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The protective role of ethanolic extract of ginseng (Panax ginseng) against adverse physiological and histological alterations in the liver of female rabbits exposed to chlorine

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Ginseng, chlorine, MDA, liver enzymes and blood parameters.

Abstract

Background: Ginseng (*Panax ginseng*) has garnered considerable attention due to its medicinal properties. This study aimed to assess the protective effects of ginseng ethanolic extract against chlorine-induced toxicity in female rabbits.

Methods: Thirty-two female rabbits were randomly divided into four groups: a negative control group receiving chlorine-free water, a positive control group receiving tap water with chlorine (0.05 ppm/L), a group exposed to chlorine (5 ppm/L) in water, and a group exposed to chlorine (5 ppm/L) along with ethanolic ginseng extract (200 mg/kg body weight). Treatment lasted for four weeks.

Results: revealed that oral exposure to chlorine led to oxidative stress, evidenced by elevated levels of malondialdehyde (MDA) and liver enzymes (AST & ALT), as well as suppressed blood parameters (PCV, HB, and RBC count), and histological liver alterations compared to the normal control group. Conversely, administration of ginseng extracts alongside chlorine for four weeks reduced MDA levels and liver enzyme activity while enhancing blood parameters, accompanied by decreased inflammatory histological changes.

Conclusion: These findings suggest that ginseng extract possesses potent antioxidant activity, mitigating chlorine-induced toxicity and its associated pathophysiological consequences.

Introduction

Chlorine (Cl₂) is widely employed as a water disinfectant due to its effectiveness in eradicating waterborne diseases. However, its use raises concerns regarding the formation of disinfection by-products (DBPs) and potential adverse health effects [1]. Chlorine plays a critical role in developed countries for water sanitation, effectively combating waterborne diseases like cholera, typhoid, and dysentery [2]. However, its volatile nature poses inhalation risks, and its reaction with organic materials in water generates harmful disinfection by-products (DBPs). These DBPs, including trihalomethanes, chloroform, bromoform, haloacetic acid, among others, are known to have toxic effects on health, physiology, and can even contribute to cancer development. Trihalomethanes and haloacetic acid are commonly found in treated water and are monitored in various countries to assess the level of DBP contamination and its adverse effects on bodily functions [3]. Furthermore, exposure to chlorine and its by-products can induce oxidative stress, disrupt metabolic pathways, and lead to dysfunction in multiple organs. Thus, while chlorine is essential for water disinfection, its potential health risks, including DBP formation and oxidative stress induction, highlight the need for appropriate management and alternative approaches to ensure water safety without compromising public health [4,5]. Ginseng, a widely recognized medicinal plant, is known for its numerous bioactive compounds and natural antioxidants. These substances play a crucial role in strengthening the body's endogenous antioxidant defenses against reactive oxygen species (ROS), thereby restoring the optimal balance and aiding in disease prevention. Among the thousands of potential medicinal plants, *Panax ginseng* stands out as one of the most economically significant. This plant has been revered for centuries in America for its nutritional and medicinal values, with both its roots and leaves being edible [6]. *Panax ginseng*, also known as Korean ginseng or Asian ginseng, is primarily found in eastern Asia, including regions such as China, Korea, Bhutan, and eastern Siberia [7]. The active compounds in ginseng, including ginsenosides (ginseng saponins), phenolic compounds, polysaccharides, and polyacetylenes, contribute to its pharmacological activities [8]. These activities include antioxidant properties, immune enhancement, anti-aging effects, stress reduction, anti-inflammatory effects, anti-neoplastic effects, and anti-tumor activity. Given the diverse therapeutic potential of ginseng, the current study aimed to assess the protective efficacy of ginseng ethanolic extract against the adverse physiological effects induced by chlorine exposure in female rabbits [9].

This investigation seeks to leverage the antioxidant properties of ginseng to counteract the oxidative stress and other detrimental effects triggered by chlorine exposure, thereby offering potential therapeutic benefits in mitigating chlorine-induced toxicity.

Methods

Procurement of *Panax Ginseng*:

Panax ginseng roots were obtained from the market in Thi Qar city, Iraq.

Preparation of Roots

Washed the ginseng roots thoroughly with distilled water to remove any impurities. Then, carefully dried the roots at room temperature for 5-7 days under shade until they were completely dried.

Cleaning and Grinding

Cleaned the dried roots to remove any remaining debris or dirt. Grind the cleaned roots into a fine powder using a grinder.

Extraction of Leaves:

50g of the powdered ginseng leaves were taken. The measured quantity of leaves was placed into a round-bottom flask.

Extraction Solvent Preparation

200ml of 70% ethanol was added to the round-bottom flask containing the powdered ginseng leaves.

Extraction Process

The mixture was allowed to stand for 12 hours using a reflux extractor setup. This allowed for efficient extraction of bioactive compounds from the ginseng leaves into the ethanol solvent.

Filtration

After the extraction period, the mixture was filtered using Whatman filter paper (No. 31) to separate the extracted solution from the solid residue. This step helped to obtain a clear extract.

Drying of Extract

The filtered extract was transferred into a clean petri dish and left to dry naturally under shade. This process helped to evaporate the ethanol solvent, leaving behind the concentrated ginseng extract.

Collection and Storage

Once dried, the ginseng extract was collected and stored in a tightly closed container at 4°C until further use. Proper storage helped to maintain the stability and potency of the extract over time.

Dosage Preparation

The desired dosage for the experiment was determined. In this study, doses of 100mg/kg and 200mg/kg of the ginseng extract were utilized.

Usage in Experiment

The prepared ginseng extract was then used in the experiment, as described in the methodology, to evaluate its protective efficacy against chlorine-induced adverse effects in female rabbits [10]. Treatment and Animal Housing Conditions: Thirty-two female rabbits, each weighing approximately (grams), were housed in the departmental Animal House in pairs within plastic cages. The animals were acclimated for 14 days prior to the experiment under controlled conditions (temperature: 25±2 °C, 12-hour light/dark cycle) with free access to food and water. The rabbits were randomly divided into four experimental groups, with eight rabbits per group, as follows:

- Group 1 (Negative Control): Rabbits received chlorine-free drinking water.
- Group 2 (Positive Control): Rabbits received tap water with chlorine estimated at 0.05 ppm/L.
- Group 3: Rabbits received water with chlorine at a concentration of 5 ppm/L.
- Group 4: Rabbits received water with chlorine at 5 ppm/L, along with ethanolic ginseng extract (200 mg/kg body weight), administered one hour after water intake.

According to [11], the treatment continued for four weeks.

Blood samples, traits, and physiological assay:

Hemoglobin (Hb) Measurement: Hemoglobin levels were determined spectrophotometrically using a suitable method described in reference [12].

Packed Cell Volume (PCV): The packed cell volume, also known as hematocrit value, was measured using a spectrophotometric method according to the procedure outlined in reference [12].

Red Blood Cell (RBC) Count: The RBC count was determined using a spectrophotometric method as per the protocol described in reference [12].

Malondialdehyde (MDA) Measurement: Levels of malondialdehyde, a marker of oxidative stress, were assessed using an Enzyme-Linked Immunosorbent Assay (ELISA) method following the procedure outlined in reference [13].

Transaminase Enzymes (AST, ALT) and Alkaline Phosphatase: Levels of transaminase enzymes (aspartate transaminase - AST, alanine transaminase - ALT) and alkaline phosphatase were measured using a Reflotron apparatus with commercially available kits according to the manufacturer's instructions, as referenced in [14]. These analytical methods were

chosen to assess various physiological parameters, including blood traits and oxidative stress markers, to evaluate the impact of ginseng ethanolic extract on mitigating the adverse effects induced by chlorine exposure in female rabbits.

Tissue Samples: liver tissues were collected from rabbits, washed with normal saline and prepared then were fixed by using 10% formalin for histological examination.

Chemical implementation:

Chlorinated water at concentration (5 ppm/L) prepared previously before each use for animals' groups were treated with chlorinated water [15]. Tap water quality criteria investigate to assess the liberated chlorine levels as(ppm/L) by gathering samples of water and using pocket digital colorimeter that analysis water samples for liberated chlorine by following the instruction of using, likewise the device is modern technology with high sensitivity about range from 0.01 up to 1.00 ppm/L [16]. The Statistical Analysis: Mean values of serum indices were analyzed by one-way analysis of variation (ANOVA), obtained mean differences and standard deviations (Mean ± SD) between treated and control groups, P-values (P<0.05) are considered statistically significant. Table (1) serum MDA and blood parameters in control group and treated groups with Ginseng extract plus chlorine in female rabbits. Data are expressed as mean ± SD (n=8). The different letters refer to significant difference (P< 0.05).

Results

The results table (1) indicates that the exposure to tap water 2nd group and Chlorine 5ppm water 3rd group induced remarkable alteration in blood indices, there is a significant reduction $p \leq 0.05$ in the average of RBC, Hb and PCV compared with control 1st group, While the treatment with ethanolic extract ginseng and chlorine resulted in notable increase in RBC, Hb and PCV values comparison with group exposed to chlorine alone the decline in those components mostly in the Second, third groups, also the data illustrated in the table (1) revealed remarkable elevation in MDA level to Tap water (2nd group) and Chlorine 5 ppm water (3rd group) compared to normal rabbits, whereas the receiving animals ethanolic extract ginseng and chlorine (group 4th) prominent reduction in MDA level as compared to the chlorine group.

Data obtained from the table (2) the outcomes exhibited that the oral exposure of Tap water (2nd group) and Chlorine 5ppmwater (3rd group) induced prominent increase $p \leq 0.05$ in levels of liver enzymes (AST and ALT) compared to the corresponding control, whereas the animals received ethanolic extract ginseng

and chlorine (group 4th) produced a notable decrease in levels of the above enzymes comparison to chlorine. These results clarified that the hepatotoxicity was artificial in normal, healthy rabbits with metabolic activity stable, so any effect of chlorine or the indirect influence of liver enzymes on hepatocytes activity.

Parameters Groups	RBC (10 ⁶ cell/mm ³)	Hb g/100ml	PCV%	MDA μ mol/L
Chlorine free water 1st group	6.76±0.33a	12.94±0.57 a	43.78±2.14 a	0.54±0.03 b
Tap water 2nd group	4.87±1.21b	10.87±0.36 b	36.16±2.12 b	2.18 ±0.10 a
Chlorine 5ppm water 3rd group	3.92±0.34 b	9.01±1.08 c	26.89±1.99 c	3.13 ±1.86 a

Table 1: Serum MDA and blood parameters in control group and treated groups with Ginseng extract plus chlorine in female rabbits. Data are expressed as mean ± SD (n=8). The different letters refer to significant difference (P< 0.05).

Parameters Groups	ALT unit/L	AST unit/L
Chlorine free water (1st group)	52.9 ±2.68c	38.17±3.29c
Tap water (2nd group)	74.87±1.21b	57.87±0.36 b
Chlorine 5ppm water 3rd group	83.92±0.34a	72.01±1.08 a
Chlorine 5ppm water GN(200mg/kg) 4th group	54.81±0.22 c	45.82±0.83c

Table 2: liver enzymes in control group and treated groups with Ginseng extract plus chlorine in female rabbits. Data are expressed as mean ± SD (n=8). The different letters refer to significant difference (P< 0.05).

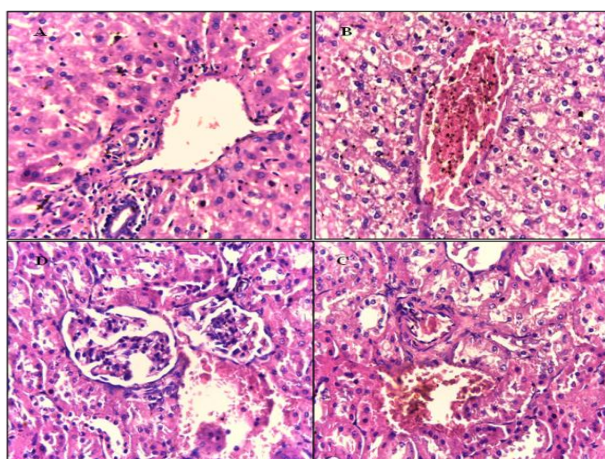


Figure 1: (A, B, C and D): Liver section of female rabbits (control group), the normal structure of the hepatic lobule with normal central vein (CV) and portal vein (PV). (Fig :B and c) female rabbits treated with chlorine for four weeks, sever congestion and dilated in portal vein, and sinusoid extended (thin arrow), with fibrosis, aggregation in inflammatory cells around bile duct and vein (thick arrow) and hyperplasia of bile duct (BD). H&E. X40 I. (Fig: D) female rabbits treated with ginseng extract (200mg/kg B.W) and chlorine for 4 weeks, sinusoid less dilated (thick arrows) with regeneration in some area which appeared with early hyperplasia (head arrow), inflammatory cells filtration (thin arrow) and less congested in central vein.40 X H&E.

Discussion

As shown in table (1) the results indicate that the exposure to Tap water 2ndgroup and Chlorine 5ppm water 3rd group induced remarkable alteration in blood indices, there is a significant reduction $p < 0.05$ in the average of RBC, Hb and PCV compared with control 1st group, While the treatment with ethanolic extract ginseng and chlorine resulted in notable increase in RBC, Hb and PCV values comparison with group exposed to chlorine alone the decline in those components mostly in the Second, third groups could attributed to the high portions of chlorine and due to disinfection the water by chlorine and overlapping or suppression the excretion of erythropoietin hormone from the kidney and with a low portions from the liver, which the first and most responsible for synthesis of erythrocytes from bone marrow, may be due to hematotoxicity caused by chlorine treatment which lead to decrease synthesis of red blood cells in bone marrow. Chlorine caused a significant decrease in the levels of RBC count, Hb and PCV indicating anemia. This anemia may come in part from the effects of free radicals generated by the chlorine on RBC. Several studies have suggested that RBC are particularly vulnerable to oxidative stress because they are exposed to higher concentrations of oxygen and, thus, Hb can easily be oxidized [17]. On the other hand, the activity of ALAD (d-aminolevulinic acid dehydratase which represents a key regulatory enzyme of heme synthesis pathway) is highly sensitive to the presence of stressful chemical; ALAD is a sulfhydryl-containing and its inhibition can be attributed to the binding of chlorine with sulfhydryl groups), the inhibition of ALAD enzyme and the reduction of Hb and RBC led to decreased heme synthesis and ultimately anemia and therefore lowering in Hb, PCV estimates this a greed and confirmed with [1,18], increased in estimates of blood traits Hb and PCV table (1) in the treated with ethanolic extract ginseng and chlorine group could be attributed to The extract of *P. ginseng* possesses antioxidant properties was explained by the presence of ginsenosides which are the main active compounds in ginseng, that reducing oxidative stress [18]. This protective effect may be due to the higher ability to eliminate free radicals and protect the cell membranes from the lipid peroxidation [19], The data illustrated in table (1) revealed remarkable elevation in MDA level to Tap water(2nd group) and Chlorine 5ppmwater (3rd group) compared to normal rabbits, whereas the receiving animals ethanolic extract ginseng and chlorine (group 4th) prominent reduction in MDA level as compared to the chlorine group.

The outcomes of this study (Table 1) revealed significantly ($p < 0.05$) higher in malondialdehyde concentration especially in female rabbit serum that

treated Tap water and Chlorine 5ppmwater compared with another groups could be attributed to the portability of (chlorine) to activating the oxidation and producing the free radicals and increasing fat peroxidation leading to oxidative stress, therefore increasing malondialdehyde concentration as an important production of peroxidation or (chlorine) may be cause activating to enzymes that in change of unsaturated fatty acid oxidation, which lead to producing hydrogen peroxide and therefore disorder in balance between production of free radicals and antioxidant defenses which caused tissues damage and increased MDA production, this outcomes agreed with [20,21]. The present study showed that the co-administration of ginseng combined with chlorine may have beneficial role and protective effects against chlorine intoxication. That role played by ginsenosides free phenolic fractions have been shown to induce the cytosolic antioxidant enzyme superoxide dismutase by enhanced nuclear protein binding to its gene regulatory sequences. Ginseng contains polyphenols, flavonoids, saponins, these properties effects might be due to the potential antioxidant, free radical scavenging radical mechanism and detoxification effected, these results similar to those [7, 9]. Data obtained from table (2) the outcomes exhibited that the oral exposure of Tap water (2nd group) and Chlorine 5ppmwater (3rd group) induced prominent increase $p \leq 0.05$ in levels of liver enzymes (AST and ALT) compared to the corresponding control, whereas the animals received ethanolic extract ginseng and chlorine(group 4th) produced a notable decrease in levels of the above enzymes comparison to chlorine. The progressively increased in the outcomes of (AST and ALT) in second and third groups could be attributed to the oxidative stress in liver and the injury done to tissues that contains those enzymes especially liver cells resulting in production free radicals (ROS) that be impact in cell activity especially cell wall functions, as considered chlorine as toxic and harmful impact materials to cell functions from through the elevation in the biomarkers of liver (Transaminases activity) indicator to cellular damage of liver hence released these enzymes in to circulation, the oxidative stress and hepatic injury induced by this by- products, similar results were agreed and confirmed by some researches [22]. On the other hand, Co- administration of ginseng extract combined with chlorine restored the levels of enzymes in the serum of the rabbits as an indication of protective effect of ginseng extract against liver damage induced by chlorine. This is consistent with [26], who stated that ginseng has a productive effect on the hepatotoxicity The hepatoprotective effects of ginseng extract by diminishing AST and ALT activities

in serum due to three of its components (falcarinol, octadecadienoic acid and acetate [23,24].

Histopathological Study of Liver

A histopathological assessment in the liver of the control groups were evaluated, consist of a repeated hexagonal unit called hepatic lobules, each lobule is occupied by center vein, hepatocytes and sinusoid are arranged in cords radiating from the central hepatic (Fig. 1A) the inter lobular in the portal vein where the branches of a hepatic artery, hepatic portal vein and bile ducts, blood sinusoids were lined with Kupffer cells and endothelial cells (Fig. 1A).

After 4weeks from orally given chlorine, the liver of rabbits showed alteration in structure, dilation and congestion of central vein and sinusoid capillaries, moderate on largamente of hepatic cell, which containing to the portal triad to the portal triad could be revealed to the formation of fibrosis, infiltration of mononuclear inflammatory cells in the portal area and central vein, thickening in the lining endothelial layer, some nuclei chromatin was fragmented and cytoplasm contained many vacuoles and necrotic in some area (Fig 1B,1C). When rabbits treated with ginseng extract (200mg/kg) plus chlorine the liver examination revealed amelioration section from treated chlorine 4 weeks alone clear central and portal veins were filled with erythrocytes, all hepatocytes had eosinophilic cytoplasm, some of these vacuolated cytoplasm and sinusoid were narrowed and irregular shaped (Fig 1D.), This results clarified as the hepatotoxicity was artificially in normal, healthy rabbits with metabolic activity stable, so any effect of chlorine or the indirect influence of liver enzymes on hepatocytes activity. Previous suggest on showed clear histological changes included damaged of the hepatocytes, wider sinusoid spaces and presence of binucleated cells indicating proliferation and regeneration. inflammatory cells in the hepatic tissue and necrosis due to chlorine exposure may suggest that chlorine might interact with proteins and enzymes of liver interstitial tissue interfering with the antioxidant defense mechanism and leading to generation ROS, which in turn may imitate an inflammatory. The ameliorating effects of ginseng which significantly inhibits liver fibrosis by inhibiting activation, proliferation and expression of collagen, transforming growth factor and tissue inhibitor of metalloproteinase-1 in hepatic stellate cell, the major cause of liver fibrosis [20-24]. However, the ginseng extract regulating inducible hepatic enzymes, and might be associated with modulating liver cytochrome P450 activation and protein 152 phosphorylation, similar have also been reported by [6].

Author Contributions

Shireen Ali Hasan conceptualized, supervised the study, and collected data, performed experiments, did data analysis and reviewing manuscript, Alyaa Abdalazq Abass helped in computational analysis and Layth Jabbar was involved in proofreading the manuscript

This study used the procedures agreed upon by the Standing Committee for Scientific Research Ethics at University of Thi-Qar/ College of Pharmacy.

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