PROTECTIVE EFFECTS OF WHITE TEA EXTRACT AGAINST MERCURIC CHLORIDE INDUCED HEPATOTOXICITY IN MICE

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Keywords: White tea, Mercuric chloride, hepatotoxicity, flow cytometry

ABSTRACT: The present work was aimed to evaluate the potential protective effect of white tea's extract against the liver injury in an experimental model of hepatotoxicity induced by HgCl₂ in mice. Histopathological investigations, liver functions and flow Cytometrical analyses were estimated. The experimental mice were divided into four groups, each of eight mice (each mouse weighed 25 - 30gm): Group 1 was served as the control group, the mice were injected i.p. with saline solution (1ml/ day). In group 2, mice were orally administered with freshly prepared aqueous extract of white tea (100 mg / kg / day). Mice of group 3 were i.p. injected with HgCl₂ (1mg/ kg / day); The experimental periods of the mentioned three groups were lasted for fourteen days. On the other hand, mice of group 4 were injected i.p. for 14 days with HgCl₂ then administered with white tea extract for another 14 days. The aspartate amino transaminase, alanine aminotransferase levels and alkaline phosphatase level are significantly lower (P<0.05) in both groups of the control group, and those treated with mercury and white tea extract than the mice treated only with mercury. Histopathological examination of liver showed that white tea extracts reduced fatty degeneration, cytoplasm 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 extracts possesses hepatoprotective effects on acute liver injuries induced by mercury, and these results may be related to the anti-oxidant, anti-toxic and anti-apoptotic properties of white tea.

INTRODUCTION: Heavy metals 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, results in a variety of adverse health effects, including the neurological, respiratory, immune, dermatologic, reproductive and development sequel. 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 used widely in agriculture as the anti-fungal agent, in medicine as a topical purificator, disinfectant and insecticide for parasites. The most frequent chemical form to which humans and animals are exposed to mercury is elemental mercury vapor, mercury salts as mercuric chloride and organic mercury compounds such as methyl mercury. Poisoning can result from inhalation, ingestion, or absorption through the skin.

Mercury can cause damage to vital tissues by different chemical mechanisms such lipid peroxidation, high level of malonaldehyde, created...
types of oxygen-free radicals and through binding to the thiol sets. Treatment of rats with Hg showed a significant increase in liver enzymes and damage of liver cells.

Tea from the young buds and leaves of *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.

In animal studies, tea and its individual constituents have been reported to inhibit cancers of the skin, lung, esophagus, stomach, 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. White and green teas are the least refined types of tea and contain the highest levels of epigallocatechin-3-gallate (EGCG) and other monomeric catechins, whereas the more highly refined oolong and black teas have high levels of complex polyphenol called theaflavins and thearubigins.

Epidemiological studies have indicated that green and white teas reduced the risk many types of cancer, including the stomach, lung, colon, rectum, liver, breast, and pancreatic cancer, etc.

In view of these considerations, 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 cytometrically 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. All mice were kept under the same laboratory conditions for one-week, as an acclimatization period.

**The experimental studies:**

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

**Group 1, control:** Each animal in this group was injected i.p. daily 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 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 cytometrically analysis. Furthermore, blood serum was collected for biochemical examination.

**Histopathological studies:** Liver specimens of all groups were collected and fast dissected, then they were fixed in buffered neutral formalin, dehydrated through alcohols, cleared in xylene and embedded in paraffin wax according to the method described by Drury and Wallington (1967). Five micrometer thickness paraffin sections were prepared and mount on clean slides. For histopathological studies, such as sections were stained with Ehrlich's hematoxylin 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 (Hirsch et al., 1993). 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 10^6 nuclei per ml., then divided into two 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, two mg/ml ribonuclease and 1 percent Triton. After the 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: Re 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 were considered statistically significant.

RESULTS:

Histopathological observations: The liver sections of the control group exhibited normal architecture where it consists of a roughly hexagonal arrangement of plates of hepatocytes radiating outward from a central vein in the center (Fig. 2A). Light microscopic examinations demonstrated that liver tissue of the animals administered white tea extract had a view similar to normal (Fig. 2B), while examinations of the liver obtained from mice treated with HgCl2 showed destruction of the normal hepatic architecture and severe pathological alterations, and many hepatocytes showed vacuolar degenerative changes in their cytoplasm (Fig. 2C). In addition, focal necrotic areas infiltrated with mononuclear leukocytes were observed to contain pyknotic and karyolitic nuclei of necrotic hepatocytes. Furthermore, central veins, portal veins and sinusoids were severely damaged; they appeared dilated and congested (Fig. 2D & E). On the other hand, treatment with white tea simultaneously after administration of HgCl2 revealed marked restoration of the hepatic configuration. Most nuclei exhibited normal shape, being rounded and centrally located except for few pyknotic ones. No inflammatory changes were observed (Fig. 2F).

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 (Fig. 1). In HgCl2 - treated group, the liver function tests revealed a significant increase in serum ALT (125.45±9.21, Fig. 1A), AST (80.8±6.23, Fig. 1B) and ALP (115±7.8, Fig. 1C) activities and a decrease in ALB (2.04±0.16, Fig. 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 level.

Flow cytometric observation: (DNA content in liver cell's 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 dyes 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 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 the 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.

![Graphs showing level concentration of Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Albumin (ALB) in different experimental animal groups.](image-url)
FIG. 2: MICROGRAPH OF HISTOLOGICAL SECTIONS EXHIBITED (A) GROUP 1, NORMAL HEPATOCYTES. (B) GROUP 2, LIVER TISSUES OF THE ANIMALS ADMINISTERED WHITE TEA EXTRACTS. (C, D & E) GROUP 3, THE LIVER OF MICE TREATED WITH HgCl₂. (F) GROUP 4, THE MICE TREATED WITH WHITE TEA SIMULTANEOUSLY AFTER ADMINISTRATION OF HgCl₂. (H&E, X 250).
Phases in different experimental groups

- Results showed that Hg induced hepatic injury is a common pathological feature which exists in many liver diseases. Liver injury induced by HgCl₂ is the best characterized system of hepatotoxicity and is considered a new used model for the study of plant potential hepatoprotective activities. Therefore, prevention and treatment of hepatic injury are a key to treating liver diseases clinically. In humans, the antioxidant 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 *Camellia sinensis* plant 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 is higher in white tea compared to green tea.

In the present study, the histological features of mercury exposure were further measured by H&E staining. The results 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 a good sign of regeneration. Sohle *et al.* demonstrated that treatment with epigallocatechin gallate, the major flavonoid component of white and green 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 antioxidant.

**DISCUSSION:** Hepatoprotective potentials of medicinal plants against drug models of hepatotoxicity remain an area that needs comprehensive scientific research. Hepatic injury is a common pathological feature which exists in many liver diseases. Liver injury induced by HgCl₂ is the best characterized system of hepatotoxicity and is considered a new used model for the study of plant potential hepatoprotective activities. Therefore, prevention and treatment of hepatic injury are a key to treating liver diseases clinically. In humans, the antioxidant 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 *Camellia sinensis* plant 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 is higher in white tea compared to green tea.

Hg is not able to induce free radicals directly, but it indirectly influences the processes of lipid peroxidation through damage the protective antioxidant barrier. Like cadmium and Pb; Hg possesses a strong affinity to thiol groups of amino acids, especially cysteine.

Where the best-known enzyme, being inhibited via Hg binding to thiol groups of its activity's center and this led to dehydrate the delta-aminolevulinic acid (ALAD), and an inhibition of ALAD activity led to an accumulation of amino levlunic acid (ALA), which undergoes auto-oxidation inducing free radicals and in this, way induces lipid peroxidation.

Tea and its polyphenol may have a promising role in that the substrate of SOD is the superoxide radical anion (O₂⁻) 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. The recorded results explained certain consistencies regarding the role of flow cytometry in the assessment of large hepatic injury induced by HgCl2. 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, showing a significant increase in the percent of apoptotic cells in HgCl2-induced hepatocellular toxicity, and this group was reversed with white tea treatment. In this respect, Carter, et al., mentioned that, administration of white tea significantly inhibited the development of colonic aberrant crypt's carcinomas and liver carcinoma. Since green and white tea catechins partially protect DNA from OH radical-induced strand breaks and base damage through fast chemical repair. In addition, tea polyphenol provide cytoprotective and DNA protection against oxidative stress may involve the following suggested mechanism, that the hydroxyl group in the aromatic B ring of polyphenol are considered important in scavenging free radicals, where the additional hydroxyl groups in tea polyphenol make it the most effective in reactive oxygen species (ROS) scavenging.

Furthermore, a recent study demonstrated that tea polyphenol might reduce ROS formation by blocking the ROS-generating enzymes and related oxidative signal transducers.

CONCLUSION: We have clearly demonstrated for the first time that white tea polyphenol possess potent preventive effects against HgCl2-induced hepatotoxicity, apoptosis, serum ALT, AST, ALP and decrease ALB activities and flow cytometrically analysis to predict or discover HgCl2-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 extracts 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 antioxidant effects of white tea.

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How to cite this article:
Abdella, IJPSR, 2017; Vol. 8(2): 603-610. E-ISSN: 0975-8232; P-ISSN: 2320-5148

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