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Protective role of propolis on mercuric chloride induced neurotoxicity in rats

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ABSTRACT

This study quantified the protective role of propolis on mercuric chloride induced neurotoxicity in the brain tissue of rats, *Rattus norvegicus*. Mercury poisoning induced oxidative stress leading to generation of free radicals and simultaneous alterations in antioxidant mechanism in animals. In the present research, the level of lipid peroxidation (LPO) was increased in the brain tissue of rats at sub-lethal 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 period an altered level of antioxidant status was restored to near normal level in the brain tissue of mercury intoxicated animal, when treated with propolis (200 mg/ kg body wt.) for another 15 days. Stastical significance was evaluated using ANOVA followed by Duncan Multible Range Test (DMRT). The analysis of the results showed that the Propolis play a vital role to detoxify mercury toxicant in the mercury intoxicated animals.

KEYWORDS

$HgCl_2$;
Propolis;
Rats;
Antioxidants;
LPO.

INTRODUCTION

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[23]. 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[30]. Mercury and its compound are used widely in industries and their hazards to animal have been well documented[12,17,20,21]. Although people know the adverse effect of mercury they used 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 denstistry for fillings[6,7,12,17,20,21]. The toxic effect of mercury varies according to the chemical composition.

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Propolis is a resinous material, collected by honey bees from plant exudates that is used for construction and repair of honey comb. It has been used for thousand of years in folk medicine It has pleasant aromatic odor and yellow-green to dark brown color depending on its source and age[10].It has a broad spectrum of biological activities against hepatitis[35,36], arthritis[13] and as hepatoprotective agent againstgalactosamine[41] econazole[19] tert-butyl hydroperoxide[46] paracetamol[3,27,28], ethanol[37,39] and carbon tetrachloride[3,27,28] induced toxicity.Synergism between propolis and antibiotics[14] antibacterial agents[43] and with chelators against light metal[3,27,28] and heavy metal intoxication[11] has also been proposed.

The main objective of this research is to evaluate the effect of mercury on brain tissue of rats and simultaneously to find out efficacy of propolis 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. The animals were procured from the central animal house. 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. The experimental design is shown in TABLE 1.

Total weight of diet was kept constant throughout the experimental period. The chemicals ($HgCl_2$ and Propolis) 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 lipid peroxidation[29], superoxide dismutase[15], catalase[40], reduced glutathione[2] and glutathione peroxidase[34]. Stastical significance was evaluated using ANOVA followed by Duncan Multible Range Test (DMRT)[9].

RESULTS

In the normal untreated control rat, *Rattus norvegicus,* the level of LPO and GSH content were 1.817±0.10 (μmoles/g wet wt. tissue) and 30.588±0.93 (μ moles/g wet wt. tissue). The level of GPx, CAT and SOD activities were 0.13±0.03 (μMoles/mg protein/min), 45.493±0.64 (μMoles/mg protein /min) and 8.954±0.49 (Units/mg protein).

At sub- lethal dose of $HgCl_2$ fed animal shows the increased level of LPO content and simultaneously decreased level of GSH, GPx CAT, and SOD activities in brain tissue ($p<0.05$) but during the recovery period ($HgCl_2$ followed by Propolis treatment), the altered level of LPO and antioxidants enzymes were restored to reach normal level ($p<0.05$). Propolis alone treatment also show the maintained level of LPO and antioxidants enzymes respectively TABLE 2.

DISCUSSION

The accumulation of heavy metal in the brain region may interfer 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[5] and other neuro degenerative disorders[4,33]. 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[6,7] and mercury induced oxidative stress[31,48].

The existence of oxidative stress in the brain following mercury poisoning[47,48] 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)[47,48].

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[32].

In the present investigation, the level of LPO content was increased and simultaneously GSH, GPx, CAT and SOD activities were reduced in the brain tissues of

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rats treated with mercuric chloride for 30 days (TABLE 1). These results suggested that mercury mainly induced oxidative damage in the brain, because mercury probably act as inducer of P_{450} iso enzyme which is secreted by liver organ. Mercury not only affect the neurons but also affect the hepatocytes. Induction of P_{450} enzyme system by mercury may be responsible for its increased bio transformation to P=0 analogue in hepatic cells[16]. 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 P_{450} would lead to high rates of radicals production, which, inturn, would favour increased rates of lipid peroxidation. After biotransformation, an enhancement of oxygen radical production may be ensured; leading to an increased level of LPO content was noticed[37,39].

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[45]. Moreover, in spite of high amount of oxidative metabolism, brain has a relatively low antioxidant defence system[22].

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 catalyzed by super oxide dismutase (SOD)[22] 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[37,39].

During the recovery period (administration of propolis 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. Propolis supplementation not only promotes the regenerative capacity and also eliminates and nullify the mercury toxicity in rats.

Post-treatment of propolis supplementation on mercury fed animals iso-enzymes (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)[22].

Induction of LPO by mercury suggest that cell membrane permeability may be affected by this process[24,26]. Administration of propolis protected the brain markedly against mercury induced toxicity by diminishing LPO. Propolis contains a wide variety of phenolic compounds mainly flavonoids. Phenolics provides protection as good antioxidands against LPO induced pathogenesis[25] and also act as effective chelators for several toxic metal ions[1]. Antioxidants have a proactive effect against tissue injury in the pathogenesis of

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	2 mg/kg body weight. Oral administration daily up to 30 days
Group III	Post treatment of propolis	200 mg/kg body weight. Oral administration daily up to 15 days
Group IV	Propolis alone treatment	200 mg/kg body weight. Oral administration daily up to 15days

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

Parameters	Control	$HgCl_2$	$HgCl_2$+Propolis	Propolis
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 observation, *(P<0.05) group I compared with group II, ** (P<0.05) group II compared with group III

which LPO may be involved .Quercetin, a major component of propolis, is well known to inhibit LPO by scavenging free radicals and/or transition metal ions[44]. This may be due to the favorable capacity of propolis to pass through the membrane and to accumulate in both hydrophilic and hydrophobic environments for protecting cells against oxidative stress and scavenging free radicals[38,42]. The present study demonstrates that administration of propolis has a therapeutic role in preventing mercury induced oxidative stress in brain tissue.

From these results we conclude that Propolis play a protective role in brain damage against mercury induced oxidative stress in animals.

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