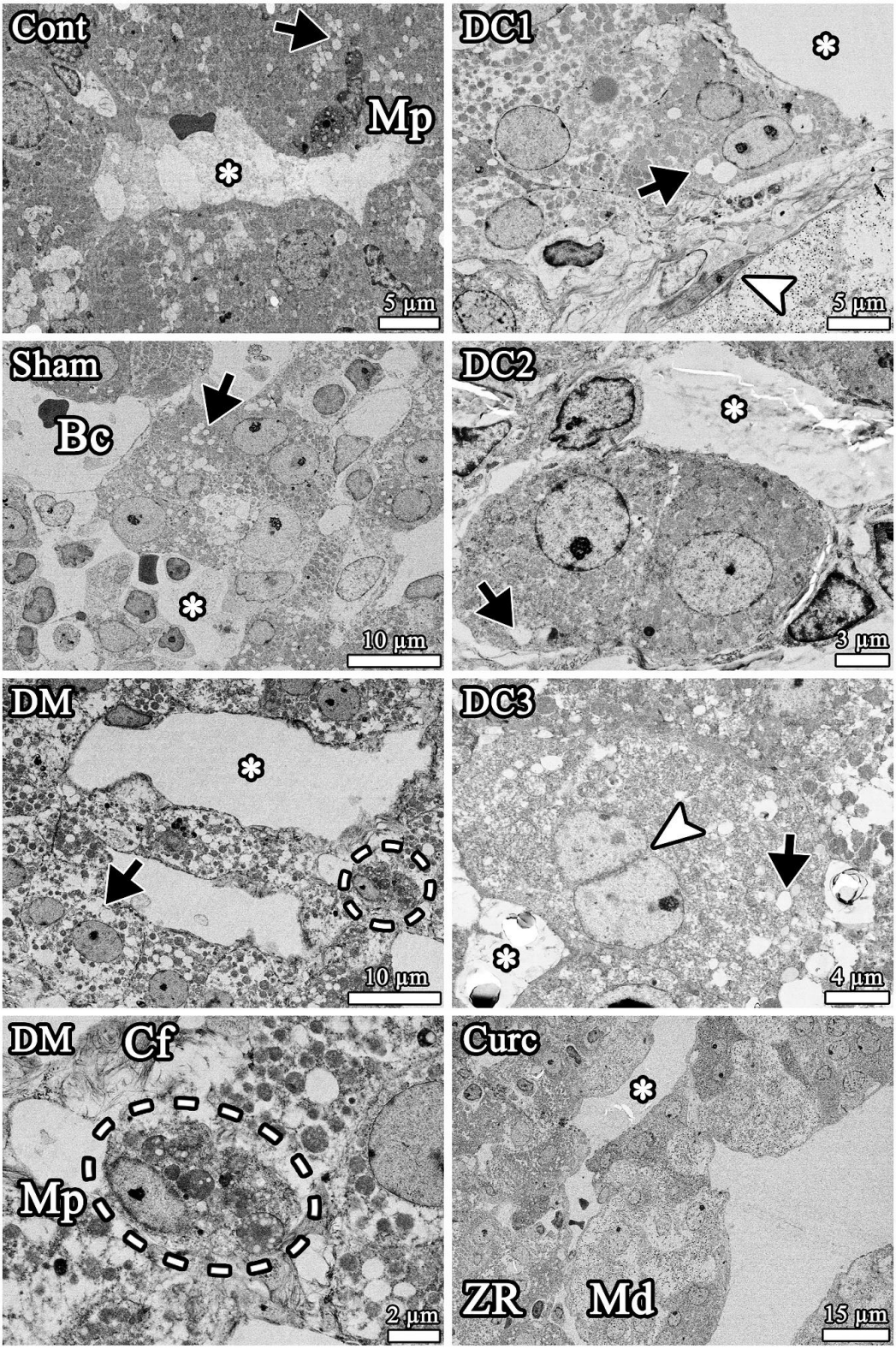


Figure 3. *Images from the zona reticularis of the adrenal gland are seen. In the Cont group, the general morphology of the cells appears healthy. The cytoplasmic density of the cells in this zone is more significant than in the ZF, as shown by the number of lipid droplets. A macrophage-like cell can be seen around the sinusoid. All cells in the Sham group have a normal structure. Most cells in the zona reticularis of the DM group had many lipid droplets, some of them large, and the number of mitochondria had also decreased. The dashed circle at high magnification shows a macrophage containing much phagocytic debris in the DM group. In the DC1 group, although the cells in the zona reticularis are tiny in size, most have a well-protected structure. The arrowhead indicates a fibrocyte of connective tissue. The cells in the DC2 group were healthy in appearance. An arrowhead indicates a cell division of densely stained cells in the DC3 group. In the Curc group, the border of the zona reticularis and medulla can be seen. All structures, cells, and sinusoids are normal in appearance. Connective fibers (Cf), lipid droplets (arrow), macrophage (Mp), medulla (Md), sinusoids (*), and zona reticularis (ZR).*

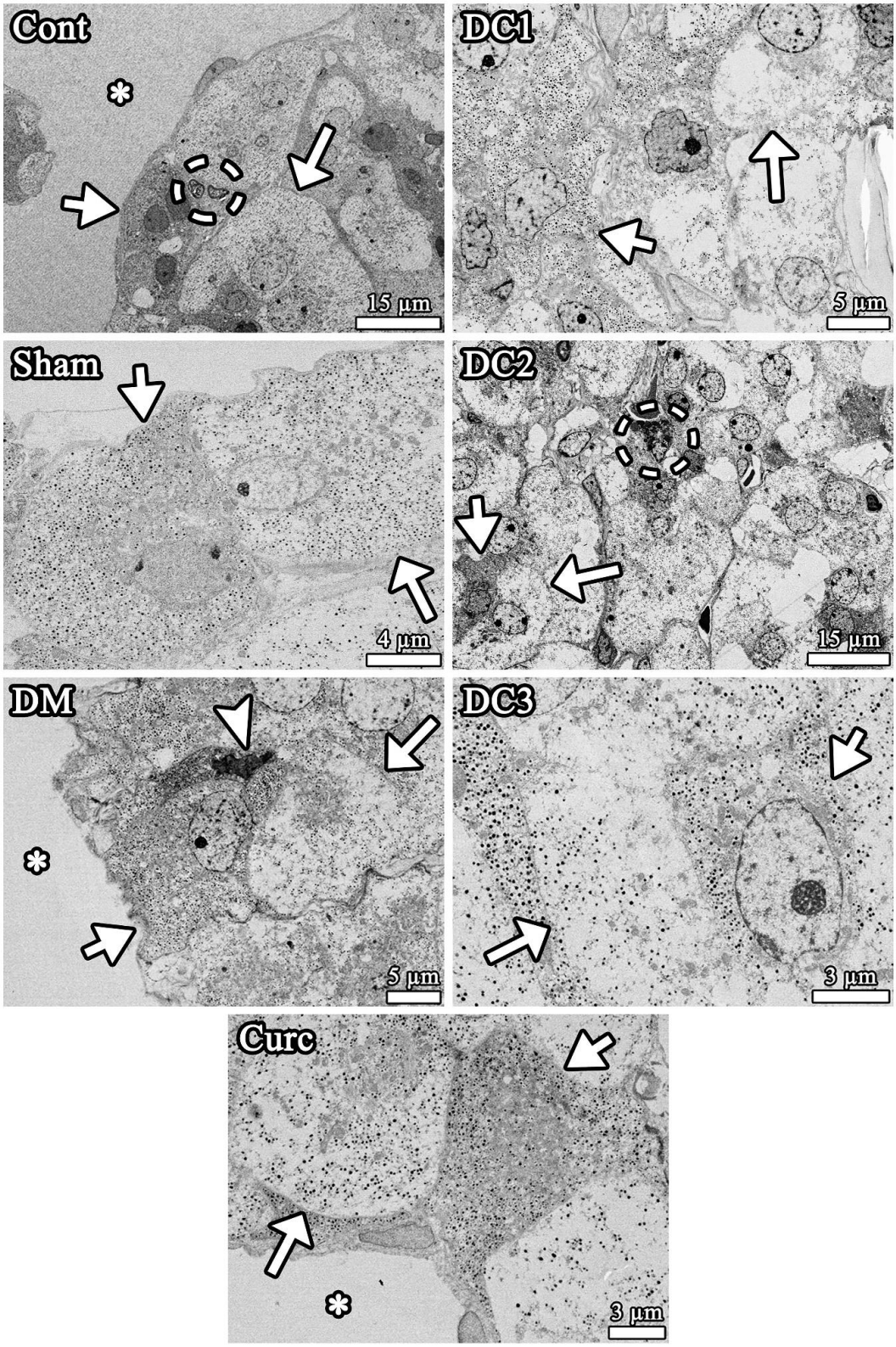


Figure 4. *Images from the medulla of the adrenal gland are seen. The Cont group revealed a healthy general cellular morphology. Two types of cells can be seen: light and dark. A collection of myelinated and non-myelinated nerve fibers can be seen in a dashed circle. The cells in the Sham group exhibit a healthy structure. In the DM group, the shape of the densely stained cell nucleus is partly impaired. Also, a dark-stained cell with a shrunken nucleus, as well as dense cytoplasm, is observed. In the DC1 group, although lightly stained cells have a well-preserved nucleus, most densely stained cells have a deformed nucleus that may show the non-functional state of those cells. Densely and lightly stained cells are seen as healthy in the DC2 group. A mega-sized macrophage stands out in a dashed circle. This cell has numerous phagosomes of different sizes. In the DC3 group, the cells in this region appear healthy. In the Curc group, two types of lightly and dense stained cells and sinusoidal capillaries display a healthy appearance. Dark-stained cell (long arrow), lightly stained cell (short arrow), shrunken nucleus (arrowhead), sinusoids (*).*

Immunohistochemical Findings

Immunostained sections of the adrenal glands were examined regarding anti-caspase-3 activity based on the cell densities that were positively stained in the evaluated sections. The anti-caspase-3 activity in the cortex of the adrenal gland belonging to the Cont group is relatively low; in the medulla, immunolocalization of anti-caspase-3 was observed in the nucleus and the cytoplasm of some cells. When sections of the Sham group were examined, higher anti-caspase-3 activity was observed in the nucleus and cytoplasm of the medullar region compared to the Cont group. A decreased expression in the cortex was observed, like in the Cont group. An increased expression of anti-caspase-3 in the cortex and medulla of the adrenal gland belonging to the DM group was remarkable. While intense positive staining was observed in the zona glomerulosa and zona fasciculata layers in the cortex region, weak staining was in the zona reticularis layer. Overexpression of caspase-3 was seen in the sinusoidal area of the zona fasciculata of this group. A similar finding was also observed in the DC2 group. Compared to the Cont and Sham groups, the cortical layers were more intensely stained in the DM group's tissues. The anti-caspase-3 activity was more predominant in cell nuclei in the zona glomerulosa region and the cell nucleus and cytoplasm of the medullar region. The staining intensity in the medullar region was higher compared to the Cont group.

When the sections of the DC1 group were examined, there was a nuclear and cytoplasmic immunolocalization in the medulla. The findings of the DC1 and DC2 groups were similar regarding anti-caspase-3 immunoreaction in the medulla. In both groups, increased anti-caspase-3 expression in the medulla was remarkable. However, while less staining was found in the cortex in the DC1 group, intense anti-caspase-3 immunoreaction was observed in the DC1 group, especially in the zona glomerulosa and part of the zona fasciculata. Similarly, intense positive staining was found in the cortex's zona glomerulosa and zona fasciculata layers in the DC3 region. Additionally, the fierce cytoplasmic localisation of anti-caspase-3 in the medulla was remarkable. In the medulla of the DC2 group, anti-caspase-3 positive activity was at nuclear and cytoplasmic levels, while positive staining was not observed in the nuclei of cells in the medulla of the DC3 group. In the examination of the immunohistochemical sections of the Curc group, a low anti-caspase-3 expression was seen in the medulla compared to the Cont group. Similarly, a decreased immunoreaction was found in the cortex. Positive staining belonging to the Curc group was widely located in the cytoplasm of cells **(Figure 5).**

Discussion

DM is a metabolic disease that causes a series of changes in various body organs, including neuroendocrine dysfunctions [2]. In this context, the adrenal gland is critical in symptoms of DM disease. The adrenal gland has a critical role in the symptoms of DM disease. Structural changes in the adrenal gland due to DM have been previously reported [18]. However, since no enough works regarding the quantitative and qualitative structural changes of diabetes and Curcumin used for diabetes treatment on the adrenal gland, our study would make an important contribution in the subject.

When histopathological data were evaluated in the current study, the general structures of the cells in the electron microscopic sections from the control and sham groups were healthy. Numerous lipid droplets and cytoplasmic density were noted in most cells in the zona fasciculata from the DM group. The diameter of the sinusoid lumen had increased, and the nuclear boundaries of some parenchymal cells in this group were disrupted. The cells in the zona reticularis in the DM group were tiny. A study investigating ultrastructural changes in adrenocortical and adrenomedullary cells in DM reported decreased lipid droplets, and membrane disruptions in cortical cells were found. The filopodia associated with these cells increased, and the gap junctions were lost. The authors suggested that the degradation of cell membranes might occur because of hyperglycemia, which induces lipid peroxidation by increasing ROS [18]. In the sections from the DC1 group, lipid droplets increased significantly, and the lysosomes and mitochondria in the cells were well preserved. The diameter of the sinusoids in the gland was partially reduced to normal size in the DC1 group. The general structure of the gland in the DC2 group was partly healthy. Most cells in the zona fasciculata were dark-stained. The structure of the sinusoids, as well as their diameters, was normal in the cortex. Mega-sized macrophages with numerous phagosomes of different sizes in the connective tissue of the medulla were observed. The lumen diameter in the sinusoids and the density of the cytoplasm increased significantly in the zona fasciculata of the adrenal gland from the DC3 group. These cells also contained numerous small-sized lipid droplets and heavily stained mitochondria. Norepinephrine and epinephrine cells in the medulla of the DC3 group were healthy in appearance. In the curcumin group, cells and sinusoids in the zona fasciculata had a normal structure.

Badawy (2018) showed that adrenal gland cells in their curcumin-treated betamethasone group exhibited a structure close to normal compared to the control group. However, lipid-like inclusions and a small number of mitochondria forming

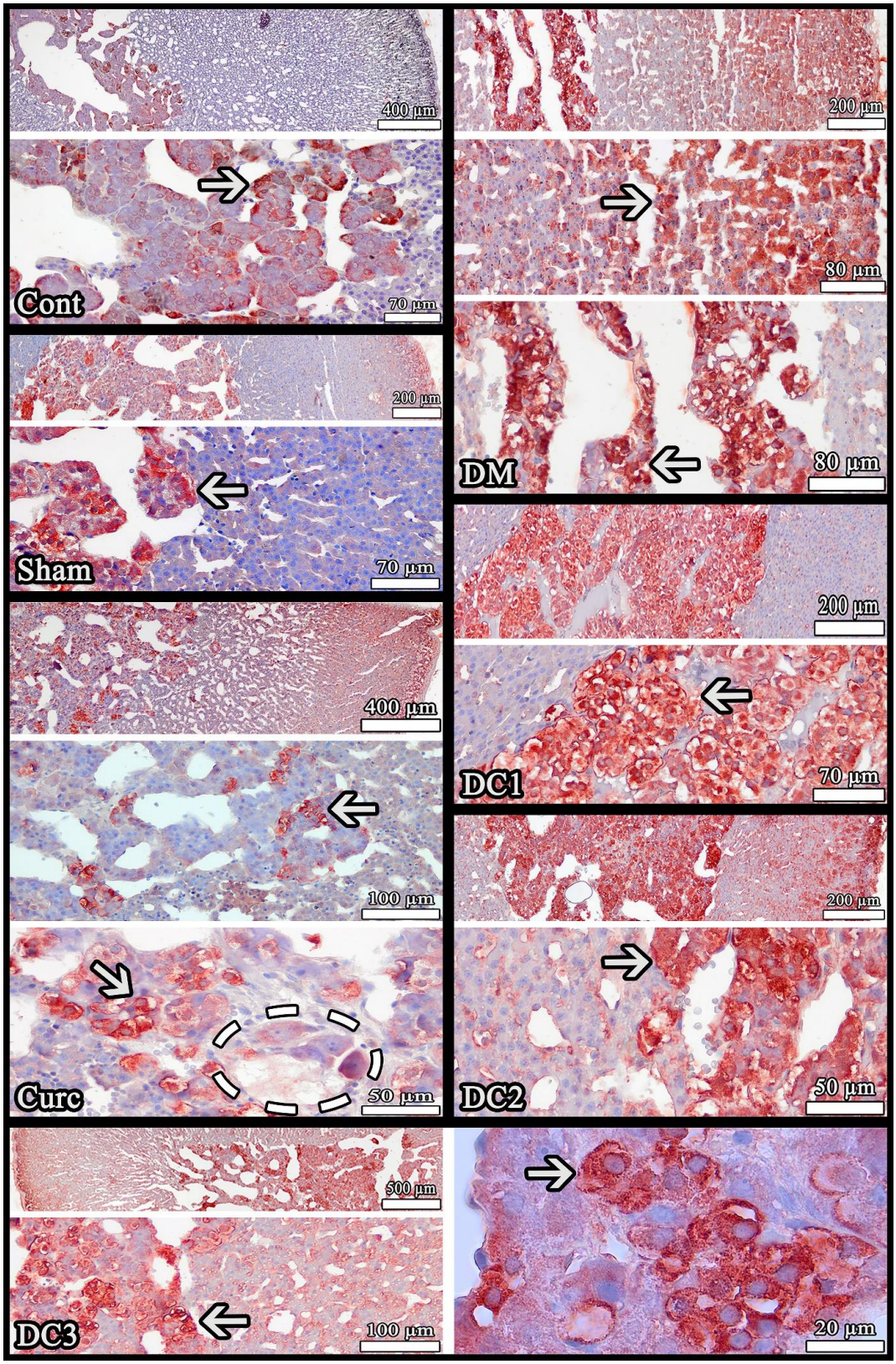


Figure 5. *Light microscopic images are observed from the sections of the adrenal glands belonging to the groups, which are stained immunohistochemically with the anti-caspase-3 antibody.* ***(Cont)*** *Low magnification of the gland belonging to the Cont group is shown (above). At the high magnification (below), a low-intensity anti-caspase-3 antibody staining is observed in the cortex, while high-intensity positive staining is in the medulla (arrow).* ***(Sham)*** *Anti-caspase-3 positive staining is seen in the zona glomerulosa and medulla of the gland. In a high magnification of the gland tissue (below), anti-caspase-3 antibody staining was seen in the medulla (arrow).* ***(DM)*** *When the general view of the DM group is examined, the staining density in the cortex and medulla regions is remarkable (above). An increased anti-caspase-3 (+) activity in the zona glomerulosa and zona fasciculata (arrow) of the cortex (middle) is remarkable.* ***(DC1)*** *General views of glands at low (above) and high magnification (below) belonging to the DC1 group are observed. A pronounced anti-caspase-3 (+) staining activity (arrow) is high in the medulla, but a low activity of anti-caspase-3 (+) staining in the cortex.* ***(DC2)*** *At the low magnification of the gland section (above), anti-caspase-3 (+) activity was weak in the zona reticularis. In contrast, an increased anti-caspase-3 (+) activity in the medulla was observed (below).* ***(DC3)*** *Anti-caspase-3 (+) activity is high in the zona glomerulosa and the medulla. In contrast, anti-caspase-3 (+) activity is low in the zona fasciculata and zona reticularis. At the high magnification (below, left), the positively stained cells (arrow) in the medulla are remarkable. In another high-magnification image (right), increased anti-caspase (+) activity (arrow) is observed in the medulla.* ***(Curc)*** *Low-intensity-stained anti-caspase-3 (+) cells are seen in the cortex and medulla of the group (above). The arrow indicates anti-caspase-3 (+) cells in the medulla (below). Sympathetic ganglion cells are marked with a dashed circle. Mayer's hematoxylin was used as a counterstain on sections.*

"myelin-like" structures by penetration of mitochondria into lipid droplets were also shown in zona reticularis cells [19]. Since the number of lipid droplets and mitochondria play a vital role in steroidogenesis, the protective role of curcumin against adrenal cortex toxicity was significant in the DC1 group. However, no similar protective role was observed in the DC2 and DC3 groups, which is consistent with the results from our stereological analysis. Therefore, we concluded that curcumin exhibits a protective activity in the early stage of DM, but late treatment has no effect. Although the exact mechanism involved is unknown, curcumin is thought to prevent the degradation of cytochrome P450 enzymes. Further studies are now needed on the efficacy of curcumin in different stages of diabetes to elucidate the effect mechanisms involved.

It has been suggested that autoimmune destruction is observed in the adrenal medulla, especially in type 1 diabetes [20]. It has been reported that the sympathochromaffin activity of the adrenal gland is impaired in diabetic humans and rats [21]. In the presented study, an intense apoptotic process was observed morphologically and immunochemically in the DM group in adrenocortical and chromaffin cell structures. The apoptosis caused by the caspase-3 pathway in chromaffin cells in the Cont, Sham, Curc, and DC3 groups was observed at a deficient level in the adrenocortical region. This may indicate the anti-apoptotic effect of Curc. Recently, in a study conducted by Tülüce et al. (2024), 100 mg/kg curcumin used in treating ultraviolet A and B-induced damage in rat skin was found to suppress the number of Tunel-positive cells due to its antiapoptotic properties [22]. At the same time, it was observed that simultaneous Curc treatment was more effective in preventing/treating the apoptotic effect caused by DM compared to early and late curcumin treatments. The presence of caspase-3 positive cells was lower in the DC3 group compared to the DC1 and DC2 groups. This shows that the simultaneous administration of curcumin in treating diabetes may have an anti-apoptotic effect. In addition, the apoptotic effect observed in the ZG and subcapsular regions in the DM group was highly intense compared to the other groups. This side effect, observed in zona fasciculata, may indicate that diabetes mellitus negatively affects corticoid production. Atrophic changes created by the STZ-induced diabetes model in the zona glomerulosa led to a decrease in the basal plasma level of aldosterone [23]. In this case, biochemical studies are needed to evaluate the reflection of diabetes-induced apoptotic changes in the adrenocortical and medullar regions to hormonal processes.

In the present study, hyperplasia and severe morphological changes occurred in the adrenal glands of diabetic rats, as seen in the adrenal cortex and medullar volumes. The adrenal hypertrophy that formed in the DM group was higher than in the other groups. Our findings are broadly consistent with previous studies [24, 25]. Compared to the control group, the volumes of the zona glomerulosa, zona fasciculata, zona reticularis, adrenal cortex, and medulla were increased in the DM group. Compared to the other groups, only the increases in volume in the adrenal cortex, medulla, and zona fasciculata layer were statistically significant. Our finding that diabetes causes an increase in adrenal cortex volume is consistent with previous investigations [24, 26]. Studies have shown that HPA axis activity and, consequently, the hormonal outputs of the adrenal cortex are affected under hyperglycemic conditions, primarily type 2 diabetes [27, 28]. The results may be due to increased HPA activity in the diabetic group. Godoy-Matos et al. found a significant increase in total adrenal volume in type-2 diabetic patients compared to a non-diabetic control group [6]. In addition to this finding, based on HPA activity, diabetic patients had significantly higher total adrenal gland volumes than non-diabetic patients [7]. Hyperglycemia has been reported to cause increased HPA activity, leading to adrenal hypertrophy and increased hormonal output of the adrenal gland in experimentally induced type 1 and 2 diabetic rats [25]. These results may be due to increased HPA activity in patients with diabetes.

Changes in hormonal levels may be due to negative feedback against exogenous and endogenous glucocorticoids and changes in 11-beta-hydroxysteroid dehydrogenase enzyme activity [2, 28, 29]. Under stress conditions, the adrenal gland plays a defensive role and increases the synthesis of glucocorticoids (corticoids) and catecholamines [30]. In this study, we did not measure cortisol levels. Still, it would be suggested that an increase in the medullar volume in diabetic rats might result from morphological and microvascular changes due to hormonal impairment.

Disorders of the adrenal cortex and medulla may occur as a source of glucose intolerance, i.e., diabetes mellitus. In the current study, there was also a substantial increase in volume in the DM group adrenal medulla compared to the sham group. Diabetes causes autonomic nervous system damage and adrenal medullary responses, and sympathetic ganglion cells show pathologies such as chromaffin degeneration, hypertrophy, leukocyte infiltration, macrophage, and amyloid deposition [3, 31]. Catalano-Iniesta et al. reported that diabetes caused adreno-medullar hypertrophy in an experimental rat study in which they investigated the effect of diabetes on adreno-medullar chromaffin cells [32]. Similarly, long-term, i.e., chronic diabetic rats with renal failure, have been shown

to exhibit degeneration of chromaffin cells and hypertrophic sympathetic ganglion cells in the adrenal medulla, together with lymphocyte infiltration, macrophages, and amyloidosis. The capillaries in the DM group were dilated in all regions and were observed at scanning electron microscopy examinations [31]. Histopathological examinations in the present study revealed a high degree of enlargement of the vessels in both the cortex and medulla due to hyperglycemia in the DM group. It may be suggested that an increase in the medulla volume in diabetic rats might result from morphological and microvascular changes due to hormonal impairment.

In the presented study, curcumin was protective against adreno-medullary hypertrophy due to diabetes. Diabetes was observed to cause hypertrophy in the adrenal gland by increasing HPA activity. Previous studies have shown that curcumin regulates HPA activity when administered orally, providing cortisol balance and reducing hypertrophy in the adrenal gland [33, 34]. Curcumin normalizes hyperactivity of the HPA axis and reduces adrenal gland hypertrophy and oxidative stress [35]. Xu et al. (2006) also showed that chronic curcumin administration could alleviate physiological changes in adrenal cortex thickness and serum corticosterone levels [36]. The multifaced therapeutic effects of curcumin may contribute to its role in maintaining hormonal balance [37]. Curcumin administration in the present research may alleviate adrenal hypertrophy by regulating HPA activity, serum corticosterone, and hormone levels. In the present study, based on the stereological analysis, the protective effect of curcumin was only observed in the DC3 group. No protective effect of curcumin was found in the other two groups that started treatment. We concluded that if curcumin is used in the early stage of DM, it exhibits protective/antioxidant activity and a protective effect on the adrenal medulla, whereas the stereological analysis revealed no antioxidant effect with late treatment. Considering its pharmacokinetic properties, its poor bioavailability and instability under physiological conditions have been revealed in the application of curcumin treatment [38]. In this context, clinical studies are needed to determine the pharmacological efficacy of curcumin in different doses and durations.

To our knowledge, this study is the first to investigate the effect of curcumin against adrenal hyperplasia/hypertrophy developing due to DM using stereological techniques. Curcumin exhibited a partial protective effect against adreno-medullary hypertrophy caused by diabetes but no protective effect against adrenocortical hypertrophy based on our stereological analysis. This study was designed to provide a theoretical basis for the therapeutic efficacy of curcumin in the adrenal gland, which is known to exhibit hypoglycemic activities against diabetic conditions. However, this substance also appears unstable, easily degradable, and quickly metabolizes to other forms. The optimum dose of curcumin and duration of administration are also unclear. The mechanism by which curcumin ameliorates diabetes and regulates the expression of genes is also unknown. All these can be considered limitations of this study. More detailed studies are needed to determine curcumin's effect on the preservation of diabetes-induced adrenal hyperplasia and adrenal impairment.

In conclusion, the presented results demonstrate that early curcumin treatment has a potent protective effect on the structure of the adrenal gland. However, treatment with curcumin in the late period of diabetes does not provide a sufficient impact on the light and electron microscopic structure of the gland.

Conflicts of interest

The authors report no conflicts of interest.

Ethics committee approval

The tissue samples used in this study are cadaver samples of the study carried out with the permission numbered 2017-53 and dated 30.03.2018, approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (HADYEK) (with the approval numbered 100-E.25374 dated 05.03.2018). Ethical rules performed experimental procedures according to the guidelines of the Local Animal Experiments Ethics Committee of Ondokuz Mayıs University. Experimental procedures on animals were carried out in the Experimental Animal Application and Research Center.

References

1. Parsamanesh N, Moossavi M, Bahrami A, Butler AE, Sahebkar A. Therapeutic potential of curcumin in diabetic complications. Pharmacol Res. 2018; 136:181-193. https://doi.org/10.1016/j.phrs.2018.09.012.
2. Barber M, Kasturi BS, Austin ME, Patel KP, MohanKumar SM, MohanKumar PS. Diabetes-induced neuroendocrine changes in rats: role of brain monoamines, insulin and leptin. Brain Res. 2003; 964(1):128-35. https://doi.org/10.1016/s0006-8993(02)04091-x
3. Rudchenko A, Akude E, Cooper E. Synapses on sympathetic neurons and parasympathetic neurons differ in their vulnerability to diabetes. J Neurosci. 2014;34(26):8865-74. https://doi.org/10.1523/JNEUROSCI.0033-14.2014
4. Chan O, Inouye K, Akirav EM, Park E, Riddell MC, Matthews SG, et al. Hyperglycemia does not increase basal hypothalamo-pituitary-adrenal activity in diabetes but it does impair the HPA response to insulin-induced hypoglycemia. Am J Physiol Regul Integr Comp Physiol. 2005;289 (1):R235-46. https://doi.org/10.1152/ajpregu.00674.2004

5. Stephens MA, Wand G. Stress and the HPA axis: Role of glucocorticoids in alcohol dependence. Alcohol Res. 2012;34(4):468-83. PMID: 23584113; PMCID: PMC3860380
6. Godoy-Matos AF, Vieira AR, Moreira RO, Coutinho WF, Carraro LM, Moreira DM, et al. The potential role of increased adrenal volume in the pathophysiology of obesity-related type 2 diabetes. J Endocrinol Invest. 2006;29(2): 159-63. https://doi.org/10.1007/BF03344090
7. Carsin-Vu A, Oubaya N, Mulé S, Janvier A, Delemer B, Soyer P, Hoeffel C. MDCT Linear and volumetric analysis of adrenal glands: Normative data and multiparametric assessment. Eur Radiol. 2016;26(8):2494-501. https://doi.org/10.1007/s00330-015-4063-y
8. Yang H, Du Z, Wang W, Song M, Sanidad K, Sukamtoh E, et al. Structure-activity relationship of curcumin: Role of the methoxy group in anti-inflammatory and anticolitis effects of curcumin. J Agric Food Chem. 2017;65(22):4509-4515. https://doi.org/10.1021/acs.jafc.7b01792
9. Avila-Rojas SH, Lira-León A, Aparicio-Trejo OE, Reyes-Fermín LM, Pedraza-Chaverri J. Role of autophagy on heavy metal-induced renal damage and the protective effects of curcumin in autophagy and kidney preservation. Medicina (Kaunas). 2019;10;55(7):360. https://doi.org/10.3390/medicina55070360
10. Lao CD, Ruffin MT 4th, Normolle D, Heath DD, Murray SI, Bailey JM, et al. Dose escalation of a curcuminoid formulation. BMC Complement Altern Med. 2006; 6:10. https://doi.org/10.1186/1472-6882-6-10.
11. Nabavi SF, Thiagarajan R, Rastrelli L, Daglia M, Sobarzo-Sánchez E, Alinezhad H, et al. Curcumin: a natural product for diabetes and its complications. Curr Top Med Chem. 2015;15(23):2445-55. https://doi.org/10.2174/1568026615666150619142519
12. Kandemir FM, Ozkaraca M, Küçükler S, Cağlayan C, Hanedan B. Preventive effects of hesperidin on diabetic nephropathy induced by streptozotocin via modulating TGF-β1 and oxidative DNA damage. Toxin Rev. 2017;(37):287–293. https://doi.org/10.1080/15569543.2017.1364268
13. Breidert M, Böttner A, Möller S, Herberg L, Bornstein S. Apoptosis in the adrenal gland of non-obese diabetic (NOD) mice. Exp Clin Endocrinol Diabetes. 1998;106(6):478-83. https://doi.org/10.1055/s-0029-1212020.
14. Sharma S, Kulkarni SK, Agrewala JN, Chopra K. Curcumin attenuates thermal hyperalgesia in a diabetic mouse model of neuropathic pain. Eur J Pharmacol. 2006;536(3):256-61. https://doi.org/10.1016/j.ejphar.2006.03.006
15. Tufekci KK, Kaplan S. Beneficial effects of curcumin in the diabetic rat ovary: a stereological and biochemical study. Histochem Cell Biol. 2023;159(5):401-430. https://doi.org/10.1007/s00418-022-02171-4
16. Zengin H, Kaplan S, Tümkaya L, Altunkaynak BZ, Rağbetli MÇ, Altunkaynak ME, Yilmaz O. Effect of prenatal exposure to diclofenac sodium on the male rat arteries: a stereological and histopathological study. Drug Chem Toxicol. 2013;36(1):67-78. https://doi.org/10.3109/01480545.2011.649287
17. Zaki SM, Abdelgawad FA, El-Shaarawy EAA, Radwan RAK, Aboul-Hoda BE. Stress-induced changes in the aged-rat adrenal cortex. Histological and histomorphometric study. Folia Morphol (Warsz). 2018; 77(4): 629-641. https://doi.org/10.5603/FM.a2018.0035
18. Baimai S, Bhanichkul P, Lanlua P, Niyomchan A, Sricharoenvej S. Modifications of adrenal gland ultrastructure in streptozotocin-induced diabetic model rats. Int J Morphol. 2021;39:109-115. https://doi.org/10.4067/S0717-95022021000100109
19. Badawy GM. Curcumin ameliorates the hazard effect of prenatal betamethasone administration on the fetal adrenal gland of albino rats. EJPMR. 2018;5(12): 133-148
20. Itoh N, Hanafusa T, Katsura H, Yamamoto K, Takeda A, Kurahashi A, et al. Two types of autoantibodies to adrenal medullary cells in type 1 (insulin-dependent) diabetic patients: prevalence, properties and implications. J Autoimmun. 1991;4(5):807-18. https://doi.org/10.1016/0896-8411(91)90175-c
21. Wilke RA, Hillard CJ. Decreased adrenal medullary catecholamine release in spontaneously diabetic BB-Wistar rats. Role of hypoglycemia. Diabetes. 1994;43(5):724-9. https://doi.org/10.2337/diab.43.5.724.
22. Tülüce Y, Osmanoğlu D, Rağbetli MÇ, Altındağ F. Protective action of curcumin and alpha-lipoic acid, against experimental ultraviolet-A/B induced dermal-injury in rats. Cell Biochem Biophys. 2024; 82(4): 3535-46. https://doi.org/10.1007/s12013-024-01442-2
23. Rebuffat P, Belloni AS, Malendowicz LK, Mazzocchi G, Meneghelli V, Nussdorfer GG. Effects of streptozotocin-induced experimental diabetes on the morphology and function of the zona fasciculata of rat adrenal cortex. Virchows Arch B Cell Pathol Incl Mol Pathol. 1988;56(1):13-9. https://doi.org/10.1007/BF02889996
24. Noguchi S, Ohno Y, Aoki N. Adrenocortical insufficiency in Otsuka Long-Evans Tokushima Fatty rats, a type 2 diabetes mellitus model. Metabolism. 2007;56(10):1326-33. https://doi.org/10.1016/j.metabol.2007.05.021
25. Elahi-Moghaddam Z, Behnam-Rassouli M, Mahdavi-Shahri N, Hajinejad-Boshroue R, Khajouee E. Comparative study on the effects of type 1 and type 2 diabetes on structural changes and hormonal output of the adrenal cortex in male Wistar rats. J Diabetes Metab Disord. 2013;12(1):9. https://doi.org/10.1186/2251-6581-12-9
26. Serifoglu I, Oz II, Bilici M. The adrenal gland volume measurements in manifestation of the metabolic status in type-2 diabetes mellitus patients. Int J Endocrinol. 2016;2016:7195849. https://doi.org/10.1155/2016/7195849
27. Chiodini I, Adda G, Scillitani A, Coletti F, Morelli V, Di Lembo S, Epaminonda P, Masserini B, Beck-Peccoz P, Orsi E, Ambrosi B, Arosio M. Cortisol secretion in patients with type 2 diabetes: Relationship with chronic complications. Diabetes Care. 2007;30(1):83-8. https://doi.org/10.2337/dc06-1267
28. Inouye KE, Chan O, Yue JT, Matthews SG, Vranic M. Effects of diabetes and recurrent hypoglycemia on the regulation of the sympathoadrenal system and hypothalamo-pituitary-adrenal axis. Am J Physiol Endocrinol Metab. 2005;288(2):E422-9. https://doi.org/10.1152/ajpendo.00389.2004
29. Revsin Y, van Wijk D, Saravia FE, Oitzl MS, De Nicola AF, de Kloet ER. Adrenal hypersensitivity precedes chronic hypercorticism in streptozotocin-induced diabetes mice. Endocrinology. 2008; 149(7):3531-9. https://doi.org/10.1210/en.2007-1340
30. Kanczkowski W, Sue M, Bornstein SR. Adrenal gland microenvironment and its involvement in the regulation of stress-induced hormone secretion during sepsis. Front Endocrinol (Lausanne). 2016;7:156. https://doi.org/10.3389/fendo.2016.00156
31. Sricharoenvej S, Boonprasop S, Lanlua P, Piyawinijwong S, Niyomchan A. Morphological and microvascular changes of the adrenal glands in streptozotocin-induced long-term diabetic rats. Ital J Anat Embryol. 2009;114(1):1-10. PMID: 19845276
32. Catalano-Iniesta L, Iglesias-Osma MC, Sánchez-Robledo V, Carretero-Hernández M, Blanco EJ, Carretero J, et al. Variations in adrenal gland medulla and dopamine effects induced by the lack of Irs2. J Physiol Biochem. 2018;74(4):667-677. https://doi.org/10.1007/s13105-018-0655-8
33. Xu Y, Ku B, Cui L, Li X, Barish PA, Foster TC, et al. Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. Brain Res. 2007;1162:9-18. https://doi.org/10.1016/j.brainres.2007.05.071
34. Liu D, Wang Z, Gao Z, Xie K, Zhang Q, Jiang H, et al. Effects of curcumin on learning and memory deficits, BDNF, and ERK protein

expression in rats exposed to chronic unpredictable stress. Behav Brain Res. 2014; 271: 116-21. https://doi.org/10.1016/j.bbr.2014.05.068

35. Bhatia N, Jaggi AS, Singh N, Anand P, Dhawan R. Adaptogenic potential of curcumin in experimental chronic stress and chronic unpredictable stress-induced memory deficits and alterations in functional homeostasis. J Nat Med. 2011;65(3-4): 532-43. https://doi.org/10.1007/s11418-011-0535-9
36. Xu Y, Ku B, Tie L, Yao H, Jiang W, Ma X, et al. Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. Brain Res. 2006;1122(1):56-64. https://doi.org/10.1016/j.brainres.2006.09.009
37. Wang X, Zhang W, Zhou S. Multifaceted physiological and therapeutical impact of curcumin on hormone-related endocrine dysfunctions: A comprehensive review. Phytother Res. 2024;38(7):3307-3336. https://doi.org/10.1002/ptr.8208
38. Wei Y, Li H, Li Y, Zeng Y, Quan T, Leng Y, et al. Advances of curcumin in nervous system diseases: the effect of regulating oxidative stress and clinical studies. Front Pharmacol. 2024;15:1496661. https://doi.org/10.3389/fphar.2024.1496661