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HEPATO-PROTECTIVE POTENTIAL OF GREEN TEA EXTRACT AGAINST MERCURIC CHLORIDE INDUCED HEPATOTOXICITYIN ADULT WISTAR RATS

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ABSTRACT

Objectives: This study investigates the Hepatoprotective activity of the green tea extract against mercuric chloride induced toxicity was investigated in rats. **Methods:** Twenty four adult rats weighing between 180-200 g were used for this study. They were randomly divided four groups (A,B,C & D) whereby group A served as the control while groups B,C and D served as test groups. The parameters studies were liver and kidney marker enzymes and bilirubin in serum. **Results:** The alterations in lipid profile, lipid peroxidation and antioxidant status in liver & kidney. All these levels are increased in mercuric chloride induced rats in green tea extract treated rats. wistar strain rats were pretreated with mercuric chloride, the levels of enzymes were significantly decreased. these alterations were prevented by green tea extract. Showed the above parameters attained near normal levels and the hepatoprotective activity of green tea extract. **Conclusion:** The conclusion of this study strongly indicated that Green tea extract has a hepatoprotective action against mercuric chloride induced hepatic damage in experimental animals.

KEYWORDS: Mercuric chloride, Green tea extract, Liver and body weight, Liver and Kidney Marker enzymes, Antioxidant enzymes, Lipid profile Histopathology.

ABBREVIATION

GTE- Green tea extract

$HgCl_2$- Mercuric chloride

INTRODUCTION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism.[1] Diverse homeostatic mechanisms are affected if liver function is impaired, with potentially serious consequences. About 20, 000 deaths occur every year due to liver diseases. Hepatocellular carcinoma is one of the ten most common tumours in the world with over 2, 50,000 new cases each year. Although viruses are the main cause of liver diseases, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon. Liver disease is a worldwide problem; Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.[2] Free radicals are highly reactive atoms or molecules characterized by one or more unpaired electrons in their outer orbital. In every cell, during aerobic oxidation, a small amount of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed. ROS include superoxide anion (O2•), hydroxyl radicals (OH•), hydrogen peroxides (H2O2), singlet molecular oxygen (•O2), alleoxyl (RO•) and peroxyl radicals (ROO•). RNS include nitric oxide, nitrogen dioxide and peroxy nitrite.[3]

Heavy metals are placed under environmental pollutant category due to their toxic effects on plants, animals and human beings. Heavy metals are persistent in nature, therefore get accumulated in soil and plants. Dietary intake of many heavy metals through consumption of plants has long-term detrimental effects on human health.[4] Heavy metals can be toxic if consumed in large amount. Therefore, a continuous intake of low amounts of heavy metal over a long period could cause metal accumulation in the kidney and liver leads to organ damage.[5] The main threats to human health from heavy metals are associated with exposure to lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (Ar). These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO.[6] Mercury, a highly toxic metal, which induces oxidative stress in the body and results in a variety of adverse health effects including renal, neurological, respiratory, immune, dermatologic, reproductive and developmental sequel.[7] Although the mechanism underlying mercuric chloride (HgCl2) toxicity is not clearly understood, several hypotheses have been proposed. It is well known that inorganic mercury causes severe kidney damage after acute and chronic exposure.[8] Chronic exposure to inorganic mercury salts

primarily affects the renal cortex and may manifest as renal failure (dysuria, proteinuria, hematuria, oliguria and uremia) or gastrointestinal problems (colitis, gingivitis, stomatitis and excessive salivation). Irritability and occasionally acrodynia can occur.[9] The distribution metabolism and toxicity of mercury are largely dependent upon its chemical form.[10] Inorganic mercury has a non-uniform distribution after absorption being accumulated mainly in the kidneys causing acute renal failure.[11] Once absorbed into the bloodstream, inorganic mercury combines with proteins in the plasma or enters the red blood cells. It does not readily pass into the brain or fetus but may enter into other body organs. The liver is a major site of metabolism for mercury, and all mercury absorbed from the stomach and intestine is carried in blood directly to the liver. It accumulates in the kidneys, and may cause severe damage. Poisoning can result from inhalation, ingestion, or absorption through the skin.[10]

Green tea (Camellia sinensis) extract is fast becoming ubiquitous in consumer products supplemented with green tea such as shampoos, creams, soaps, cosmetics, vitamins, drinks, lollipops and ice creams.[12] Fresh tea leaves are rich in flavanol monomers known as catechins such as epicatechins[13], which are 13.6 g/100 g in green tea and 4.2 g/100 gm dry weight in black tea.[14] Tea, a product made up from leaf and bud of the plant *Camellia sinensis*, is the second most consumed beverage in the world, well ahead of coffee, beer, wine and carbonated soft drinks.[15] Originating from China, tea has gained the world's taste in the past 2000 years. The economic and social interest of tea is clear and its consumption is part of many people daily routine, as an everyday drink and as a therapeutic aid in many illnesses. Depending on the manufacturing process, teas are classified into three major types: 'non-fermented' green tea (produced by drying and steaming the fresh leaves to inactivate the polyphenol oxidase and thus, non oxidation occurs); 'semi-fermented' oolong tea (produced when the fresh leaves are subjected to a partial fermentation stage before drying); and 'fermented' black and red teas which undergo a post-harvest fermentation stage before drying and steaming, although the fermentation of black tea is due to an oxidation catalyzed by polyphenol oxidase, and that of red tea is attained by using microorganisms[16] reported a per capita mean consumption of tea in the world of 120 ml/ day. Therefore, based on the above literature, the present study investigates the effect of green tea extract on mercuric chloride induced toxicity in rats by evaluating circulatory lipid peroxidation products and antioxidants.

MATERIALS AND METHODS

Animals

Adult male albino rats (Wistar strain of body weight (180-200 g) bred in the Central Animal House, Rajah Muthiah Medical College, IAEC NO: 02/005/2014. Annamalai University, Tamil Nadu, India were used in this study. The animals were housed in polypropylene cages and provided with food and water and libitum. They were maintained in a controlled environment under standard conditions of temperature and humidity with alternating light/dark (LD 12:12) cycle. All animals were fed standard pellet diet (Hindustan Lever Ltd., Bangalore, India). composed of 21% protein, 81% ash, 5% lipids, 4% crude, 3.4% glucose, 2% vitamins, 1% calcium, 0.6% phosphorus and 55% nitrogen free extract (carbohydrates). It provides a metabolisable energy of 3600 Kcal.

During the experimental period, food and water consumption were measured every day and the body weight was measured at the initial (day 1) and final day (day 46) of the experiment. At the end of the experimental period, animals in different groups were sacrificed by cervical decapitation under pentobarbitone sodium (60 mg/kg body weight) anesthesia. Blood was collected and serum was separated by centrifugation and used for various biochemical estimations.

Chemicals

Green Tea was purchased from Sigma Aldrich, U.S.A. Mercuric chloride, other chemicals and biochemicals used in this study were of analytical grade.

Experimental design

Animals were divided into 4 groups, of albino of animals (n = 6) each.

Toxicity was induced in Wistar rats by daily intraperitoneal injections of a freshly prepared solution of mercuric chloride at a dose of 1.25mg/kg body weight for 45 days.[17]

Group I	:	Normal untreated rats.
Group II	:	The GTE was made according to (Maity *et al*., 1998)[18] by soaking 15 g of instant green tea powder in 1 L of boiling distilled water for 5 minutes. The solution was filtered to make 1.5% green tea extract (GTE). This solution was provided to rats as their sole source of drinking water for 45 days.
Group III	:	Rats were injected with mercuric chloride (i.p)(1.25mg/kg body weight) (Sharma *et al*., 2007).[19]
Group IV	:	Rats were tr Rats were treated with mercuric chloride (1.25mg/kg body weight) as in group III and Green T Green t Green tea extract (1.5%) as in group II rats.

During the experimental period, food and water consumption were measured every day and the body weight was measured at the initial (day 1) and final day (day 46) of the experiment. At the end of the experimental period, animals in different groups were sacrificed by cervical decapitation under pentobarbitone sodium (60 mg/kg body weight) anaesthesia. Blood was collected and serum was separated by centrifugation and used for various biochemical estimations. The tissues were collected and used for the various estimations and for the histopathological studies.

Biochemical Analysis

Biochemical analysis were carried out to determine the serum concentrations of protein, albumin and total bilirubin and the activities of liver enzymes such as AST, ALT and ALP using diagnostic kits (Randox laboratories Crumlin, Co. Antrim, UK). Bilirubin was estimated by the method described by Jendrassik and Grof (1938).[20] Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated by the method of Reitman & Frankel (1957)[21] alkaline Phosphatase (ALP) method of Kind and Kings (1954).[22] GGT was determined by Rosalki *et al.*, (1970)[23], Urea was estimated by Fawcett and Scott, (1960)[24], uric acid Caraway, (1955),[25] Creatinine method by Tietz (1987).[26] Lipids Folch et al.(1957),[27] total cholesterol, Zlatkis et al., (1953)[28], HDL-cholesterol Burnstein *et al*., (1970)[29], Triglycerides Fossati and Lorenzo(1982)[30], Phospholipid (Zilversmit and Davis, 1950).[31] Free fatty acids Falholt *et al*.,(1973).[32] (TBARS) Niehius and Samuelsson(1968)[33], lipid hydroperoxides (LOOH) Jiang *et al*., (1992)[34] ascorbic acid Omaye *et al*., (1979).[35] Reduced glutathione (GSH) (Moran *et al*., 1979).[36] superoxide dismutase (SOD) Kakkar *et al*.,(1984)[37], catalase (CAT) Sinha, (1972)[38] Glutathione peroxidase (GPx) Rotruck *et al*.,(1973).[39]

Histopathological studies

For histological studies, the liver tissues were fixed with 10% phosphate buffer neutral formalin, dehydrated in graded (50- 100%) alcohol and embedded in paraffin, thin sections (5 µm) were cut and stained with routine hematoxylin and eosin (H & E) stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.[40]

Statistical Analysis

All data from *in vivo* studies were expressed as mean ± SD (n=6). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 9 (SPSS, Cary, NC, USA) and the individual comparison were obtained by Duncan's Multiple Range Test (DMRT). A value of $p<0.05$ was considered to indicate a significant difference between groups. Values sharing a common superscript letter do not differ significantly with each other at $p<0.05$.

RESULTS

The present study was carried out to evaluate hepatoprotective activity of green tea extract. The observations made on different groups of experimental and control animals were compared as follows.

Effect of GTE on body weight, hepatic markers, renal-function markers in $Hgcl_2$ hepatotoxic and control rats

Table 1 & Figure 1 represents the changes in body weight in control and Hgcl2 hepatotoxic rats between 0 and 21 days. Weight gain was loss in the Hgcl2 hepatotoxic rats when compared with control rats. Administration of GTE (1.5%) to Hgcl2 treated rats significantly increased the body weight. Control rats treated with GTE (1.5%) showed no alteration in body weight when compared with control rats. **Table 2 & figure 2** shows the activities of plasma hepatic marker enzymes such as AST, ALT, ALP, GGT and bilirubin in Hgcl2 hepatotoxic and control rats. Increased activities of AST, ALT, ALP, GGT and bilirubin levels were observed in Hgcl2 induced rats. Oral administration GTE improved these parameters towards normalcy. The effect of various doses of GTE on renal function markers levels are shown in **Table 3 & figure 3**. The levels of urea, uric acid and creatinine significantly increased in Hgcl2induced rats and treatment with GTE significantly decreased the levels of urea, uric acid and creatinine.

Effect of GTE on lipid profile of $Hgcl_2$ hepatotoxic and control rats

Table 4 represents the levels of total cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C in the serum of control and Hgcl2 treated rats. A significant elevation of serum TC, TG, LDL-C, VLDL-C and reduction in HDL-C were observed in Hgcl 2 induced rats. The administration of GTE decreased the levels of TC, TG, LDL-C and VLDL-C and increased the level of HDL-C. The tissue (liver, kidney) concentration of total cholesterol, triglycerides,

free fatty acids, phospholipids and liver glycogen of experimental rats are shown in **tables 5** and **6**. In Hgcl2 treated rats, the levels of these lipid parameters increased and the level of liver glycogen decreased. Treatment with GTE decreased the levels of lipid parameters to near normal and increased the levels of liver glycogen.

Effect of GTE on lipid peroxidation and antioxidant status in $Hgcl_2$ hepatotoxic and control rats

Table 7 represents the measurement of lipid peroxidation by the concentrations of (TBARS and lipid hydroperoxides) in the serum and tissues (liver and kidney) of Hgcl2 hepatotoxic and control rats. Increased levels of TBARS and lipid hydroperoxides were observed in the plasma and tissues of Hgcl2 administered group. Treatment with GTE significantly decreased the levels of TBARS and lipid hydroperoxides.

The activities of SOD, CAT and GPx in the erythrocytes and tissues are given in **Tables 8**, **9** and **10** respectively. The activities of these antioxidant enzymes were remarkably decreased in erythrocyte and tissues of Hgcl2 administered group. In response to GTE treatment, the activities of these enzymatic antioxidants significantly increased near normalcy. **Tables 11**, **12** and **13** show the levels of non-enzymatic antioxidants such as reduced glutathione, ascorbic acid and α-tocopherol in the serum and tissues of Hgcl2 hepatotoxic and control rats. The levels of non-enzymatic antioxidants in Hgcl2 hepatotoxic rats significantly decreased. Treatment with GTE, significantly increased antioxidant levels.

Histopathological changes

Histological changes in the liver are shown in **Fig**. **4**. Control rats showed central vein surrounded by normal hepatocytes. Control rat supplemented with green tea extract showed Central vein surrounded by normal hepatocytes. Mercuric chloride fed rats showed micro and macro vesicular fatty changes. Mercuric chloride with green tea extract treated rat showed normal hepatocyte with congested central vein and sinusoidal dilatation.

Table 1: Effect of GTE on body weight in $Hgcl_2$-hepatotoxic and control rats.

Groups	Body weight (g)		
	Initial (0 day)	Final (21 day)	Net gain (g)
Control	164.60 ± 4.08	188.55 ± 3.39[a]	24.66 ± 3.26
Control + GTE (1.5%.)	167.69 ± 5.84	189.11 ± 3.81[a]	23.50 ± 4.42
$Hgcl_2$+control (1.25 mg/kg BW.)	174.10± 7.07	179.12 ± 8.75[b]	6.66 ± 0.58
$Hgcl_2$ + GTE (1.5%.)	166.72 ± 5.84	181.10 ± 3.74[cb]	11.50 ± 3.39

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

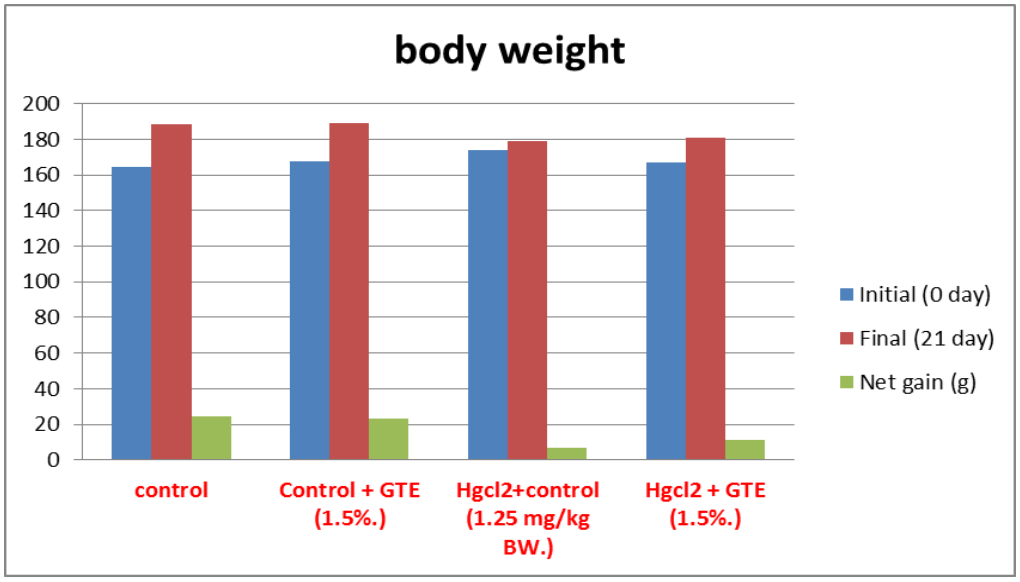

Fig. 1: Effect of GTE on body weight in $Hgcl_2$-hepatotoxic and control rats.

Table 2: Effect of GTE on hepatic marker enzymes and bilirubin in the serum of Hgcl2-hepatotoxic and control rats.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Bilirubin (mg/dL)
Control	74.28 ±7.38[a]	34.92± 2.62[a]	86.19 ± 7.01[a]	2.67 ± 0.16[a]	0.53 ± 0.05[a]
Control + GTE (1.5%.)	75.72± 6.57[a]	23.26 ±1.71[a]	85.59 ± 6.99[a]	2.31 ± 0.14[a]	0.52 ± 0.05[a]
$Hgcl_2$+control (1.25 mg/kg BW.)	120.47± 9.79[b]	72.24 ± 6.75[b]	129.71 ± 12.62[b]	4.90 ± 0.40[b]	1.17 ± 0.07[b]
$Hgcl_2$ + GTE (1.5%.)	102.17 ± 8.48[c]	62.27± 3.74[c]	121.98 ± 9.76[c]	4.11 ± 0.34[c]	1.10± 0.08[c]

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 3: Effect of GTE on the levels of Urea, Uric acid, Creatinine in the serum of Hgcl2- hepatotoxic and control rats.

Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	23.16 ± 1.87[a]	1.16 ± 0.09[a]	0.89 ± 0.06[a]
Control + GTE (1.5%.)	25.56 ± 1.65[a]	1.27 ± 0.09[a]	0.87 ± 0.06[a]
$Hgcl_2$+control (1.25 mg/kg BW.)	44.15 ± 3.28[b]	2.21 ± 0.17[b]	1.69 ± 0.14[b]
$Hgcl_2$ + GTE (1.5%.)	39.68 ± 2.81[c]	2.34 ± 0.21[c]	1.29 ± 0.10[c]

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 4: Effect of GTE on lipid profile in the plasma of $Hgcl_2$-hepatotoxic and control rats.

Groups	Serum (mg/dL)						
	Total cholesterol	HDL-C	LDL-C	VLDL-C	Triglycerides	Phospholipids	Free fatty acids
Control	77.70± 5.85[a]	49.26± 3.60[a]	16.41 ± 1.33[a]	10.92 ± 0.87[a]	58.63± 4.37[a]	112.29± 8.00[a]	51.67± 3.75[a]
Control + GTE (1.5%.)	72.80± 5.51[a]	47.88 ± 3.66[a]	12.96 ± 1.01[a]	10.75 ± 0.83[a]	53.78± 4.16[a]	101.20± 7.21[b]	53.73± 4.06[a]
$Hgcl_2$+-control (1.25 mg/kg BW.)	131.47± 8.86[b]	26.67± 2.06 [b]	84.59 ± 7.20[b]	25.85 ± 1.95[b]	126.81± 9.63[b]	169.35± 11.98[c]	116.99± 6.91[b]
$Hgcl_2$ +GTE (1.5%.)	113.73± 7.76[c]	34.80± 2.75[c]	65.18 ± 7.20[c]	26.81 ± 1.82[c]	20.06± 9.10[c]	149.62 ± 10.68[d]	96.03± 6.79[c]

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 5: Effect of GTE on lipid profile and glycogen in the liver of $Hgcl_2$-hepatotoxic and control rats.

Groups	Liver (mg/g of tissue)				
	Total cholesterol	Triglycerides	Phospholipids	Free fatty acids	Glycogen
Control	3.27 ± 0.29[a]	3.44± 0.37 [a]	17.95 ± 1.39[a]	7.32± 0.56[a]	49.66± 3.81[a]
Control + GTE (1.5%.)	2.87± 0.24[a]	2.99 ± 0.35[a]	17.22 ± 1.40[a]	6. 95± 0.63[a]	51.87± 4.00[a]
$Hgcl_+$-control (1.25 mg/kg BW.)	6.32 ± 0.45[b]	7.32 ± 0.72[b]	27.06 ± 2.17[b]	15.33 ± 0.99[b]	19.69 ± 1.55[b]
$Hgcl_2$ + GTE (1.5%.)	5.83 ± 0.39[c]	6.13 ± 0.56[c]	28.21 ± 1.92[c]	11.21 ± 0.91[c]	27.90 ± 2.16[c]

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT)

Table 6: Effect of GTE on lipid profile in the kidney of $Hgcl_2$- hepatotoxic and control rats.

Groups	Kidney (mg/g of tissue)			
	Total cholesterol	Triglycerides	Phospholipids	Free fatty acids
Control	3.69 ± 0.32[a]	4.35 ± 0.33[a]	14.21 ± 1.13[a]	4.81 ± 0.29[a]
Control + GTE (1.5%.)	3.62 ± 0.33[a]	9.16 ± 0.37[a]	13.91 ± 1.09[a]	4.21 ± 0.27[a]
$Hgcl_2$ +control (1.25 mg/kg BW.)	6.14 ± 0.58[b]	5.95 ± 0.59[b]	23.90 ± 1.98[b]	7.95 ± 0.59[b]
$Hgcl_2$ + GTE (1.5%.)	5.31 ± 0.39[c]	5.94 ± 0.46[c]	21.65 ± 1.67[c]	6.13 ± 0.59[c]

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 7: Effect of GTE on TBARS and lipid hydroperoxides in the serum and tissues of $Hgcl_2$-hepatotoxic and control rats.

Groups	serum (mmoles/dL)		Liver (mmoles/100 g wet tissue)		Kidney (mmoles/100 g wet tissue)	
	TBARS	LPO	TBARS	LPO	TBARS	LPO
Control	0.14 ± 0.02[a]	8.98 ± 0.83[a]	0.79 ± 0.05[a]	96.18 ± 6.82[a]	1.63± 0.13[a]	73.93 ± 5.58[a]
Control + GTE (1.5%)	0.14± 0.01[a]	9.19 ± 0.66[a]	0.78 ± 0.04[a]	82.71 ± 6.47[a]	1.33± 0.11[a]	69.53 ± 5.42[a]
$Hgcl_2$+ control (1.25 mg/kg BW)	0.38 ± 0.03[b]	19.44 ± 1.52[b]	1.83 ± 0.11[b]	158.96 ± 12.02[b]	2.43± 0.21[b]	148.57 ± 11.28[b]
$Hgcl_2$ + GTE (1.5%)	031± 0.03[c]	17.81± 1.39[c]	1.32 ± 0.12[c]	143.18 ± 10.91[c]	2.37 ± 0.21[c]	131.38 ± 10.69[c]

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT

Table 8: Effect of GTE on the activities of SOD, CAT and GPx in the serum of $Hgcl_2$- hepatotoxic and control rats.

Groups	Serum		
	SOD (/mg Hb)	CAT (/mg Hb)	GPx (/mg Hb)
Control	7.32± 0.63[a]	172.21 ± 10.23[a]	12.56± 1.34[a]
Control + GTE (1.5%.)	7.27 ± 0.54[a]	175.92 ± 15.12[a]	12.24 ± 0.80[a]
$Hgcl_2$+ control (1.25 mg/kg body wt.)	4.93 ± 0.06[b]	119.69 ± 9.16[b]	6.92± 0.67[b]
$Hgcl_2$ + GTE (1.5%)	6.10 ± 0.90[c]	140.14 ± 10.36[c]	8.90 ± 1.12[c]

Values are given as means ± SD for six rats in each group. Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 9: Effect of GTE on the activities of SOD, CAT and GPx in the liver of $Hgcl_2$ hepatotoxic and control rats.

Groups	Liver		
	SOD (/mg protein)	CAT(/mg protein)	GPx (/mg protein)
Control	8.35 ± 1.47[a]	77.09 ± 5.75[a]	9.16 ± 0.46[a]
Normal + GTE (1.5%.)	9.57± 0.85[b]	81.92 ± 8.11[b]	9.77 ± 0.80[b]
$Hgcl_2$+control (1.25 mg/kg BW.)	4.12± 0.45[c]	51.23 ± 3.82[c]	5.32 ± 0.58[c]
$Hgcl_2$ + GTE (1.5%.)	5.85 ± 0.87[d]	61.46 ± 4.58[d]	6.51 ± 0.30[d]

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 10: Effect of GTE on the activities of SOD, CAT and GPx in the kidney of $Hgcl_2$-hepatotoxic and control rats.

Groups	Kidney		
	SOD (/mg protein)	CAT (/mg protein)	GPx (mg protein)
Control	12.16 ± 1.59^a	34.99 ± 2.71^a	7.80 ± 0.59^a
Control + GTE (1.5%.)	17.17 ± 1.34^a	37.15 ± 2.74^a	8.56 ± 0.84^a
$Hgcl_2$+control (1.25 mg/kg body wt.)	7.14 ± 0.10^b	19.98 ± 1.64^b	4.22 ± 0.32^b
$Hgcl_2$ + GTE (1.25%)	9.85 ± 1.07^c	25.56 ± 2.99^c	5.44 ± 0.39^c

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 11: Effect of GTE on vitamin C in the plasma and tissues of $Hgcl_2$-hepatotoxic and control rats.

Groups	Vitamin C		
	Serum (mg/dL)	Liver (mg/100 g wt tissue)	Kidney (mg/100 g wt tissue)
Control l	2.30 ± 0.22^a	0.70 ± 0.06^a	0.61 ± 0.06^a
Control + GTE(50 mg/kg BW.)	2.44 ± 0.23^a	0.68 ± 0.06^a	0.65 ± 0.05^a
$Hgcl_2$+control (1.25 mg/kg BW.)	0.86 ± 0.08^b	0.28 ± 0.03^b	0.31 ± 0.02^b
$Hgcl_2$ + GTE (1.5%.)	1.44 ± 0.10^c	0.40 ± 0.08^c	0.40 ± 0.10^c

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT)

Table 12: Effect of GTE on vitamin E in the plasma and tissues of $Hgcl_2$- hepatotoxic and control rats.

Groups	Vitamin E		
	Plasma (mg/dL)	Liver (mg/100 g wet tissue)	Kidney (mg/100 g wet tissue)
Control	1.87 ± 0.15^a	5.82 ± 0.37^a	3.78 ± 0.19^a
Control + GTE(1.5%.)	1.91 ± 0.14^a	5.99 ± 0.41^a	3.55 ± 0.34^a
$Hgcl_2$+control (1.25 mg/kg BW.)	0.73 ± 0.05^b	2.30 ± 0.26^b	1.48 ± 0.18^b
$Hgcl_2$ + GTE (1.5%.)	1.07 ± 0.07^c	3.76 ± 0.37^c	2.25 ± 0.25^c

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

Table 13: Effect of GTE on Reduced glutathione in the plasma and tissues of $Hgcl_2$-hepatotoxic and control rats.

Groups	Reduced glutathione		
	Plasma (mg/dL)	Liver (mg/100 g wt tissue)	Kidney (mg/100 g wt tissue)
Control	33.37 ±1.99[a]	114.62 ±8.56[a]	108.81 ± 8.12[a]
Control l + GTE (1.5%.)	30.44 ±2.27[a]	111.36 ±8.23[a]	114.24 ± 8.47[a]
$Hgcl_2$+control (1.25 mg/kg BW.)	13.54 ±1.01[b]	73.89 ±5.51[b]	67.74± 5.06[b]
$Hgcl_2$ +GTE (1.5%.)	18.94 ±1.41[c]	84.88 ±8.38[c]	77.70 ± 5.76[c]

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at $p < 0.05$. (DMRT).

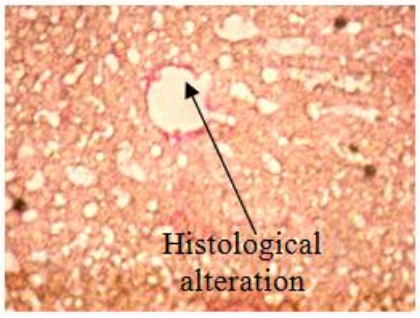

Fig: 1a, Group-1 Control rat liver shows no histological alteration

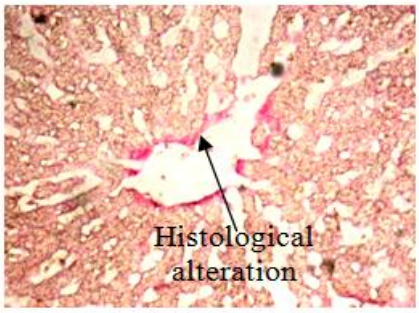

Fig: 1b, Group-2 Control +Drug treated rat liver shows no histological alteration

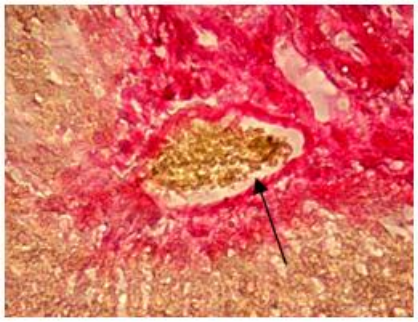

Fig: 1c, Group-3 Liver of Mercuric Chloride –treated rats shows the thickening markedly increased.

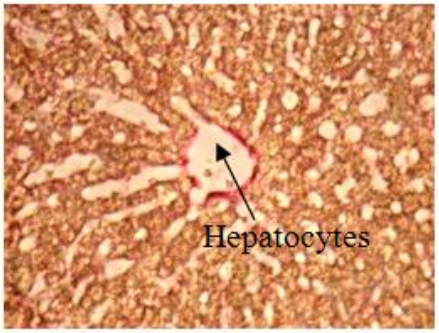

Fig: 1d, Group-4 Mercuric Chloride +Drug treated liver shows normal hepatocytes.

Fig. 2: Histological changes of liver.

DISCUSSION

A number of evidences advocate the role of oxidative stress in mercury induced renal and liver toxicity. Eventhough mercury induced neurotoxicity is not well understood; it has been shown that alterations in calcium and glutamate homeostasis, oxidative stress represent mercury may cause toxicity.[41,42]

The liver response to chemical injury can be extremely varied, reflecting the chemical's proper-ties, the exposure regimen and animal species examine.[43] Historically, identification and classification of hepatotoxic chemicals has been based upon morphologic changes, in which both the location and type of lesion are considered. However, serum enzyme tests, hepatic excretory tests or alterations in the chemical constituents have proved more sensitive indicators of damage. This classification is not to be confused with mode of action, which is best established by associating molecular and biochemical changes to hepatocellular dysfunction. The liver is also a major inflammatory organ and inflammatory processes participate in a number of pathological (i.e., necrosis and fibrosis), protective and repair events following exposure to hepatotoxic chemicals[44] determined the kidney function impairment and oxidative stress induced by $HgCl_2$ in rats. Oxidative stress and the production of free radicals have been suggested to be involved in mercury-induced renal injury[45] and the high affinity between mercury and endogenous thiol-containing molecules, such as GSH and δ-aminolevulinate dehydratase seems to contribute to this process. This finding suggested that mechanisms other than oxidative damage may also contribute to the renal cellular-injury induced by $Hgcl_2$. An obvious sign of hepatic injury is the leakage of cellular enzyme such as AST, ALT and ALP into the plasma significantly elevated levels of AST, ALT and ALP activity were observed levels in HgCl2 treated rats.[46] The increased activity of AST, ALT and ALP may be due to cellular necrosis of hepatocytes, which causes increase in permeability of cell resulting release of transaminases in the blood stream.[47] Administration of GTE caused a decrease in the activity of the above enzyme which may be consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by $Hgcl_2$. Reports suggested that green tea polyphenol could prevent liver damage by enhance cellular antioxidants by free radical scavenging activity.[48] Further research is needed to evaluate molecular mechanisms underlying protective effect of GTE against $Hgcl_2$ induced toxicity.

In our study administration of $Hgcl_2$ caused significant increase in the levels of free fatty acids, triglycerides, phospholipids and cholesterol reports.[47] The levels of cholesterol, free fatty acids phospholipids and triglycerides were significantly reduced in rats treated with GTE. It has been shown that GTE could lower the levels of lipid profile in plasma and liver.[49,50] Chronic administration of $Hgcl_2$ caused serious kidney injury. The involve move of the hydrogen peroxide and Nitrous oxide induce nephrotoxicity. Mercury induced oxidative stress is an important contribution to molecular mechanism in liver and kidney injury. Oral supplementation of GTE attenuates LDL, HDL, VLDL, triglycerides and phospholipids by unknown mechanism.[51]

Enhanced levels of TBARS in serum, liver and kidney of HgCl2 treated rats indicated the increased levels of lipid peroxidation. Reports have shown that mercury 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 highly reactive hydroxyl radical.[52,53,54]

The decrease in activities of antioxidant enzymes (SOD, CAT, GPx) in hemolysate and tissues (liver, kidney and brain) in mercuric chloride treated rats may be due to the inhibition of these enzymes by H2O2 and nitric oxide (NO). It is known that NO and H2O2 mercury induced acute renal injury.[55] Both GSH and cellular antioxidant enzymes play an important role in HgCl2 induced nephrotoxicity and liver injury[56] have shown HgCl2 inhibit the activities of renal SOD, CAT, GR, GPx in addition to depleting GSH content of kidney tissues.

Green tea is considered a dietary source of antioxidant nutrients: green tea is rich in polyphenols (catechins and gallic acid, particularly), but it also contains carotenoids, tocopherols, ascorbic acid (vitamin C), minerals such as Cr, Mn, Se or Zn, and certain phytochemical compounds. These compounds could increase the GTE antioxidant potential. GTP present antioxidant activity in vitro by scavenging reactive oxygen and nitrogen species and chelating red ox active transition metal ions; GTP can chelaete metal ions like iron and copper to prevent their participation in Fenton and Haber-Weiss reactions.[57,58]

The liver histology of the mercuric chloride administered rats showed pathomorphological alterations These changes were predominant in the centrilobular region having reduced oxygen perfusion. Hepatic damage may be partially attributed to cytochrome P450-dependent

enzyme activities in the liver; that tends to be present in the greatest concentration near the central vein and lowest near the peripheral sites.[59] In this context showed that Injury in liver and kidney due to mercury exposure was also supported by the finding from histological alterations.

Thus, green tea extract would appear to alleviate the adverse effects of mercuric chloride ingestion by enhancing the hepatic antioxidants effectively. Although green tea extract supplementation was clearly beneficial for mercuric chloride-treated rats, the detoxification mechanism at the pharmacological and biochemical level still needs to be elucidated; therefore, further studies to identify the effective hepatoprotective mechanism by this compound appears to be warranted.

CONCLUSION

Taken together, this study indicates that the administration of GTE has the capability to alleviate the toxic effects of mercuric chloride ($Hgcl_2$). GTE exhibited its protective effect by reducing oxidative stress induced lipid peroxidation, abnormalities in lipid metabolism as well as histopathological changes. Hence, the present study strengthen the observation that naturally occurring bioactive compounds of plant origin could be a potential therapeutic agent for Hgcl2 and other related heavy metals induced toxicity. However, more precise action mechanism of green tea extract on molecular targets should be delineated in future study.

Conflict of interest

The authors declare that they have no conflict of interest.

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