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Research paper

Evaluation of the protective effect of *Cinnamomum Zeylanicum* on cadmium testicular toxicity and Nrf2 gene expression in albino rats

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Abstract

Cadmium is an industrial pollutant that may exert specific toxic effect on mammals. The aim of study was to investigate the protective effect of Cinnamon on male reproductive system of albino rat induced by cadmium toxicity. Forty male albino rats were divided into 4 groups. 1st Group administrated saline and kept as control.2nd Group administrated 200mg/kg / day Cinnamon extract. 3rd Group administrated 20mg/kg/day cadmium chloride.4th Group administrated 200mg/kg/day Cinnamon extract and 20mg/kg/day cadmium chloride orally for 8 weeks. . Our results revealed significant decrease in testosterone hormone level, sperm viability, and significant increase in sperm abnormality. An increase in expression of Nrf2 gene was recorded. Pathological changes in testes showed focal degeneration with loss of spermatogenic series in the seminiferous tubules. All of the above mentioned results were significantly improved in 4th group. In conclusion, Cinnamon extract has a protective effect on the testicular damage induced by cadmium chloride.

Keywords:*Cadmium Toxicity; Cinnamon Extract; Nrf2; Antioxidants.*

1. Introduction

Infertility is a problem which affect one in six couples (Balen and Rutherford 2007). However, male factor considers solely responsible in about 20% of infertile couples and contributory in another 30–40% (Wang et al. 2013). One of the most toxic industrial and environmental heavy metals is cadmium (Cd) which act as oxidative stress inducer and an endocrine disruptor in humans and rodents (Takiguchi and Yoshihara 2006, Siu et al. 2009). Cadmium has long been known to damage the reproductive, hepatic, respiratory systems (Who 1992). Cd has extremely toxic to the testicular tissues of mice, rats and several morphological and biochemical changes in the mammalian testis (Acharya et al. 2008, Siu et al. 2009). Cadmium has effect on testicular spermatogenic and steroidogenic functions which impair male fertility, degrade semen quality and induce testicular degeneration, seminiferous tubular (ST) damage and ultimately, reproductive failure (El-Demerdash. et al. 2004, Thompson and Bannigan. 2008,Siu et al. 2009 , Pandyaet al. 2012). The pathogenesis of Cd on testicular dysfunction is result of a complex network of causes including modulation of apoptosis and inhibition of DNA repair enzymes and induction of oxidative stress (Thompson and Bannigan. 2008, Bu et al. 2011, Kalender et al. 2012, Oguzturk et al. 2012). Cadmium induced testicular oxidative stress is mediated through depletion of reduced glutathione (GSH),generation of reactive oxygen species (ROS), altered antioxidant enzymes and elevated lipid peroxidation (LPO) which lead to male infertility (Sen Guptaet al. 2004, Karaet al. 2005, Siu et al. 2009,Bu et al. 2011) . Administration of vitamin E, vitamin C, α-lipoic acid and beta-carotene, which act as antioxidants and free radical scavengers, has effective against Cd-induced testicular damage (El-Demerdash. F et al. 2004, Obianime and Roberts 2009). Therefore, it is conceivable that antioxidant agents might prevent or at least reduce Cd-induced testicular toxicity.

Generally Oxidative stress defined as an imbalance which favors the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) over antioxidant defenses (El-Nekeety et al. 2009). Cd was unable to directly generate free radicals; however, indirect mechanisms involved the generation of various radicals including nitric oxide, the superoxide radical and hydroxyl radical (Bernhoft 2003). ROS is the major consequence induced by Cd through oxidative stress which -mediated attack of double bonds in membrane lipids that results in increased lipid peroxidation (LPO) as well as interference with the endogenous antioxidant defenses in different systems and several organs (Valko et al. 2006). ROS were predominantly implicated and it was been clearly demonstrated that oxidative stress interfered with the expression of genes as well as several transcriptional factors such as AP-1 upstream stimulator factor (USF), NF-E2-related factor (Nrf2) ,metal regulatory transcription factor 1 (MTF1) and nuclear factor-B (NF-κB). ROS may function as secondary messengers that deregulate gene expression and induce cell transformation when cells were exposed to Cd (Yang et al. 2007). The exposure to Cd in many cell types induced oxidative stress; this phenomenon, in turn, has been associated with increase Nrf2 activity that results in increased expression of some antioxidant enzymes(Chenand Shaikh . 2009, Simmonset al. 2011).

Herbal medicines are increasingly being used worldwide. There has been increased interest among phytotherapy researchers to use medicinal plants with antioxidant activity for protection against heavy metal toxicity (Valko et al. 2006).Cinnamon (Cinnamomum

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zeylanicum), a medicinal plant belongs to Luaraceae family which contain mucilage, oxalates volatile oils, tannins, terpenoids, and starch. Different chemical constituents of Cinnamomum zeylanicum were known to have significant germicidal, antiulcerogenic and cytotoxic effects. This plant has many therapeutic effects. Increase of sexual ability is one of its most important effects (Shagauoand Davidson 2006). Limited data is available on the protective effect of this substance against the toxicity of heavy metals on male reproduction. In a study, the aqueous extract of Cinnamon increased the weight of testes, caudae epididymidies and seminal vesicles in the treated male mice, explaining a possible stimulation of hormonal levels in the animals. Also, the sperm count and motility of the treated animals have significantly higher than the control group (Shah et al. 1998).

Therefore, the aim of this study is investigation of toxic effect of cadmium chloride on testes with possible ameliorating effect of aqueous cinnamon extract of adult male albino rats.

Hypothesis, supplementation of Cinnamon extract at a dose 200mg/kg may improve toxic effect of cadmium chloride on testes which improve infertility which maybe induced as a result of toxicity in adult male albino rats.

2. Material and methods

2.1. Cinnamon aqueous extract

The plant was identified by Botany department of faculty of Science , Benha University. Cinnamon extract was extracted according to method of (Shenget al. 2008). Briefly, cinnamon powder (100gm) was dissolved in 1000 ml double distilled water then was subjected for vacuum state of revolving evaporator using vacuum pump for reduce the water volume to 50%. The supernatant was filtered by Whitman paper No. 1 for obtaining cinnamon water extract. Measuring the final concentration by Lowry method for protein concentration. Cinnamon extract was given in a dose of 200 mg/kg body weight orally ,daily for 8 weeks as stated by(Kim et al. 2006, Kimand Choung 2010) .

2.2. Animal experiment

Forty apparently healthy albino rats were obtained from Veterinary Serum And Vaccine Research Institute, Abbasia, Cairo, Egypt. Housing the animals in stainless steel wire bottom cages and were kept under constant environmental conditions and were fed on fresh standard pellet and were given tap water *ad libitum* throughout the study. All animals were acclimatized before the beginning of the experiment for 1 week.

2.3. Experimental design

In this study forty albino rats were divided in to four groups each one has ten rats.

1stGroup administrated saline and kept as control.

2ndGroup administrated Cinnamon extract at a dose 200mg/kg / day.

3rd Group administrated cadmium chloride obtained from (El-Gomhorya Company, Egypt) at a dose of 20mg/kg/day (Doaa et al. 2014).

4th Group administrated Cinnamon extract at a dose 200mg/kg and cadmium chloride at a dose of 20mg/kg orally and daily for 8 weeks, all groups kept under observation all over the duration of experiment (8 Weeks).

2.4. Organ relative weights

At the end of the study period, rats were euthanized and organs were dissected. Testes and tail of the epididymis, were removed and weighed form each rat in treated and control groups.

2.5. Sperm concentration and morphology assay

The content of epididymis were obtained through cutting of the cuda epididymisbyusing surgical blades then squeezed in sterile clean watch glass. This content was diluted 5 times by 2.9% sodium citrate dehydrate solution and was mixed thoroughly to estimate the sperm concentration. One drop from suspension was smeared on a glass slide and stained by Eosin Nigrosin stain to determine the sperm abnormalities according to (Okamura et al. 2005).

2.6. Measurements of serum testosterone levels

Testosterone determination using ELISA explained by (Ekins 1998).

2.7. Gene expression

Total RNA isolated from testis tissue using kits of total RNA purification, then total RNA concentration was determined by Nano drop. c DNA was synthesized from RNA by using Reverse transcriptase Kits. At the end two-Step RT-PCR performed by using The amount of change in gene expression was calculated from the cycle threshold (CT) values provided from real time PCR instrumentation using the 2-ΔΔCT calculation, where ΔCT indication for the CT changes in target gene Nrf2 in comparison with the reference (house-keeping) gene, which is GAPDH as cleared by (Livak and Schmittgen 2001).

a) The primer used to amplify nuclear related factor Nrf2 gene was: -F: 5- GGG AGG AAT TCT CCG GTC TC -3, R: 5- CCT CAC CTC TGC GCC AGT -3
b) The primer used to amplify GAPDH (housekeeping gene) was: F: 5- AGC TTG TCA TCACG GGA AG -3, R: TTT GAT GTT AGT GGG GTC TCG -3

2.8. Histopathological examination

The testis of rats in all groups were taken as samples and were used for histopathological examination according to (Bancroft and Gamble 2008).

2.9. Statistical analysis

Statically the data were analyzed by using SPSS for windows (Version 18). Using (ANOVA) test and DUNCAN test for evaluating the significance of differences between groups according to (Bailey 2008).

3. Results

The relative weights of testes and tail of the epididymis in 3rd group were significantly ($P < 0.05$) decreased in compared with 1st, 2nd and 4th groups. While the relative weight of these organs of group administrated cinnamon with cadmium chloride (4th group) was not significantly differs than 1st and 2ndgroupin (Table 1).

The sperm cell count were significantly ($P < 0.05$) reduced in 3rd group in compared with that in 1st, 2nd and 4th groups. Sperm abnormalities were significantly ($P < 0.05$) increased in rats of 3rd group in compared with that in 1st, 2nd and 4th groups. While the sperm cell count and abnormalities were not significantly differs than 1st and 2nd group as shown in (Table 1).

Regarding to testosterone hormone in serum there were significant decrease on its level in rats of 3rdgroup compared to 1st group. While, no significance difference in 2ndgroup and4th group compared to 1stgroup as shown in (Table 1).

There were significant up-regulation of Nrf2 gene expression in group 3rd treated to 1st group. While, no significant difference in Nrf2 gene in 2nd group and 4th group compared to 1stgroup as shown in (Fig 1, Table .2.).

Table 1: Effect of Oral Administration of Cinnamon Extract and Cadmium Chloride on Relative Weight of Reproductive Organs, Sperm Cell Count, Sperm Abnormalities and Testosterone Level

Groups	Testis weight (gm)	Tail of epididmyis (gm)	Sperm cell Conc %	Abnormalities (%)	Testosterone (ng/ul)
1st group	2.1±0.3 [a]	0.7±0.01[a]	23.6±0.5 [a]	14.8 ±0.3 [a]	20.1±4.2[a]
2nd group	2.5 ±0.4[b]	0.6±0.03[a]	20.5 ± 0.3 [a]	12.3 ±0.7 [a]	17.2±.3.5[a]
3rd group	1.9±0.3[b]	0.4±0.01[b]	16.5 ±0.4 [b]	20.7 ±0.9 [b]	7.5±2.1[c]
4th group	2.00±0.9[a]	0.5±0.02[a]	19.3 ±0.3 [a]	18.5 ±0.3[a]	15.8±3[b]

Mean with different litters at the same raw differs significant (P <0.05).

Table 2: Effect of Administrated Cadmium Chloride and Cinnamon on Expression of Nuclear Related Factor (Nrf2) of Male Albino Rat (Mean ±SD)

Groups	Nrf2 (%)
1st Group (control)	0.5±0.10[a]
2nd Group (cinnamon)	0.7±0.07[ab]
3rd Group (cadmium)	1.34±0.14[c]
4th Group (cd + cinnamon)	0.82±0.11[ab]

Mean with different litters at the same raw differs significant (P<0.05).

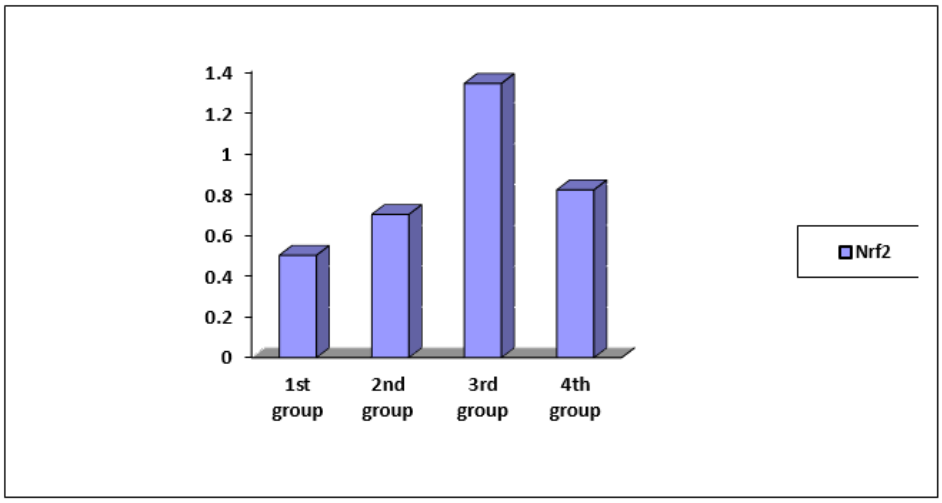


Fig. 1: Effect of Administrated Cadmium Chloride and Cinnamon on Expression of Nuclear Related Factor (Nrf2) of Male Albino Rat.

3.2. Histopathological results

Testis of control rat showing normal histopathological structure of mature active seminiferous tubules with complete spermatogenic series (Fig 2) and testis of rat administrated extract of cinnamon showed homogenous eosinophilic materialin interstitial stroma associated with and congestion in the blood vessels dilatation (Fig3). Testis of rat administrated of cadmium chloride showing Coagulative necrosis was noticed in diffuse manner all over the seminiferous tubules with inflammatory cells infiltration, edema and dilatation of the blood vessels in the interstitial stroma (Fg 4). While testis of rat administrated of cadmium plus cinnamon showing normal histological structure of seminiferous tubules (Fig 5).

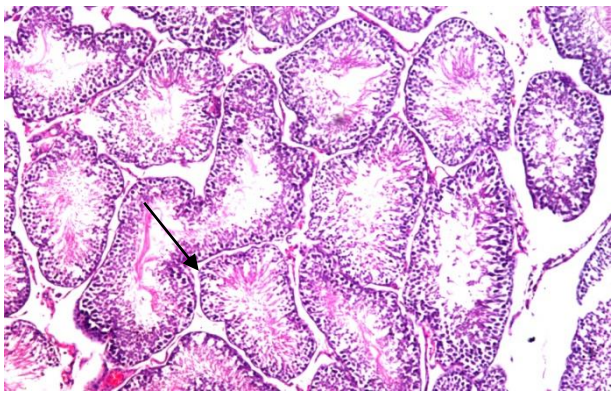


Fig. 2: The Arrow Refers to testis of control rat showing Normal Histopathological Structure of Mature Active Seminiferous Tubules with Complete Spermatogenic Series. H&E Stain X10.

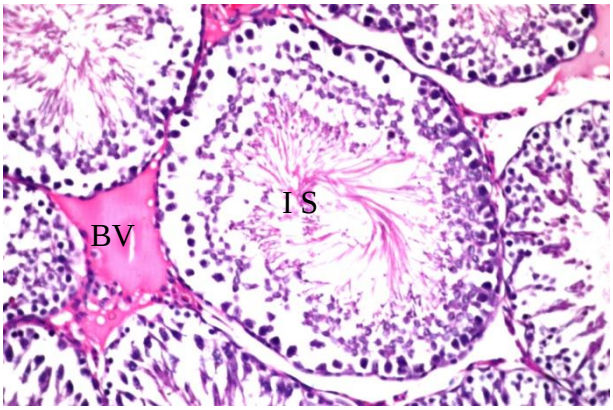


Fig 3: Testis of rat administrated extract of Cinnamon showing the Interstitial Stroma (IS) showed Homogenous Eosinophilic Material Associated with and Congestion in the Blood Vessels (BV) Dilatation H&E Stain X16.

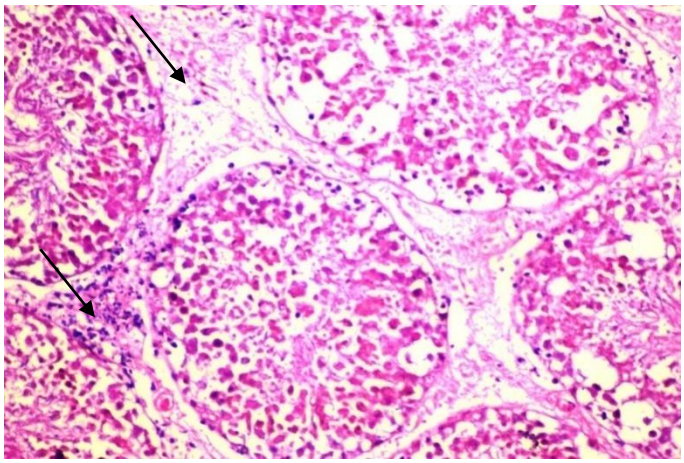


Fig. 4: The Arrows Refer to testis of rat administrated of Cadmium Chloride Showing Coagulative Necrosis Was Noticed in Diffuse Manner All Over the Seminiferous Tubules with Inflammatory Cells Infiltration , odema and dilatation of the Blood Vessels in the Interstitial Stroma H&E Stain X16

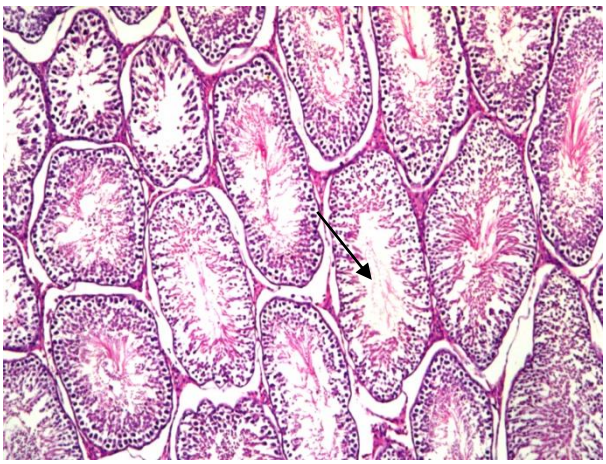


Fig 5: The Arrow Refers to Testis of rat administrated of Cadmium Plus Cinnamon Showing Normal Histological Structure of Seminiferous Tubules. H&E X 10

4. Discussion

Generally the effect of cinnamon has not yet been fully identified on reproductive system. This study concentrated on the effect of cinnamon extract on some reproductive parameters after cadmium exposure and its ability to correct the adverse effect of cadmium on seminal picture and testicular structure in rats.

The present results indicate that Cd lowered the epididymal sperm concentration and increased the percentage of sperm abnormalities. Our findings were consistent with previous reports (Acharya et al. 2008, El-Shahat et al. 2009); confirm that Cd has spermiotoxic and organotoxic effects in rats. In addition to Cadmium has necrotic degenerative effect in the testis (El-Shahat et al. 2009), which may participate in reduction of testicular weight and decreased sperm count levels (Who 1992, Yang et al. 2007). The male reproductive toxicity by Cd developed from the testis is very sensitive to Cd, which induce profound testicular damage and irreversible infertility (Blanco et al. 2009), This result supported by histopathological finding (fig 4),Our findings were consistent with (Henson and Chedrese 2004,Darbre 2006) studies which reported that Cd has endocrine disruptor by affecting the regulation and/ or synthesis of several hormones. In addition to some studies were shown that Cd may activate the estrogen receptor (ER) α and/or mimic estrogen effects in uterus and mammary gland (Johnson et al. 2003).

The effect of cadmium chloride(Cd) on Nrf2 gene expression of treated testes , our results showed significant increase in gene expression of Nrf2 up-regulation in 3rd group compared to 1stgroup, these results compatible with (Rubiolo et al. 2008) who reported that oxidative stress which.Nrf2-dependent Antioxidant Response Element (Nrf2-ARE) pathway induced due to exposure to cadmium which was activated because of it is regulation of the cellular oxidative stress response (Wang et al. 2013), as Nrf2-ARE is a basic leucine zipper transcription factor that binds to antioxidant responsive element (ARE); this factor has cellular protection to oxidative stress in addition it has a critical regulator of effective cellular response (Kensleret al. 2007). The nuclear accumulation of Nrf2 is an essential signaling step for its function as a transcription factor (Nguyen et al. 2004)while no significant difference in Nrf2 gene expression of2nd group and 4th group compared with 1st group.

The present data revealed that oral administration of Cinnamon zeylanicum extract showed increases in testosterone levels and improvement of sperm abnormality and induced marked degenerative changes lowered semen quality and rats. These findings are similar to those reported by (Khan et al. 2003).According to the Cinnamon zeylanicum extracts have an antioxidant effect in CCL4 – intoxicated rats,(Yang et al. 2006)concluded that the natural antioxidants could protect DNA and other molecules from cell damage induced by oxidation and can increase reproductive efficiency of males with improvement of sperm quality. Moreover, (Jedlinska et al. 2006) reported that supplementation of antioxidants and vitamins A; B, C and E can protect sperm DNA and may increase stability of testicular blood barrier against oxidative stress caused by active free radicals. In addition, the enhancement of fertility properties which produced by Cinnamon zeylanicum extract could be explained by its direct effect on the testes causing an increase in testosterone secretion which reported in this study.

The improvement of reproductive parameters after cinnamon administration could be explained. One of the possible explanations is that concentration of testosterone hormones were been increased significantly after cinnamon administration (Jahromiet al. 2011), this effect may be due to the presence of ingredients in cinnamon affect the hypothalamus pituitary axis and has thus increased concentrations of these hormones. (Shagauoand Davidson 2006)also showed that cinnamon is capable of releasing LH hormone by affecting hypothalamus axis which increase secretion of gonadotropin hormone (GnRH) hormone. Also, they proposed that GnRH cause proliferation of sex cells by elevating the Leydig cell activities in adult rats. In another explanation,(Parivziand Ellendorff 1982) showed that cinnamaldehyde extracted from cinnamon increase norepinephrine and this hormone can increase of nitric oxide level. Cinnamaldehyde help in exit cyclic adenosine monophosphate (CAMP) with connecting calcium in cell membrane and cause elevation of norepinephrine level. Norepinephrine elevates LH secretion with activation of nitric oxide. Nitric oxide affects hypothalamus axis and exit (GnRH). Gonadotorpin hormones increase secretion of other hormones such as LH and FSH of pituitary gland. Lactating hormone affects Leydig cells and this cells release testosterone hormone. Testosterone was the most important hormone in sex cells proliferation(Parivziand Ellendorff 1982; Sata and Tsukanmamoto 2000).

5. Conclusion

It can be concluded that cinnamon may improve the reproductive parameters in male rats after cadmium chloride exposure.

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