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journal homepage: <http://ees.elsevier.com/apjtm>Original research <https://doi.org/10.1016/j.apjtm.2017.10.019>Rescue effects of aqueous seed extracts of *Foeniculum vulgare* and *Carum carvi* against cadmium-induced hepatic, renal and gonadal damage in female albino ratsAhmed Abdel-Wahab¹, Abdel-Razik Hashem Abdel-Razik², Rabie Lotfy Abdel Aziz³¹Physiology Department, Faculty of Veterinary Medicine, Minia University, 61519 El-Minia, Egypt²Histology Department, Faculty of Veterinary Medicine, Beni-Suef University, 62512 Beni-Suef, Egypt³Department of Theriogenology, Faculty of Veterinary Medicine, Beni-Suef University, 62512 Beni-Suef, Egypt

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ABSTRACT

Objective: To investigate the protective effects of aqueous seed extracts of fennel "*Foeniculum vulgare*" (FVE) and caraway "*Carum carvi*" (CCE) on liver, kidney and reproductive organs in female rats against cadmium chloride (CC) intoxication.

Methods: A total of 36 adult female rats were divided into six groups, six in each group. Control group (fed normal diet), CC-treated group (50 mg CC/kg diet), CCE-treated group (150 mg CCE/kg diet), CCE + CC group, FVE (150 mg/kg diet) and FVE + CC. One month later, all rats were sacrificed and all samples were collected at proestrus phase.

Results: The toxic effects of CC were confirmed biochemically by significant increase of serum concentration of liver enzymes ($P < 0.05$), and creatinine ($P < 0.001$). Moreover, CC increased significantly the serum level of malondialdehyde (MDA) and decreased the total antioxidant capacity (TAC) ($P < 0.001$). In addition, serum concentrations of estrogen, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were significantly decreased ($P < 0.01$). Histopathologically, CC-treated group revealed marked pathological changes in renal, hepatic, ovarian and uterine tissues. All toxic findings observed in liver and kidney with CC treatment were found to be ameliorated markedly after co-treatments with FVE or CCE. Furthermore, co-treatment of FVE with CC improved significantly all studied reproductive parameters ($P < 0.01$).

Conclusions: Both FVE and CCE could be used as efficient treatments for liver and kidney against CC intoxication. Moreover, FVE could be utilized as a potent treatment to protect and improve female fertility from cadmium intoxication.

1. Introduction

Cadmium (Cd) is one of the common environmental pollutants and animals are at increased risk of exposure to cadmium [1]. Cadmium was found to induce oxygen free radical production mainly the hydroxyl radicals [2]. Oxidative stress affects many physiological processes, including reproduction [3]. In addition, administration of male rats with 5 mg/kg bw cadmium chloride (CC) was observed to degenerate the testicular tissues and to decrease the enzymatic and none enzymatic antioxidants [4].

Also, cadmium is a known endocrine disruptor by influencing the regulation of many hormones in embryos [5]. Also, it was found that liver, kidney and gonads of rats were significantly damaged with cadmium exposure [6,7].

Herbal medicine today became an important approach in solving many healthy problems instead of using traditional medicine due to its lesser side-effects and lower costs [8,9]. *Foeniculum vulgare* (*F. vulgare*), known as fennel, is a medicinal plant belong to family Apiaceae and it is local to the Mediterranean zone [10] including Egypt [11]. Biochemical screening of the *F. vulgare* revealed its containing for high concentrations of phytoestrogens including isoflavones, coumestans, and lignans [12]. *F. vulgare* has several medicinal properties including carminative, diuretic, expectorant, laxative, analgesic and stimulant of gastrointestinal mobility [13]. Additionally, it was reported that *F. vulgare* has an

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antioxidant properties and has the power to keep the normal activities of liver and kidney [14,15]. *F. vulgare* is highly recommended for diabetes, respiratory disorders and for kidney stones [16]. In addition, Nazari and Roozbehani found that feeding fish with different concentration of *F. vulgare* seed extract (75, 100 and 125 $\mu\text{L/g}$ diet) resulted in significant increase in weight gain and fertility rate as well as enlarging granulosa layer with increase in number of ova in different evolution stage [17]. In human, *F. vulgare* was found to support milk production during lactation, stimulate menstrual flow, facilitate birth and improve libido in males [11].

Carum carvi (*C. carvi*) or caraway is a medicinal plant belonging to family Apiaceae and it is located to Asia, Africa and Europe [18]. It was documented that *C. carvi* could be used as a treatment for oxidative stress disorders [19], microbes [20], cancer [21] and stressful conditions [22]. In addition, it has great benefits in treatment of gastrointestinal disorders [23]. *C. carvi* could be used as diuretic without any renal side effects [24]. Also, it acts as a protective medicinal plant against many disorders including ulcers [25], neoplastic disease [26], proliferative disorders [27] and hyperglycemic conditions [28]. Moreover, Thakur *et al.* reported that *C. carvi* showed a significant decrease in the levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) while amount of estrogen was found to be increased [18].

In Egyptian folk medicine, it has been claimed that sexual function and fertility in females are modulated by *F. vulgare* or *C. carvi*; however, the exact mechanism of their action is still blurred. Additionally, their effect on kidney and liver functions is needed to be more understood. Moreover, the effects of *F. vulgare* and *C. carvi* aqueous seed extracts (FVE and CCE for short respectively) against cadmium toxicity on liver, kidney and reproductive capacity are not willful till now. Thus, this study aimed to investigate the effects of FVE or CCE administration in female albino rats on the liver, kidney and reproductive activities under the normal conditions. Additionally, this study examined the efficiency of FVE or CCE to protect liver, kidney and fertility against cadmium toxicity which could provide cheaper and potent treatments to protect animals from the increased risk of cadmium exposure.

2. Material and methods

2.1. Ethics statement

All of the experimental procedures involving animals were implemented according to the US guidelines (NIH publication#85-23, revised) [29].

2.2. Rats

The present work was completed on 36 matures female albino rats with weight of (150 ± 10) g. They were purchased from the Physiology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. Before starting the experiments, the rats remained for 3 weeks for adaptation.

2.3. Preparation of plant extracts

The seeds of *F. vulgare* and *C. carvi* were purchased from herbal medicine store in EL-Minia, Egypt. The seeds were

identified by Department of Botany of Science at Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

With an electrical grinder (Moulinex, France), *F. vulgare* seeds were grounded into powdered materials. To get the aqueous extract, 200 g of the obtained powdered materials were dissolved in 800 mL of distilled water and then were kept in a refrigerator for 24 h. The extract is then filtered and dried in vacuum till obtaining 6.4 g of dried powder per 200 g *F. vulgare* seeds. FVE was administered at a dose of 150 mg/kg diet according to European Food Safety Authority (EFSA) [30]. The CCE was prepared according to Thakur *et al.* [18]. Briefly, the seeds of *C. carvi* were ground by an electrical mill. The powder was extracted with distilled water. Then, the extracts were concentrated and dried in a vacuum evaporator. Extract were weighed and kept at 4 °C in refrigerator until use. CCE treatment was administered at dose of 150 mg/kg diet.

2.4. Experimental design

The 36 female rats were divided into six groups, six rats in each group. The six treatment groups were 1) control (C group) fed a daily normal diet; 2) CC group fed a daily normal diet with CC at dose of 50 mg/kg diet (Sigma Aldrich, USA) [31]; 3) CCE group fed a daily normal diet with CCE at dose of 150 mg/kg diet; 4) CCE + CC group fed a daily normal diet with CCE at dose of 150 mg/kg diet plus 50 mg CC/kg diet; and 5) FVE group fed a daily normal diet with aqueous seed extract of FVE at dose of 150 mg/kg diet. 6) FVE + CC group fed a daily normal diet with FVE at dose of 150 mg/kg diet plus 50 mg CC/kg diet.

In all groups, animals were fed their corresponding diet daily for 4 successive weeks. Then, all animals were sacrificed 1 month later (6 estrus cycles) at proestrus stage, determined by the vaginal smear method described by Sahar *et al.* [32].

2.5. Collection of serum and tissue samples

At the end of the experiment, rats were sacrificed during proestrus phase. Serum samples were collected and kept at -20 °C until using for analysis.

In addition, the liver, kidney, ovary and uterus were excised and fixed using Bouin's fixative. Then, the organs were routinely processed and sectioned at 4–5 mm thickness. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin stain, and Periodic acid–Schiff (PAS) technique [33]. The sections are then examined and observed under light microscope at 100, 200, 400 and 1000 \times magnification.

2.6. Detection of malondialdehyde (MDA) and total antioxidant activities

Serum level of MDA and total antioxidant activities were determined using commercial kits (Sigma–Aldrich, USA) according to Valko M *et al.* [34] and Qiao YF *et al.* [35].

2.7. Detection of liver enzymes

The levels of aspartate transaminase (AST) and alanine aminotransferase (ALT) enzymes were estimated in serum according to Reitman and Frankel [36] using the colorimetric kits (Sigma–Aldrich, USA).

2.8. Determination of serum levels of sex hormones and gonadotropins

With the radioimmunoassay technique, sex steroid hormones (estrogen and progesterone) in serum were assessed as defined by Jaffe and Behrman [37]. FSH and LH in serum were measured using rat FSH and LH kits (Elabscience Biotechnology Co., Wuhan, China) using the guide of the manufacturer's protocol.

2.9. Statistical analysis

All values were expressed as mean \pm standard error (SE). The differences were analyzed using one-way analysis of variance (ANOVA). The P -value < 0.05 reflects significant differences.

3. Results

3.1. Effects of FVE and CCE either alone or in combination with CC on serum levels of liver enzymes

Data showing the effects of CCE and FVE either alone or in combination with CC on serum levels of liver enzymes are represented in Table 1. It was noticeable that CC treatment significantly increased the levels of AST and ALT enzymes in comparison to control and other treatments ($P < 0.05$). On the other side, CCE and FVE significantly reduced the levels of AST and ALT in comparison to CC-treated group ($P < 0.05$). Moreover, co-administration of CCE or FVE with CC ameliorated significantly the toxic effect of CC ($P < 0.05$) and restored the levels of the AST and ALT enzymes to normal levels.

3.2. Effects of FVE and CCE either alone or in combination with CC on serum levels of creatinine

Effects of CCE and FVE either alone or in combination with CC on serum levels of creatinine are also expressed in Table 1. Serum creatinine level was increased significantly in the CC-treated group in comparison to control and other treatments ($P < 0.001$). On the other side, there were not any significant differences between the values of CCE and FVE treatments and control values. In addition, co-administration of CCE or FVE with CC reduced significantly the elevated creatinine serum level observed after CC administration toward normal values ($P < 0.001$).

Table 1

Effects of aqueous seed extracts of *C. carvi* and *F. vulgare* (CCE and FVE for short respectively) alone or in combination with cadmium chloride (CC) on serum levels of aspartate transaminase (AST), alanine aminotransferase (ALT) and creatinine (mean \pm SE).

Treatment	AST (U/L)	ALT (U/L)	Creatinine (mg/dL)
Control	140.75 \pm 3.41 ^a	28.54 \pm 0.65 ^a	0.70 \pm 0.01 ^a
CC	174.11 \pm 1.57 ^b	49.82 \pm 2.59 ^b	4.60 \pm 0.02 ^b
CCE	101.75 \pm 3.69 ^d	29.38 \pm 1.48 ^a	0.76 \pm 0.02 ^a
CCE + CC	134.05 \pm 4.07 ^{ac}	33.59 \pm 0.82 ^a	1.20 \pm 0.02 ^c
FVE	133.19 \pm 3.57 ^{ac}	29.38 \pm 1.19 ^a	0.71 \pm 0.02 ^a
FVE + CC	142.86 \pm 2.15 ^{ac}	32.01 \pm 1.69 ^a	1.92 \pm 0.03 ^d

In the first and second columns, values with different superscript letters revealed significant difference from each other ($P < 0.05$). In the third column, values with different superscript letters revealed significant difference from each other ($P < 0.001$).

3.3. Effects of FVE and CCE either alone or in combination with CC on serum levels of total antioxidant capacity (TAC) and MDA

Data in Table 2 depicts that serum levels of TAC were reduced significantly after CC administration ($P < 0.001$) while the serum levels of MDA were elevated significantly when compared to control and other treatments ($P < 0.001$). On the contrary, administration of CCE or FVE either alone or in combination with CC resulted in a significant increase of serum levels of TAC ($P < 0.001$) and significantly decrease the serum levels of MDA in comparison to the CC group ($P < 0.001$). Also, administration of CCE or FVE either alone or in combination with CC induced a significant improvement in the serum levels of TAC when compared with the corresponding values of control group ($P < 0.001$).

Table 2

Effects of aqueous seed extracts of *C. carvi* and *F. vulgare* (CCE and FVE for short respectively) alone or in combination with cadmium chloride (CC) on serum levels of total antioxidants capacity (TAC) and malondialdehyde (MDA) (mean \pm SE).

Treatment	TAC (mmol/L)	MDA (nmol/mL)
Control	2.21 \pm 0.05 ^a	1.15 \pm 0.02 ^a
CC	1.64 \pm 0.01 ^b	1.60 \pm 0.04 ^b
CCE	2.50 \pm 0.01 ^c	0.86 \pm 0.01 ^c
CCE + CC	2.49 \pm 0.01 ^c	1.02 \pm 0.06 ^d
FVE	2.51 \pm 0.02 ^c	0.65 \pm 0.01 ^c
FVE + CC	2.48 \pm 0.03 ^c	0.73 \pm 0.01 ^{ce}

Values with different superscript letters revealed significant difference from each other ($P < 0.001$).

3.4. Effects of FVE and CCE either alone or in combination with CC on liver and kidney histopathological findings

Histologically, with light microscopic examination, the liver structure appeared normally in case of control (Figure 1A), CCE- or FVE-treated groups (Data not shown). The hepatocytes and intervening blood sinusoids arranged in strands radiating from the central vein. The blood sinusoids appeared to be lined by Van-Kupffer cells contained large nuclei. Hepatocytes are large and polyhedral in shape with slightly acidophilic granular cytoplasm. Their nuclei are large, rounded, vesicular and contain prominent nucleoli. Also, the control (Figure 2A), CCE- or FVE-treated groups (Data not shown) showed a strong fuchsinophilic reaction in the hepatocytes.

The cortex of kidney in the control (Figure 3A), CCE- or FVE-treated groups (Data not shown) was mostly occupied by renal corpuscles and the surrounding convoluted tubules (proximal and distal tubules). The renal corpuscle is formed of the glomerular blood capillaries surrounded by capsular space and Bowman's capsule. The proximal convoluted tubules are lined with large cuboidal cells with deeply stained acidophilic cytoplasm with apical brush border and rounded vesicular nuclei. The distal convoluted tubules are lined by low cuboidal cells with distinct cell boundaries and less acidophilic cytoplasm. Also, the sections of renal tissue showed strong periodic acid–Schiff positive (PAS +ve) material in the basement membrane and brush borders of the tubules of control groups (Figure 4A).

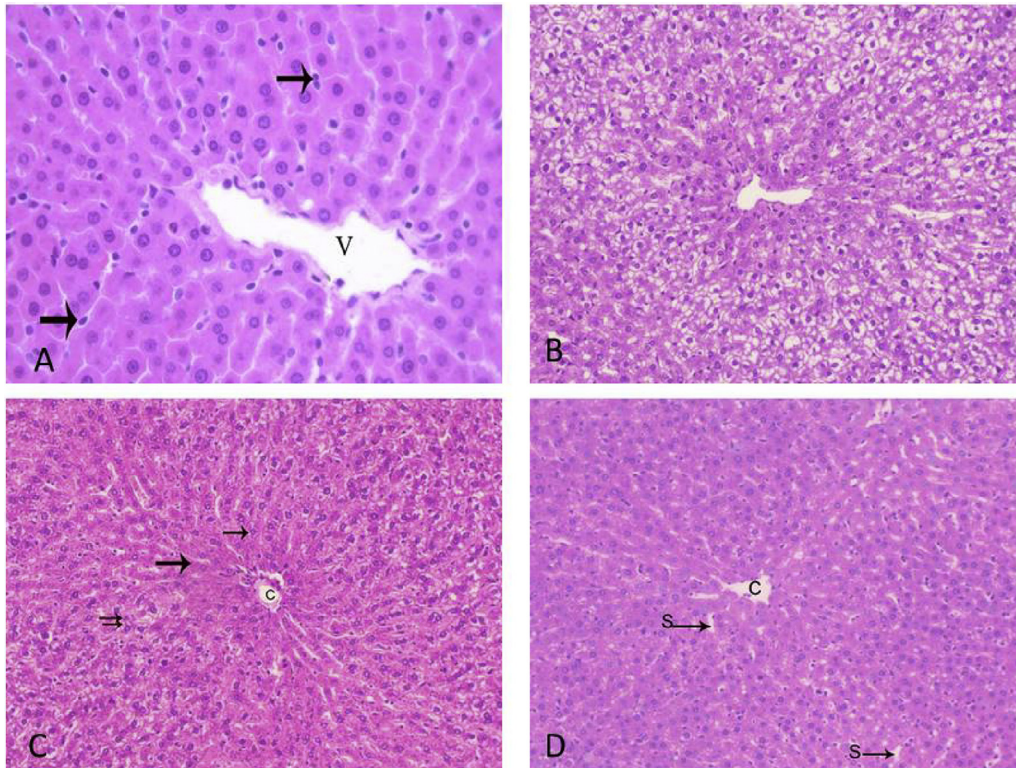


Figure 1. Photomicrograph of liver in adult female albino rats (H&E stain).

A: Control group showed normal liver lobule architecture with central vein (v), normal hepatocytes, blood sinusoids and central vein. Note Kupfer cell (arrow) $\times 400$. B: CC (Cadmium chloride)-treated group showed liver lobule with dilated central vein and portal area showing congestion of blood vessels, proliferation of fibrous tissue, vacuolated and degenerated hepatocytes, dilated and congested blood sinusoids and central vein. Note the pyknotic cell nuclei $\times 200$. C: CCE (aqueous seed extract of *C. carvi*) + CC-treated group showed normal hepatocytes (single arrow), blood sinusoids and central vein (c). Note the degenerated hepatocytes (double arrows) $\times 400$. D: FVE (aqueous seed extract of *F. vulgare*) + CC-treated group showed normal hepatocytes, blood sinusoids (s) and central vein (c) $\times 400$.

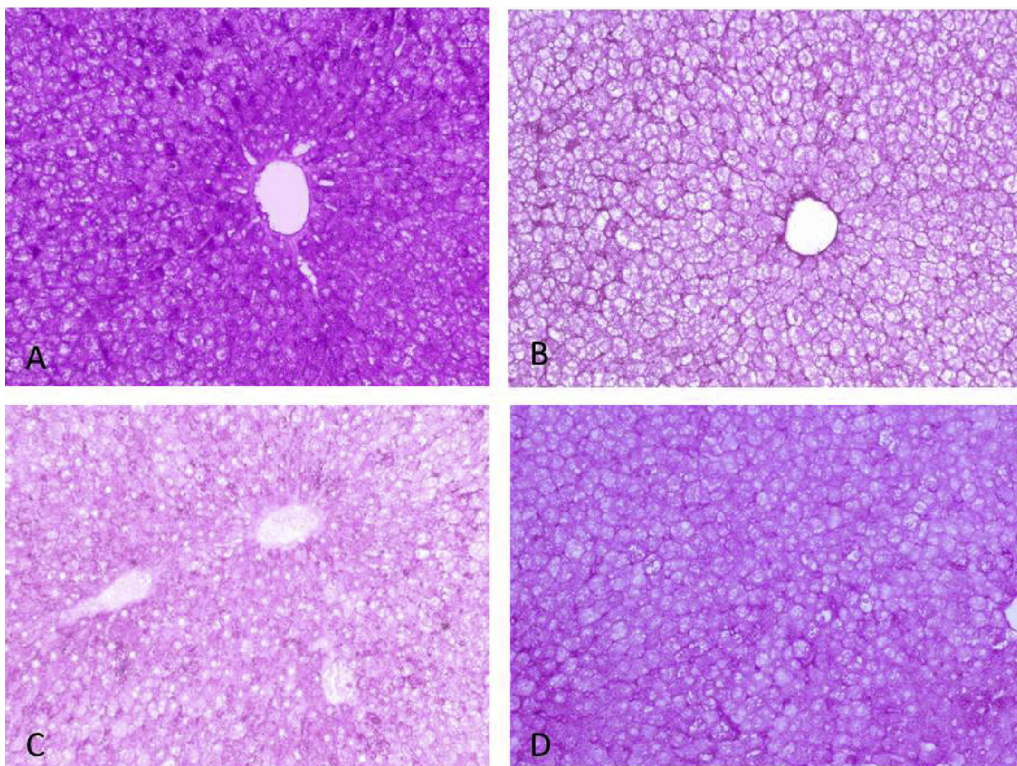


Figure 2. Photomicrograph of liver in adult female albino rats (Periodic acid-Schiff reaction, $\times 200$).

A: Control group showed a strong fuchsinophilic reaction in the hepatocytes. B: CC (Cadmium chloride)-treated group showed a weak fuchsinophilic reaction in the hepatocytes. C: CCE (aqueous seed extract of *C. carvi*) + CC-treated group showed a moderate fuchsinophilic reaction in the hepatocytes. D: FVE (aqueous seed extract of *F. vulgare*) + CC-treated group showed a strong fuchsinophilic reaction in the hepatocytes.

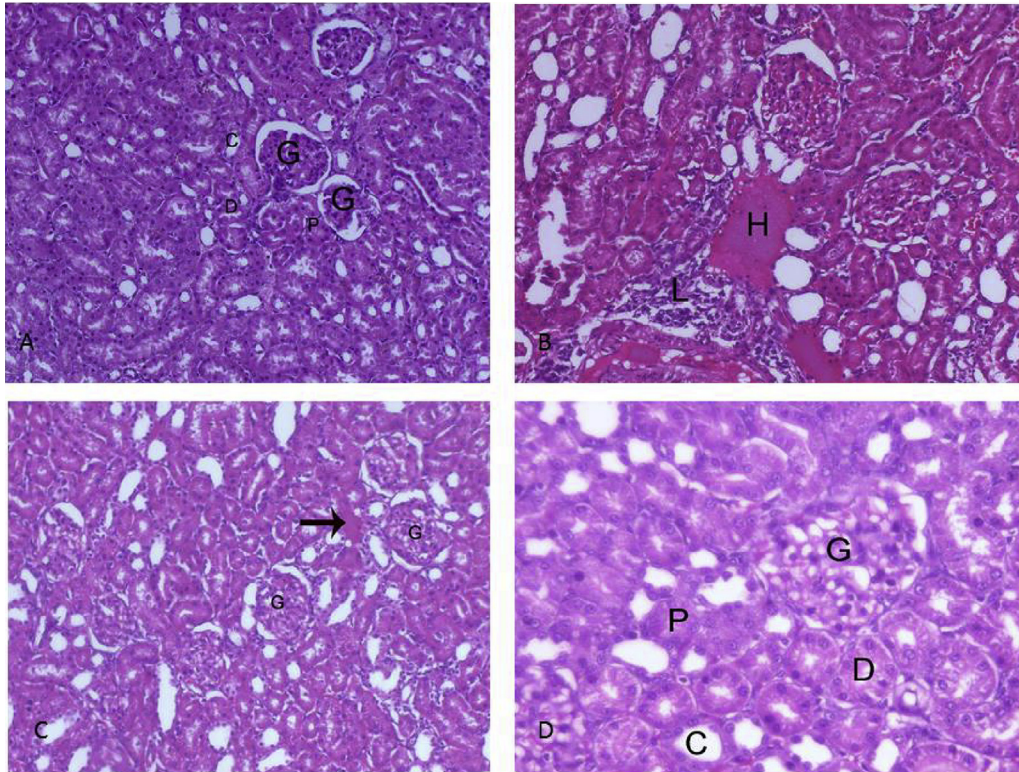


Figure 3. Photomicrograph of kidney in adult female albino rats (H&E stain, ×200).

A: Control group showed normal renal corpuscle (G), proximal convoluted tubules (P), distal convoluted tubule (D) and renal tubules (C). B: In CC (Cadmium chloride)-treated group, the renal cortex showing swelling and congestion in the renal corpuscle, degeneration of convoluted tubules and have severe hemorrhage (H) and lymphocytic infiltration (L). C: CCE (aqueous seed extract of *C. carvi*) + CC-treated group showed swelling and vacuolation of renal corpuscle (G), convoluted tubules and renal tubules (C) showing degenerative changes. Note the congestion of blood vessels (arrow). D: In FVE (aqueous seed extract of *F. vulgare*) + CC-treated group, renal corpuscle (G), convoluted tubules (P&D) and renal tubules (C) appeared more or less normal.

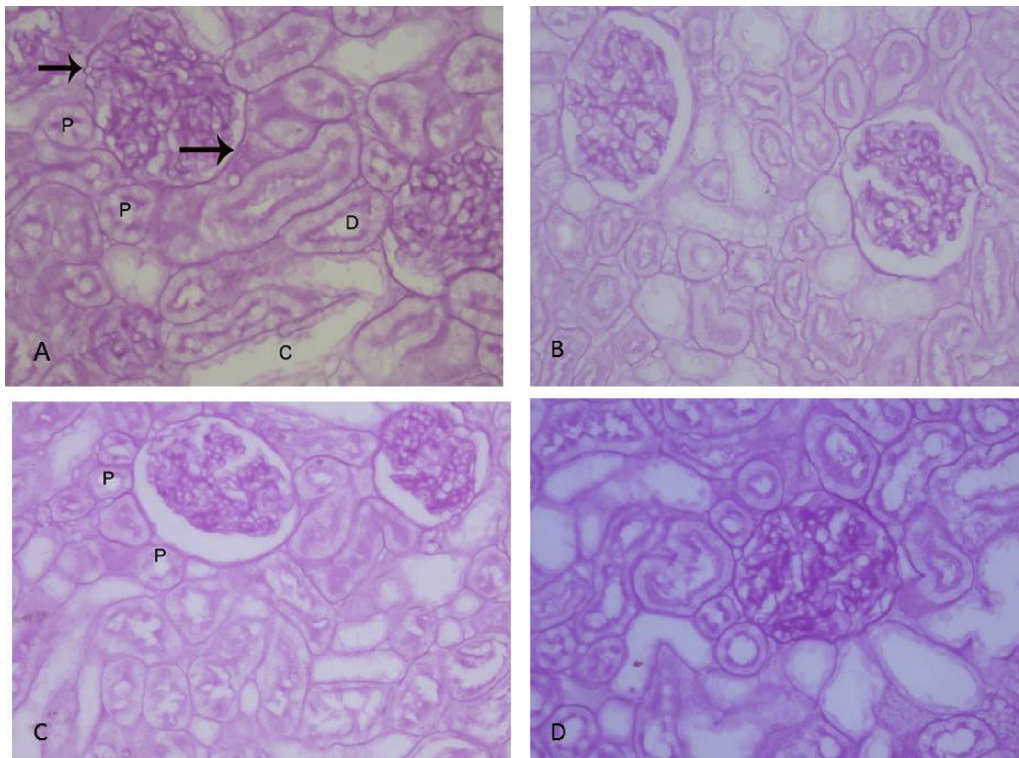


Figure 4. Photomicrograph of kidney in adult female albino rats (Periodic acid–Schiff reaction, ×200).

A: Control group showed strong reactions in the basement membranes, apical brush border of renal corpuscle, proximal convoluted tubules (P) and distal convoluted tubule (D) while a weak reaction in collecting tubules (C). B: In CC (Cadmium chloride)-treated group, the renal cortex showed a moderate reaction in the basement membranes and weak reactions in apical brush border and the cytoplasm. C: CCE (aqueous seed extract of *C. carvi*) + CC-treated group showed a moderate reaction in the basement membranes and weak reactions in apical brush border and the cytoplasm in some tubules (P). D: FVE (aqueous seed extract of *F. vulgare*) + CC-treated group was as normal group.

In CC-treated rats (Figure 1B), there were severe structural damage in the liver cells. The hepatocytes appeared irregularly arranged with disorganization of hepatic architecture. The hepatocytes appeared large with light acidophilic and foamy cytoplasm. The hepatocytes appeared with pyknotic nuclei. Other hepatocytes appeared with hyalinized cytoplasm and pale nuclei with prominent nucleoli. The central vein appeared dilated and congested. Mild fibrosis exhibited around bile duct in the portal area. There was inflammatory cell infiltration. In addition, CC-treated group showed a weak fuchsinophilic reaction in the hepatocytes (Figure 2B). Moreover, In CC-treated rats, Figure 3B displays that the glomerular capillaries showed congestion and dilatation in some renal corpuscles. The cells of the proximal convoluted tubules were swelled resulting narrowing or obliteration of the tubular lumen. The cells lining proximal convoluted tubules appeared with vacuolated cytoplasm and deeply stained nuclei. The brush borders of the proximal convoluted tubules cells were destructed. The distal convoluted tubules showed degenerative changes in the form of pyknotic nuclei and vacuolated cytoplasm. Also, CC-treated rat showed a decrease in the stainability of PAS +ve materials (Figure 4B).

The co-administration of CCE with CC (Figures 1C and 2C) improved the morphology of hepatocytes to some extent toward normal. The hepatocytes revealed very mild degenerative changes. The central vein appeared normal. The hepatocytes also showed a moderate fuchsinophilic reaction. In case of kidney, Figure 3C shows mild cellular infiltration in the interstitial tissue. Some glomeruli showed degeneration with wide urinary space. The glomerular degeneration was thickening of Bowman's capsule and cell debris in tubular lumen. In addition, rats subjected to CCE with CC showed mild increase of PAS +ve materials (Figure 4C).

However, in rats that co-administered FVE with CC, the central vein appeared more or less normal. The hepatocytes regained their normal organization and architecture and the hepatocytes showed a strong fuchsinophilic reaction (Figures 1D and 2D). Also, there was an improvement in the histological structures of the kidney compared to CC group (Figure 3D). The renal corpuscles appeared nearly similar to the control. Most of kidney tubules exhibited acidophilic cytoplasm and rounded vesicular nuclei. The PAS +ve materials were mainly distributed at the brush border and basement membrane (Figure 4D).

3.5. Effects of FVE and CCE either alone or in combination with CC on serum levels of estrogen and progesterone at proestrus stage

Data in Table 3 showed that administration of CC resulted in significant decrease in the serum level of estrogen and

progesterone levels at proestrus phase in comparison to control group ($P < 0.01$). Moreover, administration of FVE either alone or in combination with CC increased significantly the serum levels of both estrogen and progesterone at proestrus phase when compared to CC and control groups ($P < 0.01$). Also, administration of CCE alone or in combination with CC increase significantly the serum levels of estrogen, whereas the progesterone serum level was significantly reduced in comparison to control values (both $P < 0.01$).

3.6. Effects of FVE and CCE either alone or in combination with CC on serum levels of FSH and LH at proestrus stage

The results in Table 3 clarified that administration of CC treatment reduced significantly the serum levels of FSH and LH at proestrus phase than corresponding values for control group ($P < 0.01$). Moreover, CCE or CCE + CC treatments decreased markedly the serum level of FSH ($P < 0.01$) but did not affect the serum level of LH when compared to control values. On the other side, administration of FVE either alone or in combination with CC increased significantly the serum levels of FSH and LH at proestrus phase in comparison to corresponding values for CC and control groups ($P < 0.01$).

3.7. Effects of FVE and CCE either alone or in combination with CC on histopathological findings in reproductive organs

The data of the effects of CCE and FVE either alone or in combination with CC on histopathological findings in ovary are expressed in Figures 5 and 6. Figure 5A and Figure 6 showed normal mature follicles in the control group with mature ovum connected to granulosa layers and surrounded by antrum which engorged with acidophilic antral fluid. The mature follicles are enclosed by theca layers. The follicles appeared in growing state with degenerated oocytes and granulosa cells in case of CC-treated group as well as congested ovarian blood vessels (Figure 5B). Concerning the ovary of FVE + CC treatment exhibited normal growing follicles (Figure 5C). On the other side, the ovaries of animals fed on CCE + CC treatment showed atretic follicles and minor degenerative changes in granulosa cells (Figure 5D).

In addition, the data of the effects of CCE or FVE either alone or in combination with CC on histopathological findings in uterus are represented in Figures 7 and 8. Uterus of control group showed normal uterine epithelium (simple columnar epithelium) and normal endometrial glands and normal uterine epithelium, collagen endometrial connective tissue and normal

Table 3

Effects of aqueous seed extracts of *C. carvi* and *F. vulgare* (CCE and FVE for short respectively) alone or in combination with cadmium chloride (CC) on serum levels of estrogen, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) at proestrus stage (mean \pm SE).

Treatment	Estrogen level (pg/mL)	Progesterone level (ng/mL)	FSH level (ng/mL)	LH level (mIU/mL)
Control	57.50 \pm 0.68 ^a	15.20 \pm 0.39 ^a	3.16 \pm 0.02 ^a	1.26 \pm 0.05 ^a
CC	47.17 \pm 0.50 ^f	8.07 \pm 0.41 ^b	2.17 \pm 0.03 ^b	0.83 \pm 0.04 ^b
CCE	77.04 \pm 0.74 ^b	5.83 \pm 0.35 ^{bc}	2.05 \pm 0.02 ^b	1.41 \pm 0.06 ^a
CCE + CC	80.90 \pm 0.50 ^c	5.70 \pm 0.62 ^c	1.59 \pm 0.04 ^c	1.29 \pm 0.03 ^a
FVE	98.78 \pm 0.66 ^d	39.72 \pm 0.69 ^d	9.49 \pm 0.15 ^d	6.94 \pm 0.10 ^a
FVE + CC	74.10 \pm 0.42 ^c	42.28 \pm 0.60 ^c	8.05 \pm 0.16 ^c	5.60 \pm 0.13 ^c

Values with different superscript letters revealed significant difference from each other ($P < 0.01$).

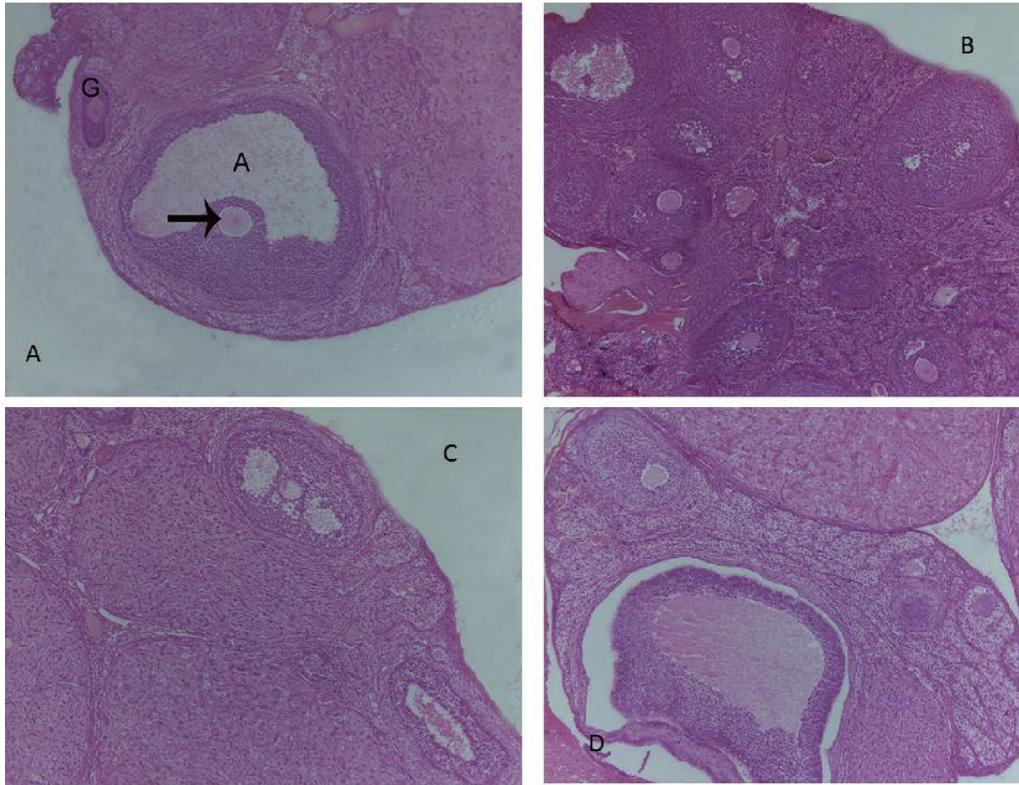


Figure 5. Photomicrograph of ovary in rats showing mature ovarian follicle in normal rats (A), degenerated follicles in CC (Cadmium chloride)-treated group (B), growing follicles in FVE (aqueous seed extract of *F. vulgare*) + CC-treated group (C), and atretic follicle in CCE (aqueous seed extract of *C. carvi*) + CC-treated groups (D). (H & E stain, ×400).

endometrial glands (Figure 7A) as well as moderate PAS reaction in uterine epithelium and endometrial glands (Figure 8A). CC treatment showed degenerated uterine epithelium and endometrial glands (Figure 7B) and weak PAS reaction uterine epithelium and endometrial glands (Figure 8B). Concerning

CCE + CC treatment, it exhibited stratified uterine epithelium and cystic endometrial glands (Figure 7C) and cystic endometrial gland with weakly reacted glands (Figure 8C). Regarding FVE + CC treatment, it showed hypertrophied endometrial folds with cystic glandular hyperplasia and the endometrium

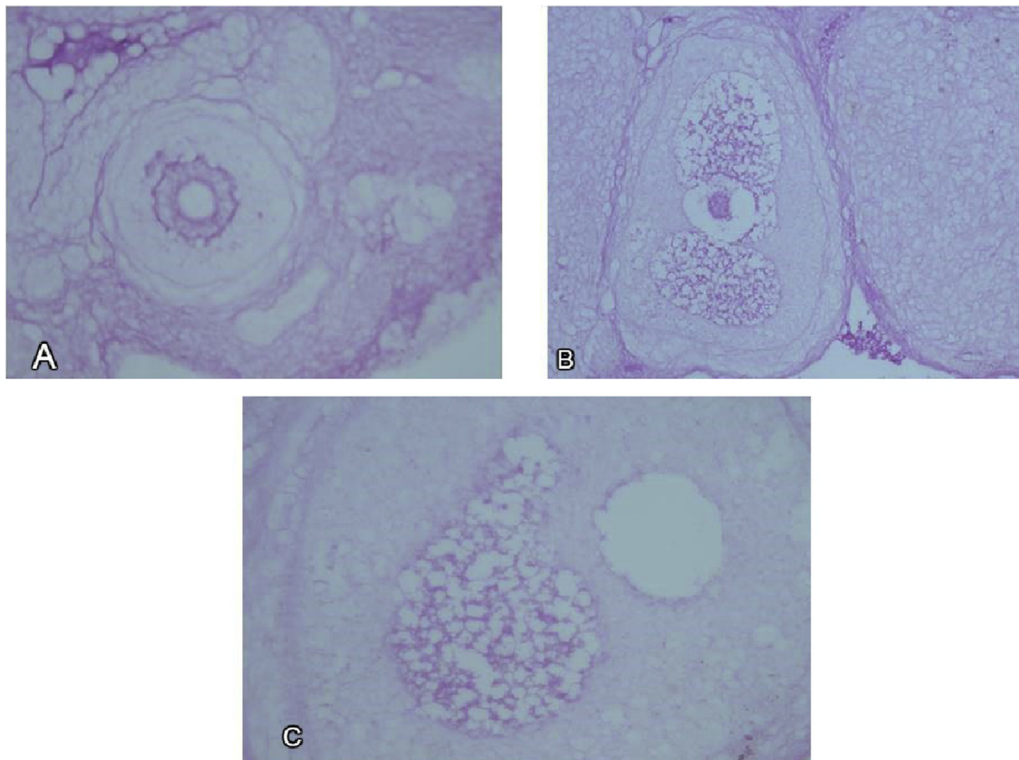


Figure 6. The ovary of normal group showing acidophilic follicular fluid and zona pellucida strong reaction in antral fluid (A) and atretic follicle (B and C). (PAS reaction, ×200).

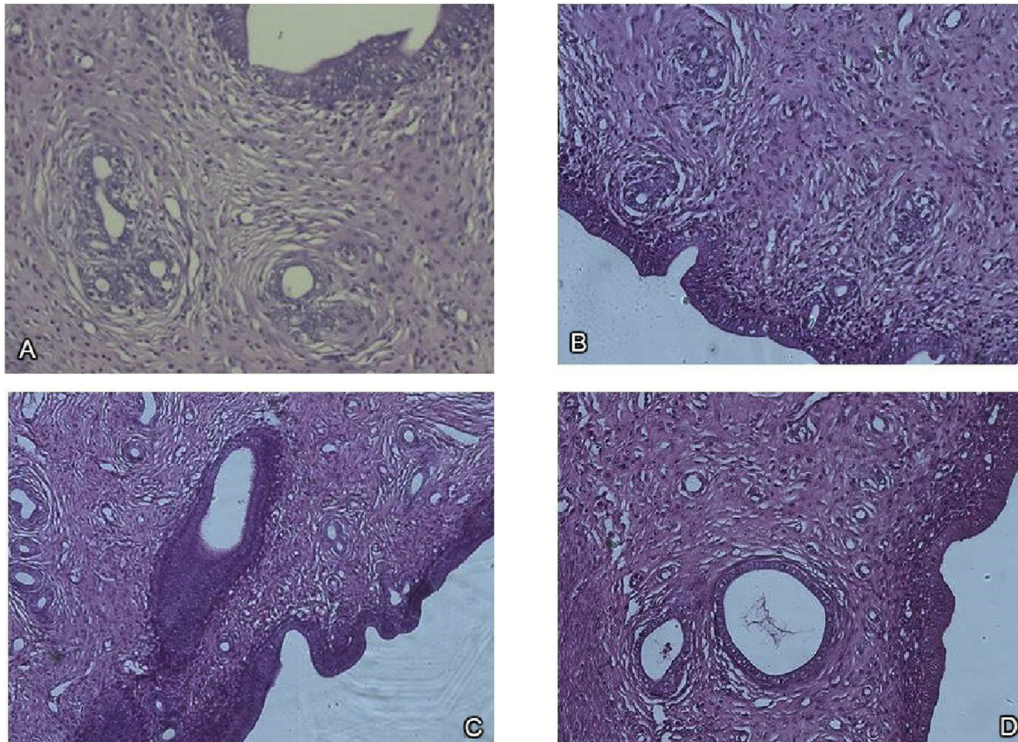


Figure 7. Photomicrograph of uterus in adult female albino rats (H&E stain, $\times 200$).

A: Control group showed showing normal uterine epithelium (simple columnar epithelium) and normal endometrial glands. B: CC (Cadmium chloride)-treated group showed degenerated uterine epithelium and endometrial glands. C: CCE (aqueous seed extract of *C. carvi*) + CC-treated group showed stratified uterine epithelium and cystic endometrial glands. Note the invaginations of endometrium. D: FVE (aqueous seed extract of *F. vulgare*) + CC-treated group showed hypertrophied endometrial folds with cystic glandular hyperplasia and the endometrium displayed more glandular.

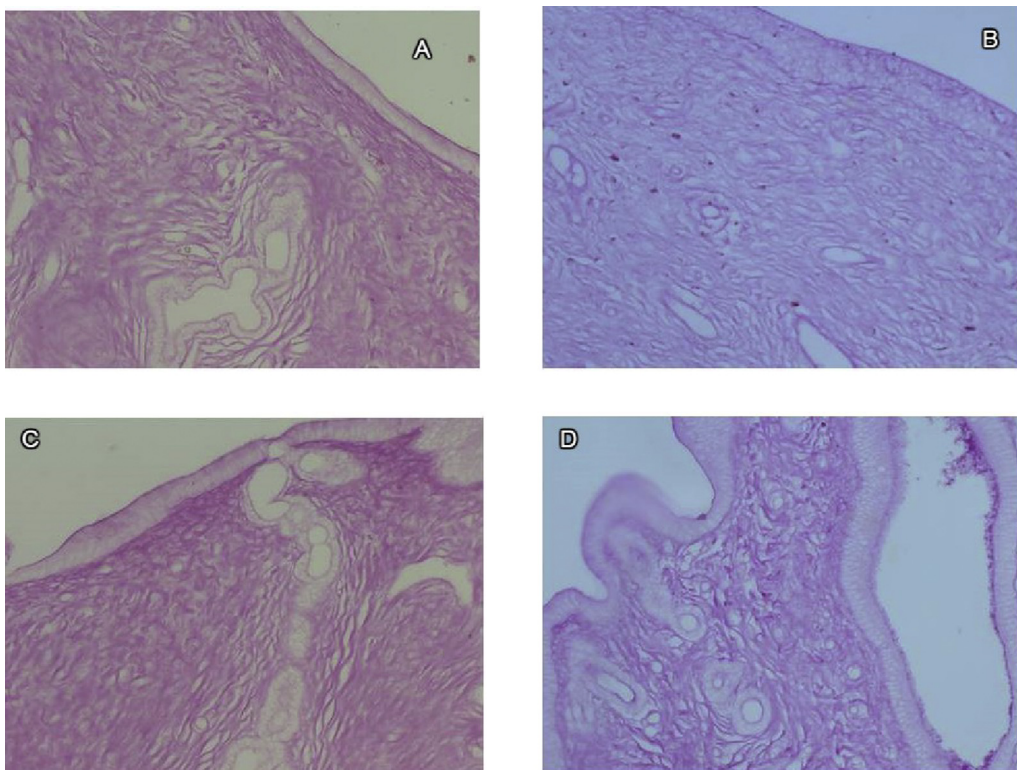


Figure 8. Photomicrograph of uterus in adult female albino rats (Periodic acid–Schiff reaction, $\times 200$).

A: Control group showed moderate PAS reaction in uterine epithelium and endometrial glands. B: CC (Cadmium chloride)-treated group showed a weak PAS reaction of uterine epithelium and endometrial glands. CCE (aqueous seed extract of *C. carvi*) + CC-treated group showed cystic endometrial gland with weakly reacted glands. D: FVE (aqueous seed extract of *F. vulgare*) + CC-treated group showed normal uterine tissue and more active glands.

displayed more glandular (Figure 7D) as well as normal uterine tissue and more active glands (Figure 8D).

4. Discussion

Cadmium is one of the common environmental pollutants and can induce severe oxidative damage [38]. *F. vulgare* and *C. carvi* plants have a long history of use in herbal medicine due to their antioxidant activity [15]. Thus, this study aimed to investigate the effects FVE and CCE against cadmium-induced hepatic, renal and gonadal damage in female albino rats.

The data of the current study clarified that CC administration induced severe structural damage in the liver cells. The hepatocytes appeared irregularly arranged with disorganization of hepatic architecture. The central vein appeared dilated and congested. Mild fibrosis exhibited around bile duct in the portal area. Histochemically, it showed a very weak PAS reaction in the hepatocytes. These data come in accordance with a study of Metwally and Hashem [39] who found that treatment of rat with CC (1.5 mg/100 g bw) once daily for 4 weeks induced an enlargement, severe hydropic degeneration and coagulative necrosis of hepatocytes. In addition, congestions and leukocytic infiltrations were observed in the portal areas. The central veins appeared congested and multiple hemorrhagic foci were observed in many cases. Moreover, it was documented that cadmium intoxication in male rabbits caused severe hepatic tissue damages, including central vein inflammation, dilation of sinusoids, inflammatory cell infiltration, necrosis, and fibrosis [40].

It was evidenced that the activities of ALT, AST in the serum of rats are tested as biomarker enzymes for normal hepatic function [41]. The biochemical analyses of the present study clarified that CC increased significantly the levels of AST and ALT enzymes greater than the control values ($P < 0.05$). These findings come in agreement with Haouem *et al.* [42] who reported that CC administration to rats for 4 weeks in drinking water at dose of 150 mg/L increased the damage of the membrane coat of hepatocytes which consequently leads to leakage of these enzymes from the cytoplasm into the blood circulation and finally increases significantly the serum levels of AST and ALT enzymes. High levels of AST are commonly observed with viral or cardiac infarction induced-liver damage [43]. The remarkable histopathological changes in hepatic tissues together with the elevated hepatic enzymes that reported in the current study proved the hepatotoxic effects of CC treatment.

On the contrary, CCE or FVE improved the liver functions and maintain normal level of liver enzymes (ALT and AST). Moreover, their co-administration with CC restored the level of ALT and AST enzymes to normal values. These results were supported by the positive histopathological findings from this study. It was found that FVE and CCE maintained normal liver architecture as well as partially ameliorated the damage induced by CC administration. These results meet agreement with the previous report of El Baz *et al.* [44] in rats who found that FVE at doses 100 and 200 mg/kg bw maintained liver from CCl₄ intoxication as well as induced a potent protection action against CCl₄-induced liver fibrosis. Moreover, these results coincide with the study of Al-Amoudi [14] who found that treatment of albino rats with 1 mL/kg bw fennel oil was effective to ameliorate the hepatic histopathological changes induced by sodium-valproic administration. Also, the author clarified that fennel oil has potent effects to restore levels of AST and ALT enzymes to normal values.

Regarding the effect of studied treatments on kidney, it was found that CC administration increased significantly the serum level of creatinine and induced many histopathological changes in renal tissues. These results are coincided with the results of Kowalczyk *et al.* [45] who found that administration of 50 mg/L CC to rats in drinking water resulted in elevation of creatinine levels. Moreover, Hussain *et al.* found that treatment of rats with cadmium produced severe damage of proximal and distal tubules, hydropic swelling, and degeneration of tubular epithelium and elevated significantly the serum levels of urea and creatinine [40]. All of these obtained data reflected the toxic effects of CC on kidney.

On the other side, administration of CCE and FVE maintained the serum level of creatinine near the control values and when administered in combination with CC, the elevated values of serum creatinine declined significantly toward normal in comparison to CC group ($P < 0.001$). This data is reinforced by the positive histological findings. In this regard, Shaffie *et al.* found that CCE and FVE significantly ameliorate the histopathological and histochemical changes in rat kidney [46]. Moreover, Al-Amoudi clarified that co-treatment of albino rats with sodium-valproic and fennel oil restored the histological structure of kidney cortex to its normal structure and also, it decreased the normal values of creatinine and urea nitrogen toward normal values [14].

The detrimental effect of CC on liver and kidney functions might be due to the high concentration of reactive oxygen species produced by cadmium and resulted in severe oxidative stress. In this respect, it was evidenced that cadmium induced a significant decrease in enzymatic antioxidants activities and a significant increase of H₂O₂ and MDA which suggest the higher oxidative stress of CC in animal cells [38]. In the current study, CC administration elevated the serum level of MDA and lowered the serum level of TAC in comparison to control group. Thus, it was suggested that the protective effect of CCE and FVE against cadmium toxicity in liver and kidney is contributed to their potent antioxidant activity. The potent antioxidant activities of FVE and CCE are previously reported [14,15]. Moreover, this protective effect of CCE and FVE on liver and kidney plausibly due to their anti-inflammatory properties [12,28,47].

The results of the current study also showed that CC administration resulted in significant reduction of the serum levels of estrogen, progesterone, FSH and LH hormones ($P < 0.01$). It is plausible that CC could impair the reproductive capacity of female rats and thus it could be considered as one of the potent anti-fertility environmental pollutants. The anti-fertility effects of CC might be due to its endocrine disruption action and thus, it interferes with synthesis of FSH and LH and accordingly affects the synthesis of sex steroids [2]. Also, the present results showed that CC administration resulted in degeneration of ovarian follicles and uterine epithelium and endometrial glands. These degenerative changes of ovary and uterus observed with CC administration might be due to creation of high percentage of free radicals that have the opportunity to react easily with lipid molecules in ovarian and uterine epithelium and resulted in increase the level of lipid peroxidation and many degenerative changes [48]. This comes in agreement with the results of the current study that revealed high serum concentration of MDA and low serum level of TAC after CC administration. Also, these results were in accordance with previous report of Nad *et al.* [49] who found that cadmium administration for Japanese quails resulted in

negative changes in ovary and markedly increase the number of atretic follicles when compared to control. Furthermore, CCE was showed to have a significant antifertility activity. Administration of CCE alone or in combination with CC increased significantly the serum levels of estrogen while level of progesterone was significantly reduced ($P < 0.01$). Also, they reduced significantly the serum levels of FSH at proestrus phase ($P < 0.01$). In this respect, Thakur *et al.* found that oral administration of different doses of aqueous and ethanolic extracts of *C. carvi* showed a significant decrease of FSH and LH levels while amount of estrogen was found to be increased [18]. This is confirmed by the histopathological findings which showed atretic follicles on ovaries as well as stratified uterine epithelium and cystic endometrial glands and proliferation of connective tissue and less active glands as well as cystic endometrial gland with weakly reacted glands. These findings reflect the antifertility effect of CCE and CC treatments.

On the other side, administration of FVE either alone or in combination with CC increases significantly the serum levels of estrogen and progesterone, FSH and LH at proestrus phase when compared to control group ($P < 0.01$). These finding proved that FVE has great impact on improvement of female fertility as well as high efficiency to relieve the deleterious effects of CC treatment. This is supported by the histological finding that showed normal growing follicles on ovaries. In addition, it showed hypertrophied endometrial folds with cystic glandular hyperplasia and the endometrium displayed more glandular as well as normal uterine tissue and more active glands. Thus, FVE can be introduced as a novel medicine for treatment of infertility. The results of the present study come in accordance with a study of Sadeghpour *et al.* [50] who found that fennel extract can increase the serum level of estrogen, progesterone, and prolactin in female mice. Also, these results meet agreement with a previous report of Khazaei *et al.* [13] who showed that FVE considerably increased the number of graafian, antral and multilaminar follicles at concentration of 100 and 200 mg/kg. The authors also found that FVE extract analysis identified the presence of diosgenin, which is an estrogenic compound and it was considered as precursor for synthesis of progesterone hormone. Moreover, Azam *et al.* found that treatment of mice with fennel at different doses (100, 200 or 400 mg/kg d) succeeded to ameliorate the toxic effects of cyclophosphamide on the ovaries as it elevated the serum levels of estrogen and progesterone hormones in addition to the increase of the number of ovarian follicle [51].

It was concluded that FVE or CCE are efficient treatment for liver and kidney against cadmium intoxication. Moreover, FVE is a potent treatment to improve female fertility and efficient treatment against cadmium-induced gonadal damage.

Conflict of interest statement

The authors declare they have no conflict of interest.

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