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Hepatoprotective effect of pycnogenol in gentamicin-induced liver injury in rats

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Abstract

Aim: The present study aimed to investigate the possible hepatoprotective effect pycnogenol (PYC) in gentamicin (GEN)-induced liver injury in rats.

Materials and Methods: The study consisted of four groups of seven rats; control, PYC (20 mg/kg for 8 days), GEN (80 mg/kg for 8 days) and GEN+PYC. At the end of the experiment, liver tissues of all groups were collected for histopathological and immunohistochemical (fibrillin-1, caspase-3, and betatrophin antibodies) evaluation.

Results: Histopathological analysis revealed that the GEN treated group had liver damage as a result of the GEN administration compared to the control group. Additionally, fibrillin-1, caspase-3, and betatrophin expression all increased in rat liver tissues. However, PYC pretreatment significantly reduced the expression of the aforementioned antibodies and mitigated liver damage.

Conclusion: The anti-apoptotic and antioxidant properties of PYC may help to preserve liver histoarchitecture against GEN-induced hepatotoxicity.

Introduction

Gentamicin (GEN), an antibiotic from the aminoglycoside family, is frequently used to treat infections caused by gram-negative bacteria [1]. Due to the progression of inflammation and oxidative stress, which causes hepatic fibrosis, hepatotoxicity is regarded as a major side effect of this drug [2]. Cell damage is brought on by GEN's increased activity of reactive oxygen species (ROS) metabolites, ATP depletion, and induction of apoptosis [3,4]. According to prior research, the liver damage caused by GEN has been successfully mitigated by the administration of antioxidant and anti-apoptotic drugs [1,4].

Pycnogenol (PYC), a strong antioxidant, is obtained from the bark of the French maritime pine (*Pinus maritima*). Polyphenolic monomers (taxifolin, epicatechin, and catechin), flavonoids, and phenolic acids (ferulic, caffeic, and gallic acid) constitute the chemical composition of PYC [5]. PYC, when taken as a dietary supplement, has been shown to protect body cells from damage caused by reactive oxygen and nitrogen species, as well as having anti-inflammatory, antioxidant, and a very potent free radical scavenging properties. This natural compound prevents hepatotoxicity, nephrotoxicity, diabetes, hypertension, inflammatory diseases, and cardiovascular disease [5-7].

Fibrillin-1 is a large glycoprotein which assembles to form microfibrils in the extracellular matrix (ECM) of connective tissue. The liver, as well as the kidney, lung, eye, skin, vasculature, and tendons, have all been found to have this glycoprotein [8]. In the connective tissue of the portal region of the liver and vessel walls, fibrillin-1 coexists with elastin [9]. It coordinates between hepatic stellate cells and hepatocytes by providing a proper extracellular microenvironment and a network [10].

Betatrophin, also known as lipasin, angiopoietin-like protein 8, re-feeding induced fat and liver protein, and hepatocellular carcinoma-associated protein, is a novel protein that is mostly produced from hepatic and adipose tissue [11,12]. Betatrophin has a significant part in the modulation of lipid and glucose metabolism as well as a number of other functions in non-alcoholic fatty liver disease, re-

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nal dysfunction, metabolic disorders, adriamycin-induced cardiomyopathy, and polycystic ovarian syndrome (PCOS) [13].

The present study designed to examine the effects of PYC on fibrillin-1, apoptosis and betatrophin through histological and immunohistochemical evaluation in an experimental model of GEN-induced liver injury.

Materials and Methods

Animals and experimental design

The current experimental study used 28 Wistar albino male rats that weighed 250–300 g. The rats were kept in cages at a controlled laboratory temperature of 22 ± 3 °C with a 12-hour light/dark cycle, with access to unlimited standard rodent food and water. The Institutional Research Ethics Board of Experimental Animals of Kahramanmaras Sutcu Imam University permitted the experimental protocols (Approval number: 2022/11-07).

After the stabilization period, the rats were divided randomly into 4 equal-sized groups (7 rats per group). The experiment was as follows:

- Control group; did not receive any solvent or drug for 8 days.
- PYC group; PYC was administered orally to animals at a dose of 20 mg/kg for 8 days [6].
- GEN group; received intraperitoneal (ip) injection GEN (Genta, Ulagay, Turkey) at a dose of 80 mg/kg for 8 days for induction of hepatotoxicity [2].
- GEN + PYC group; treated with PYC (20 mg/kg, orally) 1 h before GEN (80 mg/kg, ip) for 8 days.

The study was terminated by sacrificing the rats under anesthesia using 10% ketamine (Alfamine; Alfasan IBV, Woerden, The Netherlands) and 2% xylazine (Alfazine; Alfasan IBV, Woerden, The Netherlands) 24 h after the last injection. The liver tissues were collected in fixative solution for histopathologic and immunohistochemical analysis.

Histopathological analysis

To prepare the liver tissues for light microscopy, the samples were fixed with a 10% formalin buffered solution, embedded in paraffin, and then 5-µm thick sections were cut using a Leica 2125RT rotary microtome. Hematoxylin and eosin (H&E) staining was applied to the deparaffinized sections, and a blind histologist analyzed the histopathological findings under a Carl Zeiss Axio Imager A2 microscope (Zeiss Instruments Inc., Germany). The leukocyte infiltration, cellular degeneration, sinusoidal dilatation, and vascular congestion were evaluated in ten fields for each slide as follows: grade 0 = no damage, grade 1 = 1–20%, grade 2 = 21–40%, grade 3 = 41–60%, grade 4 = 61–80%, and grade 5 = 81–100% [14].

Immunohistochemical analysis

The liver sections taken on polylysine-coated slides were immunostained with anti-FBN1/fibrillin (1:200, bs-1157R, Bioss), anti-caspase-3 (1:200, ab184787, Abcam), and betatrophin (1:200, PA5-38043, Invitrogen) primary antibodies using the avidin-biotin-peroxidase complex (ABC) and analyzed using a Carl Zeiss Axio Imager A2 microscope in accordance with the published literature [15]. The histoscore was calculated using the following rating scale to represent the occurrence of fibrillin-1, caspase-3, and betatrophin immunoreactivity in liver tissue: prevalence of immunoreactivity (0.1: < 25%, 0.4: 26—50%, 0.6: 51—75%; 0.9: 76—100%), and intensity of immunoreactivity were as follows 0: unstained, +0.5: very low, +1: low, +2: moderate, +3: severe. The histoscore was calculated as follows: prevalence x intensity [15].

Statistical analysis

Data were analyzed using the SPSS software package, version 25.0 for Windows (SPSS Inc., Chicago, IL, USA). Power calculations for testing the sample size were performed using the PASS software package program (NCSS, LLC, UT, version 11.0 for Windows; desired study power, 80%; α error = 0.05, two-tailed). As a result, the minimum required sample size was calculated as 8 animals each group. Shapiro-Wilk test was used to determine whether the data was distributed normally. One-way analysis of variance (ANOVA) was carried out to compare fibrillin-1, caspase-3, and betatrophin findings of the groups and followed by post hoc analysis with the Tukey's test. Moreover, Kruskal-Wallis and Mann-Whitney nonparametric tests were used to evaluate the total histopathological score. Mean differences were considered significant at $p<0.05$. The data were reported as mean±SD.

Results

Histopathological analysis

The liver sections of the control (Figure 1a) and PYC (Figure 1b) groups exhibited normal hepatic histoarchitecture. In comparison to control group, derangement of hepatic

Table 1. The effect of PYC on histopathological damages induced by GEN.

Histopathologic damages	Experimental groups			
	Control	PYC	GEN	GEN + PYC
Leukocytes infiltration (Mean)	0	0	2.9	0.4
Cellular degeneration (Mean)	0	0	3.1	1.5
Sinusoidal dilatation (Mean)	0.3	0.5	2.6	1.3
Vascular congestion (Mean)	0.4	0.3	3.6	1.4
Total histopathologic score (Mean)	0.7	0.8	12.2*†	4.6*†#

*p < 0.01, in comparison with control group, †p < 0.01, in comparison with PYC group, and #p < 0.001, in comparison with GEN group.

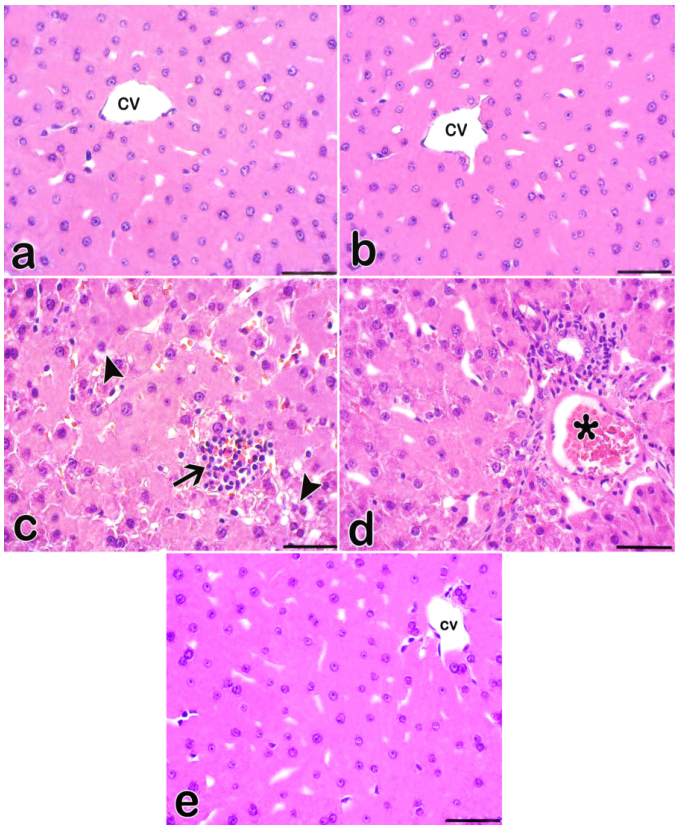

Figure 1. Representative liver sections photographs of groups stained with H&E (400x). (a) control group, (b) PYC (20 mg/kg), (c,d) GEN (80 mg/kg); inflammatory cell infiltration (arrows), degenerative changes in the cytoplasm of the hepatocytes (arrowheads), congested blood vessel (asterisk), and (e) GEN + PYC (80 mg/kg+20 mg/kg). CV; central lobular vein.

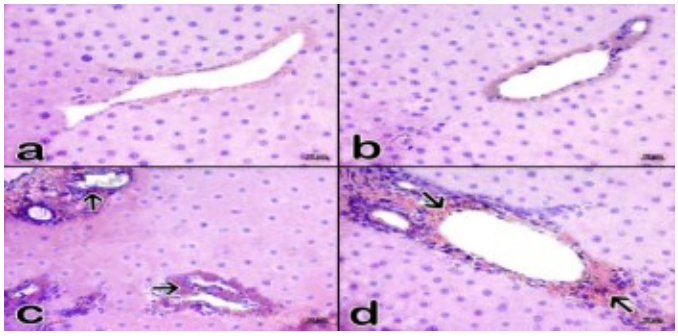

Figure 2. Immunohistochemical staining photomicrographs with fibrillin-1 of rat liver tissue (400x). (a) Control group, (b) PYC group, (c) GEN group; intense fibrillin-1 expression (arrows), and (e) GEN + PYC group; mild fibrillin-1 expression (arrows).

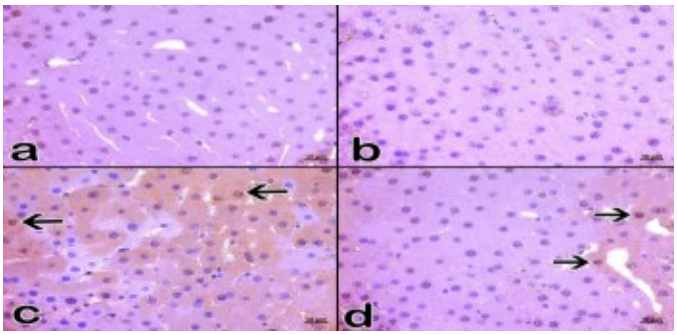

Figure 3. Immunohistochemical staining photomicrographs with caspase-3 of rat liver tissue (400x). (a) Control group, (b) PYC group, (c) GEN group; intense caspase-3 expression (arrows), and (e) GEN + PYC group; mild caspase-3 expression (arrows).

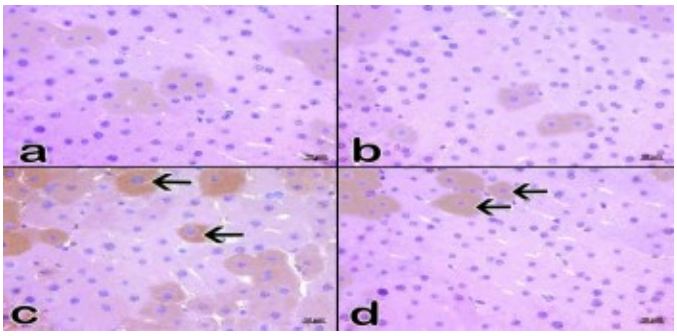

Figure 4. Immunohistochemical staining photomicrographs with betatrophin of rat liver tissue (400x). (a) Control group, (b) PYC group, (c) GEN group; intense betatrophin expression (arrows), and (e) GEN + PYC group; mild betatrophin expression (arrows).

Table 2. Histoscores of fibrillin-1, caspase-3 and betatrophin immunoreactivities of the experimental groups.

Groups	Mean±SD Fibrillin-1	Mean±SD Caspase-3	Mean±SD Betatrophin
Control	0.267±0.332	0.303±0.454	0.226±0.454
PYC	0.245±0.372	0.285±0.714	0.190±0.414
GEN	1.230±0.729 ^{a,b}	1.113±0.422 ^{a,b}	0.985±0.734 ^{a,b}
GEN + PYC	0.865±0.631^{a,b,c}	0.665±0.631 ^{a,b,c}	0.798±0.130 ^{a,b,c}

Data are expressed as mean±SD.
$^{a\ b\ c}$ The means in the same row with different superscripts differ significantly ($p < 0.05$, Tukey's test) *:One way ANOVA. ^{a} $p<0.001$ compared to control group, ^{b} $p<0.001$ compared to PYC group, ^{c} $p<0.05$ compared to GEN group.

cords, granular changes and vacuolar degeneration in the cytoplasm of the hepatocytes, inflammatory cell infiltration around hepatocytes and in the portal area, congested portal blood vessels were observed in the GEN-treated group (Figure 1c, d). The degree of leukocyte infiltration, cellular degeneration, sinusoidal dilatation, and vascular congestion in the GEN group, on the other hand, were significantly higher than in the control group ($p < 0.05$). Pre-treatment with PYC reversed most of the pathological changes in the GEN + PYC group when compared to the GEN group (Figure 1e), (Table 1).

Fibrillin-1, caspase-3 and betatrophin expression

The control and PYC groups showed nonsignificant difference in group comparisons involving fibrillin-1 (Figure 2a and 2b), caspase-3 (Figure 3a and 3b), and betatrophin (Figure 4a and 4b) expression, respectively ($p > 0.05$). Expression of fibrilin-1 (Figure 2c), caspase-3 (Figure 3c), and betatrophin (Figure 4c) increased significantly in the GEN-treated group as compared to the control group ($p<0.05$). When PYC was combined with GEN, the expression of these antibodies was significantly reduced in comparison to the GEN-treated group ($p<0.05$), (Figure 2d,3d and 4d), (Table 2).

Discussion

The application of most commonly used aminoglycoside antibiotics such as GEN has remained the mainstay treatment of serious infections [4,16]. Hepatotoxicity and nephrotoxicity are two main side effects of GEN that can be brought on by increased oxidative stress, the depletion of antioxidant defenses, triggered apoptosis, and inflammation [1, 2, 4]. Hepatic necrosis, apoptosis, inflammation, and free radical generation have all been identified as potential pathogenic pathways of GEN-induced hepatotoxicity [2, 17]. The histopathological findings of the present study revealed that the typical architecture of the hepatocytes was disturbed, and inflammatory cell infiltration appeared in the GEN-treated group which is consistent with earlier research in rats [16,17]. The hepatic damage that we determined in the liver tissue may be the result of reduced antioxidant activity as well as excessive ROS and free radical generation [4,18].

The hepatoprotective effects of PYC have been reported in hepatotoxicity induced by acetaminophen [19], cisplatin [20], carbon tetrachloride (CCl_4) [21] and methotrexate [6]. PYC attenuated oxidative hepatic damage in rat liver tissue [20, 21] and inhibited pro-inflammatory mediators (tumour necrosis factor-α, interleukin 1β, interleukin 6, intercellular adhesion molecule-1 and perilipin 2) in murine BV2 microglial cells [22]. PYC administration could prevent lipid accumulation in the mouse liver nonparenchymal cell line [23]. Pre-treatment with PYC reversed GEN-induced hepatic damage in this study, supporting the prior results that PYC has antioxidant properties [6, 19, 20, 21].

Myofibroblasts and subsequently fibroblasts are activated in liver injury, resulting in ECM deposition. Hepatic stellate cells, which are located in the perisinusoidal area, become activated as a result of the disturbed microenvironment, which in turn causes an excessive ECM accumulation. Liver fibrosis and inflammation may potentially begin to develop as a result of an imbalance in ECM synthesis-degradation turnover [24]. Abnormal fibrillin expression is accompanied by oxidative stress and increased apoptosis in the tight-skin mouse due to abnormalities in ECM structure and function [25]. Previous research revealed that myofibroblasts, portal fibroblasts, and hepatic stellate cells all express fibrillin-1. Fibrillin-1 is involved in the pathogenesis of rat liver fibrosis [9] and has been found to be highly localized in the fibrous septa that surround the cirrhotic nodules in cirrhosis [26]. Children with cholestatic disorders exhibited increased fibrillin-1 expression in fibrous septa, portal tracts, and ductular proliferative regions [27]. Additionally, after bile duct ligation (in the connective tissue of enlarged portal zones) or CCl_4 administration (around centrilobular vein), the expression was found to be elevated [9]. In line with earlier studies [9, 26, 27], the current research found that GEN significantly increased fibrillin-1 expression in the liver tissue of the GEN-treated group as compared to the control group. The elevated expression of fibrillin-1 in portal area may be a sign of oxidative stress induced by GEN. However, compared to the GEN group, PYC therapy significantly reduced fibrillin-1 immunoreactivity with its anti-oxidative [19-21] potential.

GEN has been shown to activate apoptotic pathways in hepatic tissue via excessive free radical generation [4,17]. According to the immunohistochemical analysis of the present study, the GEN group exhibited significantly increased caspase-3 expression, which was in line with earlier results in rats [1,16]. The cytotoxic potential of GEN on the rat hepatic tissue was confirmed by histological analysis. Comparing the GEN+PYC group to the GEN group, however, revealed a substantial decrease in caspase-3 immunoreactivity. The strong anti-apoptotic properties [6,28] of PYC may be responsible for this result.

More research on the prevention of diseases appears to be focusing on betatrophin [29]. According to a study, betatrophin helps to control inflammation in both mice and humans [30]. Betatrophin plays a critical role in ECM degradation and inflammation [31]. Previous research has found that betatrophin levels are elevated in non-alcoholic fatty liver disease, cirrhosis, type 2 diabetes, PCOS, and gestational diabetes [32-35]. A previous study revealed that betatrophin levels were increased in impaired glycometabolism [36]. In this study, increased betatrophin expression in the liver tissue of the GEN group was significantly higher than that of the control group. However, PYC significantly reduced betatrophin expression. The self-defense of liver in response to inflammation and oxidative stress may be consider the reason for the increase in expression. Inflammation and oxidative stress may result in the impaired glycometabolism because one of the important functions of betatrophin, is to control glucose and lipid metabolism [36].

Conclusion

To our knowledge, this is the first study to reveal that PYC pre-treatment protected the hepatoarchitecture of rats from GEN-induced damage due to its antioxidant and anti-apoptotic effects. Therefore, we propose that PYC could be taken as a dietary supplement in conjunction with GEN treatment to mitigate its side effects.

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Ethical approval

Local Ethics Committee of Experimental Animals (Kahramanmaras Sutcu Imam University) approved this experimental research (Approval number: 2022/11-07, date: November 22, 2022).

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