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Effect of spinach-derived glutathione against carbon tetrachloride-induced stress in rats

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ABSTRACT

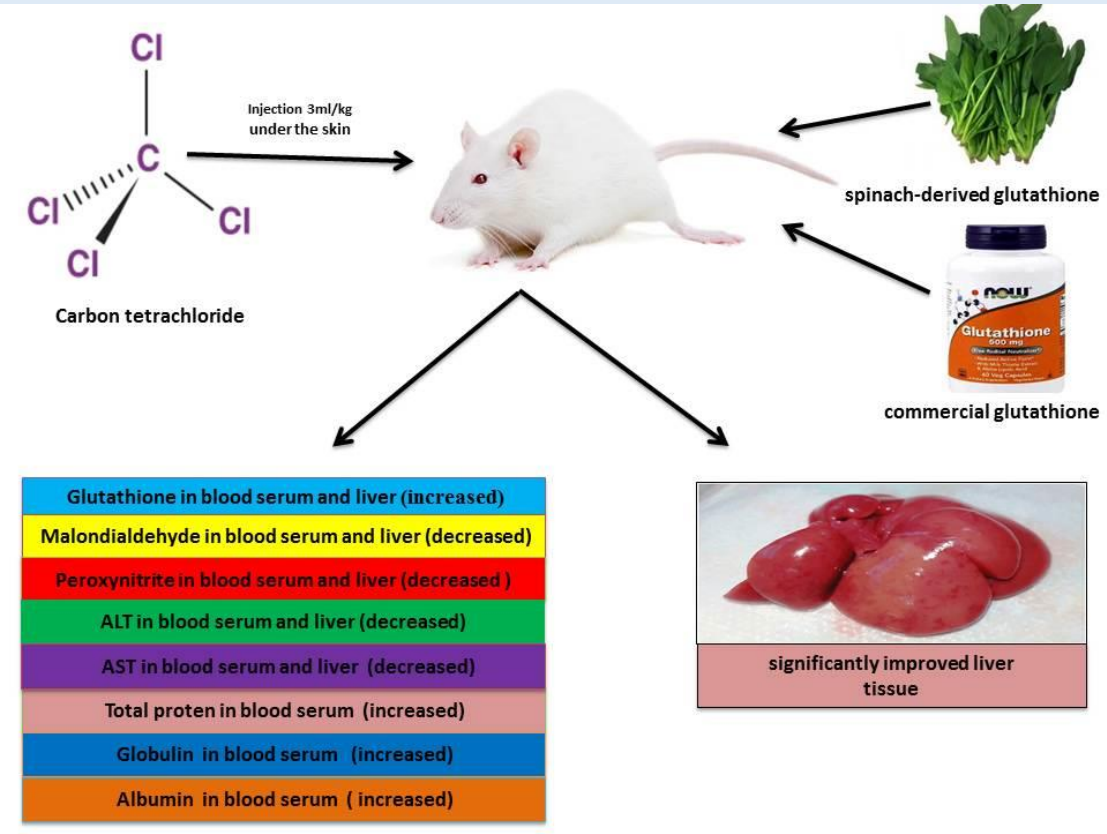
Background: Increased consumption of natural antioxidants found in vegetables and fruits has been linked to a lower risk of disease caused by oxidative stress.

Objective: The current study's focus was to compare the antioxidant properties of synthetic glutathione and glutathione derived from spinach against CCl4-induced stress in female albino rats.

Materials and Methods: Cold chloroform extraction was used to prepare the spinach leaf extract. Glutathione was then extracted from the spinach leaf extract using preparative HPLC afterward when the concentration of glutathione was measured in the extract. Biological preventive and therapeutic experiments were conducted, where laboratory rats were divided into 6 groups, the first group G1 (positive control group), the second group G2 (negative control group), the third group G3 preventive experiment 1 (group glutathione extracted from spinach + CCl4), the fourth group G4 therapeutic experiment 1 (Group CCl4+ Glutathione extracted from spinach), the fifth group G5 Preventive experiment 2 (Group of synthetic glutathione + CCl4), the sixth group G6 therapeutic experiment 2 (Group CCl4+ Glutathione extracted from spinach).

Results: Treatment of rats with spinach-derived synthetic glutathione, before or after they were given CCl4 subcutaneous, improved the values of parameters near to normal levels in the positive control animals in groups treated with spinach-derived glutathione (G3 and G4) compared to treatment with synthetic glutathione (G5 and G6). Administration of milk with glutathione derived from Iraqi spinach leaves exhibited favorable effects on oxidative stress, greatly enhanced the antioxidant system, and protected rats from liver damage brought on by carbon tetrachloride compared to milk with synthetic glutathione.

Keywords: Iraqi spinach, Functional food, Natural antioxidant, Oxidative stress, synthetic glutathione.



INTRODUCTION

With extensive use and repeated exposure to toxic chemicals in everyday life, the human body absorbs exotoxins through drinking, the skin, and respiration [1], those toxins get into the bloodstream, causing oxidative stress [2]. Carbon tetrachloride appears as a colorless and volatile liquid, the hepatotoxic effect of CCl_4 is largely due to its active metabolites, trichloromethyl free radicals such as CCl_3 and CCl_3OO- [3]. Subsequent damage caused by CCl_4 has been linked to many mechanisms, including the disruption of redox equilibrium and the induction of programmed cell death and liver cirrhosis in experimental animals and humans [4]. Functional foods are vegetables, fruits, and foods that offer health benefits, that contain vitamins, amino acids, fatty acids, fiber, antioxidants, minerals, and probiotics, which has been thought to reduce oxidative stress in the body, enhance immune function, and improve human health [5]. Antioxidants may provide some protection against oxidative stress by neutralizing free radicals and suppressing lipid peroxidation [5– 6]. The antioxidant properties of the phytochemicals in our health foods are gaining more attention [8]. Spinach (*Spinacia oleracea* L.) as a functional food is an important food vegetable and a commonly used feedstock in the food industry [9]. While glutathione is synthesized by the body, the majority of antioxidants are present in the diets we eat [10]. The three amino acids cysteine, glycine, and glutamine make up the majority of glutathione, which is one of the body's most significant and potent antioxidants [10–11].

Glutathione levels in the body can decrease for a variety of reasons, such as oxidative stress [10]. Numerous disorders linked to oxidative stress may be prevented or treated with glutathione supplementation [13]. While several studies have been conducted with respect to antioxidant activity, a review of published articles revealed that there is a need to investigate the effect of spinach-derived glutathione against CCl_4-induced damage to bridge the gap between studies in recent years, especially since spinach leaves are used in food processing. The current study was designed to know the preventive and therapeutic effect of synthetic and spinach-derived glutathione against the harmful effects of carbon tetrachloride in female laboratory rats by measuring a number of biochemical and histological variables in the blood serum and liver.

MATERIALS AND METHODS

***Chemicals and reagents*:** We obtained Iraqi spinach from a local supermarket in Erbil, Iraq. Malondialdehyde, thiobarbituric acid, and Ellman's reagent were all bought from Sigma Company (USA). All additional chemicals, including CCl4, were purchased from Scharlab Company (Spain), and synthetic glutathione was bought from America Medic and Science Company (USA).

Preparation of spinach leaf extract: The extraction of spinach leaf was conducted according to the method provided by Susanti et al. with some modifications [20]. Fresh spinach leaves obtained from the local supermarket were dried for 4-5 hours at a temperature of 35–40 °C. Following crushing, 100 g of the sample powder was macerated for 24 hours in 1000 mL of 80 % chloroform before being shaken with a magnetic stirrer for the same amount of time. Following that, Whatman filter paper (Size 0.15 cm) was used to filter the solution. Using a rotary evaporator and a vacuum, the obtained clear solution was concentrated to dryness at temperatures below 40 °C. The concentrated extract was stored in vials for later application.

Quantification of glutathione in spinach leaf extract: The modified method of [21], has been used to quantify glutathione in the spinach extract. The sample was prepared by dissolving the spinach extract in ethanol at a concentration of 1000 µg/mL, then 20 µL of the sample was injected into a high-performance liquid chromatograph (HPLC; model SYKAM, Germany) through a C18 column (50 × 4.6 mm I.D., particle size 5 µm; Agilent Technologies, USA). The glutathione was detected at 254 nm at room temperature and the flow rate was 0.8 mL/min for glutathione. The mobile phase for glutathione consisted of 40% methanol and 60% 0.01 M KH_2PO_4 at 2.5 pH, which was filtered using a 0.22 mm filter. Comparing unidentified extract samples to the standard glutathione solution given by the Sigma Company (USA).

Separation of glutathione from spinach extract using preparative HPLC: The modified method of [22], has been used to separate glutathione from the spinach extract. For pre-processing, 25 g of crude sample was dissolved in 500 mL of a mixture that contained 20 % methanol, 30% acetonitrile, and 50 % water. After shaking for 15 minutes three times, No. 1 filter paper was used for filtration, and the mixture was centrifuged at 1000 rpm for 30 minutes before being filtered with a 0.22 m Millipore filter syringe and injected by HPLC 25 times. For each separation, a type 4015 pump injection was used, 20 µL of the sample was injected, and a YMC C18 separation column (250 ×10.0 mm I.D., particle size 5 µm) was used with solvent composed of acetonitrile-water containing 0.1% HCOOH (25 : 75, v/v) and methanol-water containing 0.1% HCOOH (25 : 75, v/v) at a flow rate of 3.0 mL/min and monitored at 280 nm; at 25 °C, the separation process was maintained. The effluents were

manually collected and continually monitored with a UV detector at 280 nm.

Animals and experimental protocol: Female albino rats that were 12 to 14 weeks old, weighing 180–220 g, nulliparous, and not pregnant were obtained from Mosul University's primary animal house facility. The rats were kept in an air-conditioned, filter-protected space that was constantly between (21_25C°), 50 to 60 % humidity, and had a 12h photoperiod. Five rats were kept in each cage and given unrestricted access to a normal diet. Throughout the experiment, all efforts were taken to reduce the number of animals used as well as any unnecessary stress or discomfort the animals experienced. The 30 rats were randomly separated into six groups after a week of acclimatization.

Group 1: (positive control) rats served as controls.

Group 2: (negative control) rats were given 3 mL/kg/b.wt of CCl_4 subcutaneous[23].

Group 3: (spinach-derived glutathione (GSD) + CCl_4) animals received 14.2 mg/kg b.wt/day of GSD for 3 weeks orally with milk before being given 3 mL/kg/b.wt of CCl_4 subcutaneous.

Group 4: (CCl_4 + GSD) animals received 3 mL/kg/b.wt of CCl_4 subcutaneous, then were treated with 14.2 mg/kg b.wt/day of GSD for 3 weeks orally with milk from week 4 up to the end of the study.

Group 5: (synthetic glutathione (SG) + CCl_4) animals received 14.2 mg/kg/day of SG for 3 weeks orally with milk before being given 3 ml/kg/b.wt of CCl_4 subcutaneous.

Group 6: (CCl_4 + SG) animals received 3 ml/kg/b.wt of CCl_4 subcutaneous, then were treated with 14.2 mg/kg b.wt/day of SG for 3 weeks orally with milk from week 4 up to the end of the study.

After the final treatment on day 62, all rats were given complete access to water while fasting for 24 hours. Following chloroform anesthesia, blood samples were obtained intracardially, centrifuged for 15 minutes at 4000 rpm to receive the serum, and stored at -80 °C, after which the liver was excised to prepare for measuring the study's parameters. Then 0.1 g of the liver was then taken, along with 1.0 mL of an extract solution (the manufacturer is Solarbio), to fully break up the cells and release their component by use of an ultrasound and then centrifuged at 10000 rpm for 5 minutes at 4°C, and the supernatant was used to measure the study's parameters. Serum and the liver's biochemical parameters were then assessed. Transferring the liver to a 10 % formalin solution in order to prepare it for histology and afterward prepare the histological slices.

Study parameters and histological preparations

Study parameters measurement: Total protein, alanine aminotransferase, albumin, and aspartate aminotransferase concentrations were measured using manual kits in accordance with the manufacturer's instructions. (BIOLABO, France). Glutathione was evaluated following the method of Sedlak and Lindsay [24], malondialdehyde concentration was evaluated following the method of [25], and peroxynitrite determination followed the method of [26].

Calculation of study parameters: The following equations were used to estimate the globulin concentrations.

$$Globulin(g/dL) = total\ protein\ concentration - albumin\ concentration\ [20\text{-}21]$$

Histological preparations: Rat liver tissue was processed for histological analysis in accordance with [29].

Ethical aspects: IACUC at the University of Mosul's College of Veterinary Medicine has approved the study (No. UM.VET.2021.014). Instructions were followed regarding the handling and usage of experimental animals.

Statistical process: The mean ± standard deviation is used to express the study's results, and a probability of $p \leq 0.05$ was determined significant. One-way analysis of variance (ANOVA) was used to calculate the statistical significance of group differences.

RESULTS AND DISCUSSION

Quantification of glutathione in spinach extract: To evaluate the quantity of glutathione in the spinach extract, HPLC analysis was carried out. The data showed that extracts of spinach contained 460.9 mg/g glutathione. Compounds such as glutathione are known to be involved in redox activity because they are hydrogen donors, reducers, and oxygen radical scavengers [30]. The findings demonstrate that compared to other vegetable sources, spinach leaf contains a high content of glutathione. The effect of chloroform upon extraction is caused by an increase in the polarity of the extraction solution, which separates and precipitates glutathione from the other compounds into the extraction solution.

Effectiveness of oral dosage of glutathione spinach-derived and synthetic on Some Oxidation Evidence in blood serum and liver tissue: The current study results revealed that giving CCl_4 subcutaneous (G2) (Table 1, Figure 1) caused a notable decrease in glutathione levels in the blood serum and liver tissue, where glutathione was 2.223; 1.840 µmol/L, respectively in the group of animals that exposed to oxidative stress with CCL4 (G2). Glutathione is one of the natural antioxidants for the removal of free radicals and protecting cell membranes from free radical damage [31]. Numerous factors contribute to the decrease in glutathione levels, including an increase in the rate of consumption of glutathione, one of the most significant endogenous non-enzymatic antioxidants for reducing free radicals and their byproducts, and then converted of glutathione from its active form to glutathione disulfide, which is inactive (GSSG). Since the weak bond between sulfur and hydrogen (S-H) and the strong bond between carbon and hydrogen (C-H) in free radicals actually make the sulfur group in the structure of glutathione an efficient reducing agent that easily releases a hydrogen atom, protecting cell membranes from free radical damage [31-32]. The cause might be due to the occurrence of oxidative stress that leads to the oxidation of glutathione as a result of its antioxidant activity, and then transform it into the toxic oxidized form of disulfide, which helps to stimulate the production of new forms of free radicals. Glutathione is also consumed in rather high amounts due to its role in restoring some other antioxidants' efficacy in cases of oxidative stress[34–36]. When spinach-derived glutathione was administered to rats orally before or after they were given CCl_4 subcutaneous (G3 and G4), glutathione levels were found to have significantly increased, where was 8.8200; 8.2113 µmol/L, in blood serum and 5.376; 5.503µmol/L, in the liver, respectively compared to the group of animals in the synthetic glutathione groups (G5 and G6, Where was 6.523; 5.960 µmol/L, in blood serum and 3.370; 2.876 µmol/L, in liver respectively). This outcome is in conformity with Reddy et al. (2014) findings; they observed an increased glutathione level in the blood serum of male rats exposed to oxidative stress after treatment with ginger extracts. The spinach-derived glutathione is able to raise the level of glutathione, an exogenous food antioxidant, as it works to remove the constantly formed free radicals, which protects from the danger of these radicals as a line of defense against oxidative stress [38].

One of the by-products of polyunsaturated fatty acid peroxidation in cells is malondialdehyde [39]. An increase in free radicals leads to an excess synthesis of malondialdehyde. Free radicals initiate the lipid peroxidation process in an organism, which results in the oxidative degradation of polyunsaturated fatty acids [40].

The findings demonstrate a considerable impact on various oxidative stress parameter values in rats ($p \leq 0.05$; Table 1, Figure 1). Malondialdehyde levels considerably rose in the group given CCl4 beneath the skin (G2), where was 4.119: 6.678 µmol/L,in blood serum and liver tissue respectively compared to the value in the control group, which is a symptom of the oxidative stress of lipids in body cells. Treatment of rats with both spinach-derived and synthetic glutathione before or after they were given CCl_4 subcutaneous improved the malondialdehyde values to significantly closer to those in the control group animals (G3 and G4), compared to the animals in G5 and G6. These results indicate that compared to synthetic glutathione, spinach-derived glutathione has a greater potential to decrease the imbalance caused by CCl4 by combating free radicals and reducing the malondialdehyde levels in G3 and G4; thereby, according to our findings, it can be concluded that the levels of malondialdehyde, which had been raised in CCl_4 groups, can be lowered with spinach-derived glutathione.

A significant member of the family of reactive oxygen and nitrogen species which has evolved is peroxynitrite (ONOO−) [41]. Tyrosine residues in proteins can be nitrated, and redox metal centers can be oxidized, once ONOO2 reacts with biological substrates [42]. Numerous clinical problems, such as neurological disorders and the activation of cancer cells, have been linked to ONOO2 [43]. Peroxynitrite concentration, a marker of oxidative stress in rats, considerably increased ($p \leq 0.05$; Table 1, Figure 1) in the group given CCl_4 subcutaneous(G2) compared to that in the control group. Treatment of rats with both spinach-derived and synthetic glutathione before or after they were given CCl_4 subcutaneous improved peroxynitrite values to significantly closer to those in the control group animals (G3 and G4), compared to the animals in G5 and G6. These results indicate that compared to synthetic glutathione, spinach-derived glutathione has a greater potential to decrease the imbalance caused by CCl4 compared with synthetic glutathione by combating free radicals and reducing the peroxynitrite levels in G3 and G4.

Table 1. Effectiveness of oral dosage of glutathione spinach-derived and synthetic on Some Oxidation Evidence in blood serum.

Groups	Glutathione µmol/L	Malondialdehyde µmol/L	Peroxynitrite µmol/L
G1	10.416 ± 0.121^{a}	1.396 ± 0.246^{b}	29.316 ± 0.732^{b}
G2	2.223 ± 0.591^{d}	4.119 ± 0.640^{a}	69.237 ± 0.255^{a}
G3	8.820 ± 0.895^{b}	1.450 ± 0.030^{b}	31.406 ± 0.731^{b}
G4	8.213 ± 0.238^{b}	1.729 ± 0.467^{b}	33.733 ± 0.596^{b}
G5	6.523 ± 0.147^{c}	3.198 ± 0.892^{a}	40.740 ± 0.117^{ab}
G6	5.960 ± 0.510^{c}	3.878 ± 0.556^{a}	45.740 ± 0.707^{ab}

Different letter within each column indicates significant differences ($p\leq 0.05$), (G1) Positive Control, (G2) Negative Control, (G3) spinach-derived glutathione + CCl4, (G4) CCl4 + spinach-derived glutathione, (G5) synthetic glutathione + CCl4, (G6) CCl4 + synthetic glutathione.

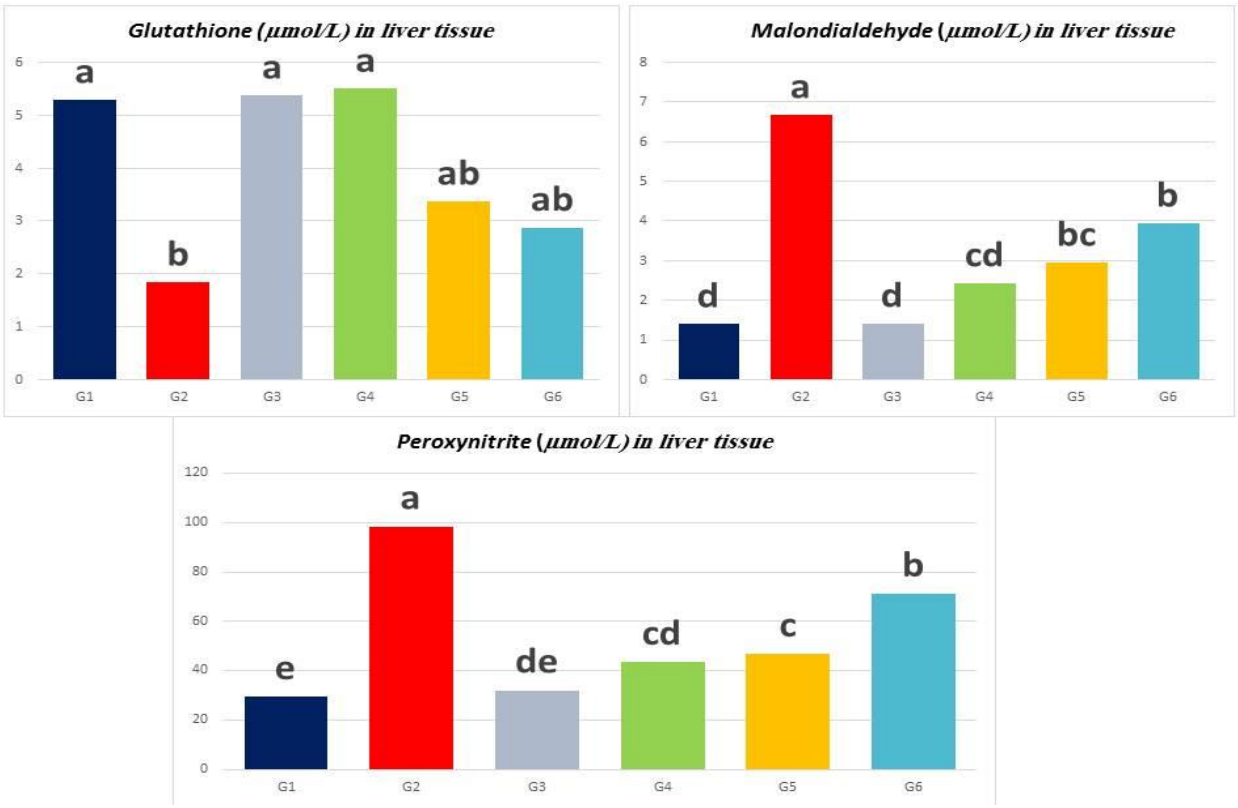


Figure 1. Effectiveness of oral dosage of glutathione spinach-derived and synthetic on Some Oxidation Evidence in liver tissue. Different letter within each column indicates significant differences (p≤ 0.05), (G1) Positive Control, (G2) Negative Control, (G3) spinach-derived glutathione + CCl4, (G4) CCl4 + spinach-derived glutathione, (G5) synthetic glutathione + CCl4, (G6) CCl4 + synthetic glutathione.

Effectiveness of oral dosage of glutathione spinach-derived and synthetic on total protein, globulin, and albumin in blood serum: It was found through the current study that giving the rats CCl_4 subcutaneous(G2) caused a significant decrease in the concentration of total proteins, globulin, and albumin in the blood serum (Table 2). This decrease may be explained by the fact that the animals under oxidative stress turned to their fat and protein reserves as alternate sources of energy, such as amino acids catabolism, which results in an increase in energy, as well as gluconeogenesis, the process of building glucose from non-carbohydrate sources [44]. The decrease in albumin is caused by the peptide bonds being broken by the impacts of oxidative stress and the production of free radicals, this causes albumin to decompose and decreases its level in blood serum [45]. Globulins are found in different forms, including alpha 1 globulins, which transport fats, steroids, and phospholipids in particular, and alpha 2 globulins which work to transport fats and hemoglobin, which is broken down from red blood cells, as it transports copper ions. Beta globulin works to transport iron ions and gamma-globulin and is considered an antibody which performs defensive functions [46]. When spinach-derived glutathione was administered to rats orally before or after they were given CCl_4 subcutaneous (G3 and G4), comparing the total protein, globulin, and albumin levels in animals to those in the synthetic glutathione groups (G5 and G6), a considerable improvement was seen. The rise in these cases may be attributed to several reasons, including the effectiveness of spinach-derived glutathione. It inhibits protein oxidation and eliminates free radicals and reduces the filtration of proteins from the blood and their excretion with urine.

Table 2. Effectiveness of oral dosage of glutathione spinach-derived and synthetic on total protein, globulin, and albumin in blood serum.

Groups	Total Protein *(g/dL)*	Albumin *(g/dL)*	Globulin *(g/dL)*
G1	6.680±0.233^{a}	4.474±0.100^{a}	2.206 ±0.236^{a}
G2	3.858±0.656^{b}	2.776±0.491^{d}	1.082±0.015^{d}
G3	6.667±0.744^{a}	4.731±0.665^{a}	1.936±0.383^{a}
G4	6.123±0.355^{a}	4.130±0.675^{ab}	1.993±0.916^{a}
G5	4.562±0.073^{b}	3.108±0.126^{b}	1.454±0.156^{c}
G6	3.553±0.840^{b}	2.270±0.609^{c}	1.283±0.359^{b}

Different letter within each column indicates significant differences ($p \leq 0.05$), (G1) Positive Control, (G2) Negative Control, (G3) spinach-derived glutathione + CCl4, (G4) CCl4 + spinach-derived glutathione, (G5) synthetic glutathione + CCl4, (G6) CCl4 + synthetic glutathione.

Effectiveness of oral dosage of glutathione spinach-derived and synthetic on ALT and AST levels in blood serum and liver tissue: AST and ALT enzymes are important and necessary enzymes in biological processes, a rise in AST and ALT enzyme levels in blood serum is a sign of tissue damage that causes these enzymes to leak into the bloodstream [47]. The results showed a rise in the level of the enzymes ALT and AST in rats given CCl_4 subcutaneous (G2) (Table 3; Figure 2). The free radicals formed by oxidative stress led to stimulation of lipid peroxidation in liver cells, causing the release of these enzymes into the bloodstream [48], which leads to an increase in their concentration compared to the normal levels in the standard groups. When spinach-derived glutathione was administered to rats orally before or after they were given CCl_4 subcutaneous (G3 and G4), ALT and AST levels were close to normal in comparison to the rats in the synthetic glutathione groups (G5 and G6). This result is consistent with the findings of [49]; they observed reduced AST and ALT levels in the blood serum of rats exposed to oxidative stress by nicotine after treatment with neem leaf aqueous extract. These results indicate that compared to synthetic glutathione, spinach-derived glutathione has a greater potential to decrease the imbalance caused by CCl4 compared with synthetic glutathione by combating free radicals and boosting ALT and AST levels in G3 and G4 rats.

Table 3. Effectiveness of oral dosage of glutathione spinach-derived and synthetic on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in blood serum.

Groups	Alanine aminotransferase *(U/L)*	Aspartate aminotransferase *(U/L)*
G1	33.312±0.655^{c}	34.912±0.928^{b}
G2	84.223±0.895^{a}	153.102±1.071^{a}
G3	36.891±0.392^{c}	38.718 ±0.045^{b}
G4	37.473±0.983^{c}	43.743 ±0.792^{b}
G5	43.389 ±0.636^{bc}	57.802 ±0.578^{b}
G6	54.649±0.394^{b}	67.444 ±0.165^{b}

Different letter within each column indicates significant differences ($p \leq 0.05$), (G1) Positive Control, (G2) Negative Control, (G3) spinach-derived glutathione + CCl4, (G4) CCl4 + spinach-derived glutathione, (G5) synthetic glutathione + CCl4, (G6) CCl4 + synthetic glutathione.

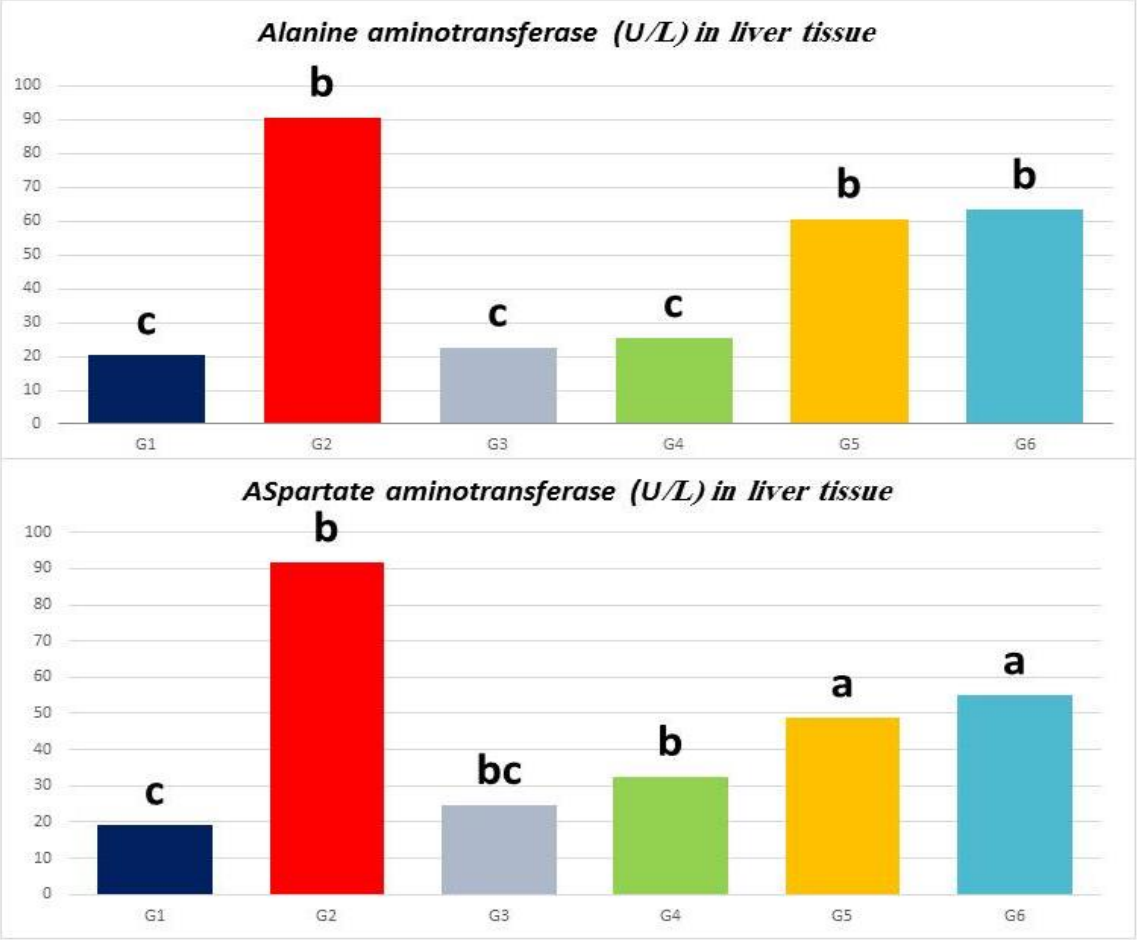


Figure 2. Effectiveness of oral dosage of glutathione spinach-derived and synthetic on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in liver tissue. Different letter within each column indicates significant differences ($p \leq 0.05$), (G1) Positive Control, (G2) Negative Control, (G3) spinach-derived glutathione + CCl4, (G4) CCl4 + spinach-derived glutathione, (G5) synthetic glutathione + CCl4, (G6) CCl4 + synthetic glutathione.

Evaluation of histopathological: Histological examination demonstrated that carbon tetrachloride has an effect on the liver, it causes observed effects in the liver tissues that occur in cases of oxidative stress because the liver is the most sensitive and most affected organ, these changes appear as severe cloudy degeneration, infiltrates, or lysis and destruction of tissues, and as the case progresses, it results in cirrhosis of the liver tissues [47]. The histopathological evaluation in Fig. 3 demonstrates that the rats in Group 1 (positive control) had sinusoids, central veins, and intact hepatocytes; rats in Group 2 had sinusoidal congestion, severe cloudy degeneration, cellular swelling, and hepatocyte coagulative necrosis. Free radicals increase the oxidation of cell wall fats, which lose their functional feature, this has a number of effects on cells, including the assembly of structural or functional proteins by joining proteins together at the sulphahydryl bond, it may interact with fats, particularly those forming cellular membranes, which are primarily composed of unsaturated fats and contain double bonds that can be attacked by free radicals, leading to the breakdown of cells and blood vessels [50–52]. Increased pressure inside the blood vessels causes a rupture in the vessel wall, which permits red blood cells to flow outside the blood vessels and causes cellular swelling, the cause of thrombosis of hepatocytes may be due to interactions between toxic substances and glutathione, which

produce compounds that lead to thrombosis. Hepatic failure results in congestion of the sinusoids and hepatic veins, which creates pressure on hepatocytes and causes an imbalance in the distribution of enzymes, effecting hepatocyte function[53–55]. Rats in Group 3 had mild sinusoidal congestion with intact hepatocytes and central vein. Group 4 demonstrated intact hepatocytes, a central vein, and sinusoidal dilatation and congestion; Group 5 demonstrated intact hepatocytes, a central venous congestion, and sinusoids; and Group 6 demonstrated the presence of cloudy hepatocyte degeneration and a central venous congestion. Rats given spinach-derived glutathione orally either before or after receiving CCl4 subcutaneous (G3 and G4) had shown significantly improved liver tissue compared to the rats given synthetic glutathione groups (G5 and G6). This result can be associated with a number of factors, including the efficiency of spinach-derived glutathione. It eliminates free radicals and reduces the risk of oxidative stress caused by free radicals.

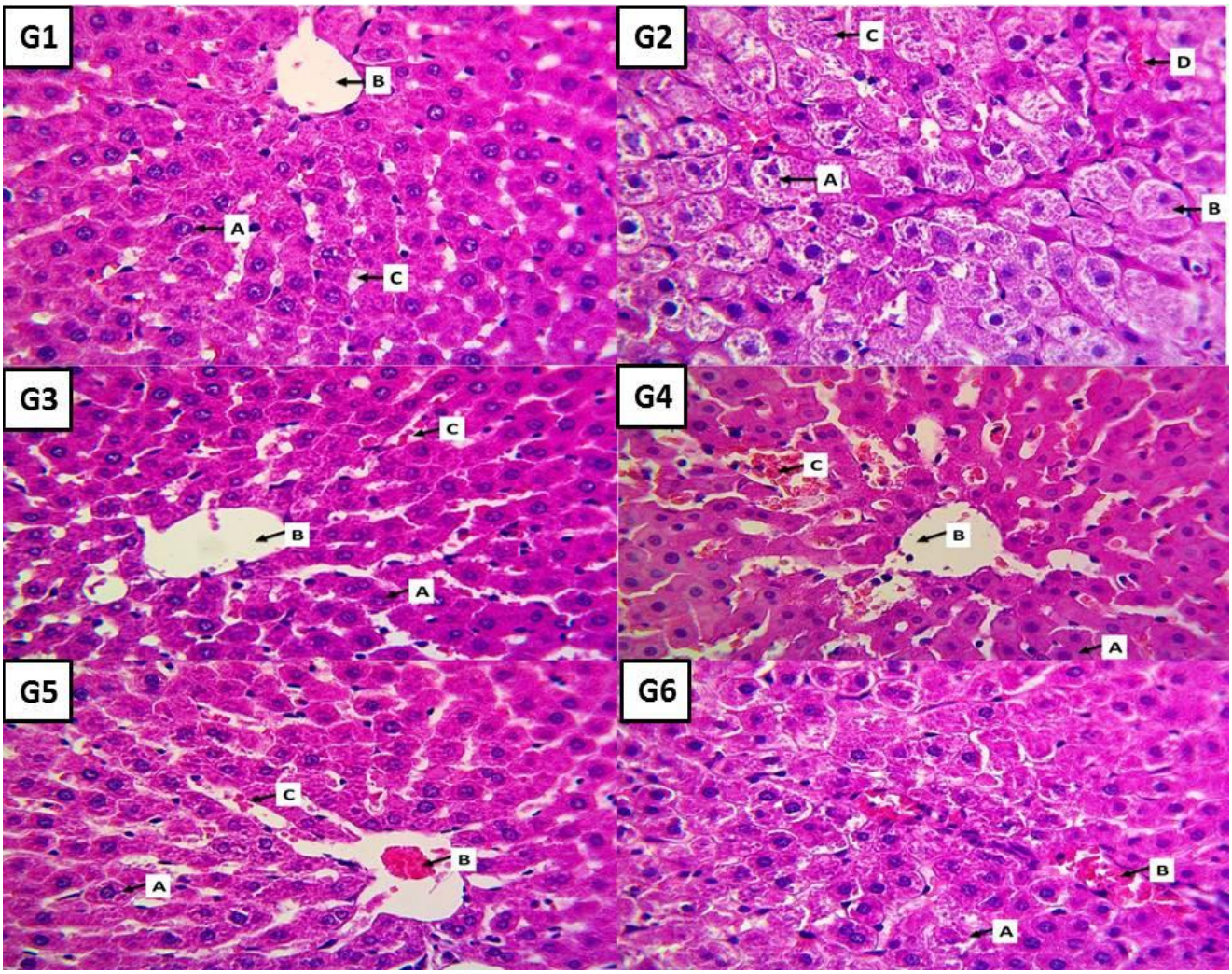


Figure 3. Histopathological evaluation, G.1: Control rats showing intact hepatocytes (A), central vein (B), and sinusoids (C), G.2: showing severe cloudy degeneration (A), cellular swelling (B), hepatocyte coagulative necrosis (C) and sinusoidal congestion (D), G.3: showing intact hepatocytes (A), central vein (B) with mild sinusoidal congestion (C), G.4: showing intact hepatocytes (A), central vein (B) with sinusoidal dilatation and congestion (C), G.5: showing intact hepatocytes (A), with central venous congestion (B) and sinusoids (C), G.6: showing the existence of hepatocyte cloudy degeneration (A), with central venous congestion (B), 400XX.

CONCLUSION

Glutathione was isolated from leaves of Iraqi spinach; the total extract of this plant possesses a powerful antioxidant activity. Administration of milk with glutathione extracts from natural sources had favorable effects on oxidative stress and significantly boosted the antioxidant system in comparison to milk with synthetic glutathione. Increasing the antioxidant efficiency of synthetic glutathione by combining it with spinach-derived glutathione may contribute to the capture of free radicals caused by oxidative stress. Iraqi spinach is utilized as a dietary vegetable. It is important to perform thorough research in order to fully explore the potential of this plant through free radical scavenging activity, from the point of view of affordability and accessibility for individuals at all socioeconomic levels.

Abbreviations: CCl_4: Carbon tetrachloride, GSD: spinach-derived glutathione, SG: synthetic glutathione, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Authors Contribution: Qaswaa Yousif Jameel: Formal analysis; Methodology; Project administration; Validation; Writing-original draft. Enas Abdulkareem Abdulrazak: Data curation; Formal analysis; Writing-review and editing.

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