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Protective Effect of Ellagic Acid on Epididymal Sperms Quality and DNA Deformity in Mature Rats Exposed to Cadmium Chloride

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Abstract

This study was designed to investigate the protective effect of Ellagic Acid on Epididymal Sperms Quality and DNA Deformity in mature rats exposed to Cadmium Chloride. The experiment includes a total number of Sixty (60) male Albino Wistar rats weighting (180-220 g) were used in this experiment. Their ages ranged between (10-14) weeks. Rats were divided into four equal groups which administrated orally and daily for six weeks as follows: 100mg EA Kg B.wt, 5mg /Kg B.wt Cd, 100mg EA + 5mg Cd Kg B.wt, and tap water for group 1 (T1), group 2 (T2), group 3 (T3) and control group (C) respectively. The experiment was lasted for 6th weeks. Semen samples were collected after 2, 4 and 6 weeks for estimation sperms parameters. The results revealed a significant decrease of Sperm concentration and Sperms Motility in T2 as compared with control increase significantly of Sperm concentration and Sperms Motility in T1 and the results showed a significant increase of Sperms Abnormalities and Sperms Immotility in T2 with a significant decrease of Sperms Abnormalities and Sperms Immotility in T1 as compared with control. Depending on the current results and discussion, Conclusions are: Ellagic acid is an effective antioxidant against the toxic effect of Cadmium chloride on testicular function and Sperm Parameters.

Keywords: Rats, Ellagic Acid, Sperms Quality, Cadmium Chloride

I. INTRODUCTION

Heavy metals have the potential to contaminate water sources through various means, including industrial and consumer waste, as well as acidic rain causing heavy metals to leach into streams, lakes, rivers, and groundwater. Heavy metal exposure can result in negative health impacts such as reduced mental and central nerve function, decreased energy levels, and damage to vital organs such as the lungs, kidneys, liver, and blood **(Zango *et al.*, 2020).** Degenerative processes in the muscles and nervous system may develop over time after chronic exposure to heavy metals, similar to those seen in Alzheimer's, Parkinson's, muscular dystrophy, and multiple sclerosis. Prolonged contact with some metals or their compounds can induce allergies or even cancer in rare situations **(Bakulski *et al.*, 2020 and Hanaa, 2007).**

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For up to forty years, cadmium may build up in the body and cause serious health issues (**Wang *et al.*, 2016).** Its biological half-life is between 17 and 30 years, making it more likely to accumulate and be harmful over time **(Shukla *et al.*, 2009).** Cadmium has been designated as a human mutagen since 1993 (IARC, 1993) by the International Agency for Research on Cancer. In addition, cadmium is listed as the eighth most dangerous material by the Agency for Hazardous Substances and Disease Registry's Priority List of Hazardous Substances **(Al-Okaily, 2017).** Experiments show that cadmium negatively affects the reproductive and immunological systems, kidneys, and liver **(Ogawa *et al.*, 2013).** Recent studies have confirmed that male rodents exposed to CdCl2 for short periods suffer significant reproductive damage **(Oguzturk *et al.*, 2012).**

A flavonoid molecule called ellagic acid (E.A.; C14H6O8; M.W.: 302.202; 3,7,8-tetrahydroxy[1]-benzopyrano[5,4,3-cde] Various berries and plants contain the compound [1] benzopyran-5,10-dione, which has attracted interest for its potential antioxidant properties (**Wang *et al.*, 2017; Syed *et al.*, 2017).** The antioxidant effects of E.A., or the activation of cellular antioxidant enzyme systems, are thought to mitigate the harmful consequences of oxidative stress **(Atessahin *et al.*, 2010; Tasaki *et al.*, 2008).** Antiapoptotic, chemopreventive, and radical scavenging effects of E.A. have been discovered **(Ceribasi *et al.*, 2010; Turk *et al.*, 2010).**

II. MATERIALS AND METHODS

All procedures used in this study were reviewed and approved by The Scientific Committee of the College of Veterinary Medicine, University of Baghdad in compliance with the ethical principles of animal welfare. Sixty adult male rats were randomly divided into four groups (ten animals per group) and subjected to the following adaptation period: **Control Group:** Every day, tap water was administered orally. T1 received Ellagic acid by oral gavage at 100 mg/kg B.W. daily (**Arrak, 2010 and Al-Hussain, 2009). Group T2:** daily oral gavage of cadmium chloride at a dosage of 5 mg/kg B.W. **(Alkhedaide *et al.*, 2016). Group T3:** Ellagic acid at a dose of 100 mg/kg B.W. and cadmium chloride at a rate of 5 mg/kg B.W. was administered orally daily. The duration of the study was six weeks. Sperm samples were taken at 2, 4, and 6 weeks for parameter estimation.

Statistical analysis:

SAS (Statistical Analysis System, version 9.1) was used for the statistical analysis of the experiment outcomes. To find significant mean differences, two-way ANOVA and LSD post hoc tests were used. Data were reported as mean ± standard errors (S.E.) with a P-value < 0.05 (SAS, 2021).

III. RESULTS AND DISCUSSION

Sperm concentration:

Table (4.1) shows the influence of Cadmium Chloride and Ellagic acid, Cadmium Chloride alone, and Ellagic acid alone on mean values of Sperm concentration. In comparison to the T1, T3, and control groups, the concentration of sperm was consistently lower in the T2 treatment group throughout the experimental periods, as shown in Table 1. Meanwhile, T1 has the highest Sperm concentration compared to the other groups. No

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statistically significant difference (P > 0.05) in the sperm concentration between the T2 and T3 groups at weeks 2, 4, and 6. However, in T1, this variation is statistically significant (P > 0.05). Ellagic acid substantially increased (P < 0.05) sperm concentration compared to the T2 group. However, when comparing the T3 group to the control group, no significant differences were found.

Cadmium Chloride treatment promotes metallothionein synthesis, according to **Siu *et al.* (2009)** and **Xu *et al.* (2005).** However, this is confined to a specific level in the testis. If insufficient M.T. is present to bond with the Cd, oxidative stress and impairment of spermatogenesis may result in toxic effects **(Xu *et al.*, 2005 and Obaid *et al.*, 2022).**

Gupta *et al.* (2003), El-Demerdash *et al.* (2004), and Khazaal *et al.* (2022) previously found weight decreases in accessory sex organs following Cd treatment. In the current investigation, the seminal vesicle weighed less when exposed to a larger dose of cadmium. Furthermore, testosterone levels in plasma and testes were reduced after chronic exposure to Cd, indicating interference with spermatogenesis and steroidogenesis.

In a previous study, a negative correlation was found between lipid peroxidation and sperm function characteristics, confirming that oxidative stress has a detrimental influence on sperm function. In the current study, E.A. supplementation consistently improved the functional characteristics of sperm, which is consistent with previous findings. The results imply that E.A. protects against TAM-induced testicular toxicity in a rodent model. Testicular health may be improved in healthy and TAM-injured animals if E.A. can enter the circulation, as has been hypothesized. TAM decreases testicular function and sperm quality, whereas E.A. increases spermatogenesis in animals with damaged sperm and improves the performance of reproductive hormones. The improvement in testosterone, FSH, and L.H. levels, as well as the increase in the number of germinal cells following E.A. therapy, may be attributable to the antioxidant properties of E.A. E.A. may exert its effects by facilitating the passage of certain amino acids, peptides, or whole polypeptide chains through the gut-blood barrier (**Olfati & Khamisabadi, 2020).**

The function of the EA-mediated pathway in E.A.'s antioxidant actions and the resultant alterations in spermatogenesis following chemotherapy remains uncertain. However, oral supplementation of E.A. at a dose of 5.0 mg/kg/day for 48 days improved spermatogenesis rate, hormone levels, and spermatogenic cell production in both normal and TAM-injured rats. More spermatogenic cells and other testicular cells, such Sertoli and Leydig cells, are consistent with these findings. It has been proven that eating foods high in antioxidants increases the chance of getting pregnant in infertile couples and lowers the risk of pregnancy losses. As a result, administering E.A. may be a viable technique for improving sperm quality and increasing the odds of conception in infertile couples.

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Table (4.1): Sperm concentration (per million sperm/ml) in response to oral administration of Ellagic acid only (T1), Cadmium Chloride only (T2), and Ellagic acid with Cadmium Chloride (T3). (at Ellagic acid 100 mg/Kg B.w. and Cadmium Chloride 5 mg/Kg B.wt). In adult male rats for six weeks.

Group / Week	Control	T 1	T 2	T 3
2	7.44 ± 0.34 Ab	7.25 ± 0.32 Ab	6.36± 0.08 Ba	7.17 ± 0.25 Aa
4	8.03 ± 0.15 A ab	7.58 ± 0.24 Ab	6.55 ± 0.22 Ba	7.53 ± 0.07 A a
6	8.41 ± 0.31 A a	8.51 ± 0.21 A a	6.81 ± 0.23 Ba	7.83 ± 0.04 Aa

Mean ± S.E. (n=15 rats/ group). LSD: 0.6671
Capital letters refer to considerable ($P < 0.05$) difference between groups.
Small letters point out to considerable ($P < 0.05$) difference within group.

Sperms Abnormalities:

Table (4.2) shows the influence of Cadmium Chloride and Ellagic acid, Cadmium Chloride alone, and Ellagic acid alone on mean values of Sperm Abnormalities. In comparison to groups T1, T3, and the control, treated group T2 demonstrates a statistically significant ($P < 0.05$) increase in Sperms Abnormalities across all experimental time points. In contrast, group T1 only displays a statistically significant ($P < 0.05$) decrease in Head Sperms Abnormalities relative to groups T2 and the control. Additionally, the T3 group experienced a significant decrease ($P < 0.05$) in the fourth week of the investigation. Moreover, T2 has the highest rate of sperm abnormalities compared to the other groups.

Sperm Abnormalities demonstrate a non-significant difference ($P > 0.05$) between the control and T2 groups at the second, fourth, and sixth weeks. This difference, however, is considerable in T1 and T3. T1 and T3 differ significantly. The fourth week had fewer sperm abnormalities ($P< 0.05$) than the second and sixth weeks in T3. T1 also experienced this in the sixth week compared to the second and fourth weeks.

The study's findings show that modest dosages of Cd can produce gradual morphological and morphometric changes in the rat testis due to its direct action. The results indicate a direct correlation between dose and latency to morphological changes. Moreover, even a minor difference in dose can result in substantially different degrees of tissue damage, which may overcome the tissue's natural defenses. These results emphasize the importance of monitoring and minimizing exposure to Cd and other toxic substances to prevent adverse effects on reproductive health (**De Souza *et al.*, 2010).**

The study's results indicate a significant decline in sperm abnormalities in rats exposed to Cd. These results are in line with earlier studies on animals (**El-Demerdash et al., 2004; Amara et al., 2008)** as well as human research **(Benoff et al., 2008; Wang et al., 2016).** The decreased sperm quality brought on by Cd may be explained by

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several processes. De Souza et al. (2010) claim that the change in sperm parameters that leads to reproductive failure, including reduced sperm count, motility, and morphology, may be caused by the direct effect of Cd on testicular tissue. Cd particularly damages Sertoli-germ cell tight connections, which prevents spermatogenesis from occurring. Seminiferous tubules are destroyed, and immature germ cells are lost one by one in severe testicular injury, causing developmental defects in the sperm (**Siu *et al.*, 2009; Zhang *et al.*, 2010).** In addition, low-dose Cd exposure influences the actions of steroid hormones implicated in regulating reproductive processes. For appropriate testicular steroidogenesis and spermatogenesis, normal steroidogenic enzyme activity must be maintained. Reduced antioxidant enzyme activities show that oxidative damage and testosterone levels are associated with declining sperm quality **(Pandya *et al.*, 2008; Acharya *et al.*, 2008).** These findings emphasize the importance of limiting Cd and other toxic substance exposure to avoid negative effects on reproductive health. It is unclear how the EA-mediated pathway contributes to E.A.'s antioxidant impacts and subsequent changes in spermatogenesis after chemotherapy. However, the findings of this study revealed that taking 50 mg/kg/day E.A. orally for 48 days improved spermatogenesis rate and spermatogenic cell production. These findings agree with the hypothesis that the number of spermatogenic cells and other testicular cells like Sertoli and Leydig has increased. Antioxidant consumption has been shown to improve fertility in infertile couples and decrease reproductive losses **(Gamidov et al., 2019).**

The findings of antioxidant rescue tests indicate that oxidative stress, primarily ROS generation, is the primary cause of Cd toxicity during spermatogenesis. Previous research suggested that consuming E.A. regularly might protect the damaged testis. **Ceribaşi *et al.* (2010)** found that E.A. reduces oxidative stress in rats by reducing plasma lipid peroxidation and enhancing erythrocyte antioxidant enzyme activity.

The findings of this investigation back with earlier studies that E.A. protects against Cd-induced testicular damage in a rat model. Therefore, it has been hypothesized that E.A. can be absorbed by the body and used to improve sperm quality. In conclusion, Cd reduces sperm quality and testicular function, but E.A. treatment improves sperm parameters, sexual hormone function, and spermatogenesis in animals with damaged sperm. E.A., a new antioxidant, may raise testosterone, FSH, L.H., and germinal cell count following treatment. E.A. may work by promoting the flow of specific amino acids or peptides over the gut-blood barrier or by increasing the activity of whole polypeptide chains **(Olfati *et al.*, 2020).**

Table (4.2): Sperms Abnormalities (per million sperm/ml) in response to oral administration of Ellagic acid only (T1), Cadmium Chloride only (T2) and Ellagic acid with Cadmium Chloride (T3). (At Ellagic acid 100 mg/Kg B.W. and Cadmium Chloride 5 mg/Kg B.W.). in adult male rats for six weeks.

Group / Week	Control	T 1	T 2	T 3
2	3.56 ± 0.11 Ba	2.27 ± 0.03 Ca	4.86 ± 0.32 Aa	3.67 ± 0.11 Ba
4	3.88 ± 0.13	2.51 ± 0.04	5.22 ± 0.48	3.13 ± 0.24

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	Ba	Da	Aa	Cc
6	3.81 ± 0.25 Ba	1.89 ± 0.01 Cb	5.31 ± 0.41 Aa	3.54 ± 0.12 Bab

Mean ± S.E. (n=15 rats/ group). LSD: 0.3771

Capital letters refer to a considerable ($P < 0.05$) difference between groups.

Small letters indicate a considerable ($P < 0.05$) difference within the group.

Sperms Motility and Sperms Immotility:

Table (4.3) shows the influence of Cadmium Chloride plus Ellagic acid, Cadmium Chloride only, and Ellagic acid only on mean sperm motility values. Throughout the experimental period, this table displays a significant decrease (P 0.05) in Sperm Motility in treated groups T2 and T3 compared to T1 and the control group. In comparison to groups T2 and T3, only group T1 treated with Ellagic acid showed a significant improvement in sperm motility ($P < 0.05$). T2 had the lowest sperm motility when compared to the other groups. The sperm motility showed a non-significant difference ($P< 0.05$) between the control and T1 groups in the second, fourth, and sixth weeks. This difference, however, is considerable in T2 and T3.

Table (4.4) displays the mean values of sperm immotility before and after treatment with cadmium chloride and ellagic acid, cadmium chloride alone, and ellagic acid alone. There was a statistically significant ($P < 0.05$) increase in the percentage of immotile sperm in the T2 and T3 treatment groups compared to the T1 and control groups across all time points in the trial. In contrast, the immotility of sperm in group T1, which was treated with Ellagic acid alone, decreased significantly ($P < 0.05$) compared to groups T2 and T3. On the other hand, T2 has the largest rate of immotile sperm compared to the other groups. At weeks 2, 4, and 6, there is no statistically significant difference ($P > 0.05$) between the immotility of sperm in the control, T1, and T3 groups. This difference, however, is considerable in T2. The data revealed a substantial increase (P 0.05) in Sperm Immotility in the second, fourth, and sixth weeks of T2. Hypogonadism and infertility have been linked to cadmium's gonadotoxic and spermiotoxic effects, in which the metal either damages reproductive structures and functions at the testicular level or alters post-testicular processes including sperm progression motility and/or function **(El-Demerdash *et al.*, 2004; Yang *et al.*, 2006; Akinloye *et al.*, 2006).**

Cadmium Chloride treatment decreased sperm motility and testosterone levels in this study. Cd-challenged rats exhibited considerably decreased sperm motility, similar to Oliveira et al. (2006), who found that exposing mice to CdCl2 for 35 days dramatically reduced sperm motility. This decrease might be attributed to Cd competing with calcium for calmodulin binding, which is necessary for sperm motility. Sperm motility is reduced when calmodulin is inhibited (Schlingmann et al., 2007; Alharris et al., 2022). The study also showed that low testosterone levels in plasma and testes resulted from prolonged exposure to Cd. The number and motility of epididymal spermatozoa were negatively affected (Amara et al., 2008). The negative effects of Cd on sperm quality and testosterone production are highlighted by these findings, highlighting the necessity of limiting exposure to Cd and other hazardous compounds to protect reproductive health.

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Human and rat sperm that were exposed to Cd in vitro did not perform as well as those that were not. Reduced fertility may result from cadmium's effect on sperm motility and progression **(Zhao *et al.*, 2017).** Short-term (30-minute) exposure to Cd does not affect the movement of sperm, but it greatly lowers the rate of in vitro fertilization of eggs and slows early embryonic development in mice. This suggests that Cd works by changing how genes work **(Zhao *et al.*, 2017).** Additional evidence of Cd's negative effects on sperm quality is that it reduces human sperm motility and forward motility **(Juma, 2011).**

Acute CdCl2 poisoning significantly reduced sperm motility and concentration. It significantly increased the rate of aberrant sperm, particularly the rate of abnormal morphology in the tail and head of the sperm compared to the control. These findings support previous findings (Acharya et al., 2008; Ola-Mudathir et al., 2008; Yari et al., 2010) that CdCl2 toxicity causes dangerous changes in sperm parameters. It is important to limit exposure to Cd and other hazardous compounds to protect reproductive health since their oxidative and histopathologic effects are suspected to be responsible for CdCl2's spermatologic effects.

Ellagic acid (E.A.) has been found to suppress the production of O2-. and. O.H. in both enzymatic and non-enzymatic systems via its metal-chelating capability, protecting lipids against peroxidation (Yüce et al., 2008; Kadhim Al-Rekabi, 2014). Previous research has shown that E.A. can protect against testicular and spermatozoal damage by lowering lipid peroxidation in the testes (Türk et al., 2008). E.A. improved sperm parameters and testicular histopathology in this investigation, completely or partially. E.A. strongly scavenged free radicals and inhibited oxidative DNA damage, as evidenced by decreased lipid peroxidation in testicular tissue. Furthermore, E.A. has been shown to improve testicular function and structure in a rat model. The effects of E.A. on sperm count, morphology, and motility were explored in a study, and it was discovered that E.A. enhanced sperm count, morphology, and motility (Rostami et al., 2008). These results indicate that EA could be a potential therapeutic agent for enhancing sperm quality and testicular function by reducing oxidative stress and lipid peroxidation. E.A. may be useful in treating male infertility, but further study is needed to prove this and establish the best treatment course, including dose and duration.

By protecting against the damaging effects of free radicals, elagic acid may increase the antioxidative capacity. The motility of sperm depends on three primary factors: regulation, structural integrity, and energy continuity. While the flagellar portion of spermatozoa is responsible for motility, the major portion of spermatozoa is in charge of hyperactivation (Turner *et al.,* 2005; Suarez *et al.,* 2007). Ellagic acid is believed to protect this functional structure of the spermatozoa's central section and increases sperm motility when the sperm is stored in liquid. These findings corroborate those of Türk et al. (2008), who found that ellagic acid increased sperm motility in the epididymis of rats. Ellagic acid, a potent antioxidative agent, may shield the cell membranes from cryoinjury. These results indicate that ellagic acid may have therapeutic potential for enhancing sperm quality and function. To confirm these findings and ascertain the optimal dosage and duration of treatment with ellagic acid for enhancing sperm quality and fertility, additional research is required.

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Table (4.3): Sperms Motility in response to oral administration of Ellagic acid only (T1), Cadmium Chloride only (T2) and Ellagic acid with Cadmium Chloride (T3). (At Ellagic acid 100 mg/Kg B.W. and Cadmium Chloride 5 mg/Kg B.W.). in adult male rats for six weeks.

Group / Week	Control	T 1	T 2	T 3
2	94.27 ± 1.64 A a	92.31 ± 1.02 A a	74.56 ± 2.97 Ca	85.05 ± 1.21 B a
4	91.62 ± 1.31 A a	95.43 ± 1.60 A a	68.38 ± 1.40 C b	79.28 ± 2.17 B b
6	95.68 ± 1.61 A a	95.52 ± 0.84 A a	68.24 ± 1.39 C b	78.44 ± 1.14 B b

Mean ± S.E. (n=15 rats/ group). LSD: 4.74

Capital letters refer to a considerable ($P < 0.05$) difference between groups.

Small letters point out to a considerable ($P < 0.05$) difference within group.

Table (4.4): Sperms Immotility in response to oral administration of Ellagic acid only (T1), Cadmium Chloride only (T2) and Ellagic acid with Cadmium Chloride (T3). (At Ellagic acid 100 mg/Kg B.W. and Cadmium Chloride 5 mg/Kg B.W.). in adult male rats for six weeks.

Group / Week	Control	T 1	T 2	T 3
2	39.25 ± 0.81 A a	40.41 ± 1.53 A a	42.01 ± 0.89 A b	42.01 ± 1.93 A a
4	37.53 ± 0.86 C a	40.21 ± 1.15 C a	57.93 ± 2.41 A a	44.19 ± 0.61 B a
6	39.82 ± 0.37 C a	38.65 ± 0.54 C a	48.72 ± 1.31 A c	43.12 ± 2.33 B a

Mean ± S.E. (n=15 rats/ group). LSD: 3.87

Capital letters refers to a considerable ($P < 0.05$) difference between groups.

Small letters point out to a considerable ($P < 0.05$) difference within group.

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Live and dead sperms:

Table (4.5) displays the effect of Cadmium Chloride and Ellagic acid, Cadmium Chloride only, and Ellagic acid only on the mean values of Live sperms. Table 1 shows that compared to the control group and group T2, the proportion of live sperm in treated groups T1 and T3 increased significantly ($P > 0.05$) throughout the experiment. In contrast, the proportion decreased significantly ($P > 0.05$) in treated group T2 with Cadmium Chloride. T1 contained the greatest concentration of live sperm relative to the other categories. No statistically significant difference ($P > 0.05$) in the number of viable sperm in the control, T1, and T3 groups between the second, fourth, and sixth weeks. However, this difference is significant in T2. Live sperm increased significantly ($P < 0.05$) during the second, fourth, and sixth weeks of T2.

Table (4.6) shows the influence of Cadmium Chloride and Ellagic acid, Cadmium Chloride only, and Ellagic acid only on mean values of Dead sperms. This table demonstrates a substantial decrease (P 0.05) in Dead sperms in treatment groups T1 and T3 during the trial compared to T2 and the control group. Compared to the control, T1, and T3 groups, group T2 treated with Cadmium Chloride only exhibits a significant increase ($P < 0.05$) in Dead sperms. T1 has the fewest dead sperms. Dead sperms for the control, T1, and T3 groups show no statistically significant change (P 0.05) between the second, fourth, and sixth weeks. Nonetheless, this difference is substantial for T2.

The results show that cadmium may cause testicular toxicity. However, the flavonoid molecule ellagic acid can mitigate the damage to the testis and help rebuild its histoarchitecture, saving both healthy and damaged sperm. Ellagic acid's antioxidant and antiapoptotic properties may explain this effect. More research is needed to determine E.A.'s protective mechanism. If clinical trials show efficacy, ellagic acid may benefit epileptic patients on long-term cadmium treatment (Fouad et al., 2013; Abbas, 2023). Cd may disturb cellular processes, triggering free radical generation and lipid peroxidation in the testis. Ellagic acid's antioxidant and free radical scavenging abilities may reduce medication toxicity. PCNA activation by CdCl2 induces apoptosis in human and rat granulosa cells. Anti-apoptotic compounds like E.A. show promise as a protection against testicular damage. To fully understand the E.A.'s protective effects, more research is required **(Eleawa *et al.*, 2014; AL-Chalabi, 2014).** These results point to the therapeutic potential of ellagic acid in the prevention and treatment of cadmium-induced testicular damage. However, more study is required to confirm these findings and to establish the best ellagic acid treatment dosage and duration.

Table (4.5): Live Sperms in response to oral administration of Ellagic acid only (T1), Cadmium Chloride only (T2), and Ellagic acid with Cadmium Chloride (T3). (At Ellagic acid 100 mg/Kg B.W. and Cadmium Chloride 5 mg/Kg B.W.). in adult male rats for six weeks.

Group / Week	Control	T 1	T 2	T 3
2	75.01 ± 0.41	92.43 ± 0.79	69.86 ± 0.32	78.97 ± 0.58

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	Ba	**Aa**	**Ca**	**Ba**
4	**78.67 ± 0.59 Ba**	**95.09 ± 0.84 Aa**	**64.22 ± 0.48 Cb**	**81.93 ± 0.64 Ba**
6	**77.89 ± 0.57 Ca**	**96.88 ± 0.91 Aa**	**61.31 ± 0.41 Db**	**83.31 ± 0.68 Ba**

Mean ± S.E. (n=15 rats/ group). LSD: 3.78

Capital letters refer to a considerable (P < 0.05) difference between groups.

Small letters point out to a considerable (P < 0.05) difference within group.

Table (4.6): Dead Sperms in response to oral administration of Ellagic acid only (T1), Cadmium Chloride only (T2), and Ellagic acid with Cadmium Chloride (T3). (At Ellagic acid 100 mg/Kg B.W. and Cadmium Chloride 5 mg/Kg B.W.). in adult male rats for six weeks.

Group / Week	Control	T 1	T 2	T 3
2	**24.99 ± 0.11 Ba**	**7.57 ± 0.09 Da**	**30.14 ± 0.32 Ab**	**21.03 ± 0.18 Ca**
4	**21.33 ± 0.13 Ba**	**4.91 ± 0.06 Ca**	**35.78 ± 0.48 Aa**	**18.07 ± 0.24 Ba**
6	**22.11 ± 0.25 Ba**	**3.12 ± 0.04 Da**	**38.69 ± 0.41 Aa**	**16.69 ± 0.12 Ca**

Mean ± S.E. (n=15 rats/ group). LSD: 3.75

Capital letters refer to a considerable (P < 0.05) difference between groups.

Small letters point out to aconsiderable (P < 0.05) difference within group.

DNA normality of sperm:

Table (4.7) shows that DNA Damage was significantly higher in the T2 group of rats given 5 mg/Kg B.W. of cadmium chloride compared to the T1 and T3 groups, as well as the control group (P 0.05). DNA damage in the treated T2 group was (59.71 ± 0.185), while in the control group it was (15.69 ± 0.031). Cadmium significantly increased (P < 0.05) the quantity of DNA damage (T2) compared to the other groups, but Ellagic acid (T1) improved the image of DNA damage compared to the other groups. However, administering cadmium with Ellagic acid (T3) significantly decreased (P < 0.05) the quantity of DNA damage compared to T2 and significantly improved the control group.

The mechanism of Cd-induced DNA base oxidation remains unknown. However, evidence suggests that Cd exposure causes oxidative DNA damage due to excess oxygen-derived free radicals. The testis is the only organ that suffers from exposure to the environmental toxin cadmium. Cadmium's detrimental effects on the testis

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include the degradation of germ cells and disruption of steroidogenesis (Sadik, 2008). Cd causes hypothalamic-pituitary-testicular axis disruption in rats due to hypothalamic-pituitary-testicular axis accumulation and decreased plasma follicle-stimulating hormone (Lafuente et al., 2000). The DNA in primary spermatocytes can be damaged by cadmium's direct toxicity. Damage to the DNA of germ cells can lead to infertility and other aberrant reproductive outcomes such spontaneous miscarriage, genetic diseases, and an increased risk of cancer (Brinkworth, 2000; Spano et al., 2000; Coddington et al., 2004). It is essential for reproduction that the paternal genome be kept intact **(Cordelli *et al.*, 2003).**

Mature spermatozoa's DNA fragmentation and damage mechanisms are unclear. DNA fragmentation can occur directly in mature spermatozoa due to exposure to endogenous (reactive oxygen species) or exogenous (chemicals or radiation) mutagens or alterations in nick production and ligation during spermiogenesis (Aitken et al., 1998; Ahmadi & Ng, 1999; Alvarez et al., 2002; Sakkas et al., 2002). DNA fragmentation in the primordial spermatocyte after exposure to certain toxic metals is the likely cause of DNA fragmentation in spermatozoa. If the DNA in a developing male germ cell is damaged, the resulting sperm may still be able to fertilize an egg, but the damage will be passed on to the offspring (Shelby et al., 1993). DNA damage induced by oxidative stress is believed to be one of the most important parameters for biomonitoring the effect of dietary antioxidants on human health. The comet assay is commonly used to quantify oxidative and other DNA damage (Hamza and Al-Baqami, 2019). These results stress the significance of reducing exposure to Cd and other toxic substances to protect one's DNA and reproductive health from potential harm.

Table (4.7): DNA Damage in response to oral administration of Ellagic acid only (T1), Cadmium Chloride only (T2) and Ellagic acid with Cadmium Chloride (T3). (At Ellagic acid 100 mg/Kg B.W. and Cadmium Chloride 5 mg/Kg B.W.). in adult male rats for six weeks.

Control C	T1 E	T2 Cd	T3 E+ Cd
15.69 ± 0.031 C	10.98 ± 0.022 D	59.71 ± 0.185 A	25.16 ± 1.49 B

Mean ± S.D. (n=15 rats/ group). LSD= 4.43

Capital letters refer to a considerable ($P < 0.05$) difference between groups.

Conclusions:

Depending on the current results and dissension, Conclusions are Ellagic acid is an effective antioxidant against the toxic effect of Cadmium Chloride on testicular function and Sperm Parameters. The results should harmfully effect of Cadmium Chloride on pituitary-testicular axis and quality of sperm.

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Conflict of interest:

The research declares that there is no conflict of interest in the publication of this paper.

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الخلاصة

صممت هذه الدراسة لمعرفة التأثير الوقائي لحمض Ellagic على جودة الحيوانات المنوية البربخية وتشوه الحمض النووي في الجرذان الناضجة المعرضة لكلوريد الكادميوم. تضمنت التجربة عدد ستين (60) ذكور من ذكور جرذان ألبينو ويستار بوزن (180-220 جم) تم استخدامها في هذه التجربة. تراوحت أعمارهم ما بين (10-14) أسبوع. تم تقسيم الجرذان إلى أربع مجموعات متساوية تعطى عن طريق الفم واليوم لمدة ستة أسابيع على النحو التالي: 100 مجم EA Kg B.wt، 5 مجم / كجم وزن Cd، 100 مجم EA + 5 مجم (Cd Kg B. T1) ، المجموعة 2 (T2) ، المجموعة 3 (T3) ومجموعة التحكم (C) على التوالي. استمرت التجربة لمدة 6 أسابيع. جمعت عينات السائل المنوي بعد 2 و 4 و 6 أسابيع لتقدير معاملات الحيوانات المنوية. أظهرت النتائج انخفاضًا معنويًا في تركيز الحيوانات المنوية وحركة الحيوانات المنوية في T2 مقارنة مع السيطرة على زيادة معنوية في تركيز الحيوانات المنوية وحركة الحيوانات المنوية في T1 وأظهرت النتائج زيادة معنوية في تشوهات الحيوانات المنوية وعدم حركة الحيوانات المنوية في T2 مع انخفاض معنوي في تشوهات الحيوانات المنوية. وعدم حركة الحيوانات المنوية في T1 بالمقارنة مع السيطرة. اعتمادًا على النتائج والمناقشات الحالية، الاستنتاجات هي: حمض Ellagic هو أحد مضادات الأكسدة الفعالة ضد التأثير السام لكلوريد الكادميوم على وظيفة الخصية والحيوانات المنوية.