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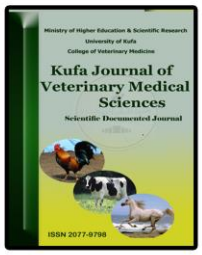

The Impact of *Eruca sativa* Seeds on Leydig's Cells Number and Hormonal Profile In Cadmium Exposed Rats

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Abstract

This study was aimed to investigate the protective role of *Eruca sativa* seeds extract on the number of Leydig's cells and hormonal profile in cadmium chloride treated rats.Forty adult male rats were randomly divided to four equal group and handled as follows for 56 days : Control group administered tap water ; group G1 administered tap water containing 30 ppm/ L of cadmium chloride; group G2 received tap water containing 30 ppm/ L of cadmium chloride and administered 250 mg/Kg.B.W of ethanolic extract of *Eruca sativa* seeds ; group G3 administered 250 mg/Kg.B.W of ethanolic extract of *Eruca sativa* seeds only.Collecting of blood samples were obtained at 0, 28 and 56 days for estimation of serum testesterone (T) , follicule stimulating hormon(FSH) and leutinizing hormon (LH) concentrations. At the end of experiment body weight and testicular weight were measurment. Furthermore, histological sections of testes were prepared for counting of Leydigs cells. A significant decrease in serum testesterone (T) , follicule stimulating hormon(FSH) and leutinizing hormon (LH) concentrations and decrease in body weight to testes weight% in group G1 at concentration of $CdCl_2$ 30 ppm/ L, as well as, statistical analysis and examination of histological sections of testes revealed a significant decrease in the number of Leydig's cells. Whereas, The results pointed the beneficial effects of *Eruca sativa* seeds extract to improvement the previous parameters, against cadmium chloride (G2), through a significant increase in hormonal profile concentration and numbers of Leydigs cells parallel with alleviating the testicular toxicity induced by $CdCl_2$.

Key Words: Cadmium, *Eruca sativa* ,Leydigs cells, hormonal profile, rats.

تأثير بذور الجرجير على أعداد خلايا ليدك وصورة الهرمونات في ذكور الجرذان المعرضة للكادميوم

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الخلاصه

أستهدفت هذه الدراسه للتعرف على الدور الوقائي للمستخلص الكحولي لبذور نبات الجرجير في الحد من تأثير كلوريد الكاديوم على عدد خلايا ليدك وصورة الهرمونات في ذكور الجرذان البالغ . تم استخدام 40 جرذ بالغ، قسمت إلى أربع مجاميع متساوية وعوملت لمدة 8 أسابيع كالتالي : .جرّعت المجموعة الأولى ماء

الشرب واعتبرت مجموعة سيطرة، أما المجموعه الثانية (G1) فقد أعطيت كلوريد الكادميوم بتركيز 30جزء بالمليون /لتر من ماء الشرب. في حين اعطيت المجموعه الثالثة (G2) كلوريد الكادميوم في ماء الشرب كما في الجموعة الأولى أضافة الى تجريعها المستخلص الكحولي لبذور الجرجير بجرعه 250ملغم /كغم من وزن الجسم ،بينما جرعت المجموعه الرابعه (G3) بالمستخلص الكحولي لبذور الجرجير بجرعه 250ملغم /كغم من وزن الجسم.جمعت عينات الدم للفترات 0و 28و56 يوم من التجربه وتم عزل السيرم لغرض تقدير تركيز هرمون التستستيرون ، الهرمون المحفز للجريب والهرمون الليوتيني . في نهاية التجربه وزنت الحيوانات ، وتم أخذ وزن الخصيتين ثم أخذت عينات من الخصية اليمنى وعمل لها مقاطع نسيجيه لغرض حسابأعـداد خـلايا ليدك .أظهرت النتائج وجود انخفاض معنوي في معايير الدراسه في الجرذان المعاملة بكلوريد الكادميوم وبتركيز 30جزء بالمليون مقارنة بالمجاميع الأخرى. فضلا عن ذلك أظهرت نتائج الفحص النسيجي حدوث اخفاض معنوي في عـدد خلايا ليدك للجرذان المعاملة بكلوريد الكاديموم. وعلاوة على ذلك،فقدأشارت النتائج الى الدور الأيجابي المستخلص الكحولي لبذور الجرجير في تقليل حدة الآثار الضارة لكلوريد الكادميوم وتحسين المعايير المدروسه من خلال الزيادة المعنوية في تركيز الهرمونات وزيادة اعداد خلايا ليدك . نستنتج من ذلك أن المستخلص الكحولي لبذور الجرجير يمتلك خصائص مضادة لتقليل تسسم الخصى عن كلوريد الكادميوم.

الكلمات المفتاحيه: كادميوم، الجرجير، خلايا ليدك، صورة الهرمونات،الجرذان

Introduction

The main natural sources of cadmium include volcanic activity, sea spray, soils, sediments and from rain getting into our water supplies (1) , besides, using of phosphate fertilizers will increasing the compounds of cadmium in ecosystem(2). Occupational exposure in metal industries (3) and nonsmoking population consider as a sources for Cd toxicity (4). At early date cadmium was proved to be induced physiological disruption of spermatogenesis and damage sperm DNA (5) leading to decline of fertility in several mammalian species. Cadmium affect the male reproductive system particularly testes causing testicular degeneration (6). However, several studies suggested that exposure to Cd caused spermatological damage and histological alteration in testes , epididymis and accessory glands with disruption of blood-testes-barrier (BTB) leading to testicular toxicity and reduced testicular size (6,8 and 9).

Some research stated that cadmium can induce neuroendocrine toxicity by disrupting the pattern of hypothalamic-pituitary –gonadal axis in male leading to acquired malformations such as cryptorchidism, as well as, testicular cancer may occur (10). Many organisms has ability to generate reactive oxygen species (ROS) in responses to cadmium exposure (11). Over-accumulation of ROS leads to oxidative stress (OS) which, in turn, causes oxidative damage of proteins and DNA leading to increased rate of mutations as well as, lesions in various biological molecules such as peroxidation of lipids (LPO) (12). Besides ROS caused an apoptosis in the testis may be mediated through alterations of mitochondrial apoptotic pathway (13). Nevertheless, spermatozoa can generate ROS normally within limited levels for its normal functions(14). Whereas, excess production of ROS in some conditions such as in cadmium toxicity caused adversely effect on sperm functions (15)**.** Consequently, the presence of dysfunctional spermatozoa in the semen caused a significantly elevation of ROS production (16). It has been known that antioxidants protect the cells directly or indirectly from the damage caused by toxic radical reactions through different mechanisms (17 and 18).*Eruca sativa* (ES) commonly known as rocket. The phytochemical composition of *Eruca seeds* and revealed the existence of glucosinolates such as allyl

sulphocyanate (19), in addition, isolated and identified three new types of quercetins derivatives from its *E.sativa* leaves(20). Aliva, (21) found that (4 methylthiobutylisothiocyanate) and Erucin are the major isothiocyanates in *E. sativa* seeds . Evidently in alternative medicine, rocket had been used as medicinal plant for different purpose due its antioxidant properties (22,23 and 24).Considering these phytochemical compounds have an antioxidant activities, so most attention has been focused on its seeds, therefore, this experiment was designed to investigate the effect of alcoholic extract of *Eruca sativa* seeds on hormonal profile and Leydigs cells against cadmium chloride in adult rats.

Materials and Methods

Eruca sativa seeds were purchased from the local market (Shorje - Baghdad). The ethanolic extract of *Eruca sativa* was prepared as described by(25) as follows : 100 g of *Eruca sativa* seeds were ground into powder then put it in a volumetric conical flask 1000 ml of 70 % ethyl alcohol was added to the powder and mixed by using magnetic stirrer apparatus for 24hr, filtration of the mixture by using 4 layers of medical gauze, then was filtered again using Whatman (No.1) filter paper. The filtered mixture was evaporated by using incubator at 40°C for 72hr to obtain crude extract. The yield equal to 3g**.**

Forty adult mature - week old male rats were randomly divided into four equal groups and one group was considered as control group ,whereas, the other groups were handled as follows for 56 days : group G1 administered tap water containing 30 ppm/ L of cadmium chloride; group G2 received tap water containing 30 ppm/ L of cadmium chloride and administered 250 mg/Kg.B.W of ethanolic extract of *Eruca sativa* seeds ; group G3 administered 250 mg/Kg.B.W of ethanolic extract of *Eruca sativa* seeds only.Collecting of blood samples were obtained via cardiac puncture from each anesthetized animal at 0, 28 and 56 days of the experiment then centrifuged at 3000 rpm for 15 minutes, and sera was isolated and frozen at -18 Cº till analysis of serum testesterone (T) , follicule stimulating hormon(FSH) and leutinizing hormon (LH) concentrations by using immunoenzymometric assay Kits (Monobind Inc, USA). Body weight and testicular weight were estimated. Furthermore, testis were excised and fixed in 10% formalin buffer solution for histological examination. Tissue were embedded in paraffin blocks and several tissue sections with thickness of 6 µ was prepared and stained by hematoxylin and eosin according to (26) to study the Leydig cells .Counting of Leydigs cells was done by reading the cells between each three seminiferous tubules,10 cross-section per rat were recorded by using light microscope (under 40X) and calculation the mean number of Leydigs cells cell / μmm^2 according to Java-based image processing program (27). Statistical analysis of data was performed on the basis of One-way and Two-Way Analysis of Variance (ANOVA) using a significant level at ($P<0.05$)and using Least Significant Differences (LSD) test for specific group differences (28).

Results

After 28 and 56 days of the experiment, cadmium treated group (G1) recorded a significant ($P<0.05$) decrease in serum testosterone concentration as compared to other groups (figure 1-A) . Whereas, the

testosterone concentration in G_2 and G_3 groups significantly ($P<0.05$) elevated after 28 and 56 days of experiment period comparing to the control and G1 groups. On the other hand, serum testosterone concentration of group G_3 at 28 and 56 days of treatment showed a significant ($P<0.05$) increase comparing to other treated groups . Besides, time dependent , a significant ($P<0.05$) highest level of serum testosterone concentration were recorded in groups G2 and G3 at two experimental periods (28 and 56 days) as compared to zero time .

The data show a marked significant ($P < 0.05$) reduction in serum FSH concentration in G1 treated group after 28 and 56 days of experiment compared with the others experimental groups (figure 1-B).While, at the end of experiment the results recorded a significant ($P < 0.05$) increase in serum FSH concentration in group G2 (received $CdCl_2$ plus E.S.) as compared with control and G1 groups. Besides, there was no significant ($P> 0.05$) differences in FSH concentration between G2 and G3 at the end of the experiment when compared among them.

Figure 1-C illustrates the mean value of LH hormone. In comparison between groups the results showed a general trend for the LH value to decrease significantly ($P<0.05$) after 56 days in Cd treated group (G1) as compared to control and other treated groups. In addition , the results showed that rats received $CdCl_2$ concurrently with *Eruca sativa* extract (group G2) exhibited a significant ($P<0.05$) increase in LH at days 28 and 56 days of the experiment comparing to group G1 . It was also noted the existence of a significant ($P<0.05$) elevation in serum LH concentration in group G3 (received E.S) as compared to control and G1 groups. Within the time, with exception to group G3, the results recorded absence of statistical ($P>0.05$) differences in all groups along the experimental period comparing to the pretreatment period.

Data pertaining, in figure 2-A, recorded a significantly ($p<0.05$) decrease in testicular weight to body weight ratio in Cd treated group (G1) as compared with the control , G2 and G3 groups. On the contrary , the results showed a significant($p<0.05$) increase in this parameter group G3 as compared with the control ,G1 and G2 groups . It was also noted the absence of statistical differences between the two G2 and G3 when compared with each other. Statistical analysis of the number of Leydig's cells showed a significant decrease ($P<0.05$) in rats exposed to CdCl2 at concentration 30Pppm/L at end of the experiment (figures 2-B, 4-A and 4-B) as compared to other groups. Besides, the histological section of testes of rats received $CdCl_2$ plus E.S extract (group G2) revealed a significant increase ($P<0.05$) the number of Leydig's cells (figures 2-B and 5) as compared to G1 (figures 4-A and 4-B). On the other hand , the number of Leydig's cells of ES treated group only (figures 2-B and 6) showed significant ($P<0.05$) increase in this parameter as compared with control, G1 and G2 groups (figures 3-B, 4-B and 5).

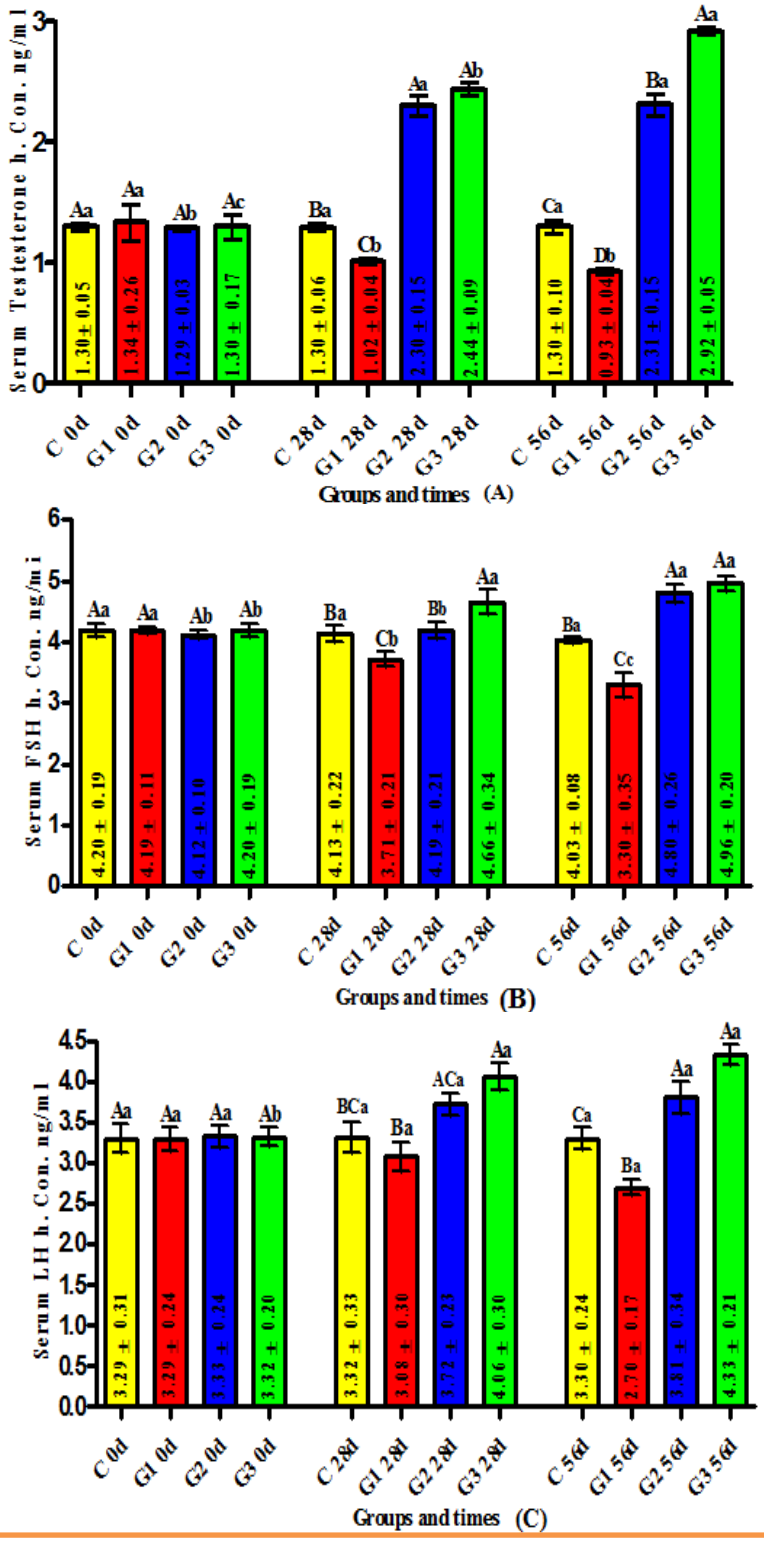

Figure.(1): Effect of alcoholic extract of *Eruca sativa* seeds and cadmium chloride on hormonal testosterone (A) , FSH (B) and LH (C) concentrations in adult male rats exposed to cadmium chloride.
Value are expressed as mean ± SE , n=10 each group
C: control , G1: administration of 30 ppm/L of CdCl2 . G2: administration 250 mg /kg B.W. ethanolic extract of *Eruca sativa* seeds plus 30 ppm/L of CdCl2 . G3: administration 250 mg /kg B.W. ethanolic extract of *Eruca sativa* seeds.
Capital letters denote differences between groups P<0.05 Vs. control.
Small letters denote differences within group P<0.05 Vs. control.

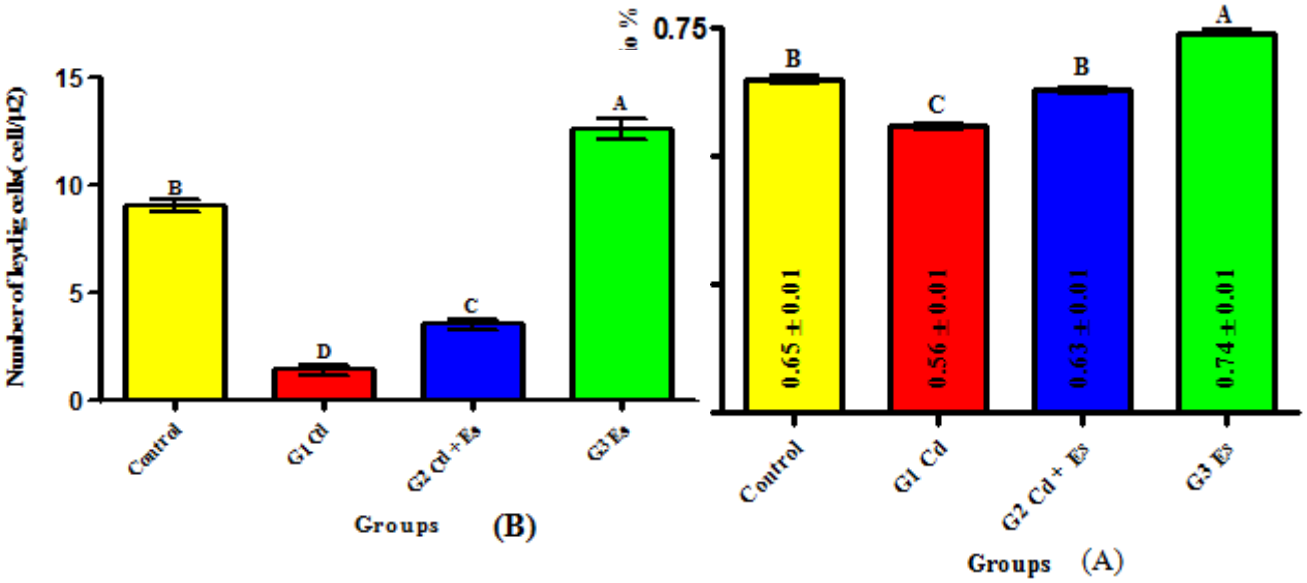

Figure .(2): Effect of *Eruca sativa* extract and cadmium chloride on testicular weight to body weight ratio (A) and on number of Leydig cells (B) in adult male rats.
Value are expressed as mean ± SE , n=10 each group
C: control , G1: administration of 30 ppm/L of CdCl2 . G2: administration 250 mg /kg B.W. ethanolic extract of *Eruca sativa* seeds plus 30 ppm/L of CdCl2 . G3: administration 250 mg /kg B.W. ethanolic extract of *Eruca sativa* seeds.
Capital letters denote differences between groups P<0.05 Vs. control.

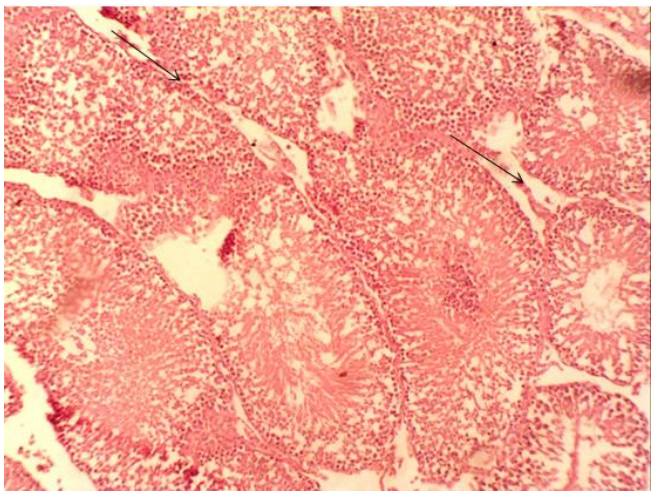

Figure. (3- A) : Cross section of rat testis of control group . note :- leydig cells with normal structure ⟶ (H &E stain 10 X)

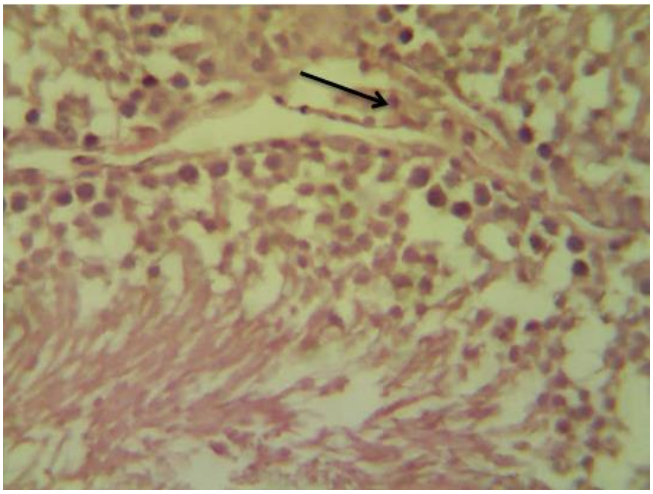

Figure.(3-B): Cross section of rat testis of control group . note:- leydig cell ⟶ with normal structure (H &E stain 40 X)

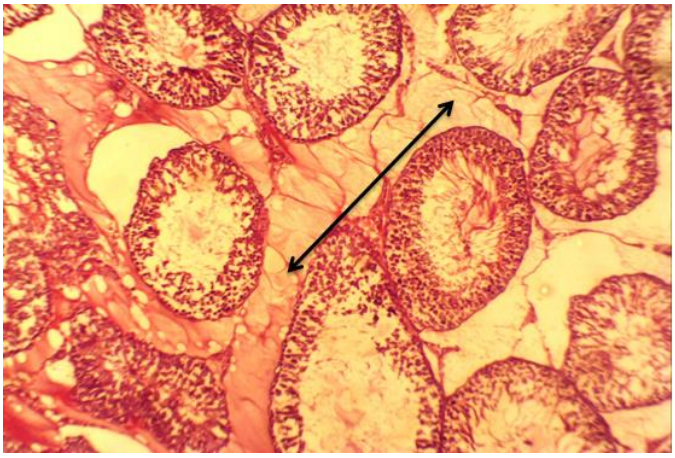

Figure.(4-A):Cross section of rat testis of group G1 treated with CdCl2 (30 ppm/L) for 56 days . note:- show vacoulation ⟷ and absent of leydig cell (H &E stain 10 X).

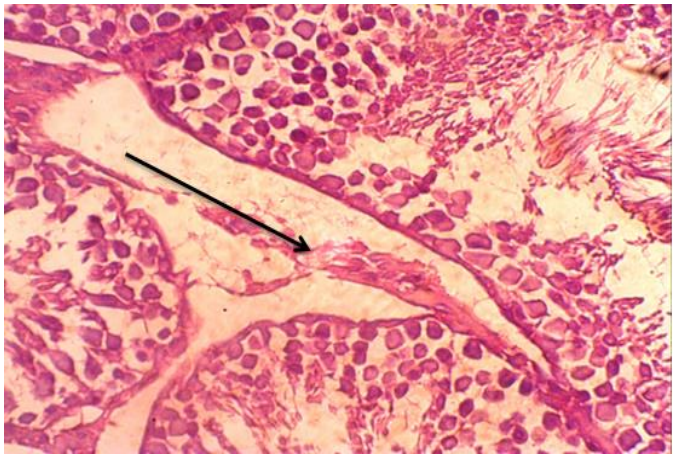

Figure. (4-B):Cross section of rat testis of group G1 treated with CdCl2 (30 ppm/L) for 56 days . note :- few numbers of leydig cells → (H &E stain 40 X)

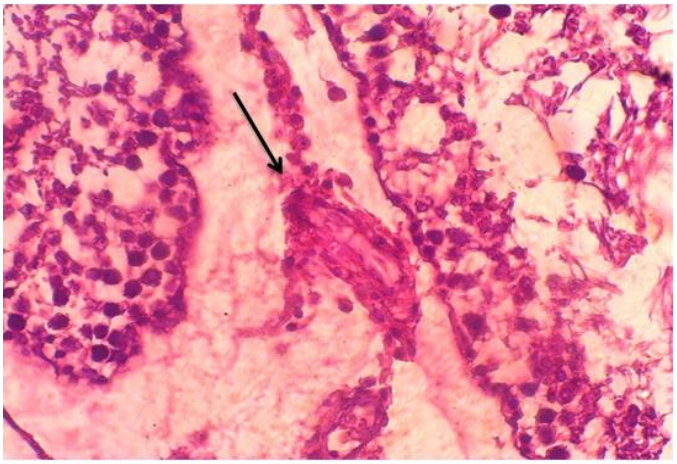

Figure.(5): Cross section of rat testis of group G2 treated with CdCl2 plus 250 mg of *Eruca Sativa* extract for 56 days . note :- show proliferation of leydig cell → (H &E stain 40 X).

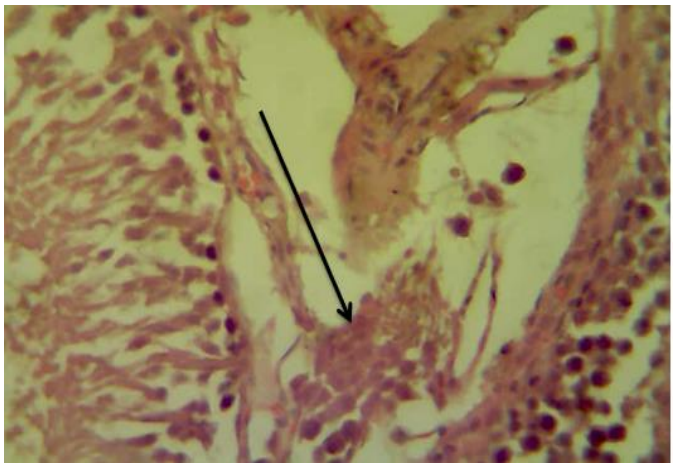

Figure.(6): Cross section of rat testis of group G3 treated with 250 mg of Eruca Sativa extract for 56 days . note :- show normal proliferation of leydig cell → (H &E stain 40 X)

Discussion

Testosterone is a vital male sex hormone produced by Leydig cells for maintenance spermatogenesis process along with FSH (29). Indeed , the result of this study finding that treatment of rats with cadmium chloride at concentration of 30 ppm/L (G1) caused decrement in hormonal profile including testosterone, FSH and LH concentrations and numbers of Leydig cells in comparison with other groups. Many studies manifested that exposure of rats to Cd induced oxidative stress through stimulating the synthesis of cadmium binding proteins (metallothioneins) and heat proteins (30) . This decrement may be due to oxidative damage induced by cadmium chloride (11). Asadi *et al* ., (31) reported that Cd caused inhibition of the serum levels of FSH and LH which induced the signals for inhibition of testosterone synthesis led to reproductive dysfunction , cell death and apoptosis **.** The reduction of testosterone level in G1 treated rats may be due to reduction or impaired the utilization of cholesterol by the Leydig cells (32) . Moreover , any chemical agent impaired or suppressed the secretion of pituitary gonadotropins will produce antifertility effects with deficient androgen secretion by the testes , cessation of spermatogenesis and loss of libido (33 and 34) **.**

Cd has been considered as an important environmental endocrine disrupter (35), this may leads to variations or modifying the hormones secreted daily by the pituitary gland (36). Elgawish and Ghanem (37), also noted that Cd cause devastation the hypothalamus-pituitary gonadal axis thus, destroying the secretory organs of hormones and compromising hormonal gland . Thereby, the disruption of this axis may contribute to a reduction in gonadotropins level FSH and LH leading to inhibition of steroid biosynthesis (38). It is well known that small amounts of reactive oxygen species (ROS) are produce normally in the steroidogenic pathway and are necessary for fertilizing capabilities (14). Also (13) reported that exposure to Cd could induced oxidative stress through decrease the activities of SOD, GPx and CAT with increasing Cd concentrations, which was accompanied with the increase in malondialdehyde (MDA) and H_2O_2 and hydroxyl radicals content in a concentration-dependent manner and these radicals promote oxidative damage as well as apoptosis in the testis, and the apoptotic processes may be mediated via mitochondria-dependent apoptosis pathway by regulating the activities of caspase-3 and caspase-9 (17).

From the results, it seem that alleviating of hormonal profile in G2 and G3 treated groups by ethanolic extract of *E. sativa* may be due to its antioxidant defense mechanism and free radicals scavenger activity (39) of many phytochemical compounds including glucosinolate and flavonoids which possess a potent antioxidant activity (19 and 40) against oxidative damage induced by Cd and thereby improving the pituitary-testes axis and fertility(41 and 42)**.** *E. sativa* seeds extract exhibited an evidence for a stimulatory effects on reproductive gonadal system through androgenic activities through increase the number of Leydig cells in G2 and G3 groups could be due to the free radical scavenging ability through excluding the Fe3+ (43 and 44), or may be due to an increasing the number and/or the sensitivity of receptors of the Leydig cells to LH which led to

increase testosterone biosynthesis (45). According to the results exposure of rats to CdCl2 at concentration 30 ppm/L caused decreased in body weight to testicular weigh ratio as compared to other groups, which are in agreement with other studies (32 and 46). These changes suggested that ROS induced and released from cadmium such as hydrogen peroxide from anions and peroxide of hydrogen radicals leading to OS and testicular degeneration (47). Adamkovicova and his collages(48) stated that Cd induced testicular pathogenicity includes edema, cellular degeneration, necrosis of testicular tissue and leading to infertility (6) . Our findings in this study showed a decrease in testicular weight to body weight ratio in Cd treated rats may be due to a possible alteration in androgen status most likely due to a decrease in serum testosterone, these results are in line with (49). Also a significant decline of gonadotropins depicted in the present study might indicate due to testicular necrosis and degeneration of seminiferous tubules as a result of contraction of myoid cells (50) or a change in the proportion of collagen mediated Cd toxicity can affect the structures of testis(46,51 and 53) and contributed to weight loss of testis.

On the other hand, Eruca extract was effective in ameliorating most of the toxic effects induced by Cd on body and testicular weights in G2 and G3 groups as compared to G1 . It is well known that maintenance of the testicular weight with the accessory reproductive glands depends on testosterone level (53).Accordingly, the significant increase in weights of testis of rats that treated with ES may be due to an improvement in testosterone and / or FSH levels or may be due to the anabolic effects of testosterone leading to raising the body weight (54), also this findings are in agreement with(41) who revealed that administration of *Eruca sativa* extract protected the testis of rats exposed to hydrogen peroxide evidenced by appearance of normal structures of seminiferous tubules of testis and preserve spermatogenesis in addition, testosterone could increase the number and functions of somatic and germinal cells of testis followed by testes weight improvement (55). This increment may be due to the effects of ES phytochemical substances in ES including; proteins, vitamins like A, and C, in addition to presence of important minerals (21 and 56). Therefore, treatment of rats with ES extract can protect pituitary-testicular axis and preserve hormonal profile from the detrimental effects of Cd but its effectiveness is dependent on the dose of Cd exposed.

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