An *in vitro* study on the interactions of pycnogenol® with cisplatin in human cervical cells

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Abstract

Objectives: In the treatment of cancer, it is intended to increase anticancer effect and decrease cytotoxicity using various plant-derived phenolic compounds with chemotherapeutic drugs. Pycnogenol® (PYC), a phenolic compound has been the subject of many studies. Since the mechanisms of the interactions of PYC with cisplatin needs to be clarified, we aimed to determine the effects of PYC on cisplatin cytotoxicity in human cervix cancer cells (HeLa), as well as the genotoxicity of PYC was also evaluated.

Materials and Methods: The cytotoxicity of cisplatin and PYC was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in HeLa cells for 24 h and 48 h. The effect of PYC against oxidative DNA damage was evaluated using the comet assay.

Results: The IC_{50} values of cisplatin were 22.4 μM and 12.3 μM for 24 h and 48 h, respectively. The IC_{50} values of pycnogenol were 261 μM and 213 μM for 24 h and 48 h, respectively. For 24 h exposure, PYC significantly reduced the IC_{50} value of cisplatin at the selected concentrations (15.6-500 μM). For 48 h exposure, PYC did not change the cytotoxicity of cisplatin at concentrations between 15.6-125 μM, but significantly reduced at the concentrations of 250 μM and 500 μM. PYC alone did not induced DNA damage at the concentrations of 10 μM and 25 μM, however it significantly induced DNA damage at the higher concentrations (50-100 μM). It also significantly reduced H_2O_2-induced DNA damage at all studied concentrations (10-100 μM).

Conclusion: Our results suggest that PYC may increase the cisplatin cytotoxicity in HeLa cells at its non-genotoxic doses. The results might contribute to the anticancer effect of cisplatin with PYC in cervical carcinoma, but in order to confirm this result, the further *in vitro* studies with cancer cell lines and *in vivo* studies are needed.

Key words: *Pycnogenol, cisplatin, cytotoxicity, genotoxicity, HeLa cells*

Özet

Amaç: Kanser tedavisinde antikanser etkiyi artırmak ve sitotoksisiteyi azaltmak amacıyla kemoterapötik ilaçlar ile birlikte çeşitli bitkisel kökenli fenolik bileşikler kullanılanımı hedeflenmektedir. Pinus pinaster'den elde edilen piknogenol® (PYC), güçlü antioksidan etkisinden dolayı birçok çalışmanın konusu olmaktadır. PYC'nin sisplatin ile etkileşme mekanizması tam olarak aydınlatılamadığı için insan serviks kanser hücrelerinde (HeLa) sisplatin sitotoksisitesi üzerine PYC'nin etkilerini belirlemeyi ve PYC'nin sitotoksik olmayan dozlarında PYC'nin genotoksisitesini değerlendirilmeyi hedefledik.

Gereç ve Yöntem: HeLa hücrelerinde, 24 ve 48 saatlik maruziyetlerde, PYC varlığında ve yokluğunda sisplatinin sitotoksisitesi 3-(4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolyum bromür (MTT) yöntemi ile ölçüldü. Oksidatif DNA hasarına karşı PYC'nin etkisi Komet yöntemi ile değerlendirildi.

Bulgular: Sisplatinin IC_{50} değeri 24 saat ve 48 saat için sırasıyla 22.4 μM ve 12.3 μM idi. PYC'nin IC_{50} değerleri 24 saat ve 48 saat için sırasıyla 261 μM ve 213 μM idi. 24 saatlik maruziyet için, PYC'nin, seçilen konsantrasyonlarda (15.6-500 μM) sisplatinin IC_{50} değerini önemli ölçüde azalttığı. 48 saat maruziyet için, PYC sisplatinin sitotoksisitesini 15.6-125 μM arasındaki konsantrasyonlarda değiştirmedi, ancak 250 uM ve 500 uM konsantrasyonlarda önemli ölçüde azalttı. PYC tek başına 10 μM ve 25 μM konsantrasyonlarında DNA hasarına neden olmadı, ancak daha yüksek konsantrasyonlarında (50-100 μM) DNA hasarını önemli ölçüde indükledi. Ayrıca, çalışılan tüm konsantrasyonlarında (10-100 μM) 50 μM H_2O_2 tarafından indüklenen DNA hasarını önemli ölçüde azalttı.

Sonuç: Sonuçlarımız PYC'nin genotoksik olmayan dozlarında HeLa hücrelerindeki sisplatin sitotoksisitesini arttırabileceğini göstermektedir. Bu sonuçlar, sisplatinin PYC ile birlikte servikal karsinomadaki antikanser etkisine katkıda bulunabilir, ancak bunu doğrulamak üzere kanser hücre hatları üzerinde daha ileri *in vitro* çalışmalara ve vivo çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Piknogenol, sisplatin, sitotoksisite, genotoksisite, HeLa hücresi*

INTRODUCTION

Oxidative stress is one of the hypotheses involved in the etiology of a number of diseases, including cancer. Considerable attention has been focused on antioxidant agents such as phenolic compounds in recent years, because it is stated that the development of oxidative stress-related diseases may be prevented or delayed by using them.[1-4]

Pycnogenol® (PYC) is a phenolic compound and a natural dried extract obtained from the bark of French maritime pine (*Pinus pinaster*). It, is a proprietary mixture of procyanidins containing %65-75 catechin and epicatechin subunits.[5,6] It is commonly consumed as a dietary food supplement owing to its strong antioxidant activity. As shown in many researches, PYC has potent to have therapeutic and protective effects against cancer.[6] However, there are not sufficient studies on the interactions between antineoplastic drugs and PYC.

Antineoplastic drugs are clinically used in therapy of cancers aiming to reduce tumor cell growth. Cisplatin (CIS) is a powerful antineoplastic drug to treat many types of cancer including esophageal, lung, breast, ovarian, bladder, cervical, and prostate cancers.[7] Nowadays, the combinatorial therapies have been investigated with the aim of increasing anticancer activity and minimizing drug resistance. As a result of recent studies, positive findings were obtained by using various phenolic compounds in combined with antineoplastic drug.[8-12] Nevertheless, further investigations are needed to clarify the effects of phenolic compounds on cancer and the effects of using combined with antineoplastic drugs in different doses.

The aim of this study was to determine the effects PYC on the cytotoxic profile of CIS in human cervical carcinoma (HeLa) cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The genotoxic/anti-genotoxic effects of PYC against oxidative DNA damage was evaluated using the alkaline single cell gel electrophoresis techniques (comet assay).

MATERIALS and METHODS

Chemicals

The chemicals used in the experiments were purchased from the following suppliers: cisplatin (Koçak Farma, Turkey); dimethyl sulfoxide (DMSO), Dublecco's modified Eagle's medium (DMEM), ethanol, ethidium bromide (EtBr), ethylenediamine tetra acetic acid disodium salt dihydrate (Na2-EDTA), fetal bovine serum (FBS), hydrogen peroxide (35%) (H_2O_2), low melting point agarose (LMA), MTT, n-lauroyl sarcosinate, normal melting point agarose (NMA) , penicillin-streptomycin, sodium chloride (NaCl), sodium hydroxide (NaOH), Tris, Triton X-100, trypan blue, trypsin–EDTA, RPMI 1640 medium, Dulbecco's phosphate buffered saline (PBS) from Sigma (St. Louis, MO, USA); millipore filters from Millipore (Billerica, MA, USA), all other plastic materials from Cornings (Corning Inc., NY, USA).Pycnogenol® (PYC) was purchased from Horphag Research Ltd., (UK, Geneve, Switzerland), The quality of standardized PYC extract is specified in the United States Pharmacopeia (USP 28).[5]

Cell culture

HeLa cells were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). HeLa cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, and 1% penicillin- streptomycin solution (10000 units of penicillin and 10 mg of streptomycin in 0.9 % NaCl), and 2mM L-glutamin at 37°C in a humidified atmosphere of 5% CO2. The cells were subcultured in 75 cm^2 cell culture flasks. The medium was changed every 2-3 days. The passage numbers used in our study for both cell lines were between passage 8 and passage 10.

Determination of cytotoxicity

After growing for 2 weeks, the cells were plated at 1×10^4 cells/well by adding 200 µL of a 5×10^4 cells/mL suspension to each well of a 96-well tissue culture plate and allowed to grow for 24 h before treatment. The number of cells was calculated by trypan blue dye exclusion.

The stock solution of PYC was freshly prepared in PBS and filtered with millipore filters (0.20 µm). The cells were treated with PYC at a wide range of concentrations (1.95-2000 µM) or CIS (0.49-500 µM) in the related culture medium for 24 h and 48 h. Control experiments were carried out with the culture medium containing only PBS (1%). After the values of IC_{50} were determined, the cytotoxic profiles of PYC on the IC_{50} of CIS were evaluated at a wide doses of PYC in HeLa cells for 24 h and 48 h.

The cytotoxicity of PYC and CIS were measured in HeLa cells using MTT assay, which is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase.[13,14] At the end of the incubation (24 h and 48 h), 5 mg/mL MTT solution was added to

each well and incubated for another 4 h at 37°C. Then the medium was discarded. The formazan crystals were dissolved in 100 µL of DMSO and absorbance of each sample was measured at 570 nm using the microplate reader (SpectraMax M2, Molecular Devices Limited, Berkshire, UK).

The percentage of cell viability was calculated using the formula:

"Percentage of cell viability = (The absorbance of sample/ control) x 100"

The cytotoxic concentration that killed cells by 50% (IC_{50}) was determined from absorbance versus concentration curve.

Determination of the genotoxicity

HeLa cells were incubated with PYC at non-cytotoxic doses (0, 10, 25, 50 and 100 µM) for 2 hours (preincubation) with/without the genotoxic doses of H_2O_2 (50 µM) for 5 minutes. Thus, the possible protective effect of PYC against oxidative DNA damage induced by H_2O_2 was also evaluated. 50 µM H_2O_2 was applied as a positive control. Medium containing %10 PBS was applied as a negative control.

The comet assay was performed to assess DNA damage. The basic alkaline technique of Singh et al.[15] was used for the detection of DNA damage in the cells. The concentrations of the cells were adjusted to $2x10^5$ cells/mL, suspended in 5% LMA, and were then embedded on slides precoated with a layer of 1% NMA. Slides were allowed to solidify on ice for 5 min. Cover slips were then removed. All slides were immersed in cold lysing solution (pH 10), for a minimum of 1 h at 4 ºC.

The slides containing the cells were removed from the lysing solution, drained and then placed in a horizontal gel electrophoresis tank filled with freshly prepared alkaline electrophoresis solution (300 mmol/L NaOH, 1 mmol/ EDTA-Na_2, pH 13.0) for 20 min at 4ºC to allow unwinding of the DNA and expression of DNA damage. Electrophoresis was then conducted at 4ºC for 20 min at 24 V/300 mA. The slides were neutralized at room temperature by washing 3 times in neutralization buffer (0.4 mol/L Tris-HCl, pH 7.5) for 5 min. After neutralization, the slides were then incubated in 50%, 75%, and 98% of alcohol for 5 min, successively.

The dried microscope slides were stained with EtBr (20 µg/ml in distilled water, 60 µl/slide), covered with a cover-glass prior to analysis with a fluorescence microscope (Leica DM1000, Wetzlar, Germany) equipped with an excitation filter of 515–560 nm. The microscope was connected to a charge-coupled device camera and a personal computer-based analysis system (Comet Analysis Software, Version 3.0, Kinetic Imaging Ltd., Liverpool, UK) to determine the extent of DNA damage after electrophoretic migration of the DNA fragments in the agarose gel.

In order to visualize the DNA damage, slides were examined at 400x. For each condition, 100 randomly selected comets from each of two replicate slides were scored (without the knowledge of the group codes). DNA damage parameters was expressed as DNA tail intensity %.

Statistical analysis

All experiments were carried out in quadriplicate. The results were given as the mean ± standard deviation. The statistical analysis was performed with software programs *"SPSS 10.5"* (Statistical Package for the Social Sciences, Chicago, IL, USA). The distribution of the data was checked for normality using the Kolmorog-Smirnov test. The means of data were compared by the one-way variance analysis test and post hoc analysis of group differences was performed by least significant difference (LSD) test. A *p* value of less than 0.05 was considered as statistically significant.

RESULTS

Pycnogenol cytotoxicity

The results of PYC cytotoxicity are given in Table 1 and Figure 1. It was found that PYC did not cause significant cytotoxic effect at the concentration range of 1.95-125 µM when compared to the negative control for 24 h and 48 h incubation; however, the cell viabilities were significantly decreased above 250 µM concentrations of PYC ($p < 0.05$) (Table 1). The IC_{50} value of PYC were found to be 261 µM and 213 µM for 24 h and 48 h, respectively (Figure 1).

Cisplatin cytotoxicity

The results of CIS cytotoxicity are given in Table 2 and Figure 2. It was found that CIS did not cause significant cytotoxic effect at the concentration range of 0.49-7.81 µM and at the concentration range of 0.49-3.91 µM when compared to the negative control for 24 h and 48 h, respectively; however, the

cell viabilities were significantly decreased above 15.2 μM and 7.81 μM of CIS for 24 h and 48 h incubation, respectively ($p < 0.05$) (Table 2). The IC_{50} values of CIS were found to be 22.4 μM and 12.3 μM for 24 h and 48 h, respectively (Figure 2).

Effects of PYC on Cisplatin Cytotoxicity

The effects of PYC at the concentration range of 15.6-500 μM on CIS cytotoxicity in Hela cells are shown in Figure 3, for 24 h and 48 h incubation.

As shown in Figure 3a, at all studied concentrations (15.6-500 μM) PYC significantly decreased the IC_{50} value of CIS (20 μM, approximately) in a dose dependent manner (1.53 fold, 1.84 fold, 1.87 fold, 1.94 fold, 2.28 fold, 2.86 fold for 15.6 μM, 31.3 μM , 62.5 μM, 125 μM, 250 μM, and 500 μM, respectively, vs. positive control) when compared to the negative control for 24 h incubation ($p < 0.05$).

As shown in Figure 3b, when compared to the negative control, PYC did not change the IC_{50} value of CIS (10 μM, approximately) at the concentration range of 15.6-125 μM for 48 h incubation; however, the IC_{50} value of CIS was significantly reduced at concentrations of 250 μM and 500 μM of PYC (1.11 fold, 1.57 fold for 250 μM and 500 μM, respectively, vs. positive control) ($p < 0.05$).

Effect of Pycnogenol against DNA damage

The results of genotoxicity and antigenotoxicity of PYC at non-cytotoxic doses (10 μM, 25 μM, 50 μM and 100 μM) in HeLa cells using comet assay were evaluated. DNA damage, expressed as DNA tail intensity in the HeLa cells, are shown in Figure 4. In our study, we observed that PYC did not significantly increase DNA damage at all studied concentrations when compared to the negative control ($p > 0.05$). In additionally, PYC significantly decreased the DNA damage induced by H_2O_2 (50 μM) in a dose dependent manner at all studied concentrations (10 μM=%36.6; 25 μM=%36.7; 50 μM= %40.1; 75 μM=%50,8; 100 μM=%58,6) when compared to the positive control ($p<0.05$).

DISCUSSION

As is well-known, CIS is clinically used in therapy of many types of cancers (including esophageal, lung, breast, ovarian, bladder, cervical and prostate cancers esc.) aiming to reduce tumor cell viability. However, it has important side effects, mainly nephrotoxicity.[7]

Side effects and drug resistance are two of the major problems in the antineoplastic therapy; hence recent studies have focused on new approaches, like combinational therapies with phenolic compounds, in order to prevent drug resistance, minimize side effects and increase anticancer activity.[8-12]

PYC, a phenolic compound, is commonly consumed as a dietary food supplement because of its strong antioxidant activity. It has potent to have therapeutic and protective effects against cancer, as shown in many researches.[5,6] However, there are not sufficient studies on the interactions between antineoplastic drugs and some natural phenolic compounds including PYC.

In this study, after the determination of the cytotoxicities of PYC and CIS alone, the effects of PYC in combination with CIS was evaluated. The cytotoxicity of PYC and CIS increased approximately 1.22-fold and 1.82-fold, respectively, for the 48 h incubation, when compared to 24 h incubation. The cytotoxicity profiles of PYC and CIS alone were found to be different. It seems that the cytotoxicities of PYC and CIS are a dose and time dependent manner.

In our study, PYC (15.6-500 μM) significantly decreased the cytotoxicity of CIS in a dose dependent manner for 24 h incubation. But for 48 h incubation; PYC did not increase the cytotoxicty in the cells treated with CIS (10 μM, approximately) at the concentration range of 15.6-125 μM when compared to negative control; however, the cell viability was found to be reduced significantly at concentrations of 250 μM and 500 μM of PYC in the CIS treated cells ($p<0.05$). According to our results, PYC seems to have desired effect on the cytotoxic profile of CIS in HeLa cells for anticancer activity in time and dose dependent manner.

Possible mechanism underlying the cytotoxic effect of PYC have been associated with apoptosis.[16,17] In the study investigating the apoptotic effects of PYC, PYC induced apoptosis in human fibrosarcoma cells (HT1080), using flow cytometric analysis and RNA microarray (16/Harati). In another study, it was reported that PYC significantly decreased cell viability and also induced caspase-independent apoptosis. Also, PYC induced the translocation of apoptosis-inducing factor into nucleus and regulated apoptosis.[17]

In a study investigating the antitumor effect of PYC, the IC_{50} values of PYC in human leukemia cells (HL-60, U937 and K562) were detected to be 150 µg/ml (~516.8 µM), 40 µg/ml (~137.8 µM) and 100 µg/ml (~344.5 µM), respectively, for 24 h incubation, by propidium iodide exclusion method.[18] In another study, in which the apoptotic effect of PYC in human oral squamous carcinoma (HSC-3) cells was investigated by the MTS assay, the IC_{50} value of PYC was reported as 20 µg/ml (~ 68.9 µM) for 24 h incubation.[19] However the IC_{50} value of PYC was determined to be 285 µg/ml (~982 µM) for 24 h incubation, in Chinese hamster ovary (CHO) cells, by NRU test.[12]

The genotoxicity and antigenotoxicity potential of PYC was evaluated with commonly used the alkaline comet assays, at non-cytotoxic doses in the HeLa cells. In this study, we observed that PYC alone did not induced DNA damage at the concentrations below 50 µM. However, it significantly reduced H_2O_2-induced DNA damage at all studied concentrations (10-100 µM). Our study using comet assay show that PYC might have protective effect against H_2O_2-induced DNA damage in the cells.

The results were in good correlation with the studies done before. The antigenotoxic studies using comet assay show that PYC may have protective effect against oxidative DNA damage. For instance, Taner et al.[12] reported that PYC caused no genotoxic effects alone at low concentrations (5-50 µg/ml) as compared with the controls, as well as it might reduce H_2O_2 induced chromosome breakage and loss and DNA damage in cultured human lypmphocytes in the comet assay. It seems that PYC may be hopeful on the treatment of the diseases related to oxidative DNA damage.

The IC_{50} value of CIS in the selected human cancer cells was reported to be 54.07 µM and 96.38 µM in cervical cancer cells (HeLa and Caco-2), respectively; 97.20 µM and 85.66 µM in pancreatic cancer cells (MIA PaCa-2 ve BxPC-3), respectively; 14.87 µM and 77.89 µM in hepatocellular carcinoma cells (Hep-G2 ve SK-HEP-1), respectively, for 24 h incubation, using MTT method.[20]

Although there are some in vivo studies on the protective effect of PYC on CIS cytotoxicity, there are limited *in vitro* studies on the chemotherapeutic activity of PYC.[21-23]

It has been reported that in CIS cytotoxicity, CIS-induced prooxidant enzymes (myeloperoxidase, xanthine oxidase), malondialdehyde and nitric oxide levels were corrected by PYC and chromosomal defects were reduced. These findings suggest that PYC may be a protective agent against CIS-induced oxidative, inflammatory and genotoxic damages.[24] It has also been suggested that increased oxidative damage through radiotherapy can be prevented by strong antioxidant activity of PYC.[25]

It was shown that grape seed extract (GSE) a polyphenolic compound like PYC exerted synergistic anti-cancer effects with doxorubicin in human breast carcinoma (MCF-7 and MDA-MB468) cells.[26] In this study, GSE and doxorubicin alone and in combination strongly inhibited cell growth but did not show any increase in apoptotic cell death caused by doxorubicin. These results suggest a strong possibility of synergistic anticancer effects of GSE and doxorubicin combination for breast cancer treatment and also promising effects of combination of PYC and CIS for cancer.

In the resent studies, it has been aimed to decrease the cytotoxicity and to increase the anticancer activity by using various phenolic compounds with antineoplastic drugs.[8-10,19] Many researchers have revealed that CIS has positive effects in combination with antioxidants to increase its efficacy in cancer chemotherapy, reduce resistance development and reduce toxicity. Nevertheless, more investigations are necessary to clarify the effects of phenolic compounds on cancer and the effects of using combined with antineoplastic drugs in different doses.[11, 24]

CONCLUSION

At the end of the study, it was considered that the use of PYC in the treatment of CIS revealed its positive aspects on HeLa cells. These findings suggest that PYC might contribute to the anticancer effect of CIS in cervical carcinoma. Therefore, combinatorial therapy may therapeutically use in order to increase anticancer activity and minimize drug resistance and side effects. It will be a new point of view to anticancer treatment, but further *in vitro* studies with other cancer cell lines as well as *in vivo* studies are suggested.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Tables

Table 1. Effects of pycnogenol on the cell viability of HeLa cells for 24 h and 48 h*

Group	24 h (%)	48 h (%)
(-) Control	100.0±0	100.0±0
1.95 µM PYC	91.9±12.9	94.4±6.2
3.91 µM PYC	84.1±9.6	87.0±3.3
7.81 µM PYC	84.0±6.9	86.6±11.9
15.63 µM PYC	92.2±7.6	89.9±8.2
31.25 µM PYC	91.8±4.7	92.3±7.9
62.5 µM PYC	89.6±9.7	86.2±12.9
125 µM PYC	86.2±10	84.3±10.4
250 µM PYC	52.2±10.8 [a]	36.1±5.5 [a]
500 µM PYC	7.0±1.6 [a]	3.8±0.3 [a]
1000 µM PYC	6.6±0.7 [a]	3.7±0.4 [a]
2000 µM PYC	5.7±0.8 [a]	2.8±0.7 [a]

* Values were given as the mean ± standard deviation (n=4). [a] $p < 0.05$, compared to negative control (PBS). PYC, pycnogenol.

Table 2. Effects of cisplatin on the cell viability of HeLa cells for 24 h and 48 h*

Treatment group	24 h (%)	48 h (%)
(-) Control	100.0±0	100.0±0
0.49 µM CIS	100.1±4.2	94.2±6
0.98 µM CIS	100.4±9.1	93.0±7.4
1.95 µM CIS	99.5±7.5	93.2±13.5
3.91 µM CIS	100.1±4.6	94.3±14.2
7.81 µM CIS	86.5±14	77.6±7 [a]
15.63 µM CIS	64.7±6.6 [a]	29.4±2 [a]
31.25 µM CIS	31.6±5.6 [a]	6.7±0.9 [a]
62.5 µM CIS	21.0±4.5 [a]	5.9±0.7 [a]
125 µM CIS	12.3±6.1 [a]	6.3±0.7 [a]
250 µM CIS	7.8±0.8 [a]	6.9±0.9 [a]
500 µM CIS	5.8±0.6 [a]	7.3±1.7 [a]

* Values were given as the mean ± standard deviation (n=4). [a] $p < 0.05$, compared to negative control (PBS). CIS. cisplatin.

Figures

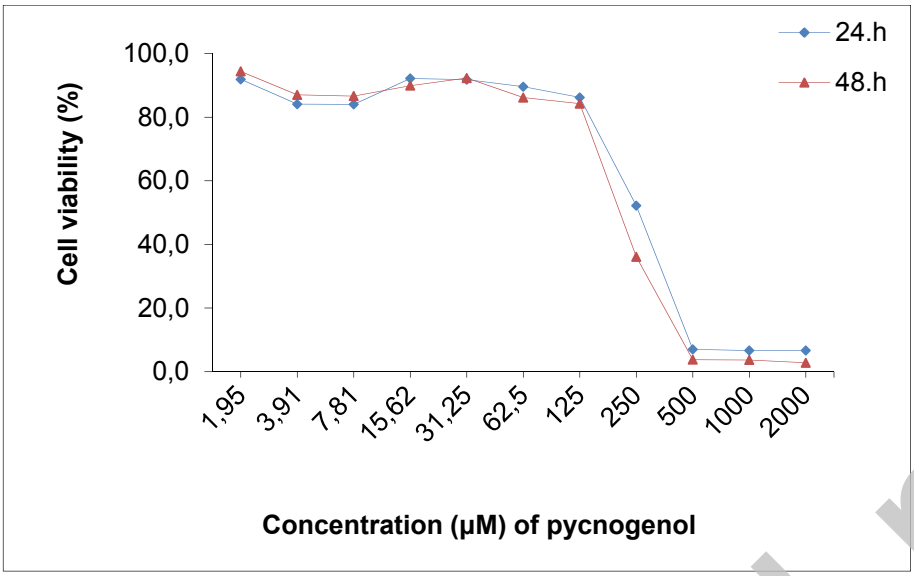

Figure 1. Effects of pycnogenol on the cell viability of HeLa cells for 24 h and 48 h*

* Values were given as the mean ± standard deviation (n=4). [a] p<0.05, compared to negative control (PBS).

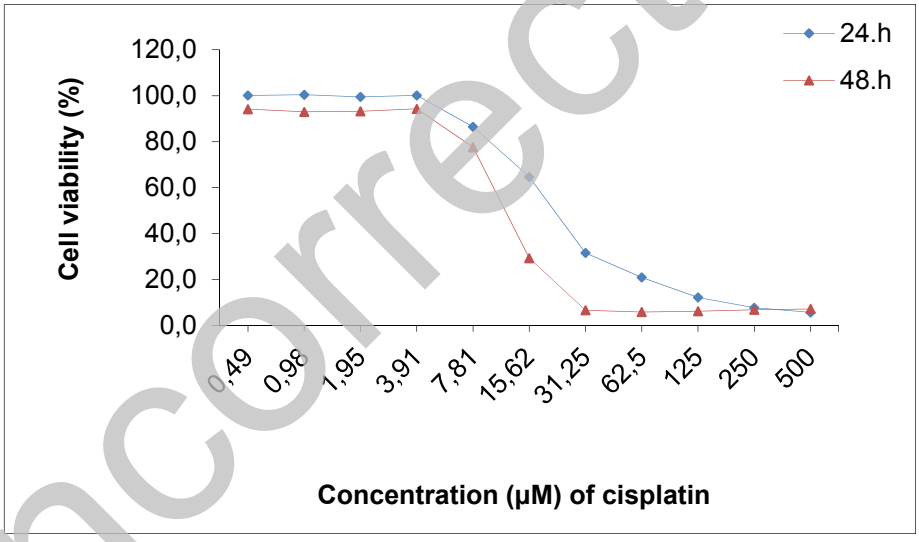

Figure 2. Effects of cisplatin on the cell viability of HeLa cells for 24 h and 48 h*

* Values were given as the mean ± standard deviation (n=4). [a] p <0.05, compared to negative control (PBS).

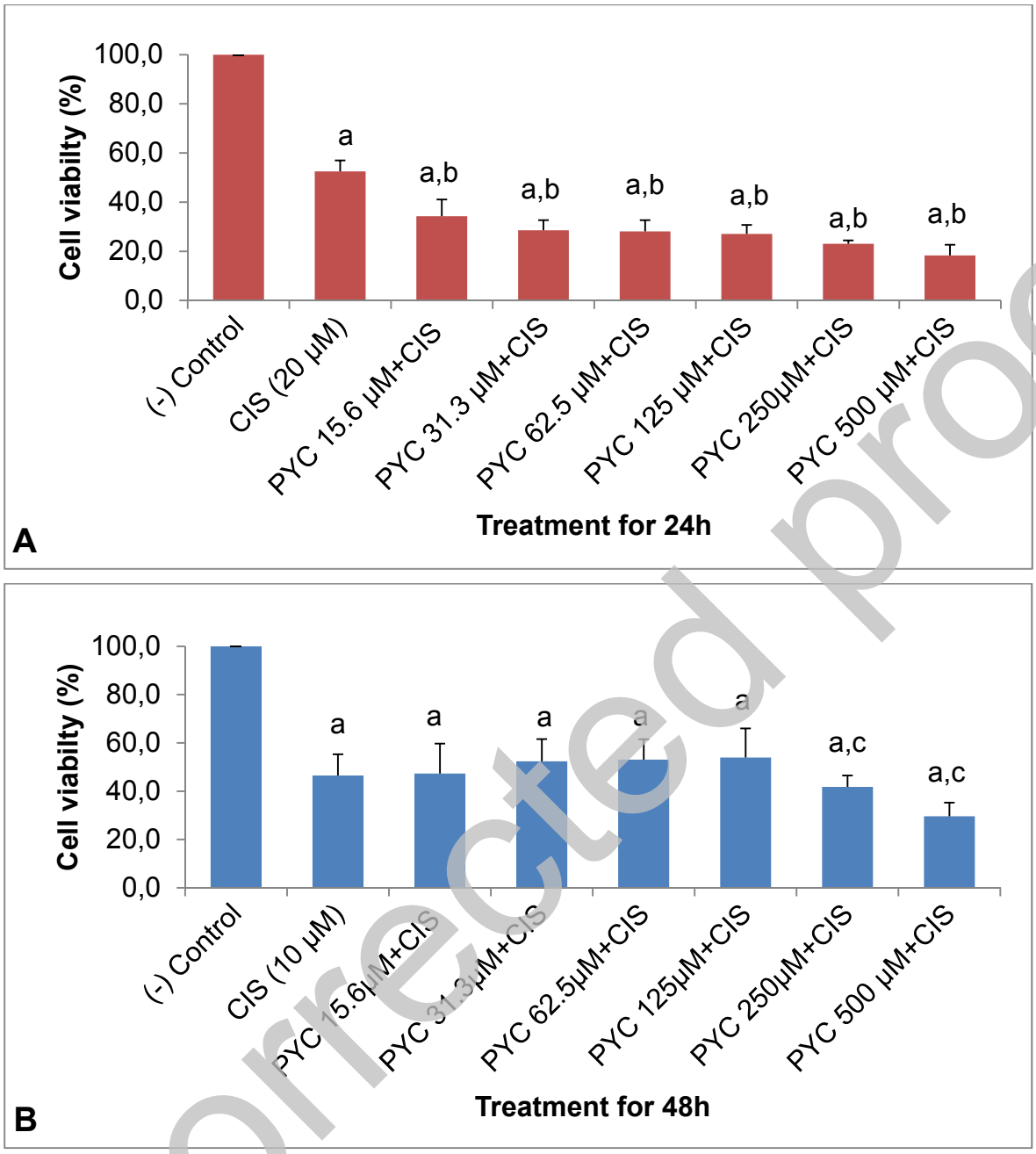

Figure 3. Effects of pycnogenol on the cisplatin cytotoxicity in HeLa cells for 24 h (A) and 48 h (B). Values were given as mean ± standard deviation (n=4). [a] $p < 0.05$, compared to negative control (PBS); [b] $p < 0.05$, compared to cisplatin (20 µM); [c] $p<0.05$, compared to cisplatin (10 µM). PYC,pygnogenol; CIS. cisplatin.

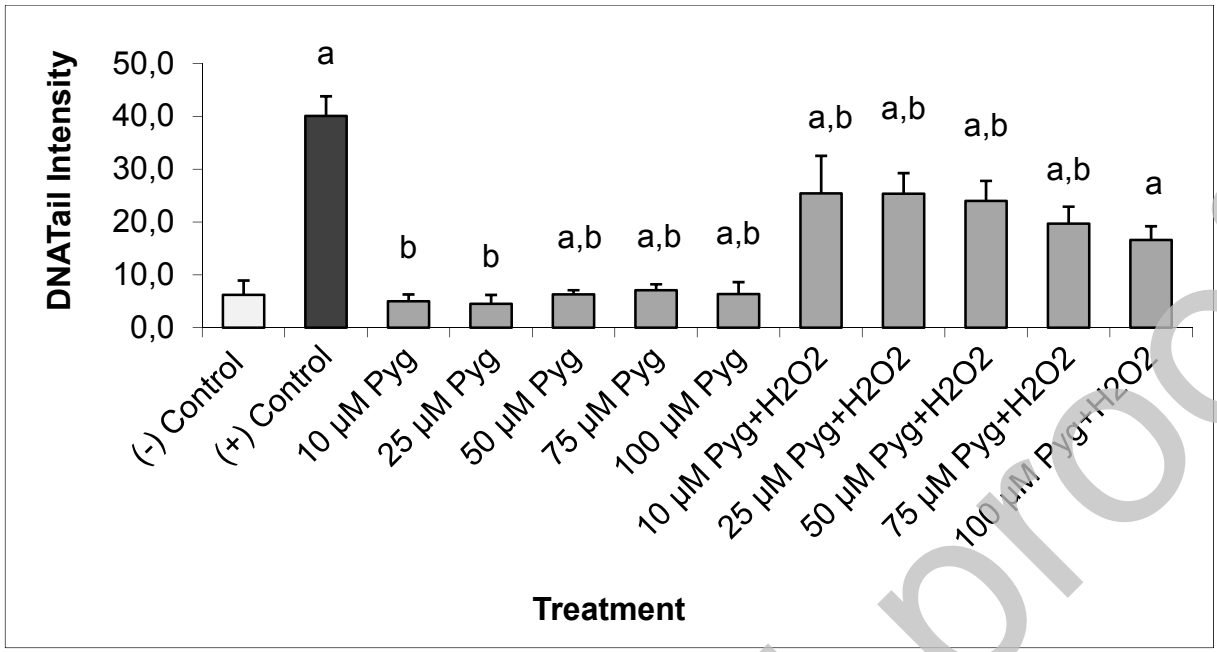

Figure 4. Effect of pycnogenol against oxidative DNA damage in HeLa cells. DNA damage was expressed as DNA tail intensity. Values are given as the mean ± standard deviation (n = 4). [a]*p<0.05*, statistically different from negative control (1% PBS). [b]*p<0.05*, statistically different from positive (50 µM H_2O_2) control. Pyc, pygnogenol.