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Panax ginseng is superior to vitamin E as a hepatoprotector against cyclophosphamide-induced liver damage

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ABSTRACT

Background and aims: Cyclophosphamide (CPh) is a frequently used drug, in human and animals for its immunosuppressive and anticancer potential. However, it is metabolized by the liver yielding damaging toxicants (to the liver itself and other non-target vital organs) via oxidative stress, apoptosis induction and finally necrosis. Since there is no escaping of using such harmful medications, we focused on alleviating its side-effects. *Panax ginseng* Meyer is a potent candidate, and we still lack adequate information on its hepatoprotective role against cyclophosphamide-induced liver-damage.

Methods: Here, we used *P. ginseng* (Korean Red Ginseng) compared to vitamin-E (natural antioxidant) in combating CPh-induced liver damage. Forty-eight albino rats were divided into 6 groups, Control, Ginseng, Vitamin E, Cyclophosphamide (CPh), CPh + Ginseng or CPh + Vitamin-E. Blood samples were taken for biochemical analyses and liver samples were collected for histopathology, oxidative stress evaluation, and gene expression analyses.

Results: In CPh group, typical CPh-liver damage was evident (higher levels of AST, ALT, ALP; lower albumin and total proteins levels; lower liver tissue concentrations of SOD, GPX and CAT and higher MDA; injured liver histopathological picture; and finally increased TNF-α, IL-1β and Caspase3 and decreased BCL-2 genes expression). All these were abolished with either *P. ginseng* or vitamin-E administration. However, *P. ginseng* was overall superior to vitamin-E, especially in restoring blood biochemical findings and damaged histopathological picture.

Conclusions: Therefore, *P. ginseng* is a potent hepatoprotector (vitamin-E to a lesser extent) and should be considered where liver damage is expected secondary to damaging medications; as cyclophosphamide.

1. Introduction

The liver is one of the most vital body organs, and a good understanding exists of the pathogenesis of liver diseases and its protecting role by detoxification of chemicals and drugs. [1,2] Liver diseases' burden is described to be increasing in humans and it often remain undiagnosed until it is too late. [3] It represents a serious health problem worldwide and, despite current advanced medical approaches, we are still far from curing it. Moreover, some drugs that are used nowadays are described as hepatotoxic agents (such as carbon tetrachloride, nitrosamines and cyclophosphamide … etc.), they exert their damaging effects on liver tissue by giving rise to intermediary reactive oxygen species that, after exhausting liver antioxidant store, lead to liver damage. [4] Furthermore, malignancies might result as a consequence to liver's higher regeneration capacity, especially in advanced conditions of fibrosis and cirrhosis. Hindering or minimizing these damaging effects on the liver is a goal for research combating primary liver damage or damage secondary to drugs and chemotherapeutics. Consequently,

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proposing hepatoprotective strategies to accompany such hepatotoxic, but necessary, medications is very important.

Cyclophosphamide (CPh) is a member of the oxazaphosphorines (also known as, alkylating nitrogen mustards), it is a drug that is frequently used for its immunosuppressive and anticancer characteristics; used in human for treating different forms of cancer as breast cancer, lymphoma and leukemia. [5,6] It is also used for treating immune-mediated hemolytic anemia, in dogs, along with different cancer types in dogs [7–10] and cats. [11,12] After CPh administration, it is metabolized by liver enzymes, mainly cytochrome P450, yielding highly toxic metabolites [13,14]; such as acrolein. [15] These harmful metabolites are detoxified by liver endogenous detoxification mechanisms until they are depleted. With depletion of liver antioxidant defenses the CPh-associated hepatotoxicity begins to develop, in addition to other toxicities of various body tissues, for example the kidneys and nervous system. [16] Also, another associated effect is production of reactive oxygen species that lead to increased membrane lipid peroxidation and malondialdehyde (MDA) release. [17] In addition to upregulation of nuclear factor-κB (NF-κB) that leads to increased production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). [18] Overall, CPh leads to oxidative damage and tissue wide inflammatory reaction accompanied with apoptosis and necrosis. Its dose-dependent side effects are well-recognized, especially in bone marrow suppression and hepatic and renal injury. [19] These undesirable and harmful side effects limit CPh medical use especially in patients with liver or kidney diseases, putting these patients at higher risks.

Ancient Asian medicine introduced the wonders herb, Ginseng, to the world; which has been widely used to treat various illnesses since then. Now, a valuable amount of scientific information exists and confirms ginseng's various beneficial effects. [20,21] In short, these include protection against cardiovascular diseases [22–24] neurodegenerative diseases such as Alzheimer's disease, Parkinsonism, stroke and Huntington's disease (reviewed in [25]). In addition to various effects against different cancer types; including liver, ovarian, gastric, lung, colon and breast cancers (summarized in [26,27]), radiation-induced liver injury [28] and even on obesity and adipogenesis. [29] Also, it plays an important role in modulating the inflammatory responses (reviewed in [28]). There are plenty of other beneficial effects that can be found elsewhere in the literature. All of the ginseng's desirable effects are due to its active ingredients, known as ginsenosides, of these approx. 40 types were successfully identified and isolated. [28] Freshly collected ginseng roots are white but after steaming they turn red in color, and the red ginseng is traditionally described to be more powerful than the white or fresh one. [30,31]

Several studies investigated the ameliorative effects of ginseng on alcoholic and non-alcoholic fatty liver disease, [32,33] carbon-tetrachloride hepatotoxicity, [34] aflatoxin B1 [35] and hepatocellular carcinoma in rat [36] and in human. [37] However, we still lack adequate information on its hepatoprotective role in case of cyclophosphamide-induced liver damage. Therefore, we herein describe the use of *Panax ginseng* (Korean Red Ginseng) compared to a natural antioxidant (vitamin E) in combating liver damage induced by cyclophosphamide.

2. Material and methods

2.1. Study animals

In this study we used male Albino rats (body weight 210–225 g) obtained from the animal house at the Faculty of Medicine, Zagazig University. The rats were kept under controlled temperature (24 ± 1 °C), relative humidity (50 ± 5%) and lighting conditions (12 h light /12 h dark), rat feed and filtered tap water were freely provided. A one-week acclimatization period was allowed before commencing with the experiments. All experiments described herein were following the guidelines of the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Zagazig University.

2.2. Experimental design

Study rats were allocated randomly to one of these groups (n = 8 per group): Control (C), Ginseng (G), Vitamin E (VE), Cyclophosphamide (CPh), Cyclophosphamide + Ginseng (CPh + G) or Cyclophosphamide + Vitamin E (CPh + VE) groups.

Control group received intraperitoneal injection of sterile physiological saline daily for 7 consecutive days. In the cyclophosphamide group, cyclophosphamide (Sigma-Aldrich, USA) was dissolved in sterile physiological saline and given as an intraperitoneal injection of 20 mg/kg/day for 2 weeks. [38] Whereas, in the Cyclophosphamide + Ginseng group, in addition to cyclophosphamide administration, ginseng (*Panax ginseng* C.A. Meyer G115 extract) was administered orally at a dose of 100 mg/kg/day, also for 2 weeks. [39] Concentrated and standardized Korean Red Ginseng (*Panax ginseng* C.A. Meyer G115) extract preparation and ginsenoside contents are previously reported, [20,21,40] this extract was purchased from EIPICO (10th of Ramadan City, Egypt). Vitamin E treated groups were given 100 mg/kg vit E (400 mg VITAMIN E capsules, PHARCO, Egypt) orally once daily for 2 weeks. [41] Vitamin E and ginseng were administered 2 h before CPh administration in their respective treated groups.

Blood samples were collected from the tail vein of three rats for biochemical analyses. The study rats were sacrificed by cervical dislocation, and liver tissue specimens were collected for tissue assays, histopathological examination and gene expression analyses.

2.3. Biochemical analyses

Blood samples were collected in plain Vacutainer tubes, then serum was separated by centrifugation at 1500 g for 10 min at RT and stored at −20 °C until analyses. Levels of aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total proteins, albumin and globulins were all measured using commercially available kits, following manufacturer's instructions and in triplicates.

2.4. Oxidative stress evaluation in liver tissue homogenate

Liver tissue was immediately collected post-sacrifice and washed twice in PBS + heparin (0.16 mg/ml) to wash away blood and/or blood clots. After that liver tissue was homogenized in ice-cold PBS, followed by centrifugation at 2000 g and 4 °C for 15 min. The supernatant was collected for assessing liver lipid peroxidation by measuring malonyl dialdehyde (MDA). Also, the levels of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and Catalase (CAT) were assessed using commercial kits (Bio-diagnostics Co., Cairo, Egypt and BioVision, Inc., California, USA). All assays were performed following manufacturer's instructions and in triplicates.

2.5. Histopathological examinations

Additional liver-tissue samples were gently washed with warm saline and immediately fixed in 10% neutral buffered formalin solution, then histologically prepared using standard techniques. Tissue sections (5 μm thick) were obtained and standard hematoxylin and eosin staining (H&E) was performed. The extent of liver damage was blindly assessed by a pathologist for hepatocyte vacuolization, inflammatory-cells infiltration, blood-vessels congestion, and sinusoids dilatation, as previously reported. [42]

2.6. Real time PCR (RT-qPCR)

Total RNA was extracted from liver tissue specimens, using RNeasy mini kit (Qiagen, Cat. No. 74104) following manufacturer's protocol. The obtained total RNA concentration and purity were measured

Table 1
List of primers used in qPCR.

Gene	Oligonucleotide sequence	Reference
TNF-α	F 5'-ACCACGCTCTTCTGTCTACTG-3' R 5'-CTTGGTGGTTTGCTACGAC-3'	Li et al., 2015
IL-1β	F 5'-GCAATGGTCGGGACATAGTT-3' R 5'-AGACCTGACTTGGCAGAGGA-3'	Li et al., 2015
BCL-2	F 5'-GATTGTGGCCTTCTTTGAG-3' R 5'-CAAACTGAGCAGAGTCTTC-3'	Zucchini et al., 2005
Casp3	F 5'-TGTATGCTTACTCTACCGCACCCG-3' R 5'-GCGCAAAGTGACTGGATGAACC-3'	Liu et al., 2013
β-actin	F 5'-AGCCATGTACGTAGCCAT-3' R 5'-CTCTCAGCTGTGGTGGTGAA-3'	Batalha et al., 2016

TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin 1β and BCL-2, B-Cell Lymphoma 2; Casp3, Caspase 3.

spectrophotometrically at 260 and 280 nm wavelengths using nanodrop (Quawell Q5000, Quawell Technology, Inc., San Jose, USA). The obtained mRNA was reverse transcribed into cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Cat. No. 1622).

The evaluation of gene expression was done with quantitative real-time PCR (qPCR), using QuantiTect SYBR® Green PCR Kit (Qiagen, Cat. No. 204141) on StepOnePlus™ Real-Time PCR system (Applied Biosystems, USA) the reaction cycles were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s followed by 60 °C for 1 min, then 72 °C for 1 min. The qPCR reaction (25 μL total) included 2 μL cDNA, 12.5 μL of SYBER green master mix, 8.5 μL of RNAse-free water and 1 μL of each primer (forward and reverse). The samples were normalized against β-actin gene using $2^{-\Delta\Delta Ct}$ method as previously described. [43] Primers for genes selected for the qPCR reactions are shown in Table 1.

2.7. Statistics

The obtained data was analyzed through one-way ANOVA followed by post-hoc comparisons using (SPSS statistical package v17, SPSS Inc., USA). All data is shown as mean ± SD. Significance was considered when P-value ≤ 0.05.

3. Results

3.1. Biochemical findings

The present biochemical analyses results for the control groups fell within normal rodent reference ranges, published in the website of the School of Veterinary Medicine, University of Pennsylvania (http://cal.vet.upenn.edu/rodentrr.htm, last accessed on December 11th, 2018). Data for serum biochemical findings and liver function markers in control and treated rats are presented in Table 2.

Serum levels of albumin ($P < 0.01$, $F = 66.3$) and, hence, total proteins ($P < 0.01$, $F = 52.9$) were roughly four times lower in cyclophosphamide treated rats compared to control, ginseng or vitamin E groups. And, after administration of ginseng their levels increased again and were comparable to the control groups. While in vitamin E treated rats, albumin and total proteins were also increased but less than that observed in ginseng group. The previous results were also reflected in albumin/globulin ratio ($P < 0.01$, $F = 91.1$). In case of globulins, by merely looking at their values, their serum levels were lower in cyclophosphamide liver-injured group and restored to the control levels in both ginseng and vitamin E treated groups (however, statistically, the difference between all groups was non-significant, $P = 0.63$, $F = 0.28$).

Additionally, the activities of liver function markers aspartate aminotransferase (AST; $P < 0.01$, $F = 24.4$), alanine aminotransferase (ALT; $P < 0.01$, $F = 111.5$) and alkaline phosphatase (ALP; $P < 0.01$, $F = 84.5$) were assessed in serum (Table 2), and their levels in the Control, Ginseng and Vitamin E groups were fairly similar and, hence, were statistically non-significant. Whereas, rats treated with cyclophosphamide showed higher levels of ALT, AST and ALP (approx. 5x, 2x and 2x times increase, respectively) compared to the control groups. While after administration of ginseng or vitamin E their serum levels dropped, again, this was better observed in ginseng treated group than in vitamin E treated group.

3.2. Oxidative stress findings

Levels of Malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT) were measured in liver tissue samples (Fig. 2). Cyclophosphamide administration led to significant decrease of liver tissue concentrations of SOD, GPX and CAT and increase in MDA compared to the control groups ($P < 0.01$, $F = 5.3$, 3.2, 15.8 and 5.6, respectively). Whereas, administration of ginseng or vitamin E restored their concentrations to approximate the normal levels found in control groups.

3.3. Histopathological findings

In control, ginseng and vitamin E groups, the liver showed normal histological structure with roughly hexagonal-shaped classic hepatic lobules and no apparent pathologies (Fig. 1 A, B and C, respectively). While, in the cyclophosphamide group, the liver showed numerous large focal areas of coagulative necrosis invaded with numerous leukocytes; mostly mononuclear cells (Fig. 1, D), significant fatty changes visible as sharp clear round vacuoles inside signet ring shaped hepatocytes (Fig. 1, E) and portal fibrous connective tissue proliferation infiltrated with numerous mononuclear cells (Fig. 1, F). However, in case of cyclophosphamide with vitamin E group, there were less numerous and small focal areas of coagulative necrosis invaded with mononuclear cells in addition to apparent proliferation of Von Kupffer cells (Fig. 1, G), fatty changes are visible in fewer hepatocytes (Fig. 1, H) and portal congestion with mild fibrous connective tissue

Table 2
Effects of ginseng (*Panax ginseng meyer*) or vitamin E administration on serum biochemical parameters that reflect liver condition after cyclophosphamide-induced injury.

	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	8.20 ± 0.20 $^{a, b}$	5.12 ± 0.10 ^{a}	3.08 ± 0.10	1.65 ± 0.03 ^{a}	14.00 ± 2.00 ^{d}	22.33 ± 2.52 ^{c}	74.67 ± 5.51 ^{d}
Vit E	8.25 ± 0.13 ^{a}	5.18 ± 0.10 ^{a}	3.07 ± 0.05	1.68 ± 0.03 ^{a}	13.33 ± 3.51 ^{d}	23.33 ± 4.04 ^{c}	73.67 ± 2.52 ^{d}
Ginseng	8.15 ± 0.94 $^{a, b}$	5.25 ± 0.65 ^{a}	3.23 ± 0.58	1.63 ± 0.11 ^{a}	12.33 ± 2.31 ^{d}	23.33 ± 2.52 ^{c}	75.00 ± 11.14 ^{d}
CPh	3.11 ± 0.10 ^{d}	1.22 ± 0.18 ^{c}	2.89 ± 0.60	0.44 ± 0.15 ^{c}	67.00 ± 2.65 ^{a}	54.67 ± 3.06 ^{a}	164.00 ± 4.58 ^{a}
CPh + G	6.85 ± 0.74 ^{b}	3.76 ± 0.58 ^{b}	3.09 ± 0.18	1.21 ± 0.14 ^{b}	28.67 ± 3.06 ^{c}	35.00 ± 6.08 $^{b, c}$	100.33 ± 7.51 ^{c}
CPh + V	5.12 ± 0.12 ^{c}	2.08 ± 0.14 ^{c}	3.04 ± 0.06	0.68 ± 0.05 ^{c}	43.00 ± 6.24 ^{b}	43.33 ± 7.37 $^{a, b}$	124.33 ± 6.66 ^{b}

CPh, Cyclophosphamide; CPh + G, Cyclophosphamide + Ginseng and CPh + V, Cyclophosphamide + Vitamin E. Dose of Ginseng is 100 mg/kg/day for 14 consecutive days, Vitamin E is 100 mg/kg/day for 14 consecutive days and Cyclophosphamide is 20 mg/kg/day for 14 consecutive days.
Different superscript letters within columns denote statistical significance ($P < 0.01$, group comparison was done using Tukey's HSD test). Data shown as mean ± SD.

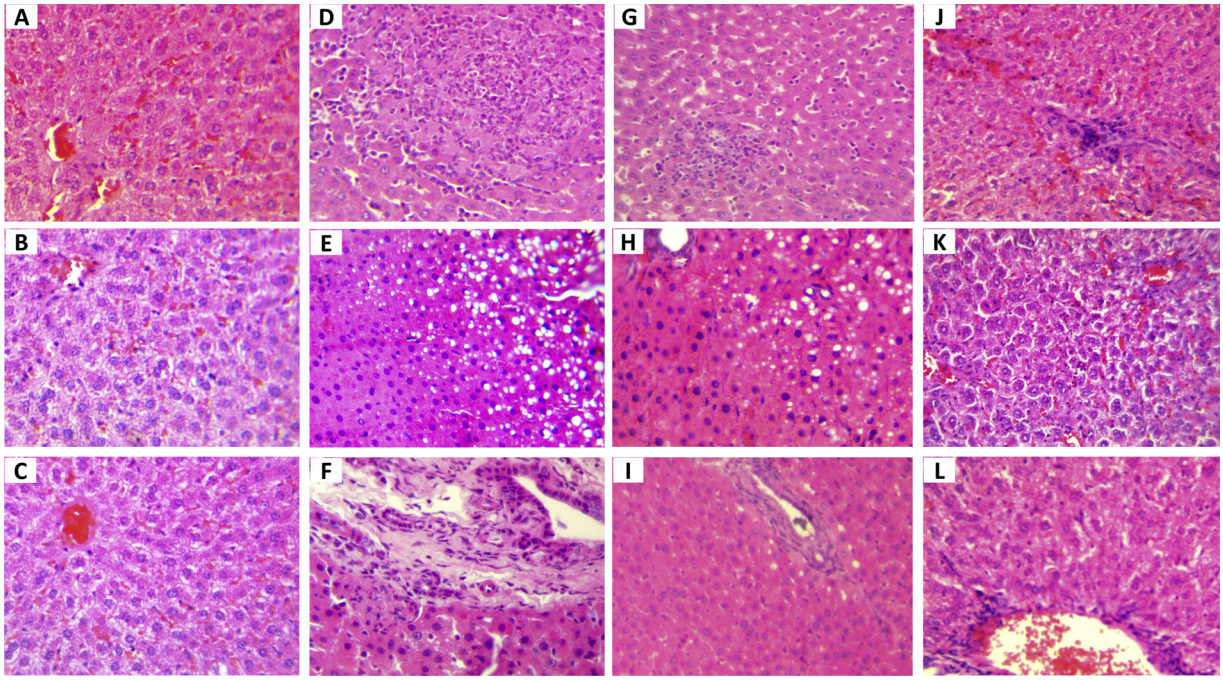

Fig. 1. Representative histopathological examination of degree of liver tissue damage in case of Control (A), Vitamin E (B, 100 mg/kg/day for 14 consecutive days), Ginseng (C, 100 mg/kg/day for 14 consecutive days), Cyclophosphamide (D, E & F, dose 20 mg/kg/day for 14 consecutive days), Cyclophosphamide + Vitamin E (G, H & I) and Cyclophosphamide + Ginseng (J, K & L) administration. In control, Ginseng and Vitamin E groups, rat's liver showed normal histological structure with roughly hexagonal-shaped classic hepatic lobules. In the Cyclophosphamide group rat's liver showed numerous large focal areas of coagulative necrosis invaded with many leukocytes; mostly mononuclear cells (D), significant fatty changes visible as sharp clear round vacuoles inside signet ring shaped hepatocytes (E) and portal fibrous connective tissue proliferation infiltrated with numerous mononuclear cells (F). In case of Cyclophosphamide with Vitamin E group, rat's liver showed less numerous and small focal areas of coagulative necrosis invaded with mononuclear cells in addition to apparent proliferation of Von Kupffer cells (G), fatty changes are visible in fewer hepatocytes (H) and portal congestion with mild fibrous connective tissue proliferation infiltrated with less mononuclear cells (I). In case of Cyclophosphamide with Ginseng, rat's liver picture was much improved and showed dilated sinusoids with proliferated Von Kupffer cells besides focal aggregation to few numbers of mononuclear cells (J), few vacuolated hepatocytes (K) and low portal congestion with less detectable fibrosis and mild mononuclear cell infiltration (L). H&E, X400.

proliferation infiltrated with less mononuclear cells (Fig. 1, I). Finally, in case of cyclophosphamide with ginseng group, liver's picture was much improved and showed dilated sinusoids with proliferated Von Kupffer cells besides focal aggregation of few numbers of mononuclear cells (Fig. 1, J), few vacuolated hepatocytes (Fig. 1, K) and insignificant portal congestion with less detectable fibrosis and mild mononuclear cell infiltration (Fig. 1, L).

3.4. Gene expression (qPCR) findings

To test the effect of cyclophosphamide on liver proinflammatory response and apoptosis, gene expression of interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNF-α), for proinflammatory response, and B-Cell Lymphoma 2 (BCL-2) and Caspase 3, for apoptosis, was performed (shown in Fig. 3).

Proinflammatory genes expression (TNF-α and IL-1β) significantly

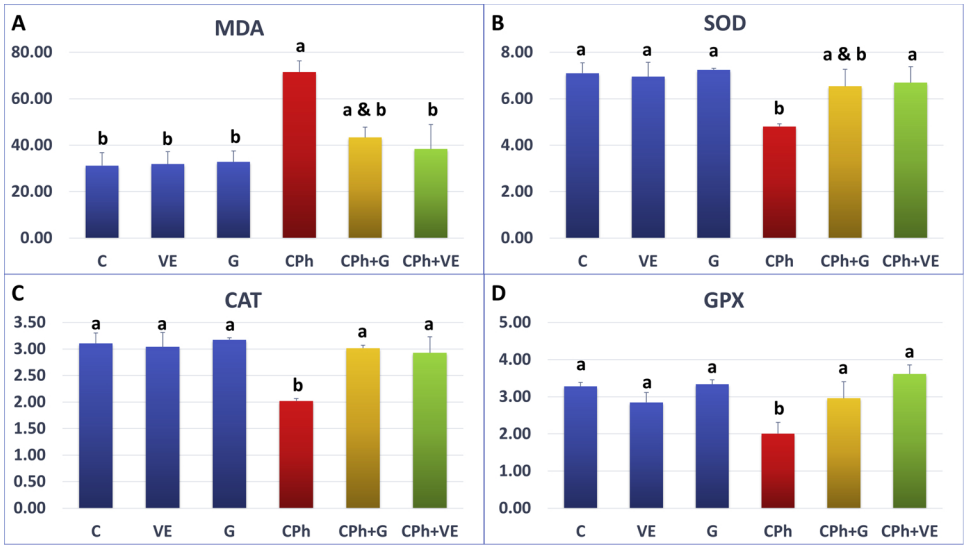

Fig. 2. Tissue levels of Malondialdehyde (MDA, nmol/g), Glutathione peroxidase (GPX, U/mL), Superoxide dismutase (SOD, U/mL) and Catalase (CAT, U/g tissue) in case of Control (C), Vitamin E (VE, 100 mg/kg/day for 14 consecutive days), Ginseng (G, 100 mg/kg/day for 14 consecutive days), Cyclophosphamide (CPh, 20 mg/kg/day for 14 consecutive days), Cyclophosphamide + Vitamin E (CPh + VE) and Cyclophosphamide + Ginseng (CPh + G) administration. Different letters denote statistical significance ($P < 0.01$, group comparison was done using Tukey's HSD test). Data shown as mean ± SD.

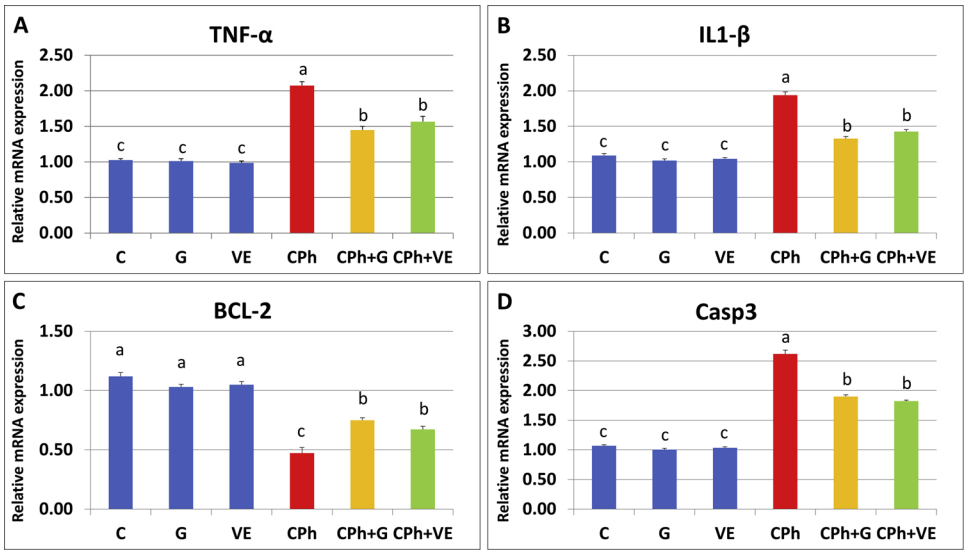

Fig. 3. Relative mRNA expression levels (qPCR) of TNF-a, IL1-B, BCL-2 and Caspase 3 (Casp3) in case of Control (C), Vitamin E (VE, 100 mg/kg/day for 14 consecutive days), Ginseng (G, 100 mg/kg/day for 14 consecutive days), Cyclophosphamide (CPh, 20 mg/kg/day for 14 consecutive days), Cyclophosphamide + Vitamin E (CPh + VE) and Cyclophosphamide + Ginseng (CPh + G) administration. Different letters denote statistical significance ($P < 0.01$, group comparison was done using Tukey's HSD test). Data shown as mean ± SD.

increased in cyclophosphamide rats ($P < 0.01$, $F = 166$ and $F = 257$, respectively), while after administration of either ginseng or vitamin E their level of expression decreased towards the normal range of expression (Fig. 3 A & B). Whilst, expression of Caspase 3 (apoptosis executioner, $P < 0.01$, $F = 297.6$) increased and BCL-2 (anti-apoptotic, $P < 0.01$, $F = 143$) decreased in cyclophosphamide rats compared to the control ones. Again, Caspase 3 levels decreased and BCL-2 levels increased, both approaching normal expression levels, with administration of either ginseng or vitamin E (Fig. 3 C & D).

4. Discussion

In the present study we targeted the exploration of the hepatoprotective effects of Korean Red Ginseng in case of cyclophosphamide-induced liver injury and compared its enhancing effect with a potent antioxidant (vitamin E). Cyclophosphamide (CPh) has a wide range of clinical applicability as anticancer and immunosuppressive agent, and there are no available clinically-approved safe alternatives in the market. So, since there is no escaping of CPh use, our focus was on alleviating its side effects by protecting various important non-targeted tissues, such as the liver.

As an alkylating agent, CPh was held responsible for multiple toxicities [44–46] The effect is pronounced when liver microsomal P450 oxidases bioconvert cyclophosphamide into various metabolites, which were unexpectedly found very harmful to the liver itself; and various body tissues. Of these metabolites the most harmful is acrolein. [47,48] Surprisingly, acrolein can also be found in cigarette smoke, car exhaust, overheated or burnet cooking oil or grease and pesticides, and its aspiration causes lung damage as well (DHHS, CDC, USA https://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=555&tid=102, last accessed on 14th of January 2019 [47]). Although it is not classified as a direct carcinogen by the DHHS, occurrence of carcinogenesis after CPh administration is possible, [48,49] especially in organs with higher regeneration rate as liver. In fact, few secondary malignancies were already reported following CPh administration. [50,51]

As previously reported, typical liver damage associated with CPh administration was evident in this study. [38,41,52,53] The damage was perceived by increased serum levels of ALT, AST and ALP (liver function markers), decreased serum albumin and albumin/globulin ratio and increased oxidative stress in liver tissue (lower tissue levels of SOD, GPX and CAT and increased MDA). In addition to the increased gene expression of proinflammatory cytokines (upregulation of IL-1β and TNF-α) and apoptotic pathway (upregulation of Casp3 and downregulation of BCL-2) indicating inflammation and apoptosis within the hepatic tissue. Furthermore, this was confirmed by the pronounced histopathologic picture which reflected liver tissue damage and inflammatory cells infiltration.

Administration of vitamin E reduced CPh-caused liver damage. It was previously reported that serum ALT and serum MDA, liver histopathology and TUNEL-positive apoptotic hepatocytes were all improved following vitamin E administration. [41] However, in the latter study, the authors failed to notice changes in serum AST between control, CPh or Vitamin E treated groups. In the present study vitamin E nullified CPh toxic effects by combating the oxidative damage exerted on liver tissue when it metabolized CPh. In addition to the results reported by Cuce and colleagues, [41] we found that vitamin E reduced mRNA levels of IL-1β and TNF-α; decreasing the pro-inflammatory responses within the liver. This effect was also demonstrated in the histopathological examination of the liver, where few necrotic areas with mild fatty changes and few mononuclear cells infiltration were observed in the liver; in comparison with CPh alone group. Also, tissue levels of oxidative stress markers (SOD, GPX, CAT and MDA) were all restored towards their normal levels in vitamin E treated group compared to the CPh group, while Cuce and colleagues only measured MDA in the serum. Finally, levels of liver damage markers (ALT, AST, ALP) and serum albumin and albumin-globulin ratio were also restored towards their control values.

However, taking in consideration that liver damage is caused mainly by oxidative damage, then antioxidants alone should prevent and diminish the extent of the damage; which was the reason for vitamin E selection for comparison with P. ginseng in this study. However, this was not totally true since we showed that ginseng had superior outcomes in terms of liver protection than vitamin E; the latter is a potent natural antioxidant. Additionally, so far, there is no therapy of choice in the literature for CPh-induced hepatotoxicity. Some studies used hesperidin, [54] punicalagin [38] and gamma-glutamylcysteine ethyl ester (GCEE) [53] to reduce this CPh-induced hepatotoxicity. However, all these have antioxidant in addition to other effects (anti-inflammatory and anti-apoptotic), so they do not fulfil our study's antioxidants alone criteria. Therefore, vitamin E was selected to be compared with P. ginseng.

Ginseng's antioxidant properties were similar to vitamin E, and this was evidenced by nearly no differences in liver tissue MDA, SOD or CAT levels in both treated groups, only vitamin E was better in case of GPX levels. Thus, antioxidant-wise, vitamin E might be slightly better than ginseng due to its direct antioxidant effects on liver tissue; hence preserving local tissue levels of MDA, SOD, CAT and GPX, as seen in our results. Still, ginseng is also famous for its great antioxidant properties

(reviewed in [55]).

Nevertheless, *Panax ginseng* was obviously more effective than vitamin E in restoring serum levels of albumin, total proteins, A/G ratio, AST, ALT and ALP. Also, ginseng was more effective in restoring the histopathological picture of the affected liver to its normal histological picture. Similarly, in acute carbon tetrachloride (CCl4) toxicity in rats, ginseng was able to restore serum biochemical parameters (AST, ALT, GGT, cholesterol, triglycerides, total protein) related to liver damage. [4] In addition, in aged rats, ginseng minimized the oxidative stress (MDA, SOD, GPX, CAT, glutathione reductase, glutathione-S-transferase and, also, on non-enzymatic antioxidants: reduced glutathione, ascorbic acid and α-tocopherol) in most organs thus helped in reducing age-related disorders caused by oxidative stress. [56] Therefore, the observed effects herein could possibly be due to ginseng's other beneficial effects (other than antioxidant effects), such as anti-inflammatory and tissue regenerative properties caused by ginseng's various active ingredients.

By fractioning ginseng's extract, the acidic polysaccharide fraction was found to have immunomodulating properties [57,58] and can stop the immunotoxicity of CPh. [59] More recently, with the ability to separate individual ginsenosides, it was found that ginsenoside Rh2 was able to reduce the genotoxic effect of CPh [60] and total saponins from *Panax ginseng* C.A. Meyer [61] and ginsenoside Rb1 [62] were able to diminish CPh genotoxicity in mouse bone marrow and peripheral lymphocytes. Furthermore, ginseng [63] and in particular ginsenoside Rh2 [60,64,65] show an enhancing antitumor effect when administered with chemotherapeutics; meaning that ginseng administration will not reduce their chemotherapeutic power. Likewise, numerous studies highlighted the critical roles of numerous ginsenosides (such as: G-Rb1, compound K, G-Rb2, G-Rd, G-Re, G-Rg1, G-Rg3, G-Rg5, G-Rh1, G-Rh2 and G-Rp1) in various anti-inflammatory activities (elegantly reviewed in [28]). All this, together with this study's results, highlights the possible mechanisms by which P. ginseng improved CPh-induced liver damage. In addition, it confirms that ginseng should be superior to vitamin E in correcting the CPh-induced liver damage.

Indeed, a research group from National Research Center and National Liver Institute in Egypt went directly to test the therapeutic potential of Korean red ginseng extract on human patients suffering from cirrhotic livers without or with hepatocellular carcinoma; secondary to hepatitis C infection. [37] They found that ginseng administration improved serum levels of liver function markers (ALT, AST, bilirubin and albumin), reduced serum levels of alfa fetoprotein (AFP; tumor marker for hepatocellular carcinoma) and reduced the viral load in treated patients. However, the possible mechanisms by which ginseng exerted these beneficial effects were not examined in their study. We herein, report some of the possible mechanisms (namely reduction of oxidative stress, inflammation and apoptosis as discussed above) that could have led to these ginseng beneficial effects in treated human patients. Other mechanisms for this hepatoprotective effects were also recently reported. Where, P. ginseng led to upregulation of Nrf2 gene which controls many antioxidant genes and guards against oxidative damage caused by polychlorinated biphenyls (PCBs) in cultured rat pheochromocytoma (PC12) cells. [66] Also, *P. ginseng* administration upregulated Nrf2 and corrected the disturbed homeostasis of glutathione and bile acids seen with CPh-induced liver damage. [67] In addition, in dogs, ginseng was found to enhance liver regeneration rate after partial hepatectomy; mainly by reducing numbers of degenerated cells and areas of connective tissue. [68]

Finally, a question arises about the safety of *P. ginseng* use as a therapy. While most reports highlighted its potential benefits, few focused on its adverse effects. Therefore, a systematic review on *P. ginseng* adverse effects was performed on data from clinical trials, case reports, epidemiological studies and reporting schemes (WHO, FDA, UK Medicine Control Agency and German Federal Institute for Drugs and Medical Devices) and the authors found that ginseng monopreparation's rarely show adverse effects; most reported ones were headache, sleep disorders and GIT disorders. [69] They, also, found that in isolated cases, more serious adverse effects (such as: nausea, diarrhea, vomiting, blood pressure changes, vaginal bleeding, cerebral arteritis … etc.) were found especially in combination products (i.e. drug interactions) but no clear causality was linked to *P. ginseng* in these cases. [69] The authors further state that data presented in their review should be evaluated with a degree of caution. Others, however, linked these serious side effects with "ginseng abuse syndrome". [21] Moreover, regarding the liver, ginseng's hepatoprotective and hepatotoxic effects were also reviewed. [70] All this highlight two points, one is the contradictory data presented on P. ginseng adverse effects and the other is the importance of considering more evaluations of possible adverse effects, especially when P. ginseng is used in combination with other medications (i.e. considering drug-drug interactions). However, in our study, we did not observe side effects to ginseng's administration, either alone or with CPh, which could be due to the small, but still effective, dose used in this study (100 mg/kg) compared to reported 2000 mg/kg [71] or LD_{50} of 5000 mg/kg orally in rats. [72]

In conclusion, so far there is no escaping of the clinical use of cyclophosphamide. This led us to focus on alleviating its side effects by protecting various important non-targeted tissues, such as the liver. Here, we reported the hepatoprotective effect of Korean Red Ginseng (*Panax ginseng meyer*) compared to vitamin E in cyclophosphamide-induced liver injury. The effects were lower inflammation and less apoptosis which led to lower levels of liver damage markers in serum and oxidative stress markers inside liver tissue. Ginseng was superior to vitamin E in terms of overall hepatoprotection. In addition, both are easily found in the market as nutritional supplements, compared to other chemical substances (such as GCEE) that are difficult to obtain or need strict medical supervision. It is therefore recommended to take ginseng (or vitamin E) as a hepatoprotective therapy with cyclophosphamide, or similar damaging drugs, to alleviate the associated hepatotoxic side-effects.

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Declaration of Competing Interest

None declared.

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