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Protective Effect of Curcumin on Mercuric Chloride Induced Oxidative Stress in Rats

KEYWORDS: Curcumin, mercuric chloride, oxidative stress, rat

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ABSTRACT *Curcumin, the yellow pigment in turmeric, is present in the rhizomes of the plant Curcuma longa. Mercury (HgCl2) induces various toxic effects in different organs of the body. The present work was undertaken to evaluate the beneficial effect of curcumin following mercuric chloride induced oxidative stress in rat brain. The antioxidative indices assayed were superoxide dismutase, glutathione peroxidase, glutathione reductase, thiobarbituric acid-reactive substances and total –SH groups. Metabolic parameters like total protein, cholesterol, succinate dehydrogenase, adenosine triphosphatase and mercury levels were also measured. Mercury exposure resulted in a significant increase in thiobarbituric acid-reactive substances and mercury levels. Contrarily a decrease in the activities of most of those enzymes, total –SH groups, protein and cholesterol levels were noted. Curcumin administration reduced TBARS and mercury levels, whereas it enhanced the other enzyme activities, total –SH groups, proteins and cholesterol levels comparable to controls. In summary, curcumin administration protects different parts of the brain against mercuric chloride induced neurotoxicity.*

Introduction
Mercury is released into the environment through various natural geological processes, such as volatilization of rocks, dissolution, and volcanic eruption as well as due to some anthropogenic activities like combustion of fossil fuels, incineration of waste, mining and industrial discharge (Agrawal and Baheri, 2007). The cellular mechanisms by which mercury compounds exert their neurotoxic action were obtained from in vitro studies. Sorg et al. (1998) proposed that the mechanism of mercury toxicity could be via binding to thiol groups. Mercury compounds can inactivate a number of enzymes by blocking the functional sites binding to -SH groups, which are part of the catalytic or binding domains. Mercury treatment induced the dramatic increase in reactive oxygen species accumulating in rat brain cell cultures, leading to increased lipid peroxidation, protein degradation, and finally to cell death (Sorg et al., 1998). Rats and mice injected with inorganic mercury were detected with mercury granules in the different regions of the nervous system (Neustadt and Pieczenik, 2007). Mercury treatment exerted significant increase in ROS only in the cortical region and marked dose dependent increase (2500-5600-fold) in total Hg in the different brain regions (Goering et al., 2002). Morphological changes and Hg accumulation are different between cerebral hemisphere and cerebellar astrocytes after mercury treatment cultured from newborn rats (Adachi and Kunimoto, 2005). Pregnant female rats exposed to a very low dose of inorganic mercury from prenatal day 0 continued to postnatal day 20, the highest Hg content was present in the infant hippocampus and cerebellum, whereas its content in maternal brain regions like cerebrum, cerebellum, brain stem, hippocampus and thalamus (Feng et al., 2004).

However, the role of herbal antioxidants on metal exerted neurotoxicity is attained less attention. Hence, this study was proposed to investigate the ameliorative effect of curcumin powder on regional brain toxicity induced by mercury in the rat. Rao et al. (2009) also studied effects of Hg in different regions of male rat brain affecting it in a dose dependent manner and role of melatonin on its toxicity.

Materials and Methods
Male Wistar strain Albino rats (Rattus norvegicus), weighing 200-250gms were procured from Cadila Pharma, under the Animal maintenance and Registration No.167/1999/CPCSEA from the ministry of Social Justice and empowerment, Govt. of India. The rats were fed on the standard commercial laboratory chow and distilled water ad libitum and were housed in the plastic cages with good ventilation. Light dark conditions as well as temperature was maintained (12h: 12h and 26±2oC respectively) throughout the seasons. Animals were assigned to 5 groups of 8 rats each. Group I served as control and animals were provided with distilled water. Group II animals received (low dose) HgCl2 (2mg/kg body weight) orally. Group III was administered with 4 mg/kg body weight (high dose) of mercuric chloride. Group IV received curcumin alone (80mg/kg body weight) and Group V received curcumin along with high dose of HgCl2. All the treatments were administered for 2 months and on the 61st day the animals were weighed and necropsy was performed. The brain was dissected carefully and weighed. Experiments were carried out on cerebral hemisphere and cerebellum.

Antioxidant parameters
The antioxidant enzyme activities like superoxide dismutase (SOD, EC:1.15.1.1), glutathione reductase (GR, EC:1.6.4.2), glutathione peroxidase (GPx, EC:1.11.1.9) were analysed by the spectrophotometric method of Kakkar et al. (1984), modified method of Pagila and Valentine (1967) and the method of Carlberg and Mannervik (1985) respectively. Thiobarbituric acid-reactive substances (TBARS) were determined by Ohkawa et al., (1979).

Biochemical parameters
The methods of total (-SH), succinate dehydrogenase (SDH, EC:1.3.99.1) and adenosine triphosphatase (ATPase, EC:3.6.1.3) was carried out by the methods of Sedlak and Lindsey (1968), Beatty et.al., (1966) and Quinn and White (1968) respectively. Total proteins and Cholesterol were determined by the methods of Lowry et al., (1951) and Zlatkis et. al., (1953) respectively. Mercury levels in the brain were estimated using mercury analyzer (MA 5840, Electronic Corporation of India Ltd., Hyderabad) using acid digestion method followed by cold vaporization of the sample. Data were statistically analyzed by Student's t-test and ANOVA.

Results
Antioxidant system.
In the present study antioxidant enzymes such as SOD, GPx, and GR were significantly ($P<0.001$) decreased in both regions of brain by mercury ingestion (Table 1). The treatment contrarily brought about a significant ($P<0.001$) enhance-

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ment in the levels of thiobarbituric acid-reactive substances (TBARS) followed by a depletion (P<0.001) in total –SH groups in the two regions of the rat brain (Table 2). Administration of curcumin alone and in combination with the high dose of mercuric chloride did not reveal any significant difference when compared to control. Levels of TBARS increased by lipid peroxidation and total –SH groups also registered a recovery (Table 2).

Other biochemical studies.
The mercuric chloride treated groups had a significant (P<0.001) reduction in the SDH and ATPase activities when compared to control. Total proteins as well as cholesterol levels were also reduced (P<0.01, P<0.001) by mercury feeding. In contrast mercury levels were increased (P<0.001) in a dose dependent manner in cerebral hemisphere and cerebellum of the brain as compared to control (Table 3). The treatment of the curcumin alone or in combination with high dose of mercury revealed no significant alterations in the activities of these enzymes in treated rats. Total proteins, cholesterol and mercury levels were also recovered and compared to those of control. (Table 3).

Discussion
Production of Reactive Oxygen Species (ROS) and free radicals is an integral part of animal metabolism (Sundal et al., 2005). When ROS levels exceed the antioxidant capacity of a cell, results into a deleterious condition known as oxidative stress. Unchecked excessive ROS can lead to the destruction of cellular compounds including lipids, proteins and DNA resulting into changes in cell permeability, ultimately leading to cell death (Klein and Ackerman, 2003). In the present study, chronic administration of HgCl2 to rats caused toxic effects via generating the reactive oxygen species in different parts of the brain affecting their functions. One of the factors that induced free radicals is lipid peroxidation which breaks unsaturated fatty acids of the cell membrane. Due to continuous peroxidation of lipids, the membrane permeability and function gets altered by increasing TBARS levels as a result of toxicant intoxication. Hence in our study, these levels were increased in the brain due to Hg++ poisoning. Increase in the levels of TBARS due to mercury intoxication was also observed in different rat tissues as well as in isolated rat hepatocytes (Hijova et al., 2002; Sener et al., 2003) in support of our data.

The first line of cellular antioxidant defense enzymes includes SOD, GR and GPx. The enzyme SOD catalyses the dismutation of superoxide to O2 and H2O2. H2O2 is further converted to H2O by GPx and CAT (Ray and Husain, 2002). Thus SOD acts as a primary defense and prevents further generation of free radicals. The decreased SOD activity in different parts of the brain and other organs suggests that the accumulation of superoxide anion radical might be responsible for increased lipid peroxidation following mercury treatment. The reduction in GR and GPx of brain also supported changes in glutathione metabolism leading to oxidative stress of brain. The role of glutathione and its related enzymes like GR and GPx is well known in stress condition and are responsible for defense mechanism in the brain (Gutierrez et al., 2006). Nielsen et al. (1994) also reported that the amount of glutathione and the glutathione peroxidase activity decreased with increasing doses of mercuric chloride in light of our data. Mercury exposure thus brought about an imbalance in intracellular redox status leading to oxidative stress in the brain. As a result of this stress, sensory and motor as well as other functions of cerebral hemisphere and cerebellum of brain are inhibited by Hg poisoning.

HgCl2 treatment brought a decrease in protein levels due to its oxidation via ROS affecting thiol (-SH) groups containing molecules such as glutathione, cysteine as well as -SH groups of proteins. Further the treatment inhibited the activity of carbohydrate metabolic enzymes such as SDH and ATPase. Rao and Sharma (2001) reported that mercuric chloride treatment exerted a significant reduction in the SDH and ATPase activities in tissue of mice. Inorganic mercury is suggested to increase H2O2 production by impairing the efficiency of oxidative phosphorylation and electron transport. By accelerating electron transfer rates in electron transport chain in mitochondria, premature shedding of electrons to molecular oxygen increases and generates O2o and H2O2 (Ercal et al., 2001) lead to oxidative stress affecting its energy production in the rat brain probably due to its accumulation. Similar results are also reported by Agrawal and Behari (2007) in rats by mercury exposure.

Co-administration of curcumin with high dose of HgCl2 exposed groups exerted amelioration in treated rats. This antioxidant and ROS scavenging effects of curcumin is only due to its phenolic (-OH) group, which would inhibit the -SH group oxidation (Pari and Amali, 2005) and block thiol depletion (Chattopadhyay et al., 2005) and thus it protects the oxidation of protein (Priyadarshini et al., 2003). Further it also enhances the activities of some antioxidant enzymes such as SOD, catalase and GPx is in agreement with the previous findings of Irulappan and Natarajan (2007), Corona-Rivera et al. (2007). Curcumin inhibits H2O2 induced injury in renal cell line (Cohly et al., 1998) and significantly decreased the levels of TBARS by increasing the levels of glutathione reductase reported by others (Pari and Amali, 2005; Biswas et al., 2005; Chen et al., 2005; Tirkey et al., 2005). Further curcumin might induce Hg++ affinity to –SH groups leading to Hg–SH complexes directing to its elimination from the brain as reported by Gangadharan and Rao (2008). As a result of it, there is a significant reduction of mercury retention levels in different parts of the brain in our study.

Conclusion
In conclusion, our results suggested that curcumin treatment mitigates the HgCl2 induced oxidative damage in cerebral hemisphere and cerebellum of rat brain, which could be due to its strong antioxidant properties.

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Table 1
Changes in the activities of antioxidant enzymes glutathione peroxidation (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) in cerebral hemisphere (CH) and cerebellum (C) of male rats treated with curcumin, mercuric chloride and curcumin + mercuric chloride (HgCl2).

Parameters	Brain regions	Control	HgCl2		Cur	Cur + HD
			LD	HD		
Superoxide dismutase (SOD) (units SOD/mg protein)	CH	13.34 ± 0.29	10.58 ± 0.31▪	5.97 ± 0.25▪	14.06 ± 0.34	11.93 ± 0.29*
	C	11.36 ± 0.26	7.69 ± 0.23▪	3.33 ± 0.20▪	13.42 ± 0.32	10.56 ± 0.28
Glutathione peroxidation (GPx) (n moles of NADPH oxidised/min/mg protein)	CH	7.55 ± 0.28	5.64 ± 0.27**	3.25 ± 0.25▪	7.97 ± 0.30	6.23 ± 0.25*
	C	6.55 ± 0.30	5.42 ± 0.29	2.65 ± 0.29▪	7.27 ± 0.27	5.85 ± 0.32
Glutathione reductase (GR) (n moles of NADPH oxidised/min/mg protein)	CH	5.13 ± 0.11	4.69 ± 0.13	3.04 ± 0.28▪	5.41 ± 0.26	4.98 ± 0.14
	C	4.99 ± 0.13	4.32 ± 0.18▪	2.43 ± 0.22▪	5.08 ± 0.23	4.74 ± 0.18

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Values are Mean ± SE, *P<0.05, **P<0.01, ▪P<0.001.

Table 2
Changes in the levels of thiobarbituric acid-reactive substances (TBARS) and total sulfhydryl groups (total SH-groups) in cerebral hemisphere (CH) and cerebellum (C) of male rats.

Parameters	Brain regions	Control	HgCl2		Cur	Cur+ HD
			LD	HD		
thiobarbituric acid-reactive substances (TBARS)	CH	27.64 ± 0.53	39.00 ± 0.53▪	50.19 ± 0.44▪	28.67 ± 0.54	25.19 ± 0.58*
(n moles of TBARS formed/100 mg tissue weight)	C	25.82 ± 0.50	38.08 ± 0.51▪	49.26 ± 0.45▪	27.67 ± 0.54	24.42 ± 0.56
Total –SH groups	CH	13.44 ± 0.20	9.79 ± 0.15▪	4.95 ± 0.12▪	14.16 ± 0.14	12.68 ±0.25
(mg/100mg tissue weight)	C	8.79 ± 0.17	5.82 ± 0.09*	3.25 ± 0.20▪	9.36 ± 0.22	7.45 ± 0.11

Values are Mean ± SE, *P<0.05, **P<0.01, ▪P<0.001.

Table 3
Changes in the activities of enzymes succinate dehydrogenase (SDH) and adenosine triphosphataase (ATPase), cholesterol, protein concentration and mercury levels in cerebral hemisphere (CH) and cerebellum (C) of male rats.

Parameters	Brain regions	Control	HgCl2		Cur	Cur + HD
			LD	HD		
Succinate dehydrogenase (SDH)	CH	50.91± 0.37	35.69 ± 0.17▪	18.26 ± 0.22▪	52.16 ± 0.45	48.70 ± 0.69
(µg formazan formed/mg protein)	C	45.70 ± 0.69	34.67 ± 0.52▪	17.51 ± 0.61▪	47.29 ± 0.58	43.09 ± 0.47*
Adenosine triphosphatase (ATPase)	CH	1.44 ± 0.06	0.81 ± 0.03▪	0.44 ± 0.03▪	1.59 ± 0.12	1.41 ± 0.08
(µ moles of inorganic phosphate released/mg protein)	C	1.07 ± 0.06	0.54 ± 0.09**	0.13 ± 0.09▪	1.20 ± 0.11	0.88 ± 0.12
Cholesterol	CH	2.11 ± 0.14	1.69 ± 0.14	1.27 ± 0.13**	2.15 ± 0.15	1.68 ± 0.10
(mg/100mg tissue weight)	C	1.37 ± 0.16	1.08 ± 0.13	0.62 ± 0.10*	1.42 ± 0.07	0.99 ± 0.13
Protein	CH	7.67 ± 0.17	6.61 ± 0.21**	3.54 ± 0.20▪	8.14 ± 0.15	6.95 ± 0.14
(mg/100 mg tissue weight)	C	7.13 ± 0.16	5.73 ± 0.20▪	2.97 ± 0.11▪	7.68 ± 0.20	6.24 ± 0.14**
Mercury levels	CH	1.7 ± 0.14	5.59 ± 0.17▪	9.67 ± 0.18▪	0.99 ± 0.21	2.72 ± 0.28*
(ng mercury/g tissue weight)	C	1.47 ± 0.11	4.89 ± 0.20▪	8.73 ± 0.23▪	1.09 ± 0.19	2.57 ± 0.21

Values are Mean ± SE, *P<0.05, **P<0.01, ▪P<0.001.

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