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Protective Effect of Green Tea Extract on Heavy Metals-Induced Oxidative Testicular Damage in Rats

KEYWORDS: Cadmium; Lead; Green Tea Extract; Rat Testicles.

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ABSTRACT *Background: Exposure to Lead and Cadmium is associated with various pathological conditions that include reproductive dysfunction. Generation of oxidative stress is one of the possible mechanisms for Lead and Cadmium induced cellular deteriorations. Green Tea Extract (GTE) is an antioxidant and free radicals scavenger and has a chelating property.*

Aim and Methods: The present study investigates the efficacy of GTE to attenuate Lead and Cadmium- induced oxidative damage in adult rat testicles. A total of 56 male albino rats were divided into eight groups (13rats each). The first group served as control and received Distilled Water only. Rats of the second group received GTE (1.5%) only. Rats of the third group received Lead acetate at the dose of 1g/L of drinking water. Fourth group received GTE (1.5%) 2 weeks before receiving Lead acetate (1g/L) plus GTE (1.5%). Fifth group received Lead acetate (1g/L) plus GTE (1.5%), Sixth group received Cadmium chloride at the dose of 4g/L of drinking water. Rats of seventh group received GTE (1.5%) 2 weeks before receiving Cadmium chloride (4g/L) plus GTE (1.5%). The eighth group received Cadmium chloride (4g/L) plus GTE (1.5%) for 8 weeks the duration of the experiment.

Results and Conclusion: The use of GTE in attenuating the damaged effects of Lead and Cadmium on reproduction of male rats, improved its testicular damage, decreased sperm count, testosterone level and inducing antioxidant defense. The present study demonstrated that, administration of GTE attenuated the adverse effects of Lead and Cadmium in rats by inducing antioxidant defense mechanism.

INTRODUCTION:

Heavy metals are widely distributed in the environment among of them is Lead and Cadmium (Walker et al., 1995). The most common heavy metals, implicated in acute and / or chronic intoxication can effect the development and the overall health. These metals may cause cell damage, impairment of enzymes, functions or alter genetic material (DNA) (David, 2001). They can be found in soils because insecticides, fungicides, sludge and commercial fertilizers containing these heavy metals that are used in agriculture (Robards & Worsfold, 1991).

Lead (Pb) is a heavy soft metal, occurs in nature as an oxide or salts. Lead is a ubiquitous environmental and industrial pollutant that has been detected in every facet of environmental and biological systems (Juberg et al., 1997). Reproductive dysfunction by Lead has distinct morphological and biochemical features such as disorganized epithelia, decrease sperm quality, and alter sperm morphology, and low androgen levels (Alexander et al., 1996 & Hsu et al., 1997). In the animal model, Lead has a primary toxic affect on the hypothalamic pituitary unit, a primary effect on the testes, and acts at all levels of the reproductive axis (Sokol et al., 2002).

Cadmium (Cd) is carcinogenic metal to which humans are exposed through contaminated foods, water or air. Chronic Cd poisoning can result in nephrotoxicity, osteoporosis, cardiovascular diseases, testicular necrosis, prostatic and testicular cancers, renal failure and neurodegenerative conditions (Yu et al., 2007 & Diana, 2008). Moreover, it was reported that spermatogenesis is disturbed by free radical toxicity (Aruldhas et al., 2005) as Cd depletes many essential metal antioxidants including selenium in the body (Sato & Takizawa, 1982). Also Cd exposure results in decreases in glutathione (GSH) levels which causes an increase in reactive oxygen species leading to increase lipid peroxidation, change intercellular stability, damage deoxyribonucleic acid (DNA), membranes and consequently inducing cell death (Stohls et al., 2001).

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases (Gupta et al., 2004). More attention has been paid to the protective effects of natural antioxidants against toxicities especially whenever free radical generation is involved (Frei & Higdon, 2003). Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress (Okada et al., 2001 & Babich et al., 2005), especially in case of cancer. Flavonoids are group of polyphenolic compounds that occur widely in Green Tea (Camellia sinensis) Extract. Green Tea polyphenol can scavenge oxygen free radicals and lipid radical, prevent lipid peroxidation, it has also been shown to inhibit tumorgenesis and delay aging (Chung et al., 2003).

Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

Green tea has been found to aid in heavy metal detoxification by inhibiting its absorption and promoting excretion. Another potential effect due to the antioxidant activities of green tea polyphenols such as catechin, which binds with heavy metals ions to form an insoluble complex –ionic salt that was used to remove them (Paul, 2008).

The aim of the present study was to throw a light on cytoprotective effects of Green Tea Extract as a model of powerful antioxidant against the toxic effects of Lead acetate and Cadmium chloride on the testes of male albino rats.

MATERIAL AND METHOD

Chemicals:

Lead acetate and Cadmium chloride were purchased from Oxford lab Company. Green tea was purchased from Lipton Company. The rest of the chemicals were of analytical grade.

Animal treatment

56 healthy male Sprague-Dawley rats about 150-200g body weight were

Purchased from Animal House, Animal Health Research Center in Dokki. All animals were conditioned at room temperature (22-25o C) at a natural photoperiod for two week before experiment execution. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 8 weeks.

They were randomly divided into 8 groups (7 rats each) as the following:

- Group (A):-Served as control group and given distilled water as sole source of water all over the experimental period (8 weeks).

- Group (B):

-Given distilled water as sole source of water 2 weeks before receiving Green Tea Extract (1.5%) as sole source of water and continuous till the end of experimental period (8weeks).

- Group (C):-

Given distilled water as sole source of water 2 weeks before receiving Lead acetate dissolved in drinking water at the dose of (1g/L) per day and continuous till the end of experimental period.

- Group (D):-

Given Green Tea Extract (1.5%) as sole source of water 2 weeks before receiving mixture of Lead acetate at the dose of (1g/L) per day and Green Tea Extract (1.5%) continuously till the end of experimental period .

- Group (E):-

Given distilled water as sole source of water 2 weeks before receiving mixture of Green Tea Extract (1.5%) and Lead acetate at the dose of (1g/L) per day as sole source of water continuously till the end of experimental period.

- Group (F):-

Given distilled water as sole source of water 2 weeks before receiving Cadmium chloride dissolved in drinking water at the dose of (4g/L) per day and continuous till the end of experimental period.

- Group (G):-

Given Green Tea Extract (1.5%) as sole source of water 2 weeks before receiving mixture of Cadmium chloride at the dose of (4g/L) per day and Green Tea Extract (1.5%) continuously till the end of experimental period.

- Group (H):-

Given distilled water as sole source of water 2 weeks before receiving mixture of Green Tea Extract (1.5%) and Cadmium chloride at the dose of (4g/L) per day as sole source of water continuously till the end of experimental period.

The Green Tea Extract was made according to Maity et al., 1998 by soaking 15 g of instant green tea powder in 1 Litter of boiling distilled water for 5 minutes. The solution was filtered to make 1.5% Green Tea Extract (GTE).This solution was provided to rats as their sole source of drinking water.

Table(A)show experimental design:-

groups	No. of animals	2 weeks before	EXP.PERIOD 8 WEEKS
A (Control)	7		Distilled water
B (G.T)	7		GTE (1.5%)
C (Pb acetate)	7		Lead acetate (1g/L)
D (G.T+Pb)	7	GTE (1.5%)	Lead acetate (1g/L) and GTE (1.5%)
E (Pb+G.T)	7		Lead acetate (1g/L) and GTE (1.5%)
F (Cd Cl)	7		Cadmium chloride (4g/L)
G (G.T+Cd)	7	GTE (1.5%)	Cadmium chloride (4g/L) and GTE (1.5%)
H (Cd+G.T)	7		Cadmium chloride (4g/L) and GTE (1.5%)

Collection of blood serum:

Blood samples are collected at the end of 8weeks from heavy metals administration from all groups after over night fasting from the medial canthus of eye using microhematocrite tubes. The blood samples was taken into a clean and dry screw capped centrifuge tubes and left to clot at room temperature, then centrifuged at 3000 r.p.m for 15 minutes to separate clear serum samples.

Biochemical analyses:

Testosterone, Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) were determined by commercial kits (Roche Diagnostics) according to methods described by Arlt, 2006, Scott et al., 1989, Tietz, 1995, Smith and Norman, 1990 respectively.

Estimation of oxidative stress parameters:

The testes were dissected and separated then washed with normal saline for determination of reduced glutathione (GSH), Catalase, Superoxide dismutase (SOD) and Malondialdhehyde (MDA) by commercial kits (Biodiagnostic, Egypt) according to methods described by Beutler et al., 1963, Aebi, 1984 and Fossati et al., 1980, Nishikimi et al., 1972,Satoh, 1978 and Ohkawa et al., 1979 respectively.

Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

Preparation of testicular tissue homogenate:

1- Prior to dissection, perfuse tissue with a PBS (phosphate buffer saline) solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells.
2- Homogenize the tissue in 5-10 ml cold buffer (i.e., 50 mM potassium phosphate, pH 7.5, containing 1mM EDTA) per gram tissue.
3- Centrifuge at 4,000 rpm for 15 minutes at 4° C.
4- Collect supernatant for assay and store on ice, if not assayed immediately store the supernatant at – 80° C. The sample will be stable for at least one month.

Sperm Analysis and Evaluation:

I-Epididymal sperm cell concentration:-

Epididymal spermatozoa were counted by a modified method of Yokoi et al., (2003). Briefly, the epididymis was minced in 5 ml of saline, placed in a rocker for 10 min and incubated at room temperature for 2 min. The supernatant fluid was diluted 1:100 with a solution containing 5 g NaHCO3, 1 ml formalin (35%) and 25 mg eosin per 100 ml distilled water. About 10 microlitre of the diluted semen was counted by hemocytometer. The sperm cell concentration was calculated from the following equation:

Sperm cell concentration / ml = N x 125 x 10000.

II- Epididymal alive sperm percent:-

A drop of epididymal contents of each rat was mixed with an equal drop of eosin–nigrosin stain. The semen was carefully mixed with the stain and thin films was spread on a clean slide. Two hundred sperms were randomly examined per slide at ×400 magnification according to Bearden et al., (1980). this method also used for estimation sperm abnormalities %.\

Statistical analysis

Analysis was carried out by using SPSS program for windows version 20 (SPSS, Chicago, USA). All results are presented as "Mean ± SE", One-way analysis of variance (ANOVA) was used for comparison between different groups and treatments. Differences were considered statistical significance at ($P < 0.05$).

RESULTS:

Table I showed that, the levels of MDA in the tissues homogenates of testes were significantly higher in Lead acetate and Cadmium chloride treated groups than control group. In the GTE+ Pb or Cd group, the levels of MDA in the studied tissues were significantly reduced comparing to Cd and Pb treated groups with no significant difference between groups received GTE 2 weeks prior to treatment with Pb and Cd plus GTE and groups received GTE within heavy metal treatment at the same time.

Also, The levels of GSH, SOD and CAT activities in the tissue homogenates of testes (Table I) were significantly reduced in heavy metals treated rats when compared to control rats, their levels came to normal in the group of rats administered a solution of GTE and Cd or Pb. Groups which received GTE 2 weeks prior to treatment with Pb plus GTE show improvement in enzyme activities than groups received GTE within Pb treatment at the same time

Results in Table II showed that, administering of Cadmium chloride and Lead acetate to rats resulted in a statistically significant decreased in testosterone hormone level in the rat's serum comparing with the control group and significant increase in FSH and LH. On contrast, adding GTE to Lead acetate and Cadmium chloride produced a significant decrease in the levels of FSH, LH and significant increase in testosterone level in relation to rats received heavy metals alone. Groups received GTE 2 weeks prior to treatment with Pb and Cd plus GTE show improvement in androgen level than groups received GTE within heavy metal treatment at the same time.

Table III showed spermatogenic damage in both Lead acetate and Cadmium chloride treated groups as significant decrease in sperm count, alive sperm% and significant increase in sperm abnormalities % on other side groups which received GTE showed improvement sperm count, alive sperm% as significant decrease sperm abnormalities % especially groups received GTE 2 weeks prior to treatment with Pb and Cd plus GTE.

Table I: Levels of MDA, GSH, SOD and CAT in testicular tissue homogenate in control and experimental rats:

Parameters / Groups	MDA nmol /g.tissue	GSH mg/g. tissue	**SOD** U/ g.tissue	T U/ g.tissue
A (control)	$6.30^{c} \pm 1.53$	$0.495^{a} \pm 0.01$	$913.56^{ab} \pm 36.80$	$0.233^{a} \pm 0.013$
B (G.t)	$8.36^{c} \pm 0.69$	$0.462^{ab} \pm 0.07$	$1095.99^{a} \pm 75.37$	$0.255^{a} \pm 0.011$
C(Pbcetate)	$31.83^{b} \pm 4.47$	$0.217^{cd} \pm 0.02$	$398.34^{d} \pm 85.49$	$0.138^{b} \pm 0.016$
D (G.t +Pb)	$7.94^{c} \pm 1.84$	$0.323^{b} \pm 0.02$	$761.32^{bc} \pm 90.05$	$0.255^{a} \pm 0.012$
E (Pb+G.t)	$7.95^{c} \pm 1.02$	$0.247^{cd} \pm 0.05$	$665.71^{c} \pm 46.94$	$0.177^{b} \pm 0.014$
F (Cd Cl)	$190.59^{a} \pm 15.24$	$0.148^{d} \pm 0.008$	$223.08^{d} \pm 29.24$	$0.050^{c} \pm 0.028$
G (G.t+Cd)	$17.09^{bc} \pm 2.63$	$0.299^{bc} \pm 0.05$	$610.83^{c} \pm 62.92$	$0.226^{ab} \pm 0.043$
H (Cd+G.t)	$37.29^{b} \pm 2.48$	$0.291^{c} \pm 0.03$	$649.78^{c} \pm 57.39$	$0.208^{ab} \pm 0.044$

Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

Values are means ± SE

Means carrying different superscripts considered significant ($P \leq 0.05$).

Groups D & G: - Rats received Green Tea Extract 2 weeks before heavy metals treatment.

Table II: Levels of Testosterone H, FSH, LH and Prolactin H. in serum of control and experimental rats:

Param-eters / Groups	Testosterone ng/ml	FSH ng/ml	LH ng/ml
A (control)	$2.07^{a} \pm 0.18$	$5.60^{e} \pm 0.25$	$3.64^{d} \pm 0.27$
B (G.t)	$2.19^{a} \pm 0.12$	$3.92^{f} \pm 0.22$	$3.56^{d} \pm 0.25$
C(Pbcetate)	$0.72^{c} \pm 0.14$	$8.35^{c} \pm 0.23$	$5.13^{b} \pm 0.32$
D (G.t +Pb)	$2.00^{a} \pm 0.05$	$6.22^{de} \pm 0.23$	$4.26^{cd} \pm 0.22$
E (Pb+G.t)	$1.35^{b} \pm 0.06$	$6.86^{d} \pm 0.31$	$5.08^{bc} \pm 0.38$
F (Cd Cl)	$0.13^{d} \pm 0.02$	$11.37^{a} \pm 0.58$	$7.20^{a} \pm 0.34$
G (G.t+Cd)	$0.57^{c} \pm 0.04$	$7.80^{c} \pm 0.23$	$5.08^{bc} \pm 0.29$
H (Cd+G.t)	$0.16^{d} \pm 0.01$	$10.14^{b} \pm 0.52$	$6.4^{a} \pm 0.25$

Values are means ± SE

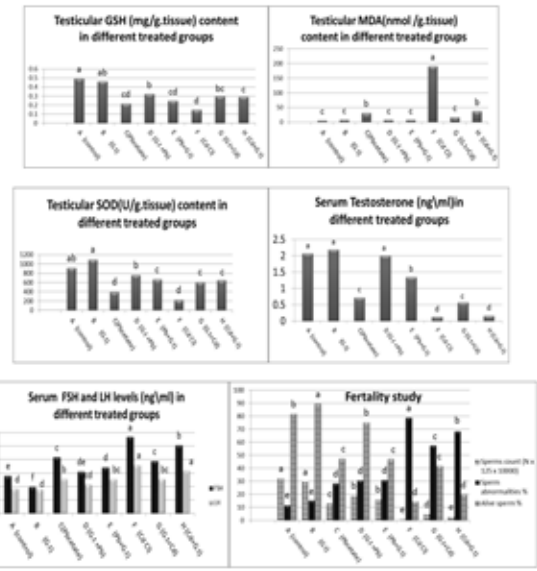
Means carrying different superscripts considered significant ($P \leq 0.05$).

Groups D & G: - Rats received Green Tea Extract 2 weeks before heavy metals treatment.

Table III: Sperms count, Sperm abnormalities % and alive sperm % in control and experimental rats:

Parameters / Groups	Sperms count (N x 125 x 10000)	Sperm abnormalities %	Alive sperm %
A (control)	$32.6^{a} \pm 4.7$	$11.6^{e} \pm 1.1$	$82^{b} \pm 2.7$
B (G.t)	$30^{a} \pm 2.34$	$15.2^{e} \pm 1.5$	$89.6^{a} \pm 2$
C (Pbcetate)	$13^{c} \pm 0.9$	$28.5^{d} \pm 0.8$	$47.5^{c} \pm 2$
D (G.t +Pb)	$19.4^{b} \pm 2.5$	$30.8^{d} \pm 1.4$	$75.6^{b} \pm 3$
E (Pb+G.t)	$16.5^{bc} \pm 2$	$31^{d} \pm 0.7$	$47.2^{c} \pm 2$
F (Cd Cl)	$0.6^{e} \pm 1.01$	$79^{a} \pm 2.5$	$14.6^{d} \pm 2$
G (G.t+Cd)	$5.2^{d} \pm 1.3$	$57.4^{c} \pm 0.8$	$41.8^{c} \pm 1.7$
H (Cd+G.t)	$3^{d} \pm 0.01$	$68.4^{b} \pm 1.2$	$20^{d} \pm 0.7$

Values are means ± SE
Means carrying different superscripts considered significant ($P \leq 0.05$).
Groups D & G: - Rats received Green Tea Extract 2 weeks before heavy metals treatment



DISCUSSION:

The goal of the present study was to investigate the effect of Green Tea Extract (GTE) in Lead and Cadmium- induced testicular toxicity in rats as The effect of this heavy metals on male reproduction has been studied in details in various experimental species caused alteration in sperm morphology, count, motility as well as biochemical disruptions of enzymes and hormones with reactive oxygen species (ROS) and neuro-endocrine mechanism are highly affected by these heavy metals exposure (Roy, 2009).

Lead as one of the environmental pollutants can threats the life of living creatures in many ways. Lead as a toxic metal is not essential for nutrition. (Goyer et al., 1995). As environmental exposure to Lead has increased, the toxic effects of it on various organ systems in the body have been recognized (Lyn, 2006). Some studies suggested that low doses of Lead affect reproduction and sexual development in small mammals either directly or indirectly (Mc Murry et al., 1995 and Junaid et al., 1997).

This study showed that Lead treated group (group C) which received Lead acetate at the dose of (1g/L) per day significantly decline in the levels of GSH activity in the testicular tissue homogenate when comparing with control animal as shown in table (I) With a significant decrease in the activity of antioxidative system elements (superoxide dismutase and catalase) These results correlate well with (Roy, 2009 and Mohsen et al., 2012) and also significantly elevation in levels of MDA activity in the testicular tissue homogenate when compared with control group as the results are in accordance with those of other workers (**Patra** et al., 2001 and Lyn, 2006) as Gurer et al., (2004) and Ahamed et al., (2005) reported that, Lead binds to enzymes that have functional sulfhydryl groups like glutathione, rendering them nonfunctional and further contributing to impairment in oxidative balance. Lead effectively inactivates the glutathione molecule so it is unavailable as an antioxidant or as a substrate in liver metabolism (Christie and Costa, 1984).

Lead toxicity leads to the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide, and this make direct depletion of antioxidant reserves as Lead has also been shown to sup-

Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

press blood levels of the antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) (Sugawara et al., 1991 and Han et al., 2005). In any biological system where ROS production increases, antioxidant reserves are depleted and this resulted in oxidative stress and this was well extrapolated from the increase in lipid peroxidation products (LPP). An increase in LPP damages various cellular components of tissues as which occurred in testicular tissue (Batra et al., 1998).

Also Lead treatment resulted in a statistically significant decrease in testosterone hormone other than those of control group as shown in table (II). these result come in agreement with the finding of (Batra et al., 2004; Biswas and Ghosh, 2004) with significant increase in the levels of Serum LH, FSH and Prolactin H. these results are in accordance with those of other workers (Roy, 2009 and Mohsen, 2012) associated with a significant decrease in sperm count , alive sperm % and significant increase in sperm abnormalities as shown in table (III). These results are in accordance with (Piao et al., 2007 and Alhassan et al., 2010) as they indicate bad effect of Lead acetate on reproductive hormonal response.

These findings suggest that it might involve other hormonal and/or hormonal feedback pathway(s) than disruption of testosterone secretion in the reproductive hormonal axis by Lead, such as a lack of reflex in response to plasma testosterone, direct inhibitory androgen biosynthesis in Leydig cells (wiebe et al., 1983), or defects in LH regulation at the pituitary level (sokol et al., 1985). Molecular mechanisms underlying histopathological examination have revealed disturbance degeneration in Leydig cells among rats (Saxena et al., 1986), thereby suggesting Leydig cells as a target for Lead intoxication.

On the other hand, due to imbalances in the HPT hormonal axis induced by Lead exposure, pituitary cells release inappropriate levels of LH and change the steroid negative feedback loop (Ronis et al 1996), usually at the hypothalamus level. Increased concentrations of other reproductive hormones, such as follicle stimulation hormone (FSH), secreted from the pituitary gland, have been observed following Lead exposure in men (Ng et al., 1991) and in Lead treated rats (Petrusz, 1979).

Lead has spermicidal effect on high exposure causing decrease in sperm count supports the finding of Xu et al. (2006), that the testicular sperm count are important indicators of adverse effect of Lead on spermatogenesis, and this can be accounted for by direct influence of Lead on testicular tissues. However, the impairment of spermatogenesis appeared to be as a consequence of the decline of testosterone serum in treated rats since the androgen is clearly essential to the gametogenesis (Martin et al., 1996). The administration of Lead acetate resulted in significant increase sperm abnormalities. This could be accounted for by the Lead's ability to cross the cell membrane in various ways that causes structural abnormality (Thoreux et al., 1995).

Cd is used industrially to manufacture electroplates, batteries, alloys and fuels (Waisberg et al., 2003). The increasing industrial use of Cd causes soil, air and water contamination. Exposure to Cadmium is associated testicular necrosis. Moreover, it was reported that spermatogenesis is disturbed by free radical toxicity (Aruldhas et al., 2005).

The results of these study showed that Cadmium chloride treated group (group F) at the dose of (4g/L) per day significantly decline in the levels of GSH activity in the testicular tissue homogenate when comparing with control as shown in table (I) with a significant decreased in the activity of antioxidative system elements (superoxide dismutase and catalase) these results correlate well with other reports (Stohs et al., 2001 and Al-Hashem et al., 2009) also significantly elevation in levels of MDA activity in the testicular tissue homogenate when compared with control and the results are in accordance with those of other workers (El-Maraghy et al., 2001and EL-Shahat et al., 2009) which reported that, Cd induces its effect by affecting tissue antioxidant enzyme systems and elevation of lipid peroxides (LPO) level.

Cd exposure results in decreases in glutathione (GSH) levels via inhibiting the functional thiol groups of antioxidant enzymes and reduce activities of several enzymes including enzymes antioxidants as the decrease in SOD and catalase activities (Xiao et al., 2002) and this causes an increase in reactive oxygen species like hydrogen peroxide, hydroxyl radicals and superoxide radical ions, Leading to increase lipid peroxidation, change intercellular stability, damage deoxyribonucleic acid (DNA), membranes and consequently inducing cell death (Stohs et al., 2001).

According to our study which show that Cd treated group at dose (4g/L) per day resulted in a statistically significant decrease in testosterone hormone other than those of control group as shown in table (II).these result come in agreement with the finding of (Salama and El-Bahr, 2007; Obianime and Roberts, 2009) with significant increase in the levels of Serum LH and FSH respectively. These results are in accordance with those of other workers (Lafuente et al., 2002)which suggest that, the decrease in serum testosterone levels observed in Cadmium treated rats may reflect direct effects of the metal at the testis as this metal accumulates in this tissue causing damage caused to the testicular tissue and a disruption of the regulatory mechanism of the hypothalamus-pituitary gonadal axis (Massanyi et al., 2007 and Stoh et al., 2001) resulting in testes and accessory sex tissues atrophy such as the prostate and compromising hormonal release, this will result in reproductive dysfunction (Waalkes et al., 1997a).

Furthermore the loss of testosterone feedback can result in pituitary cell hypertrophy; hyperplasia and eventually pituitary neoplasia (Nyska et al., 1998). Which responsible for abnormal endocrine response as increase FSH, LH and prolactin hormone .Thus the disruption of the testes-pituitary axis may contribute to the causation of both testicular and pituitary destructions in this study (Waalkes et al., 1997b).

Also Cd treated rats show a significant decrease in sperm count and alive sperm % associated with significant increase in sperm abnormalities as shown in table (III) .These results are in accordance with (Salama and El-Bahr, 2007). Its may be due to the effect of Cadmium on germ cells as be attribute to disturbance in seminiferous tubules especially spermtogenic cells (long et al., 2002).whereas secondary abnormalities such as bent tails and abnormal acrosome are believed to arise after spermatogenesis is completed due to epididymal dysfunction. Thus, the increase in abnormal sperms in the Cadmium-treated rats may be due to both testicular dysfunction and impairment of epididymal function (Zemjanis, 1970).

Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

In the current study, the administration of Green Tea Extract (1.5%) 2 weeks before or at the same time with heavy metals show significant improvement in The levels of GSH and activity of antioxidative system elements (superoxide dismutase and catalase) and also significant decrease in MDA levels if comparing with Lead acetate and Cadmium chloride treated groups. These results are in agreement with the report of (EL-Shahat et al., 2009 and Vinoth et al., 2010) as Green Tea Extract (GTE) is an antioxidant and free radicals scavenger and has a chelating property (EL-Shahat et al., 2009). Also improved serum level of testosterone and reduce the levels of FSH and LH and has good impact on sperm count and sperm alive % with decreasing the sperm abnormalities % and this in agreement with Jassem et al., 2008 and Azza et al., 2010.

Green tea has been found to aid in heavy metal detoxification by inhibiting its absorption and promoting excretion (Paul, 2008). Another potential effect due to the antioxidant activities of green tea polyphenols such as catechin (antioxidant present in green tea) chelate metal ions, especially iron and copper, which, in turn inhibit generation of hydroxyl radicals and degradation of lipid hydroperoxides which causes reactive aldehyde formation (Azram et al., 2004). It prevents the loss of lipophilic antioxidant a tocopherol, by repairing tocopheryl radicals and protection of the hydrophilic antioxidant ascorbate (Skrzydlewska et al., 2002). Indeed, tea extract enhances the expression of intracellular endogenous antioxidants such as SOD, catalase and GPX by maintaining their activities higher and other antioxidants enzymes such as glutathione, glutathione reductase, glutathione-S reductase, and quinone reductase (Khan et al., 1992 and Valerio et al., 2001).

Also the results show that groups which received GTE 2 weeks before administration of heavy metals plus GTE show improvement in antioxidant parameters and androgen hormones than groups which received GTE at the same times with heavy metals and this may due to tea polyphenols have ability to scavenge free radicals constantly generated due to oxidation process of drugs and food, physical stress, environmental pollutants, radiation, chemicals and toxins so it strength antioxidant defense system against ROS (Amie et al., 2003) and at the same time chronic exposure to Lead acetate and Cadmium Chloride is associated with reduced appetite and off food in treated rats (McDowell, 1992; Lyn, 2003 and 2006).

CONCLUSION:

Our results indicate that Green Tea Extract has a protective effect against Lead and Cadmium induced testicular toxicity in rats.

Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

REFERENCE

Aebi, H. (1984): "catalase in vitro". Methods enzymol 105:121-126. | Ahamed, M.; Verma, S.; Kumar, A. and Siddiqui, M. K. (2005): "Environmental exposure to Lead and its correlation with biochemical indices in children". Sci Total Environ 346:48-55. | Alexander, B. H.; Checkoway, H.; Van Netten, C.; Muller, C. H.; Ewers, T.G. and Kaufman, J. D. (1996). "Semen quality of men employed at a Lead smelter". Occup Environ Med; 53 (6): 411-6. | Al-Hashem, F.; Dallak, M.; Bashir, N.; Abbas, M. and Elessa, R. et al. (2009): "Camel's Milk Protects Against Cadmium Chloride Induced Toxicity in White Albino Rats". Am. J. of Pharm. and Toxicol. 4 (3):107-117. | Alhassan, A.; Mabrouk, M.; Idris, R.; Salawu, E.; Oyerinde, A. and Bauchi, Z. (2010):"Aqueous Extract of Juglans Nigra Prevents Lead Induced Testicular Toxicity in Rats". Macedonian J. of Med. Sci. 1857-5773. | Amie, D.; Amie, D. D.; Beslo, D. and Trinajstie, N. (2003). "Structure-Radical Scavenging Activity Relationships of Flavonoids". Croat. Chem. Acta, 76: 55-61. | Arlt, W. (2006): "Androgen theraby in woman". Eur J Endocrinol; 154 (1):1-11. | Aruldhas, M. M.; Subramanian, S.; Seker, P.; Vengatesh, G.; Chandrahasan, G.; Govindarajulu, P. and Akbarasha M A. (2005): "Chronic chromium exposure-induced changes in testicular histoarchitechitecture are associated with oxidative stress: study in a non-human primate (Macaca radiata Geoffroy)". | Azram, S.; Hadi, N.; Khan, N. and Hadi, S. (2004):"Prooxidant property of green tea polyphenols, epicatechin and epicatechin- 3-gallate: implications of anticancer properties". Toxocol. InVitro 18: 555-561. | Azza M. Gawish, Aliaa M. Issa, Aziza M. A., and Sherin Ramadan (2010): | "Morphometrical, Histopathological, and Cytogenetical ameliorating Effects of Green Tea Extract on Nicotine Toxicity of the Testis of Rats". J.of Amer. Sci.; 6(11). | Babich, H.; Gold, T. and Gold, R. (2005): "Mediation of the in vitro cytotoxicity of green tea and black tea polyphenols by cobalt chloride". Toxicol. Lett. 155, 195-205. | Batra, N.; Nehru, B. and Bansal, M. P. (2004): "Reproductive potential of male Portan rats exposed to various levels of Lead with regard to zinc status".Br. J. of Nut. 91: 387–391. | Batra, N.; Nehru, B. and Bansal, M.P. (1998): "The effect of zinc supplementation on the effects of Lead on the rat testis". Reprod Toxicol 21: 507-512. | Bearden, H.; Fuqua Bearden, H. and Fuquary, J. (1980): "Applied reproduction". Reston, Virginia: Reston Publishing Co. Inc, 158–160. | Beutler, E.; Duran, O. and Kelly, B. (1963): "improved method for the determination of blood glutathione". J. of lab. And clinic. Med. 61:882. | Biswas, N.M. and Ghosh, P. (2004):"Effect of Lead on male gonadal activity in Albino Rats". Kathmandu Univ. Med.J. 2(1): 43-46. | Christie, N.T. and Costa, M. (1984): "In vitro assessment of the toxicity of metal compounds. IV. Disposition of metals in cells: interaction with membranes, glutathione, metallothionein, and DNA".Biol. Trace Elem. Res. 6:139-158. | Chung, F. L.; Schwartz, J.; Herzog, C. R. and Yang, Y. M. (2003): "Tea and cancer prevention: Studies in animals and humans". J Nutr 133:3268–3274. | David, J. F. (2001): "Symptoms toxicity of heavy metals". J. American Med. Asso. 260: 1523 – 1533. | Diana, P. H. (2008): "Effect of green tea polyphenols on Cadmium toxicity in Coenorhabaditis elegons". NCDR, Poster session, No. 2. | El-Maraghy, S. A.; Gad, M. Z.; Fahim, A. T. and Hamdy, M. A. (2001):" Effect of Cadmium and aluminum intake on the antioxidant status and lipidperoxidation in rat tissues". J. Biochem. Mol. Toxicol 15: 207–221. | EL-Shahat, A. E.; Gabr, A.; Meki, A. R. and Mehana, E. S. (2009). "Altered Testicular Morphology and Oxidative Stress Induced by Cadmium in Experimental Rats and Protective Effect of Simultaneous Green Tea Extract" Int. J. Morphol., 27(3):757-764. | Fossati, P.; Prencipe, L. and Berti, G., (1980): "Use of 3, 5- Dichloro- 2-hydroxybenzen sulfonic acid and 4 aminophenazine chromagenic system in direct enzymic assay of uric acid in serum and urine". Clin. Chem. 26 (2):227-231. | Frei., B. and Higdon, J. (2003):" Antioxidant activity of tea polyphenols in vivo: evidence from animal studies". J. Nutr. 133, 3275-3284. | Goyer, R. (1995): "Toxic effect of metal": Casarett and doult's toxi¬col. 691-736. | Gupta, M.; Mazumder, U.; Kumar, T.; Gomathi, P. and Kumar, R. (2004): "Antioxidant and hepatoprotective effects of Buhinia racemosa against paracetamol and carbon tetrachloride induced liver damage in rats". Iranian J. Pharma. Therapeutica 3, 12-20. | Gurer-Orhan, H.; Sabir, H. U. and Ozgunes, H. (2004):"Correlation between clinical indicators of Lead poisoning and oxidative stress parameters in controls and Lead exposed workers". Toxicol. 195:147-154. | Han, S.G.; Kim, Y.; Kashon, M. L. and et al. (2005): "Correlates of oxidative stress and free-radical activity in serum from asymptomatic shipyard welders'. Am. J. Respirr Cit Care Med. 172:1541-1548. | Hsu, P. C.; Liu, M. Y.; Hsu, C. C.; Chen, L. Y. and Leon Guo, Y. (1997): "Lead exposure causes generation of reactive oxygen species and functional impairment in rat sperm. Toxicol. 122 (12): 133-43. | Hum. Reprod., 20: 2801-13. | Jassem, H. M.; Ismaiel, H. K.; Jasem, A. Y. (2008): "Effect of aqueous extract of green tea on sexual efficiency in adult male rats treated with alloxan". Mosul, J. of Iraq Vet. Science 2: 75-82. | Juberg, D. R.; Klieman, C. F. and Simons, C. K. (1997):"Position paper of the American council on sciences and Health. Lead and human health". Ecotoxicol Environ Safety 38:162–80. | Junaid, M.; Chowdhuri, D. K.; Narayan, R. and Saxena, D. K. (1997): "Lead-induced changes in overian follicular development and maturation in mice". J. Toxicol. Environ Health. 50: 31-40. | Khan, S. G.; Katiyar, S. K.; Agarwal, R. and Mukhtar, H. (1992): "Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention". Cancer Res. 52: 4050– 4052. | Lafuente, A. ; Cano, P. and Esquifino, A. I. (2002):"Are Cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent?"Dep. of Pharm., Univ. of Brescia Italy. | Long, C.; Wen-Hua, R.; Shan-Liang, Z.; Wei, G.; Juan, Z.; Ying-Zi, J. and G. Y. (2002): "Effects of chronic Cadmium loading on the tests and endocrine function of reproduction in male rats". Acts Physiol. Sinica., 54: 258-262. | Lyn, P. (2003): "Toxic metals and antioxidants: part 2. The role of antioxidants in arsenic and Cadmium toxicity". | Lyn, P. (2006):"Lead toxicity, a review of the literature. Part 1: Exposure, evaluation and treatment". Altern. Med. Rev. 11: 2-22. | Maity, S.; Vedasiromoni, J. R. and Ganguly, D. K. (1998): "Role of glutathione in the antiulcer effect of hot water extract of black tea". Jpn. J. Pharmacol., 78: 285-92. | Martin, J.; Ronis, J.; Thomas, M.; Badger, Sarah, J.; Shema; Paula, K.; Roberson and Fatima Shaikh. (1996):"Reproductive toxicity and growth effects in rats exposed to Lead at different periods during development". Toxico. and Applied Pharma. 136: 361-171 | Massanyi, P.; Lukac, N.; Slivkova, J.; Kovacik, J.; Makarevich, A.V.; Chrenek, P.; Toman, R.; Forgacs, Z.; Somosy, Z.; Stawarz, R. and Formicki, G. (2007): "Mercury-induced alterations in rat kidneys and testes in vivo". J. Environ. Sci. Health. 42(7):865-870. | Mc Murry, S. T.; Lochmiller, R. L.; Chandra, S.A. and Qualls, C. W. (1995): | "Sensitivity of selected immunological hematological, and reproductive parameters in the cotton rat (Sigmodon hispidus) to subchronic Lead exposure". | J.Wildl. Dis. 31: 193-204. | McDowell, L.R. (1992)."Minerals in animal and human Nutrition. Academic press, New York, ISBN: 0-12-483369-1, pp. 524 | Mohsen Vigeh, M. D.; Derek R. S. and Ping-Chi, H. (2012):"How does Lead induce male infertility?" Enviro. Research 112: 225–229. | Ng, T. P.; Goh, H. H.; Ng, Y. L.; Ong, H. Y.; Ong, C. N.; Chia, K. S. and et al. (1991): "Male endocrine functions in workers with moderate exposure to Lead". Br. J. Ind. Med. 48: 485-491. | Nishikimi, M.; Rao, N.A. and Yogi, K. (1972): "The occurance of super oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen.Biochemical and Biophysical Research". Common. 46: 849 – 854. | Obianime, A.W. and Roberts, I. I. (2009): "Antioxidants, Cadmium-induced toxicity, serum biochemical and the histological abnormalities of the kidney and testes of the male Wistar rats". Niger. J. Physiol. Sci.24: 177–185. | Okada, K.; Wangpoengtrakut, C.; Tanaka, T.;Tomoyuki, T.;Toyokuni, S.; Uchida, K. and Osawa, T. (2001): "Curcumin and especially tetrahydrocurcumin ameliorate oxidative stress induced renal injury in mice". J. Nutr. 131, 2090-2095. | Patra, R. C.; Swarup, D. and Dwived, S. K. (2001):"Antioxidant effects of α tocopherol, ascorbic acid and -methionine on Lead induced oxidative stress to the liver, kidney and brain in rats". Ind. Vet. Research Instit. Izatnagar, India. | Paul, D. H. (2008): "Effect of green tea polyphenols on Cadmium toxicity in rats". | Dominican. Edu. J. 6(2):1-10, 2008. | Petrusz, P.; Weaver, C. M.; Grant, L. D.; Mushak, P. and Krigman, M. R. (1979): "Lead poisoning and reproduction: effects on pituitary and serum gonadotropins in neonatal rats". Environ. Res. 19: 383-391. | Piao, F.; Cheng, F.; Chen, H.; Li, G.; Sun, X.; Liu, S.; Yamauchi, T. and Yokoyama, K. (2007):"Effects of Zinc Coadministration on Lead Toxicities in Rats". Indus. Health 45:546–551. | Robards, K. and Worsfold, P. (1991): "Cadmium: toxicology and analysis". a review. Analyst 116:549-68. | Ronis, M. J.; Badger, T. M.; Shema, S. J.; Roberson, P. K. and Shaikh, F. (1996): "Reproductive toxicity and growth effects in rats exposed to Lead at different periods during development". Toxicol. Appl. Pharmacol. 136: 361-371. | Roy Chowdhury, A. (2009):"Recent Advances in Heavy Metals Induced Effect on Male Reproductive Function". J. Med. S c i. 2 (2): 3 7 -4 2. | Salama, A. F. and El-Bahr, S.M. (2007): "Effect of Curcumin on Cadmium-Induced Oxidative Testicular Damage in Rats". J.of Med. Research Institute JMRI, Vol. 28 (2): 167-73. | Sato, M. and Takizawa, Y. (1982): "Cadmium–binding proteins in human organs". Toxicol. Lett., 11(3-4): 269-73. | Satoh, K. (1978): "Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Chim. Acta, 90: 37. | Saxena, D. K.; Hussain, T.; Lal, B. and Chandra, S.V. (1986): "Lead induced testicular dysfunction in weaned rats". Ind. Health 24: 105-109. | Scott, M. G.; Ladenson, J. H.; Green, E. D. and Gast, M. J. (1989): "Hormonal evaluation of female infertality and reproduction disorders". Clin Chem; 35:620-630. | Skryzdlewska, E.; Ostrowska, J.; Stankiewicz, A. and Fabisszewski, R. (2002): "Green tea as a potent antioxidant in alcohol intoxication". Addict. Biol. 7: 307-314. | Smith, C. R. and Norman, M. R. (1990):"prolactin and growth hormone . molecular hetergensity and measurement in serum". Ann. Clin. Biochem.; 27: 542-550. | Sokol, R. Z.; Madding, C. E. and Swerdloff, R. S. (1985): "Lead toxicity and the hypothalamic-pituitary-testicular axis". Biol. Reprod 33: 722-728. | Sokol,R. Z.; Saixi, W.; Yu-Jui, Y.; frank, Z. S.; Elizabet, G. and Robert, E. C. (2002): "Long-term, low-dose Lead exposure alters the gonadotropin-releasing hormone system in the male rat". Environm.health Perspectives 110: 871-874 | Stohs, S. J.; Bagchi, D.; Hassoun, E. and Bagchi, M. (2001): "Oxidative mechanisms in the toxicity of chromium and Cadmium ions". J.Environ. Pathol. Toxicol. Oncol 20: 77–88. | Sugawara, E.; Nakamura, K.; Miyake, T. and et al. (1991): "Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to Lead". Br. J. Ind. Med. 48:239-242. | Thoreux-Manlay, A.; Vélez de la Calle, J. F.; Olivier, M. F.; Soufir, J. C.; Masse, R. and Pinon-Lataillade, G. (1995):"Impairment of testicular endocrine function after Lead intoxication in the adult rat". Toxicol. 26: 101-109. | Tietz, N. W. (1995): "Clinical Guide to laboratory tests". 3rd ed Philaadelphia, Pa:WB Saunders Co,:410. | Valerio. J. R.; Kepa, L.G.; Pickwell, J. K., G.V. and Quattrochi, L. C. (2001): "Induction of human NAD (P)H:quinone oxidoreductase (NQOI) gene expression by the flavonol quercetin". Toxicol. Lett., 119 : 49 – 57. | Vinoth kumar, P.; Amala bricey, A.; Veera thamari selvi V.; Sudheer kumar, C. and Ramesh, N. (2010):"Antioxidant Effect of Green Tea Extract in Cadmium Chloride Intoxicated Rats".Advances in Applied Sci. Research, 1 (2): 9-13. | Waalkes, M.P.; Rehm, S. and Devor D. E. (1997b): "The effects of continuous testosterone exposure on spontaneous and Cadmium-induced tumors in the male Fischer (F344/NCr) rat: Loss of testicular response". Toxicol. Appl. Pharmacol. 142: 40-4. | Waalkes, M.P.; Rehm, S.; Coogan, T.P. and Ward, J.M. (1997a):"Role of Cadmium in the etiology of cancer of the prostrate. In target organ toxicology series". Endocrine toxicol. 227-244. | Waisberg, M.; Joseph, P.; Hale, H.; and Beyersmann, D. (2003) "Molecular and cellular mechanisms of Cadmium carcinogenesis". Toxicol. 192: 95–117. | Walker, M. N; Dwan, B. A; Dovor, D. E. and Coyer, R. A. (1995): "Renal tubular tumor and a typical hyperplasia in Mice exposed to Lead acetate during gestation, lactation occurs with minimal chronic nephropathy". Cancer Res. 55: 265 – 271. | Wiebe, J. P.; Salhanick, A. I. and Myers, K. I. (1983):"On the mechanism of action of Lead in the testis: in vitro suppression of FSH receptors, cyclic AMP and steroidogenesis". Life Sci 32:1997-2005. | Xiao, P.; Jia, X. D.; Zhong, W. J.; Jin, X. P. and Nordberg, G. (2002): "Restorative effects of zinc and selenium on Cadmium induced kidney oxidative damage in rats". Biomed. Environ. Sci., 15:67-74. | Yokoi, K.; Uthus, E. O. and Nielsen, F. H. (2003): "Nickel deficiency diminishes sperm quantity and movement in rats". Biol Trace Elem Res; 93:141. | Yu, H. N.; Shen, S. R. and Yin, J. J. (2007): "Effects of metal ions, catechins, and their interactions on prostate cancer". Crit.Rev. Food Sci. Nutr., 47(8):711-9. | Zemjanis, R. (1970): "Collection and evaluation of semen". Baltimore MD (2nd Ed) In: Diagnostic and Therapeutic Technique in Animal Reproduction. William and Wilkins Company. 1970. pp 139-53. |