The Protective Effect of Aged Garlic Extract (AGE) against Genotoxic, Chromosomal Aberration and Semen Picture Alteration Effects of Cadmium Chloride on Albino Rats

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ABSTRACT

The present study was conducted to investigate the protective effect of AGE as an antidote against the toxic hazards occurred by $CdCl_2$ on immunity, genotoxicity, chromosomal aberrations, and male fertility in albino rats. One hundred male albino rats were randomly distributed into four groups each of 25. The first group was the negative control was administered saline orally. The second group was the positive control orally administrated 2 gm /kg b.wt. of AGE daily for 15 days. The third group was orally administered $^1/_{10}$ LD_{50} of $CdCl_2$ (7.5 mg/kg b.wt.) daily for 15 days. The fourth group was orally administrated $^1/_{10}$ LD_{50} of $CdCl_2$ followed by 2 gm/kg b.wt. of AGE daily for 15 days. Five animals were sacrificed from each group at 3, 5, 7, and 15 days post administration. One testis and rats femurs were collected after scarification for assessing the genotoxicity studies by alkaline comet assay and chromosomal aberrations at 3, 7, and 15 days post administration. Epididymal spermatozoa were collected rapidly after scarification for seminal picture assay at 5, 7, and 15 days post administration. The results showed that there was significant increase in TL in $CdCl_2$ group when compared with both the control groups, while after the administration of AGE caused significant decrease in TL when compared with $CdCl_2$ group. DNA% was significantly increased in $CdCl_2$ group when compared with both the control groups, while after the administration of AGE caused significant decrease in DNA% when compared with $CdCl_2$ group after 3, 7, and 15 days post administration. Also, the number of total aberrated cells was significantly increased in $CdCl_2$ group when compared with control group after 3, 7, and 15 days. After the administration of AGE, there was significant decrease in the total number of aberrated cells. In addition, there was significant increase in the total chromosomal aberration. Oral administration of AGE after $CdCl_2$ caused significant decrease in the total chromosomal aberrations. In conclusion, AGE can be used successfully in the treatment of some hazards induced by $CdCl_2$ toxicity.

INTRODUCTION

Cadmium (Cd), a heavy metal, is a member of group IIb in the periodic table of elements which is present in soils, sediments, air and water *(1)*. Occupational and environmental exposure to Cd results from nickel-cadmium batteries manufacture, pigments, plastic stabilizers and phosphate fertilizers which may contain high concentrations of Cd, application of contaminated sewage sludge as a soil amendment, in alloys, solders and electroplating showed a decreasing trends *(2)*. For non-smokers who are not occupationally exposed, diet is the main route of exposure to Cd *(3)*. Cd exposure leads to obstructive airways disease, emphysema, end-stage renal failure, diabetic and renal complications, bone disorders and immune suppression *(4)*.

There is an increasing interest towards the use of naturally occurring phytochemicals with antioxidant activity in Cd intoxication therapy. Numerous metal-chelating agents and synthetic antidotes have been employed to reduce the toxic oxidative burden by Cd. Some of them are unsuccessful due to their intrinsic limitations and their side affects. Cd intoxication therapy is now looking for therapeutic agents especially phytochemicals *(5)*. Garlic can be used in fresh and processed forms as medicinal or as supplement with food. Due to the pungent odor of fresh garlic and the unpleasant gastric side effects of garlic, this has caused many scientists to favor dietary garlic preparations as an optimal choice for increasing daily garlic intake *(6)*. One of these preparations is extract of garlic aged in dilute alcohol *(aged garlic extract, AGE) (7)*.

AGE, is an odorless garlic preparation which is produced by prolonged extraction of fresh garlic at room temperature in 20% ethanol for up to 20 months. It has been considered to be the most useful garlic product as antioxidants and effective in medicine compared with other garlic preparations *(8)*. The cold process of aging is gently modifying the harsh and irritating compounds found in the raw garlic and naturally generates unique and beneficial compounds through both enzymatic and natural chemical reactions. AGE has a greater concentration of organosulfur compounds such as S-allylcysteine and S-allyl mercaptocysteine which are a potent antioxidant and free radical scavenger *(9)*.

This study was designed to evaluate the protective effects of aged garlic extract (AGE), as a natural antidote to ameliorate some of the toxic hazards due to the exposure to the environmental pollutnt, cadmium (Cd) by studying the effect of AGE on Cd - induced genotoxicity, chromosomal aberrations and seminal picture analysis in albino rats.

MATERIAL AND METHODS

Animals and sampling

One hundred male albino rats weighting 180–200 g were used; the animals were obtained from laboratory animals Housing Unit, Animal Health Research Institute, Dokki, Cairo, Egypt. They were randomly distributed into four groups each of 25. The first group was used as negative control group which was administered saline orally. The second group was the positive control *(placebo group)* that orally administrated 2 gm /kg b.wt. of AGE daily for 15 days. The third group was orally administered $^1/_{10}$ LD_{50} of $CdCl_2$ (7.5 mg/kg b.wt.) daily for 15 days *(10)*. The fourth group was orally administrated $^1/_{10}$ LD_{50} of $CdCl_2$ followed by 2 gm/kg b.wt. of AGE daily for 15 days. Five animals were sacrificed from each group at 24h, 3, 5, 7, and 15 days post administration. Testis and bone marrow were obtained for genotoxicity studies (Comet assay and chromosomal aberrations) at 3, 7, and 15 days post administration and epididymal spermatozoa for seminal picture analysis at 5, 7, and 15 days post administration. Animals were sacrificed 90 min. after I/M colchicines 0.05% injection for genotoxic study in bone marrow by cervical dislocation and samples of testis were immediately taken and preserved in PBS and kept at -20°C until used for Comet assay.

Chemicals

Cadmium chloride ($CdCl_2$) and Aged garlic extracts (AGE). The Egyptian garlic cloves *(Allium sativum)* were sliced and soaked in 20% ethanol in dark colored glass bottles, where the concentration of garlic was 200 mg dry garlic. The alcohol found to have no effect except to inhibit bacterial growth *(11)*. The garlic was aged naturally for 20 months at room temperature. The extract was then filtered and ready to be used.

Methods

Cytotoxicity study in bone marrow cells for preparation of somatic cell chromosomes to study the cytogenic effect of $CdCl_2$, investigation was carried out on bone marrow cells of male rats at third, seventh and fifteen days post administration. Chromosomes of somatic cells (bone marrow cells) were prepared and chromosome aberrations were scored by identifying chromatin and chromosome aberrations *(12)*.

Alkaline single cell gel electrophoresis (comet assay)

A piece of testis was placed in 1 ml cold HBSS containing 20 mM EDTA / 1% DMSO, minced into fine pieces, settled, removed and mixed with 75µl LMP agarose at 37°C . The mixture was layered onto slides precoated with NMP agarose, then covered by cover slip. 0.5% LMP agarose and 1% NMP agarose were prepared, heated until near boiling and the agarose dissolves. For LMP agarose, aliquot 5 ml samples were placed into vials and refrigerate until needed. While LMP agarose is hot, conventional slides were dipped up to one – third and gently remove. The underside of slide was wipped to remove agarose the slides were laid in a tray on a flat surface to dry. After at least 2 hours at 4°C, slides were gently removed from the lysing solution and placed side by side on the horizontal gel box. The tank was filled

with freshly made PH> 13 electrophoresis buffer until the liquid level completely covers the slides (avoid bubbles over the agarose). Slides were left in the alkaline buffer for 20 minutes to allow for unwinding and the expression of alkaline–labile damage. Power was turned on 25 volts (0.74 v/cm) and was adjusted the current to 300 milliamperes. Turn off the power. The slides were gently lifted from the buffer; drop the slides with neutralization buffer, let sat for at least 5 minutes. Slides were drained and repeated two more time. Slides were stained with 80µg Ethidium Bromide, left for 5 min and then dipped in chilled distilled water to remove excess stain. The cover slip was then placed over it and the slides are scored immediately or dried before staining. For visualization of DNA damage, observation is made of Et Br–stained DNA using a 40 x objective on a fluorescent microscope *(13)*.

Semen picture analysis

The cauda epididymis of one testis was excised and received in a sterilized Petri dish containing warm normal saline at 37°C, and then it was macerated by sterilized scissors to obtain the epididymal contents in a suspension that was handled as the semen *(14)*. Sperm motility *(15)*, concentration per ml *(16)*, and abnormalities of spermatozoa *(17)* were assessed.

Statistical analysis

The obtained data were analyzed and graphically represented using the statistical package for social science *(18)* for obtaining mean data and standard error. The data were analyzed using one way ANOVA to determine the statistical significance of differences among animals groups.

RESULTS

1.Effect of $CdCl_2$ on DNA % (amount of DNA fragments in the tail) and tail length of Comet assay and the antidotal effect of AGE

• ***After 3 days post administration***

Regarding TL, there was significant increase in TL in $CdCl_2$ group when compared with both the control groups, while after the administration of AGE caused significant decrease in TL when compared with $CdCl_2$ group and both control groups. Regarding DNA%, there was significant increase in DNA% in $CdCl_2$ group when compared with both the control and placebo groups, while after the administration of AGE caused significant decrease in DNA% when compared with $CdCl_2$ group at 3, 7, and 15 days post administration. Fig. 1and 2 showed results of the previous study.

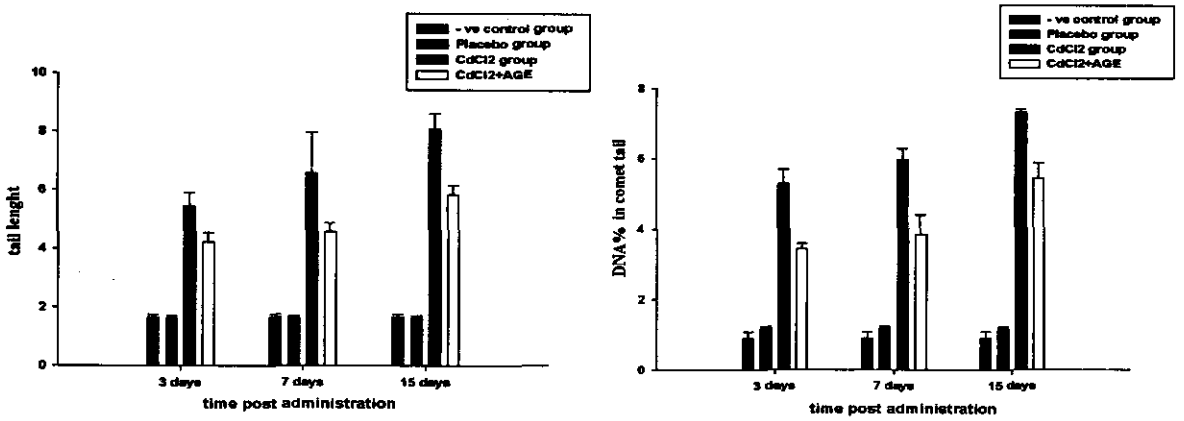

Fig. 1. Changes in tail length and DNA% in the comet tail as a result of comet assay analysis in testis of male albino rats at 3, 7, and 15 days after oral administration of $CdCl_2$ and the antidotal effect of oral administration of AGE.

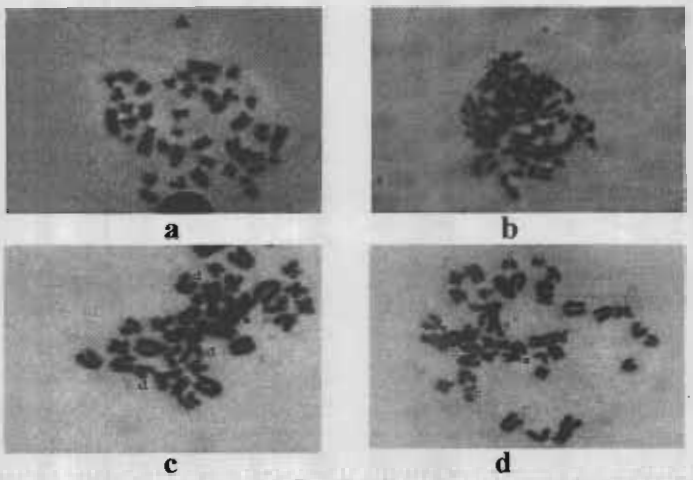

Fig. 3. Metaphase spread from bone marrow of male albino rat of control group showing normal chromosomal number and profile (2n = 42, xy) (a),metaphase spread from bone marrow of male albino rat treated with 1/10 LD50 (7.2 mg/ kg b.wt.) of $CdCl_2$ showing pulverization (b), metaphase spread from bone marrow of male albino rat treated with 1/10 LD50 (7.2 mg/ kg b.wt.) of $CdCl_2$ showing chromatid deletion (d) and sticky chromosome (s), (c), metaphase spread from bone marrow of male albino rat treated with 1/10 LD50 (7.2 mg/ kg b.wt.) of $CdCl_2$ showing centromeric attenuation (C), chromatid deletion (d) and chromatid gap (G). (Giemsa stain, x 1000).

4. Effect of $CdCl_2$ on seminal picture of male albino rats and the antidotal effect of AGE

After 5 days post administration

Regarding sperm cell concentration, there was significant decrease in the $CdCl_2$ when compared with both the control and placebo groups. On the other hand after oral administration of AGE, there was significant increase when compared with $CdCl_2$ but there was no significant increase when compared with both control and placebo groups.

Regarding sperm motility, there was significant decrease in sperm motility in $CdCl_2$ group when compared with both control and placebo groups, while after the oral administration of AGE, there was significant increase in sperm motility when compared with $CdCl_2$ group. Regarding sperm abnormalities, there was non significant increase in sperm abnormalities in $CdCl_2$ group when compared with the negative control group, while after the oral administration of AGE; there was significant decrease when compared with $CdCl_2$ group.

- ***After 7 days post administration***

Regarding sperm cell concentration, there was significant decrease when compared with the negative control group. On the other hand, after oral administration of AGE, there was significant decrease when compared with $CdCl_2$ group. Regarding sperm motility, there was significant decrease in sperm motility in $CdCl_2$ group when compared with both negative control and placebo groups, while after the oral administration of AGE, there was significant increase in sperm motility when compared with $CdCl_2$ group.

Regarding sperm abnormalities, there was no significant change in sperm abnormalities in $CdCl_2$ group when compared with control groups and the $CdCl_2$ +AGE group.

- ***After 15 days post administration***

Regarding sperm cell concentration, there was significant decrease in sperm cell concentration in $CdCl_2$ group when compared with the negative control and placebo groups, while after the oral administration of AGE, the was significant increase in the sperm cell concentration when compared with $CdCl_2$. Regarding sperm motility, there was significant decrease in sperm motility in $CdCl_2$ group when compared with both negative control and placebo groups, while after the oral administration of AGE, there was significant increase in sperm motility when compared with $CdCl_2$ group. Regarding sperm abnormalities, there was significant increase in $CdCl_2$ group compared with both the negative control and placebo groups, while after the oral administration of AGE, there was significant decrease when compared with $CdCl_2$ group. Table 2 and fig. 4showed results of the previous study.

Table 1. Changes in chromosomal aberrations in the bone marrow of male albino rats at 3, 7,and 15 days after oral administration of $CdCl_2$ and the antidotal effect of oral administration of AGE (mean ±S.E).

Parameter	Negative control (Normal saline)			$CdCl_2$ group (1/10 LD_{50} of $CdCl_2$)			$CdCl_2$+AGE group (2gm/kg B.W.AGE+1/10 LD_{50} of $CdCl_2$)		
	3 days	7 days	15 days	3 days	7 days	15 days	3 days	7 days	15 days
TAC	2.00±0.31b	2.00±0.94c	2.00±0.63c	9.00±1.09a	26.00±2.77a	36.60±2.31a	7.600±1.40a	15.20±0.86b	26.60±1.74b
CA	0.400±0.400a	0.80±0.48b	1.20±0.80a	1.60±0.81a	3.80±1.01a	2.20±1.01a	1.400±0.60a	1.20±0.48b	1.20±0.80a
R	0.00±0.00b	0.00±0.00a	0.00±0.00b	3.00±0.707a	2.60±1.16a	6.20±0.80a	1.20±0.58b	2.60±0.92a	6.40±1.02a
G	0.00±0.00a	0.60±0.40b	0.60±0.40b	1.00±0.44a	3.60±1.02a	8.40±2.42a	1.00±1.41a	2.40±1.02a	5.20±0.73a
B	0.60±0.40a	0.20±0.20b	0.40±0.40b	1.00±0.40a	3.60±0.92a	3.00±1.48a	2.40±1.16a	3.20±0.96a	3.80±0.48a
D	6.00±0.40a	0.800±0.48c	0.40±0.24c	3.00±0.89a	18.00±1.64a	27.80±2.45a	2.80±1.15a	9.80±1.15b	17.40±1.74b
S	0.00±0.00a	0.20±0.20a	0.00±0.00b	0.00±0.00a	0.00±0.00a	2.80±0.86a	0.60±0.60a	0.400±0.400a	0.00±0.00b
E	0.00±0.00a	0.00±0.00a	0.40±0.24b	0.60±0.60a	0.00±0.00a	8.60±1.24a	0.80±0.48a	0.20±0.20a	10.40±1.43a
P	0.00±0.00a	0.00±0.00b	0.00±0.00b	2.00±0.83a	3.20±0.86a	8.40±1.36a	1.60±0.67a	2.00±0.44a	2.20±0.96b
TCA	2.40±0.244b	3.00±1.37c	3.40±0.92c	13.60±2.11a	33.80±2.70a	68.20±2.45a	12.60±2.42a	22.60±1.02b	47.60±3.50b

Means within the same raw having different superscripts are significant at $P \leq 0.05$.

TAC: Total aberrated cells; CA: Centromeric attenuation; R: Ring chromatid; G: gap chromatid; B: Break chromatid; D: Deletion chromosome; S: Sticky chromosome; E: End to end association chromatid; P: pulverization; TCA: Total chromosomal aberrations

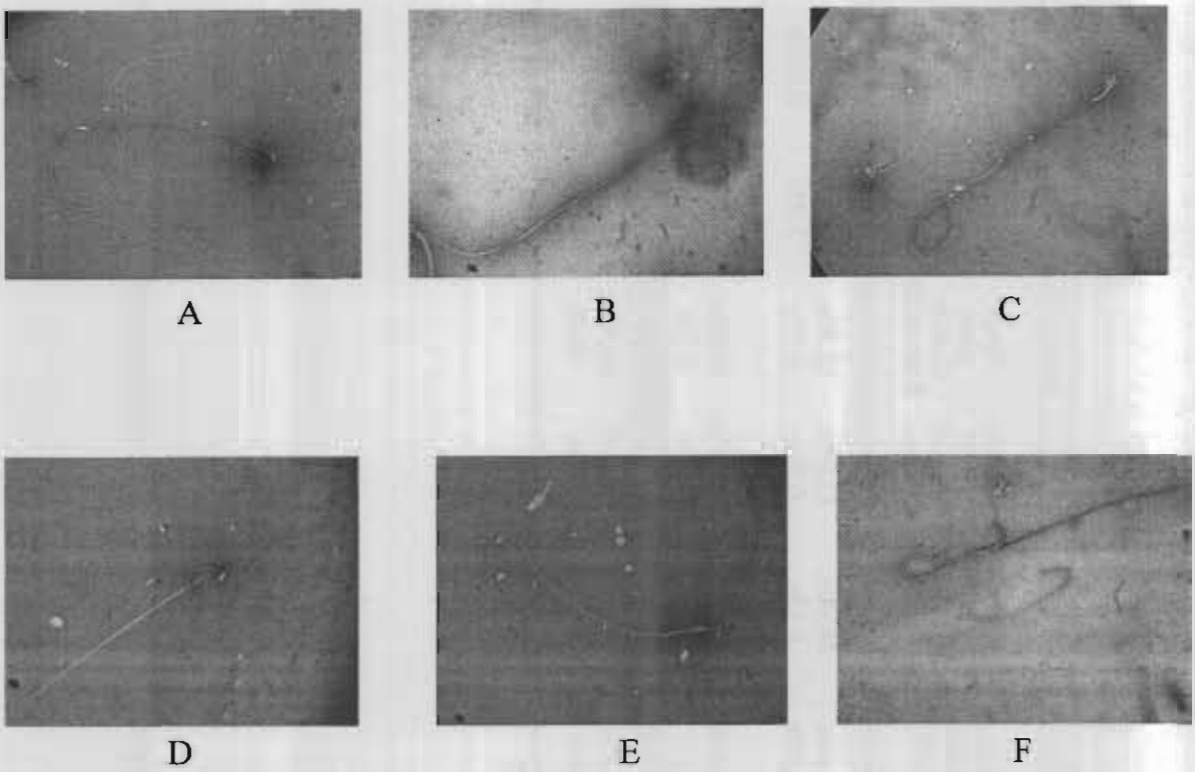

Fig. 7. Spermatozoa of male albino rat showing different abnormalities in the form of: abnormal head (A, B, C, E), abnormal middle piece (D, F) after the oral administration of 1/10 $LD_{50}CdCl_2$.

DISCUSSION

Garlic *(Allium Sativum)* is used as a vegetable spice and medicinal herb. Recent researches on garlic have used it in the form of tablets, fresh, dried raw, boiled and cooked preparation ***(19).*** Garlic, exhibits a wide range of properties including immunomodulatory, hepatoprotective, antimutagenic, anticarcinogenic and antioxidant effects ***(20).***

Cd, a widely used heavy metal, can induce several cellular dysfunction including decreased DNA repair and increased mutagenesis ***(21).*** Cd interferes with antioxidant defense mechanisms together with the production of ROS, which may act as a signaling molecule in the induction of cell death ***(22).***

Regarding the comet assay analysis (DNA % in the comet tail and TL) in testis of male albino rats revealed significant increase in DNA % in the comet tail and TL after 3, 7 and 15 days post administration when compared with the control group. This means that there was significant increase of DNA damage in testicular cells after oral administration of $CdCl_2$ for 3, 7 and 15 days. This was attributed to ROS which has been reported to injure DNA via causing single strand breaks, DNA- protein cross links and modification of base residues such as the induction of a hydroxyl group (- OH) into the c-8 position of guanidine and guanine residues forming 8- OH dg and 8- hydroxyl guanine, respectively being the commonly used markers of DNA oxidation ***(23, 24).*** Co administration of AGE with $CdCl_2$ evoked significant decrease in both DNA damage % in the comet tail and TL of comet when compared with control and $CdCl_2$ group. This was attributed to the chelating property of DTS which is present in AGE enhanced the elimination of Cd from the body, which might reduce the Cd burden with displacement of metal cofactors and Cd binding

Table 4. Changes in semen quality in male albino rats at 5, 7, and 15 days after oral administration of $CdCl_2$ and the antidotal effect of oral administration of AGE (mean ±S.E).

Time post administration (d)	Sperm cell conc. (X106)				Sperm cell Motility				Sperm cell Abnormalities			
	Negative control (Normal saline)	Placebo group (2gm/kg B.W. AGE)	CdCl2 group (1/10) LD50 of CdCl2)	CdCl2+AGE group (2gm/kg B.W.AGE +1/10 LD50 of CdCl2)	Negative control (Normal saline)	Placebo group (2gm/kg B.W. AGE)	CdCl2 group (1/10 LD50 of CdCl2)	CdCl2+AGE group (2gm/kg B.W.AGE+ 1/10 LD50 of CdCl2)	Negative control (Normal saline)	Placeb group (2gm/kg B.W. AGE)	CdCl2 group (1/10 LD50 of CdCl2)	CdCl2+AGE group (2gm/kg B.W.AGE +1/10 LD50 of CdCl2)
5	66.00± 6.20a	67.00± 6.81a	56.00± 12.08b	72.00± 12. 90a	88.00± 3.74a	91.00± 1.87a	67.00± 11.02b	88.00± 1.22a	11.00± 0.70a	13.80± 1.11b	15.40± 2.95a	9.80± 0.860b
7	76.00± 1.8a	62.00± 8.15b	65.00± 10.60b	49.00± 5.33c	90.00± 1.58a	91.00± 1.87a	65.00± 4.47c	79.00± 1.8b	12.400± 1.28a	11.60± 0.74a	12.60± 2.42a	10.20± 0.58a
15	76.00± 9.40a	67.00± 6.04b	50.00± 4.47c	66.00± 4.84b	90.00± 2.73a	92.00± 1.22a	48.00± 8.60b	89.00± 2.91a	11.60± 1.43b	10.40± .092b	17.60± 2.29a	11.60± 1.32b

Means within the same raw having different superscripts are significant at $P \leq 0.05$.

D E F

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with enzymes. In addition, the capability of DTS to react with free radical or with highly reactive by-products of lipid peroxidation as well as enhancement of tissue thiol pools may be responsible for the reduction of oxidative modification of enzymes and a reversal of the activities of antioxidant and glutathione metabolizing enzymes *(25)*. It is tempting to suggest that AGE may act against $CdCl_2$ toxicity to DNA through inhibition of protein, lipid peroxidation, and DNA damage and so, inhibits chromosome damage and protect RNA translation and protein synthesis. Also the action of AGE is thought to be due to inhibition of free radical production or Ros Scavenging and preventing DNA damage *(26)*.

Regarding the cytogenetic effect of CdCl2 and induction of chromosomal aberrations (CAs), the results showed that CdCl2 evoked significant increase in TAC % and TCA after 3, 7 and 15 days post administration. Also, the most pronounced chromosomal aberrations were centromeric attenuation which was significantly increased at 7^{th} day post administration. Gap chromatid and pulverization also were significantly increased after 7 and 15 days post administration. End to end association chromatid and sticky chromosome were also significantly increased after 15 days post administration. Deletion chromatid was significantly increased after 7 and 15 days post administration. The TCA % was significantly increased at 3^{rd}, 7^{th} and 15^{th} day post administration. This may be due to the generation of ROS and the resulting oxidative stress and not due to a direct effect of the metal *(27)*. In addition, by means of correlation, a moderate to strong direct relation between CAs in bone marrow cells and LPO and inverse relation between CAs and GST and GSH were found following Cd^{+2} treatment *(28)*. Our results were in agreement with those *(28)* who indicated that the mice received cd^{+2} at the dose of 2.5 mh/kg b.w.t. significantly increase CAs after 5 days the highest CAs in bone marrow cells were noticed after 15 days. Moreover, among different types of CAs, the structural types as fragments, breaks, gap, and deletion indicated greater value in all treatment schedules. The occurrence of chomatid break would support the view that cd^{+2} acted after the reproduction of the chromosome or at G_2 phase of DNA synthesis and had inhibitory effects on cell division and chromatid break seen as early as 5 days continued to 15 days and this would indicate not only non delayed, but also, lingering effect of cd^{+2}. The occurrence of this aberration at three time periods would also suggest that the metal acted at G_2 or late S-phase of DNA synthesis. The non random aberration in this study may be due to the existence of some localized weaker spots in the chromosomes *(29)*. According to the shorter time period (5 days) showed no significant alteration in biochemical parameters, but moderate significant increments were evidenced in CAs studies. With the advancement of time, the adverse effect of Cd^{+2} or biochemical parameters was increased and produced significant alterations in cryptogenic parameters. Thus, the suppression of endogenous protective effect of Cd^{+2} could be caused by elevation of LPO and inhibition of GST activity and depletion of GSH level. All this may explain the enhancement of clastogenic effect of Cd^{+2}*(30)*. The co-administration of AGE with CdCl2 evoked significant decrease in CAs caused by 1/10 LD50 of CdCl2. This may be attributed to the OSCs which provide strong nucleophilic centers enable them to react with carcinogens and inhibition of the genotoxic activities *(31)*. In addition, garlic and its active constituents have been reported to augment the endogenous antioxidants, which is an important event that protects cells of the body from oxidative injury *(32)*. It is well documented that garlic and its preparation scavenge ROS and inhibit oxidation of lipoproteins which prevent cellular injury *(7)*. Another possible mechanism for the reduction of DNA damage in CAs by OSCs is stimulation of DNA repair *(33)*.

In the present work it has been obvious that oral administration of $CdCl_2$ in a dose of 7.5 mg/kg daily 5, 7 and 15 days to male albino rats resulted in significant decrease in sperm cell concentration, motility percent and total sperm abnormalities after 5 and 15 days post administration as compared with the control group. Evidence at hand indicates that, the testis is extremely sensitive to Cd toxicity than other important organs and low doses have no detectable effect on general health can interfere

with testis function in rodents *(34)*. After acute Cd exposure which causes BTB disruption, germ cell loss, testicular edema, hemorrhage, necrosis and sterility in several species as rodent, rabbit, dog *(35)*. Cd has been shown to have gonadotoxic and spermiotoxic potentials *(3, 36, 37)*. It exerts adverse effects on reproductive structures and function directly at the testicular level or by altering post-testicular events such as sperm progress motility and function, all of which may culminate in hypogonadism and infertility *(3,38)*. The decrease in sperm concentration and motility (%), and the increase in abnormal sperm are in agreement with the findings of *(39)* who demonstrated that Cd can induce lipid peroxidation and testicular tissue necrosis and apoptosis in rats, which has been correlated to changed circulating androgen levels and fertility.

In the present study the oral administration of AGE with Cd Cl_2 in male albino rats abolished the adverse effects of Cd Cl_2 on semen picture. The results showed a significant increase in epididymal sperm cell count, sperm motility percent and total sperm abnormalities as compared with Cd Cl_2 group after 5, 7 and 15 days post administration. Our results are in accordance with those reported by previous investigators *(11)*. The authors reported that daily intake of garlic extract provoked significant increase in the weight of male reproductive organs, enhanced spermatogenesis and a significant elevation of sperm cell count, live sperm percentage, sperm motility and significant decrease in sperm abnormalities. Moreover, AGE was found to inhibit protein and lipid peroxidation and production of ROS and oxidative DNA damage (sperm DNA) *(8)* as well as improvement in peripheral circulation which would likely result in improved nutrition and thus, spermatogenesis *(11)*.

REFERENCES

1.Stoeppler M (1991): Cadmium. In: Meian, E.(Ed.), metals and their compounds in the environment. VCH, weinheim, New york, Basel, Cambridge, pp. 803-851.

2.Thornton I (1992): Sources and pathways of cadmium in the environmental. IARE . Sci. Publ. 118 : 162-194.

3.WHO (1992): Cadmium environmental health criteria 134. World Health Organization.

4.IARC (1993): International Agency for Research on cancer. Cadmium and certain cadmium compounds. In: IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 158. Beryllium , cadmium, mercury and exposures in the glass manufacturing industry., World Health Organization , Lyon , France.

5.Flora S J S , Mehta A , Gautam P , Jatav P C and pathak U (2007): Essential metal status, prooxidant/ antioxidant effects of MiADMSA in male rats: age-related effects. Biol. Trace Elem. Res. 120: 235-247.

6. Moriguchi T , Saito H and Nishiyama N (1997): Antiaging effect of AGE in the inbred brain atrophy mouse modle. Clin. Exp. Pharmacol. 24: 235-242.

7. Borek C (2001): Antioxidant health effects of aged garlic extract. *Nutrition*, 131 (3s), 1010-1015.

8.Banerjee S K , Dinda A K , Manchanda S C and Maulik S K (2002): Dose-dependant induction of endogenous antioxidants in rat heart by chronic administration of garlic. *Life sci.* 70:1509-1518.

9.Imai J , Ide N , Nagae S , Moriguchi T , Matsuura H and Itakura Y (1994): Antioxidant and radical scavenging effects of aged garlic extract and its constituents. Planta Med. 60: 417-420.

10.Budavari M (1996): The Merk index: an encyclopedia chemicals, drugs, and biologicals. 12th ed. Merck and Co., Inc.

11.Kasuga S , Uda N and Kyo K (2001): pharmacological activities of AGE in comparison with other garlic preparation. J. Nutr. 131: 1080S-1084S.

12.Yosida T H and Amano K (1975): Autosomal polymorphism in laboratory bred and wild Norway rats, Ratus norvagus, found in Misima Chromosoma. 16: 658-667.

13.Singh N P, McCoy M T, Tice R R, and Schneider E l (1988): A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res. 175: 184-191.

14.Hafez E S E (1970): Reproduction and breeding techniques for laboratory animals. Lea and Faiberg eds., Philadelphia, pp. 310-321.

15.Slott V, Suarez J and Perrealt S (1991): Rat sperm motility analysis: methodologic considerations. Rep. Toxicol. 5: 449-458.

16.Robb E.A, Amann R and killian G (1987): Daily sperm production and epididymal reserve of pupertal and adult rats. J. Rep. Fertil. 54: 103-107.

17.Filler R (1993): Method of epididymal sperm morphology. Cited in methods in Toxicology, Vol. 3, Part A, Male Reproductive Toxicology, Academic Press Limited, London, pp. 334-343.

18.SPSS, 0.8 software, 1997

19.Gorinstein S, Leontowicz H, Leontowicz M, Drzewiecki J, Najman K, Katrich E, Barasch D, Yamamoto K and Trachtenberg S (2006): Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats. *Life Sci.*, 78:655-663.

20.Al-Numair K S (2009): Hypocholestolemic and antioxidant effects of garlic (*Allium sativum*) extract in rats fed high cholesterol diet. *Pak. J. Nutr.* 8:161-166.

21.Bertin G and Averbeck D (2006): Cadmium: cellular effects, modifications of biomotecules, modulation of DNA repair and genotoxic consequences (a review). *Biochimie.* 88: 1549-1559.

22.Valko M, Rhodes C J, Moncol J, Izakovic M and Mazur M (2006): Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* 160: 1-40.

23.Yoshioka N, Nakashima H, Hosoda K, Eitaki Y and Omae K (2008): Urinary excretion of an oxidative stress marker, 8-hydroxyguanine (8-OH-Gua), among nickel-cadmium battery workers. J. Cooke, M.S.; Olinski, R.; and Evans, M.D. (2006): Dose measurement of oxidative damage to DNA has clinical significance. *Clin.him. Acta.* 365: 30-49.

24.Pari L, Murugavel P, Sitasawad S L, and Kumar K S (2007): cytoprotective and antioxidant role of diallyl tetrasulphide on cadmium-induced renal injury: An in vivo and in vitro study. 80:650-658.

25.Loven D P (1988): A role of reduced oxygen species in heat-induced cell killing and induction of thermotolerence. *Med. Hypotheses.* 26: 39-50.

26. Joseph P (2009): Mechanisms of cadmium carcinogenesis. Toxicol. Appl. Pharmacol. 272 -279.

27.Karmakor R, Banik S, Bandyo-Padhyay S and Chatterjee M (1998):Cadmium–induced alterations of hepatic lipid peroxidation, glutathione S-transferase activity and reduced glutathione level and their possible correlation with chromosomal aberration in mice : a time course study. Mut. Res. 397: 183-190.

28.Manna G K and Das R K (1972): Chromosome aberration in mice induced by aluminium chloride. *Nucleus.* 15:180-186.

29.Lorencz R, Nehez M and Desi I (1996): Investigations of the mutagenic effects of cadmium and lead on the chromosomes of the bone marrow cells in subchronic experiments. Abstracts of the XXVIIth Meeting of the Hungarian Society of Hygiene Balatonfoldvar, Hungry, Sptember 25-27, and p.116. (in Hungarian).

30.Le Bon A M and Siess M H (2000): Organosulfur compounds from Allium and the chemoprevention of cancer. *Drug Metab. Drug Interact.* 17: 51-79.

31.Mukherjee S, banerjee S K, Maulik M, Dina A K and Talwar K K (2003): protection against acute adriamycin-induced cardiotoxicity by garlic: role of endogenous

antioxidants and inhibition of TNF-a expression. *BMC Pharmacol.* 3(16):1-9.

32.Knowles L M and Milner J A (2000): Allyl sulphides modify cell growth. Drug Metabol. Drug Interact. 17: 81-107.

33.Laskey J W, Rehnberg G L, Laws S C and Hein J F (1986): Age- related dose response of selected reproductive parameters to acute cadmium chloride exposure in the male long-Evans rats. *J.Toxicol. Environ. Health.* 19: 393-401.

34.Li L and Heindel J (1998): Sertoli cell toxicants. In: Korach, K. (Eds), Reproductive and Developmental Toxicology. Marcel Dekker. New York.

35.El-Demerdash F, Yousef M I, Kedwany F S and Baghdadi H H (2004): Cadmium induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role & vitamin E and B-carotene. Food Chem. Toxicol., 42 : 1563-1571.

36.Yang S, Shih H J, Chow Y C, Tcai P S, Wang T W and Huang C J (2006): The protective role of heme oxygenase-1 induction on testicular tortion and distortion. *J. Urol.* 177:1928-1933.

37.Akinloye O, Arowojolu A O, Shittu O B and Anetor J L (2006): Cadmium toxicity: a possible cause of male fertility in Nigeria. *Reprod. Biol.* 6(1): 17-30.

38.El-Missiry M A and Shalaby F (2000): Role of beta- carotene in ameliorating the cadmium- induced oxidative stress in rat brain and testis. J. Biochem. Mol. Toxicol. 14:238-243.

39.Ushijima M, sumioka I, Kakimoto M, yokoyama K, Uda N, Matsuura H, Kyo E, Suzuki A and amagase H (1997): Effect of garlic and garlic preparations on physiological and psychological stress in mice. *Pytother. Res.* 11: 226-230.

الملخص العربي

التأثير الترياقي لمستخلص الثوم المعتق علي التغيرات الوراثية في كل من الحمض النووي الديوكسي ريبوزي و خلايا نخاع العظم و كذلك التغيرات في السائل المنوي نتيجة التسمم بكلوريد الكادميوم في الفئران البيضاء

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تستهدف هذه الدراسة استبيان تأثيرمستخلص الثوم المعتق في تقليل الاضرار الناجمة عن التعرض لكلوريد الكادميوم لفترات مختلفة في الفئران البيضاء. أجريت هذه الدراسة علي عدد ١٠٠ من ذكور الفئران البيضاء حيث قسمت هذه الفئران عشوائيا إلي أربعة مجموعات كل منها يحتوي علي ٢٥ من ذكور الفئران: المجموعة الأولي ضابطة سالبة. المجموعة الثانية أعطيت مستخلص الثوم المعتق يوميا لمدة ١٥ يوم بجرعة مقدارها ٢جم/كجم من وزن الجسم، المجموعة الثالثة أعطيت هذه كلوريد الكادميوم يوميا لمدة ١٥ يوم بجرعة مقدارها ٧,٢ مجم/كجم من وزن الجسم, المجموعة الرابع أعطيت كلوريد الكادميوم كما هو مبين في المجموعة الثالثة بالإضافة إلي مستخلص الثوم المعتق كما هو مبين في المجموعة الثانية. أوضحت النتائج زيادة معنوية ملحوظة في كل من طول المذنب و كذلك نسبة الحمض النووي الذي حدث به تغيرات و الموجود بالمذنب في المجموعة التي أعطيت كلوريد الكادميوم عندما قورنت بكل من المجموعة الأولي و الثانية. بعد إعطاء مستخلص الثوم المعتق لوحظ نقصا معنويا ملحوظا في كل من طول المذنب و كذلك نسبة الحمض النووي الذي حدث به تغيرات و الموجود بالمذنب بعد ٣، ٧، ١٥ يوم من التجريع. أوضحت النتائج أن هناك زيادة معنوية ملحوظة في عدد التشوهات التركيبية في كروموسومات الخلايا الجسدية في صورة فجوات أو أجزاء ناقصة في المجموعة التي أعطيت كلوريد الكادميوم عندما قورنت بالمجموعة الضابطة السالبة بعد ٣، ٧، ١٥ يوم من التجريع. بعد إعطاء مستخلص الثوم المعتق لوحظ نقصا معنويا ملحوظا في عدد التشوهات التركيبية في كروموسومات الخلايا الجسدية. أوضحت النتائج أن كلوريد الكادميوم له تأثير سلبي علي الذكورة في الفئران البيضاء فقد أحدث بعض التغيرات الملحوظة كنقص معنوي في حركة الحيوانات المنوية و نقص معنوي في عدد الحيوانات المنوية و زيادة غير معنوية في التشوهات الموجودة في الحيوانات المنوية بعد ٥، ٧، ١٥ يوم من التجريع بالمقارنة بالمجموعة الضابطة السالبة. بعد إعطاء مستخلص الثوم المعتق أوضحت النتائج وجود زيادة معنوية في حركة الحيوانات المنوية و زيادة معنوية في عدد الحيوانات المنوية و نقص معنوي في التشوهات الموجودة في الحيوانات المنوية. و من النتائج السابقة يتضح أنه يمكن استخدام مستخلص الثوم المعتق و بنجاح كترياق طبيعي ضد الاضرار الناجمة عن كلوريد الكادميوم.