



RESEARCH ARTICLE

THE EFFECT OF THE FOOD CONTAMINANT FURAN ON ADRENAL CORTEX OF ADULT MALE ALBINO RATS: HISTOLOGICAL AND BIOCHEMICAL STUDY

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ABSTRACT

Furan is produced during food processing and preservation techniques that involve heat treatment. Furan-exposed rats showed dose-dependent gross and histological changes in endocrine and reproductive organs.

**Aim:** this work was carried out to investigate the effect of furan on the histological structure of adrenal cortex in adult male rats.

**Material and methods:** twenty adult male albino rats (4 months old) were divided equally into two groups. Group I (control) and group II (Furan- treated) that was administered furan in a dose of 8 mg/kg/ day orally by dissolving it in corn oil for 90 days. The animals were sacrificed at the end of the experiment. The suprarenal glands of the two groups were prepared for light and electron microscope examination. Blood samples from animals of all groups for estimation of serum corticosterone with statistical analysis of the levels were done. Morphometric study was also performed. Serum level of corticosterone showed significant decrease after furan administration.

**Results:** The suprarenal cortex of the furan-treated group (group II) exhibited loss of architecture of both the zona glomerulosa and fasciculata with cellular infiltration. Areas of hyperplasia were found under the capsule. Also, the cells of zona fasciculata appeared with marked cytoplasmic vacuolation, whereas zona reticularis cells had condensed nuclei and congested blood sinusoids. Ultrastructurally, the cells of zona glomerulosa and fasciculata showed swollen mitochondria with disrupted cristae and many lipid droplets. Electron dense apoptotic nuclei were also seen.

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INTRODUCTION

After the discovery of the heat-induced food contaminant acrylamide in carbohydrate-rich foods, a wide range of heat-induced food contaminants such as furan began to be important for both the scientific community and the public (Glatt *et al.*, 2005; Moser *et al.*, 2009). Furan (C<sub>4</sub>H<sub>4</sub>O) is the main compound of many chemicals used in some industrial sectors. This chemical is a colorless, volatile liquid with a low boiling point (NTP, 1993). Furan and its derivatives occur naturally in many kinds of foods that treated by heat such as jarred and canned foods (<http://www.cfsan.fda.gov/about~dms/furandat.html>, [http://www.efsa.europa.eu/en/science/data\\_collection/furan.htm](http://www.efsa.europa.eu/en/science/data_collection/furan.htm)). Furan is produced by different routes: heat degradation of carbohydrates only or with amino acids (this type of reaction is called Maillard reaction), heat degradation of some amino acids or ascorbic acid and related substances, and

heat degradation of carotenoids and polyunsaturated fatty acids (Becalski and Seaman, 2005; Yaylayan, 2006; Locas and Yaylayan, 2004). It has been reported that ascorbic acid undergoes a high potential for furan production (Locas and Yaylayan, 2004; Fan, 2005). A survey was performed by the United States Food and Drug Administration (FDA) on about 300 food samples and showed that furan was present in many types of foods up to levels more than 100 mg/g (<http://www.cfsan.fda.gov/about~dms/furandat.html>). For example, it is found in canned and cooked meats, beer, roasted coffee, and wheat bread (Crew and Castle, 2007; Morehouse *et al.*, 2008; Bolger *et al.*, 2009). Worldwide, more than one thousand of food samples have been analyzed for furan (Becalski *et al.*, 2010). Furan has been detected in heat-processed food such as canned fruits and vegetables, as well as in cigarette smoke, wood smoke, and engine exhaust (<http://www.cfsan.fda.gov/about~dms/furandat.html>, Becalski and Seaman 2005; Wegener and Lopez-Sanchez, 2010). High levels have been detected in coffee, baby food, soups, sauces cereal products and meat products, (Bakhiya and Appel, 2010; Liu and Tsai, 2010). Fruit juices have high amounts of free sugars and

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ascorbic acid, both are precursors of furan through thermal decomposition. In baby food, a level of 25 ng/g was reported; this finding is important, because it can be the main diet of infants (Lachenmeier *et al.*, 2009; Ariseto *et al.*, 2010; Owczarek-Fendor *et al.*, 2010). Furan is classified as carcinogenic in rodents such as rats and mice and “possibly be carcinogenic to humans” by the IARC (<http://www.inchem.org/documents/iarc/vol63/furan.html>). In 2-year study, B6C3F1 mice and F-344 rats were orally administered furan in doses of 8, 15 mg /kg/ day in both males and females. In that study, hepatocellular adenomas, carcinomas, and benign pheochromocytoma of the suprarenal gland were increased significantly (NTP, 1993). It has been reported that furan induced a significant increase in the micronucleated cells in splenocytes of mice that were administered furan for 4 days (Leopardi *et al.*, 2009). It has also been reported that furan induced some histopathological changes in the liver and kidney of male rats, changes in serum enzyme levels, and also a dose-dependent increase in liver TNF- $\alpha$  levels (Selmanoğlu *et al.*, 2012). Toxicological research is needed because industrialized countries are suffering from the total burden of disease, due to environmental factors (Grandjean, 2005). Several kinds of chemicals are known to possibly influence the endocrine systems of mammalian animals, including the thyroid gland, adrenal glands, pancreas, hypothalamus, hypophysis, and reproductive organs (Neubert, 1997). Because furan and its derivatives are found at high levels in jarred baby foods, infants and children are possibly at risk. Children are also highly susceptible to chemicals because their metabolic pathways differ from that of adults; so, they are less capable of metabolizing toxicants (Suk *et al.*, 2003). In this respect, the purpose of our work was to evaluate the prospective effects of furan on the histological architecture of adrenal cortex of adult male albino rats.

## MATERIALS AND METHODS

Furan (CAS No. 110-00-9, >99 % purity) was obtained from Sigma Aldrich, St. Louis, Mo, USA). This study was carried out on twenty male albino rats (4 months old) obtained from the Animal House of Faculty of Medicine, Zagazig University. All animals were acclimated to the laboratory conditions for 1 week before the beginning of the experiment. The rats were randomly divided into 2 groups with 10 animals in each: control and treated group. The animals in the treated group were administered furan (was dissolved in corn oil) orally at a dose of 8 mg /kg/ day by gastric tube. After 90 days of administration, the rats were sacrificed. Mean of body weight of control and experimental group was measured (Karacaoğlu *et al.*, 2012).

**I-Biochemical study:** Venous blood samples were collected from orbital vein the day before sacrifice from animals of all groups for measurement of serum corticosterone (a.m. corticosterone was obtained at 8 a.m. and p.m. corticosterone was obtained at 8 p.m.). The results were statistically analyzed.

### II-Histological examination

#### Light microscope examination:

**1. Preparation of paraffin sections:** The adrenal gland of one side from each rat was carefully dissected then removed.

Specimens were fixed in Bouin's fixative, dehydrated in alcohol, and impregnated in paraffin blocks. Slides were prepared at a 5  $\mu$ m thickness and stained with 2 different histological stains for light microscope study: hematoxylin & eosin and Mallory's trichrome staining (Kiernan, 2000).

**2. Immunohistochemical examination for BCL-2:** BCL-2 protein (anti-apoptotic marker) was investigated using the avidin biotin peroxidase system. Anti-human BCL-2 mouse monoclonal antibody (purchased from DAKO) was the primary antibody. Paraffin section slides were incubated in the secondary biotinylated anti-mouse antibody (Zymed). Then, the slides were incubated with streptavidin horseradish peroxidase conjugate (Zymed) for 15 minutes, washed in phosphate buffered saline. Color was produced using diaminobenzidine (DAB) for 5 minutes (Kiernan, 2000).

**3. Morphometric study:** Stained sections with Mallory's trichrome and BCL-2 immune reaction were analyzed morphometrically. The data were obtained using Leica Qwin 500 image analyzer computer system in the Histology department, Faculty of Medicine, Cairo University. The area percentage of collagen fibers in the capsule and the area percentage of BCL-2 protein immunoreaction in the cytoplasm of adrenocortical cells were measured.

**III Statistical analysis:** The results of serological test and morphological study were statistically analyzed using SPSS version 14.0. and least significant difference was done to find where the significant to control group. P value < 0.05 was considered a statistically significant. The data were expressed as mean  $\pm$  S.D.

**IV Transmission electron microscope examination:** Small pieces of the suprarenal cortex from the other side of each rat were fixed in 2.5% glutaraldehyde for 24 hours, the specimens were washed in 0.1% M phosphate buffer, 7.4 at 4°C and secondary fixed in 1% phosphate-buffered osmium tetroxide for 30 min. They were dehydrated and then embedded in epoxy resin. Semithin sections (1  $\mu$ m thickness) were obtained and mounted in a drop of water on glass slide and then stained with 1% aqueous toluidine blue stain to be examined with light microscope. Ultra-thin sections were cut and put on copper grids. The grids were double stained with lead citrate and uranyl acetate (Glaurt and Lewis, 1998) for examination by a transmission electron microscope (Joel TEM), at the Histology and Cell Biology Department, Faculty of Medicine, Zagazig University.

## RESULTS

In the studied groups, the rats appeared healthy along the duration of the study. Total body weight and weight gains were comparable for controls and treated group (data not shown here).

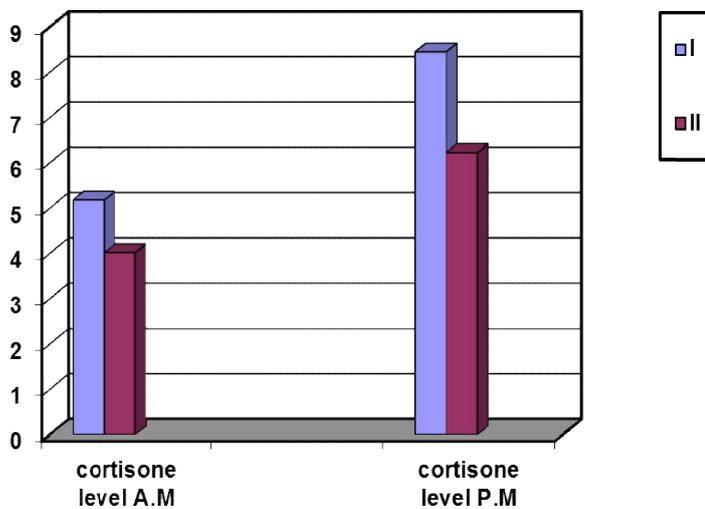
### I- Statistical analysis of biochemical and morphometrical results

#### Serum corticosterone level: –A.M. corticosterone levels

The mean values of serum a.m. corticosterone levels were 5.18  $\pm$  0.179 g/dl in control group and 5.017  $\pm$  0.123 g/dl in group II.

**Table 1. Serum A. M. and P.M. corticosterone levels**

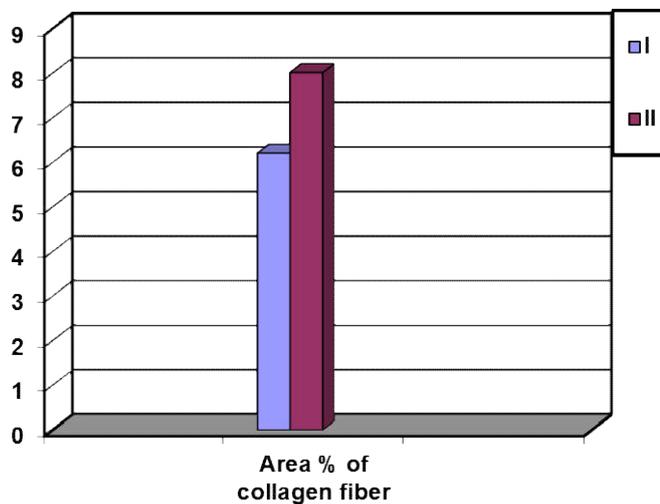
GROUPS /	PARAMETERS	Group I X±SD	GROUP II X±SD
Serum A. M. corticosterone levels	g/dl	5.18 ±0.179	5.017 ±0.123*
Serum P. M. corticosterone levels	g/dl	8.45 ±0.28	6.23 ±0.308*



**Bar chart 1. Serum A. M. and P.M. corticosterone levels**

**Table 2. Area percent of collagen fibers**

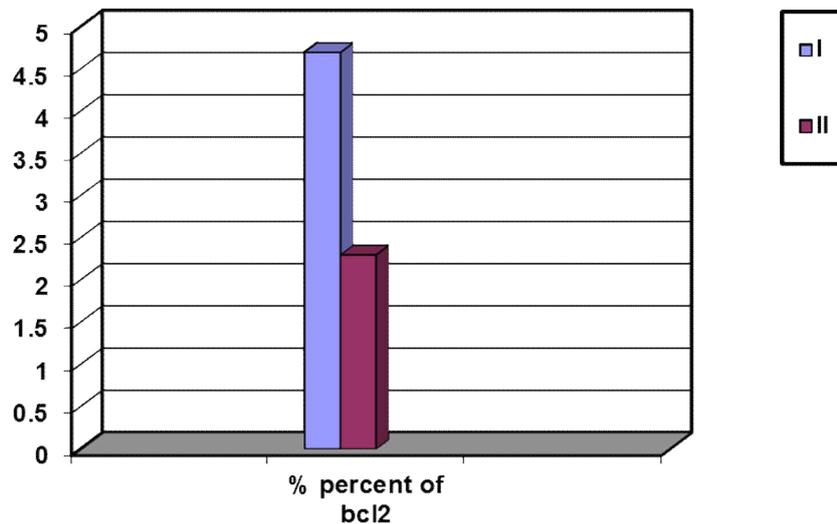
GROUPS /	PARAMETERS	Group I X±SD	GROUP II X±SD
Area percent of collagen fibers		6.2±0.11	8.01 ±0.1*



**Bar chart 2. Area percent of collagen fibers**

**Table 3. Area % of BCL-2 protein reaction**

GROUPS /	PARAMETERS	Group I X±SD	GROUP II X±SD
Area % of BCL-2 protein reaction		4.76 ±1.43	2.95 ±1.13*



Bar chart 3. Area % of BCL-2 protein reaction

**-P.M. corticosterone levels:** The mean values of serum p.m. corticosterone levels were  $8.45 \pm 0.28$  g/dl in control group and  $6.23 \pm 0.308$  g/dl in group II. Statistical analysis of these results revealed that A.M. serum corticosterone level and P.M. serum corticosterone level in group II were significantly decreased in comparison with control (Table 1 & Bar chart 1). Area percent of collagen fibers in the capsule was a significantly increased in the furan-treated group when being compared with that of control group (Table 2 & Bar chart 2). The area percentage of BCL-2 protein reactions in the adrenocortical cells was significantly decreased in the furan-treated group when being compared with that of the control group (Table 3 & Bar chart 3).

**II- Light microscopic results:** H& E stained sections of the control group showed a normal histological structure for the adrenal cortex. The gland was surrounded by a thin connective tissue capsule and contained cortex and medulla. The cortex was formed of three zones; zona glomerulosa, zona fasciculata and zona reticularis. The zona glomerulosa (area under the capsule of the adrenal cortex) was formed of small closely arranged cells with rounded densely stained nuclei. They were arranged in oval or curved clusters. The cells of zona fasciculata were longitudinally arranged in parallel cords separated by blood sinusoids. Their cytoplasm was acidophilic and vacuolated. Their cells had rounded vesicular nuclei with prominent nucleoli (Figure 1a, b). Cells of zona reticularis were arranged in anastomosing cords that were separated by blood sinusoids. The cells were small, closely packed with densely stained nuclei (Figure 1c, d). Haematoxylin and eosin-stained sections of furan- treated group revealed the three zones of the adrenal cortex. The zona glomerulosa cells were disturbed in arrangement. They contained dark nuclei and vacuolated cytoplasm. Areas of hyperplasia were also seen under the capsule (Figure 2a, b). The parallel arrangement of the zona fasciculata cells was lost. Most cells had darkly stained nuclei and their cytoplasm appeared pale and vacuolated. The nuclei of zona reticularis cells were darkly stained and their cytoplasm was moderately vacuolated (Figure 2c, d). In Mallory's trichrome stained sections of control group, few collagen fibers in the capsule were seen. Furan-treated

group revealed a moderate amount of collagen fibers in the capsule (Figure 3a, b). Immunohistochemically, positive immune reaction of BCL-2 protein was noticed in the cytoplasm of many adrenocortical cells in the control group. However, furan-treated group showed positive immune reaction of BCL-2 protein in the cytoplasm of few adrenocortical cells (Figure 3c, d). Toluidine blue stained sections of control group revealed the gland surrounded by a capsule enclosing the underlying arches of zona glomerulosa which are separated by blood sinusoids. Zona glomerulosa cells contained rounded euchromatic nuclei and some cytoplasmic lipid droplets (Figure 4a). Cell cords of zona fasciculata appeared large and polyhedral and were separated by blood sinusoids. Most of cells had rounded nuclei with prominent nucleoli and numerous cytoplasmic lipid droplets. In zona reticularis, blood sinusoids were present between its anastomosing cell cords. The cells were closely packed and their cytoplasm contained few lipid droplets (Figure 4b). Treated group revealed thick capsules. Distorted architecture of zona glomerulosa cells with numerous lipid droplets was seen. Some cells showed darkly stained irregular nuclei. Some nuclei of the zona fasciculata cells were darkly stained and shrunken. The cytoplasm contained numerous lipid droplets (Figure 4c). Zona reticularis cells had many lipid droplets. Some cells had pale nuclei with prominent nucleoli, whereas others showed irregular darkly stained nuclei (Figure 4d).

### III- Electron microscopic results:

Ultrathin sections in a control rat's adrenal cortex demonstrated rounded euchromatic nuclei with prominent nucleoli in the cells of zona glomerulosa. The cytoplasm contained numerous mitochondria, smooth endoplasmic reticulum, free ribosomes and some lipid droplets (Fig. 5a). Zona fasciculata cells showed regular outline, euchromatic nuclei. The cytoplasm contained high content of lipid droplets of different sizes. Mitochondria were also present (Fig. 5b). Cells of zona reticularis contained rounded euchromatic nuclei. The cytoplasm contained many mitochondria and few lipid droplets (Fig. 5c).

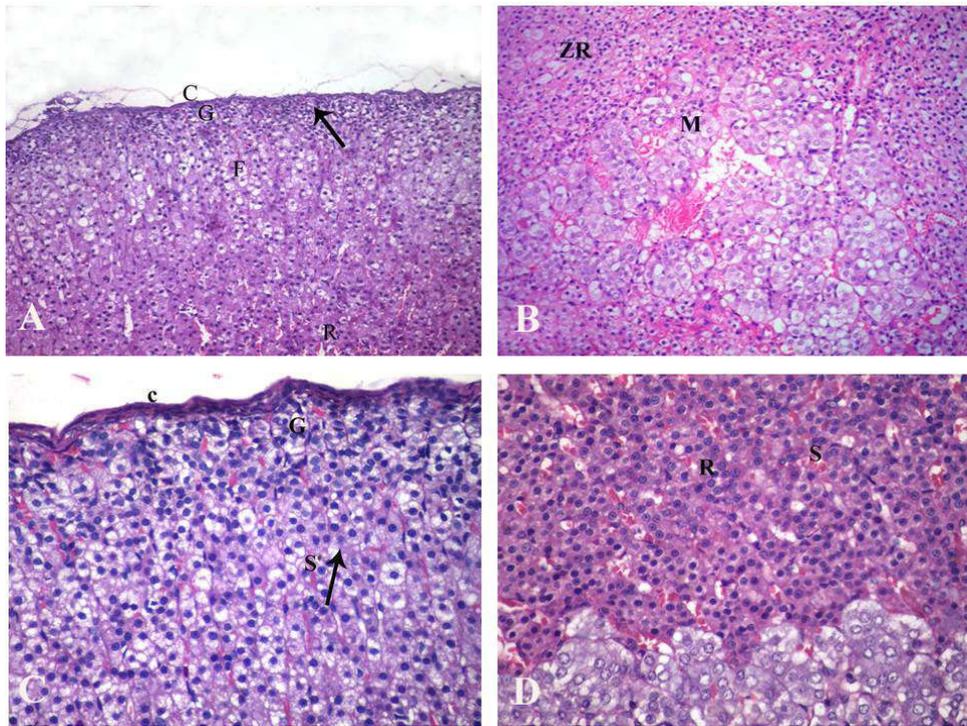


Figure (1): H&E of the control adrenal cortex showing (a) a thin capsule (C) with underlying zona glomerulosa arranged in oval (G) or curved clusters (arrow). Zona fasciculata cells (F) are arranged in parallel cords (b) cells of zona reticularis (R) with anastomosing cords and a part of the medulla (M) are also seen 200×. (c) The capsule (C) and underlying closely arranged cells of the zona glomerulosa (g) with acidophilic cytoplasm and rounded densely stained nuclei are seen. Large polyhedral fasciculata cells (arrow) are arranged in long straight columns and are separated by blood sinusoids (S). They have acidophilic, vacuolated cytoplasm and vesicular rounded nuclei with prominent nucleoli. (d) ZR cells (R) are small, closely packed with deeply stained nuclei arranged in anastomosing cords which are separated by blood sinusoids(s) 400×

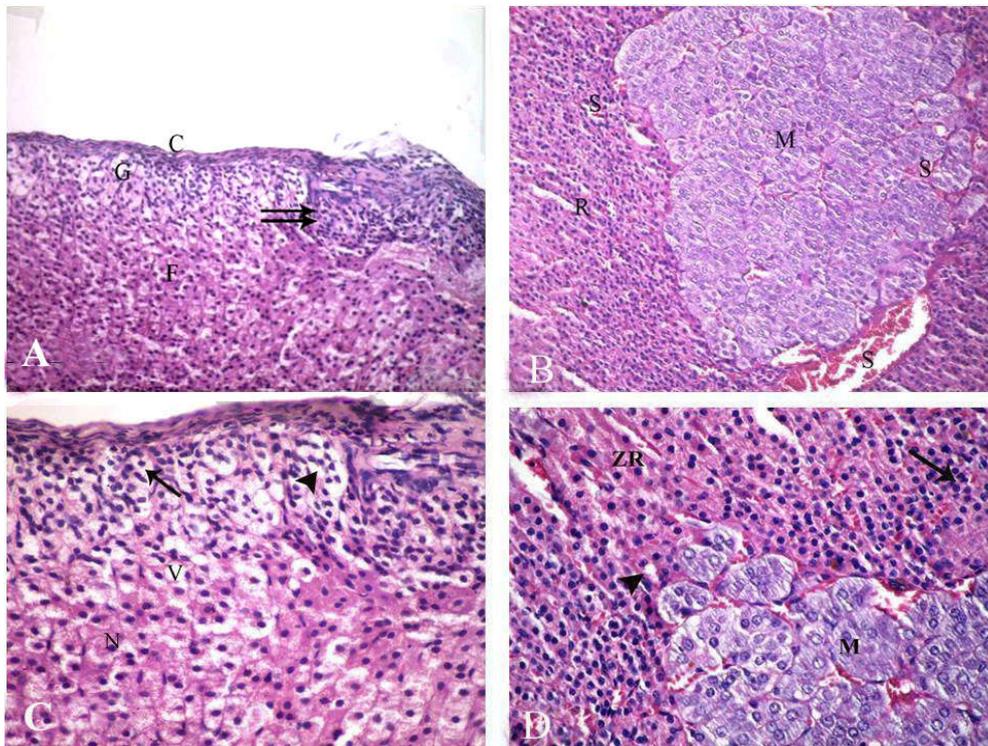


Figure (2): H&E of furan-treated group showing (a) disturbed arrangement of the cells of zona glomerulosa (G) and fasciculata (F). Areas of hyperplasia (double arrows) are seen under the capsule (C). (b) Zona reticularis (R) and medulla (M) possess congested blood sinusoids (s) 200×. Adrenal cortex of furan-treated group showing (c) cells of zona glomerulosa containing darkly stained nuclei (arrow) and vacuolated cytoplasm (arrowhead). Most cells of zona fasciculata appear pale with extensive vacuolations (V) and darkly stained nuclei (N). (d) Cells of the zona reticularis show darkly stained nuclei (arrow) and vacuolated cytoplasm (arrowhead) 400×

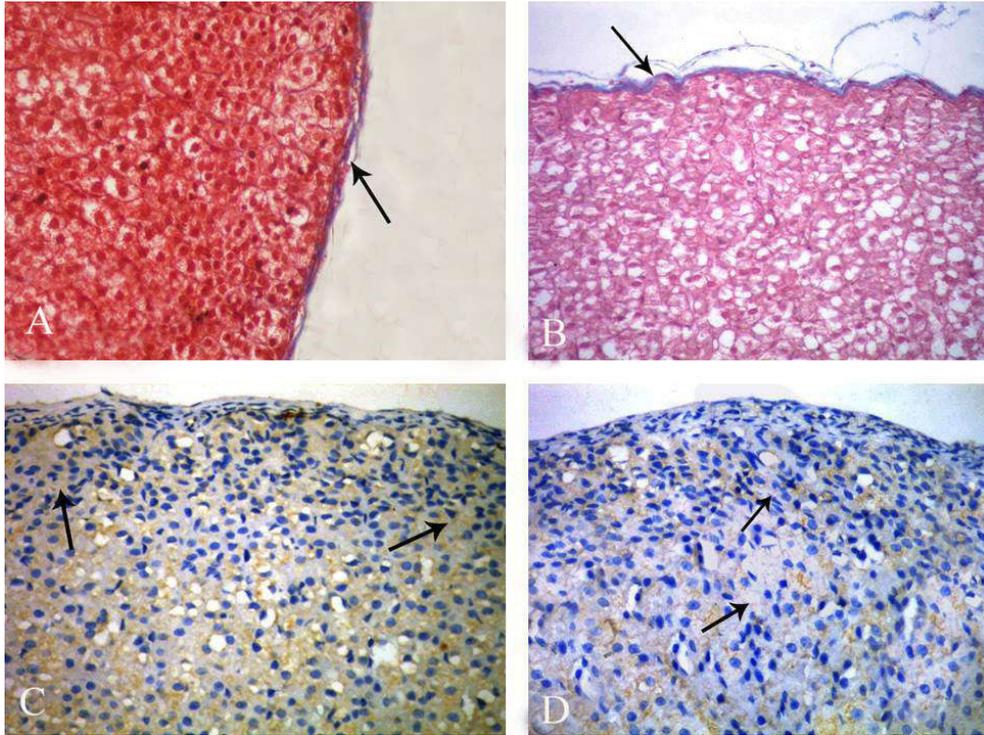


Figure (3): Mallo'ry's trichrome of (a) a control adrenal cortex showing few collagen fibers in the capsule (arrow). (b) adrenal cortex of furan-treated group showing moderate amount of collagen fibers in the capsule (arrow). (c) A control adrenal cortex showing positive immune reaction for BCL-2 protein (arrow) in the cytoplasm of many adrenocortical cells. (d) Adrenal cortex of furan-treated group showing positive immune reaction of BCL-2 protein (arrow) in the cytoplasm of few adrenocortical cells 400 $\times$

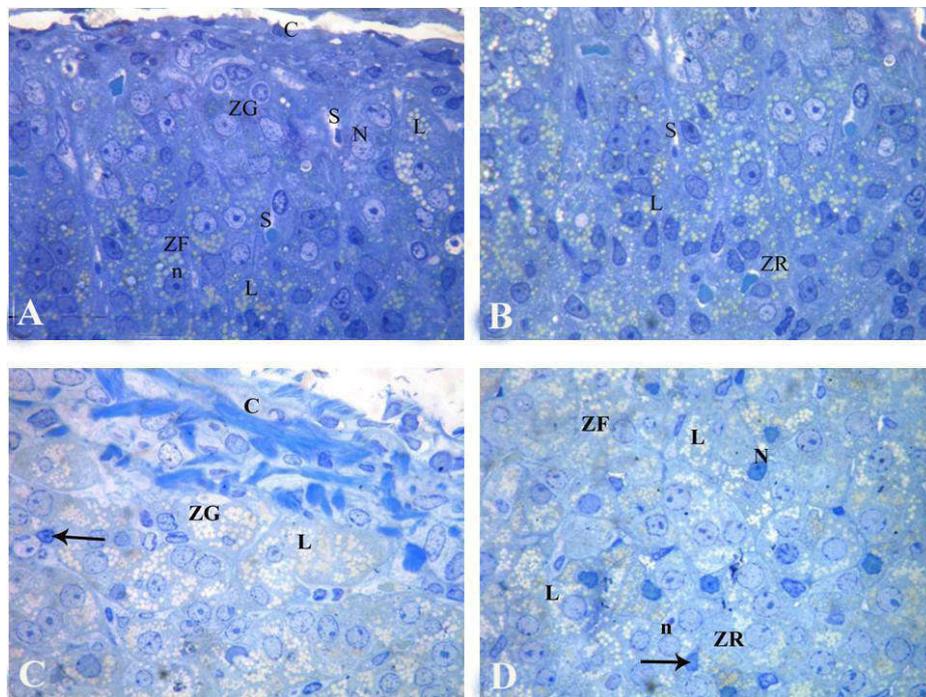


Figure (4): A semithin section in the control adrenal cortex showing (a) the capsule (C). Underlying arches of zona glomerulosa (ZG) are separated by blood sinusoids (S). Zona glomerulosa cells contain rounded euchromatic nuclei (N) and some cytoplasmic lipid droplets (L). Parallel cords of zona fasciculata (ZF) separated by blood sinusoids (S) are seen. The cells are large and polyhedral. Most of cells have rounded nuclei with prominent nucleoli (n) and numerous cytoplasmic lipid droplets (L). (b) anastomosing cords of the zona reticularis cells (ZR) and blood sinusoids (S) are present between the cords. The cells are closely packed and their cytoplasm contains few lipid droplets (L). A semithin section in adrenal cortex of furan-treated group showing (c) thick capsule (C) and zona glomerulosa (ZG) cells with numerous unstained lipid droplets (L). Some cells show darkly stained irregular nuclei (arrow). (d) the zona fasciculata (ZF) cells revealing some darkly stained shrunken nuclei (N). The cytoplasm contains numerous unstained lipid droplets (L). Zona reticularis (ZR) cells have many lipid droplets (L). Some cells have pale nuclei with prominent nucleoli (n), whereas others show irregular deeply stained nuclei (arrows) Toluidine blue 1000 $\times$

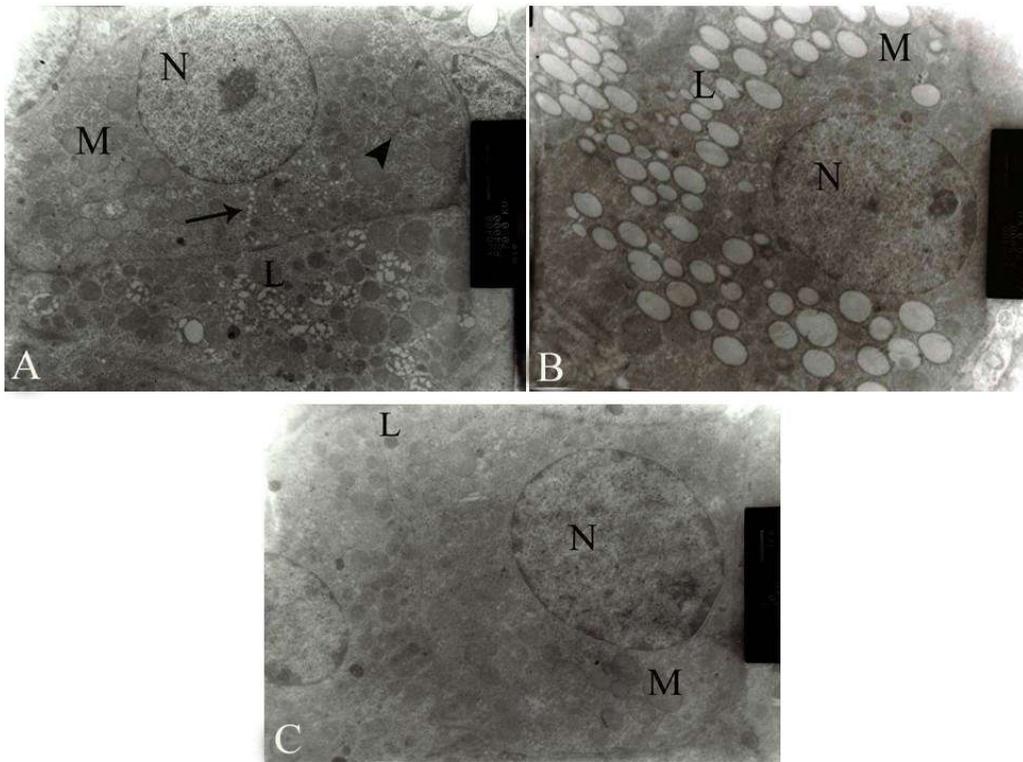


Figure (5): A transmission electron micrograph of a rat's adrenal cortex showing (a) cells of zona glomerulosa. They had rounded euchromatic nuclei (N) with prominent nucleoli. The cytoplasm contains numerous mitochondria (M), smooth endoplasmic reticulum (arrow), free ribosomes (arrowhead) and some lipid droplets (L) 5000 $\times$ . (b) zona fasciculata cells with regular outline, euchromatic nuclei (N). The cytoplasm contains high content of lipid droplets in different sizes (L). Mitochondria (M) are also seen 4000 $\times$ . (c) cells of zona reticularis cells with rounded euchromatic nuclei (N). The cytoplasm has many mitochondria (M) and few lipid droplets (L) 5000 $\times$ .

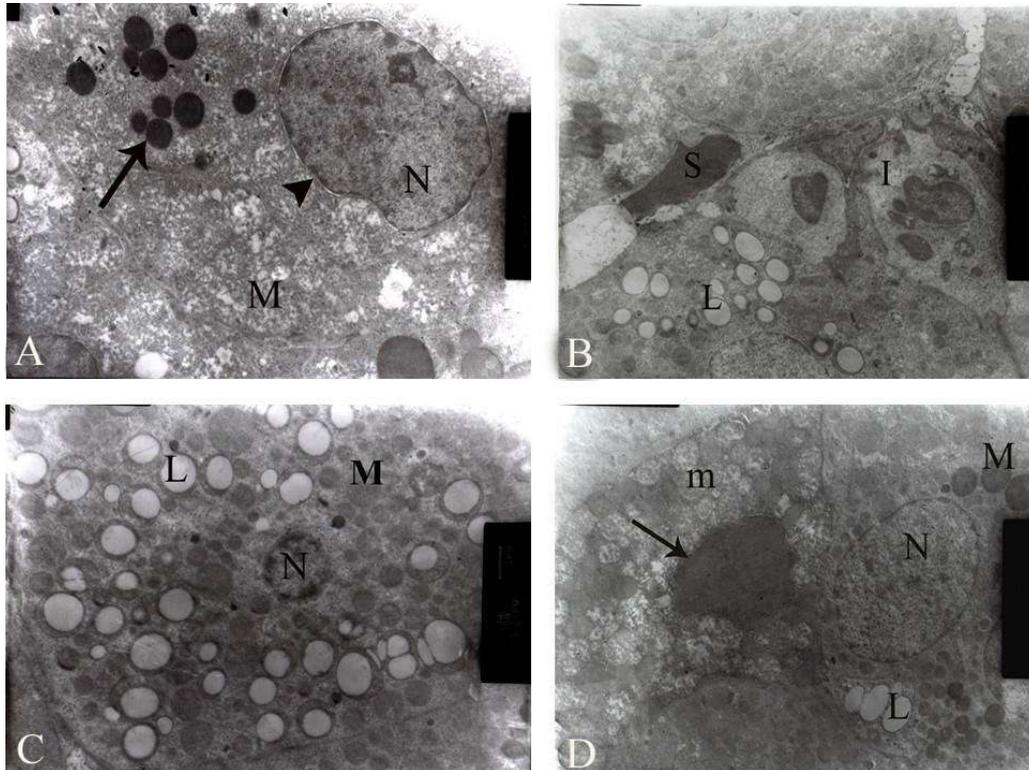


Figure (6): A transmission electron micrograph of furan- treated group showing (a) cells of zona glomerulosa with numerous swollen mitochondria (M) with disrupted cristae and lipid droplets (arrows). Nucleus (N) with irregular nuclear envelope and widened perinuclear space (arrowhead) is also seen 5000 $\times$ . (b) parts of adrenal cortical cells that contain some lipid droplets (L). Infiltrating cells (I) are located near to a blood sinusoid (S) 4000 $\times$ . (c) cells of zona fasciculata with an electron dense apoptotic nuclei (N). The cytoplasm contains mitochondria (M) and many lipid droplets (L) 5000 $\times$ . (d) zona reticularis cells with one of them has euchromatic nuclei (N), mitochondria (M) and lipid droplets (L). The other cell has a nucleus (arrow) with an irregular outline and condensed chromatin. Multiple swollen mitochondria (m) with disrupted cristae are also observed 4000 $\times$ .

Furan-treated group demonstrated thick capsule and zona glomerulosa cells that had many swollen mitochondria with disrupted cristae and lipid droplets. Nuclei appeared with irregular nuclear envelope and widened perinuclear space (**Fig. 6a**). Infiltrating cells were also located near to blood sinusoids (**Fig. 6b**). Zona fasciculata cells had electron dense apoptotic nuclei. The cytoplasm contained mitochondria and multiple lipid droplets (**Fig. 6c**). Some of cells of zona reticularis had euchromatic nuclei, mitochondria and lipid droplets. Others had nuclei with irregular outline and condensed chromatin. Multiple swollen mitochondria with disrupted cristae were also seen (**Fig. 6d**).

## DISCUSSION

The endocrine system is an essential system of the body that plays an important role in the regulation of metabolic processes such as maintenance of homeostasis, regulation of growth maturation, reactions to exterior stimulations (stress, infection, etc.), and regulation of reproduction (Clark and Van Leeuwen, 1990; Mayer and Hancock, 2010). Various factors (environmental factors, chemicals, physiological factors, etc.) are known to predispose the endocrine glands to toxicity. Disorders of endocrine glands result in disease, the effects of which may proceed to many organs and functions (WHO 2002). The suprarenal gland is found to be the most common endocrine organ associated with chemically induced lesions. It is important to understand the structure and function of the adrenal gland to correctly interpret the significance and mechanisms of drug-induced lesions. The adrenal cortex is required for life, especially the secretion of aldosterone (Ribelin, 1984). The adrenal gland is composed of 2 parts, an outer cortex and an inner medulla, which both secrete hormones essential for life. Catecholamines are secreted under the control of the sympathetic nervous system by the adrenal medulla (Chan *et al.*, 2011) and steroid hormones are produced by the adrenal cortex. Failure of adrenal function may result in some disorders involving the electrolyte and carbohydrate metabolism (Kempná and Flück, 2008). The present work was performed to evaluate the possible effects of a heat-produced food contaminant (furan) on the adrenal cortex of adult male albino rats. In the present work, furan administration caused disturbed arrangement of cells of zona glomerulosa and loss of parallel arrangement of the zona fasciculata cells. A significantly increased area % of collagen fibers was found in the capsule. Areas of hyperplasia were also seen. Most cells had darkly stained nuclei and their cytoplasm appeared pale and vacuolated. Similarly, some investigators demonstrated that furan induced some histopathological changes in the cortex of the adrenal gland such as mononuclear cell infiltration, fibrosis, and hyperplasia. They added that adrenal injury may influence the hormonal activity of the adrenal cortex such as changes in serum corticosterone levels (Karacaoğlu *et al.*, 2012). Also, it was reported that chronic toxicity of adrenal cortex can result in atrophy, nodular formation, fibrosis, or proliferation of cortical cells (Latendresse *et al.*, 1995; Nyska *et al.*, 1999). Furan induced increased incidences of numerous non-neoplastic lesions in treated rats. These lesions were in the form of biliary tract fibrosis, hyperplasia, chronic inflammation, and hepatocyte cytomegaly with cytoplasmic vacuolations. Moreover, the severity of the lesions were

increased with dose (Koëter, 2004; Gill *et al.*, 2011). The adrenal cortex produces both mineralocorticoids and glucocorticoids. The precursor of steroid hormones is cholesterol, which has the 17- carbon steroid nucleus. Cholesterol is converted into steroid hormone intermediates and mature hormones by cytochrome P-450 enzymes in the mitochondria and smooth endoplasmic reticulum. Synthesis starts in the mitochondria, continues in the endoplasmic reticulum, and is completed in the mitochondria. So, shuttling of steroid hormone precursors between the 2 cytoplasmic compartments is essential in the multiple steps of hormone synthesis. Secretion of steroid hormones is immediate because of their lipid solubility, and there is a lack of storage of the preformed hormone in the cortical cells. So, blood concentrations reflect the rate of hormone synthesis. Steroid hormones secretion follows a circadian rhythm (Latendresse *et al.*, 1993). It is useful to measure the function of the adrenal cortex. This can be done by measuring blood glucocorticoid hormone levels. In the present study, statistical analysis of the biochemical results revealed that a.m. and p.m. serum corticosterone level in the furan-treated group was significantly decreased when compared with the control group. Furan induced a decrease in testosterone level in a dose-dependent manner and also histopathological changes were observed in the male genital organs of rats. Apoptotic cells in testis increased significantly. The administration time may be an important factor affecting the results of toxic effects (Karacaoğlu and Selmanoğlu, 2010). Also, it is evident that subchronic furan exposure affects testicular steroidogenesis (Cooke *et al.*, 2014). At the level of the transmission electron microscope, the cells of ZF and ZR of the same group showed electron- dense nuclei with condensed chromatin. In addition, cells of ZG showed irregular nuclei with widening in the perinuclear space. This may be an indicator for apoptotic changes that were confirmed by a significantly increased area percent of BCL-2 protein immune reaction in the cytoplasm of many adrenocortical cells. It was reported that furan induced apoptosis in mice at doses of 8 and 15 mg/kg b.w., possibly due to an increase in DNA damage (Fransson-Steen *et al.*, 1997). It was demonstrated that furan is converted into cis-2-butene-1,4-dialdehyde, which is a cytotoxic metabolite and binds to proteins and nucleosides irreversibly (Burka *et al.*, 1991; Crew and Castle, 2007). In addition, the cytotoxic metabolite cis-2-butene-1,4-dialdehyde causes cell proliferation and uncoupling of mitochondrial oxidative phosphorylation (Mugford *et al.*, 1997; Kedderis and Ploch, 1999).

Hepatocytes in furan-treated rats showed 95 % loss of ATP, due to uncoupling mitochondrial oxidative phosphorylation. This leads to depletion of the energy stores, with activation of cytotoxic enzymes, including endonucleases that induce DNA double strand breaks prior to cell death (necrosis or apoptosis) (Kedderis and Ploch, 1999). In our present work, the ultrastructure of cells of ZG and ZR after furan administration showed multiple swollen mitochondria with disrupted cristae. Also, there was an accumulation of lipid droplets possibly due to impaired steroidogenesis. These findings may explain the increased vacuolation seen by LM. Adrenal cortical cells contain large lipid stores that used as substrate for steroidogenesis. Many compounds which are toxic for the adrenal cortex are lipophilic and accumulate in these cells that

are lipid-rich cells (Rosol *et al.*, 2001). An important mechanism of toxicity in the adrenal cortex is the impaired steroidogenesis. It can be resulted from disruption of cytochrome P-450 enzymes or inhibition of cholesterol biosynthesis or metabolism. Both of these mechanisms will induce the accumulation of increased cytoplasmic lipid in the form of droplets. Impaired steroidogenesis is a common toxin-induced change that can lead to excess steroid precursors and cytoplasmic vacuolation. Firstly, the cytoplasmic vacuoles are small, but then they coalesce to produce larger vacuoles (Latendresse *et al.*, 1994). Also, it was reported that furan caused an increase in free radicals and lipid oxidation (Wang *et al.*, 2014). Reduction of furan formation during food manufacture is difficult because foods must be heated to ensure the microbiological safety. However, domestic cooking procedures reduce the furan content of foods. Brewing coffee causes a dramatic decrease, and stirring food or leaving it to stand is also effective. Furan can be determined by headspace-sampling gas chromatography with mass spectrometric detection (Crews, 2014). It was reported that apigenin had protective effects against furan-induced toxicity changes mainly due to its high capability of scavenging free radicals and inhibiting lipid oxidation. This is important for considering the administration of apigenin as a dietary supplement to protect against changes of furan toxicity (Wang *et al.*, 2014). The present findings indicate that furan induced histopathological and functional changes in the adrenal cortex of adult male albino rats. So, it is recommended using freshly home prepared food to overcome the hazards of furan produced during food processing. Also, further research work is advised to evaluate the use of apigenin as a dietary supplement for protection against changes induced by furan toxicity.

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