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Influence of *s*-allyl cysteine against mercuric chloride induced nephrotoxicity in albino rats

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ABSTRACT

Mercury (II) is a highly toxic metal which induced oxidative stress in the living organism. In the present study we examined the effect of S-Allyl cysteine (SAC) against the mercuric chloride ($Hgcl_2$) intoxicated in albino rat model. The animals were treated with sub-leathal dose of mercuric chloride (1.23 mg/kg body wt) for 7 days. After scheduled treatetment the animals were decapitated and whole kidney tissue was used for the determination of biochemical and bioenzymological assays like lipid peroxidation (LPO) glutathione (GSH), Glutathione peroxidase (GPx), Catalase(CAT), and Superoxide dismutase (SOD) levels. During the mercuric chloride treatment the level of LPO content was significantly increased and simultaneously the level of reduced glutathione (GSH), Glutathione peroxidase (GPx), Catalase (CAT), and Superoxide dismutase (SOD) activities were decreased. The results revealed that $HgCl_2$ induced oxidative stress and cell damage. During the recovery period, S-Allyl cysteine (5 mg/kg body wt) administrated for another 7 days on mercury intoxicated rat kidney tissue showed the decreased the level of LPO content and also restore the antioxidant level. The result suggested that $HgCl_2$ mainly induced oxidative cell damage in kidney and administration of S-Allyl Cysteine on mercury intoxicated animal can get protection with its antioxidant effects.

Keywords: Mercuric Chloride, S-Allyl Cysteine, LPO, GSH, GPx, CAT, SOD etc.

INTRODUCTION

Health status of an animal is mainly depend upon their proper metabolic activities of its vital organs. Mercury toxicity is a significant clinical entity and as it is ubiquitous in the environment it poses serious risks to individual's health. Exposure to mercury promotes the reactive oxygen species (ROS) formation such as hydrogen peroxidises. Mercury induced oxidative stress to make an important contribution to molecular mechanism for organ injury [7, 22].

Inorganic mercury has a non-uniform distribution after absorption being accumulated mainly in kidneys causing acute renal failure. This mercury forms has great affinity for SH groups of endogenous biomolecules such as the enzyme s-aminolevution acid dehydrates which may contribute to its toxicity. Mercury exposure has been demonstrated to induce lipid peroxidation detected by increased thiobarbituric acid reactive substance in kidney and other tissues. Thus it is believed that antioxidants should be one of the important components of effective treatment for mercury poisoning [1, 17, 18],

Historically plants have been used as folk medicine against various types of disease. Remedies from plant sources (Indian system of medicine the Ayurveda) have proved to be very popular in primary health care in India for a long time. Chemical agents are avoided against heavy metal toxicity. The aged garlic extract compounds evoke antioxidant and protective responses under several experimental conditions. Among these constituents S-Allyl cysteine the most abundant organosulfer molecular with reported antioxidant properties exerts its protective actions through its ability to scavenger O_2 and H_2O_2 these preventing H_2O_2 endothelial cell damage and lipid peroxidation well as low density. The most abundant organosulfar compounds in garlic is known to possess a broad spectrum of antioxidant properties evidenced both under *in vivo* and *in vitro* condition [10, 11, 12, 15]

Despite its extensive medicinal uses limited knowledge is available regarding its role in heavy metal detoxification. However its anti oxidative potential against mercury induced oxidative stress remains unexplored. Therefore the purpose of this study was to delineate its role in mercuric chloride induced oxidative stress in rat kidney tissue [4, 7, 9].

EXPERIMENTAL SECTION

Normal adult male albino rats *Rattus norvegicus*, of the Wister strain weighing ranging from 200-250g were used in the experiments. All the animals were fed on a standard rat feed (Hindustan Lever Ltd, Mumbai) and water *ad Libitum.* Experimental protocol was approved by the intuitional Animals Ethics Committee (IAEC) of RMMCH, Annamalai University.

Group-I Untreated control provided standard diet and clear Water *ad labium* and observed for 30 days; Group-II Mercuric Chloride 1.23 mg/kg body weight, Oral administration (Dietary exposure) daily up to 30 days; Group- III Mercuric Chloride followed 1.23 mg/kg body wt.of Mercuric Chloride by S-Allyl Cysteine 5 mg/kg body wt.of SAC another 15 days; Group- IV S-Allyl Cysteine 5 mg/kg body wt Oral administration (Dietary exposure) daily up to 15 days.

Total weight of the diet was kept constant throughout the experimental period. After the scheduled treatments, the animals were sacrificed by cervical dislocation. The whole kidney tissue was isolated immediately from the animals in the cold room and then used for estimation of Lipid peroxidation, Reduced Glutathione, Glutathione peroxidase, Catalase, and Superoxide dismutase [2, 8, 14, 16, 21]

Statistical significance was evaluated using ANOVA followed by Duncan multiple range test (DMRT) [5].

RESULTS

The present work showed that the level of LPO content was significantly increased and simultaneously GSH, GPx, CAT, SOD activities were significantly decreased in mercury intoxicated kidney tissue. During the recovery period, the level of decreased antioxidant level was increased to reach near normal level. These results suggested that the mercury toxicant mainly induced nephrotoxicity and oxidative stress in animal models.

Table 1: Changes in the level of Lipid peroxidants and antioxidants in kidney tissue of experimental rats treated with mercuric chloride followed by *S-AllyleCysteine*

Parameters	Control	$HgCl_2$ Treatment	$HgCl_2$ + S-AllylCysteine	S-AllylCysteine
Protein	136.29±7.54	86.4±2.71	129.52±1.98	158.17±2.71
Amino acid	417.29±9.08	311.56±7.20	451.97±13.8	515.67±17.86
Sulfhydrol group	141.34±3.65	91.59±4.16	121.59±1.67	150.49±2.05
Lipid peroxidation	1.569±0.15	3.498±0.13	2.655±0.17	1.477±0.18
Reduced glutathione	64.56±2.27	32.54±0.54	39.04±4.01	66.35±4.89
Glutathione peroxidase	6.52±0.31	3.512±0.18	3.889±0.51	4.866±0.67
Catalase	36.21±0.01	18.75±0.26	40.94±2.09	54.39±0.95
Super oxide dismutase	4.694±0.24	3.179±0.13	4.530±0.61	4.768±0.57

DISCUSSION

Generally toxicants are promotes the formation of ROS by Fenton transition equation, such as hydrogen peroxides and enhances the subsequent iron and copper-induced production of lipid peroxides and the lightly reactive hydroxyl radical [1, 7].

It has been demonstrated that mercury (II) decreases the antioxidant systems and produces oxidative damages via H_2O_2 generation thereby leading to lipid peroxidation [9, 22]. All these possible mechanisms of mercuric chloride toxicity may lead to the formation of reactive oxygen species (ROS), as found in the present investigation. Therefore, an increase in the formation of ROS by $HgCl_2$ may induce membrane biochemical and functional alterations and thus induced kidney cell damage [1, 7]. As a free radical generating system, lipid peroxidation has been suggested to be closely related with Hg-induced tissue damage, and MDA is a good indicator of the degree of lipid peroxidation. In the present study we observed a significant increase in MDA content, during Hg toxicity, which is in agreement with the previous studies, where lipid peroxidation products were increased from 40% to 120% above basal values.

LPO alters the membrane structure and its integrity and are highly disruptive of mito radical quenching enzymes such as CAT, SOD, and perhaps the GSH, peroxides and thus resulting in cellular toxicity [19] Simultaneous administration of SAC decreased the formation of (LPO) Lipid per oxidation in the kidney tissue of mercury intoxicated animal because it possesses antioxidant activity. Thus this agent might provide medical benefit because the use of this agent could alleviate oxidative damage [7, 9, 17, 22].

GSH is a major thiol, which binds electrophilic molecular species and free radical intermediates. It plays a central role in the antioxidant defence system, metabolism and detoxification of exogenous and endogenous substances [17, 19]. Mercury has a high affinity on GSH and causes the irreversible exertion of upto two GSH tripeptides. The metal GSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. However, it depletes the GSH from the cell and they decrease the antioxidants potential. In fact, GSH serves as a primary line of cellular defense against Hg compounds. Released Hg ions form complexes with GSH and cysteine results in greater activity of the free Hg ions, disturbing GSH metabolism and damaging cells. As a result of binding of mercury to glutathione and subsequent elimination of intracellular glutathione, levels of GSH are lowered in the cell and decrease the antioxidant potential of the cell [1, 7, 9, 17, 19]

In the present experimental study indicated that exposure to Hg had altered the antioxidant defence system of rat. GSH plays a vital role in the protection of cells against oxidative stress. It can act as a non-enzymatic antioxidant taking part in cellular redox reactions, or it can be served as a cofactor or a coenzyme and involved in the enzymatic detoxification reactions for reactive oxygen species (ROS) [7]. Our present results showed both SOD and GPx increased in the kidney of Hg-exposed group comparing to the control. In the present experimental study clearly demonstrated that the exposure to Hg had greatly increased its body burden and also altered the antioxidant defense systems of rat [19]. The GSH and other thiols depletion will render cells more susceptible to oxidative damage, while elevated antioxidant enzymes activity will counteract it to a certain extent [13].

Glutathione act as both a carrier of mercury and an antioxidant and it has specific roles not only in protecting the body from mercury toxicity and also nullify the toxicity effect of mercury. Glutathione, specifically bind with mercury, forms a complex that prevents mercury from binding to cellular proteins and causing damage to both enzymes and tissue. Glutathione– mercury complexes also reduce intracellular damage by preventing mercury from entering tissue and cells [1, 4, 6, 18]

Administration of S-Allyl Cysteine (SAC) increased the level of GSH. The enhancement of GSH may be due to the presence of SAC which is a glutathione presence of SAC which is a glutathione preclusion from which GSH is formed [3, 12] Antioxidant enzymes such as superoxide dismutase (a Cu2+ dependent enzymes), catalase (in which NADPH protect it against inactivation by its substrate H_2O_2) and glutathione-S-transferase (glutathione related enzyme affected by ROS) play a major role in the intracellular defence against oxygen radical damage to aerobic cells. Superoxide dismutase catalyses the dismutation of superoxide anion the H_2O_2 which intern can be destroyed by catalase or glutathione peroxidise reactions. Catalase, which is present virtually in all mammalian cells, is responsible for the removal of H_2O_2. It plays an important role in the acquisition at tolerances to oxidative stress in adaptive response at cells. Glutathione peroxidase is the most important cellular antioxidant defence mechanising. It

catalytically removes H_2O_2 and Lipid hydroperoxidase from the cell thereby reducing the generation of the OH. In addition, GPx converts GSH to its oxidized product, GSH, disulfide (GSSQ). It has been important role in the recycling at GSH and thereby reducing free radical damage. Besides functioning in the removal at H_2O_2 from cells GPx also reduces peroxy nitrite anion. And it's having an additional catalytic function to lower the oxidative stress which is promoted by various toxicants. Both GSH and cellular antioxidant enzymes play an important role in $HgCI_2$ induced nephrotoxicity and kidney injury [9, 13, 22].

The decrease in activities of antioxidants enzymes (SOD, CAT, GPx) in kidney tissue in $HgCI_2$ treated rats may be due to the inhibition at these enzymes by H_2O_2. It is known that H_2O_2 involved in mercury induced acute renal injury [7, 21]. The inactivation or insufficient level of CAT and GPx mainly induced by $HgCl_2$ during the catalytic cycle involved in the kidney tissue of mercury intoxicated animal. SOD inhibition may be related to a covalent attachment of mercury ions to its reactive cystine residues which are involved in the detoxification of metals like mercury. Alternatively SOD inhibition might also been consequence of excess of residue which would effect on enzyme structure [22]. Mercury induced oxidative stress in the mercury intoxicated rats mainly due to the inhibition of antioxidant enzymes in kidney tissue. In the present experimental studies, we observed the level of lipidperoxidation and reduced glutathione along with kidney tissue damage with decreased level of SOD, CAT and GPx activities. In addition, these findings also indicate that free radicals generated by $HgCl_2$ exceeded endogenous antioxidant activity and induced tissue oxidative damag. The decrease in the level of CAT and GPx activities occurred probably as a defense response used against hydrogen peroxide generated by $HgCl_2$ [1, 4, 6].

Interestingly, in this experimental work, we also found depressed SOD activity in rat treated with $HgCl_2$ SOD inhibition may be related to a covalent attachment of mercury ions to the respective tissue cellular cysteine residues which are involved in the detoxification of metals like mercury. Alternatively, SOD inhibition might be also a consequence of an excess of reactive oxygen species, which would affect enzyme structure. SOD catalyzes superoxide anion radical dismutation into hydrogen peroxide. Therefore, regardless of the underlying mechanism, SOD inhibition could contribute to the enhanced oxidation observed in mercury-treated rats, since lipid peroxidation induced by $HgCl_2$ seems to be caused by increased levels of superoxide anion radical [7, 20, 21].

The elevated level of SOD, CAT and GPx by S-Allylcystein as compared to the $HgCl_2$ may have facilitated the conjugation reaction of xenobiotics metabolism and may have increased the availability of non-critical nucleophile for inactivation of electrophiles and therefore might be playing a major role in metalloprotection. Some of the active constituents of S-Allylcystein from garlic have been reported to possess a bioactive compound which is act as phytochelating substance to nullify the toxicity effect on various organisms. Reports suggested that SAC has been known to have free radical scavenging effect and it could be a potential therapeutic or modulating agent for oxidative damage induced disease [10, 22] Lin, *et al.* (2008) also reported that garlic is a potent free radical scavenger and antioxidant due to the presents of flavanoids, phenol, acids, vitamins and sulphur compound, of garlic which contain the biological properties of garlic. The free radical scavenging effect of SAC has been reported in previous studies. SAC could enhance the levels of SOD, CAT, and GSH in fact it has been shown that SAC have antioxidant properties *in vivo* conditions [1, 7, 9, 13, 18, 22].

SAC changed the rat nephrotic glutathione related antioxidant enzyme activities by increasing the GSH has been suggested that the number of sulphur atoms and allyl groups may play a determining factor on the biological activities of garlic organosulfur compounds[10, 15]

In the present experimental study we observed that S-Allylcystein when given to mercuric chloride intoxicated rats shows significantly increases in GSH contents and also increases in the level of SOD, CAT and GPx activities as antioxidant potential and thereby declines the level of lipid peroxidation, which in turn reduces the mercury toxicity.

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REFERENCES

[1]. Augusti. PR., Conterato. GMM., Somacal. S., Einsfeld.L., Amos. AT., Hosomi. FYM., Grac.DL., and T.Emanuelli., (**2007**). *Clin. Pharm.Toxicol*. 100: 398–402.

[2]. Bentler.E., and BM.Kellay., (**1963**). *Expertentia*. 29:97.
[3]. Cheng-Chin Hsu., Chun-Che Lin., Ting-Sin Liao., Mei-Chin Yin., (**2006**). *Food and Chem. Toxicol*.44:393-397.
[4]. Chung. AS., Maines. MD., and WA.Reyndds., (**1982**). *Biochem. pharm* 31:3093-3100.
[5]. Duncan,BD **1957**.Duncan's multiple range test for correlated and heteroscedastic mean.*Biometrics*.13:359-364.
[6]. Franco. JL., Braga. HC., Nunes. AKC., Ribas. CM., Stringari. J., Silva AP., Pomblum. SCG., Moro. AM., Bohrer. O., Santose. ARS., Oafr. AL., and M. Farina., (**2007**). *Neurotoxicol, Teratol*.29:360-367.
[7]. Jagadeesan.G and Sankarsamipillai.S.,(**2007**). *J.Env.Biol*.28(4):753-756.
[8]. Kakkar. P., B.Das and PN. Viswanathan., (**1984**). *Ind .J. Biochem. Biophys*. 211; 731-132.
[9]. Kavitha. A.V. and G.Jagadeesan., (**2006**). *J. Environ. Biol*. 27(2) 397-400.
[10]. Kim. KM., Chun. SB., Koo. MS., Choi. WJ., Kim. TW., Kwon. YG., Chung. HT., Billiar. TR.. and YM.Kim., (**2001**). *Free Radic. Biol. Med.*30:747–756.
[11]. Lin. CC., Yin.MC., and WH.Liu., (**2008**). *Food chem.. Toxicol.* 46(11): 3401-6.
[12]. Manivasagam. T., Subramanian. P., Suthakar. G., and M. Mohammed Essa., (**2007**). *Toxico. Mod. Meth* 17: 1-8.
[13]. Margarat. A., Jagadeesan. G and S. Sethupathy., (**2001**). *Mus musculus.Poll.Res.*,20(1);1-4.
[14]. Nichens W.G. and Samuelson B., (**1968**). *Eur. J. Biochem*. 6: 126 – 130.
[15]. Numagamami. S and T.Ohnishi., (**2001**). *J.Nutr*.131: 1100S-1105S.
[16]. Rotruck., (**1973**). *Science*.179:588-590.
[17]. Sankarsamipillai.S and G.Jagadeesan., (**2009**). *Recent Res. Sci.and Technol*.1 (2):81-87.
[18]. Sener. G., Sehidi. AO., and G.Ayanoglu-Dulger., (**2003**). *Pharm.Toxicol*. 93; 290–296.
[19]. Sharma.MK, Kumar.M., and A.Kumar., (**2005**). *Env.Toxicol and Pharm*.19:161–167.
[20]. Shimojo. N., Kumagai. Y., J.Nagafune., (**2002**). *Arch. Toxicol*. 76: 383–387.
[21]. Sinha.KA., (**1972**). *Anal Biochem*.47: 389-394.
[22]. Sugunavarman. T., Jagadeesan.G and S.Sankarsamipillai., (**2010**). *J.Ecobiotech*.2/1:59-65.