

Pretreatment with diallylsulphide modulates mercury-induced neurotoxicity in male rats

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Many studies have reported on the toxicity and related oxidative stress of mercury. Antioxidants play an important role in counteracting metal-induced neurotoxicity under *in vivo* conditions. In this study, the effect of diallylsulphide (DAS) was evaluated on mercuric chloride induced activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione content in brains of rats. Pretreatment of rats with DAS in the Hg-treated group also inhibited an increase in lipid peroxidation and elevated acetyl cholinesterase and glutathione content. Activities of antioxidant enzymes were also restored concomitantly when compared to the control rats after DAS administration. DAS also caused a decrease in tumor necrosis factor- α level which was higher in HgCl₂-treated group. The results indicate that DAS augments antioxidant defense with anti-inflammatory response against HgCl₂-induced neurotoxicity. The increased level of antioxidant enzymes enhances the antioxidant potential of the organ to reduce oxidative stress.

Key words: diallylsulphide, metal toxicity, neurotoxicity, antioxidants

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INTRODUCTION

Heavy metal pollution has increased considerably worldwide as a result of high industrial activities. Mercury (Hg) contamination of the environment has received considerable attention because of its inherent toxicity to living forms (Liu & Lewis, 2014; Grandjean & Landrigan, 2014). Exposure to mercury may occur primarily by ingestion, inhalation and through the food chain. Mercurial compounds, including organic and inorganic forms, exhibit a variety of toxicological effects, including neurotoxicity, hepatotoxicity, nephrotoxicity and gastrointestinal toxicity with ulceration and hemorrhage (LeBel *et al.*, 1992). Mercury produces strong inhibition of a large number of enzymes that have functional sulfhydryl (SH) groups. Mercury might exert its influence by combining with the SH group of the enzyme leading to conformational changes and consequent inactivation.

Past studies have already documented the deleterious effects of heavy metal toxins in humans which may induce oxidative stress, lipid peroxidation, and may promote the decline in cognitive performance (Taber & Hurley, 2008; Lucena *et al.*, 2007; Liu *et al.*, 2006; Farina *et al.*, 2005). Lead and mercury exposure, air pollution, and organic compounds all have the potential to damage brain functioning, yet remain not understudied. The mechanism by which Hg induces neurological damage is still unclear, while its ability to react with and deplete

sulfhydryl groups as well as to disrupt cell cycle progression and/or induce apoptosis in several tissues is well recognized (Sutton & Tchounwou, 2006). Moreover, Hg-induced neurotoxicity is known to be mediated by reactive oxygen species (ROS) in different models (Mieiro *et al.*, 2011) by altering Na⁺/K⁺-ATPase activity and mitochondrial function. A brain imaging study found that the areas of the brain most vulnerable to the toxic effects of mercury are the pre- and post-central gyri, and the temporal transverse gyrus including granule cells in the cerebellum are also susceptible to harm (Taber & Hurley, 2008).

Recently, attention has been focused on the protective function of dietary antioxidants against harmful effects associated with heavy metal exposure. Some studies have focused their efforts on the protective effects of plants or natural compounds on various neuropathological conditions. Particularly important is the fact that it has been evidenced that plants/natural compounds are able to counteract metal-induced neurotoxicity under *in vivo* conditions (Gupta & Flora, 2006; Xu *et al.*, 2005). Diallyl trisulphide, a naturally occurring organic sulphur compound derived from *Allium* vegetables, is shown to reduce the risk of cardiovascular disease and diabetes, to stimulate the immune system, to protect against infections and has anti-aging as well as anticancer effects (Pinto & Rivlin, 2001). Initial evidence for the anticancer effect of *Allium* constituents is provided by epidemiological studies (Rivlin, 2001; Pinto & Rivlin, 2001). Antioxidants have been shown to have protective potential in ameliorating metal induced injury either by a metal-chelating activity or by increasing the antioxidant enzyme activity (Moraes-Silva *et al.*, 2014; Li *et al.*, 2013; Chiu *et al.*, 2013; Ansar & Iqbal, 2013; Ansar, 2013; Yang *et al.*, 2012; Jindal *et al.*, 2011; Rao *et al.*, 2010; Simoes Pires *et al.*, 2014; Chai *et al.*, 2014). Therefore, the aim of the present study was to study a possible mitigating effect of DAS against acute HgCl₂ neurotoxicity in rats based on oxidative stress and inflammation induction in rats.

MATERIALS AND METHODS

Maintenance of animals. Male Westar rats weighing approximately 180–200 g were procured. The animals were acclimatized for 14 days prior to experiments. The institutional ethics committee approved the experimental

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Abbreviations: DAS, diallylsulphide; CAT, catalase; SOD, superoxide dismutase; AChE, acetylcholinesterase; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione S-transferase; LPO, lipids peroxidation; ROS, reactive oxygen species, TNF- α , tumor necrosis factor- α .

Table 1. Effect of DAS on Hg-induced antioxidant status of rat brain (mean \pm S.E.M., n=6)

Parameters	Experimental Groups			
	Control (I)	HgCl ₂ (II)	DAS (III)	DAS + HgCl ₂ (IV)
SOD (IU/mg prot)	312.73 \pm 35.60	225.23 \pm 23.49*	315.93 \pm 21.45	289.52 \pm 33.40**
CAT (IU/mg prot)	0.38 \pm 0.091	0.25 \pm 0.015*	0.37 \pm 0.057	0.32 \pm 0.055**
GPx (U/mg prot)	0.35 \pm 0.056	0.22 \pm 0.018*	0.31 \pm 0.041	0.28 \pm 0.036**

* P <0.05 compared to control group, ** P <0.05 compared to Hg-exposed group

protocols. All the animals used in this study were placed in cages in an air conditioned room maintained at 12 h light /dark schedule.

Experimental protocol. Animal treatments: Different groups of animals were used to study the effects of DAS on mercury-induced oxidative stress. Twenty-four rats were randomly divided into 4 groups (6 rats in each group). Group I received saline injection intraperitoneally (0.85% NaCl) at a dose of 10 ml/kg body weight. Group II received a single intraperitoneal injection of mercuric chloride at a dose of 50 mg/kg body weight. Groups III and IV received pretreatment with DAS once a day for a period of one week, administered through gavage at a dose of 200 mg/kg body weight. Twenty four hours after the last treatment with DAS, group IV animals also received a single intraperitoneal injection of HgCl₂ at a dose level of 50 mg/kg body weight. After 24 h of last administration of HgCl₂, the animals were euthanized under mild ether anesthesia.

Brains of rats were carefully removed, washed in ice-cold 50 mM Tris/HCl, pH 7.4 and homogenized immediately to give 10% (w/v) homogenate in ice-cold medium that contained 50 mM Tris/HCl, pH 7.4. The homogenates were centrifuged at 3000 rpm for 10 min at 4°C. The supernatants were used for the various biochemical determinations. The total protein content of the homogenized brain was determined by the method of Lowry and coworkers (1951) using bovine serum albumin as a standard.

Oxidative stress markers/enzymatic antioxidant status: Brain homogenates were used to determine lipid peroxidation (LPO) by reaction of thiobarbituric acid (Ohkawa *et al.*, 1979). Glutathione (GSH) and acetylcholinesterase activity were assayed by the methods of Ellman (Ellman, 1959; Ellman *et al.*, 1961). For enzymatic antioxidant status, brain homogenates were used for the determination of superoxide dismutase activity (SOD) according to Nishikimi *et al.* (1972), catalase activity (CAT) (Aebi, 1984), and glutathione peroxidase activity (GPx) according to Paglia & Valentine (1967).

TNF- α level determination by ELISA: Brain homogenates were prepared in lysis buffer. Brain lysates were adjusted to equal protein concentrations and analyzed for TNF- α levels with the ELISA kit (Biosource Inc., USA), according to the manufacturer's instructions. The concentrations of TNF- α in brain lysates were calculated with the help of the calibration curve generated by using known amounts of standards.

Statistical analysis. Results were expressed as the mean \pm standard error of the mean (S.E.M.). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the Statistical Package for the Social Sciences (SPSS version 17.0). All p values are two-tailed and p <0.05 was considered significant for all statistical analyses in this study.

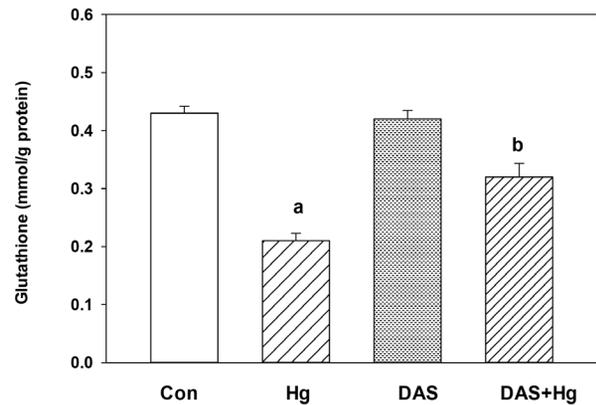


Figure 1. Effect of DAS on glutathione depletion in brain. Values are means \pm S.E.M.

(a) significant change at p <0.05 with respect to the control group; (b) significant change at p <0.05 with respect to the Hg group.

RESULTS

DAS mitigated Hg-induced neurotoxicity by modulating the antioxidant defense system

Neurotoxic effect of HgCl₂ in rats was examined by measuring activities of SOD, CAT, and GPx enzymes. As shown in Table 1, HgCl₂ administration led to modulation of antioxidant enzymes relative to the control rats. HgCl₂ administration led to a decrease in SOD, CAT and GPx activities in the brain homogenates significantly (p <0.05) compared to the control. On the other hand DAS pretreatment elevated the activities of SOD, CAT, and GPx significantly (p <0.05) compared to the HgCl₂ treated group.

The HgCl₂-induced neuronal oxidative stress was indicated by a significant reduction (p <0.05) in GSH contents of brain tissue of HgCl₂-treated rats in comparison with the control group. This reduction in GSH contents was attenuated by pre-treatment with DAS as shown in Fig. 1.

DAS ameliorated oxidative stress in Hg-induced neurotoxicity

To examine the effect of Hg on oxidative stress markers, lipid peroxidation levels in brain homogenates of rats treated with HgCl₂ were measured and its levels are shown in Fig. 2. HgCl₂ administration caused a significant (p <0.05) increase in the levels of LPO in the brain compared to the control group. DAS-pretreated group caused a significant (p <0.05) reduction in LPO levels which were increased by HgCl₂ administration as compared to control.

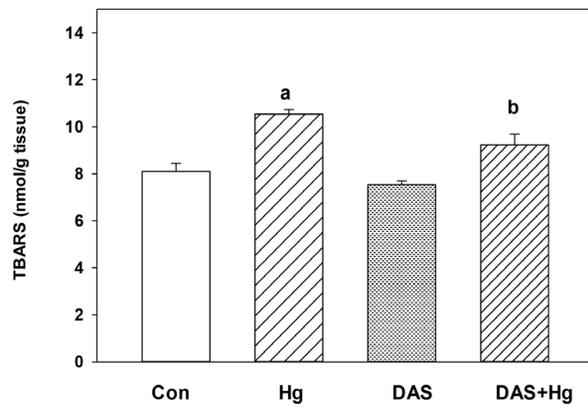


Figure 2. Effect of DAS on lipid peroxidation formation in rats treated with mercuric chloride. Values are mean \pm S.E.M. (a) Significant change at $p < 0.05$ with respect to the control group; (b) significant change at $p < 0.05$ with respect to the Hg group.

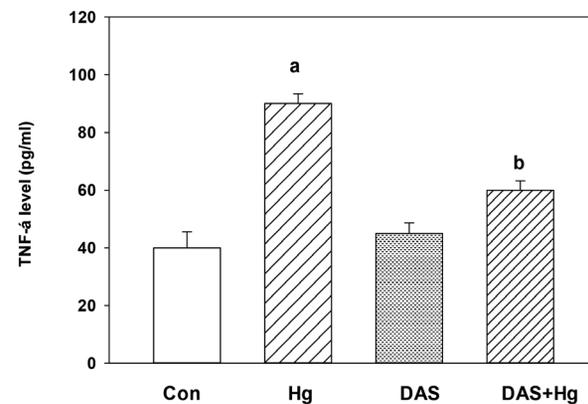


Figure 3. Effect of DAS on tumor necrosis factor- α level in rat brains. Values are means \pm S.E.M. (a) Significant change at $p < 0.05$ with respect to the control group; (b) significant change at $p < 0.05$ with respect to the Hg group.

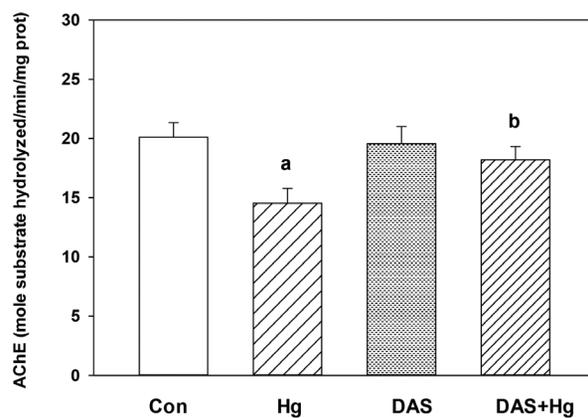


Figure 4. Effects of DAS on acetylcholine esterase (AChE) level in rat brains. Values are mean \pm S.E.M. (a) Significant change at $p < 0.05$ with respect to the control group; (b) significant change at $p < 0.05$ with respect to the Hg group.

DAS has anti-inflammatory effect in Hg-induced neurotoxicity

To study the proinflammatory effect of HgCl_2 in rats, $\text{TNF-}\alpha$ was measured in brain homogenate. HgCl_2 administration led to a significant elevation in $\text{TNF-}\alpha$ compared to the control. On the other hand, DAS pretreatment reduced the level of $\text{TNF-}\alpha$ significantly ($p < 0.05$) compared to the HgCl_2 treated rats as shown in Fig. 3

Effect of DAS on Hg-induced acetylcholine esterase (AChE) activity

The results indicated that HgCl_2 had a significant ($p < 0.05$) inhibitory effect on AChE activity in the brain, while there was no effect of DAS alone. The presence of DAS caused alleviation of the toxic effect of Hg on the activity of AChE as shown in Fig. 4.

DISCUSSION

Hg-induced neurotoxicity is a well-described phenomenon but there are still no effective treatments available for Hg poisoning. Hg-induced neurotoxicity has been considered to be either due to overproduction of ROS and/or by reducing the antioxidant defense system (Simoes Pires *et al.*, 2014; Liu *et al.*, 2006). The brain contains large amounts of polyunsaturated fatty acids and is particularly susceptible to free radical attack and, therefore, lipid peroxidation. Elimination of free radicals by treatment with antioxidants or free radical scavengers leads to a reduction in the toxicity induced by mercury exposure (Sener *et al.*, 2003; Kukreja & Hess, 1992).

The protective effects of garlic have been attributed to the presence of organosulphur compounds like diallyl sulphide (DAS), diallyl disulphide (DADS), ajoene, allixin, allyl mercaptans and allyl methyl sulphides (Shukla & Taneja, 2002). In DAS-treated rats no toxicity was seen in brain tissue. Also, DAS altered the increase in the activity of antioxidant enzymes. Glutathione S-transferase (GST) plays an important role in the detoxification of many environmental chemicals (Kramer *et al.*, 1988). The results presented here indicate that GST was markedly inhibited in the brain of animals treated with Hg. Inhibition of GST activity might potentiate the deleterious effects of many environmental toxicants and carcinogens. CAT is the enzyme responsible for dissipation of hydrogen peroxide formed during oxidative stress. It has been reported that CAT and SOD constitute a mutually supportive team of defense against ROS. The decreased activity of SOD in HgCl_2 -treated rats may be due to the enhanced lipid peroxidation or inactivation of the antioxidant enzymes. However, the significant decrease of levels of antioxidant enzymes like CAT and SOD was elevated nearly to normal levels by DAS supplementation in the HgCl_2 treated group.

Lipid peroxidation in the brain was detected by increased thiobarbituric acid reactive substances (TBARS) formation in rats treated with Hg. DAS treatment resulted in a reduction in the levels of TBARS. Previously it has been shown that exposure to mercury produced an increase in ROS in rat brains (Hussain *et al.*, 1997). The present results show elevated LPO level in the brain due to Hg exposure, indicating that Hg-induced neurotoxicity might be due to the overproduction of free radicals and lipid peroxidative products. The results presented here also corroborated with previous findings as reported by Othman and coworkers (2014) and Franco and coworkers (2010). The principal toxic effects of mercury involve

interactions with a large number of cellular processes including the formation of complexes with free thiols and protein thiol groups, which may lead to oxidative stress. Mercury produces oxidative damage *via* H₂O₂ generation thereby leading to lipid peroxidation (Alvarez & Storey, 1984).

Acetylcholinesterase (AChE) is an enzyme that is responsible for hydrolyzing and thus deactivating acetylcholine in the nervous system. It is a good indicator of sub-lethal toxicity of metals (Bocquene *et al.*, 1995). The presence of DAS caused alleviation of the toxic effect of Hg on the activity of AChE. The inhibition of brain AChE by heavy metals was also observed by several investigators (Huang *et al.*, 1997; Devi & Fingerman, 1995). Mercury produces strong inhibition of a large number of enzymes that have functional sulfhydryl (SH) groups. Mercury might exert its influence by combining with the SH group of the enzyme leading to conformational changes and consequent inactivation. In addition, changes and alterations in AChE as a result of tissue toxicity have also been suggested before (Adefegha & Oboh, 2012; Kim *et al.*, 2011; Kopecka-Pilarczyk, 2010; Xuereb *et al.*, 2009; Battisti *et al.*, 2009).

Many studies have shown that antioxidant enzyme activities are significantly decreased after Hg exposure *in vivo* (Vijayaprakash *et al.*, 2013; Branco *et al.*, 2012; Mieiro *et al.*, 2011). The present results, therefore, suggest that DAS attenuated Hg-induced oxidative stress by maintaining the balance of oxidant and antioxidant status. Therefore, DAS was able to antagonize the toxic effects of mercury.

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