

Prophylactic and Therapeutic Effects of Pycnogenol Against Doxorubicin-Induced Cardiomyopathy in Rat

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Abstract

Doxorubicin (Dox) is the most potent antitumor of wide spectrum. It is used only by developing cardiomyopathy threatening life. Cardiomyopathy is a disease of the heart muscle, categorized by abnormal wall thickness, chamber size and other functional contractile abnormalities. Pycnogenol (PYC) is an aqueous extract of the pine bark known for its content of bioactive flavonoids. The study aimed to examine the cardio-protective and therapeutic efficacy of Pycnogenol on doxorubicin brought cardiomyopathy. Fifty rats were divided into five equal groups as follow: **G1** Control, **G2** Cardiomyopathy group; doxorubicin dissolved in saline solution; 10 mg/kg; single i.p. injection. **G3**: healthy rats administered Pycnogenol treated with aqueous extract of pycnogenol (150mg /kg (b.w)/day) orally for five weeks, **G4**: Prophylactic group; rats received aqueous extract of Pycnogenol (150mg /kg (b.w)/day) orally, then received single injection of DOX (10 mg/kg b.w.i.p); **G5**: Cardio-therapeutic group; rats received single injection of DOX (10 mg/kg.i.p.) then, treated with aqueous extract of pycnogenol (150mg /kg (b.w)/day) orally. Blood samples were collected for biochemical measurements, hearts were excised for measurement of cardiac tissue oxidative stress and histological examination. Current evidence revealed that, DOX induced cardiomyopathy that confirmed in several aspects such as increasing TC, TG, and LDL-c with a reduction in HDL-c as compared to control group, it also decreased levels of oxidative stress parameters in cardiac tissues GSH and TAC and elevated NO and MDA levels. In the same way, DOX disturbs myocardial marker levels (CTnt, Acl, Ac.P, CRP, LDH). Pycnogenol treatment either as prophylactic or therapeutic improved lipid profile, oxidative stress markers and decreased myocardial marker injury. Furthermore, histological findings confirmed the biochemical aspects. Protection was more apparent in therapeutic group rather than in prophylactic group. Results

affirmed that Pycnogenol aqueous extract protected against DOX induced cardiomyopathy in experimental rats.

Keywords: Pycnogenol- Doxorubicin- Cardiomyopathy.

Introduction

Cardiomyopathies are heart muscle diseases, which are characterized in the abnormal size of the chamber and wall thickness, and systolic or diastolic dysfunction in the absence of coronary artery disease and hypertension (Elliott et al. 2008). Cardiomyopathies classification were primary or secondary. Primary cardiomyopathies include heart muscle, that caused by genetic and non-genetic causes. Secondary cardiomyopathies were syndromes characterized by myocardial damage (Maron et al., 2006). This disease can be identified as documented cardiovascular disease (Towbin et al., 2006). Dilated cardiomyopathy is related to heart failure and sudden cardiac death, thus increases cost burden due to the elevated frequency of hospital charges and possible necessity for heart transplantation.

Doxorubicin (DOX) is an anthracycline antibiotic used in the treatment of various diseases e.g. leukaemia, carcinomas, lymphoma and sarcomas (Towbin et al., 2006). The occurrence of chronic cardiomyopathy was estimated to be 1.7% and is lower than acute toxicity (Kanu et al., 2010). Doxorubicin- induced cardiotoxicity is characterized by different symptoms including: hypotension, transient arrhythmias, tachycardia, lower limb edema and fatigue, dyspnea (Lipshultz et al., 2008).

Pycnogenol (PYC) is an aqueous extract of the bark of *Pinus pinaster* (formerly known as *Pinus maritima*). It consists of flavonoids mixture as procyanidins, cinnamic acids, phenolic acids, and taxifolin (Rohdewald, 2002). Administration of pine bark extract may improve microcirculation and reduce platelet aggregation (Gabriele, 2010). The study aimed to examine the cardio-protective and therapeutic efficacy of Pycnogenol on doxorubicin brought cardiomyopathy.

Materials and Methods

Materials:

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Pycnogenol was purchased from Nature Way company, USA, as capsules. Every capsule contains 50 mg of pure powdered pycnogenol.

Chemicals

Kits of Total cholesterol (TC), Triglycerides (TG), High density lipoprotein-cholesterol (HDL), Low density lipoprotein-cholesterol (LDL) were purchased from Crescent Diagnostic Co. (Jeddah, Saudi Arabia). Kits of reduced glutathione (GSH), Nitric oxide (NO), lipid peroxides as Malondialdehydes (MDA) and Total antioxidant capacity were obtained from Bio-Diagnostic (Cairo, Egypt). Kits of Anti-cardiolipin (IgM), Acid Phosphatase, C-reactive protein (CRP) and Lactate dehydrogenase (LDH) were purchased from BioVision Company (Milpitas, USA). Troponin - T kits were purchased from INNOVABIO (Salt Lake City, USA). Doxorubicin was purchased from Sigma Chemical Co., St. Louis MO, USA.

Preparation of Pycnogenol:

Pycnogenol extract dissolved in distal water and administered at dose of 150 mg/kg, body weight/ day.

Experimental Animals

Fifty adult male albino rats weighing 120-130 g were used. Rats were supplied from Animal House Colony of King Fahd Medical Research Center. Animals were left to acclimatize for 1 week before the experiments then kept at standard housing facilities at King Fahd Medical Research Center Animal Facility Breeding Colony and provided with standard laboratory chow and water *ad libitum*. The experiment was approved by the Ethical Committee of King Fahd Medical Research Center. Jeddah, KSA. Approval number (163-19).

Experimental Design

Fifty rats were randomly divided into 5 groups, 10 / each:

Group 1 (Control group): fed on basic diet through the experimental period.

Group 2 (Cardiomyopathy group): Rats received intraperitoneal single injection of DOX at dose of 10 mg/kg body weight dissolved in saline solution, this dose is well proved to produce cardiotoxic effects (Kumaryl et al., 2015).

Group 3 (Pycnogenol group): Rats received an aqueous extract of pycnogenol at dose of 150 mg/kg body weight /day orally for five weeks.

Group 4 (Prophylactic group): Rats received aqueous extract of pycnogenol at dose of 150mg /kg b. w /day orally for five weeks, then received intraperitoneal single injection of DOX at dose of 10 mg/kg. b.w dissolved in saline solution.

Group 5 (Cardio-therapeutic group): Rats received intraperitoneal single injection of DOX at dose of 10 mg/kg. b.w dissolved in saline solution then, treated with aqueous extract of Pycnogenol at dose (150mg /kg body weight/day) orally for five weeks according to Aydin et al., (2011).

Sample Collection

At the end of the experimental period, rats were sacrificed under ether anesthesia, then blood was collected through retro-orbital puncture. Blood samples was left for 30 min. then centrifuged at 5000 r.p.m for 20 minutes, to separate serum, and divided into several aliquots and stored at -20 °C until analysis was performed. Rat hearts were directly removed, rinsed with ice cold saline and blotted dry.

Histological Examination of cardiac tissue

Myocardial tissue from all the groups was exposed to histopathological studies, by fixing in 10% formalin solution, then sections were prepared using paraffin blocks and stained with hematoxylin and eosin after dewaxing.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Science (SPSS, version 25). Significance was made using one Way Analysis of variance test ANOVA.

Results

Doxorubicin administration induced cardiomyopathy displayed significant increment in biomarkers of serum lipid profile levels. DOX administration increased TC, TG, and LDL-c and significantly decreased HDL-c compared to G1. On the other hand, administration of Pycnogenol either as prophylactic (G4) or therapeutic (G5) improved these parameters and increase the HDL-C level significantly as compared to cardiomyopathy group (G2). Comparing results of TC, HDL-C and LDL-C between control group G1 and G3 (normal healthy rats supplemented with Pycnogenol) showed no significant ($p < 0.05$) difference between these groups. Moreover, a significant ($p < 0.05$) reduction in TG level was noticed in G3 group as compared to G1. Additionally, comparing prophylactic (G4) or therapeutic (G5) groups detect that the improvement was more apparent in G5 which reflects the powerful therapeutic role of Pycnogenol rather than its prophylactic one (Table 1).

Table 1: the effect of supplementing Pycnogenol as a prophylactic or therapeutic agent in serum lipid profiles parameters.

Parameters \ Groups	Healthy Control group	Cardiomyopathy group	Pycnogenol group	Prophylactic group	Cardio-therapeutic group
Total cholesterol (TC) (mg/dl)	43.05±0.50 ^a	53.14±0.48 ^b	40.90±0.58 ^a	46.16±0.22 ^c	42.99±0.38 ^a
Triglyceride (TG) (mg/dl)	32.11±0.52 ^a	63.80±0.78 ^b	26.93±0.38 ^c	41.85±0.19 ^d	35.47±0.61 ^e
High density lipoprotein cholesterol (HDL-C) (mg/dl)	25.83±0.07 ^a	18.73±0.07 ^b	26.09±0.16 ^a	20.41±0.10 ^c	24.11±0.05 ^a
Very low density lipoprotein cholesterol (VLDL-C) (mg/dl)	6.42±0.11 ^a	12.76±0.31 ^b	5.34±0.23 ^c	8.37±0.08 ^d	7.09±0.08 ^e
Low density lipoprotein cholesterol (LDL-C) (mg/dl)	10.72±0.44 ^a	22.94±0.56 ^b	9.62±0.37 ^a	17.90±0.18 ^c	14.55±0.61 ^d

Data are expressed as mean±/ standard error. Means that have similar letters are not significant at (p≤0.05).

Table (2) showed the result of different treatments on serum GSH, NO, MDA, and TAC receptively. Rats received DOX demonstrated a significant (p < 0.05) decrease in GSH and TAC levels as compared to G1. Likewise, the cardiac tissue levels of NO and MDA were higher (p < 0.05) in the DOX Cardiomyopathy group G2 in comparison with G1, therefore reflecting increased oxidative stress following DOX administration. While results revealed that, Pycnogenol treatment either prophylactic (G4) or therapeutic (G5) attenuated the effect

of DOX by reducing NO and MDA levels significantly (p < 0.05) with a concomitant significant (p < 0.05) increase in GSH and TAC levels. The supplementation of Pycnogenol did not alter GSH and NO levels when compared to G1 group, whereas it induced a slight reduction in MDA and TAC levels compared to G1. On the same way, a comparison was made among prophylactic (G4) and therapeutic (G5) groups and comprised that the antioxidant effect was more apparent when Pycnogenol administered as a therapeutic agent (G5).

Table 2: Comparison of oxidative stress markers in different studied groups.

Parameters \ Groups	Healthy Control (G1)	Cardiomyopathy group (G2)	Pycnogenol control group (G3)	Prophylactic group (G4)	Cardio-therapeutic group (G5)
Reduced glutathione (GSH) (mg/g tissue)	342.11±1.51 ^a	270.88±6.04 ^b	340.97±8.06 ^a	305.09±11.25 ^c	322.27±10.54 ^d
Nitric oxide (NO) (μmol/L)	1.64±0.07 ^a	0.64±0.04 ^b	1.62±0.04 ^a	0.9±0.06 ^c	1.24±0.04 ^d
Malondialdehyde (MDA) (nmol/ml)	7.49±0.09 ^a	16.27±0.83 ^b	6.48±0.10 ^c	12.57±0.19 ^d	9.51±0.06 ^e
Total antioxidant capacity (TAC) (mMol/L)	1.56±0.003 ^a	0.57±0.007 ^b	1.4±0.005 ^c	0.94±0.005 ^d	1.2±0.30 ^e

Data are expressed as mean±/ standard error. Means that have similar letters are not significant at (p≤0.05).

Table (3) presented results of different treatments on serum cardiac markers Troponin T (cTn-T), Anti-cardiolipin (Acl), acid phosphatase (AP), C-reactive protein (CRP), and lactate dehydrogenase (LDH) respectively. DOX administration markedly increased serum cardiac marker enzymes significantly

(p < 0.05) indicating cardiac damage. It was observed that administering Pycnogenol as therapeutic agent could restore anti-cardiolipin and CRP levels and brought them to normal levels. On the other hand, supplementing Pycnogenol alone (G3) did not alter or induce any effect on cardiac serum enzymes markers.

Table 3: Comparison of cardiac destructive markers in different studied groups.

Parameters \ Groups	Healthy Control (G1)	Cardiomyopathy group (G2)	Pycnogenol control group (G3)	Prophylactic group (G4)	Cardio-therapeutic group (G5)
Troponin T (pg/ml) (cTn-T)	12.47±0.55 ^a	60.68±0.33 ^b	13.33±0.28 ^a	45.19±0.26 ^c	28.55±0.12 ^d
Anti-cardiolipin (Acl) (IgM) (U/ml)	1.26±0.09 ^a	3.39±0.13 ^b	1.30±0.12 ^a	1.55±0.05 ^c	1.37±0.09 ^a
Acidphosphatase (U/L) (AP)	7.36±0.33 ^a	11.66±0.11 ^a	6.97±0.17 ^a	8.96±0.22 ^c	7.54±0.18 ^a
C- reactive protein (CRP) (mg/dl)	3.38±0.18 ^a	5.63±0.18 ^b	3.38±0.18 ^a	4.21±0.16 ^c	3.00±0.18 ^d
Lactate dehydrogenase (LDH) (U/L)	130.16±5.48 ^a	211.53±19.45 ^b	134.21±9.49 ^a	180.67±0.25 ^c	155.29±0.76 ^d

Data are expressed as mean±/ standard error. Means that have similar letters are not significant at (p≤0.05).

Histopathological findings of cardiac tissues:

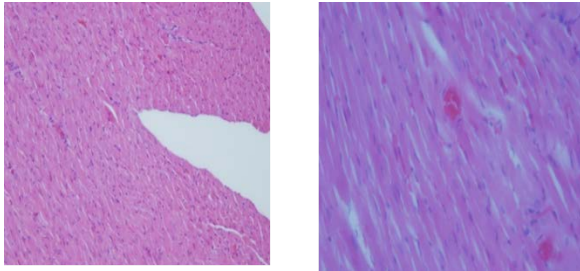


Figure 1. Represents **Group 1** of Rats with **Normal Hearts as control group**. All figures showed longitudinal orientation of myocardial muscles with different magnifications as shown at the right lower corner of each micrograph. The figures showed myocardial cells with prominent nuclei and blood vessels. Spiral shaped fibroblasts of connective tissues surrounding the myocardial smooth muscles are also clearly seen.

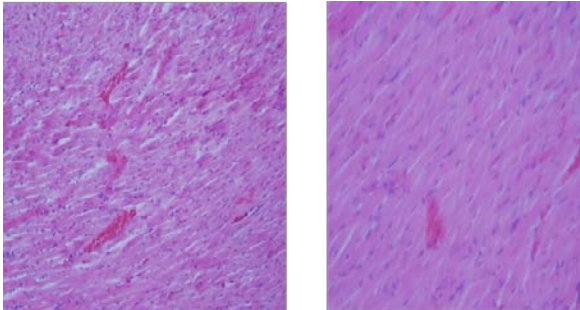


Figure 2. Represents **Group 2** of Rats with **Cardiomyopathy**. All figures showed longitudinal orientation of myocardial muscles with different magnifications as shown at the right lower corner of each micrograph. The figures showed myocardial cells with prominent nuclei and profuse bleeding. Spiral shaped fibroblasts of connective tissues surrounding the myocardial smooth muscles are also clearly seen.

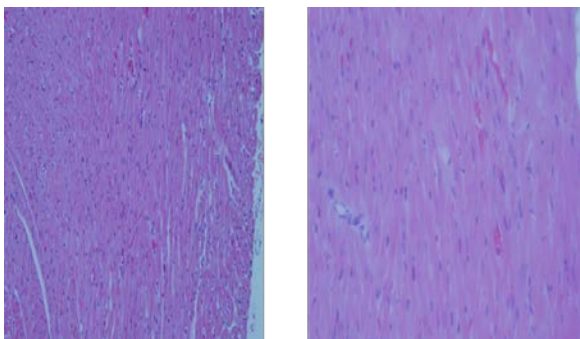


Figure 3. Represents **Group 3** of Rats with **Pycnogenol Control group**. All figures showed normal appearance with longitudinal orientation of myocardial muscles with different magnifications as shown at the right lower corner of each micrograph. The figures showed myocardial cells with prominent nuclei and blood vessels. Spiral shaped fibroblasts of connective tissues surrounding the myocardial smooth muscles are also clearly seen.

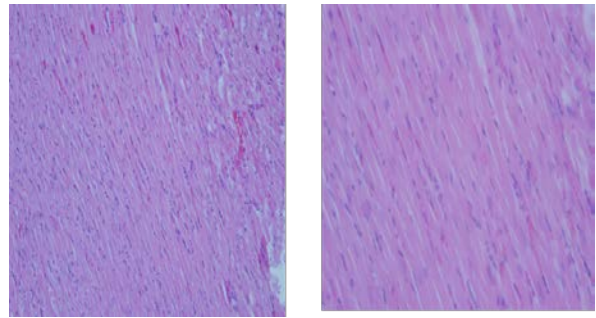


Figure 4. Represents **Group 4** of Rats as **Prophylactic group**. All figures showed longitudinal orientation of myocardial muscles with different magnifications as shown at the right lower corner of each micrograph. The figures showed myocardial cells with prominent nuclei and blood vessels.

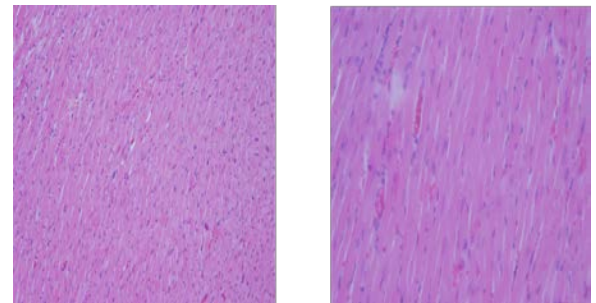


Figure 5. Represents **Group 5** of Rats as **Cardio-therapeutic group**. All figures showed longitudinal orientation of myocardial muscles with different magnifications as shown at the right lower corner of each micrograph. The figures showed myocardial cells with prominent nuclei and blood vessels. Spiral shaped fibroblasts of connective tissues surrounding the myocardial smooth muscles are also clearly seen.

Discussion

In this study, cardiomyopathy was induced by DOX injection. Doxorubicin induced cardiomyopathy has been established in numerous investigational models. A prominent function of ROS including hydroxyl radical was seen in doxorubicin induced cardiotoxicity (Warpe *et al.*, 2015). Numerous studies have stated that acute and chronic administration of doxorubicin caused a dose-dependent myopathy, that causes heart failure due to overload of calcium ions in myocytes (Raj *et al.*, 2014).

The pathways of Doxorubicin toxicity include oxidative stress, mitochondrial dysfunction and apoptosis (Hosseini *et al.*, 2017). Lately, it has been suggested that dysregulation of also contributes in DOX cardiotoxicity (Kawaguchi *et al.*, 2012).

DOX administration encouraged cardiomyopathy displayed significant increment in biomarkers of serum lipid profile levels. It is recognized that DOX treatment has toxic properties on the lipid profile (Hamza and Mahmoud, 2013). It also induces toxic effects on heart function and contribute to DOX-induced cardiomyopathy (Koti *et al.*, 2009).

Administration of Pycnogenol either as prophylactic (G4) or therapeutic (G5) improved these parameters and increase the HDL-C level significantly as compared to cardiomyopathy group (G2). The useful effect of Pycnogenol treatment on cardiovascular system and its defensive characteristics against atherogenesis and thrombus formation was previously detected (Koti et al., 2009). Pycnogenol enhanced blood lipid profile, reducing plasma concentrations of LDL-cholesterol and lipid peroxidation, and elevates HDL-cholesterol (Gulati, 2015). Moreover, due to its anti-inflammatory action, Pycnogenol has been proved to reduce development of atherosclerotic plaques by the inhibiting the appearance of proinflammatory cytokines or adhesion molecules involved in atherogenic process (Rezzani et al., 2010).

Results agreed with Koch (2002) study in which, Pycnogenol administration in obese patients, lowered LDL level with simultaneous increase of HDL this reduced risk reduction of pathological changes in patient's vascular system. Furthermore, agreed with Aydin et al (2019) study which represents that Pycnogenol supplementation attenuated lipid profile parameters in diabetic rats.

Moreover, Pycnogenol lowered LDL cholesterol levels and elevated HDL-cholesterol levels in the blood (Devaraj et al., 2002). Another study revealed that, Pycnogenol improved erectile dysfunction from moderate to mild stage. The same study reports a simultaneous significant elevation of plasma antioxidant activity was detected. The level of total cholesterol decreased from 5.41 to 4.98 mmol/L associated with a decrease LDL cholesterol from 3.33 to 2.78 mmol/L (Durackova et al., 2003).

Results of oxidative stress biomarkers represented that, DOX induced oxidative stress manifested as disturbance of normal oxidative biomarkers levels.

Exactly, DOX-derived ROS causes an imbalance between pro- and anti-apoptotic proteins, disrupting mitochondrial membrane potential, causing cytochrome c release and subsequently causing cell death through apoptotic pathway.

Pycnogenol can suppress the generation of peroxides by raising the antioxidant enzyme activity and intracellular GSH level. Parveen et al (2013) showed that, Pycnogenol treatment significantly reduced oxidative stress and increased GSH levels in liver and pancreas of the diabetic rats. However, previous study (Yang et al., 2008) represented that Pycnogenol significantly prohibited the CCl₄-induced exhaustion of hepatic GSH and representing the antioxidant effect of Pycnogenol against CCl₄ toxicities. Moreover, Pycnogenol was shown to have remarkable anti-inflammatory and wound healing functions were demonstrated subsequently (Blazso et al., 2005).

Results exposed that, Pycnogenol treatment either prophylactic or therapeutic attenuated the effect of DOX by reducing NO and MDA levels significantly. A previous study (Taner et al., 2014) agreed with our study, which demonstrated the defensive effects of PYC (100 mg/kg b.w./day) on oxidative stress parameters and

DNA damage in the septic rats. As it decreased MDA levels and increased GSH levels in the liver and kidney tissues of septic rats. Previous studies showed that PYC is a very potent antioxidant to scavenge reactive oxygen and nitrogen species such as superoxide anion radical, hydroxyl radical, lipid peroxy radical and peroxynitrite radical (Maritim et al., 2003).

Results of the present study showed that, DOX is shown to be linked to elevation of lipid peroxidation MDA in heart tissue, the elevation in serum MDA of DOX-treated groups evidence that DOX induces obvious oxidative stress injury in short term treatment. These results were in full agreement with the results of previous study by Tsuda et al., (2012).

DOX administration markedly increased serum cardiac marker enzymes significantly indicating cardiac failure. Cardio-protective strategies that mitigate myocardial damage early during treatment may aid in preventing long-term cardiotoxicity. Oxidative stress and the related apoptosis of cardiomyocyte mitochondria are primary mechanisms of anthracycline-induced cardiotoxicity (Ascensão et al., 2011).

Lipshultz et al., (2012) observed a rise in the levels of cardiac troponin in children treated with DOX, even after the cessation of treatment, indicative of cardiac damage and irreversible cardiomyocytes necrosis. Moreover, Lobna et al., (2002) observed that serial monitoring of serum troponin T could be of value as an early sensitive detector for DOX induced acute myocyte injury.

Stimulatingly, DOX has a wide empathy for anticardiolipin, which is a phospholipid located on the myocytic mitochondrial membrane, which ends up as an accumulation of DOX in the interior of cardiac cells and increases toxicity, this because DOX-cardiolipin complex aids as the substrate for the starting of lipid peroxidation (Jung and Reszka, 2001).

DOX intoxication caused a significant increase and AP activity in heart tissues. C-Reactive protein CRP is the most important assessing inflammatory markers of atherosclerosis that synthesized in liver as a result of proinflammatory cytokines, such as IL-6, IL-1 β , and TNF- α (Salazar et al., 2014). Pycnogenol has several roles in lessening CRP level and are correlated with NF- κ B gene expression suppression (Sing., 2008). The cardiac biomarker enzyme LDH is clinically used as markers for the diagnosis of cardiac toxicity. Doxorubicin induces marked cardiotoxicity that demonstrated by increase in LDH activities, on the other hand, increasing LDH serum levels have been in cardiac tissue dysfunctions was observed because these are normally positioned in the cytoplasm of cardiomyocytes and leakage happens into the serum following cardiomyocytes damage (Peng et al., 2000).

Additionally, Feng et al (2002) investigated the effect of Pycnogenol on cardiotoxicity in mice treated with antineoplastic drugs, they showed that when Pycnogenol was administered at 150 and 200 mg/kg orally, it prevents doxorubicin cardiotoxicity by inhibiting the elevation of creatine phosphokinase in the

serum. The study results agreed with previous experiments, Pycnogenol presented optimistic effects on cardiac performance in diabetic cardiomyopathy (Klimas *et al.*, 2010). Moreover, Pycnogenol also improves endothelial function in patients by reducing oxidative stress (Enseleit *et al.*, 2012).

No structural changes established microscopically in the heart of Pycnogenol supplemented rats as compared with control group, while DOX treated rats showed severe pathological lesions, which were characterized by congestion of myocardial vessels (Majzner *et al.*, 2015). The supplementary histopathological variations as myocardial degeneration, vacuolization and necrotic cardiomyocytes revealed that, cardiac enzymes activities can reveal the grade of cardiac damage in the acute stage (Goudarzi *et al.*, 2018).

In conclusion: Pycnogenol aqueous extract protected against DOX induced cardiomyopathy in experimental rats this may due to its antioxidant and anti-inflammatory properties.

References

- Ascensão, A., Lumini, O. J., Machado, N.G., Ferreira, R.M., Gonçalves, I.O., Moreira, A.C., *et al.*, Acute exercise protects against calcium-induced cardiac mitochondrial permeability transition pore opening in doxorubicin-treated rats, *Clin. Sci.*, 2011, 120, 37–49.
- Aydın, S., Bacanlı, M., Anlar, H. G., Çal, T., Arı, N., Bucurgat, Ü. Ü., & Başaran, N. Preventive role of Pycnogenol® against the hyperglycemia-induced oxidative stress and DNA damage in diabetic rats. *Food and Chemical Toxicology* 2019, 124, 54–63.
- Blazso, G., Gabor, M., Schönlaue, F., Rohdewald, P. Pycnogenol® accelerates wound healing and reduces scar formation. *Phytother Res*, 2005,18,579-581.
- Devaraj, S., Kaul, N., Schönlaue, F., Rohdewald, P., Jialal, I. Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters plasma lipoprotein profile. *Lipids*, 2002,37,931-934.
- Durackova, Z., Trebaticky, B., Novotny, V., Zitanova, I., Breza J. Lipid metabolism and erectile function improvement by Pycnogenol®, extract from the bark of *Pinus pinaster* in patients suffering from erectile dysfunction, a pilot study. *Nutr Res*, 2003, 23, 1189-1198.
- Elliott, P., Andersson, B., Arbustini, E., *et al.* Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J*, 2008, 29, 270–76.
- Enseleit, F., Sudano, I., Périat, D., Winnik, S., Wolfrum, M., Flammer, A.J., Fröhlich, G.M., Kaiser, P., Hirt, A., Haile, S.R., Krasniqi, N., Matter, C.M., Uhlenhut, K., Högger, P., Neidhart, M., Lüscher, T.F., Ruschitzka, F., Noll, G.(2012) Effects of Pycnogenol on endothelial function in patients with stable coronary artery disease: a double-blind, randomized, placebo-controlled, cross-over study. *Eur Heart J*,2012, 33,1589-1597
- Feng, W.H., Wei, H.L., Liu, G.T. Effect of PYCNOGENOL on the toxicity of heart, bone marrow and immune organs as induced by antitumor drugs. *Phytomedicine*, 2002, 9: 414–8.
- Gabriele, D'Andream, Pycnogenol: A blend of procyanidins with multifaceted therapeutic applications? *Fitoterapia* 2010, 81, 724–736.
- Goudarzi, M., Fatemi, I., Siahpoosh, A., Sezavar, S.H., Mansouri, E., Mehrzadi S., Protective effect of ellagic acid against sodium arsenite-induced cardio- and Hmatotoxicity in rats, *Cardiovasc. Toxicol.* 2018 ,4, 337–345.
- Gulati, O.P. Pycnogenol® in metabolic syndrome and related disorders. *Phytother Res*, 2015,29, 949-968.
- Hamza, A.H. and Mahmoud, R.H., Soy milk and Sesame seeds (Phytoestrogens) Ameliorate cardiotoxicity induced by adriamycin in experimental animals. *American Journal of Research Communication*, 2013,1(10), pp.1-15.
- Hosseini A., Sahebkar A., Reversal of doxorubicin-induced cardiotoxicity by using phytotherapy: a review, *J. Pharmacopuncture* ,2017, 20, 243–256.
- Jung, K., Reszka, R., . Mitochondria as subcellular targets for clinically useful anthracyclines. *Adv. Drug Deliv. Rev.* 2001,49 (1), 87–105.
- Kanu C., Jianqing Z., Norman H., Joel S., Doxorubicin Cardiomyopathy, *Cardiology* ,2010,115,155–162.
- Kawaguchi, T., Takemura, G., Kanamori, H., Takeyama, T., Watanabe, T., Morishita, K., *et al.* Prior starvation mitigates acute doxorubicin cardiotoxicity through restoration of autophagy in affected cardiomyocytes. *Cardiovasc Res* 2012,96,456–65.
- Klimas J., Kmecova J., Jankyova S., Yaghi D., Priesolova E., Kyselova K., *et al.*, Pycnogenol improves left ventricular function in streptozotocin-induced diabetic cardiomyopathy in rats, *Phytother. Res.* 2010,24, 969–974.
- Koch, R., Comparative study of Venostasin and Pycnogenol in chronic venous insufficiency. *Phytotherapy Research*, 2002, 16,1–5.
- Koti, B.C., Vishwanathswamy, A.H., Wagawade, J., Thippeswamy, A.H., Cardioprotective effect of lipistat against doxorubicin induced myocardial toxicity in albino rats, *Indian J. Exp. Biol.*, 2009, 47,41–46.
- Kumaralm, A., Giris M., Soluk-Tekke O, Dogru-Abbas U., Turkog Ü. and Uysal M.: Effect of olive leaf extract treatment on doxorubicin-induced cardiac, hepatic and renal toxicity in rats. *Pathophysiology*,2015, 22, 117-23.
- Lipshultz, S.E., Lipsitz, S.R., Sallan, S.E., *et al.* Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. *J Clin Oncol*, 2012, 23, 2629–36.
- Lipshultz S.E., J.A. Alvarez, R.E. Scully, Anthracycline associated cardiotoxicity in survivors of childhood cancer, *Heart (British Cardiac Society)*, (2008), 94,525–533.
- Lobna, S., Emad, H., Hanan, S., Mona, A., Wafaa, E., and Hussein S. the value of troponin measurement in assessment of anthracycline induced cardiotoxicity in breast cancer patient. *msc. journal of the Egyptian nat. cancer inst*, 2000, 12(1), 35-40.
- Majzner, K., Wojcik, T., Szafraniec, E., *et al.* Nuclear accumulation of anthracyclines in the endothelium studied

- by bimodal imaging: fluorescence and Raman microscopy. *Analyst* 2015,140, 2302–10.
- Maritim, A., Dene, B.A., Sanders, R.A., Watkins, J.B., Effects of pycnogenol treatment on oxidative stress in streptozotocin-induced diabetic rats. *J. Biochem. Mol. Toxicol.* 2003,17, 193–199.
- Maron, B.J., Towbin, J.A., Thiene, G., Antzelevitch, C., Corrado, D., et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology. *Circulation* ,2006,113, 1807-1816.
- Parveen, K., Ishrat, T., Malik, S., Kausar, M.A., Siddiqui, W.A. Modulatory effects of Pycnogenol in a rat model of insulin-dependent diabetes mellitus: biochemical, histological, and immunohistochemical evidences. *Protoplasma*, 2013,250, 347–360.
- Peng, Q., Wei, Z., Lau, B.H. Pycnogenol inhibits tumor necrosis factor- α -induced nuclear factor kappa B activation and adhesion molecule expression in human vascular endothelial cells. *Cell Mol Life Sci* ,2000,57,834-41.
- Raj, S., Franco, V.I., Lipshultz, S.E., Anthracycline-induced cardiotoxicity: a review of pathophysiology, diagnosis, and treatment. *Curr. Treat. Options Cardiovasc. Med*,2015, 16, 1–14.
- Rezzani ,R., Porteri ,E., De Ciuceis, C. et al . Effects of melatonin and Pycnogenol on small artery structure and function in spontaneously hypertensive rats. *Hypertension*, 2010,55(6): 1373-80.
- Rohdewaldm, P., A review of the French maritime pine bark extract(Pycnogenol), a herbal medication with a diverse clinical pharmacology, *Int. J.Clin. Pharmacol. Ther.* 2002, 40, 158–168.
- Salazar, J., Martínez, M.S., Chávez-Castillo, M., Núñez, V., Añez, R., Torres, Y., Toledo, A., Chacín, M., Silva, C., Pacheco, E., Rojas, J., Bermúdez, V. C-reactive protein: an in-depth look into structure, function, and regulation. *Int Sch Res Notices*, 2014, 653045.
- Singh S.K., Suresh M.V., Voleti B., and Agrawal A.The connection between C-reactive protein and atherosclerosis. *Annals of Medicine* 2008; 40(2): 110–120.
- Tacar, O., Sriamornsak, P., Dass, C.R., Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *J. Pharm. Pharmacol.* 2013,65, 157–170.
- Taner, G., Aydın, S., Bacanlı, M., Sarıgöl, Z., Sahin, T., Başaran, A.A., Başaran, N. Modulating effects of pycnogenol® on oxidative stress and DNA damage induced by sepsis in rats. *Phytother Res.* 2015,28, 1692–1700.
- Towbin JA, Lowe AM, Colan SD, Sleeper LA, Orav EJ, et al. (2006) Incidence, causes, and outcomes of dilated cardiomyopathy in children. *JAMA* 296: 1867-1876.
- Tsuda, H., Kawada, N., Kaimori, J.-Y., Kitamura, H., Moriyama, T., Rakugi, H., Takahara, S., Isaka, Y. Febuxostat suppressed renal ischemia–reperfusion injury via reduced oxidative stress. *Biochem. Biophys. Res. Commun.*2012, 427, 266–272.
- Warpe, V. S., Mali, V. R., Arulmozhi, S., Bodhankar, S. L., & Mahadik, K. R. (2015). Cardioprotective effect of ellagic acid on doxorubicin induced cardiotoxicity in wistar rats. *Journal of Acute Medicine*, 2015, 5,0 1-8.
- Yang, Y. S., Ahn, T. H., Lee, J. C., Moon, C. J., Kim, S. H., Jun, W., & Kim, J. C. Protective effects of Pycnogenol® on carbon tetrachloride-induced hepatotoxicity in Sprague–Dawley rats. *Food and Chemical Toxicology*, 2008, 46(1), 380-387.