Alexandria Journal of Veterinary Sciences

www.alexjvs.com

AJVS. Vol. 54 (1): 70-78. Jul. 2017

DOI: 10.5455/ajvs.268149

Garlic Powder Attenuates Apoptosis Associated with Lead Acetate-Induced Testicular Damage in Adult Male Rats

Nasr E. Nasr[1,*], Mostafa A. Elmadawy[2], Essam A. Almadaly[3], Walied Abdo[4], Mona M. Zamel[1]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish street, 33516 Kafrelsheikh, Egypt.
[2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish street, 33516 Kafrelsheikh, Egypt.
[3]Department of Theriogenology, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish street, 33516 Kafrelsheikh, Egypt.
[4]Department of Pathology, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish street, 33516 Kafrelsheikh, Egypt.

Key words:
Lead toxicity, apoptosis, male rat, testis, garlic

Correspondence to:
Nasr E. Nasr:
nasr_157@yahoo.com

ABSTRACT

Lead is a highly prevalent heavy metal pollutant in human and animals which has various toxicological effects on the different organs including reproductive system on particular aspect the male testis. Lead (Pb) exposure enhances the generation of Reactive Oxygen Species (ROS) which negatively affect testis structure and functions. The present study aimed to investigate the effect of garlic supplement on daily basis and its prophylactic possibility against lead acetate induced testicular dysfunction. Thirty two adult male rats were divided to four groups, eight animals per each. All animals were maintained under standard laboratory conditions with free access to suitable diet and water supply. The 1st group was supplied with water and diet and kept without further treatment as a negative control. The 2nd group was treated by lead acetate(50mg/L) daily in drinking water as a positive control. The 3rd group was administered commercial garlic powder preparation only (200 mg/kg b.w.) by stomach tube. The 4th group was co-treated with lead acetate in drinking water plus garlic in same doses by stomach tube. Then, all animals were scarified after 42 days for clean excision of epididymis and testes. Semen was collected and analyzed for count, motility and viability. Testicular tissues sections were prepared, stained and examined. Oxidative stress markers, SOD and MDA were determined in testicular tissue homogenate. Immunostaining was carried out on sections of testicular tissue for detection of proapoptotic markers caspase-3 and Bax expression. Finally, the level of expression of Bcl-2 and p53 genes was measured in testicular tissue samples as well. The data summary obtained from co treated group signified the protective effect of garlic against potential lead acetate induced undesired testicular apoptosis.

1 INTRODUCTION

Study of causes leading to cell death in the testis is of great importance for the improvement of male reproductive health. Thereby, it is important to know the molecules and pathways involved during development because this determines the future state of fertility. Moreover, how the toxins affect the testis is an essential research target for prevention of such effects particularly in occupational healthcare. Lead is a widely prevalent toxic heavy metal in both environment and daily human activities. Exposure to Lead has been shown to be related to adverse health effects on many organs especially testis (Akidwu et al., 2013). The mechanism of Lead toxicity is mainly due to oxidative stress since lead enhances generation of Reactive Oxygen Species (ROS) over the activity of endogenous antioxidants including Superoxide Dismutase (SOD), reduced glutathione (GSH) and catalase (CAT). ROS are mainly produced in mitochondria initiating the mitochondrial pathway of apoptosis. Since the testis is a complex organ with different cell types dependent on each other for survival, the challenge is now to identify the functional relevance of inter- and intracellular regulators of germ cell apoptosis (Shaha et al., 2010). Although apoptosis is essential for maintaining cellular homeostasis, excessive apoptosis due to lead toxicity deteriorates semen

quality and disturbs serum gonad/pituitary hormone levels Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and testosterone, which leads to male infertility (Al-Masry., 2015). Several studies recognize the critical role played by natural antioxidant in pre lead toxicity prophylaxis. Since ancient times, Garlic has exhibited pharmacological properties and medical applications. Notably, garlic contains more than 200 medicinal compounds, including volatile oil with sulphur containing allicin, allin, ajone, allinase, peroxidase, myrosinase S-methyl cysteine (SMC) (Block, 1985). Garlic has been successfully used in induction of immune function, enhancement of detoxification of foreign compounds, antimicrobial and antioxidant effects (Banerjee et al., 2001). The present study was performed to evaluate the effect of lead on the apoptotic markers related to oxidative stress in testes of adult male rats and to clarify the prophylactic effect of garlic on these biomarkers.

2 MATERIALS AND METHODS

2.1.Preparation of materials

Lead acetate ([Pb(CH3CO2)2], 99% pure) was purchased from El-Nasr pharmaceutical chemicals company (Mansoura, Egypt). Lead acetate was dissolved in distilled water at concentration of 50 mg/l and administrated to rats in drinking water. Garlic (dry garlic powder 300 mg in each tablet, 50 tablets ATOS Pharma, Cairo, Egypt) was grounded and suspended in distilled water and administered by gavage at a dose of 200 mg/kg b.w. daily. All other chemicals and reagents were of analytical grade and obtained from standard commercial suppliers.

2.2.Experimental animals

Thirty two healthy adult male albino rats of similar age and weight (200 ± 20 g) were used in this study. The animals were obtained from a farm for experimental animals (Kafrelsheikh University, Egypt). They were maintained under standard laboratory conditions (25 ± 2°C and 12 h light-dark cycle). Basal diet and clean water were provided ad libitum for two weeks as an adaptation period. All animals' procedures are in accordance with the guidelines of Ethical Committee of Kafrelsheikh University for care and use of laboratory animals

2.3.Experimental design

For 42 days, the animals were randomly divided into four groups of eight rats each:

Group 1: Rats received a daily oral administration of distilled water as normal control. Group 2: Rats was administrated lead acetate in drinking water at concentration of 50 mg/l. Group 3: Rats received garlic (200 mg/kg b.w. daily by gastric gavage). Group 4: Rats received garlic (200 mg/kg b.w. daily by gastric gavage) with lead acetate (50 mg/l) in drinking water.

2.4.Tissue samples

All animals were sacrificed and testes were immediately excised, washed several times from blood by ice-cold isotonic saline. Testes, epididymis, seminal vesicles and ventral prostate were dissected from any adhering connective tissue weighed and shock-freeze in liquid nitrogen (-170°C) and stored at -80°C. The specimens of testes were homogenized individually with tissue homogenizer to make 10% of homogenate in PBS to assay the tissue oxidants and antioxidants. The homogenates were prepared for analysis by centrifugation at 18000 rpm (4°C) for 30 min and the supernatant was kept at -2°C for analysis of oxidative stress markers such as Lipid peroxidation (LP) and Superoxide Dismutase (SOD).

2.5.Analysis of oxidative stress and lead residues

The amount of malondialdehyde (MDA) in tissue homogenate of testes as an indicator of LP was determined according to (Ohkawa et al., 1979) based on the reaction with thiobarbituric acid. SOD activity was estimated as described by (Marklund, 1985) based on inhibiting pyrogallol autoxidation by SOD. The activity of SOD in tissue is directly proportional to the inhibition rate. The concentration of lead (Pb) was estimated in testes homogenates using atomic absorption spectroscopy method as described by Beaty and Kerber, (1993). First, 0.5 - 1g testicular tissue was digested with 2 - 3ml concentrated nitric acid in a Teflon-lined bomb at 150 ºC for 1.5 hours. Diluted samples of the digested tissue were injected into the atomic absorption spectrophotometer (Perkin-Elmer Model 400, Shelton, CT), and hollow cathode lamps of Pb were used at a wavelength of 283.3 nm.

2.6.Sperm concentration and morphology assay

The content of epididymis was released by cutting of the cuda epididymis using surgical blades then squeezed in a sterile clean watch glass. This content was diluted 5 times with 2.9% sodium citrate dihydrate solution and thoroughly mixed to estimate the sperm concentration (Bearden and Fuquay, 1980). One drop of the suspension was smeared on a glass slide and stained by Eosin Nigrosin stain to

determine the viability and sperm abnormalities using the criteria of Okamura et al.,(2005).

2.7. Histopathology

Specimens from testis were collected from all experimental and control groups and fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70–100%) and then prepared using standard procedures for Hematoxylin and Eosin stain as described by Bancroft et al.,(1996).

2.8. Immunohistochemistry of Bax and caspase-3

The paraffin embedded testis were cut into 5 µm sections and mounted on positively charged slides for caspase-3 and Bax immunohistochemistry. Sections were deparaffinized in xylene twice for 5 min each .Hydrate with 100% ethanol twice for 3 min each .Hydrate with 95% ethanol for1 min then rinsed in distilled water. For pre-treatment of tissue sections (Epitope Retrieval), Slides were immersed in EDTA 5 % for 20 min. For Immunoenzyme staining, sections were rinsed in Phosphate Buffered Saline (PBS) twice for 2 min each. Background non- specific staining was blocked by incubating sections in normal goat serum blocking solution at room temperature for 30 minutes. After that, slides were incubated in primary antibody (caspase-3 polyclonal rabbit antibody, Thermo scientific, Ref PA5-23921. and Bax polyclonal antibody, Thermo scientific, Ref PA5-32269) at 4^0C overnight then rinsed three times in PBS for 20 min each. For blocking of endogenous peroxidase, slides were incubated in 3% H_2O_2 in PBS for 10 minutes at room temperature then rinsed again in PBS for three times for 2 min each followed by incubating sections in secondary antibody for 30 min at room temperature then rinsed in PBS for for three times for 10 min each. Visualizing reaction was performed by adding DAB solution to the tissue and incubation for 10 min. The slides were washed twice with PBS for 3 min each. Counterstaining was performed by adding adequate amount of hematoxylin stain to the slide to cover the entire tissue surface. For quantitative analysis, the intensity of immunoreactive parts was used as a criterion of cellular activity after subtracting background noise. Measurement was done using an image analyzer (Image J program).

2.9. Real time PCR mRNA expression for p53 and Bcl-2

Total RNA was isolated from rat testes using RNA Purification Kit (Thermo Scientific, # K0731, USA) according to the manufacturer's protocol and as previously described (El-Magd et al., 2016). RNA integrity was assessed by electrophoresis on 1% agarose gels, and purity was evaluated by determining the 260/280 ratio. cDNA was then synthesised from 3 µg of total RNA using RevertAid H Minus Reverse Transcriptase (Thermo Scientific, USA). The produced cDNA was used as a template to determine the relative expression of the p53 and Bcl-2 genes using StepOnePlus Real-Time PCR System (Applied Biosystem, USA) and using the following primer (designed by Primer 5.0 software) sequences: F: 5′ ATGGCTTCCACCTGGGCTTC 3′ and R: 5′ TGACCCACAACTGCACAGGGC3′(p53);F:5′CT GCACCTGACGCCCTTCACC 3′ R: 5′ CACATGACCCCACCGAACTCAAAGA 3′ (Bcl-2). β-actin (with primer sequences F: 5′ AAGTCCCTCACCCTCCCAAAAG 3′ and R: 5′ AAGCAATGCTGTCACCTTCCC 3′) was used as a housekeeping gene to calculate fold change in target gene expression. A 25 µl PCR mix was prepared by adding 12.5 µl of 2× Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, # K0221, USA), 2 µl of cDNA template, 1 µl forward primer, 1 µl reverse primer and 8.5 µl of nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 10 min, 40–45 cycles of amplification of DNA, denaturation at 95°C for 15 s, annealing at 60°C for 30 s, extension at 72°C for 30 s. At the end of the last cycle, the temperature was increased from 63 to 95°C for melting curve analysis. The cycle threshold (Ct) values were calculated for target genes and the housekeeping gene, and relative gene expression was determined using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.10. Statistical analysis

All data were expressed as means ± standard error of mean (SEM). The statistical significance was evaluated by Student t test using SPSS 18.0 software. Values were considered statistically significant when p <0 .05.

3 RESULTS

3.1. Seminal picture

Figure 1 demonstrated that intoxication with lead acetate reduced significantly *(P* < 0.05) sperm cell concentration and viability compared with those in other groups. Conversely, sperm abnormalities were significantly increased in lead acetate treated rats. In green tea supplemented rats, the seminal picture was improved and the percentage of sperm abnormalities was remarkably (P < 0.05) reduced.

3.2. Effect of Pb on enzymatic activities of SOD and MDA level in testis

It is evident from the data presented in Table 1 that garlic administration alone tended to decrease insignificantly the accumulation of Pb and oxidative stress (MDA) in testes tissues, and insignificantly increased the antioxidants (SOD). However, Pb intoxicated rats showed significant elevations in the accumulation of Pb (183% and 222) and MDA (71% and 40%) in testes tissues, respectively as compared to the control group. Otherwise, the antioxidants were significantly decreased by55% and 62% for SOD activity in testes tissue, with the same respect. Pre-supplementation of Pb-intoxicated rats with garlic revealed significant reduction in the accumulation of Pb and oxidative stress (MDA), while significantly increased antioxidants (SOD) in testes tissues as compared to the Pb treatment alone. Pre-treatment Pb intoxicated rats with garlic brought back the concentration of Pb, the levels of MDA and the activities of SOD to near the normal control group. It is interesting to mention that there was insignificant difference between pretreatment Pb intoxicated rats with garlic and the control group concerning the levels of Pb, MDA and the activities of SOD in testes tissues reflecting the therapeutic value of garlic.

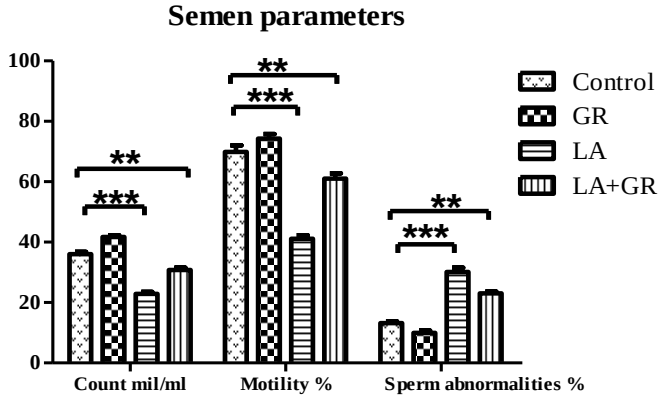

Fig. 1 Parameters of semen analysis in control, garlic treated (GR), lead acetate treated (LA) and, lead acetate and garlic co-treated (LA + GR). Values are the mean of 8 measurements ± SD. ***p ≤0.001 compared to control group, **p ≤0.01 compared to control group.

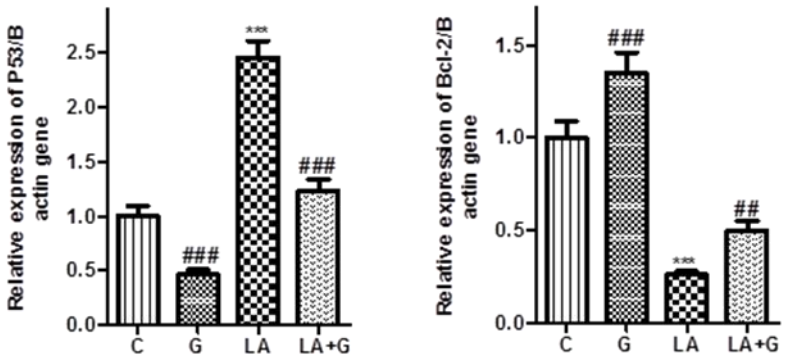

Fig. 2 Relative expression of either P53 gene (left) or Bcl-2 gene (right) to β actin gene in experimental groups including control (C), treated with garlic alone (G), given lead acetate alon (LA) and treated with garlic plus lead acetate (LA+G). Values are the mean of 9 measurements ± SD. ***p ≤0.001 compared to control group (C), ##p ≤0.01 compared to treated group (LA), ###p ≤0.001 compared to treated group (LA).

3.3.Effect of garlic on the expression of p53 and Bcl-2 genes in testis of lead acetate-intoxicated rats

Our results revealed a significant (P≤0.001) increase the expression of the apoptotic gene p53 in rat testis (Fig. 2) treated by lead acetate (LA) as compared to negative control groups (C) and other treated groups (G and LA+G). Administration of garlic (LA+G) resulted in a significant decrease in the expression level of p53 gene but with levels still higher than the control groups (C and G). On the other hand, the results showed a significant decrease in the expression of of the anti-apoptotic gene Bcl-2 in rat testis (Fig. 2) in rats intoxicated by lead acetate (LA) as compared to negative control groups (C) and other treated groups (G and LA+G).

3.2. Histopatholoy of testes

Testis of control rats (Fig.3A) as well as testis of rats treated with garlic alone (Fig.3C) or garlic with lead acetate (Fig.3D) showed normal histological structure of active mature functioning seminiferous tubules associated with complete spermatogenic series. On contrast, testis of lead intoxicated rats (Fig.3B) showed remarkable degeneration of most seminiferous tubules with absence of spermatogenic series in tubular lumen and congested testicular blood vessels.

3.3.Immunohistochemistry of androgen receptor and

3.3.a.Caspase-3 and Bax

The level of caspase-3 and Bax proteins expression were significantly increased in lead treated rats compared to the expression in other groups (Fig. 4&5). This high level of expression is represented by the intensity of activated caspase-3 immunostaining (deep brown), which is predominant on spermatogonia and seminiferous tubules of lead treated rats (Fig. 4B&5B).

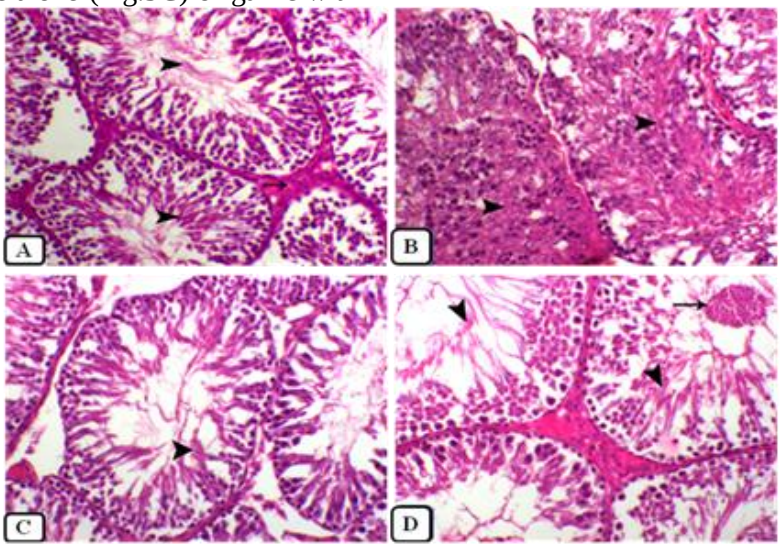

Fig. 3 (A) testis of normal rat showing normal seminiferous tubules with normal free sperm (arrowhead and normal interstitial connective tissue (arrow), H&E X200.(B) testis of rat treated with lead acetate showing degeneration and necrosis of the lining structures of seminefrous tubules (arrowhead), H&E X200.(C) testis of garlic-treated rat showing normal seminiferous tubules with normal free sperm (arrowhead), H&E X200.(D) testis of rat treated with lead acetate and administered garlic extract showing mild to moderate degree of vacuolation and desquamation of the spermatogenic cells (arrow) associated with active spermeogenesis within some tubules (arrowheads), H&E X200.

Table 1: Lead metal residue (µg/g), MDA (nmol/mg) and Lead residue (µg/g) of protein in testicular tissue following lead acetate toxicity and garlic treatment in groups of rats after 42 day.

	MDA (nmol/mg)	SOD (U/mg)	Lead residue (µg/g)
Group	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	16.23±0.8[a]	1000.03±60.52[a]	16.92±1.95[a]
GR	13.19±0.43[a]	1132.47±61.39[a]	30.0±2.0[a]
LA	26.32±1.01[b]	630.03±58.36b	763.0±11.92[b]
LA+GR	19.33±0.42[c]	798.22±49.36c	220.0±5.0[c]

a and b indicated significant change, at P < 0.0001; a and c indicated significant change, at P < 0.0001; b and c indicated significant change, at P < 0.0001 except for SOD activities and MDA at P < 0.001 .

4. DISCUSSION

In the field of toxicology, reproductive dysfunction and cancer are the adverse effects of greatest concern (Abdelrazek et al., 2016). It has been assumed that fifty percent of human male infertility of unknown cause may be due to environmental and occupational exposures (Evenson and Wixon, 2005). Therefore, male reproductive dysfunction is one of the major effects of lead toxicity (Kakkar and Jaffery, 2005). Compared to other organs, It has been proved that testis is one of main target organs for lead intoxication (Benoff et al., 2000) particularly in rodents (Perera et al., 2002), which are under investigation in the present work. The present study demonstrated the proved results of lead intoxication in lead acetate treated group. The testicular dysfunction was reflected in the semen analysis with remarkable asthenospermia, oligospermia and high percent of abnormalities, which is similar to previous study (Elgawish and Abdelrazek, 2014**).** Earlier, it has been proved that accumulation of lead in the testis has antispermatogenic effect (Fahim et al., 2013). According to our slides in figure (Tripathy), the testicular tissue of lead treated rats showed remarkable degeneration and atrophied somniferous tubules with absence of regular differentiated stages of germ cells to mature spermatozoa as described before by (Anjum et al., 2017). SOD activity and lipid peroxidation levels in tissues have been used as indicators of oxidative stress and subsequent tissue injury (Yoshida et al., 2013). In the present work, analysis of testicular tissue homogenates of lead treated animals revealed significant high levels of MDA but low values of SOD activity due to lead exposure. It has been previously reported that oxidative stress is the major mechanism underlying lead toxicity and its effect is mediated in two directions; first is the excessive generation of ROS mainly hydrogen peroxide, second one is the depletion of endogenous antioxidants including SOD(Flora, 2002). Therefore, because of its high content of polyunsaturated fatty acids spermatozoa have been considered to be highly susceptible to the damage induced by ROS. Consequently, lipid peroxidation induced by lead hampers sperm fertilizing potentials with subnormal membrane function and motility (Choudhary et al., 2010).

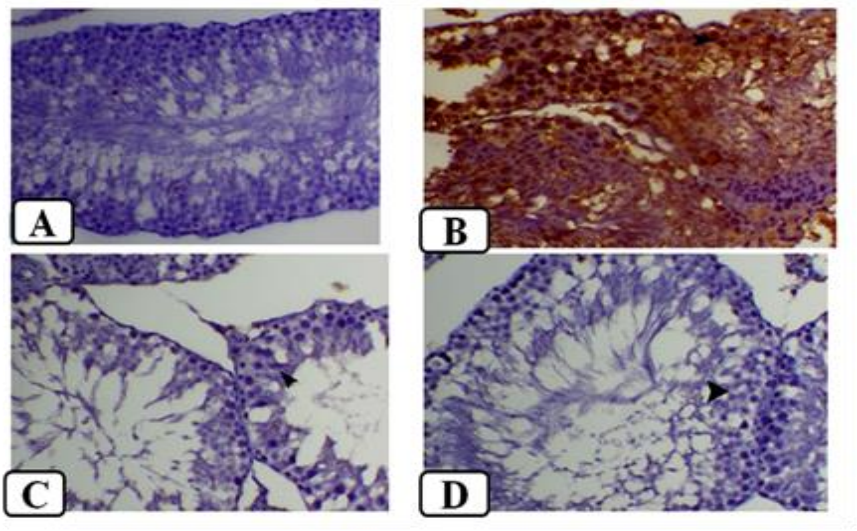

Fig. 4 Photomicrographs of testis (A) testis of normal rat showing too weak expression of Caspase-3 immunostaining within the seminiferous tubules, IHC (caspase-3), X200.(B) testis of rat treated with lead acetate showing higher expression of Caspase-3 immunostaining within the seminiferous tubules associated with degeneration and necrosis of the lining structures of seminefrous tubules (arrowhead), IHC (caspase-3), X200. (C) testis of garlic-treated rat showing too weak expression of Caspase-3 immunostaining within the seminiferous tubules (arrowhead), IHC (caspase-3), X200.(D) testis of rat treated with lead acetate and administered garlic extract showing too weak expression of caspase-3 immunostaining within the seminiferous tubules, (arrowhead), IHc(caspase-3), X200

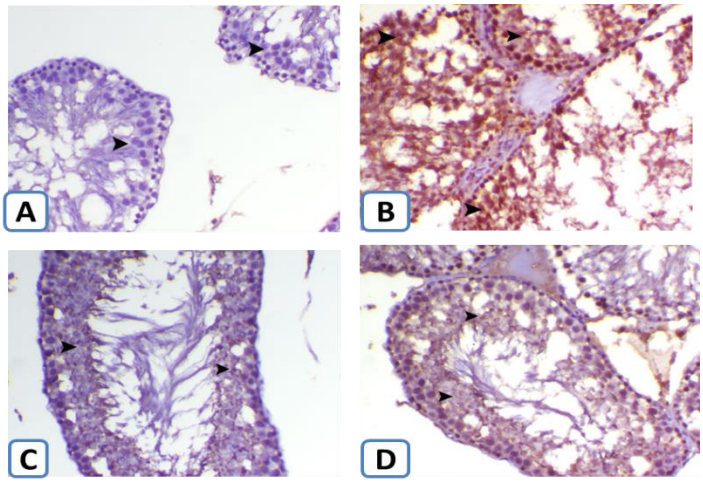

Fig. 5: Photomicrographs of testis (A) Testis of control animal showing too weak expression of Bax reactivity within the seminiferous tubules, (arrowhead), IHC (Bax antibody), X200. (B) testis of rat treated with lead acetate showing marked expression of Bax immuno-reactivity within the seminiferous tubules associated with degeneration and necrosis of the lining structures of seminefrous tubules (arrowhead), IHC (Bax antibody), X200. (C) Testis of garlic-treated rat showing weak expression of Bax reactivity within the seminiferous tubules (arrowhead), IHC (Bax antibody), X200. (D)testis of rat treated with lead acetate and administered gralic extract showing slight expression of Bax immuno-reactivity within the seminiferous tubules, (arrowhead), IHC (Bax antibody), X200.

Notably, lead has high affinity for sulfhydryl groups or metal-cofactor in the radical scavenging enzyme including SOD with inhibition of their activity(Jomova and Valko, 2011). It is worth mentioning that H2O2 is the main constituent of ROS in sperms (Aitken et al., 1996). In the mean time lead is positive for Fenton's reaction. Accordingly, H2O2 is the main form of ROS generated in lead intoxicated germ cells. H2O2 has been reported before in inducing apoptosis in germ cells through intrinsic, extrinsic and p53 pathways (Maheshwari et al., 2009). Similarly, in the present study, the expression of intrinsic proapoptotic markers Bax is increased in the testicular tissue of lead intoxicated animals compared with other groups along with increasing expression of Caspase-3 and up-regulation of p53 gene In contrast, the expression of antiapoptotic gene Bcl-2 was down-regulated in the same group. Interestingly, in the present work, garlic supplement of the prelead intoxicated rats has reversed the effects of the lead toxicity including marked improvement of semen quality similar to previous study (Obidike et al., 2007) .Additionally, garlic administration ameliorated lead induced oxidative stress as reflected by increased SOD activity and decreased lipid peroxidation indicated by reduced MDA levels in agreement with data reported before(Borek, 2001). Furthermore, in pre-lead intoxicated animals, garlic supplements protected their testicular tissue from deterioration by lead toxicity. Testicular tissue picture in those animals appeared close to that of the healthy control group. The somniferous tubules have showed normal diameter along with well differentiated stages of germ cells. The observations are in line with (Odumosu et al., 2013) who reported recovery of testicular structure and function as well as sperm count and quality after treatment with garlic. Since testes have high cellular proliferation and maturation activity apoptosis pattern should be a big concern. Notably, comparable to lead intoxicated animals, garlic administration showed testicular antiapoptotic effect. Garlic supplement significantly down regulated the expression of proapoptotic marker proteins caspase-3 and Bax as well as p53 gene but it upregulated the expression of antiapoptotic Bcl-2 gene. Indeed, the data reported about the role of garlic generally in male fertility and especially in testicular apoptosis is contradictive. For example, as previously reported, hot garlic juice has positive effect in restoring testicular hypogonadism to normal(Kasuga et al., 2001). In contrast, it has been reported that garlic powder (Dixit and Joshi, 1982) or crude garlic(Hammami et al., 2009) has negative effect on functions of testes and male genital tract. Accordingly, Garlic effect looks dependent on its preparation, dose and the cell type. In neuronal cells, It has been recorded that exposure to low concentrations of DADS has anti-apoptotic effect while high concentration has pro-apoptotic patterns (Kim et al., 2005, Koh et al., 2005). Similarly, apoptosis was induced in HL-60 cells and HepG2 after incubation with high concentration of diallyl sulfide (DADS) (Iciek et al., 2001, Kwon et al., 2002, Lin et al., 2007). In the present study,

commercial garlic preparation was used; most of which contain either garlic powder (GP) or garlic oil (GO). GP is a preparation of sliced, dried, and then pulverized garlic cloves which forms allicin upon the addition of water(Lawson et al., 2001). GO is produced commercially by heating crushed garlic cloves to 100℃ and collecting the vapor as a distillate(Lawson and Hughes, 1992). During this process, which has effects similar to those of cooking crushed garlic, allicin is converted to DADS and other garlic sulfides. Commercially available GO is normally diluted approximately 200:1 with a vegetable oil so that the final product reflects the level of allicin in freshly crushed garlic(O'Gara et al., 2000). Generally, there is a broad consent that stimulators of oxidative stress are apoptotic inducers but antioxidants are inhibitors (Buttke and Sandstrom, 1994). Accordingly, high levels of glutathione were reported after administration of GO in nicotine treated rats (Helen et al., 1999). Interestingly, glutathione depletion initiated apoptotic pathway by releasing cytochrome c and stimulation of subsequent caspase9 then caspase 3(Ghibelli et al., 1999). Therefore, increased intracellular levels of glutathione could be behind the antiapoptotic effect of garlic. (Arranz et al., 2007) supported this observation where they reported that garlic constituents DPDS and DADS were able to reduce oxidative DNA damage induced by H2O2 in HepG2 cells. Taken together, the findings of the present study demonstrated that the administration of the commercial preparation of garlic with its main constituent DADS to male rat can defeat the spermicidal effect and consequent male infertility induced by lead toxicity through inhibition of apoptotic pathways and promoting survival pathways.

5. CONCLUSION

Lead exposure is inevitable in daily basis with cumulative toxic effect on various body organs especially male reproductive system. Garlic has been popular food spices and has been added to food in daily basis as well. The present work has been carried out to evaluate the prophylactic role of widely available commercial preparation a gains potential male reproductive toxicity due to daily lead exposure.

6. REFERENCES

Abdelrazek, H. M., Helmy, S. A., Elsayed, D. H., Ebaid, H. M. Mohamed, R. M. 2016. Ameliorating effects of green tea extract on cadmium induced reproductive injury in male Wistar rats with respect to androgen receptors and caspase-3. Reprod. Biol. 16, 300-308.

Aitken, R. J., Buckingham, D. W., Carreras, A. Irvine, D. S. 1996. Superoxide dismutase in human sperm suspensions: relationship with cellular composition, oxidative stress, and sperm function. Free Radic Biol. Med. 21: 495-504.

Anjum, M. R., Madhu, P., Reddy, K. P. Reddy, P. S. 2017. The protective effects of zinc in lead-induced testicular and epididymal toxicity in Wistar rats. Toxicol. Ind. Health, 33: 265-276.

Arranz, N., Haza, A. I., Garcia, A., Moller, L., Rafter, J. Morales, P. 2007. Protective effects of organosulfur compounds towards N-nitrosamine-induced DNA damage in the single-cell gel electrophoresis (SCGE)/HepG2 assay. Food Chem. Toxicol. 45: 1662-1669.

Bancroft, J. D., Stevens, A. Turner, D. R. 1996. Theory and Practice of Histological techniques. Fourth edition. Churchill Livingstone, NewYork.

Banerjee, S. K., Maulik, M., Manchanda, S. C., Dinda, A. K., Das, T. K. Maulik, S. K. 2001. Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. Food Chem. Toxicol. 39: 793-797.

Benoff, S., Jacob, A. Hurley, I. R. 2000. Male infertility and environmental exposure to lead and cadmium. Human Reprod. Updat, 6: 107-121.

Block, E. 1985. The chemistry of garlic and onions. Sci. Am. 252: 114-119.

Borek, C. 2001. Antioxidant health effects of aged garlic extract. J. Nutr., 131: 1010S-5S.

Buttke, T. M. Sandstrom, P. A. 1994. Oxidative stress as a mediator of apoptosis. Immunol. Today 15: 7-10.

Choudhary, R., Chawala, V., Soni, N., Jayant Kumar Vyas, R. 2010. Oxidative stress and role of antioxidants in male infertility. Pak J Physiol, 6, 54-59.

Dixit, V. P. Joshi, S. 1982. Effects of chronic administration of garlic (Allium sativum Linn) on testicular function. Indian J Exp Biol, 20, 534-6.

El-Magd, M. A., Kahilo, K. A., Nasr, N. E., Kamal, T., Shukry, M. Saleh, A. A. 2016. A potential mechanism associated with lead-induced testicular toxicity in rats. Andrologia. 2016:1-9.

Elgawish, R. a. R. Abdelrazek, H. M. A. 2014. Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. Toxicol. Rep. 1: 795–801.

Evenson, D. P. Wixon, R. 2005. Environmental toxicants cause sperm DNA fragmentation as detected by the Sperm Chromatin Structure Assay (SCSA®). Toxicol. Applied Pharmacol. 207: 532-537.

Fahim, M. A., Tariq, S. Adeghate, E. 2013. Vitamin E modifies the ultrastructure of testis and epididymis in mice exposed to lead intoxication. Ann. Anat. 195; 272-277.

Flora, S. J. 2002. Lead exposure: health effects, prevention and treatment. J. Environ. Biol. 23: 25-41.

Ghibelli, L., Coppola, S., Fanelli, C., Rotilio, G., Civitareale, P., Scovassi, A. I. Ciriolo, M. R. 1999. Glutathione depletion causes cytochrome c release

even in the absence of cell commitment to apoptosis. FASEB J, 13, 2031-6.

Hammami, I., Amara, S., Benahmed, M., El May, M. V. Mauduit, C. 2009. Chronic crude garlic-feeding modified adult male rat testicular markers: mechanisms of action. Reprod. Biol. Endocrinol. 7: 65.

Helen, A., Rajasree, C. R., Krishnakumar, K., Augusti, K. T. Vijayammal, P. L. 1999. Antioxidant role of oils isolated from garlic (Allium sativum Linn) and onion (Allium cepa Linn) on nicotine-induced lipid peroxidation. Vet. Hum. Toxicol. 41: 316-319.

Iciek, M. B., Rokita, H. B. Wlodek, L. B. 2001. Effects of diallyl disulfide and other donors of sulfane sulfur on the proliferation of human hepatoma cell line (HepG2). Neoplasma, 48: 307-312.

Jomova, K. Valko, M. 2011. Advances in metal-induced oxidative stress and human disease. Toxicol. 283; 65-87.

Kakkar, P. Jaffery, F. N. 2005. Biological markers for metal toxicity. Environmental Toxicol. Pharmacol. 19: 335-349.

Kasuga, S., Uda, N., Kyo, E., Ushijima, M., Morihara, N. Itakura, Y. 2001. Pharmacologic activities of aged garlic extract in comparison with other garlic preparations. J. Nutr. 131: 1080S-4S.

Kim, J. G., Koh, S. H., Lee, Y. J., Lee, K. Y., Kim, Y., Kim, S., Lee, M. K. Kim, S. H. 2005. Differential effects of diallyl disulfide on neuronal cells depend on its concentration. Toxicol. 211: 86-96.

Koh, S. H., Kwon, H., Park, K. H., Ko, J. K., Kim, J. H., Hwang, M. S., Yum, Y. N., Kim, O. H., Kim, J., Kim, H. T., Do, B. R., Kim, K. S., Kim, H., Roh, H., Yu, H. J., Jung, H. K. Kim, S. H. 2005. Protective effect of diallyl disulfide on oxidative stress-injured neuronally differentiated PC12 cells. Brain Res. Mol. Brain Res., 133: 176-86.

Kwon, K. B., Yoo, S. J., Ryu, D. G., Yang, J. Y., Rho, H. W., Kim, J. S., Park, J. W., Kim, H. R. Park, B. H. 2002. Induction of apoptosis by diallyl disulfide through activation of caspase-3 in human leukemia HL-60 cells. Biochem. Pharmacol. 63: 41-47.

Lawson, L. D. Hughes, B. G. 1992. Characterization of the formation of allicin and other thiosulfinates from garlic. Planta Med. 58: 345-50.

Lawson, L. D., Wang, Z. J. Papadimitriou, D. 2001. Allicin release under simulated gastrointestinal conditions from garlic powder tablets employed in clinical trials on serum cholesterol. Planta Med. 67: 13-8.

Lin, H. T., Chen, S. W., Shen, C. J. Chu, C. 2007. Dissipation of pendimethalin in the garlic (Allium sativum L.) under subtropical condition. Bull Environ Contam Toxicol. 79: 84-86.

Livak, K. J. Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408.

Maheshwari, A., Misro, M. M., Aggarwal, A., Sharma, R. K. Nandan, D. 2009. Pathways involved in testicular germ cell apoptosis induced by H2O2 in vitro. FEBS J. 276: 870-81.

Marklund, S. L. 1985. Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice. Mutat. Res. 148:, 129-34.

O'gara, E. A., Hill, D. J. Maslin, D. J. 2000. Activities of garlic oil, garlic powder, and their diallyl constituents against Helicobacter pylori. Appl. Environ. Microbiol. 66: 2269-73.

Obidike, I. R., Maduabuchi, I. U. Olumuyiwa, S. S. 2007. Testicular morphology and cauda epididymal sperm reserves of male rats exposed to Nigerian Qua Iboe Brent crude oil. J. Vet. Sci. 8: 1-5.

Odumosu, F. M., Olokodana, O., Huthman, I. O., Akinyemi, R. A., , A., O.A., Adefule, A. K. 2013. Effect of Onion, Garlic and Aridan Aqueous Extract on Hormone Influenced Testis Weight of Adult Wistar Rats. International J. Chem. Pharmaceutical Res. 2.

Ohkawa, H., Ohishi, N. Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358.

Okamura, A., Kamijima, M., Shibata, E., Ohtani, K., Takagi, K., Ueyama, J., Watanabe, Y., Omura, M., Wang, H., Ichihara, G., Kondo, T. Nakajima, T. 2005. A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats. Toxicol. 213; 129-137.

Perera, F. P., Illman, S. M., Kinney, P. L., Whyatt, R. M., Kelvin, E. A., Shepard, P., Evans, D., Fullilove, M., Ford, J. Miller, R. L. 2002. The challenge of preventing environmentally related disease in young children: community-based research in New York City. Environmental Health Perspect. 110: 197.

Shaha, C., Tripathi, R. Mishra, D. P. 2010. Male germ cell apoptosis: regulation and biology. Philos Trans R Soc Lond B Biol Sci, 365, 1501-15.

Tripathy, D. N. R., W.M. 1998. In D.E. (Eds.), a Laboratory Manual for the Isolation and Identification of Avian Pathogens, Pox 4th Edition (Pp. 137–140). Kenneth Square, Pa: American Association of Avian Pathologists, New Bolton Center. In: SWAYNE, J. R. G., M.W. Jackwood, J.E. Pearson, W.M. Reed (Ed.).

Yoshida, H., Shoda, T., Yanai, H., Ikewaki, K., Kurata, H., Ito, K., Furutani, N., Tada, N., Witztum, J. L. Tsimikas, S. 2013. Effects of pitavastatin and atorvastatin on lipoprotein oxidation biomarkers in patients with dyslipidemia. Atherosclerosis, 226: 161-164.