ORIGINAL ARTICLE

The Ameliorative Effects of Pycnogenol® on Liver Ischemia-Reperfusion Injury in Rats

Sıçanlarda Karaciğer İskemi Reperfüzyon Hasarında Piknogenol®'ün İyileştirici Etkileri

Mehmet TOKAÇ[1], Merve BACANLI[2], Ersin Gürkan DUMLU[3], Sevtap AYDIN[2], Merve ENGİN[4], Birkan BOZKURT[5], Abdüssamed YALÇIN[3], Özcan EREL[6], Mehmet KILIÇ[3], Nurşen BAŞARAN[2]*

[1]Yeni Yüzyıl University, Faculty of Medicine, Gaziosmanpaşa Hospital, Clinic of General Surgery, İstanbul, Turkey
[2]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkey
[3]Yıldırım Beyazıt University, Faculty of Medicine, Department of General Surgery, Ankara, Turkey
[4]Ankara Atatürk Training and Research Hospital, Clinic of Biochemistry, Ankara, Turkey
[5]Cumhuriyet University, Faculty of Medicine, Department of General Surgery, Sivas, Turkey
[6]Yıldırım Beyazıt University, Faculty of Medicine, Department of Biochemistry, Ankara, Turkey

ABSTRACT

Objectives: Pycnogenol® (PYC®), a standardized extract from the bark of *Pinus maritima*, consists of different phenolic compounds. PYC® has shown to have protective effects on chronic diseases such as diabetes, asthma, cancer, and immune disorders. The aim of this study was to determine the effects of PYC® against the DNA damage and biochemical changes in blood, liver, and lung tissues of ischemia-reperfusion (IR)-induced Wistar albino rats.

Materials and Methods: A sham group, IR injury-induced group, and IR+PYC® group were formed. Ischemia was induced and sustained for 45 min, then the ischemic liver was reperfused, which was sustained for a further 120 min at the end of this period. After anesthesia and before the IR inducement, 100 mg/kg PYC® was given to the IR+PYC® group through intraperitoneal injections. The total oxidant (TOS) and total antioxidant status (TAS), total thiol levels (TTL), advanced oxidation protein products (AOPP), and biochemical parameters [myeloperoxidase (MPO), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH)] in the rats were analyzed using spectrophotometric methods and DNA damage was assessed using single-cell gel electrophoresis.

Results: The levels of TOS, TTL, MPO, AOPP, ALT, AST, and LDH were significantly decreased in the IR+PYC® group compared with the IR group (p<0.05). The levels of TAS were significantly increased in the IR+PYC® group compared with the IR group (p<0.05). PYC® reduced the DNA damage when compared with the IR group (p<0.05).

Conclusion: The present results suggest that PYC® treatment might have a role in the prevention of IR-induced oxidative damage by decreasing DNA damage and increasing antioxidant status.

Key words: Pycnogenol, ischemia reperfusion injury, DNA damage

ÖZ

Amaç: Piknogenol® (PYC®), *Pinus maritima* bitkisinin kabuğundan elde edilen ve birkaç tip fenolik içerikten oluşan, suda çözünür standardize bir özüttür. Piknogenolün diyabet, astım, kanser ve immün hastalıklar gibi farklı hastalıklar üzerinde koruyucu etkilerinin olduğu gösterilmiştir. Bu çalışmanın amacı PYC®'nin karaciğer iskemi-reperfüzyonu yapılan sıçanların kan, karaciğer ve akciğer dokularında DNA hasarı ve biyokimyasal değişiklikler üzerindeki etkisinin değerlendirmektir.

Gereç ve Yöntemler: Sham grubu, iskemi-reperfüzyon (IR) ve (IR+PYC®) grubu olmak üzere 3 grup oluşturuldu. Karaciğerde 45 dakika iskemi ardından 120 dk reperfüzyon yapıldı. IR+PYC® grubunda PYC® anestezi sonrası iskemi yapmadan önce 100 mg/kg dozunda intraperitoneal verildi. Sıçanlarda total oksidan durum (TOS), total antioksidan durum (TAS), total tiyol düzeyleri (TTL), ilerlemiş oksidasyon protein ürünleri (AOPP) ve biyokimyasal parametreler [miyeloperoksidaz düzeyi (MPO), aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), laktat dehidrogenaz (LDH)] spektrofotometrik yöntemle, ayrıca tek hücre jel elektroforezi tekniğiyle de DNA hasarı analiz edildi.

Bulgular: TOS, TTL, MPO, AOPP, ALT, AST and LDH düzeyleri IR+PYC® grubunda IR grubuna göre anlamlı ölçüde düşük bulundu (p<0.05). TAS düzeyi IR+PYC® grubunda IR grubuna göre anlamlı ölçüde yüksek bulundu (p<0.05). DNA hasarı değerlendirildiğinde PYC® verilen grupta DNA iskemi grubuna göre anlamlı ölçüde azaldığı saptandı (p<0.05).

Sonuç: Mevcut bulgular, piknogenol tedavisinin IR ile indüklenmiş oksidatif hasarı DNA hasarını azaltarak ve antioksidan durumu arttırarak da önlediğini göstermektedir.

Anahtar kelimeler: Piknogenol, iskemi reperfüzyon hasarı, DNA hasarı

INTRODUCTION

Liver surgical options applied for intrahepatic lesions or liver transplantation require a period of ischemia.[1,2] When blood flow is restored, injury to the liver may occur. This phenomenon is called ischemia-reperfusion (IR) injury.[3] IR, one of the main causes of hepatic failure, occurs in different situations including heart failure, liver transplantation, liver trauma, and blood occlusion to the liver.[4-6] Free oxygen radicals and various cytokines, which are produced after reperfusion, play a pivotal role in IR injury.[7,8] The infiltration of polymorphonuclear leukocytes in a tissue is characteristic of acute inflammation and indicates the collective action of chemotactic mediators.[9] The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is possible in IR. These compounds may act on proteins, enzymes, nucleic acids, cytoskeleton, and lipid peroxides, leading to mitochondrial dysfunction and lipid peroxidation.[10] Additionally, ROS and RNS may also damage endothelial cells and destroy the integrity of the microvasculature.[11]

Although there is evidence about the administration of drugs to protect the liver in animals, even today there is little evidence about the use of these substances in human IR.[12,13] Prevention of oxygen radical release via administration of radical scavengers has been found to be beneficial against IR injury.[7,8] Antioxidants are known as potential scavengers of ROS, so that they protect biologic membranes against oxidative damage. Natural products are widely used as dietary supplements because of their potential antioxidant properties. Plant polyphenols may act as antioxidants by different mechanisms such as free radical scavenging, metal chelation and protein binding.[14]

Pycnogenol® (PYC) is a standardized extract from the bark of the French maritime pine (*Pinus maritima).*[15] Studies indicate that PYC® components are highly bioavailable and it is assumed to display greater biologic effects as a mixture than its individual purified components, which indicates that there are synergistic interactions between its components. Previous studies demonstrated that PYC® was a very strong antioxidant at scavenging reactive oxygen and nitrogen species.[16] It is used in dietary supplements and health protective products because of its direct and strong antioxidant activity.[15] It has beneficial effects on various diseases such as diabetes, asthma, hypertension, cancer, and immune diseases.[17]

The aim of this study was to evaluate the effects of PYC® against the DNA damage and biochemical changes in blood, liver, and lung tissues of IR-induced rats.

MATERIALS AND METHODS

Ethics

The study was approved by the Local Ethical Committee of Laboratory Animal Research of Ankara Training and Research Hospital (2014/291).

Chemicals

The chemicals used in the experiments were purchased from the following suppliers: PYC®, a registered trade mark of Horphag Research Ltd., (Geneva, Switzerland), was provided by Henkel Corporation (La Grange, IL, U.S.A.); normal melting agarose (NMA) and low-melting-point agarose (LMA) from Boehringer Manheim (Mannheim, Germany); 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), chloramine-T, acetic acid, potassium iodide (KI), hydrogen peroxide (H_2O_2), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), sodium chloride (NaCl), sodium hydroxide (NaOH), and potassium chloride from Merck Chemicals (Darmstadt, Germany); dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), Triton-X-100, phosphate-buffered saline (PBS) tablets, from Sigma-Aldrich Chemicals (St Louis, Missouri, USA); ethylendiaminetetraacetic acid disodium salt dihydrate (EDTA-Na_2), natrium lauroyl sarcosinate, and Tris from ICN Biomedicals Inc. (Aurora, Ohio, USA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and albumin kits from Roche Diagnostics (Mannheim, Germany).

Animals

A total of 24 Wistar albino rats, male, weighing 200-300 g, were used in the study. The rats were kept in rooms with automatically adjusted temperature (23±2°C) and humidity (50%) and a rotation of light and dark illumination for 12 hr each. They were housed in plastic cages with stainless steel grid tops. Animals were fed with standard laboratory chow and allowed to access feed and drinking water ad libitum.

Experimental protocol

The rats were randomized into three groups of 8 rats each.

- Group 1 (Sham group) (n=8): Only hepatoduodenal ligament dissection was performed and no drug was given.

- Group 2 (IR group) (n=8): Forty-five min after the Pringle maneuver, reperfusion was generated for 120 min and no drug was given.

- Group 3 (IR+PYC® group) (n=8): PYC® was given at a dose of 100 mg/kg intraperitoneal (i.p.) after anesthesia

was performed. Forty-five min after the Pringle maneuver, reperfusion was generated for 120 min.

Rats were anesthetized with 80 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı-Warner Lambert, İstanbul, Turkey) plus 10 mg/kg xylazine (Rompun, Bayer, İstanbul, Turkey) via intramuscular (i.m.) injection. A midline incision was performed and rats underwent either sham surgery or IR. Ischemia was induced with the Pringle maneuver and sustained for 45 min after this time, the ischemic liver was reperfused, and this was sustained for a further 120 min at the end of this period, the animals were sacrificed by taking blood from the heart. After anesthesia and before the IR inducement, 2 mL 0.9% NaCl was given intraperitoneally (i.p.) to group 1 and group 2, and 100 mg/kg PYC® in 2 mL 0.9% NaCl i.p. was given to group 3. The dose of PYC® was selected according to our previous study.[18]

After the end of this procedure, blood samples obtained through cardiac puncture were collected into preservative-free heparin tubes for the biochemical and the DNA damage evaluations. The heparinized blood samples were kept in the dark at 4°C and processed within 6 hr. The liver and kidney tissues were carefully dissected from their attachments and totally excised. Excised tissues were divided into three parts for biochemical analysis, DNA damage analysis, and assessment of antioxidant and oxidant parameters.

Biochemical analysis

For biochemical analysis, the heparinized blood samples were centrifuged at 800 g for 15 min. The plasma was collected and examined for total antioxidant status (TAS), total oxidant status (TOS), total thiol levels (TTL), myeloperoxidase (MPO), advanced oxidation protein products (AOPP), albumin, ALT, AST and LDH.

The liver and kidney tissues were extracted following the homogenization and sonication procedure as previously described by Sier et al.[19] (1996). TOS, AOPP, ceruloplasmin and MPO levels were examined.

Determination of oxidative stress parameters

Measurement of TAS

Plasma and tissue homogenate TAS levels were measured using a novel automated colorimetric method developed by Erel[20] (2004). In this method, antioxidants in the sample reduce the dark blue-green-colored ABTS radical to a colorless reduced ABTS form. The change of absorbance at 660 nm is related with the total antioxidant level of the sample. This method determines the antioxidative effect of the sample against the potent free radical reactions initiated by the produced hydroxyl radical. The results are expressed as mmol Trolox equivalent per liter (mmol Trolox Eq/L).

Measurement of TOS

Plasma and tissue homogenate TOS levels were measured using a novel automated colorimetric method described by Erel[21] (2005). In this method, oxidants present in the sample oxidize the ferrous ioneo-dianisidine complex to the ferric ion. The oxidation reactions enhanced by glycerol molecules are abundantly present in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with H_2O_2, and the results are expressed in terms of micromolar H_2O_2 equivalent per liter (µmol H_2O_2 Eq/L).

Measurement of TTL

TTL or sulfhydryl (SH) groups in plasma and tissue homogenates were measured using the methods originally described by Ellman[22] (1959) and modified by Hu[23] (1994). Here, thiols interact with DTNB, forming a highly colored anion with maximum peak at 412 nm (e412= 13.600 M/cm). The result is expressed in µmol/L.

Measurement of MPO levels

Serum MPO activity was determined using a modification of the o-dianisidine method[24] based on kinetic measurement at 460 nm with the rate of the yellowish-orange product formation from the oxidation of o-dianisidine with MPO in the presence of H_2O_2. One unit of MPO was defined as that degrading 1 µmol of H_2O_2 min^{-1} at 25°C. A molar extinction coefficient of 1.13x104 of oxidized o-dianisidine was used for the calculation. MPO activity is expressed in units per liter serum.

Measurement of AOPP levels

The quantification of AOPP in plasma used the method described by Witko-Sarsat et al.[25] (1996). Two hundred microliters of plasma diluted 1:5 in PBS, or chloramine-T standard solutions (0 to 100 µmol/liter), followed by 20 µL of acetic acid. Ten microliters of 1.16 M potassium iodide were then added, followed by 20 µL of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm against a blank containing 200 µL of PBS, 10 µL of KI, and 20 µL of acetic acid. The chloramine-T absorbance at 340 nm was linear within the range of 0 to 100 µmol/liter. AOPP concentrations are expressed in µmol/liter of chloramine-T equivalents.

Measurement of albumin, ALT, AST and LDH levels

The levels of ALT, AST, LDH, and albumin were determined using commercially available assay kits (Roche) with an autoanalyzer (Roche/Hitachi Cobas C501).

Determination of DNA damage

Whole blood samples were used for evaluation of DNA damage. The liver and lung tissues were carefully dissected from their attachments and totally excised. Preparation of single-cell suspension from the organs was performed according to standard procedures.[26-28] In brief, approximately 0.2 g of each organ was placed in 1 mL chilled mincing solution [Hank Balanced Salt Solution (HBSS) with 20 mM EDTA and 10% DMSO] in a petri dish and chopped into pieces with a pair of scissors. The pieces were allowed to settle and the supernatant containing the single-cell suspension was taken. The concentrations of renal and hepatic tissue cells in the supernatant were adjusted to approximately 2x10⁶ cells/mL in HBSS containing 20 mM EDTA/10% DMSO.

The Alkaline Comet assay technique of Singh et al.[29], as further described by Collins[30] and Bacanlı et al.[31] was followed. The cells were suspended in 75 µL of 0.5% LMA. The suspensions were then embedded on slides precoated with a layer of 1% NMA. Slides were allowed to solidify on ice for 5 min. Coverslips were then removed. The slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 100 mM Tris, 1% sodium sarcosinate, pH 10.0 with Triton-X-100 and 10% DMSO) for a minimum of 1 h at 4˚C. The slides were then removed from the lysing solution, drained, and left in the electrophoresis solution (1 mM sodium EDTA and 300 mM NaOH, pH 13.0) for 20 min at 4˚C to allow unwinding of the DNA and expression of alkali-labile damage. They were then left in the electrophoresis solution (1 mM sodium EDTA and 300 mM NaOH, pH: 13) for 20 min at 4˚C to allow unwinding of the DNA and expression of alkali-labile damage.

Electrophoresis was also conducted at a low temperature (4˚C) for 20 min using 24 V and adjusting the current to 300 mA by raising or lowering the buffer level. The slides were neutralized by washing three times in 0.4 M Tris-HCl (pH: 7.5) for 5 min at room temperature. After neutralization, the slides were incubated in 50%, 75%, and 98% of alcohol for 5 min each.

The dried microscopic slides were stained with EtBr (20 µg/mL in distilled water, 60 µL/slide), covered with a cover-glass prior to analysis with a Leica® fluorescence microscope under green light. 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 DNA damage, 100 nuclei per slide were examined at x400 magnification. Results are expressed as the length of the comet (tail length), the product of the tail length, and the fraction of total DNA in the tail (tail moment) and percent of DNA in the tail (tail intensity).

Statistical analysis
Statistical analysis was performed using the SPSS for Windows 20.0 computer program. Differences between the means of data were compared using the ANOVA test, and post hoc analysis of group differences was performed using the least significant difference test. The Kruskal-Wallis H test was used to compare parameters that displayed abnormal distribution between groups. The results are given as the mean ± standard deviation. P values of less than 0.05 were considered as statistically significant.

RESULTS

Biochemical parameters in plasma
The plasma biochemical parameters are shown in Table 1. TAS levels in the IR group were found to be significantly lower than the sham group ($p<0.05$). The levels of TAS were found to significantly increase in the IR+PYC® group compared with the IR group ($p<0.05$). There was no significant difference in terms of TAS levels between the IR+PYC® group and the sham group. TOS, TTL, MPO, AOPP, ALT, AST, and LDH levels in the IR group and IR+PYC® group were found significantly higher than the sham group ($p<0.05$). The levels of TOS, TTL, MPO, AOPP, ALT, AST, and LDH were found to significantly decrease in the IR+PYC® group compared with the IR group ($p<0.05$). There was no significant difference with regards TTL and MPO levels between the IR+PYC® and sham groups.

Biochemical parameters in liver and lung
The TAS, TOS, and TTL levels in the lung and liver tissues are shown in Table 2 and 3, respectively. TOS and TTL levels in the lung and liver tissues were found to be significantly higher in the IR group compared with the sham group ($p<0.05$) (Table 2, 3).

In the lung samples, TOS and TTL levels were found to significantly decrease in the IR+PYC® group compared with the IR group ($p<0.05$). There were no significant differences in terms of TOS and TTL levels between the IR+PYC® group and the sham group (Table 2). TAS levels in the lung tissues were found significantly lower in the IR group compared with the sham group ($p<0.05$). There was no significant difference regarding TAS levels between the IR+PYC® group and the sham group (Table 2). In the liver samples, TOS levels were found to significantly

Table 1. Biochemical findings of plasma samples of experimental groups

	Sham group	IR group	IR+PYC group
TAS (mmol Trolox Eq/L)	4.22±1.08	1.83±0.26[a]	3.43±0.88[b]
TOS (µmol H_2O_2 Eq/L)	4.50±1.16	30.08±7.46[a]	14.34±2.81[a,b]
TTL (µmol/L)	128.98±18.81	241.13±26.66[a]	130.61±22.56[b]
MPO (U/L)	37.92±16.57	159.37±94.15[a]	55.06±21.34[b]
AOPP (µmol Chloramine T Eq/L)	141.18±37.84	299.68±62.63[a]	234.54±63.96[a,b]
Albumin (g/dL)	3.84±0.35	3.62±0.56	2.84±0.36[a,b]
ALT (mg/dL)	32±18	2997±650[a]	1380±443[a,b]
AST (mg/dL)	172±52	2747±827[a]	1525±317[a,b]
LDH (mg/dL)	325±95	8261±1947[a]	5227±1146[a,b]

IR: Ischemia-reperfusion, PYC: Pycnogenol, TAS: Total antioxidant status, TOS: Total antioxidant status, TTL: Total thiol levels, MPO: Myeloperoxidase activity, AOPP: Advance oxidation protein product, ALT: Alanine transaminase, AST: Aspartate transaminase, LDH: Lactate dehydrogenase, [a]Statistically different from sham group ($p<0.05$), [b]Statistically different from IR group ($p<0.05$)

decrease in the IR+PYC® group compared with the IR group (p<0.05). There were no significant differences in terms of TAS, TOS, and TTL levels between the IR+PYC® group and the sham group (Table 3).

Assessment of DNA damage

The DNA damage expressed as tail length, tail intensity, and tail moment in the blood, liver, and lung cells of rats are given in Figure 1, 2, and 3. In the blood and tissues studied, DNA damage was found to be significantly higher in the IR group compared with the sham group (p<0.05). PYC® was found to reduce the DNA damage significantly because the damage in the IR+PYC® group was lower than in the IR group (p<0.05). There was no significant difference in DNA damage between the sham group and the IR+PYC® group.

Table 2. Oxidative stress parameters in lung samples of experimental groups			
	Sham group	IR group	IR+PYC group
TAS (mmol Trolox Eq/L)	3.12±1.24	2.02±0.56[a]	2.97±0.70[b]
TOS (µmol H_2O_2 Eq/L)	4.78±1.15	6.20±1.54[a]	4.29±1.19[b]
TTL (µmol/L)	56.43±10.06	71.62±7.56[a]	60.87±8.30[b]

IR: Ischemia-reperfusion, PYC: Pycnogenol, TAS: Total antioxidant status, TOS: Total antioxidant status TTL: Total thiol levels, [a]Statistically different from sham group (p<0.05), [b]Statistically different from IR group (p<0.05)

Table 3. Biochemical findings of liver samples of experimental groups			
	Sham group	IR group	IR+PYC group
TAS (mmol Trolox Eq/L)	5.44±1.09	5.74±0.28	5.88±0.19
TOS (µmol H_2O_2 Eq/L)	7.05±1.61	9.68±2.41[a]	7.84±1.53[b]
TTL (µmol/L)	42.42±24.64	62.39±19.07[a]	58.59±18.95

IR: Ischemia-reperfusion, PYC: Pycnogenol, TAS: Total antioxidant status, TOS: Total antioxidant status, TTL: Total thiol levels, [a]Statistically different from sham group (p<0.05), [b]Statistically different from IR group (p<0.05)
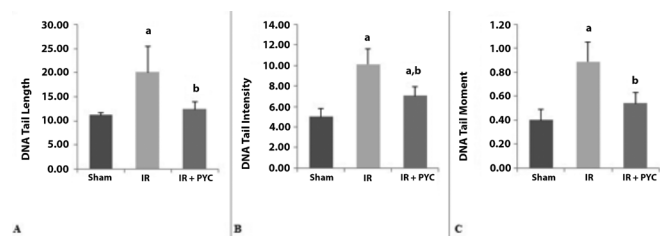

Figure 1. DNA damage in the blood cells of the experimental groups expressed as (a) tail length, (b) tail intensity, (c) tail moment. The values are expressed as mean ± standard deviation

[a]p<0.05, compared with sham group; [b]p<0.05, compared with IR group; IR: Ischemia-reperfusion, PYC: Pycnogenol

DISCUSSION

Previous studies have shown that ROS plays an important role in liver IR injury, which can result in different undesirable effects.[32,33] When tissues are exposed to ischemia followed by reperfusion, ROS are extensively generated in the early stage of reperfusion, which cause serious damage to tissues in various organs, including the liver, brain, heart, and kidney.[34] Oxidative stress contributes to the pathogenesis of liver injury. When liver is deprived of oxygen, antioxidant enzyme activities decrease, and ROS attack cellular molecules.[35] Thus, free radical scavengers are thought to be beneficial in the treatment of IR damage. A number of compounds, especially antioxidants, have been used to reduce hepatic IR injury in animal studies, but few are currently used in humans because of limited and controversial data about their efficacy.[36]

Phenolic compounds such as flavonoids found in daily diets have various beneficial effects against various diseases such as cancer, diabetes, and cardiovascular and neurodegenerative diseases, and experimental data are accumulating regarding them as natural important phytochemical antioxidants for human health.[37] Research on antioxidant substances has focused on the potential benefits of both purified phytochemicals and plant extracts such as the pine bark extract known as PYC®.[18] The protective effects of PYC® on the biochemical changes in TAS, TOS, TTL, MPO, AOPP, ALT, AST, and LDH levels in hepatic IR were investigated in our study. We used PYC® to reduce hepatic IR because it has been shown previously to have antioxidant properties.
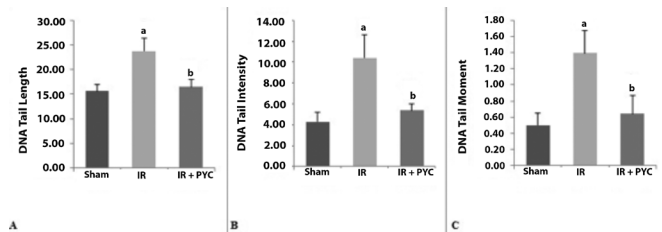

Figure 2. DNA damage in the liver cells of the experimental groups expressed as (a) tail length, (b) tail intensity, (c) tail moment. The values are expressed as mean ± standard deviation

[a]p<0.05, compared with sham group; [b]p<0.05, compared with IR group; IR: Ischemia-reperfusion, PYC: Pycnogenol
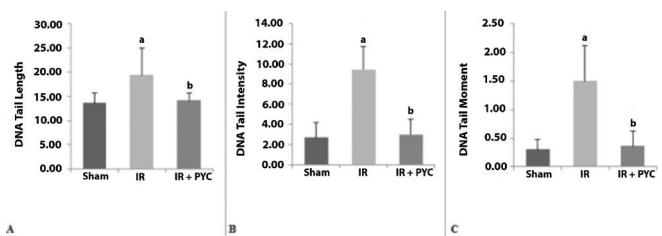

Figure 3. DNA damage in the lung cells of the experimental groups expressed as (a) tail length, (b) tail intensity, (c) tail moment. The values are expressed as mean ± standard deviation

[a]p<0.05, compared with sham group; [b]p<0.05, compared with IR group. IR: Ischemia-reperfusion, PYC: Pycnogenol

Studies indicated that PYC® components were highly bioavailable. It is a procyanidin-enriched extract of *Pinus pinaster* bark consisting of a variety of flavonoids, which are known as potent antioxidants.[38] PYC® shows various beneficial health effects against different types of diseases.[17] PYC® has been extensively used in Europe as a dietary food supplement. It has been suggested to have free radical scavenging and antioxidant properties[39,40], to protect protein oxidation[41], and to ameliorate oxidative organ injury and DNA damage. PYC® was reported to reduce IR-induced renal injury and preserve renal function.[42]

During the reperfusion phase of the liver, emerging reactive oxygen radicals activate some mediators and can cause an inflammatory response and tissue damage. For this reason, AST, ALT, and LDH levels may increase.[37,43] Atila et al.[44] (2002) showed that pretreatment with an antioxidant such as carnitine protected hepatic enzyme levels (ALT and AST) in rats with increased ischemia reperfusion. Similarly, Yang et al.[15] (2008) reported that PYC® decreased serum AST and ALT levels on CCl$_4$- induced hepatotoxicity in rats. In our study, plasma AST, ALT, and LDH levels increased in the IR group but PYC® administration decreased the levels of AST, ALT, and LDH in comparison with the IR group. These findings support the protective effect of PYC® against liver IR injury.

The decrease in TAS levels and the increase in TOS levels were reported in hepatic IR damage.[45] Tüfek et al.[46] (2013) demonstrated that the serum, liver, lung, and kidney tissues of the IR group had higher TOS values and lower TAC values when compared with the sham group. When dexmedetomidine, an antioxidant compound, was administered to the IR group, it was observed that TOS values decreased and TAC values increased. Similar with this study, our data showed that TOS values were increased and TAS values were decreased in IR group and PYC® treatment ameliorated these changes.

SH groups are known to be sensitive to oxidative damage and depleted following ischemic insult.[47] In our study, TTLs were increased following IR in plasma, liver, and lung tissues of rats. Hosseinzadeh et al.[48] (2005) reported that rats pretreated with crocin, a phenolic compound in saffron extract, exhibited higher thiol contents than their respective controls in a dose-related pattern, indicating that crocin helped to replenish the total thiol pool. However saffron-mediated SH replenishment was not as impressive as expected. Saffron pretreatment slightly increased total thiol concentration following ischemic insult, but this elevation was not significant as compared with the control group.

As far as we know, this is the first study to determine MPO and AOPP levels in IR injury- induced rats. We found that MPO and AOPP levels in the IR group were found to be significantly higher than the sham group. The levels of MPO and AOPP were significantly decreased in the IR+PYC® group compared with the IR group.

We found that the DNA damage was significantly higher in the blood, liver, and lung cells of the IR group compared with the sham group. On the other hand, the parameters were significantly decreased in the PYC® treated IR group when compared with the

IR group. PYC® treatment seemed to prevent IR- induced DNA damage in the blood, liver, and lung cells of the rats. There are limited data about the protective effects of PYC® against DNA damage. Consistent with our data, Taner et al.[18] (2014) reported the protective effects of PYC® on sepsis-induced oxidative DNA damage. The protective effects of water extracts from pine needle against DNA damage and apoptosis induced by hydroxyl radical were also demonstrated in non-cellular and cellular systems through inhibiting oxidative DNA damage induced by hydroxyl radical and preventing the cells from oxidative damage.[49]

CONCLUSION

The results of this study have shown that oxidative stress parameters are significantly altered in experimental hepatic IR injury in rats. PYC® was found to be protective against IR injury-induced oxidative damage and genotoxic effects in blood, liver, and lungs of rats. In conclusion, PYC® may protect against oxidative liver injury in rats. We also conclude that a possible protective role and clinical availability of PYC® for liver injury should be investigated in further studies.

Conflict of Interest: No conflict of interest was declared by the authors.

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