Saponin Protects Against Cyclophosphamide-Induced Kidney and Liver Damage via Antioxidant and Anti-Inflammatory Actions

Mohammad Ghasem Golmohammadi

Ardabil University of Medical Sciences: Ardebil University of Medical Sciences

Shokofeh Banaei (✉ s.banaei@arums.ac.ir)

Ardabil University of Medical Sciences <https://orcid.org/0000-0002-8742-3895>

Ali Abedi

Ardabil University of Medical Sciences: Ardebil University of Medical Sciences

Research Article

Keywords: Saponin, Cyclophosphamide, Liver, Kidney, Antioxidants

Posted Date: March 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1398111/v1>

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Abstract

Purpose

The liver and kidney are organs affected by chemotherapy drugs such as cyclophosphamide (CP). This study examined the protective effects of treatment with saponin (SP) against CP-induced nephrotoxicity and hepatotoxicity.

Methods

24 adult male mice were divided into four groups (N = 6): Control group, CP (15mg/kg), SP (2.5 mg/kg) and CP + SP. After treatment, the blood samples were collected for the determination of biochemical parameters and liver and kidney samples were taken for histological analysis and the assessment of oxidative stress and inflammatory markers.

Results

Cyclophosphamide decreased renal and liver functions and antioxidant enzymes, which significantly increased blood urea nitrogen- creatinine (BUN- Cr), liver enzyme levels, malondialdehyde, Nuclear factor kappa β (NF- κ B) and Interleukin 1 beta (IL-1B) concentrations. Moreover, histopathological findings of the CP group showed that there were acute tubular necrosis and glomerular atrophy in the renal tissues and lymphocyte infiltration in the liver samples. Treatment with saponin improved hepatic and nephric functions, the pathological changes and antioxidant capacity, also, decreased lipid peroxidation and inflammation.

Conclusion

It seems that saponin could exert a hepato-nephroprotective effect against cyclophosphamide toxicity.

Introduction

The kidney and liver are systems affected by chemical toxicity and chemotherapy drugs like cyclophosphamide (CP). The liver is one of the organs where drugs and chemical materials are metabolized then excreted. Liver cells or hepatocytes are to exposure with divers chemical agents maybe lead to hepatotoxicity, hepatic dysfunction and failure (Ribeiro, Yang, Patel, Madabushi, & Strauss, 2019). The kidney has various functions such as the regulation of water and electrolyte balance, arterial pressure, body fluid osmolarity and acid-base balance. Furthermore, the kidney excretes the metabolic waste products and exogenous chemicals and the active form of vitamin D is produced in the kidney by mitochondria of the proximal tubules. Also, the secretion and metabolism of hormones including erythropoietin and 1, 25(OH) 2D3 are associated to the kidneys. There are close relation between the liver

and kidney, which this functional interaction is pivotal to maintain internal homeostasis (Brown, Bouley, Păunescu, Breton, & Lu, 2012). Toxicity effects of chemotherapeutic agents which is widely administrated in the treatment of various types of cancers to the kidney, liver and other organs able to disrupt the physiologic functions them (Lee et al., 2008) .

These two systems (liver and kidney) metabolize and excrete chemical agents mainly involve in the material detoxification more than other organs. As a result, the toxicity of chemotherapeutic drugs can result in degenerative alterations and life-threatening kidney and liver injury (Un et al., 2020).

Cyclophosphamide is a chemotherapeutic drug, is administrated in cancer diseases, it has several side effects including genital disorders, testicular damage, cardio-renal toxicity and hepatotoxicity. CP with antineoplastic properties is associated with histological and biochemical changes in the different tissues. It is known that CP interrupts the balance of free radicals and antioxidant defenses, which produced reactive oxygen species (ROS) are responsible for DNA fragmentation, necrosis, apoptosis and cell death (Cengiz et al., 2020). Oxidative injury of the DNA plays an important role in the apoptosis of the germ cells and tissues. Despite of clinical applications of CP, it has adverse influence on the liver that decomposes CP to active metabolites, on the kidney that excretes it. The previous studies have shown that impaired humoral secretion, infertility, DNA damage, lipid peroxidation and morphological defects in the tissues are associated to the treatment with the CP (Patwa & Khan, 2020).

Therefore, the protection of organs such as the kidney, liver, cardiovascular system and etc against cyclophosphamide toxicity is inevitable. Herbal medicines have been confirm to affect in the inhibition of CP-induced tissue toxicity (El-Sebaey & Abdelhamid, 2019). Saponins (SP) are natural compounds of Ginseng, Fig and Olive which they have various biological activities including anti-inflammatory, antioxidant, anti-microbial, anti-tumor and immune-modulating properties (Shao, Wang, Zhu, & Dang, 2020; Zhao, Xue, & Zhang, 2018). Additionally, saponins improve antioxidant capacity and normalize AST (aspartate transaminase), ALT (alanine transaminase) and ALP (alkaline phosphatase) in the liver, scavenge ROS and reduce lipid peroxidation. Also, saponins decrease heat stress and suppress oxidative stress in tubular and glomerular epithelial cells in the kidney in a dose dependent manner. It has found that some saponins obtained from plants decrease tumor growth, have preventive effect on proteinuria, therapeutic effect on liver and renal function and protective effect against cardiomyopathy (Du, Wang, Qian, Zhang, & Chai, 2016; Zhou et al., 2016).

In recent decades, herbal remedies were evaluated to inhibit the toxicity of chemotherapeutic drugs, especially, to avoid the side effects caused by the chemical medicines. In the present study, we investigated the protective effects of saponin against CP-induced nephrotoxicity and hepatotoxicity using evaluation of the antioxidant capacity and inflammatory mediators.

Method And Material

For the study, 24 adult male NMRI mice (6 to 8 weeks old, weighing 20 to 25 g) were divided into 4 groups and were treated for 35 days. The mice had free access to standard feed and water during the study

period. The conditions of the animal house were under controlled temperature about $21 \pm 2^{\circ}\text{C}$ and under 12–12 hour light- dark cycle with humidity ($60 \pm 5\%$). Principles of laboratory animals ethics with regard to international standards and the ethics committee of Ardabil University of Medical Sciences were observed in all stages of research (ethics code: IR.ARUMS.AEC.1400.019).

Experimental design

Male mice were randomly divided into 4 groups (N = 6) with a treatment period of 35 days. The groups were as follow:

1. Control + Saline (0.2 ml/day, IP)
2. CP group (15 mg/kg/week, IP)
3. SP group (2.5 mg/kg/day, IP)
4. CP + SP group, received CP (15 mg/kg/week) with SP (2.5 mg/kg/day)

Cyclophosphamide (500 mg vial sigma-USA) was dissolved in normal saline in the darkness. CP was injected weekly intraperitoneally (15 mg/kg) (Mehraban, Gaffari Novin, Golmohammadi, & Nazarian, 2021). Saponin powder was obtained from the company (Sigma- Aldrich- USA), and dissolved in normal saline at room temperature, away from light and under the hood. Initially, a pilot study was performed to evaluate the dosage of saponin and effective dose was 2.5 mg/kg/day (Timar & Banaei, 2021).

Biochemical measurement

Under anesthesia, Blood samples, liver, and kidney were collected at the end of the treatment period in each group. The blood samples were centrifuged at approximately 4000 rpm for 10 min. The blood urea nitrogen (BUN) and creatinine (Cr) levels in the serum were determined to assess the renal function, using the Auto-analyser with original kits. The activities of ALP, ALT and AST were assayed using Randox assay kits.

Histological evaluation

Liver and renal tissue samples were fixed in 10% buffered formalin solution, dehydrated in ascending grades of alcohol and embedded in paraffin. Sections of $5\mu\text{m}$ were taken, stained with hematoxylin- eosin (H&E), and then examined under light microscope, in a blinded manner by pathologist. The histological score was semi-quantitatively evaluated using a scale ranging from 0 to 3 (0: none, 1: mild, 2: moderate, and 3: severe damage) (Golmohammadi & Banaei, 2021).

Tissue Malondialdehyde (MDA) and Total antioxidant capacity (TAC) measurement

The liver and renal tissues were homogenized in 1.15% KCl. The tissue levels of MDA were assessed with the ZellBio kit (Germany). The TAC levels were determined using Radox total antioxidant status kit based on the companies method. It is the production of cation ABTS^{+} and next to its inhibition via antioxidant compounds of the specimen (Ahmadiasl, Banaei, Alihemmati, Baradaran, & Azimian, 2014).

Tissue Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) assessment

To measure cytosolic enzyme activity, the liver and renal samples were homogenized in 1.15% KCl solution. SOD and GPx activity were measured according to the ZellBio kit (Germany) and the manufacturer protocol (Ahmadiasl, Banaei, Alihemati, Baradaran, & Azimian, 2014).

Assessment of tissue levels of Nuclear factor kappa β (NF-kB) and Interleukin 1 beta (IL-1B)

The liver and renal samples were collected after the treatment period and the tissue levels of NF-kB and IL-1B were measured using enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's protocol.

Statistical analysis

All the data were expressed as mean $\pm$ standard deviation, using the one-way analysis of variance (ANOVA) followed by multiple comparison procedures using post hoc complementary test, with Graph Pad Prism version 8.4.3. Statistical significance was accepted at $P < 0.05$.

Results

The aim of the current study is to determine the therapeutic and protective effects of saponin against cyclophosphamide-induced nephrotoxicity and hepatotoxicity. We hypothesized that with the intervention of appropriate therapeutics, it should be possible to manage the CP damage and provide a beneficial hepato and reno-protection. Biochemical analysis results are outlined in figures 1-5. The results of histological scores are shown in figures 7, 9.

Protective effect of saponin on CP- induced alterations in serum biochemical parameters

The levels of serum AST, ALT, and ALP enzymes were significantly elevated in the CP group in comparison with the control group ($P < 0.0001$, Figures 1-3). Also, the BUN- Cr levels were significantly higher in the CP group compared with those from the control group ($P < 0.0001$, Figures 4, 5). Saponin and co-administration of SP together with CP significantly reduced the levels of ALP, ALT, AST and BUN-Cr in comparison to the CP group.

Protective effect of saponin on CP- induced renal and hepatic histopathological changes

Histological assessment of the kidneys showed that there were no morphological changes in the control group (Figure 6A) also, healthy appearance of glomerular and tubular cells were preserved in the SP group (Figure 6C). In the CP group, acute tubular necrosis (ATN) congestion, pyknotic nuclei, glomerular atrophy and dilated bowman's space were very obvious (Figure 6B). Saponin co-administration with CP resulted in noteworthy decline in histopathological alterations induced by cyclophosphamide (Figure 6D). Correspondingly, renal histological index was significantly decreased in the saponin group in comparison to the CP group ($P < 0.01$, Figure 7).

Also, histological examination of the liver showed that there were no histological changes in the control group (Figure 8A). Moreover, SP-administrated groups showed normal hepatic structure (Figure 8C). In the CP group, the normal histological organization of the liver tissues was disrupted. There were lymphocyte infiltration, acute triaditis and neutrophil infiltration following the treatment with CP (Figure 8B). CP plus SP demonstrated the reduction of the degenerative changes in hepatocytes (Figure 8D). Also, liver histological index was significantly decreased in the SP and CP + SP groups in comparison to the CP group ($P < 0.05$, Figure 9).

The effect of saponin and cyclophosphamide on the MDA and TAC levels

As shown in the Table 1, in the CP group, the MDA levels were insignificantly increased as compared to the control group. Saponin administration significantly reduced MDA concentration than the CP group ($P < 0.01$). Besides, the CP administration decreased TAC levels in contrast to the control group. But, saponin treatment significantly elevated TAC levels in comparison with the CP group ($P < 0.05$).

The effect of saponin and cyclophosphamide on the SOD and GPx activity

The mean activity of GPx and SOD in the liver and kidney in the CP group insignificantly decreased in comparison to the control group. The saponin treated groups showed a considerable increase of SOD and GPx levels as compared to the CP group (Table 1).

The effect of saponin and cyclophosphamide on the NF- κ B and IL-1B productions

We assessed whether saponin decreases the production of the pro-inflammatory cytokines: NF- κ B and IL-1B induced by cyclophosphamide. The toxic effects of CP resulted in significant elevation of NF- κ B and IL-1B concentrations in kidney and liver tissues when compared to the control group ($P < 0.001$). However, the tissue levels of these cytokines were significantly reduced in the saponin ($P < 0.001$) and CP plus SP ($P < 0.05$) groups in comparison to the CP group (Figures 10 and 11). These results indicate that saponin alone and co-administration of CP with SP decrease the NF- κ B and IL-1B productions.

Discussion

The liver and kidney are systems affected by chemotherapy drugs like cyclophosphamide. Acute and chronic exposure to some chemical agents result in nephrotoxicity and hepatotoxicity (Bhat, Kalthur, Padmashali, & Monappa, 2018). Inflammation is characterized by the protective response of the immune system against exogenous chemicals are metabolized and finally excreted by liver and kidney. In this process, pro-inflammatory cytokines including tumor necrosis factor alpha (TNF- α), interleukins and mediators such as nitric oxide (NO) increase in the body and reactive oxygen species (ROS) are produced. Generation of ROS, including the hydroxyl radical (OH $\cdot$), hydrogen peroxide and superoxide anions or the reduction of antioxidant defense leads to oxidative stress, which plays a critical role in the development of liver and renal injury and even organ failure. Therefore, ROS scavengers and antioxidants that remove free radicals, moreover, anti-inflammatory herbal medicines (natural remedies) can protect against renal

and liver dysfunction mediated by cyclophosphamide (Kocahan, Dogan, Erdemli, & Taskin, 2017; Zhai et al., 2018).

Many investigations have been done about the antioxidant, anti-inflammatory and immune-regulator role of saponins. Saponins reduce the produced free radicals, regulate the expression of antioxidant enzymes and eliminate the toxic effects of the industrial chemicals that lead to resolution of inflammation and return to hemostasis (Ma, Jiang, Yuan, Qiu, & Zhu, 2019; Yao et al., 2019). Therefore, in the present study, we hypothesized saponins with antioxidant and anti-inflammatory capacity able to inhibit liver and kidney toxicity due to the treatment with cyclophosphamide.

Our result in the current study indicated that exposure with CP significantly increased the serum levels of ALP, ALT, AST and BUN- Cr, suggesting a liver and renal dysfunction which was greatly decreased after saponin treatment. Saponin and co-administration of SP together with CP significantly reduced these parameters. Probably, antioxidant properties of saponin could be effective in the improvement of hepatic and renal function. On the other hand, histological evaluation showed that CP caused glomerular and tubular changes, as shown by glomerular atrophy, dilated bowman's space, acute tubular necrosis and congestion. Saponin and CP plus SP attenuated the pathological changes in renal tissue caused by cyclophosphamide. Also, renal histological index was decreased by the saponin treatment. This cyto-protective effect of saponin may be due to its powerful antioxidant capacity (Hu et al., 2019).

In addition, cyclophosphamide resulted in the pathological changes in the hepatocytes, including acute triaditis and lymphocyte infiltration. As the administration of saponin and CP plus SP reduced the morphological changes of the liver tissues. Correspondingly, liver histological index was significantly decreased with the saponin treatment in the effective dose of 2.5 mg/kg. Probably, these protective effects of saponin may be due to potent anti-inflammatory and antioxidant properties (Wang, Xiang, Yi, & He, 2017), which were documented by the recovery and regeneration of pathological defects in the liver and renal structure.

The lipid peroxidation, as a free radical generating system, has been reported to be closely related to CP-induced tissue damages, and MDA is a good marker of the degree of lipid peroxidation (Mehraban et al., 2021). In the present experiment, the MDA levels were elevated and the activity of GPx, TAC and SOD were reduced by CP treatment. These data are consistent with previously published findings about the effects of CP on the organ toxicity (Duggina, Kalla, Varikasuvu, Bukke, & Tartte, 2015). The administration of CP led to oxidative stress, lipid peroxidation in renal and hepatic tissues and reduced antioxidant defenses. Increased oxidative stress is an important participant in the induced toxicity by CP. Therefore, compounds that can inhibit the production of free radicals will be of particular importance. Saponin has a more significant antioxidant effect, which our results confirmed that it decreased the amount of oxidative stress and subsequently lipid peroxidation and improved the antioxidant capacity. Thus documenting the ability of saponin's dual function as both an enhancer of antioxidative enzyme defenses and a free radical scavenger.

Exposure to chemicals and drugs result in inflammation that is characterized by the protective response of the immune system against these agents. Pro-inflammatory cytokines including tumor necrosis factor alpha (TNF- α), IL-1B and IL-6 increase in the body. Transcription of these interleukins are induced by an important protein in inflammatory process, NF-kB (Ginwala, Bhavsar, Chigbu, Jain, & Khan, 2019). Oxidative stress has been considered to be related with NF-kB. Actually, a vicious cycle has been suggested between the oxidative stress and NF-kB signaling pathway. Oxidative stress is crucial for the activation of the NF-kB cascade. On the other hand, the expression of NF-kB leads to the aggravation of free radical generation (Morgan & Liu, 2011).

Our results indicated that CP created a state of oxidative stress in kidney and liver tissues and then induced the production of pro-inflammatory cytokines such as NF-kB and IL-1B from these two organs which these findings are consistent with previous researches in this regard (Jnaneshwari et al., 2014). Usually, inhibition of inflammation is the most main actions in controlling the toxicity effect of these agents. The inflammatory conditions have been treated with the natural compounds. To prevent unwanted side effects of anti-inflammatory medicines, nonsteroidal anti-inflammatory drugs (NSAIDs), it is necessary to find out alternative anti-inflammatory agents with more efficacy than NSAIDs but with fewer side effects. Our findings demonstrated that saponin significantly could inhibit the production of inflammatory mediators induced by CP. Also, CP plus SP significantly reduced the NF-kB and IL-1B in kidney and liver tissues that were increased by CP. Probably, the high antioxidant and anti-inflammatory properties of saponin is responsible for ROS detoxification and inflammatory reduction.

In a nutshell, diagram 1 illustrates saponins-mediated hepato and renoprotection by the inhibition of oxidative stress.

Conclusion

In conclusion, liver and renal dysfunction are the most important complications of chemotherapy drugs such as cyclophosphamide. Increased oxidative stress and inflammation is an accepted participant in the induced toxicity by CP. Saponins have significant antioxidant and anti-inflammatory actions and are well-known free radical scavengers. Our findings showed that the administration of saponin or CP plus saponin appears to exert a protective role on CP-induced hepatic and nephric injury, as shown by the reduction of liver and renal dysfunction, oxidative stress, production of pro-inflammatory mediators and pathological changes and improvement of antioxidant capacity. However, further investigations is necessary to clarify the exact mechanisms mediating the therapeutic properties of saponin against cyclophosphamide toxicity.

Declarations

Funding

This study was funded by Ardabil University of Medical Sciences.

Ethical Approval

This study was approved by Ardabil University of Medical Sciences (Ethics code: IR.ARUMS.AEC.1400.019).

Authors contributions

MG.G. and Sh. B. conceived and designed research. Sh. B. did histological evaluation, statistical analysis and manuscript writing. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing interest

The authors declare no competing interests.

Data Availability

The data that support the finding of this study are available through the following link (**Supplementary files**).

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Tables

Table 1

Protective effect of saponin on cyclophosphamide- induced changes in renal and hepatic TAC, MDA and antioxidant enzyme levels.

Groups	Control	CP	SP	CP + SP
Kidney				
MDA (nmol/mg protein)	1.61 ± 0.44	1.89 ± 0.22	1.15 ± 0.14**	0.88 ± 0.10***
TAC (mmol/liter)	0.51 ± 0.07	0.43 ± 0.11	0.63 ± 0.03**	0.62 ± 0.06*
SOD (U/mg protein)	8.87 ± 1.82	7.43 ± 1.02	8.53 ± 1.67	8.38 ± 1.02
GPx (U/mg protein)	5.18 ± 1.33	4.39 ± 0.38	5.12 ± 0.83	4.70 ± 0.91
Liver				
MDA (nmol/mg protein)	0.67 ± 0.15	0.82 ± 0.21	0.68 ± 0.18	0.48 ± 0.06*
TAC (mmol/liter)	0.77 ± 0.07	0.70 ± 0.19	0.92 ± 0.16	1.01 ± 0.14*
SOD (U/mg protein)	9.47 ± 1.16	8.23 ± 0.82	10.11 ± 0.56	8.63 ± 1.39
GPx (U/mg protein)	6.26 ± 0.57	4.93 ± 1.00	6.06 ± 0.48	5.70 ± 0.77
The results were expressed as mean ± standard deviation.				
*, ** and *** show the significance of the differences to CP group (P < 0.05, 0.01 and 0.001).				
SP; saponin, CP; cyclophosphamide, MDA; malondialdehyde, TAC; total antioxidant capacity, SOD; superoxide dismutase, GPx; glutathione peroxidase.				

Figures

Figure 1

The effect of cyclophosphamide (CP) and saponin (SP) on serum levels of ALP (alkaline phosphatase) in treated mice. *P< 0.05 versus control group. **P<0.01 versus CP group.

Figure 2

Alanine transaminase (ALT) levels. ****P < 0.0001 versus control group. #####P < 0.0001, ****P < 0.0001 versus CP group. *P < 0.05 versus SP group.

Figure 3

Aspartate transaminase (AST) levels. ****P < 0.0001 versus control group. #####P < 0.0001, ****P < 0.0001 versus CP group.

Figure 4

Blood urea nitrogen (BUN) levels. ****P < 0.0001 versus control group. #####P<0.0001, ****P<0.0001 versus CP group.

Figure 5

Creatinine (Cr) levels. ***P < 0.001 versus control group. *P<0.05 versus CP group. #P< 0.05 versus SP group.

Figure 6

(A and C) Photomicrograph of the kidney of normal control and SP groups in treated mice indicates the normal renal tissue structure, healthy appearance of glomerular and tubular cells. (B) CP- administered mice had congestion (CON), pyknotic nuclei (PN), glomerular atrophy (GA), dilated bowman's space (DBS) and acute tubular necrosis (ATN). (D) Acute tubular necrosis (ATN) in CP + SP group (40 × H&E).

Figure 7

Semi-quantitative analysis of protective effect of saponin against CP- induced the kidney damage. The histological score was semi-quantitatively evaluated using a scale ranging from 0 to 3 (0: none, 1: mild, 2: moderate, and 3: severe damage). The results were expressed as mean ± standard deviation. ****P < 0.0001 versus control group. ***P<0.001 versus CP group. *P<0.05 versus SP group.

Figure 8

(A and C) Photomicrograph of the liver of normal control and SP groups in treated mice indicates the normal hepatic tissue structure. (B) CP- administered mice had lymphocyte infiltration (Lyi), acute triaditis (AT) and neutrophil infiltration (Ni). (D) Lymphocyte infiltration (Lyi) and focal necrosis (FN) in CP + SP group (40 × H&E).

Figure 9

Semi-quantitative analysis of protective effect of saponin against CP- induced the liver damage. The histological score was semi-quantitatively evaluated using a scale ranging from 0 to 3 (0: none, 1: mild, 2:

moderate, and 3: severe damage). The results were expressed as mean $\pm$ standard deviation. ***P < 0.001 versus control group. *P<0.05, ****P<0.0001versus CP group. #P<0.05 versus SP group.

Figure 10

The effect of cyclophosphamide (CP) and saponin (SP) on NF-kB and IL-B levels in renal tissue of treated mice. ***P < 0.001 versus control group. ***P < 0.001, *P<0.05 versus CP group.

Figure 11

NF-kB and IL-B levels in liver tissue of treated mice. ***P < 0.001 versus control group. ***P < 0.001, *P<0.05 versus CP group.

Supplementary Files

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