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PROTECTIVE INFLUENCE OF OLIVE OIL ON REPRODUCTIVE PARAMETERS IN MALE RAT TREATED WITH CADMIUM

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ABSTRACT
Cadmium is a no-essential toxic trace element, it affects reproductive organs. It's action may be either direct, affecting the gonads and accessory organs, or indirect via interference with the hypothalamus-pituitary-gonadal axis. Olive oil is a fat obtained from the olive, its health benefits are due to its fatty acid composition and minor compounds, tocopherols, polyphenols, ortho-diphenols carotenoids, and Vitamin E which possesses strong radical scavenging activity. The purpose of this study was to investigate the protective effect of olive oil against cadmium-induced testicular dysfunction in adult male rats. Forty five mature albino rat (*Rattus rattus* norvegicus albinos) was used as a mammalian model. These animals were divided into three groups, 15 rat/group.Group1, served as control was orally administrated with distilled water. Testicular dysfunction was induced by an oral administration of cadmium chloride (2mg/kg body weight, for four weeks daily) group2. Group3 was orally administrated with cadmium in dose of (2mg/kg body weight)plus one ml of extra virgin olive oil, for four weeks To all animals, hormones (LH, FSH and Testosterone) were measured, histological study was done and semen parameters was studded. The effects of administration of cadmium showed a significant decrease in semen parameters, highly significant increase in FSH level, significant decrease in LH level, significant decrease in testosterone level and testicular histological abnormalities. Administration of olive oil with cadmium shows caused an elevation in sperm counts, their motility, their normality, improvement in histological findings and hormonal results when compared with treated group with cadmium only. Olive oil plays a significant role in the improvement of testicular damage along with reduces oxidative stress and protect from reproductive organs injury during cadmium toxicity.

KEY WORDS: Cadmium, Olive oil, LH, FSH, Testosterone.

INTRODUCTION

It is well established that many trace elements play essential role in number of biological processes through their action as activators or inhibitors of enzymatic reaction by competing with other elements and proteins for binding sites, and influencing the permeability of cell membrane or through other mechanisms [(1)].A large number of trace elements could exert stimulatory or inhibitory effects on the sperm depending on the concentration of each divalent cations[(2)] . Cadmium (Cd) is a non-essential toxic trace element that has no known biological function. Its most abundant naturally-occurring isotope is non-radioactive. It is found in nature in mineral forms and is obtained for commercial uses principally from cadmium ore, called greenockite, which is commonly found in association with zinc ore. Commercial production of cadmium ore depends on the mining of zinc .Cd is commercially available as an oxide, chloride, or sulfide. Cd metal (Cd2+) refined from the ore is a silver-white, blue-tinged lustrous heavy metal solid at room temperature [(3)]. Cd affects reproductive organs[(4)] . It's action may be either direct, affecting the gonads and accessory organs, or indirect via interference with the hypothalamus-pituitary-gonadal axis[(5)]. Cd has been shown to concentrate in testicular tissue after occupational exposure .Acute exposure to large doses of Cd induces gonadal necrosis whereas chronic treatment with low doses has been shown to produce testicular atrophy[(6)] . Olive oil is a fat obtained from the olive, it is used throughout the world. Olive oil is composed mainly of the mixed triglyceride esters of oleic and palmitic acid along with traces of squalene (up to 0.7%) and sterol (about 0.2% phytosterol and tocosterols) [(7)].Virgin olive oil is characterized by its sensorial and nutritional properties, which are different from those of other edible oils. Its health benefits are due to both its fatty acid composition and minor compounds, tocopherols, polyphenols, sterols, and carotenoids[(8)]. A number of studies have shown that polyphenolic compounds possess strong radical scavenging activity and appear to be at least, as if not more, effective than other important dietary antioxidant [(9)]. The fatty acid composition of virgin olive oil has great importance from a health point of view.
The purpose of this study was to investigate the protective effect of olive oil against cadmium-induced testicular dysfunction in adult male rats.

MATERIALS & METHODS

All experiments were performed on 45 mature albino rat (*Rattus rattus* norvegicus albinos) as a mammalian model with a body weight (B. wt) ranging between 200-250 g. Rat were obtained from the colony of the animal house of the High Institute for Infertility Diagnosis & ART. AL-Nahrain University/Iraq. These animals were divided into three groups, 15 rat/group after labeling them with ear or tail marking and weighing them using an electrical balance, as follows: Control group (G1): This group was

orally administrated with distilled water during the four weeks period of the experiment. Experimental group (G2): This group was orally administrated with cadmium in dose of(2mg/kg body weight)[10] daily during the four weeks period of the experiment. Experimental group (G3): This group was orally administrated with cadmium in dose of (2mg/kg body weight) plus one ml of extra virgin olive oil)[11]. All treatments were given by intragastric tube daily. Blood samples were aspirated from the hart and put in plastic centrifuge tubes, left to clot and serum was obtained by centrifugation at 1500 rpm (revolution per minute) for15 min, the sample were kept frozen at-20 ^{0}C until LH, FSH and testosterone were measured. Then rats were sacrificed the weight of reproductive organs were recorded , reproductive organs (testis and epididymes) were quickly excised and immersed in few drops of normal saline which was placed in Petri dish to be cleared from surrounding adipose tissue under dissecting microscope using fine surgical scissors. Both right and left testes were fixed with 10% formal saline for a subsequent histological study. The right and left epididymes were put in a small Petri dish contained 1ml of global culture media then the epididymes were minced by microsurgical scissor about 200 times until we got a homogenized solution which contained the spermatozoa.

Statistical analyze

The data were statistically analyzed using SPSS/PC version 10 software (SPSS, Chicago). Level of FSH, LH and testosterone were analyzed using complete randomized design (CRD). The Statistical model was Yij = μ + Ti + eij.

Where Yij= dependent variables (Level of FSH, LH and testosterone), μ= overall mean, Ti= effect of treatments (cadmium), eij= error term. Differences among means were computed using the Duncan multiple ranges test)[12] .

RESULTS

The effects of administration of cadmium showed a significant decrease in sperm concentration, highly significant decrease in progressive sperm motility, significant decrease in non progressive motility, significant increase in immotile sperms and highly significant increase in sperm normality**.** While the administration of cadmium plus olive oil showed non significant differences in sperm concentration and sperm normality, with significant decrease in progressive and non progressive motility and significant increase in immotile sperm when compared with control as shown in Table 1.

TABLE 1. Effect of cadmium treatment and cadmium plus olive oil on Sperm Parameters in male rat

Sperm parameters	Control		Cadmium		Cadmium & Olive oil	
sperm concentration millions/mL	96.750 ±5.218	a	42.500 ±4.233	b	91.000 ±6.683	a
Progressive sperm motility (%)	40.750 ±2.175	a	8.500 ±1.688	c	34.833 ±1.973	b
Non progressive sperm motility (%)	32.750 ±1.109	a	21.667 ±2.929	b	20.167 ±2.197	b
Immotile sperm (%).	34.000 ±7.246	b	69.833 ±4.254	a	45.000 ±3.141	b
Sperm normality (%)	54.500 ±3.329	b	44.667 ±1.667	c	68.833 ±2.386	a

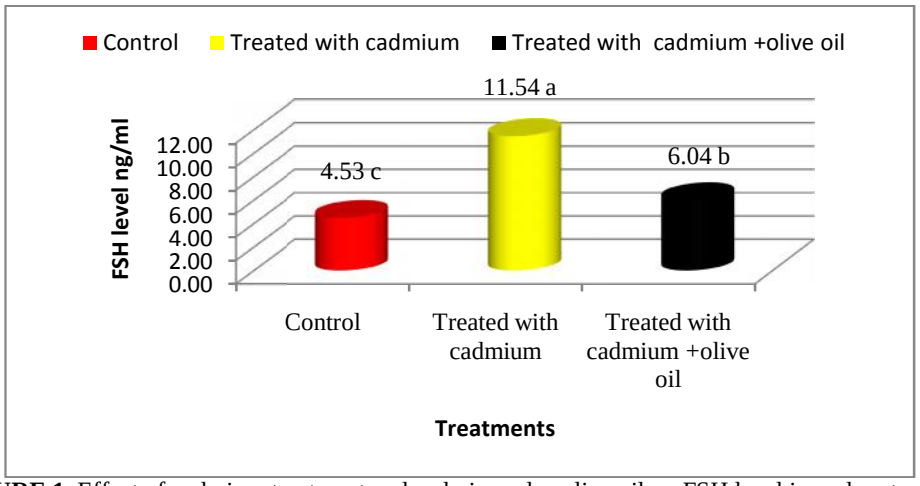

FIGURE 1. Effect of cadmium treatment and cadmium plus olive oil on FSH level in male rat serum.

* Means with different superscripts within each columns are significantly different (P<0.05)

** Means with semi superscripts within each columns are non significantly different (P>0.05)

Figure 1showed highly significant increase in FSH level after cadmium administration with significant increase in FSH level after cadmium plus olive oil administration when compared with control. Figure 2showed significant

decrease in LH level after cadmium administration with non significant deference in LH level after cadmium plus olive oil administration when compared with control. Figure 3showed significant decrease in testosterone level after cadmium administration with non significant deference in testosterone level after cadmium plus olive oil administration when compared with control.

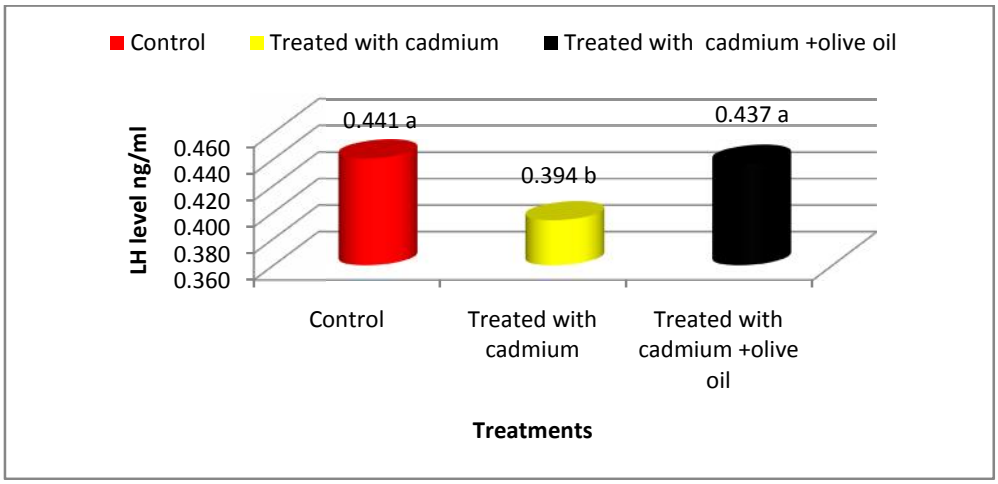

FIGURE 2. Effect of cadmium treatment and cadmium plus olive oil on LH level in male rat serum.
* Means with different superscripts within each columns are significantly different (P<0.05)
** Means with semi superscripts within each columns are non significantly different (P>0.05)

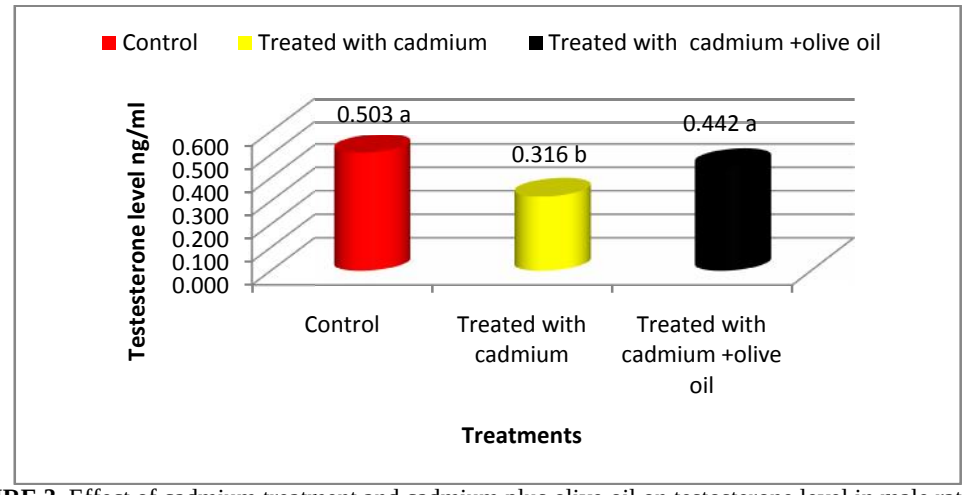

FIGURE 3. Effect of cadmium treatment and cadmium plus olive oil on testosterone level in male rat serum.
* Means with different superscripts within each columns are significantly different (P<0.05).
** Means with semi superscripts within each columns are non significantly different (P>0.05).

Histological observation

Testicular sections of the control group showed seminiferous tubules with normal germ cell population layer thickness with a normal orderly arranged pattern up to mature spermatid. No malignant or abnormal cell was seen within the germinal epithelium, also no vacuoles is present in the tubules. There were adequate Sertoli cells populations. The interstitial spaces contained adequate normal Leydig interstitial cell, blood vessels were also normal and no edema was present. The pertubular surrounding tissues were normal as shown in figure 4 and figure5 .In cadmium treated group (G2), sections showed a decrease in thickness of germ cell layer, widening of the central seminiferous tubules lumen and prominent germ cell population necrosis. Multiple rounded vacuoles were seen within the tubules, with the decreases inspermatogenesis and stromal interstitial Leydig cell hypertrophy. Also Widening of the interstitial spaces was detected and edema within the interstitial spaces was markedly present. A peritubular fibrotic change had been seen also in the testicular sections as shown in figure 6 .In the group treated with cadmium plus olive oil (G3), sections of tubules showed recovery of germ cell population, normal germinal epithelium thickness with order arrangement of the germ cell up to spermatid, adequate Sertoli and Leydig interstitial cells population. Mild edema was seen at the periphery of the testicular section in this group as shown in figure 7 and figure8.

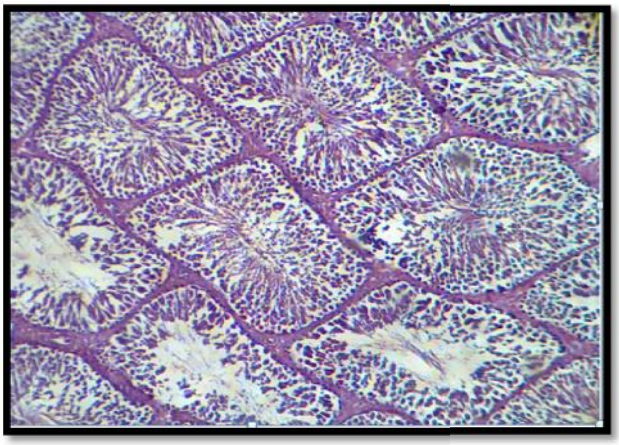

FIGURE 4. Cross sections of testes were stained with hematoxylin and eosin (H and E) of control Group 1 x10

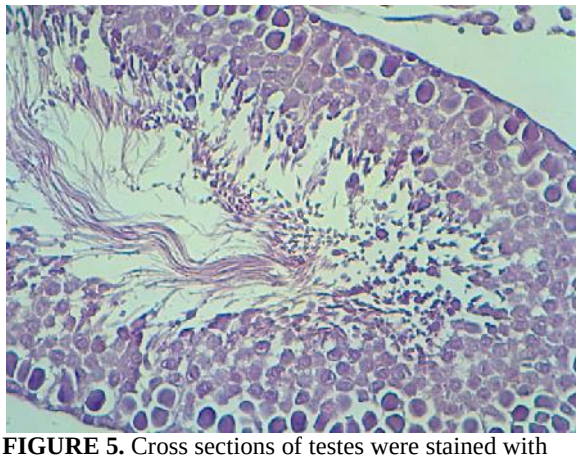

FIGURE 5. Cross sections of testes were stained with hematoxylin and eosin (H and E) of control Group 1 x 40

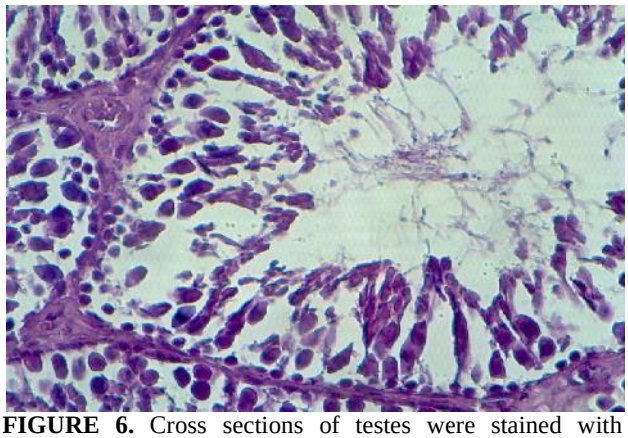

FIGURE 6. Cross sections of testes were stained with hematoxylin and eosin (H and E) of Group2 treated with cadmium x40

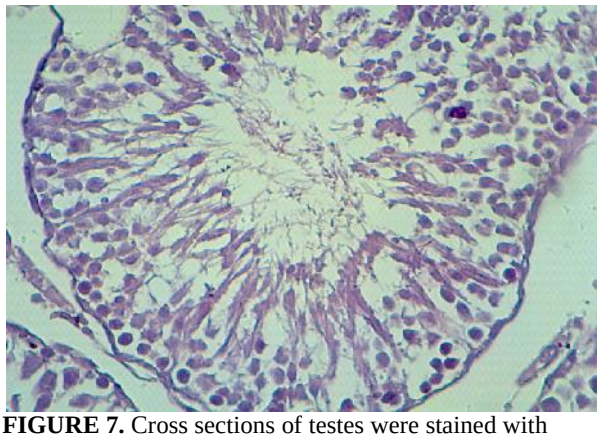

FIGURE 7. Cross sections of testes were stained with hematoxylin and eosin (H and E) of Group3 treated with cadmium plus Olive oil x4

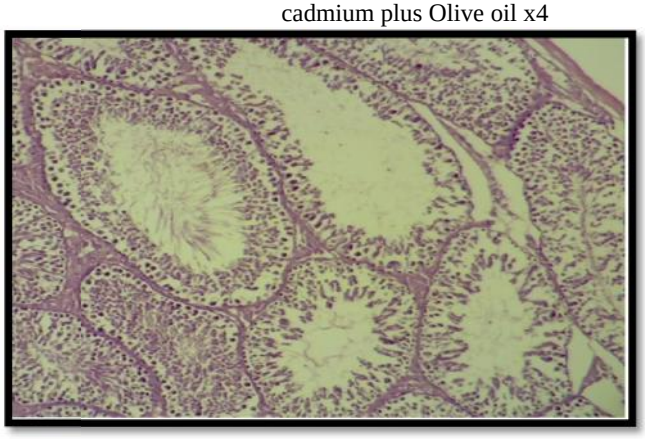

FIGURE 8. Cross sections of testes were stained with hematoxylin and eosin (H and E) of Group3 treated with cadmium plus Olive oil x10

DISCUSSION

The present study showed the efficacy of olive oil in preventing the toxic effects of Cadmium in the rat testes**.** To our knowledge, this is the first report showing these effects. Our data showed that the administration of cadmium to male rats produced generalized degeneration of the germ, sertoli and Leydig cells which agrees with the data reported by Laskey Jw *et al* and Foote RH (13,14) and also showed decrease in sperm concentration, progressive motility , sperm normality and testicular structure , this may be attributed to testicular germ cell destruction caused either by membrane damage or macromolecular degradation incurred by reactive oxygen species (ROS) leading to a significant decline in spermatogenesis or perhaps due to the low production of sperm in testes and that could be related to the low levels of gonadotrophins and/or serum testosterone level and may be due to testicular necrosis[(15)].It has been

demonstrated that Cd stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals[16]. Cadmium act on the testes, causing problems in spermatogenesis and spermiogenesis[17].The damage is due to capillary stasis followed by massive thrombosis, and is highly specific to the testes[18].The primary insult seems to be an increase in capillary permeability and a breakdown of the blood testes barrier[19]. The net effect of decreased spermatogenesis is the combination of diminished blood flow and the ability of cadmium to compete with zinc for binding to enzymes essential for cell replication[20].When Cd is administered orally and chronically, the testis accumulated about 4 times more Cd than after single bolus, high dose Cd injection. Acute exposure to large doses of Cd induces gonadal necrosis whereas chronic treatment with low doses has been shown to produce testicular atrophy[21]. The effect of cadmium on the testes appears to be manifested mainly in the Sertoli cells, which present more morphological changes under scanning electron microscopy. Cadmium can also interfere with the normal functions of mitochondrial enzymes [22].Cd decreases androgen biosynthesis, possibly by altering progesterone synthesis and metabolism through direct interaction of Cd with DNA and competitive inhibition of essential enzymes[23] . Testicular lesion from cadmium is primarily vascular, and the vascular damage determines the degree of lesion in the germ cells and Leydig cells. This lesion can generate Leydig cell tumors, tubular degeneration (in high-dose exposure), and atrophy [24], in addition to inducing tissue necrosis and deficient androgen production [25]. An increase in serum levels of FSH after cadmium administration was shown in this study . This finding could be due to the accumulation of cadmium in the testis.In this basis, it was shown that cadmium affects Sertoli cell activity by decreasing inhibin synthesis and release. Thus, the increased plasma levels of FSH could be explained, as this peptide is the main inhibitory signal for FSH secretion[26],another explanation may be because spermatogenesis is more sensitive to a toxic chemicals which disrupt spermatogenesis often cause raised FSH[27]. Metal exposure also decreased serum levels of LH and testosterone. This decrease may reflect direct effects of the metal at the testis as this metal accumulates in this tissue. This effect may explain that the metal disrupted the interrelationship mechanisms between LH and testosterone [28]. Many investigations showed that chronic treatment with cadmium induced oxidative damage causing destruction of cell membranes and increased lipid peroxidation[29].

The current study also shows that administration of Olive oil with cadmium caused an elevation in sperm counts ,their motility, their normality ,improvement in histological findings and hormonal results, The virgin olive oil,contains precious substances of high biological value such as phenols, ortho-diphenols and various natural antioxidants, tocopherol (Vitamin E), flavones as well as a high content of oleic acid, alcohols,sterols, chlorophyll, volatile aromatic substances. [30,31]. The phenolic compound, hydroxytyrosol has been identified as one of the most potent antioxidants found in olive oil[32]. The antioxidant effects of hydrophilic phenols and tocopherols on the oxidative stability in virgin olive oils and in purified olive oil have been evaluated by Baldioli M et al,[30]they found that purified olive oil had good oxidative stability. Vitamin-E present in significant amounts in olive oil.[33] , Vitamin E is the primary liposoluble antioxidant, which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes, thus maintaining its permeability.[34]. Vitamin E may also affect oxidative changes which occur in other cell organelles.[35],and prevent lipid peroxidation and cell destruction.[33] The scavenging properties of olive oil is said to be the main important effects on the sperm parameters. Our results showed the ability of olive oil to reverse the testicular dysfunction caused by cadmium, as represented by normalization of epididymal sperm counts and testosterone level and improvement of sperm motility along with restoration of spermatogenesis in seminiferous tubules as shown via the histological testicular examination.

CONCLUSION

On basis of above results and findings it is concluded that olive oil plays a significant role in the improvement of testicular damage along with reduces oxidative stress and protect from reproductive organs injury during cadmium toxicity.

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