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Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats

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Abstract:
The present study was designed to compare the protective effect of selenium and garlic against liver and kidney damage induced by (*ip*) injection of 0.5 mg/kg mercury chloride ($HgCl_2$) in rats. Thirty-six Sprague-Dawley rats were used in the present experiment and divided into six groups: one group was orally given (1 ml) saline and served as a control group; two groups of rats were given either selenium (0.1 mg/kg) or garlic (63 mg/kg) alone, once daily an oral dose for 30 successive days; other two groups of rats were given either selenium or garlic alone, once daily a dose for 15 successive days prior to $HgCl_2$ injection and on the next 15 successive days simultaneously with $HgCl_2$ injection; and the last group of rats was injected *ip* with $HgCl_2$ for 15 days and at the end of the experiment (which lasted 30 days), blood samples for the biochemical analysis were obtained from all rats after being lightly anesthetized with ether, and specimens of kidney and liver were removed and prepared for histochemical study. Computer image analysis was applied to liver and kidney tissues to evaluate the DNA density and DNA ploidy pattern in different groups. The results revealed that the rats injected with $HgCl_2$ showed a significant increase in levels of blood urea nitrogen (BUN), serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) by 29.3%, 62.5%, 29.46% and 30.61%, respectively, while alkaline phosphatase (ALP) showed a significant decrease by 22.6% as compared with saline control group. Rats that were given selenium in combination with the $HgCl_2$ injection showed a significant decrease in BUN, Serum creatinine, ALT and AST levels, while ALP was significantly increased as compared with $HgCl_2$ group. Also rats that were given garlic in combination with $HgCl_2$ injection showed a significant decrease in BUN, Serum creatinine, ALT and AST levels, although serum ALP level showed an increase as compared to $HgCl_2$ group. Rats that had been orally administered selenium or garlic alone did not show any significant changes in the serum level of BUN, Serum creatinine, ALT and AST but there was a significant decrease in ALP level as compared with saline control group. The cytometric results revealed that injection of $HgCl_2$ induced an increase in the DNA density in kidney tissues with an increase in aneuploid cells and decrease in diploid cells. However, DNA density decreased in liver tissues with mild decrease in diploid cells and little percentage of aneuploid cells. We can conclude that oral administration of either selenium or garlic produces a significant protection against liver and kidney damage induced by the $HgCl_2$ injection, but garlic appears to be more protective.

Key words:
selenium, garlic, $HgCl_2$, kidney, liver enzymes, DNA, rats

Introduction

Industrial pollution of the environment with metal compounds is becoming a significant problem. Mercury chloride (also called mercuric chloride and corrosive sublimate) is a poisonous white soluble crystalline sublimate of mercury. It was formerly used in insecticides, batteries, as an antiseptic, disinfectant, preservative, in metallurgy, and as a photographic

fixative. Mercury chloride is one of the most toxic forms of mercury because it easily forms organomercury complexes with proteins [9, 47, 48]. Mercuric chloride is highly toxic and corrosive once absorbed into blood stream: inorganic mercury combines with proteins in the plasma or enters the red blood cells. It does not readily pass into the brain or fetus, but may enter other body organs. Poisoning can result from inhalation, ingestion, or absorption through the skin [17]. The liver is a major site of metabolism for mercury, and it accumulates in the kidneys, hence, resulting in severe damage. Previous studies revealed that $HgCl_2$ caused histopathological and ultrastructural lesions evidenced by periportal fatty degeneration and cell necrosis in the liver and loss of brush border and cell loss in the cortex, tubular necrosis with casts in the kidney, and $HgCl_2$ alterations not only in the cytoplasm but also in the nucleus of proximal tubular cells [42, 43]. Schurz et al. [38] stated that DNA was a vital molecule in the cell activities and was the main target for $HgCl_2$-induced cell injury. $HgCl_2$ is a potential genotoxicant even at low doses. Furthermore, Carey [5] concluded that the abnormal DNA was a primary consequence of tumor growth and development.

Several studies were focused on the role of free radicals and oxidants in the renal injury induced by $HgCl_2$. It increases the production of many endogenous oxidants, such as hydrogen peroxide [26], depletes protective antioxidants, such as glutathione (GSH), and reduces free radical scavenging systems, such as superoxide dismutase (SOD) and GSH peroxidase (GPx) [24]. Other studies revealed that $HgCl_2$- induced injury can be ameliorated by SOD or the antioxidants N-acetylcysteine (NAC) and melatonin in some but not all studies [15, 16, 30, 32]. Selenium is a trace element that is essential at small amounts, but can be toxic at larger amount. Humans and animals require selenium for the function of a number of selenium-dependent enzymes, also known as selenoprotiens. During selenoprotiens synthesis, it is incorporated into a very specific location in the amino acid sequence in order to form a functional protein [4, 19, 34]. At least two types of selenoproteins are necessary for each animal cell, the first form is the family of GSH-peroxidase and the second form is the family of deiodinases. GSH-peroxidases are the most powerful antioxidant enzymes, which defend the cell against oxidative damage and thus oxidative stress-related diseases and disorders such as cardiovascular disease, malignancies, bacterial or viral diseases, muscle dystrophy, arthropathy, arterial plaques and others [14, 27]. GSH-PX have many other regulatory functions, such as regulation of biosynthesis of prostaglandins, prostacycline, leukotrienes, and thromboxane, while deiodinases regulate the metabolism of biologically active triiodothyronine and thus is implicated in thyroid hormone regulation of the whole organism [23, 28].

Garlic (*Alium sativum*) is a bulbous plant, whose bulb has a strong taste and characteristic odor, arising from allicin and other oil-soluble sulfur components. Typical volatiles in crushed garlic and garlic essential oil include diallyl sulfide (DAS) diallyl trisulfide, 3-vinyl-1,2-dithiin [13] and E,2-ajoene [3].

Garlic and garlic supplements are consumed in many cultures for their hypolipidemic, antiplatelet and beneficial circulatory effects. Some garlic preparations also appear to possess hepatoprotective, immune-enhancing, anticancer, chempreventive and antioxidant activities [18]. The use of herbs of medical benefit has played an important role in nearly every culture on earth. Herbal medicine was practiced by ancient cultures in Asia, Africa, Europe and the Americas. The recent popularity of use of herbs can be tied to the belief that herbs can provide some benefit over and above allopathic medicine and allow the users to feel that they have some control in their choice of medications. The widespread use of herbs either directly or as dietary supplements has raised many scientific questions. Are herbal preparations safe? Do herbs interact with pharmaceutical medications to enhance or reduce their efficacies [48]?

Therefore, the aim of the present work was to prove the possibility to use a herbal medication such as "garlic" instead of pharmaceutical medication such as "selenium" for protection of the kidney and liver from damage induced by mercury chloride ($HgCl_2$) in the rats.

Materials and Methods

Animals

Thirty-six albino Sprague-Dawley rats weighing 125–150 g were used throughout the experiments. All animals were housed in standard metal cages in

an air conditioned room at 22 ± 3°C, 55 ± 5% humidity and were provided with a standard laboratory diet and water *ad libitum* [36]. They were obtained from animal house colony of the national research center, Dokki, Giza, Egypt. All experimental procedures were conducted in accordance with the guide for the care and use of laboratory animals and in accordance with the Local Animal Care and Use Committee.

Drugs

Selenium was purchased from Mepaco Company for Pharmaceutical Industries and it was administered once daily as an oral dose of 0.1 mg/kg for thirty days. The dose was given according to Chmielnicka et al. [8].

Garlic was obtained from Sekim Company for Pharmaceutical Industries and was administered once daily as an oral dose of 63 mg/kg for thirty days. The dose was equivalent to maximum therapeutic human dose, and was calculated according to Paget and Barnes [31].

Chemical

Mercuric chloride ($HgCl_2$) was obtained from Elnasar Company for Chemical & Pharmaceutical Industries and was dissolved in distilled water and injected (*ip*) at a dose of 0.5 mg/kg once daily for fifteen days [8].

Diagnostic kits

- ALT (alanine aminotransferase) according to Bergmeyer et al. [2].
- AST (aspartate aminotransferase) according to Klauke et al. [22].
- ALP (alkaline phosphatase) according to Tietz and Shuey [45].
- Blood urea nitrogen (BUN) according to Tietz [46].
- Serum creatinine according to Mazzachi et al. [25].

All kits used were commercially available and were obtained from Roche Diagnostics.

Experimental design

Sixty rats were divided into six groups as follows: control group (orally received 1 ml of saline daily for 30 days); garlic group (orally received 63 mg/kg daily for 30 days); selenium group (orally received 0.1 mg/kg daily for 30 days); $HgCl_2$ group (*ip* injected with 0.5 mg/kg for 15 days); and the last two groups were given orally either garlic or selenium for 15 days before $HgCl_2$ injection and simultaneous with $HgCl_2$ injection for another 15 days.

Assessment of kidney and liver function

At the end of experiment, the blood was obtained from the retro-orbital plexus of all rat groups after being lightly anesthetized with ether [41]. The blood was allowed to flow into clean dry centrifuge tube and left to stand for 30 min before centrifugation to avoid hemolysis. Samples were centrifuged for 15 min at 2,500 rpm. The clear supernatant, serum was separated and collected by Pasteur pipette into a dry clean tube for the following biochemical tests: BUN and serum levels of creatinine, ALT, AST and ALP.

Histomorphometric study

All rats were sacrified after blood sampling by cervical dislocation. Their livers and kidneys were dissected out and collected in Bouin's solution for 24 h and rinsed with 70% ethanol prior to embedding in paraffin wax. Paraffin blocks were sectioned at 4–5 μm. Interrupted serial sections of each block were prepared and stained by Feulgen stain. The Feulgen stain reaction specifically stains nuclear DNA with a purple color. DNA analysis was performed by leica Qwin 500 image cytometry. The interactive measurement menu was used to detect: 1) DNA ploidy, 2) optical density. DNA ploidy was measured according to DNA index, and it classified cells into four groups, namely, diploid (2c), S-phase cells or proliferation index (3c), tetraploid (4c) and cells with more than 4c DNA content (> 4c) or (5c) indices aneuploidy cells. Optical density program was used for the quantitative analysis of DNA reaction. The intensity of the color is directly proportional to the DNA content within the nucleus of the cell.

Statistics

Data were presented as the mean ± SE. Data were analyzed by Student's *t*-test [39] and ANOVA, and differences among different group means were compared with *post-hoc* Duncan's multiple range test, where $p < 0.05$ was considered significant.

Results

Biochemical results

Results of the present study revealed that rats injected *ip* with $HgCl_2$ (0.5 mg/kg) daily dose for successive 15 days showed a significant increase in BUN and serum creatinine by 29.3% and 62.5%, respectively, as compared with control group (Tab. 1). Also $HgCl_2$ injection caused significant elevation of both ALT and AST levels by 29.46% and 30.61%, respectively, while alkaline phosphates (ALP) level significantly decreased by 22.6% as compared with control group (Tab. 2).

On the other hand, BUN and creatinine level in the rats given either selenium (0.1 mg/kg) or garlic (63 mg/kg) alone showed non-significant elevation of both (Tab. 1), while rats given selenium with $HgCl_2$ injection exhibited a significant reduction in the serum level of both BUN and creatinine by 11.15% and 13.46%, respectively (Tab. 1), whereas the rats given garlic with $HgCl_2$ had significantly reduced BUN and creatinine levels by 20.88% and 17.3%, respectively, as compared with $HgCl_2$ group. Both ALT and AST level in rats given either selenium or garlic alone showed an insignificant change in the serum level of these enzymatic activities (Tab. 2). Rats that were given selenium alone showed an insignificant change in ALP serum level, while rats given garlic alone showed a significant decrease in ALP (–7.16%) compared to the control group.

Rats given selenium in combination with $HgCl_2$ injection exhibited a significant reduction of both ALT

Tab. 1. Effect of daily oral dose of selenium (0.1 mg/kg) and garlic (63 mg/kg) alone for 30 days, and the effect of both for 15 days before *ip* injection of $HgCl_2$ (0.5 mg/kg) and simultaneously with $HgCl_2$ for other 15 days on blood urea nitrogen and serum creatinine level in the rats (N = 6)

Groups	BUN (mg/dl)		Creatinine (mg/dl)	
	X ± SE	% of change	X ± SE	% of change
Saline control	43.7 ± 0.8		0.32 ± 0.01	
Selenium	46.8 ± 1.4	7.1%	0.39 ± 0.01	21.9%
Garlic	47.3 ± 2.3	8.2%	0.38 ± 0.01	18.8%
$HgCl_2$	56.5 ± 1.5*	29.3%	0.52 ± 0.04	62.5%
Selenium + $HgCl_2$	50.2 ± 2.2˙	12.5%	0.45 ± 0.07	70.6%
Garlic + $HgCl_2$	44.7 ± 2.1¨	2.2%	0.43 ± 0.2	34.38%

* Significant as compared with saline-treated group at $p \leq 0.001$. ˙ Significant as compared with $HgCl_2$-treated group at $p \leq 0.005$. ¨ Significant as compared with $HgCl_2$-treated group at $p \leq 0.001$

Tab. 2. Effect of daily oral dose of selenium (0.1 mg/kg) and garlic (63 mg/kg) alone for 30 days, and the effect of both for 15 days before *ip* injection of $HgCl_2$ (0.5 mg/kg) and simultaneously with $HgCl_2$ for other 15 days on ALT, AST an ALP activity in the rats (N = 6)

Groups	ALT (U/l)		AST (U/l)		ALP (U/l)	
	X ± SE	% of change	X ± SE	% of change	X ± SE	% of change
Saline control	38.7 ± 0.8		112.7 ± 4.6		237.5 ± 7.9	
Selenium	40.4 ± 0.7	4.4%	118.0 ± 1.9	4.7%	234.3 ± 1.2	–1.3%
Garlic	37.3 ± 0.3	–3.62%	120.3 ± 4.3	6.74%	220.5 ± 2.3**	–7.16%
$HgCl_2$	50.1 ± 0.5**	29.46%	147.2 ± 3.8**	30.61%	183.8 ± 2.2*	–22.6%
Selenium + $HgCl_2$	42.5 ± 0.4¨	9.82%	121.4 ± 3.2¨	7.72%	200.52 ± 8.6˙	–15.68%
Garlic + $HgCl_2$	44.25 ± 0.6¨	14.34%	126.6 ± 4.8¨	12.33%	213.5 ± 5.6¨	–10.1%

* Significant as compared with saline-treated group at $p \leq 0.010$. ** Significant as compared with saline-treated group at $p \leq 0.001$. ˙ Significant as compared with $HgCl_2$-treated group at $p \leq 0.010$. ¨ Significant as compared with $HgCl_2$-treated group at $p \leq 0.001$

Tab. 3. Descriptive statistics for comparison between the mean of optical density of DNA in different treatment groups

Organs	Control	Selenium	Garlic	$HgCl_2$	$HgCl_2$ + selenium	$HgCl_2$ + garlic
	Mean ± SE					
Liver	0.1233 ± 0.0019	0.0900 ± 0.0*	0.1425 ± 0.0013*	0.0670 ± 0.0015*	0.1270 ± 0.0027*	0.1018 ± 0.0074*
Kidney	0.2030 ± 0.0054	0.1273 ± 0.0241*	0.0891 ± 0.0009*	0.2220 ± 0.0092*	0.2209 ± 0.0104*	0.2008 ± 0.0090*

Results are expressed as the mean ± SE. * Significant at $p < 0.05$

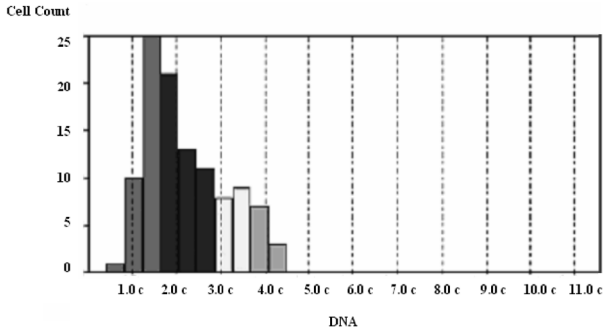

Fig. 1. Histogram of DNA cell count in the kidney of control rat showing that 47 of cells are diploid around 2c ■, 23 are in S-phase around 3c □ and 14 are tetraploid around 4c ■

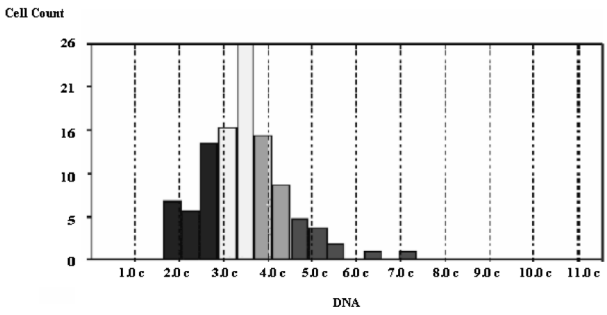

Fig. 2. Histogram of DNA cell count in the kidney of selenium-treated rats showing that one cell is diploid (non significant) around 2c, 10 cells are in S-phase around 3c, 42 cells are in tetraploid, 48 cells are > 4c and 27 cells are aneuploid > 5c ■

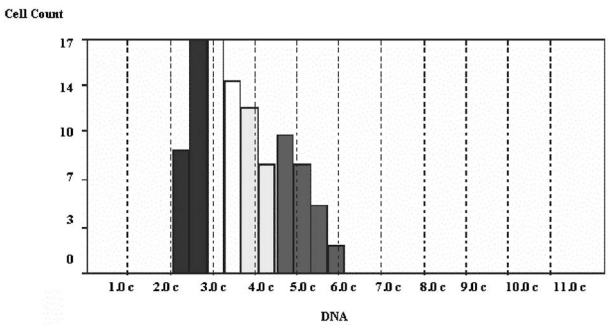

Fig. 3. Histogram of DNA cell count in the kidney of garlic-treated rats showing that there are no diploid cells, 18 cells are in S-phase around 3c, 36 cells are tetraploid, 47 cells are > 4c and 27 cells are at aneuploid > 5c

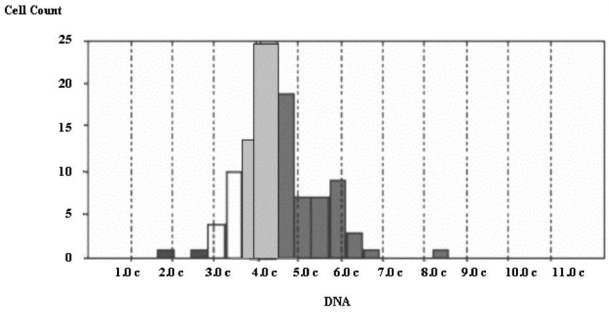

Fig. 4. Histogram of DNA cell count in the kidney of $HgCl_2$-treated rats showing few diploid cells 5 around 2c, 46 cells are in S-phase around 3c, 46 cells are tetraploid, 8 cells are > 4c and 3 cells are at aneuploid > 5c

and AST levels by –15.17% and –17.53%, respectively. Also, rats given garlic with $HgCl_2$ injection showed a significant reduction of both ALT and AST serum levels by 11.6%, 13.99%, respectively, as compared with the $HgCl_2$ group. However, rats that were given either selenium or garlic with $HgCl_2$ injection exhibited an increase in ALP activity by 9.1% and 16.2%, respectively, as compared with the $HgCl_2$ group (Tab. 2).

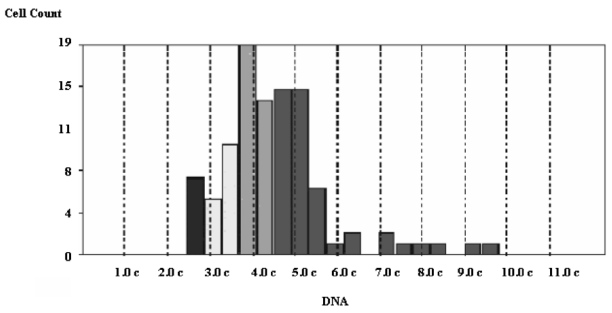

Fig. 5. Histogram of DNA cell count in the kidney of $HgCl_2$ and selenium-treated rats showing that 14 cells are diploid around 2c, 41 cells are in S-phase around 3c, 38 cells are tetraploid, 13 cells are > 4c and 7 cells are at aneuploid > 5c

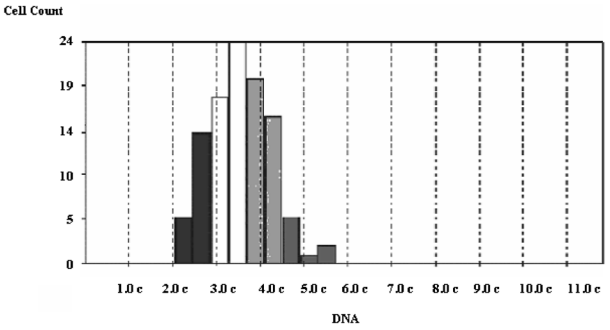

Fig. 6. Histogram of DNA cell count in the kidney of $HgCl_2$ and garlic-treated rats showing that 10 of cells are diploid around 2c, 41 cells are in S-phase around 3c, 26 cells are tetraploid, 25 cells are > 4c and 12 cells are at aneuploid > 5c

Histomorphometric results

Quantitative analysis of DNA reaction in kidney sections

Two computerized techniques were used to evaluate DNA contents by image analysis. In the optical density program, the intensity of color is directly proportional to DNA content within the nucleus of the cell. DNA contents in the control and treated groups were seen in Table 3. The density of DNA in the rats treated with $HgCl_2$ alone showed a significant increase in DNA contents compared to control group (0.2220 ± 0.0092 *vs.* 0.2030 ± 0.0054), whereas rats treated with $HgCl_2$ plus selenium showed a significant decrease in DNA intensity compared to $HgCl_2$-treated rats (0.2209 ± 0.0104 *vs.* 0.2220 ± 0.0092). Moreover, rats given garlic with $HgCl_2$ revealed an insignificant increase in the optical density of DNA contents compared to $HgCl_2$ or the treated groups (0.2008 ± 0.0090).

The normal distribution of DNA pattern in control kidney was seen in Figure 1. It is clear that selenium- or garlic-treated groups showed a marked decrease in diploid cells, measurable percentage of aneuploid cells (Fig. 2, 3). The kidneys of rats treated with $HgCl_2$ (Fig. 4) showed a significant decrease in the percentage of diploid cells reaching a value of 0.99% compared to control group (43.52%) (2c), and significant increase in aneuploid cells (47.52%) of the examined cells were > 4c.

The percentage of proliferation index of the examined cells was low (9.9%). The rats treated with $HgCl_2$ with selenium (Fig. 5) showed no diploid cells. The same percentage of aneuploid cells (46.5%) was observed as in $HgCl_2$-treated rats (> 4c), the percentage of proliferation index was medium compared to control (17.8% *vs.* 21.3%). The rats given garlic with $HgCl_2$ (Fig. 6) revealed few diploid cells 4.76% (2c), few aneuploid cells (7.62%), 43.8% were tetraploid (4c), and proliferation index was high (43.8%).

Quantitative analysis of DNA reaction in liver sections

The optical density of DNA contents in the control and treated groups is presented in Table 3. The rats treated with $HgCl_2$ alone revealed significant decrease in the optical density of DNA contents compared to control group (0.0670 ± 0.0015 *vs.* 0.1233 ± 0.0019). Rats treated with $HgCl_2$ with selenium showed a significant increase in DNA staining intensity compared to $HgCl_2$ alone-treated rats (0.1270 ± 0.0027 *vs.* 0.0670 ± 0.0015). Furthermore, the rats that were given garlic with $HgCl_2$ revealed a significantly increased optical density of DNA contents compared to $HgCl_2$-treated rats (0.1018 ± 0.0074). The normal distribution of DNA pattern in control liver was seen in Figure 7.

Selenium- or garlic-treated rats showed a marked decrease in diploid cells and a measurable percentage of aneuploid cells (Fig. 8, 9). The rats treated with $HgCl_2$ alone displayed a mild decrease in the percentage of diploid cells (2c) reaching a value of 58.3% compared to the control group (68.6%) and few aneuploid cells (1.9%) > 4c. The percentage of proliferation index was high (22.2%) (Fig. 10). Rats treated

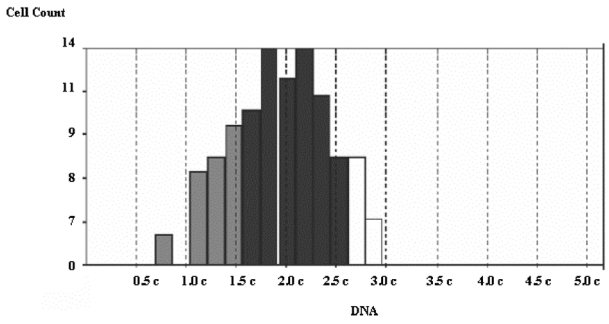

Fig. 7. Histogram of DNA cell count in the liver of a control rat showing that 70 cells are diploid around 2c, 15 of cells are in S-phase cells around 3c and no aneoploid cells 0

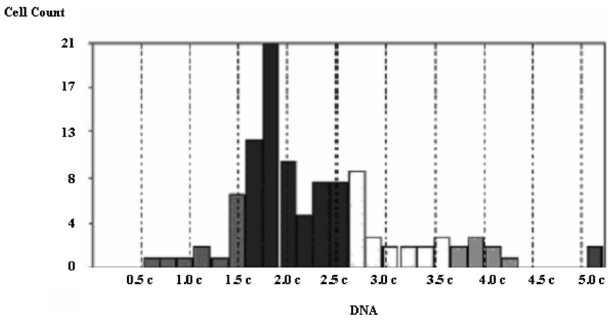

Fig. 10. Histogram of DNA distribution in the liver of $HgCl_2$-treated rats showing that 45 of the cells are diploid around 2c, 54 of cells are at S-phase around 3c and 4 cells are in tetraploid, no cells are > 4c and no cells are at aneuploid > 5c

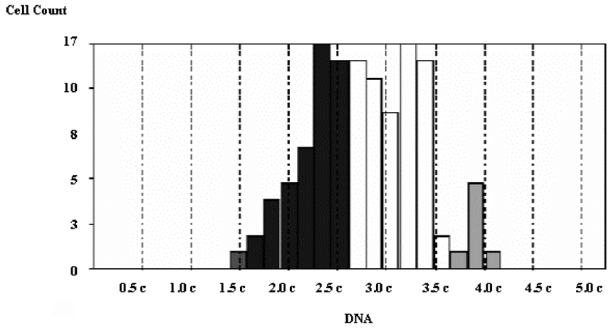

Fig. 8. Histogram of DNA cell count in the liver of selenium-treated rats showing that 63 of the cells are diploid around 2c, 24 of cells are at S-phase around 3c and 9 cells are in tetraploid, 2 cells are > 4c (non significant) and 2 cells are at aneuploid > 5c (non significant)

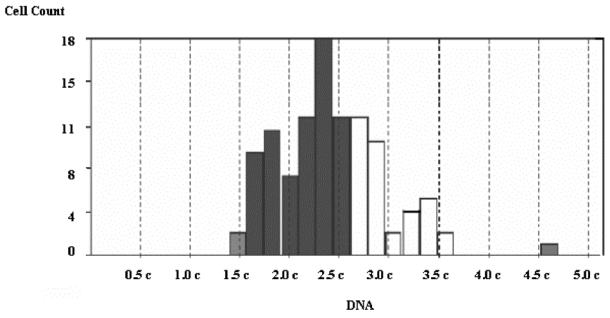

Fig. 11. Histogram of DNA cell count in the liver of $HgCl_2$ and selenium-treated rats showing that 37 of the cells are diploid around 2c, 63 of cells are at S-phase around 3c and 9 cells are in tetraploid, no cells are > 4c and no cells are at aneuploid > 5c

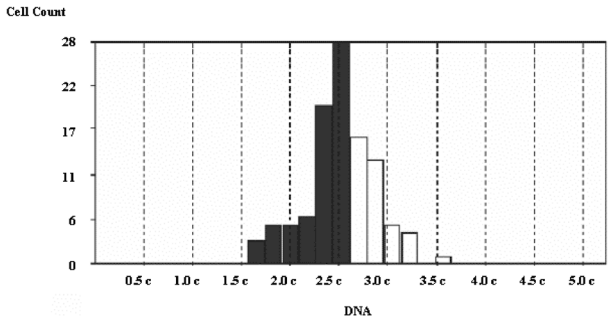

Fig. 9. Histogram of DNA cell count in the liver of garlic-treated rats showing that 63 of the cells are diploid around 2c, 42 of cells are at S-phase around 3c and 2 cells are in tetraploid, 1 cells are > 4c (non significant) and no aneuploid cells > 5c (non significant)

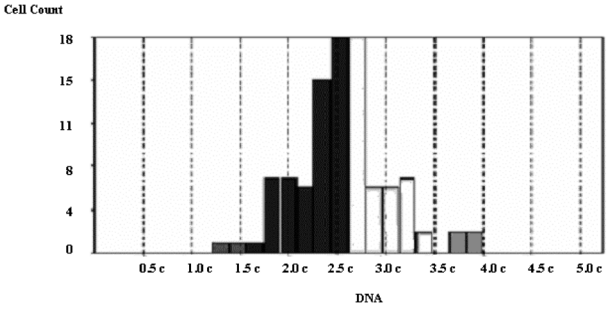

Fig. 12. Histogram of DNA cell count in the liver of $HgCl_2$ and garlic-treated rats showing that 48 of cells are diploid around 2c, 57 of cells are at S-phase around 3c and one cell are in tetraploid (non significant), no cells are > 4c and no cells are at aneuploid > 5c

with $HgCl_2$ with selenium showed a decrease in the number of diploid cells and increase in the percentage of aneuploid cells compared to the control animals or those treated with $HgCl_2$. No changes in the number of diploid cells were present but there was a decrease in the percentage of aneuploid cells. The proliferation index was high compared to control (38.8% *vs.* 14.7%) (Fig. 11).The rats given garlic with $HgCl_2$ revealed rearrangement in diploid cells (2c) (43.7%), no aneuploid cells, and high proliferation index (51.9%) (Fig. 12).

Discussion

Our results revealed that selenium and garlic ameliorated the kidney and liver damage induced by $HgCl_2$ injection. Either selenium or garlic when given with $HgCl_2$ induced a decrease in the elevated serum level of both BUN and creatinine as compared with the $HgCl_2$ group, while no marked alteration was observed in serum levels of both BUN and creatinine in rats given selenium or garlic alone as compared with saline control group. The elevated serum level of BUN and creatinine is an indicator of kidney damage as blood urea nitrogen is derived from normal metabolism of protein and is excreted in the urine. Elevated BUN usually indicates glomerular damage. While creatinine is a metabolite of creatine and is excreted completely in the urine *via* glomerular filtration, an elevation of its level in the blood is, thus, an indication of impaired kidney function.

On the other hand, the rats injected with $HgCl_2$ showed elevation of serum level of both ALT and AST and reduction of ALP serum level as compared with saline control group. As the elevation in the serum activity of ALT, a liver cytoplasmic enzyme, indicates a necrotic lesions in the liver while the decrease in serum ALP level indicates that there was no congestion or cholestasis. So, our results were in agreement with studies by El-Demerdash [11], Reus et al. [35] and Sharma et al. [40]. These authors reported that mice treated with $HgCl_2$ showed a significant elevation in serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities but significant decline in the alkaline phosphatase activity. Also, Sastry and Sharma [37] reported that alkaline phosphatase activity decreased in acute exposure of teleost fish to mercuric chloride and increased in chronic exposure but there was elevation of both SGPT and SGOT either in acute or chronic exposure.

Selenium treatment of rats injected with $HgCl_2$ in our work decreased the elevated serum levels of ALT and AST and this result confirmed the work of Farina et al. [12] who reported that selenium abolished the inhibitory effect of mercury on renal delta-aminolevulinic acid dehydratase (delta-ALA-D) at 12 h after treatment. Thus, this result suggests that selenium abolishes the interaction of $HgCl_2$ with sulfhydryl groups of protein and non-protein sources. Other studies that were done by Perottoni et al. [33] revealed that sodium selenite protected against mercury chloride effects *in vivo* (prevention of mercury interaction with thiol groups and of mercury-induced oxidative damage).

Also in the present study, the rats given garlic with $HgCl_2$ showed significant improvement of elevated serum enzymes indicative of both kidney and liver function as compared with $HgCl_2$-treated group. These results are in agreement with the results of Carmia [6], who revealed that treatment with aged garlic extract appeared to enhance the recovery from carbon tetrachloride (CCl_4) and acetaminophen induced hepatotoxity in rats. Yang et al. [49] also reported that diallyl sulfide (DAS) and related compound from garlic reduced CCl_4-, N-nitrosodimethylamine- and acetaminophen-induced toxicity in rodents. It was somewhat surprising that garlic ameliorated the toxic reactions of a wide variety of toxic agents. It seems unlikely that garlic is only an antioxidant in these situations. Garlic extracts elicit antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzyme superoxide dismutase, catalase and glutathione peroxidase, and increasing glutathione in the cells [6, 29]. Mercury is a reactive metal that has high affinity for macromolecules and binds to DNA *in vitro* leading to alterations in DNA structure [1]. Mercury chloride injection in the present work induced a mild decrease in the percentage of diploid cells, and measurable number of aneuploid cells as well as high proliferation index (S-phase) and the decrease in DNA density in the liver tissue. These results were confirmed with Homma et al. [20], who observed that $HgCl_2$ caused DNA fragmentation in the liver.

Other studies reported that injection of $HgCl_2$ (2 mg/kg) into rats caused induction of kidney necrosis and apoptosis in a dose-dependant fashion with

chromatin condensation in proximal tubules [20, 43, 44]. The apoptotic cells increased and spread over the cortex with time. Renal damage caused by $HgCl_2$ was inferred from the morphological changes in renal sections and the urinary excretion of ALP, which is a marker enzyme of manifest proximal tubular injury by mercury [45]. This study clarifies the reduction of serum ALP in $HgCl_2$-treated rats that appeared in our results. So, our results are in agreement with the results of Chatterjee et al. [7], who reported that alkaline phosphatase was involved in the synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters and in mobilizing carbohydrates and lipid metabolites to be utilized within the cells.

Ide et al. [21] reported that garlic and its major organosulfur constituents had a scavenging effect on hydrogen peroxide, and inhibited the chain oxidation induced by a hydrophilic radical initiation. Other studies demonstrated that garlic prevented tumor promotion [10] and liver damage [49], which is considered to be associated with oxygen radical injury and lipid peroxidation. In the present work, garlic administration with $HgCl_2$ better protected the liver from damage produced in DNA and from alterations in than the kidney. Garlic plus $HgCl_2$-treated animals had fewer diploid and aneuploid cells and higher proliferation index followed by a decrease in DNA density in the kidney tissue compared to $HgCl_2$-treated group. Moreover, in the liver tissue, rearrangement of diploid cells, no aneuploid cells and high proliferation index were noticed as well as an increase in DNA content. These results may be attributed to the antioxidant activity of the garlic and its organosulfur constituents. In conclusion, the findings of the present work indicated that exposure to $HgCl_2$ induced severe biochemical and histochemical changes in the liver and kidney. Both garlic and selenium had a protective effect against $HgCl_2$ toxicity. Moreover, garlic was found to be more effective than selenium. This protection may be due to the free radical scavenger effect of these antioxidants and/or their enhancing effect on the antioxidant capacity of the body.

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