Histological and flow cytometrical effects of white tea extract against mercury-induced hepatotoxicity in mice

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ABSTRACT
Liver plays an important and pivotal role in transforming and remove chemicals that may cause the hepatotoxicity such as heavy metals especially mercury. The protective effect of white tea extract against the hepatotoxicity induced by HgCl2 in mice was studied. Biochemical, histopathological and flow cytometrical investigations were estimated. The animals were divided into four groups, each of 8 animals: Group 1 was served as the control group, the mice were injected i.p. with saline (1ml/day). In group 2, mice were orally administered with freshly prepared aqueous extract of white tea (100 mg / kg / day). Animals of group 3 were i.p. injected with HgCl2 (1mg/kg/day). Animals in group 4 were injected i.p for 14 days with HgCl2 then administered with white tea extract for another 14 days. The Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) levels and Alkaline phosphatase (ALP) level are significantly lower (P<0.05) in the mice of the control group than both mice in groups 3 and 4. Liver histopathological showed that white tea extract reduced fatty degeneration, cytoplasmic vacuolation and necrosis in HgCl2-treated mice. The significant increases in apoptotic cells were observed after the animals exposed to HgCl2 and decreases in the group exposed to HgCl2 and treated with white tea extract. This study suggests that white tea possesses antioxidant, anti-toxic and anti-apoptotic properties effects in the hepatoprotective effect against mercury toxicity.

INTRODUCTION
Heavy elements are natural elements found in the Earth’s crust and are present in different concentrations in all ecosystems. Human activities play a pivotal role in the pollution of the environment through the use of industrial materials contain toxic elements and compounds that lead to pollution of the environment, which makes pollution is a serious health problem. Unlike most organic pollutants, heavy metals are not degraded, and they have a tendency to accumulate in the soil, water sources and food chain.

Mercury is a highly toxic metal, results in a variety of adverse health effects including neurological, nephrolopathay, respiratory, immune, dermatologic, reproductive and development sequel. Inorganic...
Mercury in the environment is a definite toxic substance on human\textsuperscript{50}.

Mercury is used widely in agriculture as the anti-fugual agent, in medicine as a topical purificator, disinfectant and insecticide for parasites\textsuperscript{5}, Padding used in dental amalgam\textsuperscript{23}. Metallic mercury also use in thermometers, barometers and device for measuring blood pressure. The class is more exposed to mercury compounds are the dental care employees. The most frequent chemical forms to which humans and animals are exposed are elemental mercury vapor, mercury salts as mercuric chloride and organic mercury compounds such as methyl mercury\textsuperscript{9}. All of these salts are toxic to animals and humans. From the side of nutrition, seafood, and freshwater fish are further important sources of human exposure\textsuperscript{18,34}. Poisoning can result from inhalation, ingestion, or absorption through the skin\textsuperscript{17}. It is poorly absorbed from the gastrointestinal tract; however it tends to rapidly accumulate mainly in the kidney and in the liver\textsuperscript{12,28}.

Mercury can cause damage to vital tissues by different chemical mechanisms such lipid peroxidation\textsuperscript{22}, Created types of oxygen free radicals\textsuperscript{53} and through binding to the thiol sets\textsuperscript{85}. Mercury accumulated in the liver leads to a high level of malonaldehyde, which leads to induce hepatotoxicity\textsuperscript{22}. Treatment of rats with Hg showed significant increase in liver enzymes and damage of liver cells. Previous studies have disclosed that mercury chloride caused histopathological and fine structural lesions in the liver which leads to fatty degeneration and cell necrosis\textsuperscript{111}.

Tea from the young buds and leaves of \textit{Camellia sinensis} (L.) O. Kuntze (Theaceae) is the most broad consumed beverage in the world following water and is valued for its taste, aroma, health benefits, and cultural practices\textsuperscript{29}.

There is heightened interest in the possible health benefits of\textsuperscript{30}. In animal studies, tea and its individual constituents have been reported to inhibit cancers of the skin, lung, esophagus, stomach, liver, small intestine, pancreas, colon, prostate, bladder, and mammary gland. However, the extent to which this protection might translate to the human situation remains an open question\textsuperscript{30}. During commercial production, leaves of \textit{Camellia sinensis} undergo different degrees of processing, giving rise to various types of tea. White and green teas are the least processed types of tea and contain the highest levels of epigallocatechin-3-gallate (EGCG) and other monomeric catechins, whereas the more highly processed oolong and black teas have high levels of complex polyphenols called theaflavins and thearubigins\textsuperscript{30,42}. Different content of tea polyphenols can vary greatly by season, product growth, the process of storage and fermentation conditions.

In vivo, the white and green teas are inhibiting equally effective polyps in the small intestine of mice Apomixin, was strengthened tumor suppression by the joint management of white tea with sulindac, a non-steroidal anti-inflammatory agent\textsuperscript{37}. Epidemiological studies have indicated that green and white teas reduces the risk many types of cancer, including the stomach, lung, colon, rectum, liver, breast, and pancreatic cancer, etc\textsuperscript{25,27,52,56}.

In view of these considerations, this plant; white tea; was considered to be interesting for more detailed studies. Therefore, the present study has been designed to elucidate whether the white tea when administered with mercury can ameliorate the oxidative stress-mediated hepatic dysfunction caused by mercury using biochemical, histopathological and flow cytometrical approaches.

**MATERIALS AND METHODS**

**Experimental animals**

The experimental animals used in this study were male Swiss albino mice. Male Swiss albino mice aged 9 – 12 weeks and weighing 25 - 30 gm were used throughout the study. Animals were fed a commercially prepared diet and had free access to tap water \textit{Ad libitum}.

The experimental studies

The experimental studies were carried out under the laboratory conditions. Mice of nearly similar weight (25 - 30 gm) were selected and divided into 4 groups (n=8). The selected animal groups were treated as follows:

**Group 1, control**

Each animal in this group was injected daily intraperitoneally (i.p.) with saline (1ml/ day) for successive 14 days.
Group 2, white tea treatment

For successive 14 days each mouse in this group was orally administrated with freshly prepared aqueous extract of white tea (100 mg / kg body weight / day).

Group 3, HgCl₂ treatment

Each mouse in this group was given intraperitoneally (i.p.) dose of HgCl₂ (1mg/kg / day) for successive 14 days.

Group 4, HgCl₂ - white tea treatment

Each animal in this group was injected i.p. with HgCl₂ (1mg / kg / day) for 14 days, then treated orally with white tea extract (100 mg / kg body weight / day) for another successive 14 days.

Chemicals

1. Mercuric chloride (HgCl₂), purchased from Elgomhoria Company, Cairo, Egypt.
2. White tea, from local Market, Cairo, Egypt.

Examinations

Mice of each group were scarified by cervical dislocation at the end of the experimental periods and decapitation. Liver of each animal was obtained and divided into two samples; one of them was kept in buffered neutral formalin for histological examinations, while the other was kept in liquid nitrogen for flow cytometrical analysis. Also, blood serum was collected for biochemical analysis.

Histopathological studies

Liver specimens of all groups were collected, fast dissected and then they were fixed in neutral buffered formalin, dehydrated through alcohols, cleared in xylene and embedded in paraffin wax according to the method described by Drury and Wallington[10]. Five micrometer thickness paraffin sections were prepared and mount on clean slides. For histopathological studies, such sections were stained with Ehrlich’s haemotoxylin and counterstained with eosin.

Biochemical analysis

Determination of liver enzymes, the serum aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and albumin (ALB) were determined in accordance with the methods provided by the diagnostic kits (Nanjing Jianchen Bioengineering Institute, Nanjing, China).

Flow cytometry examination

Flow cytometry analysis was carried out as described previously by[21]. Immediately after samples of liver tissue were removed, they were immersed in cold RPMI liquid. Within 1 hour, each sample was mechanically disaggregated, hand homogenized and filtered through a 50 μm nylon filter. After washing and centrifugation, the suspension was diluted to a concentration of 2 X 10⁶ nuclei per ml, and then divided into 2 samples; one of them was stained immediately, while the other was stained after addition in equal proportions of lymphocytes from healthy donors (internal reference).

For each sample a minimum of 10,000 nuclei (range 10,000 to 100,000) was stained in a solution containing 50 mg/ml propidium iodide, 2 mg/ml ribonuclease and the 1 percent Triton. After repeat filtration with 50 μm nylon filter, samples were analyzed on flow cytometer equipment. Each DNA histogram was analyzed for peak position and for the percentage of cells in the different histogram regions of different groups. A minimum of 10,000 cells was analyzed by a FACSort (Becton Dickinson, Immunocytometry Systems, San Jose, CA, USA). The excitation wavelength was 488 nm at 150 mW, 10,000 nuclei / specimen. Histogram analysis of the red fluorescence emitted by the propidium iodide was accomplished manually by setting markers around the haploid (n), diploid (2n), and tetraploid (4n) peaks and calculating the percentage of each ploidy compartment.

Statistical analysis

The present data were analyzed by using SPSS 11.0 for Windows. The significance of differences was calculated by using one-way analysis of variance (ANOVA). \( P < 0.05 \) was considered statistically significant.

RESULTS

To investigate effects of White Tea extract on mercury-induced hepatotoxicity, the histopathological, biochemical and flow cytometrically investigations were estimated.

Histopathological observations

The liver sections of control group exhibited nor-
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**Biochemical assays**

Treatment of mercuric-intoxicated mice with white tea significantly modulated the hepatic functions concerning in serum ALT, AST, ALP and ALB levels were shown in (Figure 1). In HgCl$_2$ - treated group, the liver function tests revealed a significant increase in serum ALT (125.45±9.21, Figure 1A), AST (80.8+6.23, Figure 1B) and ALP (115±7.8, Figure 1C) activities and a decrease in ALB (2.04±0.16, Figure 1D) level, compared to the control group (P < 0.05). On the other hand, animals treated only with white tea extract exhibited a significant decrease in the activities of the serum marker enzymes, combined with an elevation in ALB.

**Flow cytometric observation (DNA content in liver cell measurement)**

In liver cells, as most dividing cells, the DNA content immediately after division, represents the diploid chromosomal complement (2N); this increases during the DNA synthesis phase (S). The flow cytometer technique measures the amount of DNA per cell by quantization the intensity of the fluorescence emitted by a DNA-bound dye flow past a high-intensity laser beam. Parameters of cell cycle analysis of liver samples in all groups using flow cytometry related to histopathologic diagnosis are shown in TABLE 1.
The mean apoptosis was shown a significant decrease in mercuric chloride and white tea treated group (group 4) (35.34±3.23) when compared with mercuric chloride treated group (group 3) which showed a significant increase value (p<0.001) (90.45±11.32). G1/0 phases were showed a mild significant decrease (P<0.05) in mercuric chloride treated group than that of control and white tea groups (1.97±0.34, 3.31±0.44 and 4.21±0.23 respectively) and slightly increased in group (4) (8.45±2.56).

Mice treated with white tea for 14 days after injection by Hgcl2 for two weeks were more or less similar to white tea treated group (group 2) and control group (group 1) in both S phases and G2/M phases, but significantly increased in G0/1 phase. There are no significances in G2 of mercuric treated group (group 3), but significantly decreased in both G0/1 and S phases (1.97±0.34 and 0.28±0.02 respectively) when compared to other groups. (TABLE 1).

### DISCUSSION

Hepatoprotective of medicinal plants against drug models of hepatotoxicity remaining an area that needs comprehensive scientific research. Hepatic injury is a common pathological feature which exists in many liver diseases. Liver fibrosis, cirrhosis and even liver cancer could result from the long existence of hepatic injury. Therefore, prevention and treatment of hepatic injury is a key to treating liver diseases clinically[49].

Liver injury induced by HgCl2 is the best characterized system of hepatotoxicity and is considered a new used model for the study of plant hepatoprotective activities[9].

Mercury is a highly hazardous pollutant, with a natural Hg emission of about 5,000 tons/year in the global. Among these, the anthropogenic Hg emission to the environment was estimated to be 4,000 ton/year[31]. Overloading Hg pollution by anthropogenic activities and industrialization could result to several catastrophes of Hg poisoning in the world.

In humans, the anti-oxidant defense is influenced by dietary components. White tea is very similar to green tea but it is prepared only from the buds and young tea leaves of the Camelia sinensis plant[34] whereas green tea is prepared from the matured tea leaves. White tea is also prepared via minimal processing compared to green tea, hence, the concentration of polyphenols and catechins are higher in white tea compared to green tea[20]. Venditti et al.[49] showed that the total polyphenol content and anti-oxidant activity of white tea is higher than green tea and black tea.

In the present study, the histological features of mercury exposure were further measured by H&E staining. It was found that Hg caused necrosis, disarranged liver structure, and caused acidophilic degeneration in the liver. It was also observed that Hg induced irregular nuclei, distributed chromatin, and vacuolated cytoplasm in hepatocytes. The results suggested that apoptosis occurred in hepatocytes after HgCl2 exposure. It visually showed that Hg induced hepatotoxicity, damaged liver tissue, and induced apoptosis and necrosis. While, the group treated with Hgcl2 and white tea extract showed mild degeneration of the hepatocytes without necrosis and binucleated cells that represent good sign of regeneration[17,31]. Fiorini et al.[13] demonstrated that treatment with epigallocatechin gallate, the major flavonoid component of white tea by oral administration significantly protects the liver after ischemia/reperfusion, possibly by reducing hepatic fat content, increasing hepatic energy status, and functioning as an anti-oxidant. Thephinlap et al.[47] reported that green and white tea constituents, epigallocatechin gallate and epicatechin gallate could be natural iron chelators that efficiently decrease the levels of free radicals in iron overload. Moreover, Sinha et al.,[48] reported that tea and its polyphenols may have a promising role in counteracting the devastating effects of arsenic. The reduction of Hg concentrations in the studied tissues of the rats treated with white tea combined with HgCl2 may be due to its chelating property. The present study suggested one possibility that white tea complexes with Hg ion that decreases its lipophilicity, and thus its gastrointestinal absorption where the chelating agents form an insoluble complex.
with Hg to remove it from Hg-burdened tissues\textsuperscript{[40]}. Results obtained from present study indicated that, hepatic dysfunction was confirmed by measurement of serum ALT, AST, ALP and ALB activities following HgCl\textsubscript{2} exposure. Serum ALT, AST, ALP and ALB levels are the diagnostic indicator of many pathological conditions.

It was observed that serum ALT activities were significantly elevated in the 1 mg/kg HgCl\textsubscript{2} groups, which was similar to previous studies\textsuperscript{[38]}. The increasing ALT, AST and ALP activities indicated that HgCl\textsubscript{2} caused hepatic damage and performed to confirm the changes of tissue morphology, apoptosis, and necrosis in the liver after HgCl\textsubscript{2} exposure as mentioned above. Since aminotransferases (ALT and AST) are an important class of enzymes linking carbohydrate and amino acid metabolism, the relationship between the intermediates of the citric acid cycle is well established. These enzymes are regarded as markers of liver injury. In addition, ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. Moreover, elevated ALP activity, which was used as a marker of liver adaptation to damaging factors, has been reported frequently in Pb-exposed animals\textsuperscript{[16]}.

It is well known that Hg binds to plasmatic proteins, where it causes alterations in a high number of enzymes. It can also perturb protein synthesis in hepatocytes\textsuperscript{[44]}. Hg is not able to induce free radicals directly, but it indirectly influences the processes of lipid peroxidation through damage to the protective anti-oxidant barrier\textsuperscript{[39]}. The Hg possesses a strong affinity to thiol groups of amino acids, especially cysteine. Hg may affect the anti-oxidant barrier via inhibiting the functional thiol groups of enzymes such as superoxide dismutase. Another and the best known enzyme, being inhibited via Hg binding to thiol groups of its activities center is dehydratase of delta-aminolevulinic acid (ALAD). An inhibition of ALAD activity leads to an accumulation of amino levulinic acid (ALA), which undergoes auto-oxidation inducing free radicals and in this way induces lipid peroxidation\textsuperscript{[14]}.

Tea and its polyphenols may have a promising role in that the substrate of SOD is the superoxide radical anion (O\textsuperscript{2−}) which is generated by the transfer of one electron to molecular oxygen. This is responsible both for the direct damage of biological macro-molecules and for generating other reactive oxygen species. SOD keeps the concentration of superoxide radicals at low levels and therefore plays an important role in the defense against oxidative stress\textsuperscript{[15]}.

The recorded results explained certain consistencies regarding the role of flow cytometry in the assessment of large hepatic injury induced by HgCl\textsubscript{2}. The distribution for the control group, there was no significant of aneuploidy in G2. The mean values of the G0, S and G2 cells were 3.31±0.44, 11.51±1.23 and 8.96±1.77, respectively. The results obtained show a significant increase in the percent of apoptotic cells in HgCl\textsubscript{2}-induced hepatocellular toxicity, which was reversed with white tea treatment. In this respect, Carter, et al.\textsuperscript{[8]} mentioned that, administration of white tea significantly inhibited the development of colonic aberrant crypts carcinomas and liver carcinoma. Since the white tea catechins partially protect DNA from OH radical-induced strand breaks and base damage through fast chemical repair\textsuperscript{[44]}. In addition, tea polyphenols provide cytoprotection and DNA protection against oxidative stress. The previous action may involve the following suggested mechanism, that the hydroxyl groups in the aromatic B ring of polyphenols are considered important in scavenging free radicals\textsuperscript{[19]}. Where the additional hydroxyl groups in tea polyphenols make it the most effective in reactive oxygen species (ROS) scavenging.

Furthermore, a recent study demonstrated that tea polyphenols might reduce ROS formation by blocking the ROS-generating enzymes and related oxidative signal transducers.\textsuperscript{[92]}

In conclusion, HgCl\textsubscript{2} injection increased Hg accumulation, hepatic histological injury, apoptosis, serum ALT, AST, ALP and decrease ALB activities. The results suggested that oxidative stress played a key role in the mechanism of HgCl\textsubscript{2}-induced hepatotoxicity. Disturbing the balance between free radicals production and anti-oxidant enzyme defense would induce pathological damage and dysfunction of the liver. In addition, the results may give us a new application of traditional biomarker enzymes, histological and flow cytometrical analysis to predict or discover HgCl\textsubscript{2}-induced hepatotoxicity in the early stage and to take timely action through combined determination of serum enzymes and oxidative stress in clinics. Meanwhile, white tea extract provided protective health
benefits to the liver by preventing Hg-induced oxidative stress in the present study. Therefore, our study would be valuable and beneficial for future studies about the anti-oxidant effects of white tea.

CONCLUSION

White tea possesses anti-oxidant, anti-toxic and anti-apoptotic properties effects in the hepatoprotective effect against mercury toxicity.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES


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J.K.Lin, P.C.Chen, C.T.Ho, S.Y.Lin-Shiau; Inhibi-
P.Li, X.B.Feng, G.L.Qiu et al.; Mercury pollution inAsia: a review of the contaminated sites.J.Hazard.Mater,
S.Khokhar, S.G.M.Magnusdottir; Total phenol, cat-echin, and caffeine contents of teas commonly con-sumed in the United Kingdom. J.Agric.Food Chem,
M.Jurczuk, J.Moniuszko-Jakoniuk, M.M.Brzoska;Involvement of some low-molecular thiols in theperoxidative mechanisms of lead and ethanol ac-tion on rat liver and kidney. Toxicology,
S.K.Katiyar, H.Mukhtar; Tea in chemopreventionof cancer: epidemiologic and experimental studies.Int.J.Oncol,
M.Jurczuk, J.Moniuszko-Jakoniuk, M.M.Brzoska;Involvement of some low-molecular thiols in theperoxidative mechanisms of lead and ethanol ac-tion on rat liver and kidney. Toxicology,
M.Flanigan; Validation of flow cytometry analysisin the objective assessment of spermatogenesis: comparison to the quantitative testicular biopsy. J.Urol.,
I.H.Hirsch, P.McCue, D.Kulp Lebensm.,
G.Santana-Rios, G.A.Orner, A.Amantana, C.Provost, S.Y.Wu, R.H.Dashwood; Potent antimutagenic activity of white tea in comparison with