INTRODUCTION

Cadmium (Cd) was an environmental toxicant and an endocrine disruptor in humans and rodents. Also, Cd was known to be a carcinogenic metal that especially its compounds had sufficient evidence in both humans and experimental animals beneath its environmental effects (Ates et al., 2004). Several organs (e.g., kidney, liver) were affected by Cd and recent studies had illustrated that the testis was exceedingly sensitive to Cd toxicity (Ates et al., 2004, Martin et al., 2007 and Sadik, 2008). More important, Cd might account for the recent declining fertility in men among developed countries by reducing sperm count and testis function (Siu et al. 2009). Clinical and animal studies indicated that abnormalities of spermatogenesis resulted from exposure to three toxic metals (lead acetate, cadmium chloride, and arsenic trioxide) (Nava et al., 2009) because the testes were one of the most sensitive organs to acute cadmium toxicity (Kim and Soh 2009). Sadik (2008) showed decreased body weight, paired testicular weight, relative testicular weight, serum testosterone when male adult Wistar rats treated with cadmium (2.5 mg/kg body wt, five times a week for 4 weeks) where the testicular toxicity by Cd appeared as inhibition of androgen production in adult male rats probably by affecting pituitary gonadotrophins. So, Cd was considered gonadotoxic and spermiotoxic factor (Ola et al., 2008). Ates et al., (2004) proposed that cadmium also increased oxygen derived free radicals and lipid peroxidation which led to DNA damage.

The most important attention was paid to the search of natural dietary antioxidants and their evaluation in medicinal and food raw materials of...
plant origin. A number of plants, their extracts, food products, and medicinal preparations appeared to be the objects of scientific research (Raudonis et al., 2009). They were studied for their ability to protect cells from miscellaneous damages. Marjoram volatile oil was potent antioxidants (El-Ashmawy et al., 2007) and was known to possess various therapeutic properties (Aristatile et al., 2009). Al-Howiriny et al. (2009) used Marjoram at doses of 250 and 500 mg/kg of body weight and found significantly decreased the incidence of ulcers, basal gastric secretion and acid output. Furthermore, the extract replenished the ethanol-induced depleted gastric wall mucus. Ulcer preventing potential was further confirmed by histopathological assessment. An acute toxicity test showed a large margin of safety of the extract in mice.

El-Ashmawy et al. (2005) illustrated that the administration of Origanum majorana (volatile oil, alcoholic and aqueous extracts) with oral lead acetate in the diet of mice at concentration of 0.5% (W/W) for one month showed improvement in the liver and kidney histology in comparison with lead acetate treated group. Alcoholic extracts of majorana significantly reduced the rate of micronucleus, number of aberrant cells and different kinds of chromosomal aberrations. Aqueous extract and volatile oil of majorana significantly reduced number of gaps, ring chromosome and stickiness. It could be concluded that majorana played an important role in ameliorating liver and kidney functions and genotoxicity induced by lead toxicity.

Dorman et al. (2004) found that the Syrian oregano extract was the most effective chelator of iron(II), while Spanish and Turkish oregano extracts were the most effective inhibitors of non site-specific hydroxyl radical-mediated 2-deoxy-d-ribose degradation. All the extracts contained Folin-Ciocalteu reagent-reactive substances, which was confirmed by the presence of polar phenolic analytes.

Botoglou et al. (2002) showed that as oregano oil increased in the diet, malondialdehyde values decreased in tissue samples, suggested that the oil, particularly at 100 mg/kg of feed, exerted an antioxidant effect on chicken tissues against iron where Iron-induced lipid oxidation.

All the previous researches confirmed that the cadmium metal was highly toxic and affected both plant and animal organisms. If human exposed to very low dose of cadmium metal for long periods of time, whether through drink or food or by inhalation of polluted air, this might cause chronic toxicity led to weaken of the immune system, as a result of the accumulation within the tissues, leading to infection. Therefore the objective of this research shed light on the effects of cadmium on tissue composition of birth testicle and then to reduce and mitigate them by using the Marjoram plant, where few previous studies indicated its great protective role.

MATERIALS AND METHODS

Materials

30 male albino rats (60- days) age were reared in animal house at the center of King Fahad of Medical Researches in Saudi Arabian, divided into four groups were maintained in polypropylene cages of 50x33x20 cm at a constant temperature (21±1ºC) and 62% relative humidity, were given all the daily dose orally for 10 days as follows: the first group (control group), the second group treated with cadmium chloride (2mg/kg) (Fouad et al., 2009), the third group treated with a similar dose of aqueous extracts of Marjoram plant (Ashmawy et al., 2005), the fourth group treated with both cadmium chloride (2mg/kg) and aqueous extracts of Marjoram plant (2mg/kg).

Methods

Both the control and experimental animals were killed by cervical decapitation and dissected. Testes were extracted and histological preparation were carried according to (El- Banhawy and El-Gansory, 1989).

Specimens were fixed in 10% neutral formalin and the standard procedures of dehydration, clearing and embedding in wax were followed. They were sectioned at 3-5 µm and stained with Hematoxylin and eosin (Drury and Wallington1980).

The animals at the time of tissue collection were slightly anaesthetized, bled within two minutes by eye using a heparinized syringe, plasma samples were separated by centrifugation, frozen and stored.
at -85°C until all samples have been collected for the determination of testosterone. Plasma testosterone levels were measured by radioimmunoassay (RIA) according (Chang et al. 1995).

**Statistical analysis**

The body and tests weight in both control and experimental groups were analyzed by using the program SAS (Institute Inc.1988, Cary, NC, USA) where t-test was used to assess the significance of changes between control and treated mice (Sokal & Rohlf 1981).

**RESULTS**

During the experiment, all animals survived and this means that the doses administered, the laboratory conditions of water, food and shelter were appropriate.

**The group treated with Marjoram plant**

From table (1) homogeneity marjoram and control groups in both testes weight and body weight were noticed, where testes weight in marjoram group reach (0.957±0.62 g) and their body weight reach (251.7±4.41 g).

Examination of testicular sections of Marjoram treated group showed that the tests take circular shape surrounded by testicular capsule consisting of collagen fibers have spindle shape fibroblasts followed by internal vascular layer containing blood vessels. The testis consists of a large number of round or oval seminiferous tubules and majority of these seminiferous tubules filled with large numbers of spermatozoa in the lumen and there was an increase in epithelium height of the tubules (Fig. 2). Each seminiferous tubule had pyramidal Sertoli cells with pale triangular nucleus alternated with many spermatogonia appeared as smaller dark cells rest on the basal lamina and followed by primary spermatocytes with large nuclei and dense chromatin followed by secondary spermatocytes appeared small in size and has a faint nucleus. It was also observed that the spermatozoa filled the lumen of these tubules with high intensity (Fig. 3) compared with the control group (Fig. 1).

The level of blood testosterone reached (24.42 ± 0.257 ng / mg) that approximated the hormone level of control group (24.45 ± 0.275 ng / mg).

**The group treated with cadmium**

From table (1) cadmium group showed a clear reduction in both testes weight (0.620±.012 g.) and body weight (185.0±2.89 g.)

The volume of testes became smaller in comparison with the control group. From histological examination of testicular sections in this group, it found that sexual fertility delayed in comparison to control and other experimental group where the germ cells sloughed into the tubular lumen and the germinal epithelium was distributed (Fig. 4). Also, the tunica albuginea was disrupted, more lytic intestinal tissue with few Leydig cells were observed, the seminiferous tubules suffered from low epithelium height and surrounded by irregular basal lamina (Fig. 5). This accompanied with retardation and alteration in the histological structure of the tubules, this retardation that recognized by the separation of the necrotic dense germinal cells and a sharp decrease in both the mature spermatozoa and primary spermatocytes with central eosinoc material. Sertoli cells could not be detected but Liquid infiltration was detected in interstitial tissue

<table>
<thead>
<tr>
<th>The groups</th>
<th>The level of blood testosterone</th>
<th>Weight body</th>
<th>Testes Weight</th>
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<tbody>
<tr>
<td>Control group</td>
<td>24.45 ± 0.275 ng / mg</td>
<td>261.7±4.41</td>
<td>0.933±.033g</td>
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<tr>
<td>Marjoram treated group</td>
<td>24.42 ± 0.257 ng / mg</td>
<td>251.7±4.41</td>
<td>0.957±0.62g</td>
</tr>
<tr>
<td>Cadmium treated group</td>
<td>13.03± 0.125 ng / mg.</td>
<td>185.0±2.89</td>
<td>.620±.012g0</td>
</tr>
<tr>
<td>Cadmium and Marjoram treatment group</td>
<td>22.88±0.176 ng/mg</td>
<td>228.3±6.009</td>
<td>.850±.029g0</td>
</tr>
</tbody>
</table>
Fig. 1: Light micrograph of control rat testis section showing, seminiferous tubule (ST) with central lumen filled with sperms (S) (1000×-H α E).

Fig. 2: Light micrograph of Marjoram plant treated-rat testes section showing, tunica albuginea (arrow), vascular layer (BV) and seminiferous tubules (ST) (100×-H α E).

Fig. 3: Light micrograph of Marjoram plant treated-rat testes section showing, seminiferous tubule (ST) with basal lamina (BL), primary spermatocytes (PS), secondary spermatocytes (SS) and high sperm intensity (S) (100×-H α E).

Fig. 4: Light micrograph of cadmium treated-rat testis showing, atrophied seminiferous tubules (ST) have exfoliated cells (arrow) and large intertubular spaces with lytic interstitial tissue (IT) and Leydig cells (LC) (400×-H α E).

The group treatment with cadmium and Marjoram plant

The testes weight (0.850±0.029 g.) and body weight (228.3±6.009 g.) in this group increased than their comparable in cadmium group.
The testes sections treated with cadmium and Marjoram showed improvement in the structural organization of testes where the seminiferous tubules took the round or oval shape and the connective tissue between them appeared normal in quantity and contained leydig cells and blood.

Fig. 5: Light micrograph of the cadmium treated- rat testes section showing, disrupted tunica albuginea (arrow), seminiferous tubules (ST) with deformed germ cells and large intertubular spaces with lytic interstitial tissue (IT) (400 x-H α E)

Fig. 6: Light micrograph of the cadmium treated-rat testes section showing, seminiferous tubule with deformed sperm (S), central eosin material (star) and liquid infiltration (arrow). (1000x-H&E)

Fig. 7: Light micrograph of rat testes section treated with Marjoram plant and cadmium showing, nearly normal structure with regular seminiferous tubule (ST) suffer from liquid infiltration (arrow). (40x-H&E)

Fig. 8: Light micrograph of rat testes section treated with Marjoram plant and cadmium showing, nearly normal seminiferous tubule (ST) with regular basal lamina (BL), healthy Sertoli cell (SC), primary spermatocytes (PS), secondary spermatocytes (SS) and sperms (S). (1000x-H α E)
vessels (Fig.7). With high power, presence of well-arranged stages of spermatogenesis included spermatogonia, large primary spermatocytes (with central rounded nuclei), small size secondary spermatocytes, round spermatids and moderate amount of spermatozoa directed to Sertoli cells were recorded. (Fig.8) Still, there was low liquid infiltration level in compare with cadmium treatment group. Also, noted the high level of testosterone hormone comparable to the cadmium treatment group (22.88±0.176 ng/mg).

**DISCUSSION**

The present results showed homogeneity Marjoram and control groups in both testes and body weight but cadmium group showed a clear reduction in them, this consistent with the results of Sadik (2008) who showed that cadmium lowered rat body weight and tests, while cadmium and Marjoram group showed improvement and that may be due to the presence of the antiradical activity of Marjoram (Dambolena et al., 2010 and Botoglou et al., 2002).

The present findings indicated that exposure to cadmium affected primary spermatocytes and this agreed with (Nava-Hernández et al., 2009) who found that the chronic exposure (13 weeks) to toxic metals, affected primary spermatocytes DNA and were suggested of possible direct testicular toxicity.

From the present research, Cd intoxication significantly decreased testicular sperm and this agreed with (Ola-Mudathir et al., 2008) who recorded decreased epididymal sperm concentration and sperm progress motility, increased percent total sperm abnormalities and live/dead count.

In between seminiferous tubules, there were interstitial connective tissue and some Leydig cells which secrete the male hormone testosterone (Junqueira et al., 1998) and this might explained the low level of testosterone in cadmium treated group as it was noticed that there were a few number of Leydig cells in the interstitial tissue and this agreed with Fouad et al.,(2009) who found that testicular damage was induced by a single i.p. injection of cadmium chloride (2mg/kg) in which cadmium reduced testosterone level glutathione, catalase and superoxide dismutase activities.

Sertoli cells had many functions as feeding, supporting spermatids; hormones secretion which regulated Leydig cells function, spermatocytes division and spermatozoa formation. Also, Sertoli cells had an important role in the formation of testicular blood barrier which provided good circumstances for the developed spermatocytes and these cells were undividable so they had resistance to the harmful ecological factors as food shortage (Junqueira et al., 1998).

Cd-induced toxicity to the testis was probably the result of interactions of a complex network of causes. This was likely to involve the disruption of the blood-testis barrier (BTB) via specific signal transduction pathways and signaling molecules, such as p38 mitogen-activated protein kinase (MAPK) (Siu et al., 2009). Also, Kaisman et al.,(2009) indicated that Clusterin, is a glycoprotein produced abundantly by Sertoli cells lining the walls of the seminiferous tubules which associated with either apoptosis or cell survival, Cd(2+) influx induce expression and secretion of Clusterin, thereby linking metal homeostasis and germ cell fate. So the defects of Sertoli cells in cadmium treated group resulted in loss of spermatic cells and might lead to the destruction of this tissue and infertility as indicated before (Monsees et al., 2000).

It was summarized in this work that the harmful effect of cadmium on testis was known to be germ cell degeneration (Oner et al., 2005), Sertoli cell damage and impairment of testicular steroid genesis as indicated before (Sadik, 2008). Also, Gurel et al.,(2007) investigated that the seminiferous tubules and Leydig cells were damaged by cadmium. Martin et al.,(2007) recorded At high doses (5 mg/kg cadmium chloride or higher), testicular damage in mice, rats, and other rodents included interstitial edema, hemorrhage, and changes in the somniferous tubules affecting spermatogenesis. Yang et al.,(2006) found that, when animals received cadmium at 2 mg/kg, undifferentiated spermatids and dead Sertoli cells increased in the seminiferous tubules while interstitial cells decreased and inflammatory cells increased in the interstitial tissues. On flow...
cytometric analysis, the numbers of elongated spermatids and round spermatids decreased. Oner et al. (2005) found that spermatid cells in seminiferous tubules of CdCl2 treated animals displayed necrosis and homogenous pink particles were present in spermatid places. Also, interstitial areas were edematous and intertubular vessels were plugged.

El-Demerdash et al. (2004) found that treatment with CdCl2 caused a significant decrease in sperm concentration, motility (%), weight of testes and epididymis, and increase in dead and abnormal sperm.

The present results suggested that Marjoram plant affords a significant testoprotective and hypolipidemic effect against Cd and this agreed with Aristatile et al. (2009) who found that the administration of Marjoram for 21 days prevented and improved the histopathological parameters of Cd toward normalcy.

It may be concluded that marjoram plant was useful herbal remedies, especially for controlling oxidative damages in which, co-administration of protective plants resulted in minimizing the hazard effects of Cd toxicity on male fertility.

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REFERENCES

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