The potential protective role of grape seed proanthocyanidin extract against the mixture of carboplatin and thalidomide-induced hepatotoxicity and cardiotoxicity in male rats

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Abstract
Thalidomide is used experimentally to treat various cancers, also carboplatin is a chemotherapy drug used against some forms of cancer. Grape Seed Proanthocyanidin Extract (GSPE) has an enormously beneficial role in overcoming the adverse effects of chemotherapeutic agents due to its excellent antioxidant properties. Animals were divided into four groups as follows: The first group was used as control, the second group were treated orally for 28 consecutive days with GSPE (200 mg/kg BW), the third group were treated intraperitoneally (i.p) with thalidomide (60 mg/kg BW) for 14 consecutive days then followed by carboplatin (196 mg/kg BW) for another 14 days and the animals of the fourth group were treated with the combination of GSPE (200 mg/kg BW) and thalidomide (60 mg/kg BW) for 14-day and then followed by GSPE (200 mg/kg BW) and carboplatin (196 mg/kg BW) for other 14-day. Inflammatory cytokines, P53, oxidative stress markers, biochemical parameters, and histological analysis measured. Carboplatin and thalidomide caused oxidative stress via the elevation in free radicals and nitric oxide and the reduction in the antioxidant enzymes and glutathione in liver and heart. Tumor suppressor gene P53, tumor necrosis factor-α, and interleukin-6 were significantly increased in liver and heart. Thalidomide and carboplatin caused biochemical and histological changes in the liver and heart. Grape seed proanthocyanidin extract reduced carboplatin and thalidomide–induced liver and heart injury throughout its potent antioxidant activity. In conclusion, carboplatin and thalidomide caused hepatotoxicity and cardiotoxicity and grape seed proanthocyanidin extract showed hepatic and cardiac protective effects due to its antioxidant and anti-inflammatory potentials.

Introduction
Thalidomide was primarily used as a sedative, but it was withdrawn from the market after its teratogenic effects [1]. In spite of its teratogenicity, thalidomide possesses immune-modulatory, anti-inflammatory and anti-angiogenic properties that are potentially useful in several diseases [2]. Currently, thalidomide is used experimentally to treat various cancers, dermatological, neurological and inflammatory diseases. The immunomodulatory activities, together with the antiangiogenic, anti-proliferative and pro-apoptotic properties, are believed to mediate anti-tumor responses as observed in multiple myeloma and some solid tumors. Thalidomide and its analogs modulate the immune system in different ways [3]. Lenalidomide has shown potential in treating the bone marrow disorders multiple myeloma and myelodysplastic syndrome and is presently in Phase II and III trials [4].

Carboplatin is used against some forms of cancer, mainly ovarian carcinoma, lung, as well as endometrial, esophageal, bladder, breast and cervical; central nervous system or germ cell tumors; osteogenic sarcoma and as preparation for a stem cell or bone marrow transplant [5]. The myelosuppressive effect is the main obstruction of carboplatin. This causes the blood cell and platelet output of bone marrow in the body to decline quite massively [6].

Cancer chemotherapy induces lipid peroxidation, generates numerous electrophilic aldehydes and free radicals which can attack many cellular targets. These products of oxidative stress can delay cell cycle progression of cancer cells and cause cell cycle checkpoint arrest that may intervene with the potency of anticancer drugs to kill cancer cells [7]. The aldehydes may also inhibit drug-induced apoptosis by obstruction death receptors and suppressing caspase activity. They added, grape seed proanthocyanidin may be reduce the generation of oxidative stress-stimulated aldehydes.

Antioxidant supplements are used during treatment with chemotherapy attributable to their role in the efficacy of the chemotherapy, as well as diminish toxic side effects, allowing patients to tolerate chemotherapy for the full course of treatment and at higher doses [8]. In addition, antioxidants have several mechanisms of action depending on their use, which noted to have the potential to serve as antioxidant molecules themselves [9]. The supplements have a wide range in their antioxidant mechanisms from free radical scavengers that act by the preservation of cellular defense mechanisms or could be worked as reducers [10]. Additionally, aside from their antioxidant activities, these agents may manipulate the pharmacokinetics or pharmacodynamics of chemotherapeutic agents [11].

The beneficial role of Grape Seed Proanthocyanidin Extract (GSPE) against adverse effects of chemotherapeutic agents probably owing to its excellent antioxidant properties and high nutritional values [12].

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Also, grape seed proanthocyanidin extract (GSPE) caused enzymatic enhancement, may be due to its antioxidative phytochemicals as flavonoids [7].

Therefore, the present study was carried out to investigate the possible protective role of grape seed proanthocyanidin extract against the mixture of thalidomide and carboplatin-induced hepatic and cardiac toxicity in male rats.

Materials and methods

Tested compounds and doses

Thalidomide (C_{13}H_{10}N_{2}O_{4}) was purchased from Sigma Chemical Company (St. Louis, MO, USA). Carboplatin (C_{6}H_{14}N_{2}O_{4}Pt) obtained from Vita for NV/SA, additives and pharmaceutical, Germany (www.vitafor.com). A dried, powdered grape seed proanthocyanidin extract (GSPE) was obtained from Pharco Pharmaceuticals Company, Alexandria, Egypt. The dose of thalidomide was 60 mg/kg according to Ilona et al. [13]. The dose of carboplatin was 196 mg/kg according to Husain et al. [14]. The dose of grape seed proanthocyanidin extract (GSPE) was 200 mg/kg BW according to Yousef et al. [7].

Animals and experimental groups

Forty Wistar male rats weighing 160-180g were used in the current study. They were obtained from the Faculty of Medicine, Alexandria University, Alexandria, Egypt. Animals were housed in a stainless-steel wire cages, kept on basal diet and given feed and water ad libitum. Rats fed pellets which consisted of 30% berseem (Trifolium alexandrinum) hay, 25% yellow corn, 26.2% wheat bran, 14% soybean meal, 3% molasses, 1% CaCl_{2}, 0.4% NaCl, 0.3 % mixture of minerals and vitamins, and 0.1% methionine. The vitamin and mineral premix per kg contained the following IU/gm for vitamins or minerals: Vit. A-4000,000, Vit D3-5000, 000, vit E-16.7 g, K-0.67 g, vit B1-0.67 g, vit B2-2 g, B6-0.67 g, vit B12-0.004 g, B5-16.7 g, B1-2 g, B6-0.67 g, B9-1.67 g, Biotin-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Ca-1.67 g, Cu-1.67 g, Mg-133.4 g, and I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit Vitafor.com). A dried, powdered grape seed proanthocyanidin extract (GSPE) was obtained from Pharco Pharmaceuticals Company, Alexandria, Egypt. The dose of thalidomide was 60 mg/kg according to Husain et al. [14]. The dose of grape seed proanthocyanidin extract (GSPE) was 200 mg/kg BW according to Yousef et al. [7].

Blood samples collection and tissue preparations

At the end of the 28th day of the experimental period, all animals of each group were sacrificed. Blood samples were collected from anesthetized rats in test tubes containing heparin as an anticoagulant and placed immediately on ice. The collected blood was centrifuged at 860×g for 20 min for the separation of plasma. The plasma was kept at -80°C until analyses of the examined parameters. Liver and heart were immediately removed, washed using chilled saline solution and removed the adhering fat and connective tissues. Liver and heart were divided into two parts; one part was immersed immediately in formalin for histological analysis, another part was minced and homogenized (10%, w/v), separately, in ice-cold sucrose buffer (0.25 M) in a Potter–Elvehjem type homogenizer, the homogenates were centrifuged at 10,000×g for 20 min at 4°C, to pellet the cell debris and the supernatant was collected and stored at -80 °C for the determination of the rest of parameters.

ELISA measurements

Tumor suppressor gene p53, tumor necrosis factor- alpha (TNF-a) and interleukin-6 (IL-6) were inspected by using Enzyme-Linked Immunosorbent Assay (ELISA) kits in the liver and heart tissue homogenates [16-18].

Markers of oxidative stress

Thiobarbituric Acid- Reactive Substances (TBARS) were measured in liver and heart homogenates at 532 nm by using 2-thiobarbituric acid (2,6-dihydroxy-pyrimidine-2-thiol; TBA). Total Antioxidant Capacity (TAC) and the level of Nitric Oxide (NO) were assayed in liver and heart homogenates. Superoxide Dismutase (SOD) was determined at 480 nm. Glutathione Peroxidase (GPX) activity was established in liver and heart homogenates. The activity of GST was measured in tissue homogenates at 310 nm using UV-Double Beam Spectrophotometer. The CAT activity in tissue homogenates was measured at 240 nm. Reduced glutathione content was determined at 412 nm. All the above assays were determined according to the manual instruction of Biodiagnostic Kit, Egypt.

Biochemical parameters

Heart paraoxonase (PON1) activity, plasma total protein, and albumin were measured. The activities of Plasma Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP) And Acid Phosphatase (ACP) were measured by kits from Biosystems S.A (Costa Brava 30, Barcelona, Spain).

Histological section preparation of liver and heart

Liver and heart specimens were obtained from rats and immediately fixed in 10% formalin and then treated with a conventional grade of alcohol and xylol, embedded in paraffin and sectioned at 4.6 µm thickness. The sections were stained with Haematoxylin and Eosin (H&E) stain for studying the histopathological changes [19].

Statistical analysis

Results were reported as means ± SE. Statistical analysis for all studied parameters was performed using the General Linear Model (GLM) produced by the Statistical Analysis Systems Institute [20] Duncan’s New Multiple Range Test was used to test the significance of the differences between means [21]. Values of p<0.05 were considered statistically significant.

Results

Effect of grape seed proanthocyanidin extract, thalidomide, carboplatin and their combination on tumor suppressor p53, tumor necrosis factor- α, interleukin-6

Administration of thalidomide followed by carboplatin caused significant (p>0.05) increase in liver and heart p53, TNF-a and IL-6
compared to the control group (Table 1). The combination of GSPE with thalidomide and carboplatin significant (p < 0.05) decrease in liver and heart P53, TNF-α and IL-6 compared to a group of thalidomide and carboplatin. GSPE alone significantly (p < 0.05) decreased P53, TNF-α and IL-6 in the liver and insignificantly (p > 0.05) increased in P53, significantly (p < 0.05) increased TNF-α and IL-6 in the heart compared to the control group.

**Effect of grape seed proanthocyanidin extract, thalidomide, carboplatin and their combination of free radicals and antioxidant enzymes**

Thalidomide followed by carboplatin significantly (p < 0.05) increased in the liver and heart TBARS and NO also significantly (p < 0.05) decreased in the liver and heart GSH, TAC and antioxidant enzymes (SOD, CAT, GPx, and GST) as compared to control group (Tables 2 and 3). However, the presence of GSPE with thalidomide and carboplatin significantly (p < 0.05) decreased in TBARS and NO as well as significantly (p < 0.05) increased in GSH, TAC and antioxidant enzymes (SOD, CAT, GPx, and GST) in liver and heart compared to thalidomide and carboplatin group. On the other hand, treatment with GSPE alone significant (p < 0.05) decrease in the levels of TBARS and NO and significant (p < 0.05) increase GSH and antioxidant enzymes (SOD, CAT, GPx, and GST) in the liver and heart compared to the control group. However, TAC insignificantly (p > 0.05) increased in the liver and heart.

**Effect of grape seed proanthocyanidin extract, thalidomide, carboplatin and their combination on heart paroxanase (PON1) and plasma biochemical parameters**

The present data showed that treatment with thalidomide followed by carboplatin significantly (p < 0.05) decreased PON1 in the heart compared to the control group (Table 4). The presence of GSPE with thalidomide and carboplatin significantly (p < 0.05) increased the level of PON1 in the heart compared thalidomide and carboplatin group.

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**Table 1.** Liver and heart levels of tumor suppressor p53 (p53), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) of male rats treated with grape seed proanthocyanidin extract (GSPE), thalidomide, carboplatin and their combination (Mean values ± SE)

| Parameter | Experimental groups |
|-----------|---------------------|
| Liver | Control | GSPE | Thalidomide + Carboplatin | Thalidomide + Carboplatin + GSPE |
| P53 (pg /mg protein) | 11.3 ± 0.08b | 10.5 ± 0.10d | 18.4 ± 0.07a | 15.7 ± 0.10b |
| TNF-α (pg/g tissue) | 409 ± 95c | 3100 ± 48d | 20849 ± 35a | 11450 ± 41b |
| IL-6 (pg/g tissue) | 4095 ± 174a | 2666 ± 23c | 11236 ± 114a | 9936 ± 45a |
| Heart | Control | GSPE | Thalidomide + Carboplatin | Thalidomide + Carboplatin + GSPE |
| P53 (pg /mg protein) | 8.1 ± 0.07c | 8.7 ± 0.36d | 16.4 ± 0.09a | 11.8 ± 0.17b |
| TNF-α (pg/g tissue) | 2643 ± 247c | 2875 ± 203d | 11468 ± 219a | 7177 ± 266b |
| IL-6 (pg/g tissue) | 10314 ± 66a | 11346 ± 95c | 14428 ± 62a | 11975 ± 54b |

Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, p < 0.05.

**Table 2.** Liver free radicals, nitric oxide and antioxidant enzymes of male rats treated with grape seed proanthocyanidin extract (GSPE), thalidomide, carboplatin and their combination (Mean values ± SE)

| Parameter | Experimental groups |
|-----------|---------------------|
| Control | GSPE | Thalidomide + Carboplatin | Thalidomide + Carboplatin + GSPE |
| TBARS | 21.8 ± 1.85c | 19.4 ± 1.47d | 32 ± 2.9a | 27.5 ± 2.52b |
| NO | 0.47 ± 0.04b | 0.36 ± 0.06c | 0.71 ± 0.04a | 0.32 ± 0.02d |
| GSH | 6.1 ± 0.16b | 7.1 ± 0.23a | 4.6 ± 0.22d | 5.2 ± 0.25c |
| SOD | 8.5 ± 0.58b | 9.9 ± 0.64a | 5.4 ± 0.44d | 6.9 ± 0.58c |
| CAT | 62.9 ± 3.57b | 71.0 ± 4.77a | 40.8 ± 3.75d | 50.8 ± 3.44c |
| GST | 0.70 ± 0.036b | 0.82 ± 0.041a | 0.41 ± 0.034d | 0.57 ± 0.027c |
| GPx | 33.8 ± 1.87b | 41.9 ± 2.12a | 17.8 ± 0.98d | 27.3 ± 1.40c |
| TAC | 25.4 ± 0.80a | 26.1 ± 0.76a | 18.7 ± 0.32c | 21.1 ± 0.24b |

Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, p < 0.05.

**Table 3.** Heart free radicals, nitric oxide and antioxidant enzymes of male rats treated with grape seed proanthocyanidin extract (GSPE), thalidomide, carboplatin and their combination (Mean values ± SE)

| Parameter | Experimental groups |
|-----------|---------------------|
| Control | GSPE | Thalidomide + Carboplatin | Thalidomide + Carboplatin + GSPE |
| TBARS | 20.5 ± 1.73c | 17.3 ± 0.94d | 32.0 ± 2.42a | 24.7 ± 1.34b |
| NO | 0.14 ± 0.03c | 0.06 ± 0.01d | 0.28 ± 0.02a | 0.18 ± 0.02b |
| GSH | 5.8 ± 0.30b | 7.0 ± 0.52a | 4.3 ± 0.21c | 5.1 ± 0.12b |
| SOD | 6.5 ± 0.22b | 8.2 ± 0.29a | 4.8 ± 0.26b | 6.2 ± 0.21b |
| CAT | 61.2 ± 4.80b | 80.2 ± 2.59a | 42.5 ± 2.80d | 51.7 ± 3.29c |
| GST | 0.77 ± 0.012b | 0.86 ± 0.018a | 0.44 ± 0.014d | 0.60 ± 0.031e |
| GPx | 33.8 ± 1.9b | 42.7 ± 2.5a | 22.1 ± 1.3d | 30.4 ± 1.5c |
| TAC | 27.5 ± 0.38a | 27.5 ± 0.84a | 21.3 ± 1.52c | 26.3 ± 0.96b |

Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, p < 0.05. TBARS: Thiobarbituric acid-reactive substances (μ mol/g tissue); NO: Nitric oxide (μ mol/g tissue); GSH: Glutathione (μ mol/g tissue); SOD: Superoxide dismutase (U/g tissue); CAT: Catalase (U/g tissue); GST: Glutathione S-transferase (μ mol/g tissue); GPx: Glutathione peroxidase (U/g tissue); TAC: Total antioxidant capacity (μ mol/g tissue);
The potential protective role of grape seed proanthocyanidin extract against the mixture of carboplatin and thalidomide-induced hepatotoxicity and cardiotoxicity in male rats

Table 4. Heart paroxanase (PON1) and plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP), total protein and albumin of male rats treated with grape seed proanthocyanidin extract (GSPE), thalidomide, carboplatin and their combination (Mean values ± SE)

| Parameters | Control | GSPE | Thalidomide+ Carboplatin | Thalidomide+Carboplatin + GSPE |
|------------|---------|------|--------------------------|--------------------------------|
| PON1 (mg/mg protein) | 291 ± 7.3b | 337 ± 12.8a | 78 ± 4.0d | 191 ± 6.9c |
| AST (U/L) | 23.7 ± 1.61c | 21.9 ± 1.59d | 42.0 ± 2.11a | 30.7 ± 1.41b |
| ALT (U/L) | 22.9 ± 1.84c | 20.7 ± 1.26d | 33.5 ± 2.26a | 27.2 ± 1.74b |
| ACP (U/L) | 6.6 ± 0.36c | 6.7 ± 0.34d | 10.6 ± 1.72a | 7.7 ± 0.76b |
| ALP (U/L) | 56.2 ± 3.5c | 53.7 ± 3.3d | 95.0 ± 4.8a | 73.2 ± 4.3b |
| Total protein (mg/dl) | 6.3 ± 0.16a | 6.0 ± 0.26a | 4.5 ± 0.19c | 5.4 ± 0.11b |
| Albumin (mg/dl) | 4.3 ± 0.27a | 4.4 ± 0.19a | 2.2 ± 0.17c | 3.3 ± 0.14b |

Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, p< 0.05.

Simultaneously, treatment with GSPE alone significantly (p>0.05) increased the level of PON1 in the heart compared to the control group as shown.

Treatment with thalidomide followed by carboplatin significantly (p>0.05) increase the levels of AST, ALT, ACP, and ALP while significantly (p>0.05) decreased the levels of total protein and albumin compared with the control group. The combination of GSPE with thalidomide and carboplatin significantly (p>0.05) decreased the levels of AST, ALT, ACP, and ALP while significantly (p>0.05) increase in the concentration of total protein and albumin compared to thalidomide and carboplatin group. While, treatment with GSPE significantly (p>0.05) decreased the levels of AST, ALT and ALP and insignificant (p>0.05) increase in ACP level and albumin concentration also, insignificant (p>0.05) decrease in total protein concentration compared with the control group.

**Histopathological changes in liver and heart**

**Liver histopathological observations**

Microscopic examination of liver sections of thalidomide and carboplatin-treated group showed histopathological alterations; dilation and congestion in the portal vein and sinusoids, loss of the normal hepatocytes architecture, degenerated hepatocytes, hepatocyte vacuolization also, presence of inflammatory cells infiltrations around the portal area (Figure 1 C1&C2), compared to control. On the other hand, the histopathological alterations were noticeably reduced in thalidomide, carboplatin plus GSPE (Figure 1D) compared to thalidomide and carboplatin-treated group. However, sections of control and Grape Seed Proanthocyanidin Extract (GSPE) groups (Figure 1 A&B) depicted normal hepatocellular architecture with narrow sinusoidal spaces and a central vein.

**Heart histopathological observations**

Histological examination of tissue sections from the heart muscle in thalidomide and carboplatin-treated group (Figure 2C) showed loss of normal architecture of cardiac muscle fibers, loss of cross striations and fragmentation of sarcoplasm, cytoplasmic vacuolization in cardiac muscle cells, edema in connective tissue and congestion between myocardial fibers compared to control. Whereas, heart sections of thalidomide, carboplatin plus GSPE combination group showed less histopathological alterations compared to thalidomide and carboplatin-treated group (Figure 2D). Microscopic examination of heart sections of normal control and grape seed proanthocyanidin extract (GSPE) treated groups (Figure 2A and 2B) showed normal myofibrillar architecture with adjacent myofibrils.

Discussion

Administration of carboplatin and thalidomide caused an elevation in liver and heart proinflammatory cytokines; tumor suppressor P53 (P53), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). These results are in agreement with the report of Rehman et al. [22] and El-Awady et al. [23]. Over production of COX-2 and p53 in Cisplatin Administration of carboplatin and thalidomide caused an elevation in liver and heart proinflammatory cytokines; tumor suppressor P53 (P53), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). These results are in agreement with the report of Rehman et al. [22] and El-Awady et al. [23]. Over production of COX-2 and p53 in Cisplatin
Yousef MI (2019) The potential protective role of grape seed proanthocyanidin extract against the mixture of carboplatin and thalidomide-induced hepatotoxicity and cardiotoxicity in male rats

Increased the level of TBARS, NO and decreased the level of GSH and the Nrf2 expression led to modulation in antioxidant enzymes. They added, GSPE supplementation caused an elevation in values of various inflammatory cytokines; P53, TNF-α and IL-6 near to inflammatory stimulus can increase the IL-6 concentrations [27]. White blood cells, such as macrophages and endothelial cells and any released by innate immune cells [27]. As well as, IL-6 is secreted by the.

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Figure 2. Light micrograph of the heart tissue of rats treated as follow:

(A) Control group
(B) grape seed proanthocyanidin extract (GSPE) -treated group showing normal myofibrillar architecture with striations, branched appearance and continuity with adjacent myofibrils and oval nuclei.
(C) Section in heart tissues of thalidomide and carboplatin -treated group showing loss of normal architecture, disorganization and degeneration in myocardial fibers with pyknotic nuclei (black square), congestion between myocardial fibers (blue arrow), vacuoles (black arrow). Histological alterations induced by thalidomide and carboplatin treatment were slightly reduced in the combination group
(D) thalidomide and carboplatin + GSPE -treated rats. (H. & E, X 400).

The present study, carboplatin and thalidomide administration increased the level of TBARS, NO and decreased the level of GSH and TAC, as well as decline the activities of antioxidant enzymes SOD, CAT, GST and GPX in the liver and heart of male rats. These results are in the same line with those of El-Kholy et al. [29] who indicated that cisplatin increased oxidative stress, the level of malondialdehyde (MDA) accompanied with decline in reduced glutathione (GSH), antioxidant enzymes activities as superoxide dismutase (SOD) and catalase (CAT). This may be attributed to cisplatin inhibit the defense of anti-oxidative system and induce liver oxidative stress injury. Also, Bahadr et al. [30] reported that the increases in the levels of MDA designate the myocardial damage with a decline in the GSH level and SOD, CAT activities in the Cisplatin -treated rats. This may be due to Cisplatin-induced cardiotoxicity, increased activity of reactive oxygen species caused an elevation in MDA production.

Nannelli et al. [31] mentioned that a reduction in glutathione levels accompanied by an alteration in the cellular redox state occurred by carboplatin. This leads to a reduction in performance of the antioxidant enzyme defense system.

Furthermore, the elevation in the levels of TBARS, nitric oxide and proinflammatory cytokines in the thalidomide and carboplatin-treated group, as well as the depletion in the levels of glutathione (GST). These results are concomitant with the increases in IL-6. These may be regarded as the reduction in intracellular GSH, which has been coupled with increased cytokine biosynthesis, including the release of IL-6 [32]. The mechanism implicated a ROS-sensitive pathway since the depletion of GSH strengthened IL-6 release and the production of free radicals. Additionally, ROS-mediated activation of NF-κB can lead to upregulation of cytokine expression [33].

However, administration of GSPE caused a reduction in oxidative stress markers elevated the antioxidant enzymes activities against hepatic and cardiac damage. This may be due to GSPE increased Nrf2 nuclear translocation to promote the Nrf2 signaling pathway, thus enhancing antioxidant defense systems during hepatotoxicity [34].

In addition, Proanthocyanidins contain a large amount of H+, which can block free radical chain reaction, thus improving the activity of various antioxidant enzymes and antioxidant substances in cells [35]. GSPE considered as antioxidant, antiinflammation and antiatherosclerosis [36,37]. Also, Puiggròs et al. [38] reported that procyanidins elicit the upregulation of a sequence of antioxidant and detoxification enzymes that enhance cellular defenses.

In the current data, administration of carboplatin and thalidomide revealed elevation in PON1, ALT, AST, ACP, ALP activities, also reduction in total protein and albumin content. Similarly, Iseri et al. [39] indicated a significant disorder in the activities of plasma AST and ALT through treatment with platinum compounds. The alterations in the activity of these enzymes could be a secondary event following platinum-induced liver damage with the subsequent leakage from hepatocytes. Yilmaz et al., [40] reported that the involvement of oxidative stress, lipid peroxidation and mitochondria dysfunction in CDDP –induced liver toxicity. They added, oxidative stress is a common pathogenetic mechanism contributing to the initiation and progression of hepatic damage in a variety of liver disorders.

Transaminases are the most sensitive biomarkers directly implicated in the extent of cellular damage and toxicity because they are cytoplasmic in location and are released into the circulation after cellular damage [41]. Moreover, Rehman et al. [22] indicated that alterations in AST and ALT are reported in hepatic disease and in myocardial infarction.

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In the current data, the reduction in total protein and albumin may be attributed to the chemotherapeutic agents caused alterations in protein, albumin and liver enzymes and this may be due to the injured kidney [42]. In addition, platinum drugs diminish DNA, RNA and protein synthesis. The inhibition of protein synthesis associated with platinum compounds may be attributed to platinum-induced transcription high jacking. So, transcription high jacking refers to the consequences of the ability of certain transcription factors to join DNA adducts caused by organ platinum compounds [43].

In addition, augmented protein metabolism, albuminuria, and microproteiniuria may attribute to the reduction in serum levels of total protein and albumin. Also, high production of urea may be a result of increased proteins catabolism in the liver and plasma [44].

The presence of GSPE with carboplatin and thalidomide minimized their toxic effect on PON1, ALT, AST, ACP, ALP activities, also modulated total protein and albumin content to reach near to the control values. These results are consistent with the findings of Karthikeyan et al. [45] reported that administration of GSPE maintained the levels of the marker enzymes (AST, ALT, LDH, and CK). The maintenance of the levels of marker enzymes may be due to the free radical scavenging property of anti-oxidative phytochemicals such as flavonoids present in GSPE. A possible explanation for this is that GSPE, via its anti-lipid peroxidation activity, causes stabilization of cardiac membranes and prevents the leakage of cardiac enzymes. Moreover, the main polyphenol components in GSPE are (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG) and proanthocyanidin dimer B2 (EC-EC). Also, Phenolic antioxidants reduce lipid peroxidation through a rapid donation of a hydrogen atom to the peroxyl radical (ROO) resulting in the creation of alkyl (aryl) hydroperoxide (ROOH). The polyphenol phenoxy radical formed can be stabilized by further donation of a hydrogen atom [46].

Administration of GSPE mitigated H₂O₂ -induced oxidant stress in cardiomyocytes. This action is coupled with an increase in cardiomyocyte survival. So, the cardioprotective effects of GSPE could be realized by reduction or removal of free radicals in the myocardium [47]. Likewise, Issabeagloo et al. [48] reported that GSPE treatment improves hepatic status. This attributed to cellular regeneration and stability of cell membrane which in turn, exclude the penetration of intracellular enzymes.

The biochemical parameters are confirmed by histopathological results which showed, loss of the normal hepatocytes architecture, degenerated hepatocytes with vacuolization as well as, dilatation and congestion in the portal vein and sinusoids. Moreover, distortion and degeneration in myocardial fibers with pyknotic nuclei in carboplatin thalidomide group.

These results agreed with Kart et al. [49] who reported that cisplatin-induced hepatic damage manifested by pericentral disorganization, hepatic necrosis, and apoptotic changes.

It was shown that cisplatin induces liver cells apoptosis by cytochrome-c release and caspase 3 release activation. Also, hepatotoxicity occurs by increasing messenger Ribonucleic Acid (mRNA) expression of nuclear factor-kappa B (NF-xb) dependent Cyclo-Oxygenase (COX-II) and inducible nitric oxide synthase (iNOS) [50].

In addition, the cardiac impairment produced after cisplatin administration revealed degeneration and necrosis of cardiac muscle fiber cells [51]. Also, cisplatin treatment may lead to degenerative changes in cardiac tissues, which may point to the possible consideration of carnitine deficiency. This result is supported by Zahkouk et al. [52] who reported that cardiac tissue damage may be due to the elevation in the lipid peroxidation (MDA) and reduction in GSH and CAT levels.

The current data revealed that GSPE ameliorated the histological changes in liver and heart motivated by carboplatin and thalidomide. In general, these results were consistent with those of Kandemir et al. [53]. They reported that co-treatment with GSPE has been relieved hepatotoxicity since; histopathological changes were noteworthy less pronounced compared to animals treated with cisplatin alone. Supplementation of GSPE exhibited normal organization of the cardiac muscle fibers. The present results were in the same line with the results of Lian et al. [54] who reported the protective role of GSPE against cisplatin-induced cardiac toxicity and histological alteration in heart tissue of rats.

Conclusion

In spite of the efficacy of carboplatin and thalidomide treatment, there is a restriction for their use in order to their negative effects on most of the tissue functions. GSPE acts as a potent natural antioxidant due to its antioxidant properties; GSPE exhibited a protective effect on liver and heart tissues against carboplatin and thalidomide -induced damage in rats. It could improve the proinflammatory status in liver and heart. It also attenuated the lipid peroxidation, nonenzymatic and enzymatic antioxidants also, liver and heart functions near to the control values. The biochemical analysis confirmed by histopathological investigations. So, the present work indicates that GSPE has a promising therapeutic role as a preventive agent against hepatotoxicity and cardiotoxicity motivated by carboplatin and thalidomide.

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