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Histological and ultrastructural study on the pathogenicity of the fungicide cyproconazole and the probable protective influence of fennel oil extract on the adrenal cortex of adult rats

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ABSTRACT

Cyproconazole (CPZ) is a well-established triazole fungicide derivative applied to protect agricultural crops against various types of fungal diseases. The present investigation aims to assess its harmful influences on the adrenal cortex of adult rats and the probable protective impact of the oil extract of fennel (*Foeniculum vulgare*) against the devastated effects generated by CPZ. Sixty male rats were randomly divided into six experimental groups: the control, fennel oil, CPZ-low dose, CPZ-high dose, fennel oil + CPZ-low dose, and fennel oil + CPZ-high dose treated groups. After 15 days, the adrenal glands from all rats were removed, fixed in the adequate fixative, and processed for histological and ultrastructural examination. The current histopathological results showed that CPZ at the low and high doses significantly induced degenerative changes in the adrenal cortical tissues of the treated rats, as evidenced by reduction in the thickness of the adrenocortical zones; zona glomerulosa, zona fasciculata and zona reticularis, which resulted from the compression of the adrenocortical cells that suffered from cytoplasmic vacuolation and nuclear pyknosis, karyorrhexis and karyolysis, besides, congested and dilated cortical blood vasculatures. The ultrastructural observations showed damaged mitochondria, proliferated smooth endoplasmic reticulum, and increased lysosomes, as well as noticeable aggregation of lipid droplets. While there was an improvement in most of the hazardous impacts induced by CPZ on the histological and fine structural features of the adrenocortical tissues of rats treated with fennel oil extract alongside CPZ. In conclusion, this study evidenced that fennel oil co-treatment has obvious protective role against CPZ-triggered adrenal cortex injury in adult rats owing to its strong antioxidant and anti-inflammatory capacity.

Keywords: Triazole, fungicides, cyproconazole, fennel oil, adrenal gland, Histology, Ultrastructure, rats.

INTRODUCTION

Cyproconazole (CPZ) is one of the most widely used triazole fungicides against diverse types of fungal diseases to treat and protect a wide range of crops including cereals, soybeans, cotton, coffee, sugar beet, and other vegetables and fruit trees (Baybakova *et al*., 2019). Conazoles function by preventing lanosterol 14α-demethylase (CYP51) enzyme, that controls the production of ergosterol, a crucial constituent of the cell membrane of the fungi, causing changes in the permeability of the cell membrane, the efficiency of membrane-bound enzymes, and an increase in the saturation of fatty acids forming the lipid bilayer (Zarn *et al*., 2003). However, conazoles can interact with the cytochrome P450 (CYP450) system in mammals (Juberg *et al*., 2006). As a result, they might disturb the endocrine system by inhibiting the enzymes essential for steroid synthesis.

Triazole fungicides have large environmental impacts and are reinforced throughout the food chain as a result of their chemical persistence (Vryzas, 2018). Many triazole fungicides exhibit high oral bioavailability and have potent capability to cross the blood-brain barrier, and disrupt the hepatic cytochrome efficiency, besides they revealed teratogenic effects, cardiotoxicity, skin sensitivity, and endocrine disruption (Gridan *et al*., 2019). Additionally, triazole fungicides may have an impact on the endocrine system, according to animal research (Kjaerstad *et al*., 2010).

Fennel (*Foeniculum vulgare*) is one of Apiaceae (family Umbelliferaceae) which is an aromatic plant utilized for prolonged time as a food additive and traditional medication (Rather *et al*., 2016). Essential oils are most typically extracted from fennel seeds, even though the entire plant can be utilized for medical purposes (Malhotra, 2012). The primary constituents of the oil extracted from the fennel seeds have been identified as trans-anethole, estragol, phellandrene, and fenchone (Díaz-Maroto *et al*., 2006). Fennel oil has anti-fungal, anti-bacterial, anti-parasitic and anti-inflammatory influences, besides being a strong antioxidant agent (Malhotra, 2012; Goswami & Chatterjee, 2014).

One of the primary endocrine targets for pesticide active ingredients is the adrenal gland (Marx-Stoelting *et al*. 2014) which is crucial for the functioning of the whole endocrine system.,

The current study aimed to examine and evaluate the noxious effects of CPZ on the histological and ultrastructural aspects of the adrenal gland cortex of adult rats, as well as the probable ameliorative impacts of fennel oil extract on such hazardous consequences.

MATERIALS AND METHODS

Materials

CPZ was purchased from Syngenta (Basel, Switzerland) with CAS no. 94361-06-5, Batch no. CHF1E00042, and 96.8% purity. The fennel oil is natural and authentic essential oil with 100% purity that was purchased from SVA, Amazon. Chemicals used in this investigation were of highly analytical grades and purities.

Animal groups and treatment protocol

Sixty mature male *Rattus norvegicus* with an average weight of 200 to 250 g were brought from the confined colony of Theodor Bilharz Research Institute in El-Giza governorate, Egypt. Rats were left for one week before experiment to be adapted to the environmental condition, where they were randomly arranged into six experimental groups of ten rats each and preserved in spotless plastic crates contained wood shavings and fed on ordinary rodent pellets with tap water *ad libitum* under controlled environmental conditions (12-h light/dark period, and 25±2.0°C). They were treated daily for 15 days in the following manner;

Control group: Healthy rats were intraperitoneally (i.p.) given 1 mL DMSO (10%) as the vehicle for CPZ and orally administered 1 mL DMSO (10%) as the vehicle for fennel oil.

Fennel oil-treated group: Rats were orally received 1 mL/kg b.wt. of fennel oil suspended in 10% DMSO. This dosage was established using information from prior rat studies and in accordance with the body weight and surface area of the body (Imbabi *et al*., 2021).

CPZ-low dose-treated group: Animals were i.p. injected with CPZ at a dose equivalent to 20 mg/kg b.wt. (1/50 LD50) which was dissolved in 1 mL of 10% DMSO.

CPZ-high dose-treated group: Rats were i.p. injected with CPZ at a dose equal to 50 mg/kg b.wt. (1/20 LD50) that was dissolved in 1 mL of 10% DMSO.

The used low and high CPZ dosages were selected according to those used in previous studies (Hamdi *et al.*, 2019).

Fennel oil + CPZ-low dose-treated group: Animals were orally administered 1 mL/kg b.wt. of fennel oil suspended in 10% DMSO simultaneously with i.p. injection of CPZ at a dose equal to 20 mg/kg b.wt. dissolved in 1 mL of 10% DMSO.

Fennel oil +CPZ-high dose-treated group: Rats were received 1 mL/kg b.wt. of fennel oil suspended in 10% DMSO orally alongside with 50 mg/kg b.wt. of CPZ dissolved in 1 mL of 10% DMSO via i.p. injection.

At the terminus of the experimental interval, rats from all groups were fasted at night, and in the next morning they were anaesthetized using isoflurane anesthesia. The rats were dissected, and their adrenal glands were separated out from the surrounding adipose tissue and rapidly rinsed with saline solution (0.9% NaCl) and forthwith fixed using the suitable fixative for the histological and ultrastructural investigations.

Histological preparation

Samples of the adrenal glands from all experimental animal groups were fixed in aqueous Bouin's fixative for 24 h before processing using the previously reported procedures for paraffin sectioning (Bancroft & Gamble, 2008). A Panasonic CD-220 camera and BX-40 Olympus compound light microscope were used to analyze and take pictures of Ehrlich's hematoxylin and eosin (H&E) stained sections.

Ultrastructural preparation

Small pieces of the adrenal gland specimens obtained from all experimental animal groups were cut out and fixed for 24 h in a cold fixative of formaldehyde and glutaraldehyde (4:1) that was set to pH 2.2. After that, the specimens were post-fixed for another hour in 1% osmium tetroxide in 0.1M phosphate buffer that was adjusted to pH 7.3. After fixation, they underwent the standard transmission electron microscopy (TEM) examination processes as previously described by Dykstra *et al* (2002). In the Central Laboratory of Cairo University, semi-thin sections were examined, and the adrenocortical zones were identified, and then the ultrathin sections of these selected parts were cut, mounted on grids, stained with uranyl acetate and lead citrate, and finally examined and photographed using a JEOL.JEM-1400-EX-Electron Microscope.

Ethical approval

The current experimental protocol was carried out in strict compliance with the regulations and rules of Ain Shams university, Faculty of Science's animal care and use committee (ASU-SCI/ZOOL/ 2022/11/2).

RESULTS

Histological results

The adrenal cortices of the control (Fig. 1A-D) and fennel oil-treated (Fig. 1E-H) rats displayed intact histological architecture. The adrenal cortex is coated by a rigid capsule composed of fibrous connective tissue containing fibroblasts, fibrocytes and collagenous fibers, and it is divided into three distinct zones, namely the zona glomerulosa (ZG), fasciculate (ZF), and reticularis (ZR) (Fig.1A&E). ZG is composed of columnar or pyramidal cells that are organized into glomeruli-like formations and divided by tiny trabeculae which extended from the fibrous capsule. ZG cells possess pale vacuolated cytoplasm and rounded to oval basophilic nuclei as observed in Figure (1B&F). ZF is made up of columnar, or more appropriately polyhedral cells that coordinate in fasciculae with a thickness of one or two cells and discrete by thin blood capillaries which lined by ordinary endothelium. Additionally, the granulated eosinophilic cytoplasm of the ZF cells was seen to surround spherical basophilic nuclei (Fig. 1C&G). The ZR constituted the innermost zone of the adrenal cortex and built up of small, closely packed and eosinophilic polyhedral cells which arranged in short cords appeared as intermingled irregular networks and detached by widened blood sinusoids lined by intact endothelium. The ZR cells have small rounded basophilic nuclei and somewhat eosinophilic cytoplasm (Fig. 1D&H).

Meanwhile, the adrenal cortex of rats administered low doses of CPZ revealed degenerative alterations that were depicted as a decrease in the thickness of the adrenocortical zones resulting from the compression of the adrenocortical cells (2A-D). The majority of ZG cells showed evident signs of degenerative changes including vacuolated cytoplasm and pyknotic nuclei and seemed compressed and lost their usual glomerular structure (Fig. 2B). Additionally, the ZF cells displayed obvious evidence of pyknosis in their nuclei and numerous vacuoles of different sizes in their cytoplasm (Fig. 2C). Stagnant hemolyzed blood masses were seen in the congested blood capillaries separating these ZF cells (Fig. 2C). Also, the cells of ZR exhibited clear symptoms of deteriorated changes, where their nuclei appeared pyknotic and their cytoplasm showed noticeable signs of vacuolar degeneration, in addition, the separated blood sinusoids seemed swollen and engorged with stagnant blood masses (Fig. 2D).

Moreover, severe histopathological alterations were recorded in the adrenocortical tissues of rats treated with CPZ-high dose as manifested in Figure (3A-D). The adrenocortical zones' thickness reduced due to disintegration changes, which were brought on by the cells' compression (3A-D). The ZG cells lost their unique characteristic glomerular arrangement and showed obvious signs of necrosis represented as cytoplasmic vacuolation and nuclear pyknosis (Fig. 3B). The ZF cells contained numerous vacuoles of different sizes in their cytoplasm and clear indicative of pyknosis in their nuclei (Fig. 3C). The blood capillaries separating the cords of ZF showed stagnant blood aggregates (Fig. 3C). Additionally, ZR cells showed obvious signs of necrosis, including pyknotic nuclei and vacuolated cytoplasm. The blood sinusoids appeared severely dilated and engorged with intense stagnant blood masses (Fig. 3D).

Remarkably, the adrenal cortices of rats concomitantly treated with fennel oil extract and CPZ either the low (Fig. 4A-D) or the high (Fig. 5A-D) dose showed marked improvement of the majority of changes identified in the adrenocortical cells of ZG, ZF and ZR in comparison with those of the control specimens. ZG cells appeared with their ordinary pale vacuolated cytoplasm and basophilic nuclei and they were grouped in an almost uniform arrangement resembling glomeruli of the control group, with the thin trabeculae between them (Figs. 4B & 5B). Additionally, ZF cells were grouped in cords that were essentially identical to the typical arrangement of control cells, and most of them had spherical nuclei and granulated eosinophilic cytoplasm (Figs. 4C & 5C). In a similar manner, ZR cells largely restored their normal construction, with slightly fewer dilated blood sinusoids that were markedly restored their endothelial linings. Most ZR cells had rounded nuclei and normal eosinophilic cytoplasm, according to their appearance (Figs. 4D & 5D).

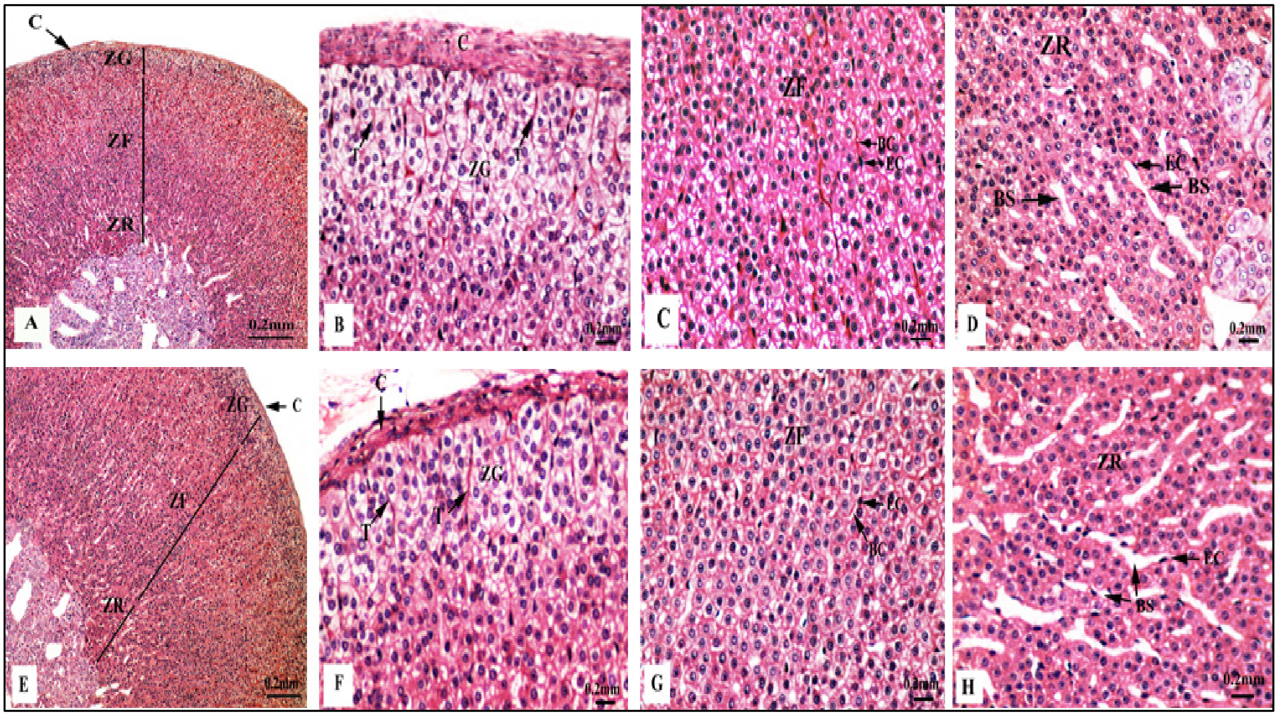

Fig. (1). Light micrographs of H&E-stained adrenal gland sections from control (A-D) and fennel oil-treated (E-H) rats illustrating **(A&E)** ordinary histological structure of the adrenal cortex which composed of three sequential zones namely; zona glomerulosa (ZG), fasciculata (ZF) and reticularis (ZR), and is coated by fibrous capsule (C); **(B&F)** ZG cells arranged in a glomerular structure and divided by tiny trabeculae (T) that protruded from the capsule (C). ZG cells possess pale vacuolated cytoplasm and rounded to oval basophilic nuclei; **(C&G)** ZF cells possess granulated eosinophilic cytoplasm surround spherical basophilic nuclei, and they are often grouped in fasciculae that are separated by tiny endothelial (EC)-lined blood capillaries (BC); **(D&H)** ZR cells are arranged in a typical network of entwined cords, with large blood sinusoids (BS) separating them that are lined with endothelial cells (EC). ZR cells have small rounded basophilic nuclei and somewhat eosinophilic cytoplasm.

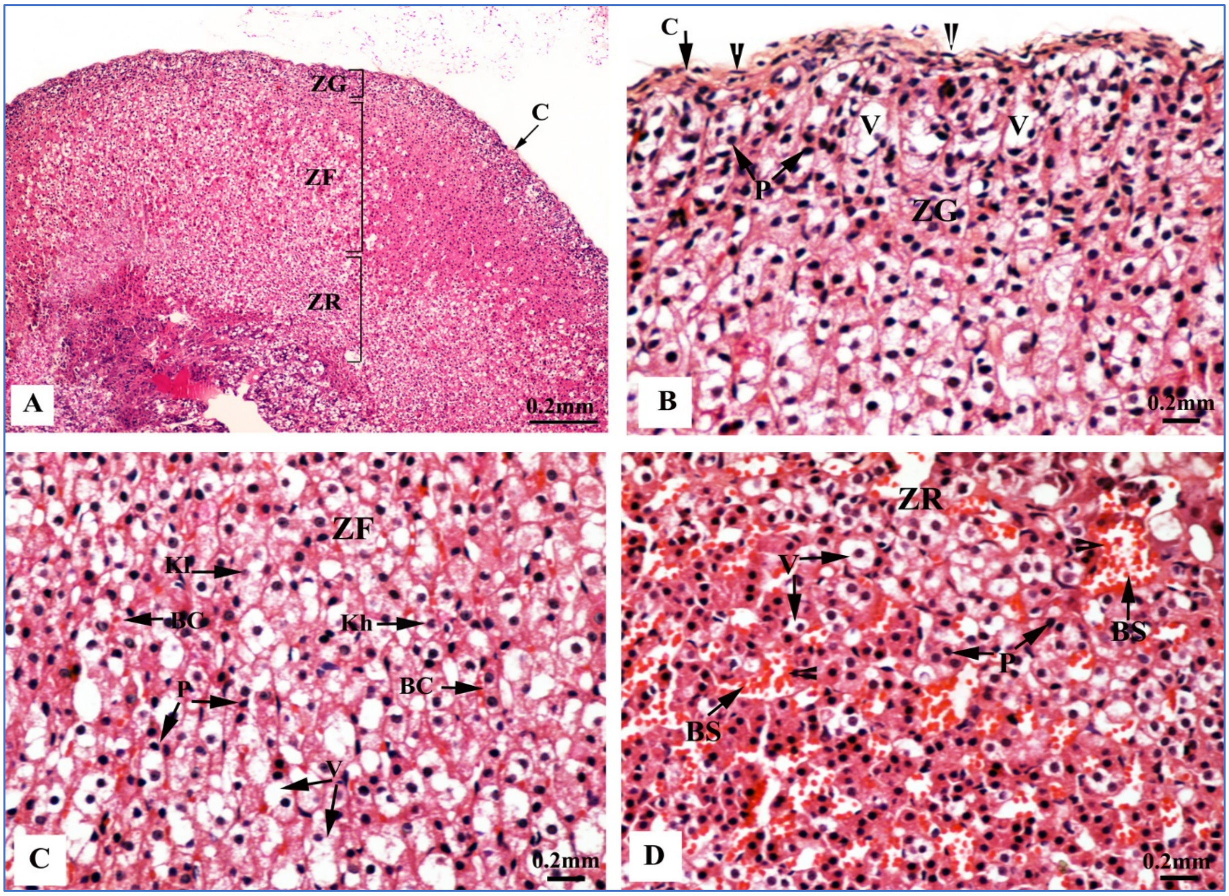

Fig. (2). Light micrographs of H&E-stained adrenal gland sections from CPZ low dose-treated rats revealing **(A)** deformed zona glomerulosa (ZG), fasciculata (ZF) and reticularis (ZR), besides irregular fibrous capsule (C); **(B)** ZG cells lost their familiar arrangement and appeared with vacuolated cytoplasm (V) and rather pyknotic nuclei (P). The outermost capsule (C) appeared irregular and compressed (arrow heads); **(C)** ZF cells suffered from degenerative changes represented by noticeable pyknotic (P), karyorrhectic (Kh) and karyolysed (Kl) nuclei, besides vacuolated cytoplasm (V). Additionally, the blood capillaries (BC) appeared congested having stagnant hemolyzed blood cells; **(D)** ZR cells appeared with pyknotic nuclei (P) and cytoplasmic vacuoles (V). Besides, the blood sinusoids (BS) appeared dilated and enclosed stagnant blood cells (arrow heads).

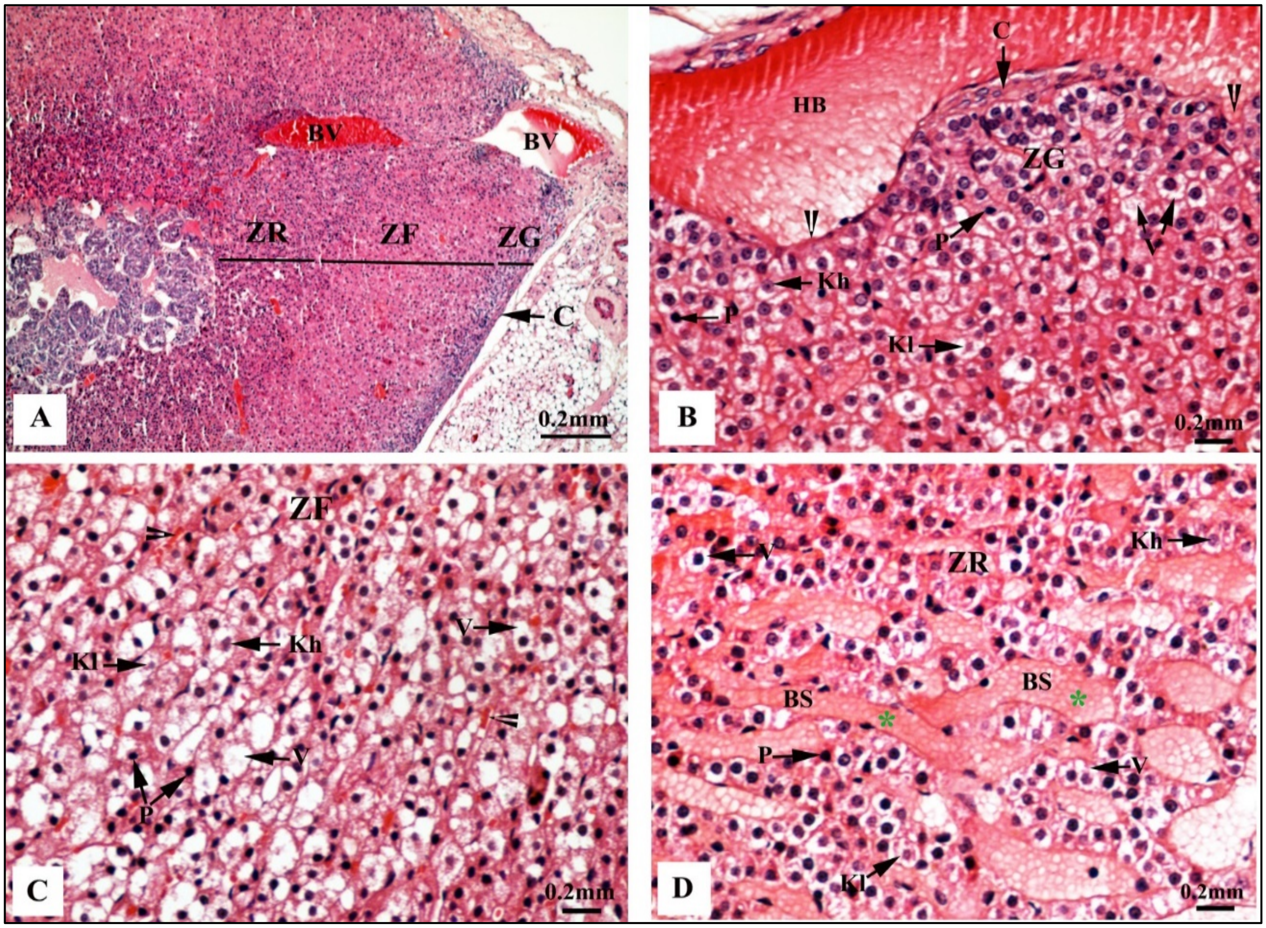

Fig. (3). Light micrographs of H&E-stained adrenal gland sections from CPZ-high dose-treated rats illustrating **(A)** deteriorated adrenocortical zonation including zona glomerulosa (ZG), fasciculata (ZF) and reticularis (ZR) which appeared surrounded by damaged fibrous capsule (C). Additionally, engorged, and dilated blood vessels (BV) are noticed; **(B)** glomerulosa cells (ZG) appeared lost their familiar organization and designating vacuolated cytoplasm (V) and necrotic nuclei having symptoms of pyknosis (P), karyorrhexis (Kh) and karyolysis (Kl). Damaged capsule (C) appeared furrowed and broken in some parts (arrow heads), besides massive hemorrhagic blood masses (HB) are obviously seen; **(C)** deteriorated ZF cells with severe cytoplasmic vacuolation (V) and nuclear pyknosis (P), karyorrhexis (Kh) and karyolysis (Kl). Additionally, the separated blood capillaries appeared with stagnant blood masses (arrow heads); **(D)** tremendous dilatation of the blood sinusoids (BS) which loaded with massive hemolyzed blood masses (green asterisks) inside their lumens. ZR cells missed their regular arrangement and appeared with vacuolated cytoplasm (V) and pyknotic (P), karyorrhectic (Kh) and karyolysed (KI) nuclei.

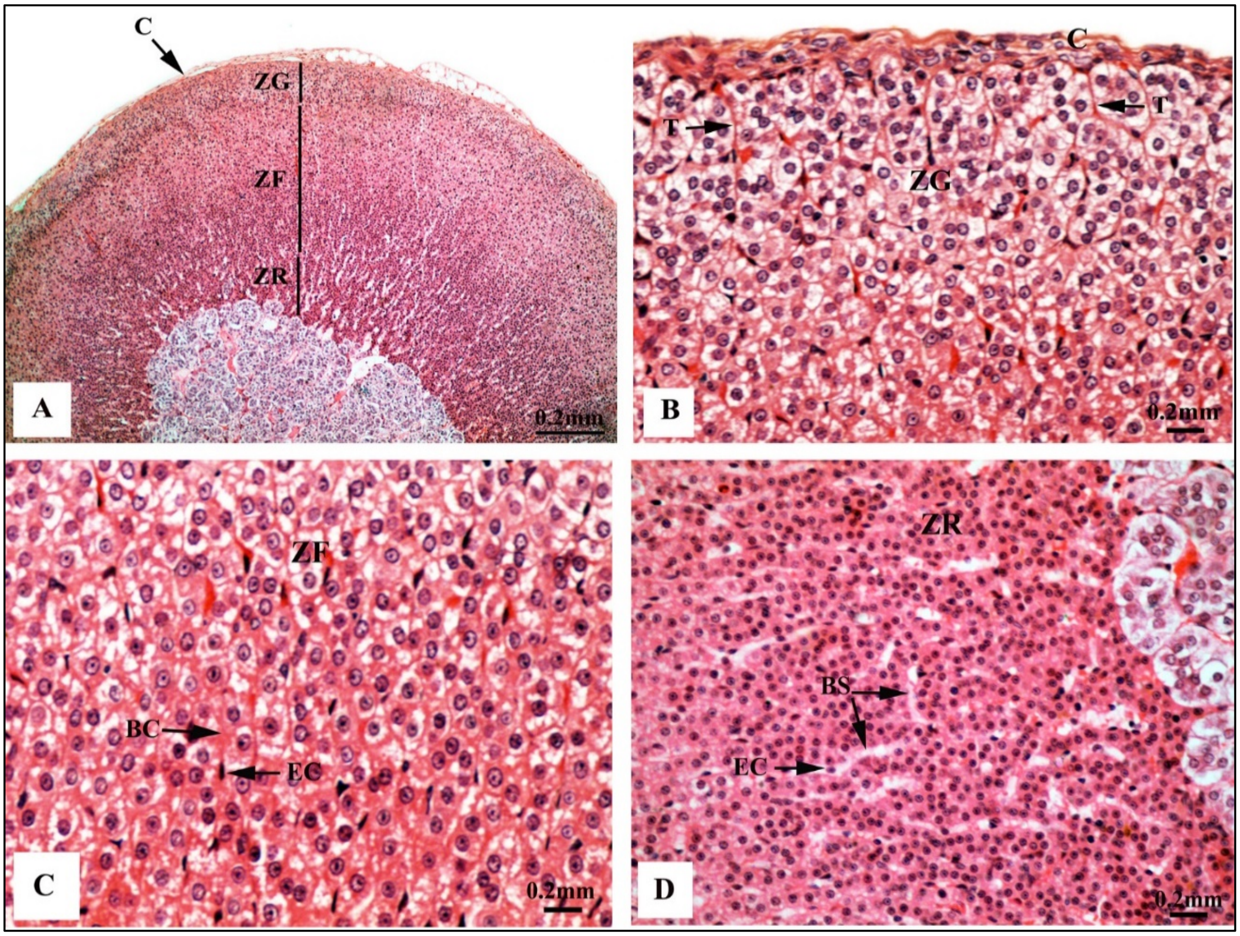

Fig. (4). Light micrographs of H&E-stained adrenal gland sections from fennel oil+ CPZ-low dose-treated rats manifesting **(A)** remarkable improvement in the histological structure of the adrenocortical tissues including zona glomerulosa (ZG), fasciculata (ZF) and reticularis (ZR), besides the surrounding fibrous capsule (C); **(B)** orderly arranged ZG cells in a glomerular structure separated by tiny trabeculae (T) which extended from the capsule (C). These cells appeared with their ordinary pale vacuolated cytoplasm and basophilic nuclei; **(C)** regular organized ZF cells having spherical nuclei and granulated eosinophilic cytoplasm and separated by tiny blood capillaries (BC) bordered with intact endothelium (EC); **(D)** regular intertwined cords of ZR cells separated by large blood sinusoids (BS) which are lined by endothelium (EC). Most of ZR cells had spherical nuclei and granulated eosinophilic cytoplasm.

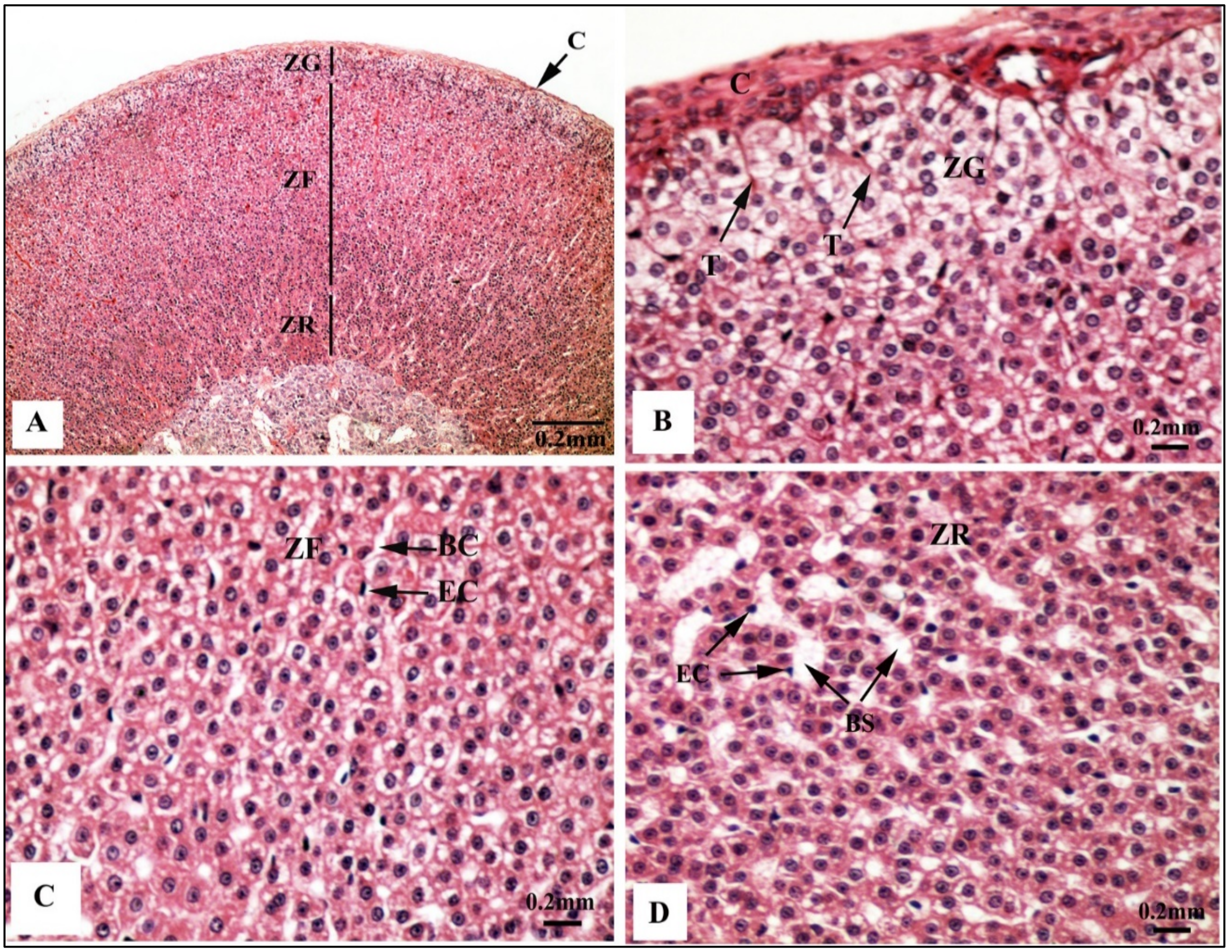

Fig. (5). Light micrographs of H&E-stained adrenal gland sections from fennel oil+ CPZ-high dose-treated rats showing **(A)** restoration of the adrenocortical tissue structure having relatively regular zona glomerulosa (ZG), fasciculata (ZF) and reticularis (ZR) and enclosed within rather regular fibrous capsule (C); **(B)** the cells of ZG appeared with their familiar architecture, separated by trabeculae (T) which radiated from the capsule (C); **(C)** ZF cells revealing a relative familiar arrangement with small blood capillaries (BC) lined with endothelium (EC) in between them; **(D)** ZR cells displaying relatively normal characters detached by widened blood sinusoids (BS) bordered by intact endothelium (EC).

Ultrastructural results

The adrenal cortices of control and fennel oil-treated rats demonstrated regular fine structural features of the adrenocortical cells of ZG, ZF and ZR as obviously noticed in Figure (6A-H). The surrounding adrenal capsules of the control and fennel oil-treated rats composed of fibroblasts appeared with regular organization and having distinct elongated nuclei, besides the collagen fibers in between them (Fig. 6A&B, respectively). ZG cells of control and fennel oil-treated rats (Fig. 6C&D, respectively) showed regular fine structural architecture as they possess oval to spherical-shaped mitochondria having unique cristae of tubule-saccular type, a few quantities of smooth endoplasmic reticula, lipid droplets and rounded to oval nuclei. Also, ZF cells of control (Fig. 6E) and fennel oil-treated (Fig. 6F) rats exhibited intact ultrastructural features including abundant round-shaped mitochondria with clear tubular cristae, distributed cisternae of smooth endoplasmic reticula, richness of lipid droplets and intact large, rounded nuclei. Similarly, ZR cells of control (Fig. 6G) and fennel oil-treated (Fig. 6H) rats were identified by their increased number of rounded mitochondria having dense tubular cristae, abundant cisternae of smooth endoplasmic reticula, large lipid droplets and oval to rounded nuclei.

On the other hand, fine structural alterations were recorded in the adrenocortical cells of ZG, ZF, and ZR of low dose CPZ-treated rats as illustrated in Figure (7A-D). The fibroblast cells that made up the fibrous adrenal capsules appeared degraded and had electron-dense nuclei and a lot of collagen fibers (Fig. 7A). The cells of ZG possess mitochondria with more electron-dense matrices, lipid globules of varied sizes, and deformed nuclei (Fig. 7B). Also, ZF cells exhibited deformed mitochondria tightly stuffed with tubular cristae, other mitochondria being destructed, besides proliferated smooth endoplasmic reticulum, increased lysosomes, aggregation of various-sized lipid globules, and destructed nuclei which illustrated symptoms of pyknosis leaving clear spaces surrounding them (Fig. 7C). Similarly, ZR cells possessed distorted mitochondria stuffed with tubular cristae, proliferated smooth endoplasmic reticulum, cumulative lipid globules of varied sizes, and rather pyknotic nuclei bordered by irregular destructed nuclear envelopes (Fig. 7D).

Moreover, the adrenal cortices of rats intoxicated with CPZ-high dose illustrated severe fine structural alterations of almost all of the adrenocortical cells of ZG, ZF and ZR and the surrounding adrenal capsules as observed in Figure (8A-D). The covering fibrous adrenal capsules appeared with deteriorated fibroblast cells which have electron-dense nuclei and high amount of collagen fibers (Fig. 8A). Part of the underlying ZG was seen. Distorted ZG cells revealed highly devastated mitochondria which lost parts of their cristae and matrices and contained tiny flocculent densities, massive lipid droplets and fragmented smooth endoplasmic reticulum, besides degraded nuclei having irregular shape and bordered by irregular ruptured nuclear envelopes which revealed signs of pyknosis as well as karyolysis as seen in Figure (8B). Also, the cells of ZF revealed massive ultrastructural changes which declared in the cytoplasm and the nuclei as manifested in Figure (8C), where mitochondria appeared with remarkable loss of cristae and matrices and clearly showed tiny flocculent densities, the cytoplasm loaded with massive groups of lipid globules, lysosomes, and smooth endoplasmic reticulum. Besides, these cells had pyknotic nuclei which surrounded by furrowed and detached nuclear envelopes. Additionally, numerous noticeable abnormalities were detected in the cells of ZR including deformed mitochondria with tightly packed cristae and electron-dense matrices, lipid droplets, lysosomes and highly pyknotic nuclei with irregular damaged nuclear envelopes as well as features of chromatolysis (Fig. 8D).

On the other side, the adrenal cortices of rats co-treated with fennel oil extract and CPZ either the low (Fig. 9A-D) or the high (Fig. 10A-D) dose manifested modulation of the majority of the fine structural changes identified in the adrenal fibrous capsules and the adrenocortical cells of ZG, ZF and ZR in comparison with those examined in the control group. The fibrous adrenal capsules appeared with regular fibroblast cells and collagen fibers in between them (Figs. 9A & 10A). ZG cells showed relatively normal fine structural features as they contained oval or rounded-shaped mitochondria having definite cristae of tubule-saccular type, a few cisternae of smooth endoplasmic reticulum, lipid globules and rounded to oval-shaped nuclei (Figs. 9B & 10B). Similarly, ZF cells showed ordinary ultrastructural organization including obvious rounded-shaped mitochondria with hollow-tubular cristae, smooth endoplasmic reticulum, increased lipid globules and large rounded nuclei. Additionally, ZR cells appeared intact with intense rounded mitochondria having dense hollow-tubular cristae, smooth endoplasmic reticulum, numerous lipid globules and oval to spherical nuclei (Figs. 9D & 10D).

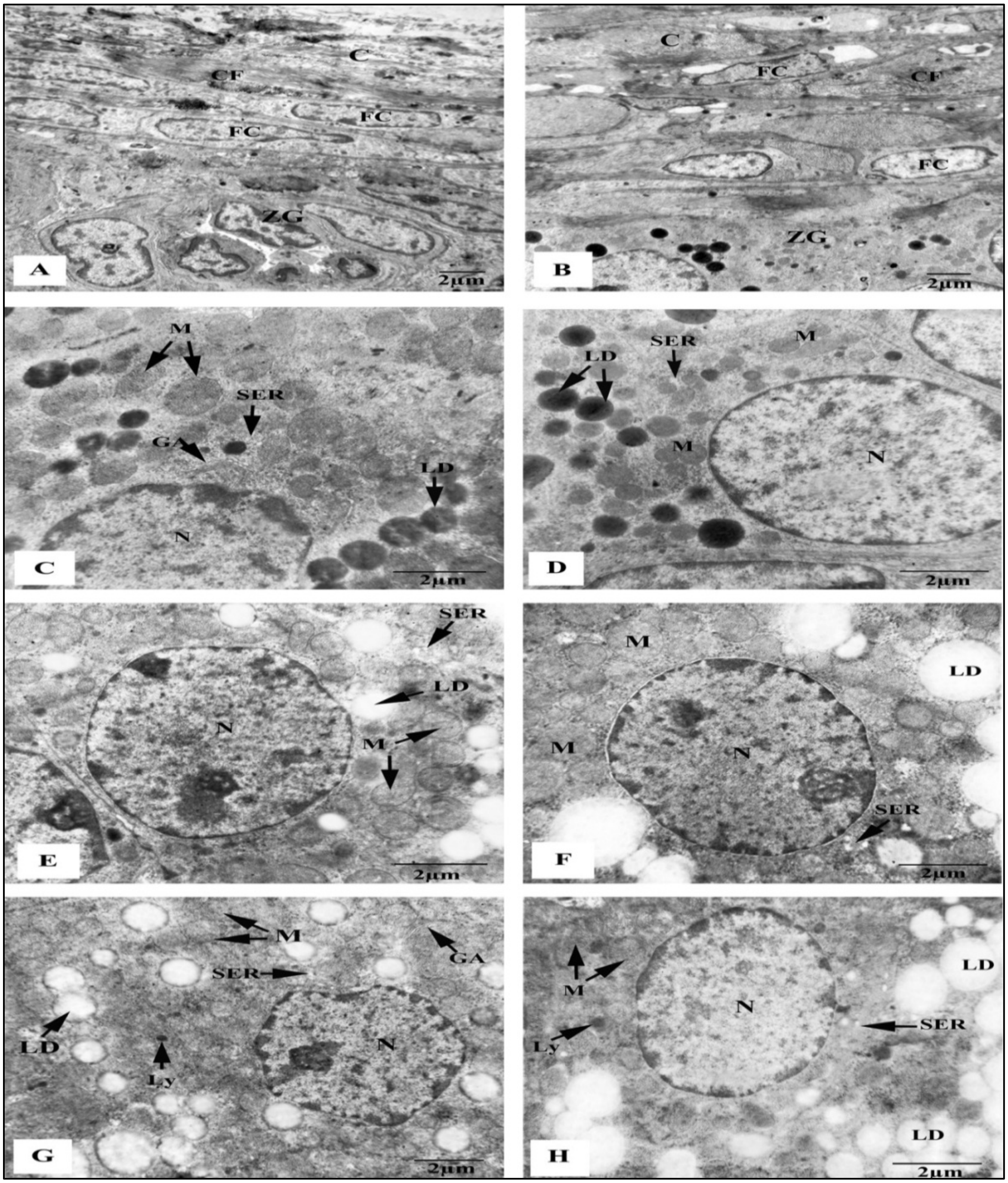

Fig. (6). Electron micrographs of the adrenal cortices of control and fennel oil-treated rats showing **(A&B)** the surrounding adrenal capsules (C) appeared formed mainly of fibroblast cells (FC) with their distinguished elongated nuclei and collagenous fibers (CF) in between them, besides zona glomerulosa (ZG) observed beneath each capsule in control and fennel oil-treated rats, respectively; **(C&D)** ZG cells possess mitochondria (M) with unique tubulo-saccular cristae, fewer cisternae of smooth endoplasmic reticula (SER), Golgi apparatus (GA), lipid droplets (LD), and distinct nuclei (N) in control and fennel oil-treated rats, respectively; **(E&F)** zona fasciculata cells crowded with numerous round-shaped mitochondria (M) having tubular cristae, well-developed smooth endoplasmic reticula (SER), abundant large rounded lipid droplets (LD) and distinguished nuclei (N) having rounded shape in control and fennel oil-treated rats, respectively; **(G&H)** zona reticularis cells have notable rounded mitochondria (M) possessing numerous cristae of tubular type, lysosomes (Ly), smooth endoplasmic reticula (SER), lipid droplets (LD), and obvious nuclei (N) in control and fennel oil-treated rats, respectively.

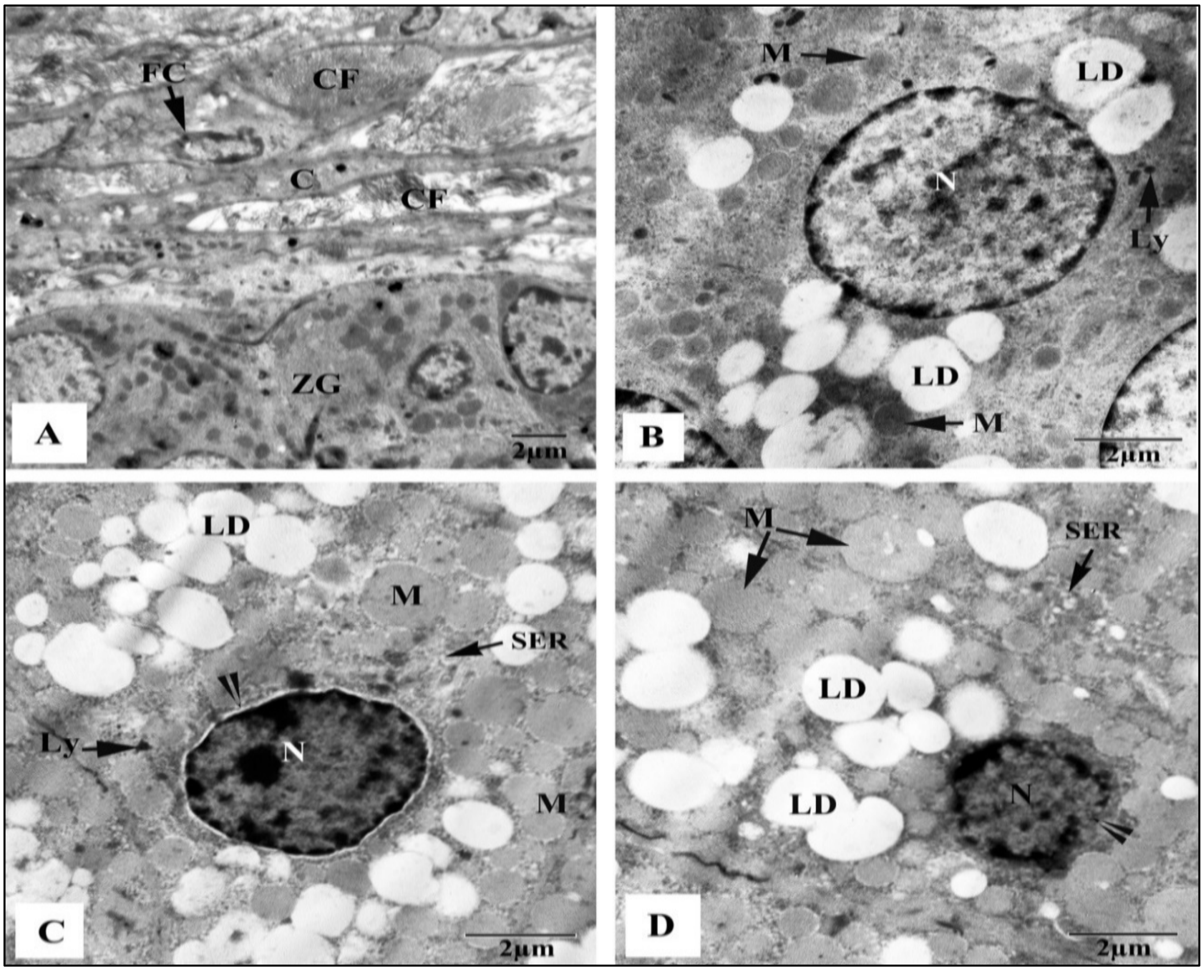

Fig. (7). Electron micrographs of the adrenal cortices of CPZ –low dose-treated rats illustrating **(A)** adrenal capsule (C) having relatively numerous intercalated fibroblast cells (FC) with rounded to oval nuclei and increased collagenous fibers (CF). Portion of the underlying zona glomerulosa (ZG) is seen; **(B)** ZG cell has more electron-dense mitochondria (M), larger sized lipid droplets (LD), lysosomes (Ly), and deteriorated nucleus (N); **(C)** zona faciculata cell has pyknotic nucleus (N) bordered by deformed nuclear envelope that appeared detached (arrow head), proliferated smooth endoplasmic reticulum (SER), mitochondria (M) stuffed with tubular cristae, massive lipid droplets (LD) and lysosomes (Ly); **(D)** zona reticularis cell appeared with pyknotic nucleus (N) bordered by fragmented irregular nuclear envelope (arrow head) and cytoplasm crowded with destructed mitochondria (M) having tightly packed tubular cristae, smooth endoplasmic reticulum (SER), and lipid droplets (LD).

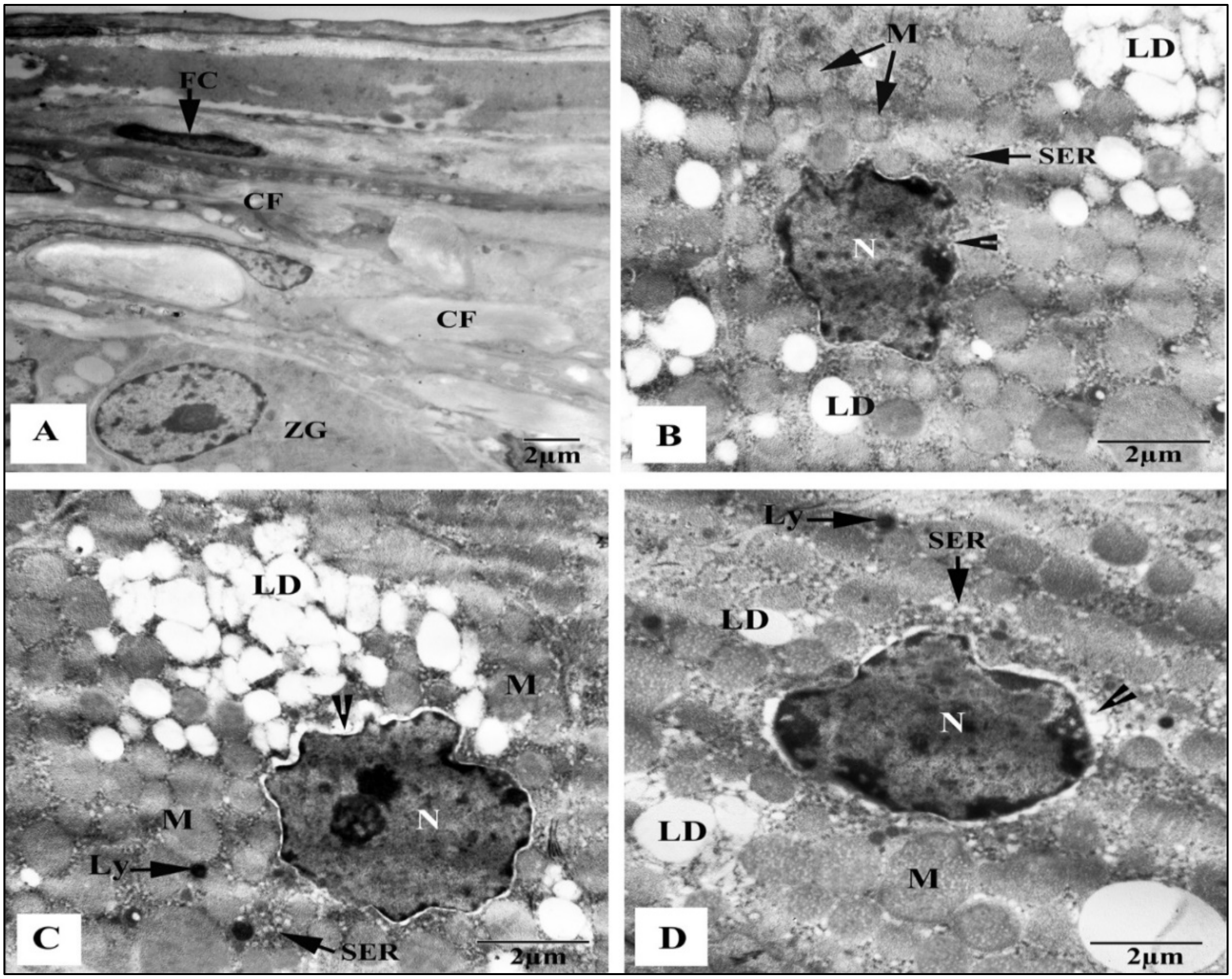

Fig. (8). Electron micrographs of the adrenal cortices of CPZ-high dose-treated rats illustrating **(A)** the covering adrenal capsules which appeared with deteriorated fibroblast cells (FC) having electron-dense nuclei and in-between them found a high density of collagen fibers (CF). The underneath zona glomerulosa (ZG) is noticed; **(B)** deformed ZG cell having noticeable crumpled nucleus (N) bordered by ruptured irregular nuclear envelope (arrow head), deformed mitochondria (M) with marked loss of their internal cristae and matrices and showed tiny flocculent densities, fragmented smooth endoplasmic reticulum (SER) and increased lipid droplets (LD); **(C)** deformed zona fasciculata cell with distinct altered mitochondria (M) having tightly packed cristae, electron-dense lysosomes (Ly), fragmented smooth endoplasmic reticulum (SER), lipid droplets (LD), and damaged nucleus (N) boarded by detached ruptured nuclear envelope (arrow head) and manifested signs of pyknosis; **(C)** Deteriorated zona reticularis cell having deformed mitochondria (M) with tightly packed cristae, lysosomes (Ly), smooth endoplasmic reticulum (SER), lipid droplets (LD) and pyknotic nucleus (N) covered with wrinkled damaged nuclear envelope (arrow head).

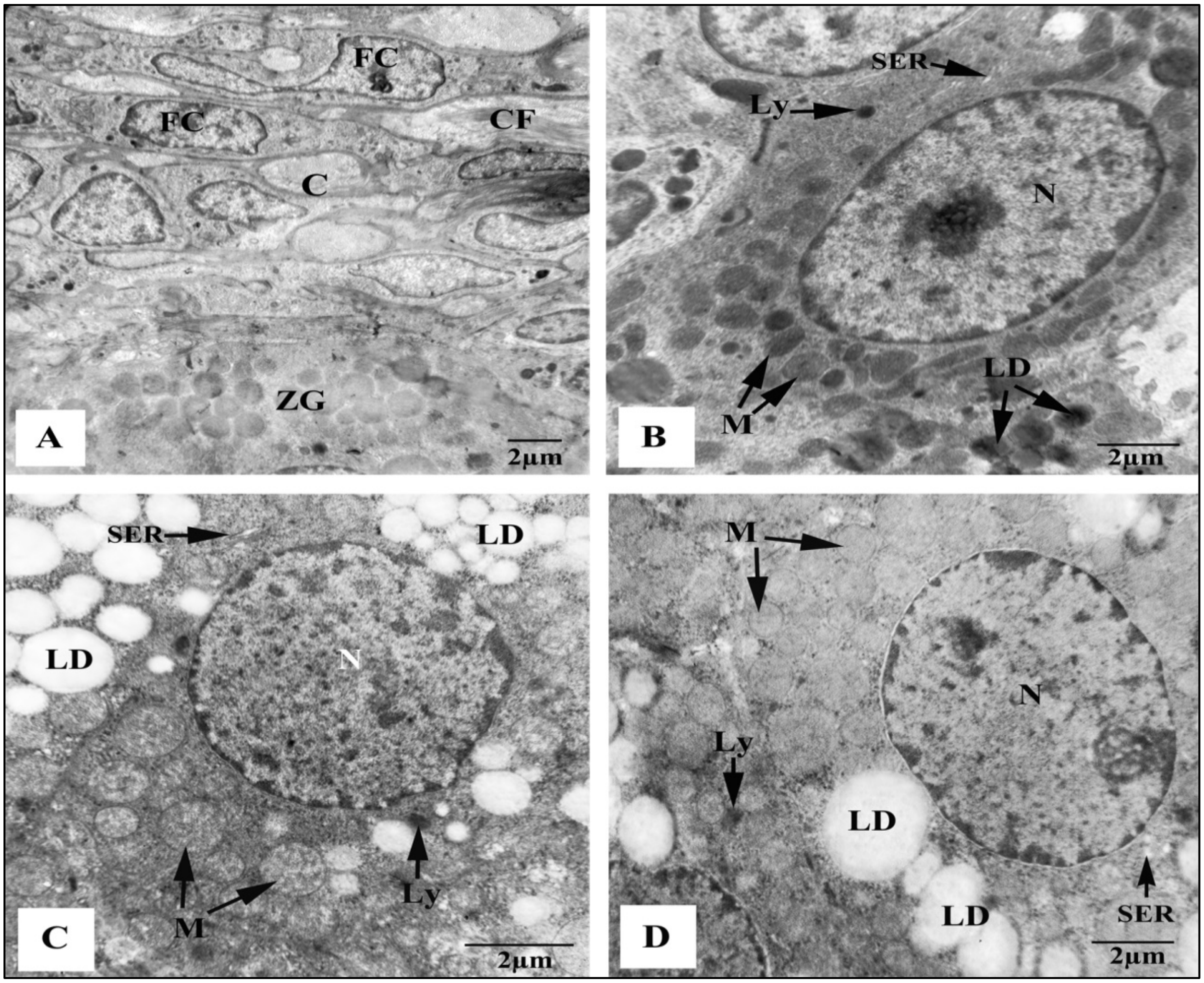

Fig. (9). Electron micrographs of the adrenal cortices of fennel oil + CPZ low-dose-treated rats showing **(A)** regular fibrous adrenal capsule (C) with fibroblast cells (FC) and collagen fibers (CF), besides portion of zona glomerulosa (ZG) is seen; **(B)** intact ZG cell having rounded to elongated mitochondria (M), smooth endoplasmic reticulum (SER), lipid droplets (LD), few electron-dense lysosomes (Ly), and an oval nucleus (N); **(C)** regular zona fasciculata cell possessing distinct nucleus (N), rounded-shaped mitochondria (M) filled with cristae of tubular type, smooth endoplasmic reticulum (SER), large lipid droplets (LD) and lysosomes (Ly); **(D)** regular zona reticularis cell possessing rounded mitochondria (M), lipid droplets (LD), lysosomes (Ly), smooth endoplasmic reticulum (SER), and intact spherical nucleus (N).

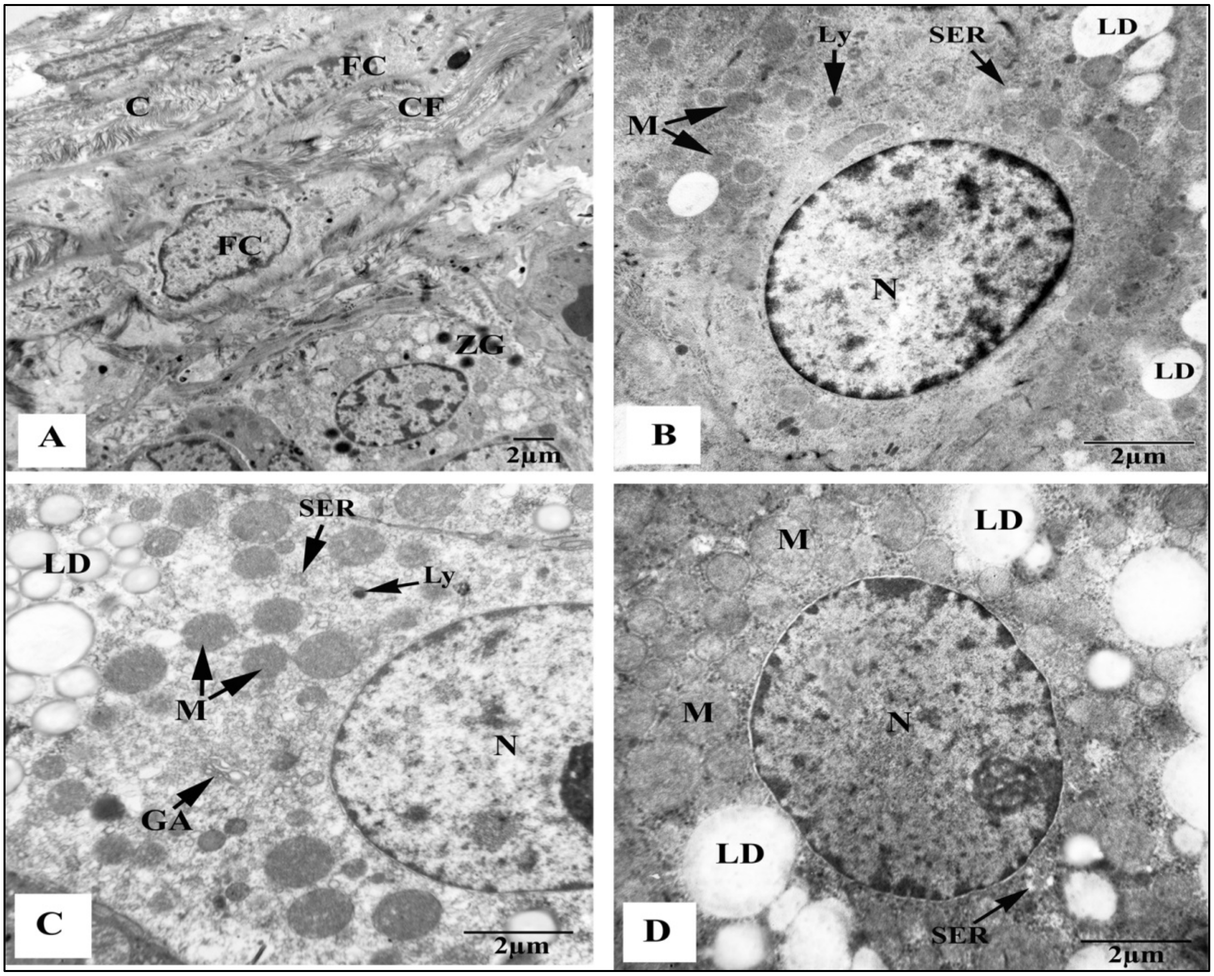

Fig. (10). Electron micrographs of the adrenal cortices of fennel oil + CPZ high -dose-treated rats showing **(A)** relatively regular adrenal capsule (C) with fibroblast cells (FC) having rounded to elongated-shaped nuclei and intense collagen fibers (CF). The neighboring zona glomerulosa (ZG) is noticed; **(B)** ZG cell having regular smooth endoplasmic reticulum (SER), mitochondria (M), lipid droplets (LD), lysosomes (Ly) and oval nucleus (N); **(C)** normal zona fasciculate cell showing distinct nucleus (N), unique mitochondria with tubular cristae, Golgi apparatus (GA), smooth endoplasmic reticulum (SER), lipid droplets (LD) and lysosomes (Ly); **(D)** intact zona glomerulosa cell having rounded nucleus (N), rounded mitochondria (M) having tubular cristae, lipid droplets (LD) and smooth endoplasmic reticulum (SER).

DISCUSSION

Globally, pesticides including fungicides are used by farmers to lessen damage from harmful organisms including fungi for the sake of improving quality and harvest of crops. Due to their effectiveness in treating a wide range of fungal infections in plant crops and vegetables, triazoles have rapidly gained popularity as lucrative and excessively utilized fungicides all over the world (Groppelli *et al*., 2005). One of these triazoles that are frequently utilized worldwide is CPZ (Baybakova *et al*., 2019).

The potential toxicity of fungicides in various internal organs is a crucial area for research. Triazoles cause several harmful adverse influences on organisms, inclusively teratogenic impacts (Groppelli *et al*., 2005), endocrine disturbance (Yu *et al*., 2013), oxidative stress (Mu *et al*., 2015), neurotoxicity (Paredes-Zúñiga *et al*., 2019), and reproductive toxicity (Shen *et al*., 2021). In this regard, the adrenal gland is considered the most frequent objective for toxicity in the endocrine system owing to its distinctive biosynthetic capability, abundant blood supply, richness of CYP450 which responsible for metabolization of xenobiotics to reactive intermediates, and lipophilicity that encourages the accumulation of lipophilic compounds (Harvey & Everett, 2003). The adrenal gland consists of two unique anatomical regions; an outermost adrenal cortex covering an innermost adrenal medulla. The adrenal cortex represents 80–90% of the gland and is made up of cords of epithelial cells which are directly connected to the blood. These epithelial cells are gathered forming three distinct zones known as ZG, ZF, and ZR (Sicard *et al*., 2007). The adrenal cortex is considered as the most significant steroidogenic tissue and survival necessity in the human body as it is the site of all steroidogenic processes (Bielohuby *et al*., 2007; Pihlajoki *et al*., 2015). According to Rosol *et al*. (2001), one of the most frequent sites for endocrine system lesions is the adrenal cortex, and more specifically the ZF.

The hazardous effects of CPZ on mammalian adrenal glands have been the subject of a few earlier studies, according to the literature. Consequently, the goal of the current investigation was to examine the toxic impacts of CPZ on the histological and fine structural architecture of rats' adrenal cortices as well as the potential protective impact of fennel oil extract against the anticipated anomalies and noxious influences induced by CPZ.

The results obtained in the present investigation showed that rats given either the low or the high doses of CPZ had severely devastating histological and fine structural changes in their adrenocortical tissues. One of these malformations was the thickening of the adrenal gland capsule, which is in line with those previously reported in both adult and senile stressed rats (Zaki *et al*., 2018). Additionally, a noticeable decrease in the thickness of ZG, ZF, and ZR was recorded in CPZ-treated rats which were resulted from the compression of their adrenocortical cells owing to the degeneration of most of their forming organelles, especially the smooth endoplasmic reticula, mitochondria, and nuclei.

Also, the present findings illustrated that the three adrenocortical zones' cells suffered from severe degenerative alterations. The majority of adrenocortical cells lacked their regular structure, illustrating obvious degeneration and necrosis manifested as vacuolated cytoplasm, and pyknotic, karyorrhectic, or karyolysed nuclei. These results are in agreement with those declared previously by

Almeida *et al.* (2006), Illera *et al.* (2007), Silvan *et al.* (2007), Ye *et al.* (2008) and Elshennawy & Aboelwafa (2011) in different experimental studies.

The most outstanding alteration observed in the adrenocortical cells obtained from the adrenal glands of animals intoxicated with CPZ was the expansion and intense accumulation of lipid globules in their cytoplasm. Such anomalies had also been formerly revealed by Almeida *et al.* (2006) and Elshennawy & Aboelwafa (2011) predominately in the cells of ZF and ZR. Since the majority of the cholesterol used in the production of steroids is stored in lipid globules, according to Hall (1995) who suggested that the aggregation of lipid globules in the adrenocortical cells might be considered as a secondary episode caused by the suppression of the reactions which transform cholesterol to progesterone. Inside these globules, the cholesterol ester is brought to the mitochondrial inner membranes and participates in the biosynthesis of steroid hormones. Therefore, we could suggest that CPZ may impede cholesterol transfer, which can cause lipid droplets to aggregate in the cytoplasm of adrenocortical cells.

The current results revealed many lysosomes of electron dense appearance in CPZ-treated rats, which showed enhanced digestion of the stored intracellular lipids. These findings were in accordance with Abd El-Gawad *et al.* (2016) who found multiple lysosomes within cells of ZG and those of ZF post exposure to stress.

The existing results manifested that CPZ induced hazardous alterations in the mitochondria and smooth endoplasmic reticulum of the adrenocortical cells of treated animals. These deteriorations may be enough to prevent the production of steroid hormones. Consistent with the existing findings, Guerrero *et al.* (2010) and Fumiko (2014) manifested that the mitochondria and smooth endoplasmic reticulum perform a significant role in the process of steroidogenesis inside the adrenocortical cells via their involvement in the linked actions of 3β-hydroxysteroid dehydrogenase (3βHSD) enzyme and CYP450, which are dispersed between the mitochondria and cisternae of smooth endoplasmic reticulum.

In addition, congested blood sinusoids were found in the CPZ-treated rats. This finding correlated with the findings obtained by other researchers in this concern, who explained the congested sinusoids as a result of increased vascular demand to accommodate for the increased endocrine activity and hormonal secretions of the adrenal cortex in response to stress (Patra *et al*., 2014).

The current structural and fine structural changes recorded in the adrenal cortices of CPZ-intoxicated animals may be related to cellular oxidative stress which has a major role in CPZ-induced adrenocortical cytotoxicity. Biochemical investigation of Yousef *et al*. (2022) illustrated that CPZ substantially induced damage to the adrenal gland which was evidenced by elevated serum levels of adrenocorticotropic hormone, total cholesterol, free cholesterol, and low-density lipoprotein cholesterol, as well as reduced serum levels of cortisol and high-density lipoprotein cholesterol. Also, high levels of malondialdehyde, decreased reduced glutathione levels, and diminished activities of superoxide dismutase, catalase, and glutathione peroxidase were recorded in the adrenal gland tissues of CPZ-treated groups.

Additionally, oxidative stress alters the structure and permeability of the cell membranes and intracellular organelles like mitochondria and the endoplasmic reticulum (Blokhina *et al*., 2003). Reactive oxygen species (ROS) formation may also have an impact on cellular DNA and lysosomes, rendering cells highly vulnerable to toxin-induced cell death (Niedowicz & Daleke, 2005). These changes detected within the adrenocortical cells resulted in lowered energy level and inhibition of steroidogenesis capacity of the adrenocortical cells as recently confirmed through biochemical assessment of the serum levels of adrenocorticotropic hormone and cortisol which showed highly significant decrease in their levels in comparison with the control group (Yousef *et al*., 2022). Numerous CYP450 subtypes were stimulated by the oxidative stress brought by CPZ, which led to increased ROS production during CYP450's catalytic cycle and the synthesis of reactive metabolites during CPZ biotransformation. Increased ROS resulted in increased lipid peroxidation, increased glutathione consumption, and decreased antioxidant enzyme effectiveness (Hamdi *et al*., 2019).

Remarkably, the present results illustrated that concomitant administration of fennel oil extract alongside with CPZ significantly improved most of the obtained histological and fine structural alterations caused by CPZ intoxication in the adrenocortical tissues of the treated rats in comparison with those obtained from the control ones. Fennel extract has been reported to contain numerous polyphenolic compounds (Chang *et al*., 2013) which have high antioxidant activity (Choi and Hwang, 2004; Chatterjee *et al*., 2012). This action is assumed to be primarily due to their redox properties which are responsible for the adsorption, neutralization and removal of free radicals, the destruction of singlet and triplet oxygen, and the solubility of peroxides (Singh *et al*., 2006). In this regard, Yousef *et al*. (2022) stated that fennel oil extract co-treatment reversed CPZ hazardous effects on the biochemical parameters and DNA structure.

In conclusion, the present histological and ultrastructural study confirmed that CPZ induced serious hazardous alterations in the adrenal cortical tissues of adult rats which can suppress the steroid hormones biosynthesis. Additionally, fennel oil co-treatment reversed CPZ hazardous influences and anomalies on the adrenal cortices of the treated rats. Therefore, the present investigation highlights the prospective hazard of the anonymous utilization of such triazole fungicide derivative in agriculture and throw a light on the protective impact of fennel oil extract supplementation against CPZ-induced adrenal gland pathogenic toxicity.

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Histological and ultrastructural study on the pathogenicity of the fungicide cyproconazole and the probable protective influence of fennel oil extract on the adrenal cortex of adult rats

دراسة هستولوجية وتركيبية دقيقة على السمية المرضية للمبيد الفطرى "سيبروكونازول" والدور الوقائى المحتمل لمستخلص زيت الشمر على قشرة الغدة الكظرية للجرذان البالغة

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المستخلص

يعتبر السيبروكونازول (CPZ) أحد مشتقات المبيدات الفطرية من مجموعة الترايازول (Triazole) المستخدمة علي نطاق واسع لوقاية المحاصيل الزراعية من مجموعة متنوعة من الأمراض الفطرية. وتهدف الدراسة الحالية إلى تقييم التأثيرات الضارة المستحثة بالسيبيروكونازول على قشرة الغدة الكظرية للجرذان البالغة والتأثير الوقائي المحتمل لزيت الشمر، وهو زيت طبيعي مستخرج من نبات الشمر *Foeniculum Vulgare* (العائلة الخيمية Umbelliferaceae) ضد الآثار المدمرة الناتجة عن استعمال السيبروكونازول. وقد استخدم في هذه الدراسة عدد ستون من ذكور الجرذان البالغة والتي قسمت الي ستة مجموعات تجريبية: المجموعة الضابطة ، مجموعة زيت الشمر، مجموعة الجرعه المخفضة من السيبيروكونازول ، مجموعة الجرعه المرتفعة من السيبيروكونازول ، مجموعة زيت الشمر + الجرعه المخفضة من السيبيروكونازول ، ومجموعة زيت الشمر + الجرعه المرتفعة من السيبيروكونازول. وبعد خمسة عشر يومًا من المعالجة، تمت ازالة الغدد الكظرية من جميع الجرذان، وتثبيتها في المثبت المناسب، واعدادها من أجل الفحوص النسيجية والتركيبية الدقيقة. وقد أظهرت النتائج الحالية أن السيبروكونازول بالجرعات المنخفضة والمرتفعة قد تسبب بشكل كبير في حدوث تغيرات تحللية مرضية في أنسجة قشرة الغدة الكظرية للجرذان المعالجة ، وقد ظهر واضحا من انخفاض سمك مناطق قشرة الكظر؛ المنطقة المكببة والمنطقة الحزمية والمنطقة الشبكية ، والتي نتجت عن ضغط خلاياها التي عانت من التحلل الفجوي في السيتوبلازم والتغلظ النووي والتفتت النووي و الانحلال النووي، بالاضافة الي احتقان الأوعية الدموية القشرية وتمددها. أظهرت نتائج البنية التركيبة الدقيقة تحلل الميتوكوندريا وزيادة توالد الشبكة الإندوبلازمية الملساء وزيادة الليسوسومات ، بالاضافة الي تجمع ملحوظ للقطيرات الدهنية. بينما عكست المعالجة المشتركة لزيت الشمر معظم التأثيرات الخطرة التي سببها السيبروكونازول فى الخصائص النسيجية والتركيبية الدقيقة لأنسجة قشرة الكظر للجرذان المعالجة . وفى الختام ، أثبتت هذه الدراسة أن العلاج المشترك بزيت الشمر له دور وقائي واضح ضد إصابة قشرة الغدة الكظرية التي سببها السيبروكونازول في الجرذان البالغة ويرجع ذلك إلى قدرتها القوية المضادة للاكسدة والمضادة للالتهابات.