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Exploring the impacts of Pycnogenol on pentraxin-3 levels in the heart tissue of rats administered with gentamicin

Tolga Çakmak

Department of Cardiology, Balıkesir Atatürk City Hospital, University of Health Sciences, Balıkesir, Turkey

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ABSTRACT

Aims: The present study explored if pentraxin-3 (PTX-3) levels, which would be boosted due to cardiac damage by gentamicin, can be regressed thanks to Pycnogenol, which was also previously shown to have desirable impacts on cardiovascular diseases.

Methods: In the study, we recruited 28 8-10-week-old male Sprague-Dawley rats into four groups: control, gentamicin, gentamycin+Pycnogenol, and Pycnogenol. We stained the tissue samples with hematoxylin-eosin and Masson's trichome dye for histopathological analysis. Then, malondialdehyde (MDA) levels were measured using the spectrophotometric technique. In addition, we measured PTX-3 levels in the heart tissues by an immunohistochemical method.

Results: We discovered the heart tissue samples of the rats in the control and Pycnogenol groups were histologically normal. As well as mononuclear cell increase and degeneration of cardiac muscle cells, we observed mild congestion in the gentamicin group compared to the control group. Despite more significant damage to the heart tissue in the gentamicin+Pycnogenol group compared to the control group, we found that the histopathological damage regressed in this group compared to the gentamicin group. While PTX-3 immunoreactivity was similar between the control and Pycnogenol groups, it was significantly elevated in the gentamicin group compared to the control group ($p < 0.001$). Moreover, the gentamicin+Pycnogenol group had decreased PTX-3 immunoreactivity than the gentamicin group. While MDA values followed a similar pattern between the control and Pycnogenol groups, these values were found to be significantly increased in the gentamicin group compared to the control group ($p < 0.001$). These values, however, were decreased in the gentamicin+Pycnogenol group compared to the gentamicin group.

Conclusion: In a nutshell, the present study was able to demonstrate that gentamicin may lead to cardiac damage by boosting PTX-3 levels and that the damage can be regressed thanks to the Pycnogenol treatment.

Keywords: Pycnogenol, gentamicin, pentraxin 3 (PTX-3), malondialdehyde (MDA), cardiotoxicity

INTRODUCTION

Pycnogenol, a phenolic compound, represents a nutritional product utilized as a bioactive phytochemical medication across the world. It was coined as a scientific term for the class of polyphenols; however, it currently refers to the extract of pine bark in France.[1] The resulting extract product is a fine, reddish-brown water-soluble powder. The consistent extract of Pycnogenol consists of phenolic components such as monomers (taxifolin, epicatechin, and catechin), condensed flavonoids (grouped as procyanidins and proanthocyanins), and phenolic acids (cinnamic acids and some glycosides). It bears a protective effect against inflammatory diseases, hypertension, diabetes mellitus (DM), and obesity.[2] In addition, it extends benefits to lung fibrosis and was previously shown to yield positive impacts on cognitive ability and cardiovascular diseases.[3-5]

Gentamicin is an aminoglycoside-group antibiotic adopted in the treatment of diverse bacterial infections. It is bactericidal that acts as a protein synthesis inhibitor by binding to the 30s subunit of the bacterial ribosome.[6] Aminoglycoside antibiotics often demonstrate three main toxic effects: nephrotoxicity, ototoxicity, and blocking neuromuscular-ganglionic transmission. The previous study also reported depression of cardiac function in various animal species, including rats, following aminoglycosides administration.[7] Acute hypotension emerging after intravenous (IV) administration of aminoglycosides leads to an adverse inotropic effect on the heart.[8,9] In addition, administration of aminoglycosides was associated with the weakening of hemodynamic parameters and even cardiovascular collapse.

Corresponding Author: Tolga Çakmak, tolgacakmak85@gmail.com

Pentraxin-3 (PTX-3), C-reactive protein (CRP), and Serum amyloid P component (SAP, an acute phase protein in rodents) are members of the pentraxin superfamily, but PTX-3 is characterized by the presence of an N-terminal domain in addition to a C-terminal pentraxin-like domain (the family's distinctive feature).[10] Expression of PTX3 is induced by proinflammatory signals such as IL-1β, TNF-α, and Toll-like receptor (TLR) agonists through both MyD88 and TRIF-dependent pathways.[10-13] Similar to CRP, plasmatic levels of PTX-3 are rapidly elevated in various pathological conditions of inflammatory and/or infectious etiology, including acute myocardial infarction, sepsis, and SARS-CoV-2 infections, and the higher levels are associated with disease severity and risk of mortality.[14-18] Therefore, the present study explored if pentraxin-3 (PTX-3) levels, which would be boosted due to cardiac damage by gentamicin, can be regressed thanks to Pycnogenol, which was also previously shown to have desirable impacts on cardiovascular diseases.

METHODS

We initiated this study with the approval of the Fırat University animal Experimentation Ethics Committee (Date: 18.04.2023, Decision No: 07-03). In all stages of the study, we strictly adhered to the rules and the principles set forth in the Declaration of Helsinki. We performed the experimental procedures on the research animals at the Experimental Research Center. At the end of the procedures, we studied the tissue samples in Faculty of Medicine, Histology and Embryology Department and serum samples in the Medical Biochemistry Laboratory of the Faculty of Medicine.

Procurement of the Rats

In this study, we studied the serum and tissue samples of 28 8-10-week-old Sprague-Dawley male rats weighing between 200-220 g. They were divided into four groups, with seven rats in each, and housed in rooms in a 12:12 light-dark (LD) cycle at 22±20 °C room temperature, with food and water ad-libitum during the 1-week adaptation and 9-day experiment period.

Experimental Groups

The groups were composed as listed below:

Group I (control group, n=7): The rats received orally 1 mL/kg saline throughout the experimental period.

Group II (gentamicin group, n=7): A single dose of 80 mg/kg gentamicin was administered to the rats at the beginning of the experiment.[19]

Group III (treatment group, gentamicin+Pycnogenol, n=7): The rats received a single dose of 80 mg/kg gentamicin at the beginning of the study19 and 50 mg/kg Pycnogenol dissolved in 1 mL of saline for 9 days.[20]

Group IV (Pycnogenol group, n=7): Rats were administered orally 50 mg/kg Pycnogenol dissolved in 1 mL of saline for 9 days.[20]

Collection of Tissue and Serum Samples

The rats in all groups were anesthetized using xylazine (10 mg/kg in a %2 solution) and ketamine (75 mg/kg in a %10 solution) and decapitated at the end of the 24-hour study period. Next, we rapidly removed their hearts. Some of the serum samples were stored at -20 ºC till biochemical analysis, and the remaining heart tissues were stored in fixative (10% formaldehyde) for immunohistochemical examination.

Immunohistochemical Evaluation

We applied the avidin-biotin-peroxidase (ABC) complex with minor modifications upon an immunohistochemical staining method. Sections of 4-6 µm thickness were taken from the tissue samples blocked with this method and deparaffinized. We used the primary antibody PTX-3 (Rabbit monoclonal IgG antibody, sc-373951 Santa Cruz Biotechnology, Inc.) diluted 1/200 with the TermoScientific™ TP-015-HA commercial kit. After applying AEC Chromogen, we performed staining using Mayer's hematoxylin and examined it under a light microscope. The preparations were then examined, assessed, and photographed using the Leica DM500 microscope (LeicaDFC295). Finally, we generated a histoscoring based on the prevalence (0.1=<25%; 0.4=26-50%; 0.6=51-75%; 0.9=76-100%) and severity of immunoreactivity in staining (0=No staining; +0.5= Very faint staining; +1=Faint staining; +2=Moderate staining, +3=Intense staining) (i.e., histoscore=prevalence x severity).[21,22]

Histopathological Analysis

We embedded renal tissues fixed with 10% formaldehyde in paraffin blocks after routine tissue follow-ups. The preparations prepared with hematoxylin-eosin (H&E) and mason trichome-staining on 4-6 µm sections from the paraffin blocks were examined through the semi-quantitative assessment proposed by Kuloğlu et al.[23] and photographed under a light microscope (Leica DFC295).

Biochemical Analysis

MDA (Malondialdehyde) Measurement: We measured MDA levels by adding 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N HCl (1:1:1,w/v) onto 500 µL of homogenate, as proposed by Placer et al. We next heated the mixture in a water bath at 100°C for 30 minutes. It was then cooled to room temperature and centrifuged at 15,000 g for 15 minutes. Finally, we determined MDA levels by analyzing the supernatant samples using a spectrophotometer at 532 nm. MDA levels are expressed as nmol/g wet tissue weight.[24]

Statistical Analysis

Initially, we resorted to the Kolmogorov-Smirnov (K-S) test to explore if the data showed a normal distribution. Accordingly, the data were presented as means and standard deviations and analyzed using one-way analysis of variance (ANOVA). We performed the pairwise analysis of the data appearing as statistically significant in ANOVA with a post-hoc test (Tukey). All analyses were performed in SPSS 26.0, and a p-value of <0.05 was considered statistically significant.

RESULTS

Histological Findings

The examination of Masson's trichrome- and hematoxylin-eosin-stained preparations of all groups showed that the heart tissue of the control (**Figure 1a**, **Figure 2a**) and Pycnogenol groups (**Figure 1d**, **Figure 2**d) had a typical appearance. Compared to the control group, the gentamicin group (**Figure 1b**, **Figure 2b**) had mild congestion (black arrow), mononuclear cell increase (red star), and degeneration of heart muscle cells (red arrow). Although the gentamicin+Pycnogenol group had greater damage to the heart tissue (**Figure 1c**, **Figure 2c**) compared to the control group, the findings yielded that the severity of histopathological damage was reduced in this group compared to the gentamicin group.

Immunohistochemical Findings

We examined immunohistochemical staining for PTX-3 immunoreactivity under light microscopy. The findings revealed PTX-3 immunoreactivity in heart tissue (black arrow), and it was similar in the control (**Figure 3a**) and Pycnogenol (**Figure 3d**) groups. Compared to the control group, PTX-3 immunoreactivity was found to be significantly increased in the gentamicin group ($p < 0.001$; **Figure 3b**). Yet, PTX-3 immunoreactivity was decreased in the gentamicin+Pycnogenol group than in the gentamicin group (**Figure 3c**; **Table 1**; **Figure 4**).

Table 1. Group Differences of PTX3 Histoscores and MDA Levels

	Control[a]	Gentamicin[b]	Pycnogenol[a]	Gentamicin-pycnogenol[c]	p
PTX3	0.34±0.12	1.05±0.18	0.31±0.13	0.70±0.12	<0.001
MDA	9.28±0.97	16.6±2.57	8.95±0.79	11.5±1.20	<0.001

Variables are expressed as means (M) and standard deviations (SD); the data were analyzed with one-way ANOVA; the Tukey test was utilized for pairwise comparisons; PTX-3=Pentraxin-3, MDA=Malondialdehyde. *a, b, are c are different in pairwise comparisons.

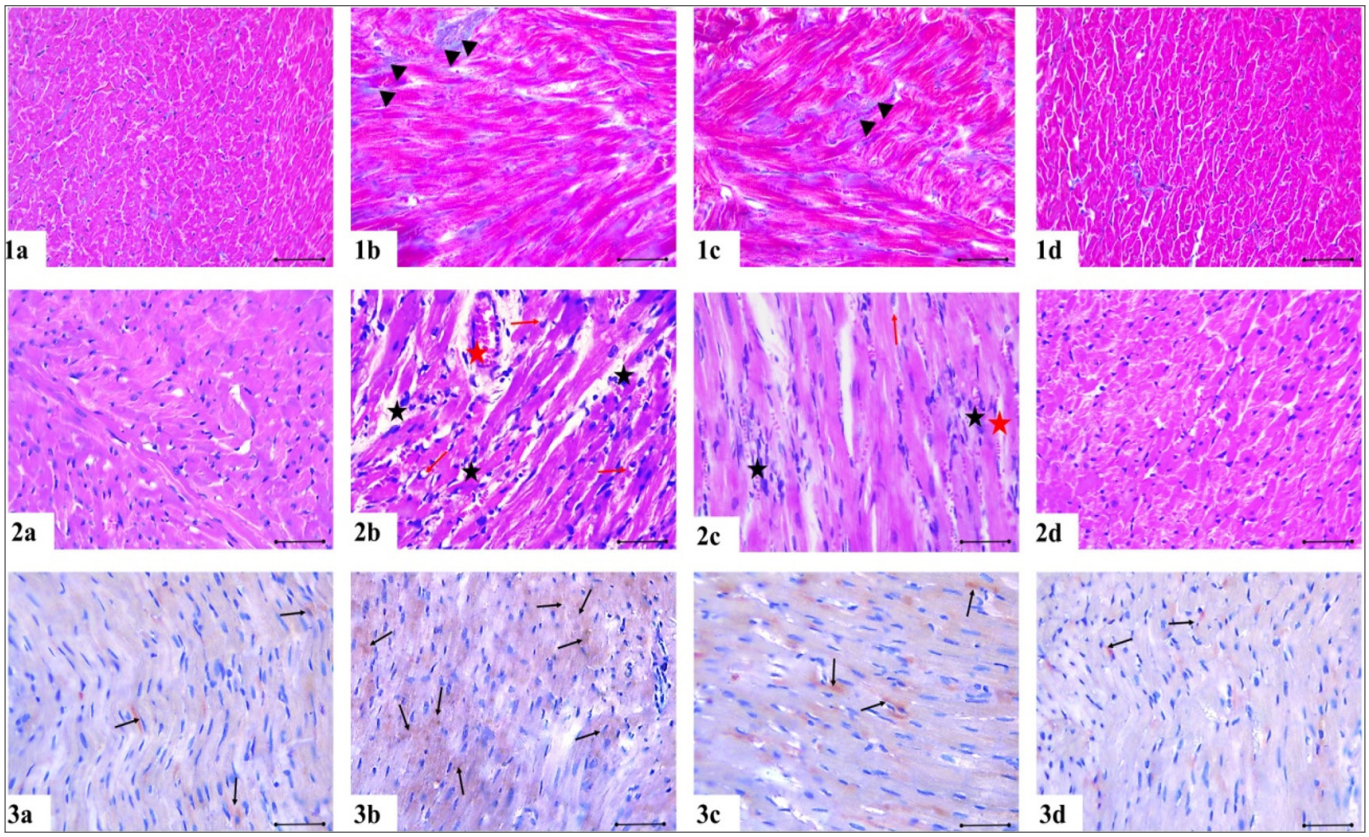

Figure 1. Cardiac tissue stained with Masson's trichome shows congestion (black arrow), mononuclear cell increase (red star), fibrosis (black star), and degeneration of cardiac muscle cells (red arrow); Scale bar=100 µm; a=control group, b= gentamicin group, c=gentamicin+Pycnogenol group, d =Pycnogenol group.
Figure 2. Heart tissue stained with hematoxylin-eosin shows mononuclear cell increase (red star) and degeneration of cardiac muscle cells (red arrow); Scale bar=100 µm; a=control group, b=gentamicin group, c=gentamicin+Pycnogenol group, d=Pycnogenol group.
Figure 3. PTX-3 immunoreactivity in heart tissue with immunohistochemical staining (black arrow); a =control group, b=gentamicin group, c=gentamicin+Pycnogenol group, d=Pycnogenol group; AEC chromogen; Mayer's hematoxylin; Scale bar=100 µm.

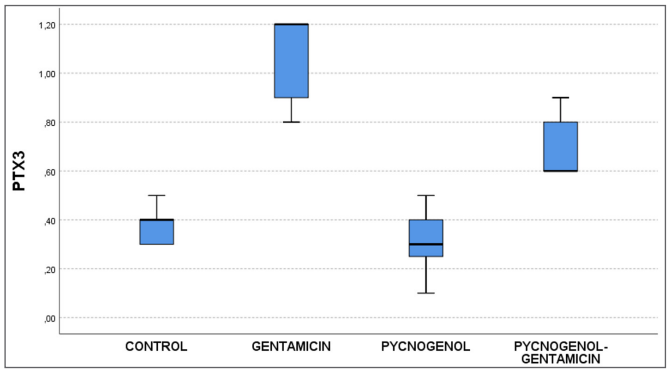

Figure 4. Box-plot of PTX3 levels of the groups (PTX-3: Pentraxin-3)

Biochemical Findings

MDA levels, a noteworthy parameter in demonstrating oxidative damage and an indicator of lipid peroxidation in tissue, were similar in the control and Pycnogenol groups. However, they were found to be significantly increased in the gentamicin group than in the control group ($p < 0.001$). We discovered that MDA levels were decreased in the gentamicin+Pycnogenol group compared to the gentamicin group (**Table 1**; **Figure 5**).

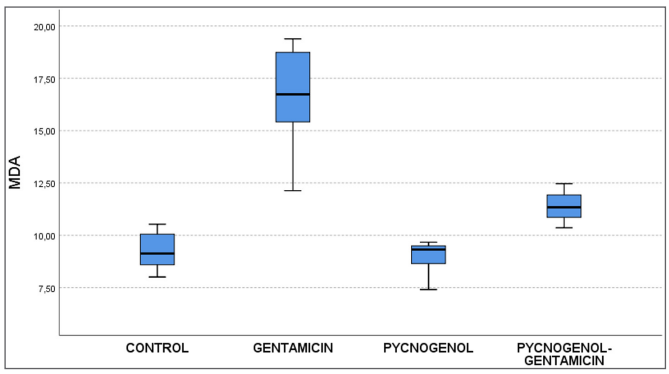

Figure 5. Box-plot of the spectrophotometrically-measured MDA levels of the groups (MDA: Malondialdehyde)

DISCUSSION

In the present study, we found that Pycnogenol, known for its protective properties, reduced PTX-3 and MDA levels in rats with gentamicin-induced cardiac damage. Therefore, we can now count cardiac protection among the protective properties of Pycnogenol.

Plant polyphenols act as natural antioxidants through a variety of processes, including free radical scavenging, metal chelation, and protein binding.[25] Among the natural polyphenols, Pycnogenol is an extract of a generic French pine bark (Pinus pinaster Aiton) containing polymeric (70%) and monomeric flavonoids (30%).[1] The high flavonoid concentration in Pycnogenol can be attributed to a wide range of antioxidant effects against both free oxygen radicals and nitrogen species.[20] In addition, Pycnogenol exhibits anti-inflammatory[26] and anticancer activities.[27,28] Orally administered Pycnogenol increases plasma antioxidant capacity, expressed as oxygen radical absorbance capacity,[29] and reduces plasma oxidative stress, measured as plasma free radicals.[30] Pycnogenol has further been shown to protect lipids from peroxidation by free radicals in the elderly and people with coronary artery disease.[31,32] Pycnogenol can improve endothelial function. It is thought that Pycnogenol exerts this effect by activating endothelial nitric oxide synthase (eNOS). In this way amplifying the nitric oxide generation from L-arginine, eventually leading to an increase in vessel lumen and adequate tissue perfusion. People with coronary artery disease, endothelial function was assessed by measuring the flow-mediated dilatation (FMD) of the brachial artery; 200 mg Pycnogenol per day was supplemented in a randomised, double-blind, placebo-controlled cross-over study for 8 weeks.[31] Several studies reported that the efficiency of Pycnogenol on blood vessels depends on the endothelium, as it could be abolished by administration of an endothelium-specific nitric oxide synthase inhibitor or by removing the endothelial lining.[33,34] Nishioka et al.[33] investigated the pharmacological effects of Pycnogenol on the endothelium-dependent vasodilation via nitric oxide production by measuring the forearm blood flow in response to acetylcholine (an endothelium-dependent vasodilator). They supported the beneficial effects of Pycnogenol on endothelial function with their study. Pycnogenol bears a protective effect against inflammatory diseases, hypertension, DM, and obesity.[2] Our findings were able to demonstrate the anti-inflammatory effect of Pycnogenol on cardiac cells, as it led to a decrease in PTX-3 levels in the treatment group; therefore, it should be noted that Pycnogenol may be adopted as a treatment option in case of cardiotoxicity.

PTX-3, identified as a cognate molecule of CRP, is a multifunctional protein with complex regulatory roles in inflammation, extracellular matrix organization, and remodeling.[10] In humans and rats, PTX-3 levels rise rapidly and dramatically in pathological conditions of inflammatory and/or infectious origin. The hallmark of PTX-3 may be that it rises more quickly than CRP (the peak within 6-8 hours for PTX-3 and within 24-48 hours for CRP), most likely due to local and systemic production of two proteins.[10] In addition, a previous study examined the heart tissues of patients who died from myocardial infarction and concluded specific immunostaining for PTX-3.[35] In our study, the high PTX-3 levels in the group administered gentamicin imply that we successfully generated cardiac damage in that group. In addition, reduced PTX-3 in the gentamicin+Pycnogenol group may be recognized as evidence that Pycnogenol alleviated cardiac damage.

MDA is the primary metabolite developing due to the oxidation and deterioration of cell lipids and is accepted as an index of lipid peroxidation.[36] Therefore, any tissue damage leads to an elevation in MDA levels. In our study, we determined that MDA levels were significantly higher in the groups receiving gentamicin compared to the others. On the other hand, reduced MDA levels in the treatment group may support the therapeutic effect of Pycnogenol.

Aminoglycosides (gentamicin and kanamycin), glycopeptide antibiotics (vancomycin), and quinolones (ciprofloxacin) are widely utilized in orthopedic surgery to prevent or treat associated infections.[37] Yet, gentamicin was previously reported to adversely affect cardiac function in various animal experiments.[7] The IV administration of aminoglycoside was also documented to cause acute hypotension and adverse inotropic effect on the heart.[8,9] It can initiate a pathological process that can lead to the weakening of hemodynamic parameters and even cardiovascular collapse. However, the mechanisms by which aminoglycoside antibiotics exert detrimental effects are largely speculative.[38] In their study, de la Chapelle-Groz and Athias[38] confirmed that gentamicin acts as a competitive inhibitor for the invasion of extracellular membrane calcium sites. This process may be the basis of early cardiac dysfunction following aminoglycoside therapy. However, penetration of gentamicin into cardiac cells may cause irreversible damage. Our study supported the previous findings that aminoglycosides cause cardiac damage by showing significant increases in PTX-3 and MDA levels in the gentamicin group. Our results also apparently underscored that gentamicin can be utilized as a cardiotoxic agent in other animal experiments and that it should be administered carefully in cardiac patients due to its cardiotoxicity. Moreover, we can assert that Pycnogenol can be considered a treatment option in clinical cases where cardiotoxicity is suspected (e.g., acute hypotension, cardiogenic shock).

Limitations

The present study is not free of a few limitations. This was an experimental study not carried out with humans. Moreover, we adopted only PTX-3 and MDA levels as indicators of cardiac damage, not other biochemical parameters (e.g., TAS, TOS, Caspaca-3, etc.).

CONCLUSION

We showed that Pycnogenol is an effective treatment method for agents causing cardiac toxicity. Furthermore, we can confidently propose that Pycnogenol should be counted in the treatment for increased proinflammatory parameters in patients treated with agents with well-known toxicity, such as gentamicin.

ETHICAL DECLARATIONS

Ethics Committee Approval: We initiated this study with the approval of the Fırat University animal Experimentation Ethics Committee (Date: 18.04.2023, Decision No: 07-03).

Informed Consent: Because the study was an animal experiment, no written informed consent form was obtained.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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