Protective Role of Diallyl Disulphide Compound (From Garlic Extract) Against Mercuric Chloride - Induced Genotoxicity and Cytotoxicity in Albino Rats

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Abstract

Mercury compounds are widely used in several industries. Such compounds can find their way to the environment causing its pollution. Mercury is considered as a powerful carcinogenic agent. On the other side, the garlic extract is characterized by having compounds of specific anticarcinogenic action. Therefore, the present study was conducted to investigate both the genotoxicity and cytotoxicity of mercuric chloride and to evaluate the therapeutic and/or prophylactic role of diallyl disulphide (DADS) on mercuric chloride-intoxicated rats. Experimental animals were divided into three main groups, keeping the 1st group as a healthy control. The 2nd group was a DADS post-treated one; receiving HgCl2 orally three times/week at a dose of 20 mg/kg bw for three weeks, then DADS three times/week at an oral dose of 80 mg/kg bw for three weeks. The 3rd group was a pre-treated one, which received DADS then HgCl2 at the same doses and the same periods mentioned in the 2nd group. The results showed that mercuric chloride has a mutagenic activity reflected in its highly significant effects on cell-cycle kinetics and frequency of chromosomal aberrations. Also, AST and ALT enzyme activities were highly significantly increased whereas ALP and AChE activities were highly significantly decreased in the serum of HgCl2-intoxicated rats. Moreover, while glucose and total cholesterol were increased after HgCl2 intoxication, total protein was decreased. These effects were much more inhibited in rats firstly treated with DADS then HgCl2 than in those treated with HgCl2 then DADS. The results reflected that DADS has a potential prophylactic activity against HgCl2 toxicity. This could be probably related to its strong antioxidant nature and Hg-binding activity.

Keywords: garlic extract, diallyl disulphide, anticarcinogenic effects, mercuric chloride, genotoxicity, cytotoxicity, albino rats

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Introduction

The Pollution, as a serious problem facing the world, has been mostly regarded. Industrialization has increased animal exposure to thousands of chemicals in environment. The most important one among these chemicals are the inorganic material such as lead compounds, mercury, cadmium, arsenic and asbestos, and particularly mercury that is deserved for special attention as potentially toxic elements causing many environmental diseases (1). The widespread use of mercury and its uncontrolled discharge into environment has resulted in increased levels of mercury in rivers and lakes, which introduces significant quantities of that metal into the body of many species of fishes (2), birds (3) and mammals (4).

Inorganic mercury is widely used in certain types of batteries (usually mercuric oxide) and continues to be an essential component of fluorescent light bulbs (5). Also, a mixed burden of Hg species (Hg vapor, inorganic mercury and methyl mercury) is assumed to be associated with human poisoning in gold mining areas (6). The Mercuric chloride is an inorganic compound that has been used in agriculture as fungicide, in medicine as a topical antiseptic and disinfectant, and in chemistry as a mediator in production of other mercury compounds. The Mercuric chloride was evaluated in toxicity and carcinogenicity studies because of its extensive use and its occurrence as environmental pollutant (7).

The effect of environmental agents entering the body depends on how they are metabolized and by which route they are excreted (8). The mechanisms of toxicity and, particularly, potential carcinogenicity of

inorganic mercury are still under debate. Results of mutagenicity and genotoxicity testing with mercury have been inconsistent. Mercury-induced DNA single-strand breaks at low concentrations in mammalian cells reported (9). Experimental studies conducted in human blood cultures showed that mercury had low effect on cell-cycle kinetics, but frequency of chromosomal aberrations and sister chromatid exchange were significantly high (10; 14). Also, many reports have shown the in vitro and in vivo exposure to mercury induced lipid peroxidation detected by increased thiobarbituric acid reactive substances (TBARS) in liver, kidney, brain and other tissues (15; 16; 16). These authors have linked lipid peroxidation and cellular toxicity directly to free radical liberation caused by inhibition of the cellular defense agents such as glutathione, superoxide dismutase and vitamin E (17; 18; 19).

In Addition, mercury in the form of water and fat-soluble salts corrodes the membranes of body and causes irreversible brain, liver and kidney damage (20). Moreover, modification of membrane thiol groups has been proved to be vital effectors producing tremendous cellular changes in permeability of water, ions, glucose and amino acids as well as inactivation of many enzymes (21, 22, 23) proposed that mercury induces its toxic influence by mercaptide formation.

Numerous scientific reports imply that vegetable intake may affect cancer incidence. In reviews of epidemiologic studies, there is convincing evidence that high consumption of certain vegetables reduces the risk of colorectal, stomach, lung and esophageal cancers; in addition, there is probable evidence for reducing the breast and bladder cancers (24). Also, in the ancient Egyptian and Indian systems of medicine, a number of plants and herbs have been indicated for amelioration of metal poisoning (25). However, these have not been scientifically updated, and their use is mainly restricted to observable recovery (26).

The Allium genus of vegetables includes garlic, onion, leeks, scallions, chives and shallots. These vegetables are characterized by a composition that is high in flavonols and organosulfur compounds. Furthermore, animal and in vitro experimental investigations have provided evidences that organosulfur compounds, present in high amount in Allium vegetables, account for their anticarcinogenic activity (27; 28; 29). Garlic (Allium sativum) is a common spicy flavouring agent used since ancient times. The traditional ethno-veterinary practitioners consider garlic as an excellent natural product that has immense therapeutic potential in many pathological conditions. It has been shown to posses many medical properties including bactericidal, hypolipidemic, hypocholesteromic and antineoplastic effects (30; 31). The bulb of garlic is used as antirheumatic and stimulant besides its use in conditions like paralysis, amnesia, tremor colicky pain and chronic fever (32). Fresh and grounded garlics have been shown to inhibit cancer, caused by polycyclic aromatic hydrocarbons and nitrosoamines (33) besides its role in affections of nervous system (27).

The protective effects of garlic has been attributed to presence of organosulphur compounds like diallyl sulphide (DAS), diallyl disulphide (DADS), ajoene, allixin, allyl mercaptans and allyl methyl sulphides (34; 35). The DADS is one of the major volatile degradative compounds of garlic. Many studies on animals showed its protective effects against chemically induced toxicity and carcinogenesis (36; 37; 38). Therefore, the present study was carried out to detect genotoxic and cytotoxic effects of HgCl2 on albino rats. In addition, the role of diallyl disulphide (DADS) in reducing and inhibiting the toxicity of mercury will be tested.

Materials and Methods

Chemicals

Mercuric chloride (purity of 99%), diallyl disulphide (80% as DADS and the remainder 20% is other allyl sulfides) and Colchicine were obtained from Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France). All other chemicals used in the study were analytical grade.

Animals

A total of 164 apparently healthy male albino rats (Rattus norvegicus) weighing about 100 -130 g were used in the present study. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. Animals were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. They were maintained in an air-conditioned animal house at a temperature of 27 °C, relative humidity of 57 ± 2 % and photo-cycle of 12:12 hours light and dark periods. Animals were fed on the standard commercial diet (ATMID Company, Egypt) and provided with tap water.

Experimental schedule

Rats were divided into three main groups: the 1st group was kept as a negative control and given DMSO (organic solvent) orally three times / week for

six weeks. The 2nd group was a post-treated one that received HgCl2 orally three times/week at a dose of 20 mg/kg bw for three weeks (39) then the DADS three times/week at an oral dose of 80 mg/kg bw (40) for three weeks. The 3rd group was a pre-treated one, which received the DADS then HgCl2 at the same doses and the same periods mentioned in the 2nd group. Ten rats were sacrificed weekly under diethyl ether anesthesia; four animals were used for cytogenetic assay and six rats were used for cytotoxic investigations.

Cytogenetic assay

The 2nd and 3rd main rat groups were divided each into six subgroups (G1 to G6) and (G7 to G12), respectively of four animals each based on time factor. In addition, two subgroups (G13 and G14) of four rats each served as negative control groups. Animals in G13 administered DMSO orally for three weeks (three times / week), while animals in G14 received saline solution.

In order to study metaphase cells, samples from bone-marrow cells of treated and control animals were prepared. The spindle inhibitor Colchicine (4mg/Kg) was injected intraperitoneally (i.p.) two hours before cervical dislocation. Bone-marrow smears of sacrificed rats were prepared according to protocol of (41). Slides were stained with Giemsa and the well spread metaphases were analyzed for chromosomal aberrations. Mitotic index and percentage of incidence of aberrant cells were analyzed. The percentage of suppressed aberrant cells was calculated according to (35) as follows:

$$100 - \left(\frac{\%\text{ of aberrant cells in groups G4-G6 and G10-G12}}{\%\text{ of aberrant cells in G3}} \right) \times 100$$

Cytotoxic assay

The collected blood samples were allowed to coagulate at room temperature and centrifuged at 3000 rpm for 30 min. The clear, non-haemolysed sera were quickly removed and kept at -20 °C for subsequent analysis.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to the method of (42) using reagent kits purchased from Randox Company (UK).

Alkaline phosphatase (ALP) activity was determined according to the method of (43) using reagent kits obtained from Bio-Merieux Chemical Company (France).

Acetylcholinesterase (AChE) activity was determined according to the method of (44) using reagent kits purchased from Bio-Merieux Chemical Company (France).

The total protein was determined according to Henry (45) using reagent kits purchased from Bio-Merieux Chemical Company (France).

Glucose concentration was determined according to the method of (46). using reagent kits purchased from Bio-Merieux Chemical Company (France).

The total cholesterol concentration was determined according to the method of (47) using reagent kits purchased from Spinreact Company (Spain).

Statistical analysis

For the cytogenetic assay, data was analyzed for mean values and standard error for all groups, which were subjected to statistical comparison using student-t-test ($P<0.05$ was considered significant).

For the cytotoxic assay, the results were expressed as mean $\pm$ S.D., and they were analyzed using one-way and two-way analysis of variance (ANOVA) followed by LSD computations to compare various groups with each other. F-values express the general effect between groups, and the effect of time, treatment and their interaction. The level of significance was expressed as $P>0.05$ for insignificantly different, while $P< 0.05$ was significantly different, whereas $P< 0.01$ and $P< 0.001$ were highly and very highly significantly different, respectively. Also, Pearson correlation coefficients were determined for the investigated parameters (48).

Results

Cytogenetic assay

The data recorded in Tables 1& 2 revealed that mercuric chloride injection (G1, G2 and G3) caused a high incidence of all types of chromosomal aberrations, including chromatid breakage, centromeric attenuation, centric fusion, end-to-end association, polyploidy and endomitosis. The mitotic index and percentage of aberrant cells were decreased by 30-46% and increased by 30-35%, respectively, compared to the control ($P<0.01$), indicating bone marrow cytotoxicity. However, the DADS alone (G7, G8 and G9) induced the lowest count of chromosomal aberrations significantly confirming its non-mutagenicity. Also, the DADS showed no cytotoxic effect, as there was no significant change in the mitotic index and incidence of aberrant cells compared to the control groups (G13 and G14).

Table 1: Curing and protecting effects of DADS pre- or post-treatment on mercuric chloride induced different types of chromosomal aberrations in albino rat bone-marrow cells.

Groups			Number of different types of structural aberrations					Number of different types of numerical aberrations		
			Chr. B.	Cen.Att	Cen.Fu.	E.E.ass	TSA	Polp.	Endom.	TNA
Post-treated (2nd group)	$HgCl_2$	G1	55 27.5%	7 3.5%	1 0.5%	9 4.5%	72 36.0%	2 1.0%	23 11.5%	25 12.5%
		G2	74 37.0%	10 5.0%	2 1.0%	12 6.0%	98 49.0%	3 1.5%	19 9.5%	22 11.0%
		G3	77 38.5%	13 6.5%	3 1.5%	5 2.5%	98 49.0%	3 1.5%	16 8.0%	19 9.5%
	DADS	G4	66 33.0%	14 7.0%	3 1.5%	11 5.5%	94 47.0%	2 1.0%	19 9.5%	21 10.5%
		G5	55 27.5%	7 3.5%	2 1.0%	7 3.5%	71 35.5%	2 1.0%	18 9.0%	20 10.0%
		G6	37 18.5%	7 3.5%	4 2.0%	5 2.5%	53 26.5%	3 1.5%	22 11.0%	25 12.5%
Pre -treated (3rd group)	DADS	G7	20 10.0%	8 4.0%	1 0.5%	2 1.0%	31 15.5%	3 1.5%	14 7.0%	17 8.5%
		G8	24 12.0%	7 3.5%	2 1.0%	1 0.5%	34 17.0%	2 1.0%	10 5.5%	12 6.0%
		G9	19 9.5%	5 2.5%	6 3.0%	1 0.5%	31 15.5%	1 0.5%	11 5.5%	12 6.0%
	$HgCl_2$	G10	30 15.0%	13 6.5%	4 2.0%	1 0.5%	48 24.0%	2 1.0%	17 8.5%	19 9.5%
		G11	34 17.0%	9 4.5%	5 2.5%	2 1.0%	50 25.0%	5 2.5%	14 7.0%	19 9.5%
		G12	39 19.5%	11 5.5%	5 2.5%	4 2.0%	59 29.5%	8 4.0%	12 6.0%	20 10.0%
Control groups		G13	23 11.5%	8 4.0%	1 0.5%	2 1.0%	34 17.0%	2 1.0%	16 8.0%	18 9.0%
		G14	17 8.5%	2 1.0%	1 0.5%	1 0.5%	21 10.5%	-	4 2.0%	4 2.0%

Number of metaphase cells analyzed per animal group = 200 cells

Chr.B. = chromatid breakage, Cen. Att= centromeric attenuation, Cen.Fu.= centric fusion, E.E.ass= end to end association, TSA= total structural aberrations, Polp.= polyploidy, Endom.= endomitosis, TNA= total numerical aberrations, G13 = DMSO control group, G14 = saline control group

Moreover, DADS administration either in the post- or pre-treated groups decreased the rates of clastogenic changes in G4-G6 and G10-G12, respectively (Tables 1& 2). Also, all types of chromosomal aberrations induced by mercuric chloride including chromatid breakages and other multiple damages were found to be reduced by DADS treatment. However, the status of mitotic index and percentage of aberrant cells were found to be increased by 5.5-22% and decreased by 10-16%, respectively over mercuric chloride-treated groups (G1, G2 and G3) indicative of DADS anticytotoxicity towards mercuric chloride.

On the other hand, the obvious anticytotoxic effect of DADS was observed in DADS pre-treated groups (G10, G11 and G12) where the different types of aberrations were highly significantly reduced in comparison with the records of post-treated groups (G4, G5 and G6) (Tables 1, 2 & 3).

Statistical analysis indicated significant differences between such data. All of the data recorded were highly significant in comparison with the control groups (G13 and G14) (Table 3).

The percentage of DADS suppressive effect from rats pre-treated with DADS and post-treated with mercuric chloride (groups G10, G11 and G12) was 34.043%, 29.787% and 31.915%, respectively, while in G4, G5 and G6, a negative suppressive effect was observed in G4 and low suppressive effects (21.276% and 25.532%) were recorded in G5 and G6, respectively.

Table 2: Preventive and suppressive effects of DADS pre- or post-treatment on mercuric chloride induced changes in number of chromosomal aberrations /cell, incidence of aberrant cells and mitotic index in albino rat bone-marrow cells.

Groups		Number of* aberrations/cell	Incidence of* aberrant cells	Mitotic index*	Suppressive (%)
Post-treated (2nd group) HgCl2	G1	0.485 + 0.020	42.25 + 1.931	61.57 + 1.396	--
	G2	0.600 + 0.036	47.00 + 2.886	56.03 + 0.387	--
	G3	0.585 + 0.033	47.00 + 1.730	46.16 + 1.450	--
Post-treated (2nd group) DADS	G4	0.570 + 0.041	52.00 + 3.559	51.74 + 0.858	--
	G5	0.455 + 0.021	37.50 + 0.500	57.30 + 0.718	21.276
	G6	0.390 + 0.019	35.00 + 1.290	57.49 + 0.547	25.532
Pre - treated (3rd group) DADS	G7	0.240 + 0.018	22.50 + 1.258	86.58 + 0.793	--
	G8	0.230 + 0.025	21.50 + 2.872	82.27 + 0.689	--
	G9	0.215 + 0.051	19.50 + 0.957	80.27 + 0.635	--
Pre - treated (3rd group) HgCl2	G10	0.335 + 0.025	31.00 + 1.732	68.79 + 0.788	34.043
	G11	0.345 + 0.022	33.50 + 1.500	60.18 + 0.601	29.787
	G12	0.395 + 0.017	32.75 + 0.750	64.80 + 0.813	31.915
Control groups	G13	0.260 + 0.014	24.00 + 0.816	73.84 + 0.469	--
	G14	0.120 + 0.018	12.50 + 1.708	91.35 + 0.437	--

Number of metaphase cells analyzed per animal group = 200 cells
* values represent mean + SE of four animals.

Cytotoxic assay

The investigated serum enzyme activity of control, post-treated and pre-treated rats are displayed in Table 4. The results showed that both aspartate and alanine aminotransferases enzymes (AST&ALT) were obviovsly elevated following mercuric chloride intoxication throughout the 1st three weeks of experiment in the post-treated group, then they were gradually decreased by DADS administration along the rest of experimental periods, but still above their level in the control ones. In the pre-treated rats, the effect of HgCl2 on AST and ALT activities appeared to be inhibited compared to their activities following HgCl2 poisoning in the post-treated group.

Contrarily, alkaline phosphatase (ALP) activity exhibited a grave decrease after HgCl2 intoxication throughout the whole experimental periods, even after DADS administration compared to the control group. For the pre-treated rats, activity of ALP enzyme appeared to be reduced by DADS treatment compared to the control values then it was further decreased after HgCl2 intoxication.

Table 3. Significancy between data of mitotic index (MI) and incidence of aberrant cells % (IAC) occurred as a result of post or pre-treating the albino rats with DADS and mercuric chloride by using student-t-test.

Groups	G1		G2		G3		G4		G5		G6		G7		G8		G9		G10		G11		G12		G13		G14	
	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC	MI	IAC
G1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
G2	++	++	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
G3	++	++	ns	ns	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
G4	++	++	++	++	++	+	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
G5	++	++	++	++	++	++	++	++	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
G6	++	++	++	++	++	++	++	++	ns	ns	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
G7	++	++	++	++	++	++	++	++	++	++	++	++	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
G8	++	++	++	++	++	++	++	++	++	++	++	++	ns	ns	--	--	--	--	--	--	--	--	--	--	--	--	--	--
G9	++	++	++	++	++	+	++	++	++	++	++	++	+	+	+	ns	--	--	--	--	--	--	--	--	--	--	--	--
G10	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	--	--	--	--	--	--	--	--	--	--
G11	++	++	++	++	++	++	++	++	++	++	++	ns	++	++	++	++	++	++	++	ns	--	--	--	--	--	--	--	--
G12	++	+	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++	++	ns	ns	ns	--	--	--	--	--	--
G13	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	+	++	+	++	++	++	++	++	++	--	--	--	--
G14	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	--	--

N.B. ns = not significant
+ = significant (P<0.05)

Table 4. Effect of DADS on serum enzymes values in male albino rats intoxicated with mercuric chloride.

Group / Parameter		W1	W2	W3	W4	W5	W6
Control group		83.70 ± 3.76 j	86.44 ± 3.73 ij	90.86 ± 3.34 ghij	88.87 ± 7.60 hij	87.08 ± 4.24 ij	89.31 ± 5.44 hij
Post-treated group	**AST U/L**	140.00 ± 15.11 cd	212.78 ± 23.59 a	164.83 ± 13.48 b	130.45 ± 8.59 d	116.27 ± 10.98 e	105.01 ± 871 ef
Pre-treated group		96.44 ± 8.09 fghi	102.98 ± 9.48 fg	99.44 ± 5.03 fgh	130.01 ± 10.14 d	145.48 ± 12.03 c	103.66 ± 10.16 f
Control group		26.20 ± 1.36 j	26.89 ± 1.18 j	27.74 ± 1.40 j	27.30 ± 1.50 j	26.92 ± 1.11 j	27.41 ± 1.13 j
Post-treated group	**ALT U/L**	48.43 ± 6.12 d	92.76 ± 8.10 a	67.11 ± 8.43 b	44.87 ± 5.34 de	39.69 ± 2.59 fg	34.49 ± 2.54 hi
Pre-treated group		27.98 ± 1.66 j	29.84 ± 1.78 ij	28.21 ± 1.14 j	42.98 ± 5.17 ef	58.98 ± 7.07 c	36.56 ± 3.46 gh
Control group		574.49 ± 29.40 a	575.48 ± 21.14 a	588.51 ± 18.74 a	599.72 ± 20.86 a	584.02 ± 18.65 a	585.43 ± 27.69 a
Post-treated group	**ALP IU/L**	413.81 ± 34.43 bc	377.14 ± 51.01 def	295.30 ± 25.82 h	417.83 ± 27.49 bc	322.08 ± 27.07 gh	368.36 ± 12.61 ef
Pre-treated group		581.80 ± 30.72 a	423.56 ± 19.31 bc	432.42 ± 19.90 b	405.67 ± 25.77 bcd	394.24 ± 18.25 cde	352.63 ± 21.07 fg
Control group		1270.73 ± 68.66 cd	1265.98 ± 60.57 cd	1205.51 ± 56.35 de	1187.42 ± 58.58 e	1235.57 ± 47.33 de	1180.80 ± 35.90 e
Post-treated group	**AChE U/L**	1571.84 ± 99.72 a	1104.84 ± 67.54 f	956.89 ± 46.69 g	1324.40 ± 22.34 c	880.00 ± 36.34 h	1268.06 ± 53.32 cd
Pre-treated group		1473.14 ± 51.82 b	981.91 ± 60.45 g	1177.03 ± 45.86 e	1491.40 ± 59.69 b	1172.67 ± 46.21 e	1097.34 ± 31.84 f

Data are expressed as mean ± S.D. Means which are not significantly different have the same symbol (s). & W= week

Table 5. Effect of DADS on serum total protein, glucose and total cholesterol concentrations in male albino rats intoxicated with mercuric chloride.

Group / Parameter		W1	W2	W3	W4	W5	W6
Control group		6.12 ± 0.20 bcd	6.20 ± 0.15 abcd	6.29 ± 0.22 abcd	6.34 ± 0.15 abcd	6.33 ± 0.16 abcd	6.44 ± 0.17 ab
Post-treated group	**TP gm/dl**	4.81 ± 0.74 f	5.45 ± 0.27 e	5.18 ± 0.40 ef	5.49 ± 0.47 e	5.43 ± 0.22 e	6.18 ± 0.37 bcd
Pre-treated group		6.23 ± 0.22 abcd	6.59 ± 0.49 a	6.00 ± 0.17 cd	5.30 ± 0.33 e	5.96 ± 0.24 d	6.37 ± 0.46 abc
Control group		78.61 ± 4.23 ghi	80.24 ± 2.29 fghi	82.21 ± 4.02 defghi	81.24 ± 3.06 efghi	83.36 ± 3.26 cdefgh	84.88 ± 3.20 cdefg
Post-treated group	**Glucose mg/dl**	99.92 ± 6.02 b	121.86 ± 10.26 a	82.01 ± 6.73 defghi	78.01 ± 7.13 hi	89.65 ± 6.36 c	87.39 ± 7.56 cde
Pre-treated group		76.45 ± 3.27 i	78.65 ± 4.74 ghi	81.85 ± 3.65 defghi	83.67 ± 5.19 cdefgh	88.05 ± 5.30 cd	85.57 ± 3.32 cdef
Control group		76.43 ± 3.66 hi	77.05 ± 3.49 hi	80.14 ± 3.34 ghi	78.96 ± 2.98 ghi	82.94 ± 3.72 efg	83.46 ± 4.34 efg
Post-treated group	**T.Ch mg/dl**	88.44 ± 5.97 de	139.42 ± 8.53 a	102.62 ± 5.73 bc	79.02 ± 6.05 ghi	81.64 ± 4.68 fgh	107.78 ± 3.77 b
Pre-treated group		75.05 ± 1.44 i	76.96 ± 2.59 hi	78.81 ± 3.88 ghi	85.98 ± 6.44 def	96.99 ± 5.87 c	89.40 ± 5.94 d

Data are expressed as mean ± S.D. Means which are not significantly different have the same symbol (s). & W= week

Table 6. Analysis of variance (ANOVA) for the effect of DADS on serum biochemical values of male albino rats intoxicated with mercuric chloride.

	S.V.	d. f.	F cal.						
			AST	ALT	ALP	ChE	TP	Glucose	T.Ch
General effect	Between groups	17	64.85***	106.97***	99.19***	63.94***	13.72***	٢٣.٠٧***	63.57***
	Within group	90							
	Total	107							
Two-way analysis of variance	Control – post-treated group								
	Treatment	1	511.42***	723.13***	1110.83***	8.64 **	116.76***	69.16***	295.50***
	Time	5	39.35***	75.75***	9.55***	62.63***	7.95***	19.96***	59.53***
	Treatment – time interaction	5	40.74 ***	75.76***	9.71***	57.80***	3.28*	26.06***	67.42***
	Error	60							
	Total								
	Control – pre-treated group								
	Treatment	1	204.36***	218.53***	0.26	0.41	10.93**	0.47	16.48***
	Time	5	21.98***	49.77***	0.93	46.01***	7.35***	8.05***	20.74***
	Treatment – time interaction	5	21.10***	49.44***	0.96	48.20***	9.77***	1.32	6.35***
	Error	60							
	Total								
LSD 1%			16.365	6.710	41.783	87.934	0.540	8.511	7.735
LSD 5%			12.152	4.983	31.026	65.295	0.401	6.320	5.744

Table 7. Pearson correlation coefficients for the effect of DADS on serum biochemical values of male albino rats intoxicated with mercuric chloride (2-tailed).

Parameter	AST	ALT	ALP	ChE	TP	Glucose	T.Ch
AST							
ALT	0.989**						
Sig.	0.000						
ALP	-0.660**	-.626**					
Sig.	0.003	0.005					
ChE	-0.126	-0.185	0.361				
Sig.	0.618	0.463	0.141				
TP	-0.701**	-0.640**	0.583*	-0.206			
Sig.	0.001	0.004	0.011	0.411			
Glucose	0.760**	0.770**	-0.372	-0.056	-0.460		
Sig.	0.000	0.000	0.128	0.825	0.054		
T.Ch	0.824**	0.860**	-0.515*	-0.194	-0.359	0.837**	
Sig.	0.000	0.000	0.029	0.441	0.144	0.000	

** *Correlation is significant at the 0.01 level.*
* *Correlation is significant at the 0.05 level.*
N=18

Activity of acetylcholinesterase (AChE) showed a transient increase after one week of the experiment in the post-treated rats then it was decreased along the 2nd and 3rd weeks of experiment. Treatment with the DADS improved the AChE activity by the end of experiment. For the pre-treated rats, alteration in the AChE activity recorded in the post-treated rats following HgCl2 administration appeared to be depleted.

As indicated in Table 5, total protein (TP) was significantly decreased by HgCl2 intoxication, compared to the control level, in both post- and pre-treated rats, but with a lower effect in the pre-treated groups. Furthermore, behaviour of both glucose and total cholesterol concentration showed a similar pattern in both post- and pre-treated rats.

One-way ANOVA analysis of investigated parameters revealed that the general effect between groups was very highly significant throughout the experiment. Regarding two-way ANOVA test of control-post-treated effect, it was noticed that the effect of HgCl2 toxicity was very highly significant for all studied parameters, except for AChE where it was highly significant. On the other hand, while doing two-ways ANOVA test of control – pre-treated effect; HgCl2 reflected a very highly significant effect on AST, ALT and total cholesterol, whereas it was highly significant on TP, AChE and glucose (Table, 6). Pearson correlation coefficients (Table, 7) showed that AST was positively correlated with ALT, glucose and total cholesterol, whereas it was not correlated with ALP, AChE and TP.

Discussion

Efforts have been done over use of dietary constituents as therapeutic agents capable of controlling or minimizing carcinogenisty and genotoxicity of various natural and man-made compounds (49;14; 29). Bone-marrow cytogenetics is a useful short-term technique for identifying the substances of clastogenic and anticlastogenic activity and elucidating the mechanism of their action (50). The majority of mutagenic/carcinogenic compounds, such as organic or inorganic heavy metals, act by generating electrophilic mediators by microsomal enzymatic reactions causing mutations (51; 9).

The present study indicated that animals treated with mercuric chloride showed several-fold increase in the frequency of aberrant cells. This agrees with the previously reported ability of inorganic mercuric compounds to produce chromosomal aberrations (10; 11). significant increase in chromosomal abnormality and significant decrease in the mitotic index may be due to that the inorganic mercuric compounds generate Hg(2+) radicals, which increase intracellular amount of reactive oxygen species and glutathione depletion. Such compounds can exert clastogenic effects, especially by acting as spindle inhibitors, thereby causing c-anaphasis (abnormal mitosis) and consequently aneuploidy and/or polyploidy (12; 13; 9;14).

The present investigation also revealed the antimutagenic potential of diallyl disulfide (DADS) against chromosomal damage induced by the mercuric chloride. The DADS, as the most important

biological active compound of garlic (52), exhibited in vivo anticarcinogenic properties (53; 54) and protected against carcinogen-induced DNA strand breaks (55).

The ability of DADS as antimutagenic agent in animals pretreated and post-treated against mercuric chloride toxicity indicated a significant decrease in chromosomal abnormalities and a significant increase in the mitotic index in both treated groups. However, high suppressive effects were observed in the DADS pretreated group (G10, G11 and G12) indicative of greater detoxification ability in such group. Earlier studies conducted with the allyl group of garlic constituents (presence of sulf-hydryl compounds) were implicated for its antimutagenic and anticarcinogenic effects (56; 57). The mechanism of protection of garlic constituent in vivo depends on potentially scavenging the toxic and mutagenic electrophiles and free radicals and modification of phase II enzymes and I profile that enhances detoxification mechanisms (54).

The cytogenetic study concludes that the DADS compounds exert antimutagenic and antineoplastic effects hilt varied potency, dependent on sampling time, since it is capable of inhibiting both cytotoxic and cytogenetic damage (chromosomal aberrations) caused by inorganic mercury.

Toxic hazards of heavy metals in relation to enzyme activities of different organisms are the main topics in toxicology. The results of the present study demonstrated that serum AST and ALT activities were highly significantly increased whereas ALP and AChE were highly significantly decreased, except a transient increase for AChE that was seen after one week of HgCl2 administration in both post- and pre-treated groups, in mercuric chloride intoxicated rats.

These results are in accordance with those of (16) who manifested that the rats receiving repeated oral doses of Hg (0.5 μmol/ml) as mercuric chloride for five consecutive days showed a significant increase in both serum ALT and AST activities and a significant decrease in both serum ALP and AChE activities. Also, (58) showed high concentrations of AST and ALT, and a diminution in ALP in sera of Aphanius dispar Rüpp (Teleostei) of the Red Sea following acute and long-term mercury exposure.

Since transaminase enzymes (AST and ALT) have their function greatest concentration within the cell, changes in serum concentration of these enzymes occur essentially as a result of some processes involving the body tissues (59,60) suggested that enzyme levels are sensitive indicators of tissue damage, since these enzymes are liberated from cells even when the magnitude of lesions is not sufficient for morphological detection. This assumption was supported by the opinion of (61) who mentioned that the elevation of serum enzymatic activity is associated with hepatic disease and necrosis in other tissues. The elevation in AST and ALT enzymes may be attributed to the accumulation of Hg in the liver tissues (62; 63 5 64) reported that Hg has a destructive cytotoxic effect on hepatocytes and other tissues. The increase in serum enzymatic activities might be due to decrease in metabolic activity, disruption of enzyme system by blocking the active sites (65) or/and increased hepatic production as a result of toxicity.

The decrease in ALP activity may be ascribed to decreased synthesis of this enzyme as evident from decreased protein concentration or deactivation of enzyme as reported by (66). Also, it may be attributed to slow but constant inhibition of this enzyme by toxicity of Hg2+ (58).

Studies carried by chang (67) and Gallagher and Lee (68) on rats indicated that mercury has neurotoxic effects reflected in its ability to penetrate and damage blood-brain barrier system. Also, electron-microscopic histochemical analysis revealed that, intracellularly, mercury was bound to the membranous organelles such as mitochondria, endoplasmic reticulum, Golgi complex and nuclear envelopes. Only very minimal amounts of mercury were found within the nucleus. In addition, biochemical and cytochemical studies indicated that drastic reduction of neuronal RNA and protein synthesis occurred in mercury-intoxicated animals. Reduction of protein synthesis was believed to elicit eventual cell death in these neurons. Also, they reported a disturbance of enzymatic systems in the brain of mercury-poisoned animals. The decrease in AChE in the HgCl2-intoxicated rats in this study may be explained on the basis of its effect on the reduction of neuronal RNA and protein synthesis in mercuric toxicity, beside its inhibitory effects on neurotransmitter high-affinity transport and release mechanisms (69).

While glucose and total cholesterol were increased after HgCl2 intoxication, total protein was decreased. significant alteration in carbohydrate, protein and lipid metabolism observed in the serum of rats exposed to metal suggests increasing energy demand to cope with mercury stress (70).

Lachapelle et al. (71) showed that the mercury affects the liver-specific functions such as albumin production, possibly through interference with ribosomal function. This may be extrapolated to explain the lowered TP production in serum in the present study. Ultrastructural analysis for rat

hepatocyte cultures exposed to increasing HgCl2 concentrations showed that hepatocytes treated with 5μM HgCl2 undergo the drastic morphological changes such as the decreased number of ribosomes associated with the rough endoplasmic reticulum, and the disappearance of latter organelle and proliferation of smooth endoplasmic reticulum (71).

Also, the mercury has long been known as a binder of proteins forming strong mercaptide bonds with sulfhydryl groups and other anionic protein chains resulting in denaturation and deactivation of protein molecules (72; Sager et al., 1984). These poisoned enzyme systems caused the inhibition of protein synthesis, resulting in a decreased rate of cell growth and development by reduced energy supply in a sub-cellular mechanism of enzymes (73; 74).

The increased glucose concentration following mercuric intoxication was supported by the results obtained by Bleau et al. (75) who showed a significant increase in plasma glucose levels and a decrease in liver glycogen reserves in juvenile rainbow trout (Oncorhynchus mykiss) exposed to mercurial compounds (HgCl2 and CH3HgCl). Also, they explained that the mercury stimulates the pituitary-interrenal and the pituitary-thyroid axes and modifies carbohydrate metabolism. Also, this may explain the present hyperglycemia on the basis of accelerated glycogenolysis by catecholamines, which are liberated from the adrenal medulla or formed as a result of acetyl choline accumulation resulting from the decreased acetylcholinesterase activity observed in this study. In addition, (16) and (63) showed that LDH activity was increased in sera of rats exposed to mercuric compounds. The elevated LDH (glycolytic enzyme) may be due to increased need for energy for metabolic process, and activation of gluconeogenic process during mercuric chloride intoxication.

Since mercuric compounds have a destructive cytotoxic effects on many tissue organs (5; 64), the hypercholesteromic effect of HgCl2 in the present study may be due to its role in inducing hyperplasia of bile duct and distension of gall bladder (76). Also, the potential carcinogenicity of inorganic mercury and its ability to induce tumors may explain the elevated cholesterol level based on the findings of Redgrave et al. (77) who showed that there is a hyperlipidemia in tumor-bearing rats resulted from an increased influx of hepatic lipids into the plasma and reduced rates of their removal from the blood compared to normal rats.

DADS administration with HgCl2, either in the post- or pre-treated groups, partially or totally alleviated the toxic effects of mercuric chloride on the investigated parameters. The inhibitory effects of DADS against the toxicity of HgCl2 may be related to presence of sulf hydryl groups, which may chelate with HgCl2 instead of enzymes (39). Also, as a very potent antioxidant, the DADS may provide protection against increasing free radical formation via inhibition of enzymes which make the HgCl2, responsible for increased DNA damage (74; 78).

The hypo-cholesterolemic effects of DADS is in accordance with the results of Adoga (79), Bordia et al. (80) and Hafez (81) who attributed that to the increase in cholesterol estrification which may lead to a better transport and utilization of lipids (82), and to the inhibition of hepatic cholesterol biosynthesis possibly via inhibition of hydroxyl methylglutaryl-COA reductase (83). Also, Hafez (81) showed that garlic has a hypoglycemic activity (as in the present study) and attributed that to increase in serum insulin (84) and increases cellular utilization of glucose (85).

It is concluded that the pre-treatment administration of DADS was more potent than post-treatment DADS in the amelioration of Hg toxicity.

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