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Antioxidant status of ginger against mercury induced toxicity in the brain of rat

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ABSTRACT

The impact of mercuric chloride on antioxidant status in the brain tissue of rats, *Rattus norvegicus*, was studied. Mercury poisoining induced oxidative stress leading to generation of free radicals and simultaneous alterations in antioxidant mechanism in animals. In the present study, the level of lipid peroxidation (LPO) was increased in the brain tissue of rats at sublethal dose of mercuric chloride (2mg/kg body wt.) treatment for 30 days and simultaneously decreased level of glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) contents were also noticed in the mercury intoxicated brain tissue. During the recovery span an altered level of antioxidant status was restored to near normal level in the brain tissue of mercury intoxicated animal when treated with ethanolic ginger extract (200mg/kg body wt.) For another 15 days. Ginger play a vital role to detoxify mercury toxicant in the mercury intoxicated animals. The results were discussed in detail.

KEYWORDS

Mercuric chloride;
Ethanolic ginger;
Rats antioxidants LPO.

INTRODUCTION

Mercury is a transition metal and it promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhances the proxides and reactive hydroxyl radicals[12]. These lipid peroxides and hydroxyl radical may cause cell membrane damage and thus destroy the cell. Mercury also inhibits the activities of free radical quenching enzymes catalase, superoxide dismutase and glutathine peroxidase[3].

Mercury and its compounds comes from weathering process of earth's crust, industrial discharge, pest or disease control agent applied to plants, urban run off, mining, soil erosion, sewage effluent[22]. It is an inorganic compound that has been used in agriculture as fungicides, in medicine as topical antiseptical and disinfectants, and in chemistry as an intermediate in the production of others compounds[24]. Mercury and its compound are used widely in industries and their hazards to animal have been well documented[13,17,19]. Although people know the adverse effect of mercury they used

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mercury in electric apparatus, choloro-alkali plants, caustic soda, and caustic potash industry etc. as well as in ayurvedic medicines, antiseptics, parasiticidal, fungicidal effects and also in the dentistry for fillings[13,19]. The toxic effect of mercury varies according to the chemical composition.

Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. Their medicinal use has been gradually increasing in developed countries. *Ginger Officinale* Roscoe, commonly known as ginger, is one of the commonly used spices in India and around the world. Ginger is example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are medicinally and wlinary useful part[20]. Many studies were carried out on ginger and its pungent constituents, fresh and dried rhizome. Among the pharmacological effects of demonstrated are antiplatlet, antioxidant, anti-tumor, anti-rhino viral, anti hepatotoxicity and anti arthritic effect[11,16] ginger may be protective effect on the tissue damage that results from oxygen free radicals in mercury induced toxicity.

Within this point of view, the present study has been designed to study the effect of mercury on brain tissue of rats and simultaneously to find out efficacy of ginger on mercury intoxicated rats, *Rattus norvegicus*.

MATERIALS AND METHODS

Normal adult healthy female rats, *Rattus norvegicus,* of the wistar strain weighing ranging from 200±5g were used in this experiments. All the animals were fed on a standard rat feed and water *ad Libitum*. Experimental protocol was approved by the institutional animals' ethics committee (IAEC) of Tamil University.

Extraction

Rhizome of *Z. officinale* was purchased from the local market. The rhizome (500 g) were cut into small pieces and homogenized in a kitchen mixer using 50%ethanol (w/v). The homogenate was kept on water bath at 70-80°C for 10-15 h with intermittent shaking. The homogenate was centrifuged at 1500g for 10 min and the supernatant was collected. Solvent in the pooled supernatant was completely evaporated at low temperature using a water bath. The residue thus obtained (6.5 g, w/w) was used for the experiment[2]. (TABLE 1).

Total weight of diet was kept constant throughout the experimental period. The chemicals (mercuric chloride and ginger) were administered orally to the experimental animals through cathedral tube. After the scheduled treatments, the animals were sacrificed by cervical dislocation and then the whole brain tissue was isolated immediately in the cold room. The isolated brain tissue was used for estimation of the following parameters.

Estimation of lipid peroxidation

The level of lipid peroxidation in brain tissue was estimated with the method of Nichans and Samuelson[23]. Whole brain tissue homogenate was prepared in Tris-HCL buffer (pH 7.5). 1 ml of the tissue homogenate was taken in a clean test tube and 2.0 ml of TBA-TCA-HCL reagent was added and then mixed thoroughly. The mixture was kept in a boiling water bath (60°C) for 15 minutes. After cooling, the mixture was centrifuged at 1000 rpm for 10 minutes and the supernatant was taken to read the absorbance of the chromophore at 535 nm against the reagent blank in a UV visible spectrophotometer 1, 1', 3, 3' tetra methoxy propane was used to construct the standard graph.

Estimation of reduced glutathione

The glutathione (reduced) in whole brain tissue was determined according to the method of Beutler and Kelley[4]. Brain tissue was homogenized in PBS buffer solution and centrifuged at 2500 rpm for 5 minutes. 0.2 ml of the sample (supernatant) was taken n a clean test tube and 1.8 ml of EDTA solution was added. To this 3.0 ml of precipitating reagent was added and mixed thoroughly and kept for 5 minutes before centrifugation at 3000 rpm for 10 minutes. In each test tube, 2.0 ml of the filtrate was taken and to this 4.0 ml of 0.3M disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added. The appearance of yellow color was read at 412 nm in UV-visible spectrophotometer. A set of standard solution containing 20-100 μg of reduced glutathione was treated similarly.

TABLE 1 : Experimental design

Group I	Untreated control	Provided standard diet and clear water *ad libitum* and observed for 30 days
Group II	Mercuric Chloride treatment	2mg / kg body weight. Oral administration daily upto 30 days
Group III	Post treatment of ethanolic ginger extract	200mg / kg body weight. Oral administration daily upto 15 days in mercury intoxicated rat
Group IV	Ethanolic ginger extract alone treatment	200mg / kg body weight. Oral administration daily upto 15days

Estimation of glutathione peroxidase

The activity of glutathione peroxidases was assayed using the method of Rotruck et al.[28]. The liver tissue was homogenized in PBS buffer and centrifuged at 2500 rpm for 5 minutes. 0.2 ml of the enzyme preparation (supernatant) was taken in a clean test tube, and then was added the following enzyme mixture: The enzyme assay mixture contained 0.2 ml of phosphate buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide. In the reaction, the mixture was mixed well and kept at 37°C for two minutes in an incubator. Then 0.2 ml of reduced glutathione and 0.1 ml of H_2O_2 were again added to the above mixture and incubated at 37°C exactly for 10 minutes. The reaction was arrested by the addition of 0.5 ml of 10% TCA. Reduced glutathione content was estimated in the supernatant obtained after centrifugation at 3000 rpm for 10 minutes. A blank was prepared similarly to which 0.2 ml of the enzyme was added after the incubation period.

Estimation of catalase

Catalase was assayed colorimetrically with the method of Sinha[31]. The whole brain tissue was homogenized in phosphate buffer solution. 0.1 ml of the homogenate was taken in a test tube and 1.0 ml of phosphate buffer was added. 0.4 ml of hydrogen peroxide was added to the above mixture. After 30 and 60 seconds 2.0 ml of dichromate acetic acid reagent was added. Test tubes were kept in boiling water bath (60°C) for 10 minutes. The mixture was cooled immediately in tap water and the color was at 620 nm against a reagent blank in UV-visible spectrophotometer.

Estimation of superoxide dismutase

The activity of superoxide dismutase was assayed with the method of Kakkar et al.[14]. The brain tissue was homogenized with 3 ml of 0.25 M sucrose solution and centrifuged at 10,000 rpm in cold condition for 30 minutes. The supernatant was dialyzed against Tris HCL buffer (0.0025M, pH 7.4). The supernatant thus obtained was used as any enzyme source.

Enzyme assay

The assay mixture (2.0 ml) contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 0.1 ml of enzyme preparation (tissue homogenate) and 0.3 ml of water. The reaction was started by the addition of 0.2 ml of NADH solution and then it was incubated at 30°C for 90 seconds. After incubation the reaction was arrested by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred and shaken with 4.0 ml of n-butanol. The mixture was allowed to stand for 10 minutes and then centrifuged for 15 minutes at 3000 rpm. After centrifugation, the butanol layer was separated. The color intensity of the chromogen was measured at 560 nm in UV visible spectrophotometer. Water was used as blank.

Statistical significance was evaluated using ANOVA followed by Duncan Multible Range Test (DMRT) Duncan[10].

RESULTS

LPO content of brain was significantly higher ($P<0.05$) in mercury treaded animal as compared to control. But supplementation of ginger significantly lower in the mercury treated animal ($P<0.05$) as compared to mercury treated animal. No significant changes was observed in the ginger alone treatment (TABLE 2).

The glutathione (GSH) content in the brain was reduced in the mercury treated animal ($P<0.05$) as compared to control. But the ginger supplementation increased significantly in mercury treated animal ($P<0.05$) as compared to mercury treated animal. Ginger alone treatment shows increased glutathione (TABLE 2).

The level of GPx was significantly declined in the brain mercury treated animal ($P<0.05$) as compared with control. The administration of ginger in mercury intoxicated animal showed enhanced level of GPx activity ($P<0.05$) as compared with mercury treated ani-

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TABLE 2 : Level of lipid peroxidation and antioxidants in the brain tissue of rats treated with mercuric chloride followed by ginger

Parameters	Control	$HgCl_2$	$HgCl_2$ + ethanolic ginger extract	Ethanolic ginger extract
Lipid peroxidation (nmoles/g wet tissue)	1.817±0.10	3.500±0.52*	1.734±0.06**	1.709±0.09
Reduced glutathione (μmoles/g wet tissue)	30.588±0.93	20.557±0.29*	30.853±0.74**	34.468±0.11
Glutathione peroxidase (μMoles/mg protein/min)	0.13±0.03	0.093±0.05*	0.135±0.03**	0.142±0.09
Catalase (μMoles/mg protein/min)	45.493±0.64	27.182±0.67*	46.250±0.69**	48.352±0.69
Super oxide dismutase (Units/mg protein)	8.954±0.49	3.242±0.22*	9.255±0.01**	12.668±0.39

Mean± S.D of six individual observations. *Significance (P< 0.05) Group I compared with group II. **Significance (P < 0.05) Group II compared with group III

mal. But ginger alone treatment shows the increased level GPx activity in the brain (TABLE 2).

The level of SOD activity was significantly decreased in the mercury treated animal (P<0.05) as compared with control. But supplementation of ginger enhanced the SOD (P<0.05) as compared with mercury treatment. Ginger alone treatment shows the increased level of SOD (TABLE 2).

In brain, mercury treatment reduced the catalase activity (P<0.05) as compared with control but ginger supplemention in mercury intoxicated animal increased (P<0.05) as compared with mercury treatment. Ginger alone treatment shows the enhanced level of catalase (TABLE 2).

DISCUSSION

Brain is the main organ for co-coordinating whole body functions. The accumulation of heavy metal in the brain region may interfere with the synthesis of specific enzymes which is responsible for brain function and in turn, produces neurological disorder including "Alzhimers disease" and encephalopathy[8]. The oxidative stress might induce a defensive antioxidant system in brain leading to neuro toxicity. Heavy metal toxicity mainly produces cellular oxidation in animals mainly due to generation of free radicals[7] and other neuro degenerative disorders[6,27]. Although the neurotoxic effect of mercury are well known, the underlying biochemical and molecular mechanism that lead to impaired cell function and nerve cell degeneration in the central nervous system and mercury induced oxidative stress[25].

The existence of oxidative stress in the brain following mercury poisoning[35] and have identified the mitochondrial electron transport chain not only as the target of mercury toxicity, but also as the most likely site of generation of excess reactive oxygen species (ROS)[34].

Mercury generates highly toxic hydroxyl radicals from the break down of hydrogen peroxide, which further deplete glutathione stores[18]. There is evidence that glutathione depletion can lead to neurological damage; Low levels of glutathione have been found in parkinson's disease and cerebral ischemia reperfusion injury[26].

In the present study, the level of LPO content was increased and simultaneously GSH, GPx, CAT and SOD activities were reduced in the brain tissues of rats treated with mercuric chloride for 30 days (TABLE 2). These results suggested that mercury mainly induced oxidative damage in the brain, because mercury probably act as inducer of P450 isoenzyme which is secreted by liver organ. Mercury not only affect the neurons but also affect the hepatocytes. Induction of P450 enzyme system by mercury may be responsible for its increased bio transformation to P = 0 analogue in hepatic cells[15]. After the bio transformation takes place the oxidative damage was also occurred in brain tissue. This result suggested that mercury induced lipid peroxidation in brain could possibly result from an enhanced microsomal oxidative capacity. Thus elevated level of cytochrome P450 would lead to high rates of radical's production, which, inturn, would favor increased rates of lipid peroxidation. After biotransformation, an enhancement of oxygen ratical production may be ensured; leading to an increased level of LPO content was noticed[29].

An increased level of LPO content has mainly due to high susceptibility of brain to oxidative disturbed. Because, it contains a large amount of PUPA and consumes 20% of the body oxygen[33]. Moreover, in spite of high amount of oxidative metabolism, brain has a

relatively low antioxidant defence system[21].

Oxygen free radicals and hydroperoxides collectively termed as reactive oxygen species (ROS). ROS are produced by the univalent reduction of dioxygen to superoxide anion (O^-_2). Superoxide disproportionative to H_2O_2 and O_2 catalysed by superoxide dismutase (SOD). Mates[21] also observed that the endogenous H_2O_2 may be converted either by catalase (CAT) or GPx to H_2O. Otherwise it may generate the highly reactive free hydroxyl radical (OH^-). At the time of mercury exposure, the brain tissue was completely damaged due to the decreased level of SOD, CAT and GPx isoenzymes evidenced in TABLE 2. Decreased level of antioxidant defense system mainly responsible for generating hydroxyl radicals leading to promote oxidative damage by Fenton reaction[29].

During the recovery period, (administration of ginger on mercury intoxicated rats) the altered level of antioxidant system was restored. The increased level of LPO content in mercury intoxicated brain tissue was significantly decreased to near normal level. It indicates that the brain tissue was slowly recovered from the effect of mercuric chloride toxicity.

Post treatment of ginger supplementation on mercury fed animals isoenzymes (GPx, CAT and SOD) are elevated to reach near normal level. The elevated level of GPx converts H_2O_2 or other lipid peroxides to water or hydroxy lipids and in the process, GSH is converted to oxidized glutathione (GSSG)[21].

Antioxidants have a proactive effect against tissue injury in the pathogenesis of which LPO may be involved. The antioxidant compounds like gingerols, shogals,ketone compounds and the phenolic compounds of ginger were responsible for scavenging the superoxide anion radicals[5,32] and also Ahmed et al.[1] explained that ginger exerts an antioxidant effect by decreasing LPO and increasing GSH levels and maintaining the normal levels of antioxidants enzymes.

The present study demonstrates that administration of ginger has a therapeutic role in preventing mercury induced oxidative stress in brain tissue. From these results we conclude that ginger may be a protective role in brain damage against mercury induced oxidative stress in animals.

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