Protective Role of Ginseng Root Aqueous Extract Administration on Antineoplastic Drug Cisplatin-induced Spleen Oxidative Stress and Injury

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors EH, MB and ET designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SE and SAF managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Background and Objective: Cisplatin (CP), a potent antineoplastic drug and its anti-cytotoxic activity is used to treat many cancer types. The present study aimed to examine the possible modifying effects of ginseng extract against spleen oxidative stress and injury induced by Cisplatin in rats.

Materials and Methods: A total 60 male albino rats were equally divided into six groups; the first and second groups were the control and ginseng groups respectively while the 3rd group was Cisplatin rat group; the 4th and 5th groups were Co- and post treated Cisplatin rat with ginseng respectively and the 6th group was Cisplatin self-healing rat group.

Results: Spleen malondialdehyde (MDA) levels in Cisplatin group showed a significant increase when compared with control group, in contrast, reduced glutathione (GSH), catalase, superoxide dismutase (SOD) and total protein levels were a significant decrease. Treatment of Cisplatin with
1. INTRODUCTION

Many different kinds of chemotherapy were used for cancer treatments [1-6]. It is therefore important to search for therapies which can reduce the side effects of anticancer treatments without altering their efficacy or increasing toxicity or damage in target organs [7-10]. Cisplatin is a non-cycle-dependent cytotoxic platinum derivative frequently and it is one of the most potent chemotherapy drugs widely used to treat many types of cancer to stop the growth and eliminate cancer cells [11,12]. Cisplatin is believed to kill cancer cells by binding to DNA and interfering with its repair mechanism, eventually leading to cell death [13]. Oxidative stress is an important contributor to the pathophysiology of a variety of pathological conditions including inflammation and drug toxicity [14-17]. The oxidative stress induced by chemotherapy is known to cause side effects in patients with cancer. However, few studies have examined whether anticancer drugs induce oxidative stress in cancer cells [18,19]. Many toxicity studies on Cisplatin have shown the oxidative stress is a common occurrence when Cisplatin was administered to rodents. Since, Cisplatin is used as an anti-cancer agent, Cisplatin’s mechanism of action is complex but effecting oxidative stress in tumours as Ehrlich solid tumour [20]. Cisplatin generates oxidative stress which is accompanied by rapid shifts in central carbon metabolism [20].

Natural medicinal plant inset along with risky medications, for enhancing their efficacy and controlling their complications, has become a global trend for pharmacological researchers [21-26]. P. ginseng has been recognized as the most prized medicine among all herbal medicine [27]. It has the most potent multiple pharmacologic for anticancer, antihypertension, anti-stress, anti-aging and anti-nociception effects and for improving weak body conditions [28,29]. Ginseng contains many physiologically important constituents that include saponins, polyacetylenes, polyphenolic compounds and acidic polysaccharides [30].

Several researches give evidences that ginseng has powerful antioxidant properties that may explain its anti-inflammatory and antineoplastic effects. Based on these evidences, the present study was conducted to examine the possible modifying effects of Panax ginseng aqueous extract against spleen oxidative stress and micro tissues alterations induced by Cisplatin in male rats.

2. MATERIALS AND METHODS

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee". The experiments were performed on 60 male albino rats (Rattus norvigicus) weighing 140-150 g and of 9-10 week’s age. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard rodent diet (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminsined starch; Egyptian Company of Oils and Soap Kafr-Elzayat Egypt) and water available ad libitum. The temperature in the animal room was maintained at 23±2°C with a relative humidity of 55±5%. Light was on a 12:12 hr light-dark cycle.

2.1 Experimental Design

Ginseng preparation: Panax ginseng aqueous extract was prepared according to the extraction method of Basuony, et al. [27].

Animal Treatments: The rats were randomly and equally divided into six groups (10 animals each).

G1: Control group in which animals did not received any treatment.

G2: Ginseng or positive control group in which animals received orally ginseng by stomach tube a dose of 100 mg/kg body weight body weight twice a week for four week according to Basuony, et al. [27].
G3: Cisplatin (Cp) rats group in which rats were injected intraperitoneally with Cisplatin administration (4 mg/kg body weight/ twice a week) for four weeks according to Basuony, et al. [27].

G4: Co-treated group in which cisplatin and ginseng extract was administered to rats simultaneously on the same day for four weeks.

G5: Post treated group in which animals injected intraperitoneally with Cisplatin administration (4 mg/kg body weight/ twice a week) for four weeks and then treated orally with ginseng (100 mg/Kg body weight/ week) for another four weeks.

G6: Self-healing rat group in which rats were injected intraperitoneally with Cisplatin administration (4 mg /kg body weight/ twice a week) for four weeks and self-treated without drugs for another four weeks according to Basuony, et al. [27].

At the end of the experimental period (8 week), Animals were euthanized with intraperitoneal injection with sodium pentobarbital and subjected to a complete necropsy.

2.2 Histopathogical Investigation

Immediately after decapitation animals were dissected, spleen from different groups were quickly removed and small parts of it were fixed in 10% neutral buffered formalin according Gazia, et al. [31] and the rest parts were homogenates for oxidative stress determinations. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin (mp. 50–58°C). Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. Sections were stained with Ehrlich's haematoxylin and counterstained with eosin as a routine method after Abou-Easa, et al. [32] and Tousson [33]. All stained slides were viewed by using Olympus microscope and images were captured by a digital camera (Cannon 620).

2.3 Oxidative Stress Investigation

Malondialdehyde (MDA) in spleen homogenates was detected by the method of Tousson, et al. [34] while the catalase (CAT) in spleen homogenates was detected by the method of Salama, et al. [35]. Also; Reduced glutathione (GSH) in spleen was detected by the method of Tousson, et al. [36] While, the total protein concentration in spleen were detected according to Beltagy, et al. [37], also; superoxide dismutase (SOD) was estimated by Salama, et al. [38] method.

2.4 Statistical Analysis

Data were expressed as mean values ± SE and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at \( p<0.01 \) for the biochemical data. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS Inc., USA).

3. RESULTS

Spleen sections in the control rat group appear to consist of two main compartments, the white and the red pulps. The red pulp occupies the distance between the white pulps (Fig. 1). No histopathological alterations in spleen tissues were observed in the control or positive control rat (ginseng group) groups (Fig. 1A&1B).

Marked disruption of spleen structure and many signs of pathological alterations were observed in the spleen sections in Cisplatin group (Fig. 1C). These alterations include the marked loss in the distinction between the white and red pulps, decrease in the lymphocyte population with marked loss in the chromatin of their nuclei, most of the lymphocytes contained pyknotic nuclei (Fig. 1C). Also; vasodilatation and moderate congestion of splenic sinusoids were seen in the spleen red pulp in Cisplatin group (Fig. 1C).

Mild histopathological alterations and marked improvement in the spleen sections were observed in Cisplatin co-treated with ginseng (Fig. 1D). In addition to, there was an obvious distinction between the white and the red pulp, however, moderate cellularity was observed in the marginal zone in the spleen in Cisplatin co-treated with ginseng (Fig. 1D). Spleen sections in post-Cisplatin co-treated with ginseng revealed moderate histopathological alterations were noticed, as a moderate to few congestion of spleen sinusoids in the red pulp (Fig. 1E). Spleen sections in Cisplatin self-healing showed a moderate disruption of spleen structure, a moderate loss in the distinction
between the white and red pulps with a moderate decrease in the lymphocyte population, in addition to most of the lymphocytes contained pyknotic nuclei were observed (Fig. 1F).

Fig. 2 indicates that MDA in spleen tissue revealed a significant increase in Cisplatin and Cisplatin self-healing groups as compared with the control and ginseng groups. In the same time, GSH, catalase, SOD and total protein levels in spleen tissues showed a significant decrease in Cisplatin and Cisplatin self-healing groups as compared with the control and ginseng groups (Figs. 3-6).

Also Figs. 2-6 indicates that treatment of Cisplatin with ginseng showed a significant decrease in MDA and a significant increase in GSH, catalase, SOD and total protein levels in spleen tissues. In the same time, Co-treatment of Cisplatin with ginseng revealed a significant decrease in MDA and a significant increase in GSH, catalase, SOD and total protein levels in spleen tissues when compared with a post treated Cisplatin with ginseng (Figs. 2-6).

Fig. 1A-1F. Photomicrographs of rat spleen sections in the different experimental groups stained with Haematoxylin & Eosin. A&B: Rat spleen sections in control and ginseng groups revealed the normal structure of the white and the red pulps. C: spleen section of Cisplatin group showed a marked loss in the distinction between the white and red pulps, decrease in the lymphocyte population with marked loss in the chromatin of their nuclei, most of the lymphocytes contained pyknotic nuclei. D: Spleen sections in co-treated Cisplatin with ginseng group revealed an obvious distinction between the white and the red pulp, however, moderate cellularity was observed in the marginal zone. E: Spleen sections in post-treated ginseng group revealed moderate histopathological alterations were noticed as a moderate to few congestions of splenic sinusoids in the red pulp, and there was an increasing number of inflammatory cell components. F: Spleen section in self-healing showed a moderate disruption of spleen structure, a moderate loss in the distinction between the white and red pulps with a moderate decrease in the lymphocyte population.
Fig. 2. Changes in splenic MDA levels in different groups under study. Where G1, Control group; G2, ginseng group; G3, Cisplatin group; G4, Co-treated Cisplatin group with ginseng; G5, Post-treated Cisplatin group with ginseng; G6, Self-healing group

(*) Significant difference compared to the control group; (#) the highly significant difference compared to Cisplatin group

Fig. 3. Changes in splenic catalase levels in different groups under study. Where G1, Control group; G2, ginseng group; G3, Cisplatin group; G4, Co-treated Cisplatin group with ginseng; G5, Post-treated Cisplatin group with ginseng; G6, Self-healing group

(*) Significant difference compared to the control group; (#) the highly significant difference compared to Cisplatin group

Fig. 4. Changes in splenic SOD activity in different groups under study. Where G1, Control group; G2, ginseng group; G3, Cisplatin group; G4, Co-treated Cisplatin group with ginseng; G5, Post-treated Cisplatin group with ginseng; G6, Self-healing group

(*) Significant difference compared to the control group; (#) the highly significant difference compared to Cisplatin group
4. DISCUSSION

Cisplatin is an inorganic platinum compound with a broad spectrum antineoplastic activity against various types of tumours. However, its clinical use is limited due to its toxic side effects including nephrotoxicity, neurotoxicity, ototoxicity and hepatotoxicity [39,40]. Our data had been suggesting with the current histopathological investigations of spleen of rats treated, was found to have an observable effect on splenocyte populations and their proliferative ability, indicating its defective functional ability. The decrease in the splenocyte population with many degenerated and vacuolated areas in the white pulps were the most prominent features indicating the partial spleen atrophy after exposure to Cisplatin. Also, there was the marked disruption of spleen organization, marked loss in the distinction between the white and red pulps, vasodilatation and congestion of spleen sinusoids in the red pulp. However, ginseng could produce a significant amelioration for these changes, and it may be considered as a potentially useful candidate in the combination of chemotherapy with to combat oxidative stress-mediated spleen injury. Also; Crăciunaş, et al. [41] and Craciun and Pasca [42] confirmed our results.

Similar results were obtained in vivo experiments when cyclophosphamide decreased almost all of the peripheral blood cell counts and lymphocyte subset counts in the thymus and spleen at all ages [43]. Many studies showed that chemotherapy administration causes a suppressive effect on the number of lymphocytes in spleen and caused induction of apoptosis.
Administration of cyclophosphamide caused a suppressive effect on the number of lymphocytes in both thymus and spleen. Also, Hermenean, et al. [44] reported that; administration cyclophosphamide of is accompanied by side effects mainly affect the lymphoid organs and it can induce apoptotic cell death in a variety of tissues, including thymus. In the present study ginseng supplementation enhancement of spleen damages induced by, and will be of major interest to be used as an adjuvant therapy under these conditions. Oxidative stress is an indicator of the damage that results from a change in the balance between oxidants and antioxidants in favour of oxidants. If the delicate balance between oxidants and anti-oxidants cannot be maintained in tissues, many pathological changes extending to cellular damage occur. Oxidative stress or oxidative cellular damage with its dual of free radical generation and profound lipid peroxidation are hallmarks of Cisplatin toxicity [45].

Cisplatin is commonly utilized in the treatment of solid tumors [20]. Cisplatin-induced changes in carbon flux can provide information potentially useful for prediction of treatment response. YU, et al. [20] reported that; Cisplatin-induced oxidative stress triggers rapid shifts in carbon flux in 3 commonly utilized catabolic pathways: glycolysis, pentose phosphate pathway and citric acid cycle.

MDA, as an end product of lipid peroxidation, usually used to estimate the extent of lipid peroxidation [46,47]. It is a stable metabolite of the free radical-mediated lipid peroxidation cascade, is used widely as a marker of oxidative stress and lipid layers destroy [48]. In the present study, there was a significant increase in MDA in spleen tissue in Cisplatin group. The high level of MDA in the Cisplatin and self-healing groups indicates that Cisplatin gives rise to oxidative stress in spleen tissue. The current results agree with Soliman, et al. [49] who found doxorubicin chemotherapy increase lipid peroxidation in Spleen tissue. It has been shown that many pathological conditions that resulted in the elevation of MDA due to lipid peroxidation were prevented by ginseng. Also in the present study, treatment with ginseng significantly decreased MDA concentration. These results were supported by our histopathological findings. Ginseng has a protective effect on lipid peroxidation by reducing the formation of hydrogen peroxide [50].

GSH is considered to be one of the most very important components of the antioxidant defence of living cells [51]. These results agree with Soliman et al. [49] who found doxorubicin chemotherapy increased GSH in Spleen tissue doxorubicin treated group. The significant reduction in GSH activity produced by Cisplatin leads to a reduction in the effectiveness of the antioxidant enzyme defense system, sensitizing the cells to ROS [52,53]. Also, the current results agree with Basuony, et al. [27] who reported that; GSH were significantly decreased in serum after Cisplatin injections and treatment with ginseng improved these conditions.

CAT and SOD are enzymes of the intrinsic antioxidant defense system, which are responsible for the dissemination of superoxide free radicals [54,55]. In the present study a significant decrease in catalase and SOD activities in spleen tissue in Cisplatin and self-healing groups, there was a significant increase in CAT and SOD in spleen tissue in treated group with ginseng when compared with Cisplatin group. These results agree with Soliman et al. [49] who reported that doxorubicin chemotherapy administration induced a significant decrease in catalase and SOD activity in spleen tissues.

A significant decrease in total protein levels in spleen tissues in Cisplatin and self-healing groups and these results agreed with Soliman et al. [49] with who reported that doxorubicin chemotherapy administration induced a significant decrease in total protein level in spleen tissues.

Ginseng extract has strong antioxidant activity on metal-induced lipid peroxidation and human low-density lipoprotein oxidation, and inhibitory activity on the scission of supercoiled deoxyribonucleic acid strands induced by peroxyl radicals [56]. In addition, many flavonoids and their related compounds are known to possess strong antioxidative characteristics [57]. Many studies have shown that ginseng attenuates free radical-induced oxidative damage [28], prevents carcinogenesis induced by toxicants [57] and possesses immunostimulating, anti-tumorigenic, and chemopreventive effects [58]. These numerous cytoprotective and chemoprotective properties attributed to ginseng might be explained in part by its ability to ameliorate oxidative or nitrosative stress [59].
5. CONCLUSION

It can be concluded that Co-treatment with ginseng has beneficial ant oxidative properties and can reduce the spleen oxidative stress and tissue injury induced by Cisplatin. Cisplatin is an inorganic platinum compound with a broad spectrum antineoplastic activity against various types of tumors. Ginseng could produce a significant amelioration for these changes, and it may be considered as a potentially useful candidate in the combination of chemotherapy with to combat oxidative stress-mediated spleen injury. So, it is, therefore, possible that ginseng could scavenge free radicals and produce beneficial effects against spleen toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Experimental procedures were performed in accordance with the Ethics Committee of the National Research Centre, Egypt, which followed the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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