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Ameliorative Effect of Cinnamon against Hypercholesterolemia Induced by High-Fat Diet in The Adult Male Albino Rat's Adrenal Gland; Histological, Ultrastructural, and Immunohistochemical Studies

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ABSTRACT

Obesity is a global health issue of great concern. It leads to hyperplasia, hypertrophy of adipocytes, and metabolic complications including coronary artery disease, and cerebrovascular disease. The effectiveness of *cinnamon zeylanicum* as a medicinal herb comes from its anti-inflammatory and antioxidant properties. The purpose of this study was to assess the potential beneficial effects of cinnamon on adrenal toxicity caused by a high-fat diet in male albino rats. Four equal groups of the experimental animals were used, the control group, the cinnamon group, the high-fat diet group, the high-fat diet, and the cinnamon group. Animals fed cinnamon showed similar results with the control group. The adrenal cortex sections of high-fat diet-fed animals showed different degenerative changes in all layers of the adrenal gland, cortex, medulla, and corrugation of the capsule. Ultra structurally, a lot of adrenal cortical cells appeared with irregular pyknotic nuclei, swollen mitochondria, dilated sER, and numerous lipid droplets with variable shapes and sizes. Moreover, a significant rise in the immunohistochemical expression of cytochrome c in a large number of cortical cells was observed. On the contrary, animals given cinnamon and a high-fat diet exhibited noticeable improvement in the structure of the adrenal gland, and the immunohistochemical expression of cytochrome c was observed in the third group. The histological structure of the adrenal gland appeared to be mostly the same as the control one. Cinnamon has a potential effect in improving adrenal toxicity against a high-fat diet because of the natural constituent capable of its pharmaceutical effects.

INTRODUCTION

Obesity is characterized by excessive fat accumulation that usually results from increased energy intake compared to that consumed (Iglesias and Díez, 2010). WHO (2021) reported that obesity is a major public health issue all over the world, not just in developed countries as was the case before, and affects adults as well as children. According to WHO (2022), 39 million kids under the age of five were either overweight or obese. Also, it added that four million people die each year because of obesity.

Obesity leads to hyperplasia and hypertrophy of adipocytes as well as some

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metabolic complications including osteoarthritis and psychosocial dysfunction, diabetes, high blood pressure, cardio disease, liver diseases, infertility, and even some cancers are all result of later (WHO, 2021 and Bray, 2004). Rats fed high-fat diet (HFD) showed a significant increase in body weight, glucose, insulin, and leptin (Caroline *et al*., 2021). Moreover, obesity leads to kidney and liver diseases (Wree *et al*., 2010 and Than *et al*., 2020).

The adrenal gland is the prime player in the endocrine system that modulates various systems and body functions like blood pressure, immunity, metabolism, and stress response (Melmed *et al.*, 2015 and Megha *et al*., 2022). The adrenal cortex produces steroid hormones while the medulla produces catecholamine hormones (Kleine and Rossmanith, 2016). All these hormones affect and interact with the adipose tissue (Kargi and Iacobellis, 2014). Mice fed HFD revealed a significant increase in the levels of leptin, inflammatory factors, and insulin (Armani *et al*., 2010 and Chakraborty *et al*., 2016). Medjerab *et al*. (2014) and Navarrete *et al*. (2018) reported that obesity produced morphological and functional changes in the adrenal cortex of *Gerbillus gerbillus* and mice that led to endocrine complications. Moreover, female mice fed HFD showed an increase in their body weight, adrenal gland weight, and intraperitoneal fat tissue (Topal *et al*., 2019).

Since ancient times, people have been using plants and herbs found in nature for medical and therapeutic purposes. With scientific development in the field of medicine, manufactured medicines have been used, but they have side effects despite their healing ability. Therefore, scientists resorted to using natural plants due to their potent pharmacological activities, low cost, and economic viability (Pracheta *et al*., 2011 and Liu *et al*., 2020). One of the most traditionally used plants is cinnamon. Cinnamon's (*cinnamon zeylanicum*) natural resource is the dried inner bark of the coppiced *Cinnamomum sp* shoots from the family Lauraceae (Vinitha and Ballal, 2008). It is composed of numerous active components, including tannins, terpenoids, anthraquinone, alkaloids, coumarins, and flavonoids (Mohamed *et al.*, 2011).

Cinnamon's main active constituents are volatile oils which include 50 to 75% cinnamic aldehyde (trans-cinnamaldehyde (E)-cinnamaldehyde), 5 to 10% eugenol, α-terpineol, L-borneol and terpene hydrocarbons like phellandrene, pinene, α-cubebene, terpinolene, α-thujene and caryophyllenes (Tung *et al*., 2010). In traditional folklore, cinnamon was used as a carminative, stomachic as well as antiseptic, and mild astringent (Navarrete *et al*., 2018 and Topal *et al*., 2019).

Cinnamomum zeylanicum (*c. zeylanicum*) was proven to be a powerful hypolipidemic and hypoglycemic agent (Tuzcu *et al*., 2017 and Mohammed and Abdel Fattah, 2018). In addition, several studies revealed the beneficial role of cinnamon on the liver (Eidi *et al*., 2012), kidney (Hussain *et al*., 2019), and intestine as anti-inflammatory agents (Kwon *et al*., 2011) and spermatogenesis (Khaki, 2015) in experimental animals. Numerous studies have focused on the biochemical or histological alteration, and a select few have examined the alteration of the adrenal's ultrastructure.

Cytochrome c is a tiny protein containing heme found in mitochondria and sER weighing 15KDa. It was identified as a mitochondrial pro-apoptotic protein. Cytochrome c is represented as a metallic color (brown color) in the cytoplasm of the adrenal cortical cells (Liu *et al*., 2020). This scientific research was established to assess the benefits of *C. zeylanicum* on adrenal toxicity caused by hypercholesterolemia in albino rats.

MATERIALS AND METHODS

Cinnamon:

Cinnamomum zeylanicum bark was bought from a shop of herbs in Giza, Egypt. The bark was recognized by specialists in botanical gardens (Orman Garden Herbarium, Egypt). Fine powder of *C. zeylanicum* was obtained by grinding the dried bark with an electric grinder. Experimental animals were supplemented with *C. zeylanicum* powder by mixing it with a diet; of fifteen w/w daily for 8 weeks (Rahman *et al*., 2013).

High-Fat Diet Formula (HFD):

The ingredients used for preparing the HFD were bought as pure powder from a chemicals and medical company (El-Gomhorya Co.) in Egypt. Preparing HFD was done as described by Jeong *et al*. (2005).

Animals:

Healthy adult male albino rats (*Rattus norvegicus*) of average body weight of 120 to 130 g were obtained from Helwan Laboratory Farms of the Egyptian Organization for Vaccine and Biological Preparations in Cairo, Egypt. Animals were kept in the laboratory under almost constant conditions of temperature (23±2°C) for 15 days before initiating the experiment. They were fed a diet specific for rodents and manufactured especially for laboratory purposes and limitless access to water was provided. The overall design of the experiment was done based on the bioethics of the Animal Care and Use Committee (Approved No. MUF/F/HI/4/23).

Animal Groups:

Rats under investigation (24) were randomly divided into four equal groups; the control group in which the animals were fed a standard rodent diet. The *C. zeylanicum* group; cinnamon was added to their standard diet (15% w/w). The HFD group was fed the HFD diet, and finally, the HFD and *C. zeylanicum* group were fed HFD and cinnamon mixed with the diet. All the experimental animals were kept on their special diet for 8 weeks then they were dissected. Adrenal glands were removed and put in 10% neutral formalin for subsequent processing.

Histological Study:

One adrenal gland from each animal from the different groups was fixed and prepared for 5µm paraffin sections to be stained with Ehrlich's hematoxylin and counter-stained with eosin (Lillie and Fulmer, 1976). Olympus microscope (CX31RTSF, Olympus Corporation, Tokyo, Japan) was used to examine the slides, and photographed by Olympus digital camera (E330, Olympus Corporation, China).

Ultrastructural Study:

The second adrenal gland was sliced into $1mm^3$ and processed according to the methods of Reynolds (1963). Sections were examined and photographed at the Electron Microscope Unit, Faculty of Science, Alexandria University (Alexandria, Egypt).

Immunohistochemical Study:

Cytochrome c was localized in the cytoplasm of adrenal gland cells according to the avidin-biotin complex technique using Thermo Fisher rabbit polyclonal anti-rat cytochrome c antibody (cat #PA5-79119) as described by Abd-El Hafeez and Soliman (2017).

Optical Density Measurement:

Six power fields from each layer of the adrenal gland (X400) per animal were imaged with an Olympus digital camera. The optical density of cytochrome c in the cytoplasm of different types of adrenal cells was quantified by Image J software compatible with Windows (11) Abd-Elhafeez *et al.* (2021).

Statistical Analysis:

Obtained data were represented as mean ± standard error and were statistically analyzed using *SPSS* software (version 18). A one-way ANOVA test was used to determine the significance of variance between different groups' means and data were considered significant when the *P* value was less than 0.05.

RESULTS

Histological Results:

Slides examined by light microscope from control and rats treated with *C. zeylanicum* revealed the normal structure of the gland. The adrenal gland is divided into two parts, an outer cortex and an inner medulla and is surrounded from the outside by a capsule formed of dense connective tissue. The cortex is formed from three layers (from outside to inside); Zona glomerulosa (ZG), a Zona fasciculate (ZF), and Zona reticularies (ZR). Zona glomerulosa is the thinnest segment of the cortex. It contains ovoid to columnar cells that cluster into spherical shapes or arcades. These cells have spherical nuclei and a small amount of cytoplasm (homogeneously, lightly stained acidophilic) containing a few small lipid droplets.

The zona fasciculate is the thickest adrenal cortex layer. It is composed of polyhedral cells organized in long, radially regular cords. These cords are separated from one another by capillary vessels (sinusoids). These cells have large darkly stained spherical nuclei, and their cytoplasm contains a large number of lipid droplets. Zona reticularies formed from an anastomosing network of cellular cords interspersed by sinusoids. Zona reticularies cells are smaller in size. It has a densely acidophilic stained cytoplasm and contains a few small lipid droplets. At the center of the gland is the medulla, which is formed of large parenchymal cells, called chromaffin cells. These cells are lightly stained basophilic cells and are arranged in ovoid clusters near capillaries (Fig. 1, a-d).

Adrenal cortex sections of HFD-fed animals showed severe degenerated changes including irregular and thick capsules. Cells in both ZG and ZF are disorganized and some cells of them appeared vacuolated with pyknotic nuclei. Vacuoles were of variable size (macro and micro vacuoles) and occupied nearly all the cytoplasm of these cells (balloon cells) (Fig. 2a&b). Moreover, the sinusoids between the cords appeared dilated. In addition, cells in ZR appeared degenerated with hyalinized cytoplasm. Similarly, sinusoids between ZR cells were dilated, and congested. In the medulla, most chromaffin cells were massively vacuolated and became more eosinophilic. Also, medullary blood capillaries and sinusoids were dilated, with obvious congestion (Fig. 2a-d).

In animals fed cinnamon + HFD, most cells of the adrenal gland retained their normal appearance. In most cells of ZG and ZF vacuoles were within normal presence and appearance. All ZR cells were nearly normal. Parenchymal cells of the medulla also retained their normal cytoplasmic appearance. However, mild congestion was still detected in medullary blood capillaries, and some sinusoids were still dilated (Fig. 3a-c).

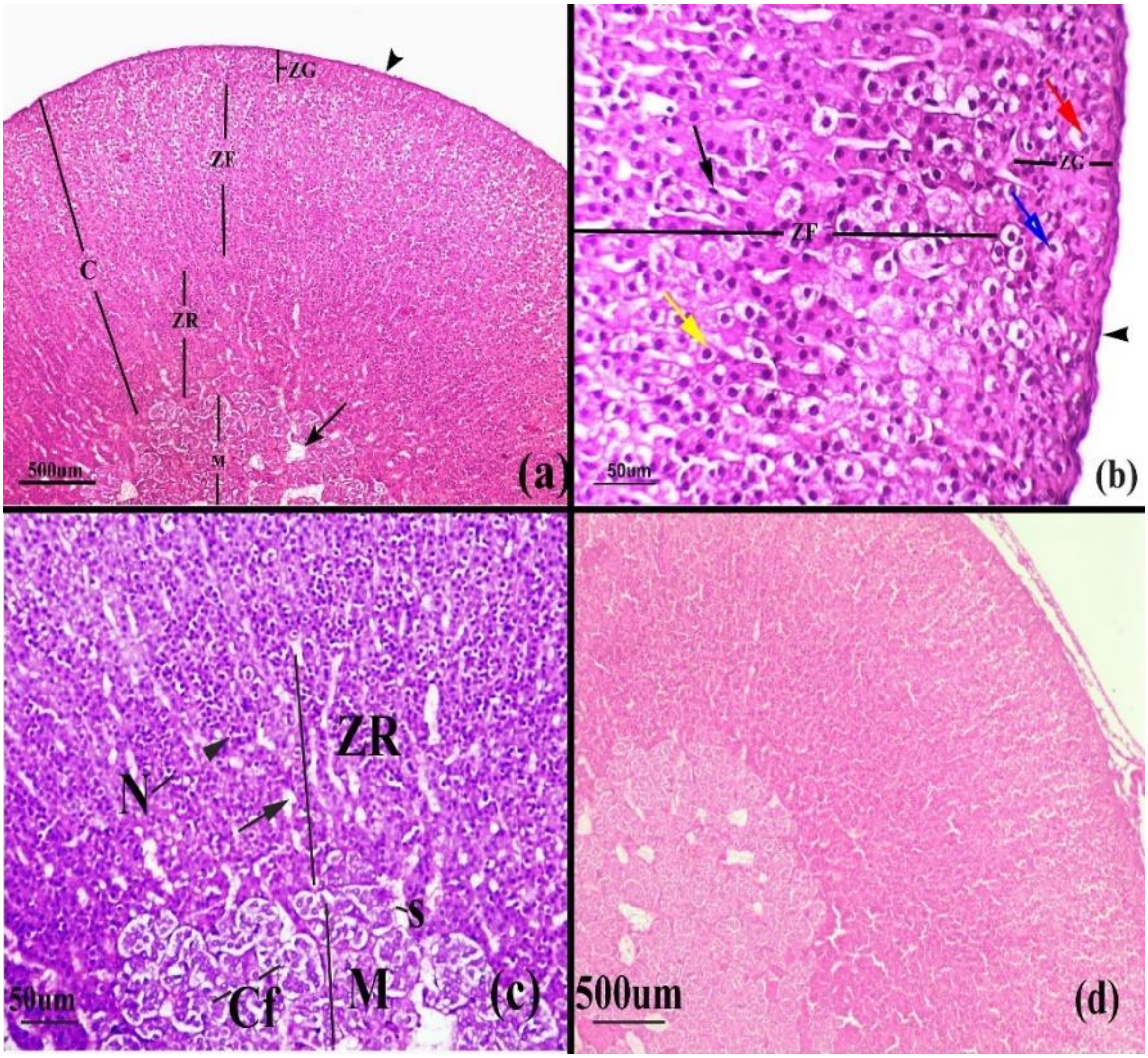

Fig.1: Photomicrographs from the adrenal gland of control rats showing: a) adrenal capsule (arrowhead), cortex (C), Zona glomerulosa (ZG), fasciculate (ZF), reticularies (ZR), and medulla (M); b) enlarged portion from the previous image showing capsule (arrowhead), spherical arcades cells of zona glomerulosa (ZG) arranged in spherical arcades and have spherical nuclei (red arrow) and homogeneously, lightly stained cytoplasm with few small lipid droplets (blue arrow), polyhedral cells of Zona fasciculata (ZF) with rounded nuclei (yellow arrow) and blood sinusoids (black arrow); c) Zona reticularis (ZR) cells (arrowhead) with deeply stained nuclei (N) and blood sinusoids (arrow), adrenal medulla (M) and chromaffin cells (Cf) surrounded by sinusoids (S); d) section obtained from rat that treated with cinnamon showing normal structure of adrenal gland.

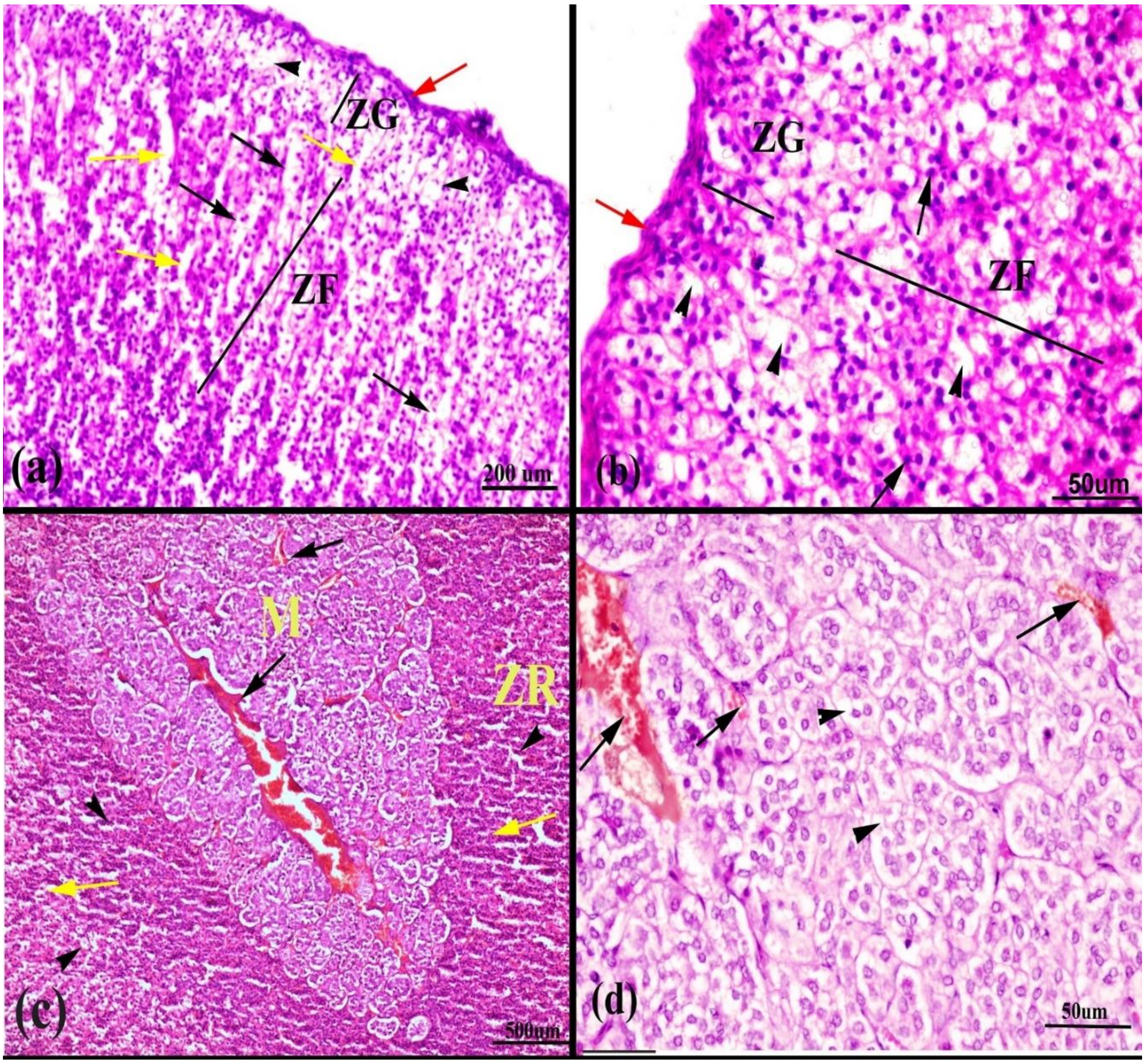

Fig. 2: Photomicrographs obtained from sections of the adrenal gland from rats fed HFD showing: a,b) degenerated capsule (red arrow), vacuolated cells (arrows head) of both Zona glomerulosa (ZG) and fasciculata (ZF), darkly stained nuclei (black arrows) and dilated blood sinusoids (yellow arrows); c) degenerated cells of zona reticularis (ZR) with hyalinized cytoplasm (arrows head) and dilated blood sinusoids (yellow arrows) and congested blood vessels (black arrows) in the adrenal medulla (M); d) vacuolated eosinophilic chromaffin cells (arrows head) and congested blood sinusoids (arrows).

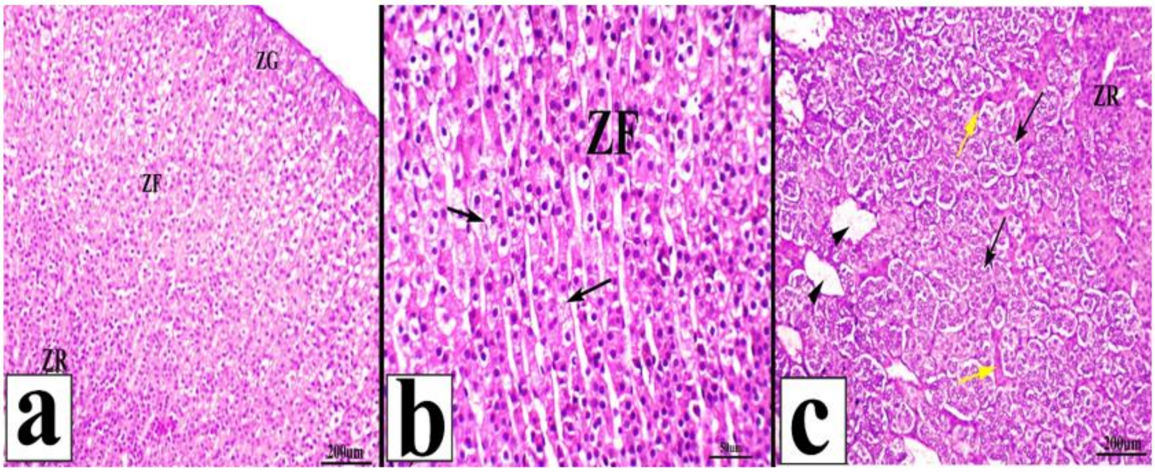

Fig.3: Photomicrographs obtained from sections of the adrenal gland from rats fed HFD+cinnamon showing a) nearly normal structure of adrenal cortical layers; Zona granulosa (ZG), fasciculata (ZF) and reticularis (ZR); b) approximately normal cells of ZF with normal vacuoles (arrows); c) normal cells of zona reticularis (ZR) and normal chromaffin cells (arrows), mild dilation (arrowheads) and mild congestion in sinusoids (yellow arrow) still appeared.

Ultrastructure Results:

Ultrathin sections of control and *C. zeylanicum* groups showed the typical appearance of the cells in all the cortical layers. Each cell of the zona glomerulosa possesses a spherical nucleus bounded by a typical nuclear envelope and contains prominent nucleoli. The cytoplasm is full of free ribosomes. Also, smooth ER (sER) and rounded mitochondria appeared. In addition, the Golgi complex with its cisternae and the associated vesicles are noticed. Moreover, a few numbers of lipid droplets were seen. The membranes of adjacent cells are connected and form gap junctions (Fig. 4a).

The most prominent ultrastructural characteristic of the zona fasciculata cells is the numerous lipid droplets, which may fill the cell. The mitochondria of these cells are distinctive, and more rounded in shape with closely packed vesicular cristae than those of the zona glomerulosa. Also, ribosomes and sER appeared in the cytoplasm. Their nuclei are spherical in shape with prominent nucleoli (Fig. 4b). Cells of zona reticularis revealed the presence of mitochondria, sER, and fewer lipid vacuoles. Their euchromatic nuclei are rounded and enclosed by nuclear envelopes. The cells of this layer were tightly connected through a junction complex (desmosome) (Fig. 4c).

On the other hand, ultra cytological examination of adrenal cortical cells from the HFD-fed group showed massive abnormalities. Thickening and irregularity in the adrenal capsule were noticed. Some cells of Zona glomerulosa appeared with irregularly shrunk nuclei filled with electron-dense material that appeared pyknotic. Mitochondria have different sizes, and most of them appear swollen with partial or complete degenerated cristae. Also, the lipid droplet accumulation was more obvious and the autophagosomes appeared. The gap junctions between cells were lost as well as dilated blood sinusoids appeared (Fig. 5a&b).

Moreover, cells of ZF showed nuclei with condensation in the chromatin materials (pyknotic nuclei) and invagination in the nuclear envelope. Numerous lipid droplets with variable shapes and sizes, and some mitochondria appeared swollen with complete or partial degenerated cristae. In addition, blood sinusoids were dilated, and its lining endothelial cell degenerated with an irregular flattened nucleus. In addition, a marked loss of junction between adjacent cells was detected (Fig. 5c-e). Like other adrenal cortical cells, cells of ZR from rats fed HFD showed pyknotic nuclei with enlargement in the perinuclear space, polymorphic mitochondria, dilated sER cisternae, and lysosomes were prominent in the cytoplasm. Moreover, the gap junction between cells was lost (Fig. 5f).

Examined adrenal cortical cells of HFD + cinnamon-fed rats revealed a nearly normal structure of most cells, where the nuclei appeared normal and regular, mitochondria were in normal shape and size and the cells appeared adjacent to each other. Moreover, the lipid droplets distribution and content were normal (Fig. 6a-d).

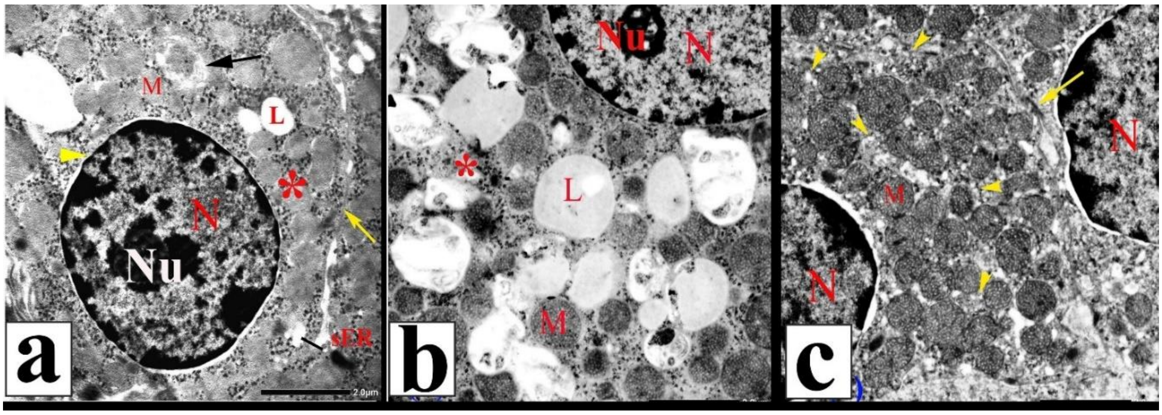

Fig. 4: Electron micrographs obtained from ultrathin sections of adrenal cortex from control rats showing: a) cell from ZG with normal nucleus (N), nucleolus (Nu), typical nuclear envelope (arrowhead), small lipid droplet (L), mitochondria (M), ribosomes (*), smooth endoplasmic reticulum (sER), Golgi complex (black arrow) and gap junction (yellow arrow); b) cell from ZF showed spherical nuclei (N), nucleolus (Nu), lipid droplets (L), mitochondria (M), and ribosomes (*); c) ZR cells containing normal nuclei (N), smooth endoplasmic reticulum (arrows head), mitochondria (M), and desmosome (arrow).

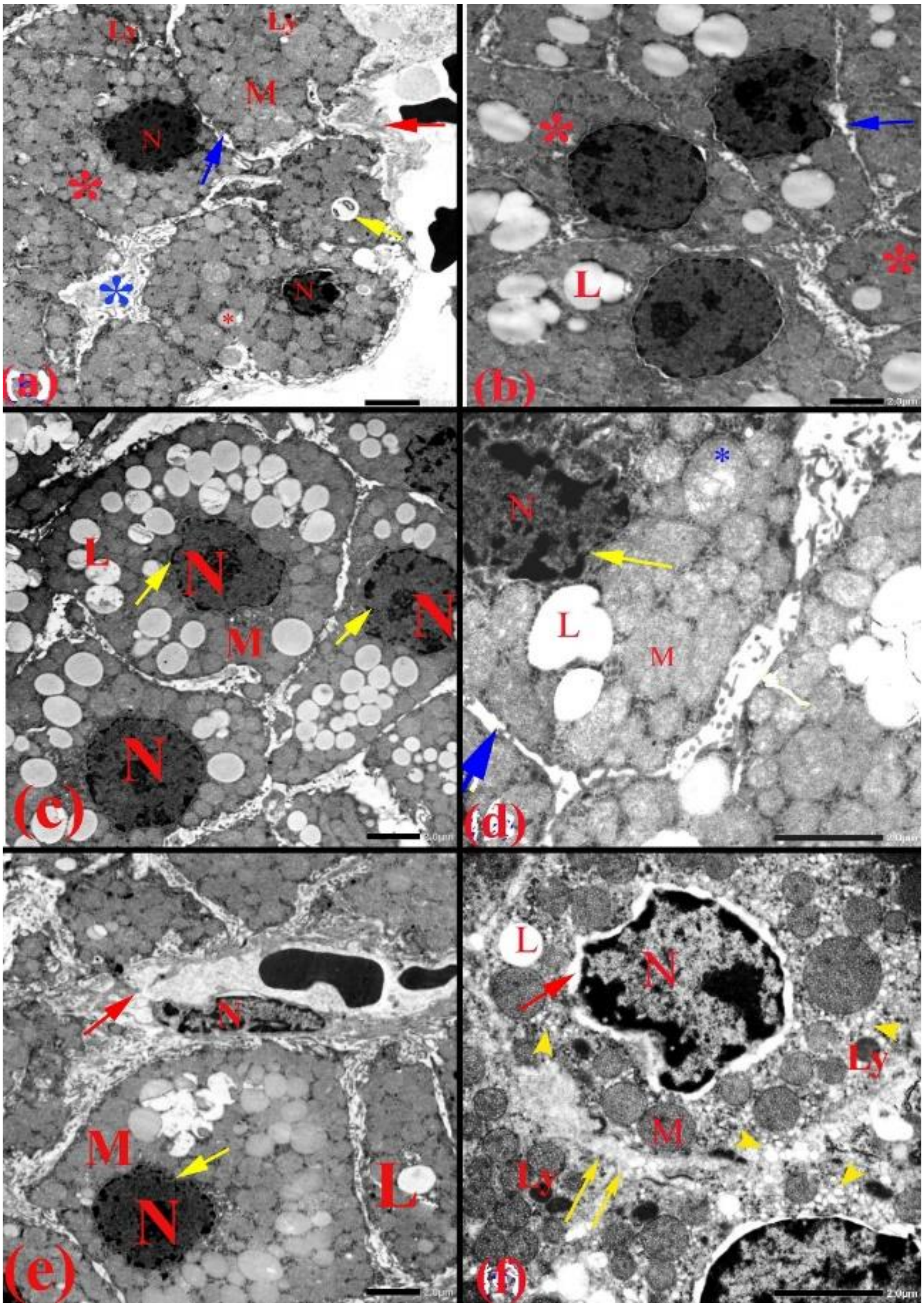

Fig. 5: Electron micrographs obtained from ultrathin sections of the adrenal gland from HFD-fed rats showing: a,b) thick and irregular adrenal capsule (red arrow), cells from ZG had pyknotic nuclei (N), swollen mitochondrion (red stars), a large number of lipid droplets (L), lysosomes (Ly), autophagosomes (yellow arrow), loss of gap junction (blue arrow) between cells and dilation in blood sinusoids (blue star); c-e) Cells from ZF showed pyknotic nuclei (N), irregular nuclear envelope (yellow arrows), lipid droplets (L), swollen and degenerated mitochondria (M), dilated blood sinusoids (red arrow), degenerated endothelial cell with an irregular flattened nucleus (N) and loss of junction complex (blue arrow); f) Cells from ZR with pyknotic nuclei (N) with enlargement in perinuclear space (red arrow), polymorphic mitochondria (M), dilated smooth endoplasmic reticulum (arrows head), prominent lysosomes (Ly) and loss of gap junction (yellow arrows).

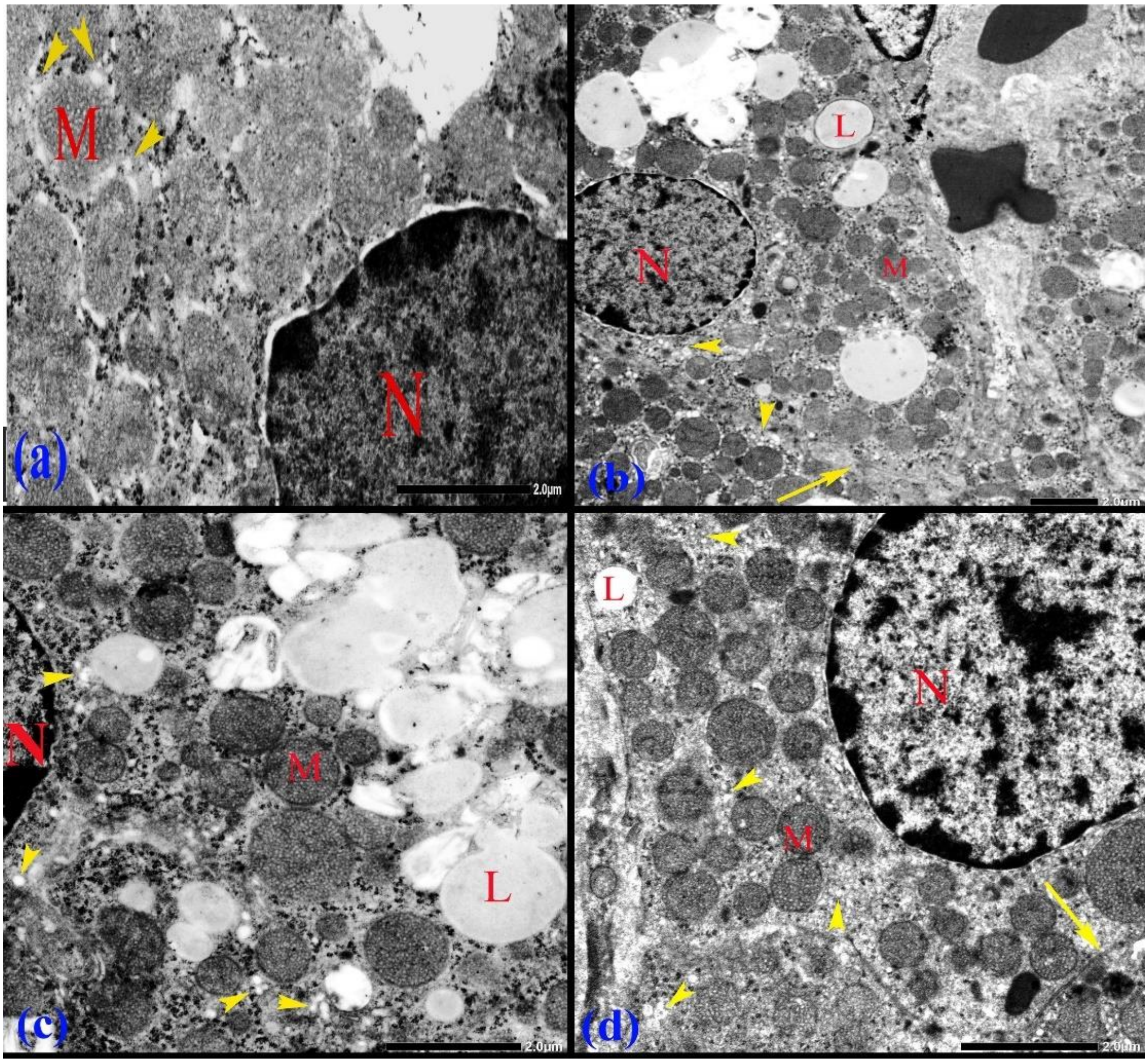

Fig. 6: Electron micrographs obtained from ultrathin sections of the adrenal gland from HFD+ cinnamon-fed rats showing: a) ZG; b), c) ZF, d) ZR with normal nucleus (N), mitochondria (M), smooth endoplasmic reticulum (arrows head), lipid droplets (L), and gap junction (arrows).

Immunohistochemical Results:

Adrenal cortical cells of control and *C. zeylanicum*-treated groups showed weak cytoplasmic immune expression of cytochrome c in a few cells of ZG, ZF, and ZR (Fig. 7a-c). On the contrary, the HFD group exhibited a positive cytoplasmic reactivity for cytochrome c in a significant number of cells of ZG, ZF, and ZR (Fig. 7d-e). The optical density of cytochrome c expression, in the same group, was significantly elevated when compared with the control group (Table 1). Examined adrenal cortical sections of rats fed HFD and *C. zeylanicum* showed moderate positive reactivity for cytochrome c in a moderate number of adrenal cortical cells when compared with the HFD group (Fig. 7f). In addition, the brown color intensity in the cytoplasm of this group was significantly lowered compared with the HFD-fed group (Table 1).

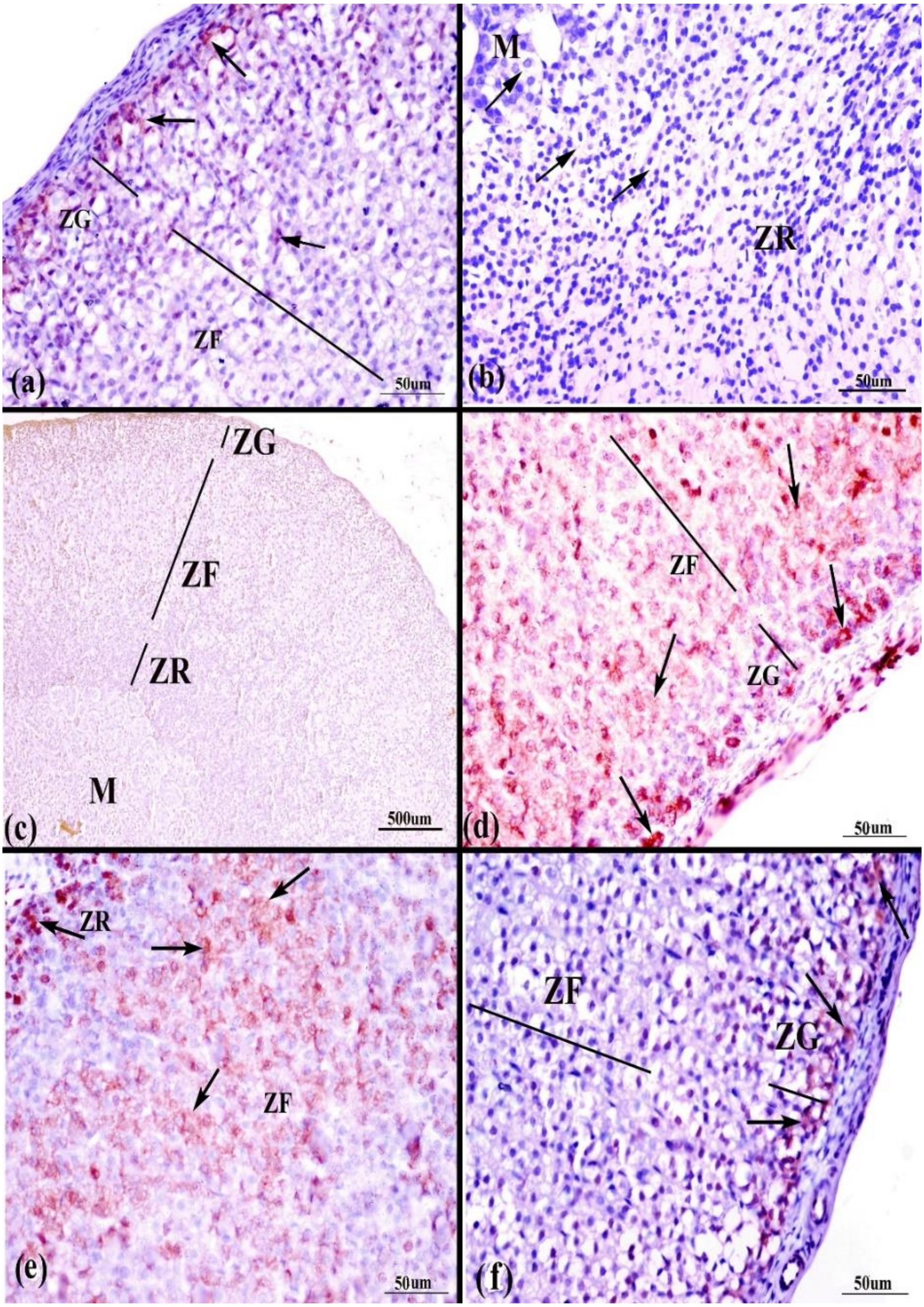

Fig. 7: Photomicrographs obtained from sections of cortical cells of the adrenal: a,b) control group, c) cinnamon group showing: normal presence of cytochrome c in some scattered adrenal cortical cells (arrows) in Zona glomerulosa (ZG), fasciculate (ZF) and reticularies (ZR); d,e) HFD-treated rats showing: expression of cytochrome c in large number of cells (arrows) in Zona glomerulosa (ZG), fasciculate (ZF) and reticularies (ZR); f) rats fed HFD+ cinnamon showing: expression of cytochrome c in a moderate number of cells (arrows) of Zona glomerulosa (ZG), fasciculate (ZF) and reticularies (ZR) (arrows).

Table 1. Cytochrome c expression in cells of the adrenal cortex in the studied groups.

Groups	Cytochrome c expression in the adrenal cortex (Mean±SE)
Control	1.86 ± 0.011
Cinnamon	1.02 ± 0.012
HFD	14.47 ± 0.030*
HFD + cinnamon	8.02 ± 0.027**

(n=6)
* Significant when *P* value is less than 0.05 against the control group.
** Significant when the *P* value is less than 0.05 against the HFD group.

DISCUSSION

Previous studies by Mohammed and Abdel Fattah (2018) and Medjerab *et al.* (2019) confirmed that rearing experimental animals on a HFD produced obesity and elevated the weight of the adrenal gland. Liu *et al*. (2020) reported a significant volume increase in obese patients' adrenal glands. Data obtained in our study revealed histological modifications in rats' adrenal glands of the HFD group compared to the control group. These changes include cytoplasmic vacuolization in cortical ZG, ZF, and medullary parenchymal cells, dilated sinusoids, and congested blood capillaries of the medulla. Besides, there were ultrastructural changes in cellular organelles of this group that include irregularity of the nucleus, swelling, and degeneration of mitochondria, sER with dilated cisternae, numerous lipid droplets with variable sizes, and loss of junction between adjacent cells. The histological and ultrastructural alterations reported in the current studies may be attributed to defects in both lipid metabolism and in the cytochrome enzymes' family, which are located in the mitochondria and sER.

Likewise, Diaz-Aguila *et al.* (2016) found that the sucrose diet induced modification in the adrenal gland tissue. The accumulation of lipids in different cortical cells, reported in the current work, was similar to Topal *et al*. (2019) who found that HFD raises the level of fat accumulation in the adrenal gland. Parallel to our findings, Melmed *et al*. (2015) and Khalaf *et al*. (2017); Abdel-Hamid (2018) retained the impairment in the corticosteroid hormones synthesis, excessive accumulation of cholesterol in cytoplasmic vacuoles, and mitochondrial degeneration to a defect in the cytochrome P450 enzyme. Similar histological and ultrastructure findings were previously reported by Medjerab *et al.* (2019) and Soliman and Noya (2020) who attributed these alterations to the elevated levels of circulating energy substrate which produced a defect in the mitochondrial function that led to disorder in lipid metabolism and lipotoxicity in adrenal cortical cells.

The observed thickening (appeared as plenty of collagen fibers formation) of the adrenal capsule, which appeared in the current results, may be attributed to the increase in collagen biosynthesis. Bouguerra *et al*. (2004) reported that there is a significant increase in total protein synthesis, particularly collagen, as a result of high glucose concentration-induced obesity in rats.

Our results showed that HFD caused congestion and dilation in the blood vessels and sinusoids. This may be due to oxidative stress caused by HFD. Kumar *et al*. (2015) and Abdel-Hamid *et al*. (2015) reported that blood vessel vasodilation happened due to a rise in the reactive oxygen species (nitric oxide) that is responsible for the deterioration of capillary endothelial as it caused vasodilation.

Moreover, the nuclei of the adrenal cortical cells appeared darkly stained (pyknotic) which could be linked to cellular oxidative stress. According to Blokhina *et al*. (2003), oxidative stress causes alteration in the molecular organization and the selective permeability of the cell membranes and organelles like mitochondria and the endoplasmic reticulum. Moreover, the generation of reactive oxygen compounds may have an impact on lysosomes and cellular DNA, increasing the opportunity for cell damage brought on by hazardous materials and ultimately leading to cellular death (Niedowicz and Daleke, 2005). In the current study, a strong positive reaction for cytochrome c was detected in the cytoplasm of adrenal gland cells (ZG & ZF) in the HFD-treated group against the control group. Our immunohistochemical changes are following those of Nežić *et al*. (2020) who reported a marked expression of cytochrome c in the cytoplasm of the renal cells. In this regard, Mantawy *et al.* (2017) demonstrated that the existence of free radicals caused damage to the membrane of the mitochondria so cytochrome c release into the cytoplasm. Bouteraa *et al*. (2020) added that mitochondrial swelling– as noticed in this study – happened due to modification in the permeability of the mitochondrial membrane so compounds diffuse into the mitochondria through osmosis leading to damage of the mitochondrial membrane and cytochrome c libration.

The present study indicated that the administration of *C. zeylanicum* with HFD ameliorated most of the adrenal gland histological, ultrastructural, and immunohistochemical changes detected in HFD-fed could be explained by the active components found in C. zeylanicum.

Kassaee *et al*. (2017) found that *C. zeylanicum* constituents like fibers, phenols, and aldehydes could be responsible for the detected improvement in the adrenal glands as they minimize intestinal absorption of dietary lipids. While the gastric empty rate significantly increased after cinnamaldehyde was given to mice (Camacho *et al*., 2015). Moreover, oral cinnamon extract therapy enhanced the severe degenerative and inflammatory changes and decreased the elevated levels of tissue malondialdehyde, and tumor necrosis factor-α induced by cecal slurry in Zona glomerulosa and Zona fasciculata (El-Safty *et al*., 2023).

Various extracts made from cinnamon prove its efficiency as a powerful antioxidant, because of the phenolic compounds present in it (Rahman *et al*., 2013 and Mousavi *et al*., 2020). In this consideration, Tuzcu *et al*. (2017), and Mohammed and Abdel Fattah (2018) reported that cinnamon has antioxidant and anti-lipidemic properties through the modulation of transcription factors including SREBP-1c, LXRs, NF-κB, and Nrf2 and several enzymes such as ACLY and FAS and insulin resistance, glucose, and lipid metabolism and antioxidant status.

The improvement observed in rats after feeding HFD+ cinnamon was confirmed by Sheng *et al*. (2008) who found that cinnamon water extract caused a significant elevation not only in the PPARs α and PPARγ levels (aid in regulation of lipid metabolism and energy homeostasis) but also in their target genes in diet-induced obesity mice models.

Medjerab *et al*. (2019) and Mousavi *et al*. (2020) attributed the reduction of lipid accumulation, after treating rats with HFD and cinnamate, to the reduction in hepatic hydroxy-3-methyl glutaryl CoA (HMG-GoA) reductase activity, which in turn decreased hepatic cholesterol content and peroxidation. The reduction in HMG-GoA reductase activity is due to antioxidant activity promotion by cinnamate. Also, Ranasinghe *et al*. (2013) and Elgendy *et al.* (2016) illustrated that eugenol and cinnamon bark oil showed potent antioxidant activity in inhibiting peroxynitrite-induced lipid peroxidation.

Concerning cinnamon's antioxidant properties, Long *et al*. (2015) reported that cinnamaldehyde activates nuclear factor erythroid 2- related factor protein, which is the main modulator of the antioxidant enzymes within cells. This activation stimulates the release of antioxidant enzymes to protect the cell against oxidative damage. Moreover, Qabaha *et al*. (2017) found that cinnamon and its cinnamic acid reduce the generation of cytokines that promote inflammation. Schink *et al.* (2018) and Ozhan *et al*. (2023) determined that trans-cinnamaldehyde and p-cymene compounds in cinnamon have strong anti-inflammatory effects that reduce interleukin-8 secretion and decrease the release of inducible nitric oxide and mitogen-activated protein kinase.

CONCLUSIONS

According to this study, *C. zeylanicum* is an effective pharmaceutical plant with numerous therapeutic activities in decreasing inflammation, improving the antioxidant enzymes' activity (antioxidant), and decreasing the accumulation of lipids (lipolytic effects). As a result, it could have a promising role in minimizing adrenal toxicity induced by HFD. To verify our findings and pinpoint, the pharmaceutical activities of the chemical components in *C. zeylanicum* that are responsible for the herb's beneficial effects must be done and more research should be carried out.

Declarations:

Ethical Approval: The overall design of the experiment was done based on the bioethics of the Animal Care and Use Committee (Approved No. MUF/F/HI/4/23).

Conflicts of Interest: The authors claim that there are no conflicts of interest.

Authors Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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REFERENCES

Abd-Elhafeez, H. H.; Soliman, S. A. (2017) New description of telocyte sheaths in the bovine uterine tube: An immunohistochemical and scanning microscopic study. *Cells Tissues Organs,* 203(5):295-315.

Abd-Elhafeez, H. H.; Soliman, S. A.; Attaai, A. H.; Abdel-Hakeem, S. S.; El-Sayed, A. M. and Abou-Elhamd, A. S. (2021). Endocrine, stemness, proliferative, and proteolytic properties of alarm cells in ruby-red-fin shark (rainbow shark), Epalzeorhynchos frenatum (Teleostei: Cyprinidae). *Microscopy and Microanalysis,* 27(5):1251-1264.

Abdel-Hamid, G. A. (2018). Ameliorative effect of vitamin C on nicotine-induced histological and ultrastructural changes in zona fasciculata in albino rats. *MOJ Anatomy & Physiology,* 5(2): 120-125.

Abdel-Hamid, G. A.; Abdel-Aal, I. H. and El-Fark, M. M. (2015). Effect of green tea on the ultrastructure of the zona glomerulosa of the adrenal cortex in diabetic rats. *The Egyptian Journal of Histology, 38*(4): 704-712.

Armani, A.; Mammi, C.; Marzolla, V.; Calanchini, M.; Antelmi, A.; Rosano, G. M.; Fabbri, A. and Caprio, M. (2010). Cellular models for understanding adipogenesis, adipose dysfunction, and obesity. *Journal of cellular biochemistry,* 110(3):564-572.

Blokhina, O.; Virolainen, E.; and Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of botany,* 91(2): 179-194.

Bouguerra, S. A.; Benazzoug, Y.; Bekkhoucha, F.; Bourdillon, M. C. (2004). Effect of high glucose concentration on collagen synthesis and cholesterol level in the phenotypic modulation of aortic cultured smooth muscle cells of sand rat (Psammomys obesus). *Journal of Diabetes Research,* 5: 227-235.

Bouteraa, Z.; Rouabhi, R.; Menaceur, F. and Gasmi, S. (2020). Cellular Apoptosis, Mitochondrial Swelling, Permeability and Cytochrome-C Level After (Feo)-Nps Nanoparticles Exposure and Protective Role of Diferuloylmethane in Rats Liver. *Acta Scientifica Naturalis,* 7(1): 140-154.

Bray, G. A. (2004). Medical consequences of obesity. *The Journal of Clinical Endocrinology & Metabolism,* 89(6):2583-2589.

Camacho, S.; Michlig, S.; de Senarclens-Bezençon, C.; Meylan, J.; Meystre, J.; Pezzoli, M.; Markram, H. and Le Coutre, J. (2015). Anti-obesity and anti-hyperglycemic effects of cinnamaldehyde via altered ghrelin secretion and functional impact on food intake and gastric emptying. *Scientific reports*, 5(1):7919-7929.

Caroline, O. B.; Ebuehi, O. A.; Cecilia, O. A. and Kayode, O. A. (2021). Effect of Allium sativum extract in combination with orlistat on insulin resistance and disrupted metabolic hormones in high-fat diet-induced obese rats. *Scientific African,* 2021;14:e00994 . doi.org/10.1016/j.sciaf.2021.

Chakraborty, T. R.; Donthireddy, L.; Adhikary, D. and Chakraborty, S. (2016). Long-term high-fat diet has a profound effect on body weight, hormone levels, and estrous cycle in mice. Medical science monitor: *International Medical Journal Of Experimental And Clinical Research,* 22: 1601-1608.

Díaz-Aguila, Y.; Castelán, F.; Cuevas, E.; Zambrano, E.; Martínez-Gómez, M.; Muñoz, A.; Rodríguez-Antolín, J. and Nicolás-Toledo, L. (2016). Consumption of sucrose from infancy increases the visceral fat accumulation, concentration of triglycerides, insulin and leptin, and generates abnormalities in the adrenal gland. *Anatomical science international,* 91(2):151-162.

Eidi, A.; Mortazavi, P.; Bazargan, M. and Zaringhalam, J. (2012). Hepatoprotective activity of cinnamon ethanolic extract against CCI4-induced liver injury in rats. *Excli Journal,* 11:495-507.

Elgendy, E. M.; Ibrahim, H. S.; Elmeherry, H. F.; Sedki, A. G. and Mekhemer, F. U. (2016). Chemical and biological comparative in vitro studies of cinnamon bark and lemon peel essential oils. *Food and Nutrition Sciences,* 8(1): 110-125.

El-Safty, F.; Issa, N. and Al-Gholam, M. (2023). The effect of cinnamon extract upon experimentally induced sepsis on the adrenal cortex of adult male albino rats. *Egyptian Journal of Histology.* doi: 10.21608/ejh.2023.197425.1865.

Hussain, Z.; Khan, J. A.; Arshad, A.; Asif, P.; Rashid, H. and Arshad, M. I. (2019). Protective effects of Cinnamomum zeylanicum L. (Darchini) in acetaminophen-induced oxidative stress, hepatotoxicity and nephrotoxicity in mouse model. *Biomedicine & Pharmacotherapy,* 109: 2285-2292.

Iglesias, P. and Díez, J. J. (2010). Adipose tissue in renal disease: clinical significance and prognostic implications. Nephrology Dialysis Transplantation, 25(7):2066-2077.

Jeong, W. I.; Jeong, D. H.; Kim, Y. K.; Park, H. Y.; Kwon, O. D.; Kim, T. H. and Jeong, K. S. (2005). Mild hepatic fibrosis in cholesterol and sodium cholate diet-fed rats. *Journal of veterinary medical science,* 67(3): 235-242.

Kargi, A. Y. and Iacobellis, G. (2014). Adipose tissue and adrenal glands: novel pathophysiological mechanisms and clinical applications. *International Journal of Endocrinology, 2014*. doi: org/10.1155/2014/614074.

Kassaee, S. M.; Goodarzi, M. T.; Roodbari, N. H.and Yaghmaei, P. (2017). The effects of Cinnamomum zeylanicum on lipid profiles and histology via up-regulation of LDL receptor gene expression in hamsters fed a high cholesterol diet. J*undishapur Journal of Natural Pharmaceutical Products,* 12(3):e37340.doi:org/10.5812/ jjnpp. 37340.

Khaki, A. (2015). Effect of Cinnamomum zeylanicumon on Spermatogenesis. *Iranian Red Crescent Medical Journal, 17*(2):e18668. doi: 10.5812/ircmj.18668.

Khalaf, H. A.; Ghoneim, F. M.; Arafat, E. A.; Mahmoud, E. H. M. (2017). Histological effect of nicotine on adrenal zona fasciculata and the effect of grape seed extract with or without withdrawal of nicotine. *Journal of microscopy and ultrastructure,* 5(3): 123-131.

Kleine, B. and Rossmanith, W. G. (2016). Hormones and the endocrine system. Cham: Springer International Publishing.

Kumar, V.; Abbas, A. K. and Aster, J. C. (2015). Robbins basic pathology". 9th edition. Philadelphia. Elsevier Saunders Inc.

Kwon, H. K.; Hwang, J. S.; Lee, C. G.; So, J. S.; Sahoo, A.; Im, C. R.; Jeon, W. K.; Ko, B. S.; Lee, S. H.; Park, Z. Y. and Im, S. H. (2011). Cinnamon extract suppresses experimental colitis through modulation of antigen-presenting cells. *World journal of gastroenterology*, 17(8):976-986.

Lillie, R. and Fulmer, H. (1976) Histopathologic Technic and Practical Histochemistry. 4th ed, McGraw-Hill, New York, ©1976.

Liu, F.; Chen, Y.; Xie, W.; Liu, C.; Zhu, Y.; Tian, H.; Ren, Y. and Chen, T. (2020). Obesity might persistently increase adrenal gland volume: a preliminary study. *Obesity Surgery*, 30:3503-3507.

Long, M.; Tao, S.; Rojo de la Vega, M.; Jiang, T.; Wen, Q.; Park, S.L.; Zhang, D. D. and Wondrak, G. T. (2015). Nrf2-dependent suppression of azoxymethane/dextran sulfate sodium–induced colon carcinogenesis by the cinnamon-derived dietary factor cinnamaldehyde. *Cancer Prevention Research*, 8(5):444-454.

Mantawy, E. M.; Esmat, A.; El-Bakly, W. M.; Salah ElDin, R. A. and El-Demerdash, E. (2017). Mechanistic clues to the protective effect of chrysin against doxorubicin-induced cardiomyopathy: Plausible roles of p53, MAPK and AKT pathways. *Scientific reports,* 7(1): 4795. doi: 10.1038/s41598-017-05005-9.

Medjerab, M.; Abdelali, M.; Khalkhal, A.; Semiane, N.; Hammadi, S.; Mallek, A.; Bellahrache, Z.; Agoun, H. and Dahmani, Y. (2019). Adrenal cortex disorders in a new model of obesity, Gerbillus gerbillus, exposed to a high carbohydrate diet. *Comptes Rendus Biologies*, 342(1-2):35-44.

Megha, R.; Wehrle, C. J.; Kashyap, S. and Leslie, S. W. (2022). Anatomy, Abdomen and Pelvis: Adrenal Glands (Suprarenal Glands). In StatPearls [Internet]. StatPearls Publishing.

Melmed, S.; Polonsky, K. S.; Larsen, P. R. and Kronenberg, H. M. (2015). Williams textbook of endocrinology E-Book. Elsevier Health Sciences.

Mohamed S. S. H.; Hansi Priscilla, D. and Thirumurugan, K. (2011). Cinnamon extract inhibits α-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. *Nutrition & metabolism*, 8 (1): 1-11.

Mohammed, H. A. and Abdel Fattah, D. M. (2018). Hypolipidemic and hypoglycemic effect of cinnamon extract in high fat diet fed rats. *Zagazig Veterinary Journal,* 46(2):160-167.

Mousavi, S. M.; Rahmani, J.; Kord-Varkaneh, H.; Sheikhi, A.; Larijani, B. and Esmaillzadeh, A. (2020). Cinnamon supplementation positively affects obesity: A systematic review and dose-response meta-analysis of randomized controlled trials. *Clinical Nutrition,* 39(1):23-133.

Navarrete, J.; Vásquez, B.; Vasconcellos, A.; del Sol, M.; Olave, E. and Sandoval, C. (2018). Effects of High-fat Diets on Biochemical Profiles and Morpho-Quantitative Characteristics of C57BL/6 Mice Adrenal Glands. *International Journal of Morphology,* 36(2):722-729.

Nežić, L.; Škrbić, R.; Amidžić, L.; Gajanin, R.; Milovanović, Z.; Nepovimova, E.; Kuča, K. and Jaćević, V. (2020). Protective effects of simvastatin on endotoxin-induced acute kidney injury through activation of tubular epithelial cells' survival and hindering cytochrome C-mediated apoptosis. *International Journal of Molecular Sciences,* 21(19):7236. doi: 10.3390/ijms21197236.

Niedowicz, D. M. and Daleke, D. L. (2005). The role of oxidative stress in diabetic complications. *Cell biochemistry and biophysics,* 43(2):289-330.

Ozhan, O.; Izci, S. F.; Huz, M.; Colak, M.; Kucukakcali, Z. and Parlakpinar, H. (2023). Therapeutic effects of cinnamon bark oil on sciatic nerve injury in rats. *European Review for Medical & Pharmacological Sciences*, 27(12):5841-5853.

Pracheta, P.; Sharma, V.; Paliwal, R.; Sharma, S.; Singh, L.; Janmeda, B.S.; Savita Yadav, S.; Sharma, S.H. (2011). Chemoprotective activity of hydro-ethanolic extract of Euphorbia nerrifolia Linn leaves against DENA-induced liver carcinogenesis in mice. *Boilogical Medecine,* 3 (2): 36-44.

Qabaha, K.; Abu-Lafi, S. and Al-Rimawi, F. (2017). Anti-inflammatory Activities of Ethanolic Extracts of curcuma Longa (Turmeric) and cinnamon (Cinnamomum verum). *Journal of Food and Nutrition Research,* 5: 668-673.

Rahman, S.; Begum, H.; Rahman, Z.; Ara, F.; Iqbal, M. J. and Yousuf, A. K. M. (2013). Effect of cinnamon (Cinnamomum cassia) as a lipid lowering agent on hypercholesterolemic rats. *Journal of Enam Medical College,* 3(2): 94-98.

Ranasinghe, P.; Pigera, S., Premakumara, G. S.; Galappaththy, P.; Constantine, G. R. and Katulanda, P. (2013). Medicinal properties of 'true'cinnamon (Cinnamomum zeylanicum): a systematic review. *BMC complementary and alternative medicine,* 13 (275):1-10.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *The Journal of cell biology,* 17(1): 208-212.

Schink, A.; Naumoska, K.; Kitanovski, Z.; Kampf, C. J.; Fröhlich-Nowoisky, J.; Thines, E.; Pöschl, U.; Schuppan, D. and Lucas, K. (2018). Anti-inflammatory effects of cinnamon extract and identification of active compounds influencing the TLR2 and TLR4 signaling pathways. *Food & function,* 9(11):5950-5964.

Sheng, X.; Zhang, Y.; Gong, Z.; Huang, C. and Zang, Y. Q. (2008). Improved insulin resistance and lipid metabolism by cinnamon extract through activation of peroxisome proliferator-activated receptors. *PPAR research,* 2008: 581348. doi: 10.1155/2008/581348

Soliman, M. A. and Noya, D. A. (2020). The possible ameliorating effect of barley grain on the histological structure of the adrenal cortex in streptozotocin (STZ)-induced diabetes in adult male albino rats. *Egyptian Journal of Histology,* 43(2): 555-568.

Than, W. H.; Chan, G. C. K.; Ng, J. K. C. and Szeto, C. C. (2020). The role of obesity on chronic kidney disease development, progression, and cardiovascular complications. *Advances in Biomarker Sciences and Technology,* 2: 24-34.

Topal, F. A. T. M. A.; Goren, H.; Yucel, F. E. R. R. U. H.; ŞAHİNTÜRK, V. and Aydar, Y. Ü. K. S. E. L. (2019). Effect of consuming high-fat diet on the morphological parameters of adrenal gland. *Bratislava medical journal-bratislavske lekarske listy,* 120(8): 593-600.

Tung, Y.T.; Yen, P.L.; Lin, C.Y.; Chang, S.T. (2010). Anti-inflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (Cinnamomum osmophloeum) leaves. Pharmaceutical Biology, 48 (10):1130–1136. In: Waly MI, Rahman MS editors. Bioactive Components, Diet and Medical Treatment in Cancer Prevention. Cinnamon as a Cancer Therapeutic Agent. Springer international publishing AG, part of Springer Nature. Midtown Manhattan, New York, United States; 2018. p 65.

Tuzcu, Z.; Orhan, C.; Sahin, N.; Juturu, V. and Sahin, K. (2017). Cinnamon polyphenol extract inhibits hyperlipidemia and inflammation by modulation of transcription factors in high-fat diet-fed rats. Oxidative medicine and cellular longevity, 2017. doi: org/10.1155/2017/1583098.

Vinitha, M. and Ballal, M. (2008). In vitro anticandidal activity of Cinnamomum verum. *Journal of Medical Sciences,* 8 (4): 425–428.

WHO 2021. obesity and overweight fact sheet. https://www.who.int/news-room/fact-

sheets/detail/obesity-andoverweight Obesity and overweight 9 June 2021.
WHO 2022. Obesity. World Health Organization. https://www.who.int/health-topics/obesity#tab=tab_1. Accessed 12/20/2022.
Wree, A.; Kahraman, A.; Gerken, G. and Canbay, A. (2010). Obesity affects the liver–the link between adipocytes and hepatocytes. *Digestion,* 83(1-2): 124-133.

ARABIC SUMMARY

التأثير التحسيني للقرفة ضد فرط كوليستيرول الدم الناجم عن اتباع نظام غذائي غني بالدهون في الغدة الكظرية لدى ذكور الفئران البيضاء البالغة. الدراسات النسيجية والكيميائية المناعية والبنية التحتية

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تعدالسمنه من المشاكل الصحيه التي تثير قلقا في جميع انحاء العالم بين الفئات العمريه المختلفه. تؤدي السمنه إلى العديد من الامراض مثل ارتفاع ضغط الدم، السكر، امراض القلب، وهشاشة العظام، والعقم، وبعض انواع السرطانات. يعتبر نبات القرفه من النباتات الطبية التي لها دور فعال في علاج الكثيرمن الامراض حيث أنها تحتوي على مضادات الإلتهابات ومضادات الأكسدة ولذلك هدفت هذه الدراسة إلى معرفة الدور التحسني المحتمل للقرفة في تسمم الغده الكظريه الناجم عن اتباع نظام غذائي غني بالدهون في الجرذان البيضاء. تم استخدام أربع مجموعات من الجرذان: المجموعة الأولى : المجموعة الضابطة، المجموعة الثانيه: تم تغذيتها لمدة ثمان أسابيع على النظام الغذائي القياسي مضافًا اليه القرفة (15٪ وزن / وزن)، مجموعة الثالثة: تم تغذيتها على نظام غذائي عالي الدهون لمدة ثمان أسابيع لإحداث فرط كوليسترول. المجموعة الرابعة: تم تغذيتها لمدة ثمان أسابيع علي نظام غذائي عالي من الدهون مضافا اليه القرفه (15٪ وزن / وزن). في نهاية التجربة تم أخذ الغده الكظريه وتجهيزها للدراسات النسيجية والتركيبية الدقيقة والكيميائية النسيجية المناعية.أظهرت الحيوانات التي تم تغذيتها بالقرفة نتائج مماثلة للمجموعة الضابطة. بينما أظهرت الجرذان التي تم تغذيتها على نظام غذائي عالي من الدهون تغيرات باثولوجيه في كلا من الكبسوله وجميع طبقات الغدة الكظرية. شملت التغيرات النسيجيه التحلليه ضهورالعديد من الفجوات السيتوبلازمية ونخر في الانويه واتساع واحتقان في الجيوب الدمويه الموجوده بين الخلايا. كما أظهر فحص العينات بالميكروسكوب الإلكترونى النافذ العديد من التغيرات الخلويه لخلايا القشرة الكظرية حيث ظهر تضخم في الميتوكوندريا و الشبكه الاندوبلازميه الخشنه والعديد من قطرات الدهون ذات الأشكال والأحجام المختلفه وكما كان هناك عدم انتظام في الغشاء النووي. كما ظهرت الخلايا الطلائيه المبطنه للجيوب الدمويه متحلله وانويتها غير منتظمه. وعلاوة على ذلك، ظهرالتعبير الكيميائي النسيجي المناعي ل cytochrome c في عدد كبير من خلايا القشره الكظريه في المجموعه التي تغذت علي نظام غذائي عالي الدهون . بينما أظهرت الحيوانات التي تم تغذيتها على نظام غذائي عالي الدهون ومضافا اليه القرفة تحسنًا واضحًا في التغييرات النسيجية والتركيبية الدقيقه والكيميائية النسيجية المناعية. خلصت هذه الدراسة إلى أن للقرفة دور محسن في علاج تسمم الغده الكظريه المحدث بواسطه نظام غذائي عالي الدهون وذلك بسبب مكوناتها الطبيعية المسؤولة عن دورها كمضادات للأكسدة وكمضادة للإلتهابات.