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In vivo cardioprotective effect of a polysaccharide from *Lycium europaeum* on cisplatin-induced heart injury in mice

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Abstract

In this study, we aimed to investigate the possible cardioprotective effect of a water-soluble polysaccharide (LEP) from the leaves of Lycium europaeum against cisplatin (CP)-induced heart injury in mice model.

Mice (31 ± 2 g) were divided into four groups (n=6) as follows: Group (1) control, Group (2) LEP alone (100 mg/kg, p.o.), Group (3) CP (10 mg/kg, i.p.), Group (4) LEP + CP and Group (5) Atorvastatin, 10 mg/kg) + CP. To assess the efficacy of LEP treatment against CP-induced cardiotoxicity, lipid peroxidation, biochemical parameters and histopathological examinations were conducted.

CP administration induced severe heart injuries and elevated lipid peroxidation levels. However, LEP pretreatment reduced cardiovascular diseases, which has been evinced by amelioration in parameters such as triglyceride, total cholesterol, lactate dehydrogenase, low-density lipoprotein-cholesterol and high-density lipoprotein-cholesterol. In addition, cardiac indexes, atherogenic indexes, and coronary artery indexes were significantly improved in Group (4) when compared to CP intoxicated animals. Moreover, LEP administration to CP-treated mice obviously mitigated the malondialdehyde level when compared to Group (3). The histo-pathological observations also demonstrated that LEP pretreatment significantly restored the damage induced by CP.

These results advised that LEP improved myocardial injury and could be recommended as a potential candidate for the development of new cardioprotective agents.

Key words: *Lycium europaeum;* Cisplatin; Cardioprotective; Polysaccharide; Heart injury.

Introduction

Cisplatin (CP) is one of the most used chemotherapeutic agents for the treatment of manifold cancer types (1). However, CP usage has been seriously associated with many undesirable side effects including nephrotoxicity (2) and cardiotoxicity (3). Indeed the cytotoxicity of CP lies in its interaction with mitochondrial DNA, which causes cardiomyopathy, bradycardia and arrhythmia (4, 5). Many studies have confirmed that cisplatin-induced cardiotoxicity stimulates the generation of free radicals (6, 7). Therefore, research has been developed to mitigate the side effects of CP without perturbing its antitumor effects. Previous studies indicated that antioxidants including L-carnitine and silymarin were able to circumvent the cardiotoxic effect associated with CP intoxication in mice (7). Numerous researches have already revealed the beneficial effects of natural plant polysaccharides in the management of many illnesses. For instance, polysaccharides extracted from *Nitraria retusa* have demonstrated potential hepatoprotective and cardioprotective effects in experimental mice (8). Cao et al. (9) proved the

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protective effect of polysaccharides extracted from *Astragalus membranaceus* against doxorubicin-induced cardiotoxicity.

In our previous study, we extracted and characterized polysaccharides from the leaves of *Lycium europaeum* (LEP) (2). Results showed that LEP was composed of 77.59% of carbohydrates and 2.25% of protein. The hepatoprotective and nephroprotective effects of LEP in mice were determined. The findings revealed that the administration of LEP at 100 mg/kg had a protective effect against hepatotoxicity and nephrotoxicity induced by CCl_4 and cisplatin, respectively. However, the effects of LEP on CP-induced cardiotoxicity have never been elucidated. Herein we examine the protective effect of LEP against CP-induced oxidative stress and cardiovascular disorders in mice.

Materials and methods

Chemicals and preparation of extract: Cisplatin was purchased from Merck (Darmstadt, Germany). Chemicals products and assay kits were purchased from Sigma Chemical Co. (St. Louis, MO). The polysaccharide from *Lycium europaeum* (LEP) was prepared according to our previous publication (2).

Cisplatin-induced cardiotoxicity *in vivo*

Experimental Animals: Male Swiss albinos mice (31 ± 2 g, 12 weeks old) were got from the animal house of the Faculty of Science Sfax, Tunisia.

Healthy animals were housed in cages, kept under standard laboratory conditions; temperature was 22 ± 2 °C with 40% humidity and allowed free access on balanced diet (according to NRC 1995) (10) and drinking water. Experimental tests were accomplished in concordance with standard ethical guidelines for laboratory animal use and care as explained in European Community Guidelines.

Experimental Setup: Cardiotoxicity in mice was induced by intraperitoneal injection (i.p.) of cisplatin (CP, 10 mg/kg, i.p.) on the 5th day (6).

In this study a total of 30 healthy animals were randomly divided into four groups (n=6 each) as follows:

Group 1 (control) mice received NaCl (0.9%, i.p) for ten days; group 2 (LEP) mice were orally administered LEP (100 mg/kg,) for 10 successive day (2); group 3 (CP) mice were intoxicated after a single injection of CP (CP, 10 mg/kg, i.p.) on the fifth day, group 4 (LEP+ CP) animals were treated with LEP at 100 mg/kg, i.p. for 5 successive days before and after a single dose of CP on the fifth day and group 5 (ATV + CP) animals received standard drug (Atorvastatin, 10 mg/kg) for 5 successive days before and after a single dose of CP on the fifth day.

Twenty four hours after the last treatment, animals were sacrificed. Blood samples were collected from the left ventricular of heart. The heart was directly removed, weighed and dissected into two halves, one for biochemical assays and the other for histopathological analysis.

Body weight and heart weight: The body weights (BW) of mice were measured at the beginning of the treatment and on the day of sacrifice and the gains (%) of BW were calculated. The hearts of animals were isolated and weighed to determine the absolute and relative weights.

Determination of cardiac biomarkers: Biomarker enzymes for cardiac function including lactate dehydrogenase (LDH), triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol were estimated using an auto-analyzer (Roche Cobas C 311, Germany).

Indexes including cardiac (CI), atherogenic (AI) *and coronary artery (CAI) were* calculated by these *formulas: (*1); (2); (3)

$$(1)\ CI = \frac{TC}{HDLc}$$

$$(2)\ AI = \frac{TC - HDLc}{HDLc}$$

$$(3)\ CAI = \frac{LDLc}{HDLc}$$

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Heart biomarkers enzymes including creatine kinase: CK-NAC and creatine kinase-MB: CK-MB were evaluated using commercial reagent kits from Biomaghreb (Tunisia) according to the manufacturer's protocol.

Lipid peroxidation assay (LPO): The LPO in tissue homogenates was evaluated using the procedure of Ohkawa et al. (11). *Heart tissues were homogenized in phosphate-buffered saline (0.1 M; pH 7.4) with an Ultra Turrax homogenizer. Next, the mixture was centrifuged at 1500 g for 15 min to give the supernatant which will be used to investigate LPO. The absorbance of each tested group was recorded at 532 nm and the results were expressed as the MDA content.*

Histopathological analysis: Heart tissues were kept 48 h into formalin solution (10%), then dehydrated in gradual concentrations of alcohol from 70 to 100% and embedded in paraffin. Finally, paraffin was cut at 5 ìm thickness, stained with hematoxylin-eosin and analyzed under a light microscope.

Statistical analysis: Data were expressed as means and standard deviation of means (±SD) and analyzed using the SPSS software program (PASW Statistics 18.0). All data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's test.

Results

Effects of LEP treatment on the body and heart weights: The effects of LEP on CP-intoxicated mice are summarized in Table 1. No changes were observed in the body and heart weights of mice treated with normal saline solution and with LEP alone. However, these parameters in CP-intoxicated mice were significantly decreased compared to the control animals. In the same way, the body weight gains and the relative heart weight were significantly reduced in CP-intoxicated mice as compared to the control group. A significant protection was recorded in the LEP+CP treated group compared with the CP group.

Effects of LEP treatment on biochemical parameters : The cardioprotective potentials of LEP at 100 mg/kg were assessed in terms of their aptitude to decrease the levels of TC, TG, LDL and LDH. Cisplatin treatment produced a significant rise in the levels of these biomarkers when compared to the normal group (Table 2). In contrast, the pretreatment of mice with LEP or either ATV significantly reduced the cardiotoxic effect of cisplatin as compared to CP-treated group. Table 2 also displayed a significant decrease in the level of HDL and increase in the ratio of AI, CI and CAI in groups treated with cisplatin in comparison to the control group. Also, significant elevations for CK-NAC and CK-MB levels, when compared to control group. However, these disorders were clearly restored in mice pretreated with LEP when compared to the CP-induced cardiotoxicity group. Interestingly, treatment of CP-intoxicated mice with *Atorvastatin* restored the normal serum levels of all aforementioned parameters.

Effects of LEP treatment on heart lipid peroxidation : As shown in Table 2, the treatment with cisplatin enhanced the level of MDA (3.82 nmol/mg protein) in comparison to the control group (1.17 nmol/mg protein). However, pretreatment of mice with LEP before CP injection resulted in a significantly down in MDA level as compared with the intoxicated mice (1.88 nmol/mg protein, versus 3.82 nmol/mg protein, $p < 0.05$).

Effects of LEP treatment on heart histopathological tissue : The cardiac tissues in the control and LEP groups showed normal histoarchitecture of the heart (Fig.1A and B). A large and irregularly shaped hypertrophic myocardial fiber and hemorrhages were observed in the cisplatin-treated group (Fig.1C). However, LEP pretreatment (100 mg/kg) prior to cisplatin administration ameliorated heart histological tissue (Fig.1D) as compared to the control group.

Discussion

Oxidative stress is one of the main mechanisms involved in the pathogenesis of heart

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failure. The use of phytochemicals as a therapeutic approach for cardiovascular disease is gaining care worldwide. In the present investigation, the protective effect of LEP was examined on oxidative stress and myocardial damage in cisplatin-induced cardiotoxicity in mice. CP treatment alone resulted in body and heart weight changes. These changes can be attributed to gastrointestinal toxicity and the minimization in the ingestion of food (12). Moreover, CP treatment may lead to hypertrophy of the heart tissue due to the increased water content, oe dematous intramuscular space (13). This result is similar to reports by Afsar et al. (14). However, LEP supplementation in the CP-treated mice restored the weight changes probably by preventing oxidative stress-induced cell death.

Results revealed that the administration of cisplatin to healthy mice caused a marked cardiac

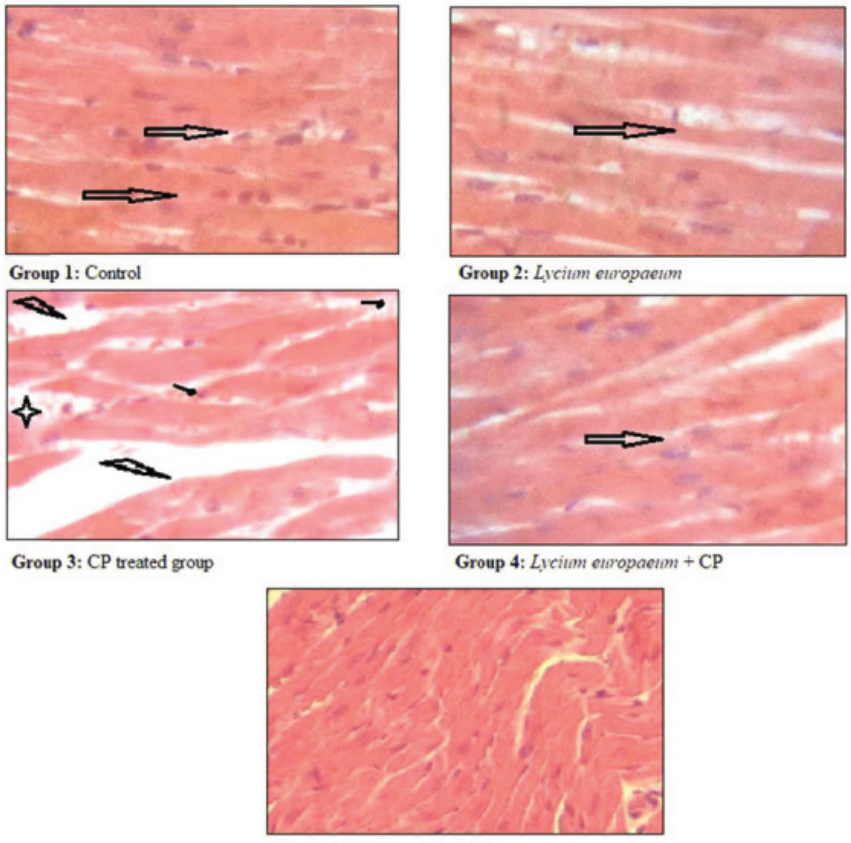

Figure 1. Photomicrographs of myocardial tissues in the control and experimental treated mice (hematoxylin–eosin, ×200). The control group and group treated with *Lycium europaeum* (LEP, 100 mg/kg, b.w.) showing normal cardiac muscle fibers. Ciplatin treated group (10 mg/kg, b.w.) showing large inflammatory cells infiltration, myocardial cells necrosis, separation of cardiac myofibrillar. LEP + cisplatin co-treated group showing normal myocardial arrangement and few inflammatory cells.

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dysfunction that was evident by increased plasma CK-NAC and CK-MB levels.

The observed troubles look to be linked to the alteration in the membrane permeability and/or the death of myocardial cells, as results of cisplatin intoxication, leading then to the release of cytosolic contents into the systemic circulation, which was in harmony with previous studies (15). Treatment of CP-intoxicated mice by either LEP or Atorvastatin significantly restored the normal plasma levels of all aforementioned parameters.

Data of the present work revealed that cisplatin treatment is associated with the alterations in lipid profile which was evident by the increase in the levels of TC, TG and LDL and the decrease in the HDL level. These abnormalities were in agreement with previous finding demonstrating hyperlipidemia in animals treated with cisplatin (6). Disorders in the lipid profile have been demonstrated for increasing the risk of myocardial infarction. Moreover, the high level of LDL-C in plasma can contribute to the formation of atherosclerosis plaques due to the lipid deposition on the arterial wall. The treatment with LEP significantly restored these parameters to normal levels in comparison to CP-treated groups, thereby reducing the risk of cardiovascular diseases. Our previous studies have confirmed that LEP protects against the toxic effects of CP via its powerful antioxidant potential (2). Likewise, pretreatment with Atorvastatin (10 mg/kg) attenuated the cardiac damage caused by CP (16).

Cardiac, atherogenic and coronary artery indices are predictive indicators of cardiac illnesses. In this study, mice treated with CP exhibited a profound increase in AI, CI and CAI as compared to the normal ones. Pretreatment with LEP (100 mg/kg, i.p.) followed by CP administration revealed a significant improvement in cardiovascular risk indices and subsequently minimized the possibility of cardiovascular disease occurrence (17). Similar effects have been recorded in previous work from our lab on polysaccharides extracted from *Nitraria retusa* fruits (8).

It has been demonstrated that cisplatin generates reactive oxygen species and stimulates lipid peroxidation that leads to the cardiotoxicity (18). In our study, the injection of CP resulted in oxidative damage, detected by the increase in the cardiac content of MDA. Our results are in line with those of Afsar *et al.* (14) that demonstrated an increase in lipid peroxidation through the high cardiac MDA levels in response to cisplatin administration. Normalization of the cardiac MDA levels in experimental groups pre-treated with LEP, suggesting its cardioprotective effect. All the above results were supported by histopathological investigations. Significant histological changes were observed in the heart of mice treated with CP including atrophic myocardial fiber and hemorrhages, which might be due to the generation of ROS that provoked damages in the histoarchitecture of cardiac tissues (14). Same toxic influences were detected using other toxic elements on cardiac tissue such as the isoproterenol (19). However, pre-treatment of mice with LEP alleviated the precedent histoarchitecture disruptions.

Conclusions

The present study demonstrated for the first time that LEP improved cardiovascular performance against cisplatin-induced heart injuries in mice. The effect is associated with a restoration of the malondialdehyde level, the histoarchitecture of cardiac tissues and the enhancement of biochemical parameters, the cardiac index and the atherogenic index. Collectively, these findings demonstrated that LEP might be a potential therapeutic medicine for the treatment of cardiovascular disease by reducing CP induced myocardial injuries.

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Table 1. Effect of CP and LEP treatment on body weight and heart weight.

Treatment (mg/kg)	Body weight (BW)		Heart weight (HW)		
	Initial (g)	Final (g)	Gains (%) w	Absolute weight	Relative weight (g) (g/100g BW)
Control	31.4 ± 1.07	37.4 ± 0.51	16.03 ± 3.03	0.168 ± 0.012	0.449 ± 0.036
LEP	30.8 ± 1.22	38.6 ± 1.07	20.15 ± 3.68	0.166 ± 0.008	0.430 ± 0.031
CP	31.0 ± 1.15	32.0 ± 1.15***	3.12 ± 0.10***	0.138 ± 0.007***	0.431 ± 0.029*
LEP + CP	30.6 ± 1.26	39.8 ± 0.78###	23.13 ± 2.15###	0.156 ± 0.008##	0.392 ± 0.023#
ATV + CP	30.4 ± 1.42	38.2 ± 0.78###	20.38 ± 4.18###	0.160 ± 0.014###	0.418 ± 0.034###

Data are expressed as means SD for six mice in each group.
* Significant difference at $p < 0.05$ and *** at $p < 0.001$ between control and CP treated group.
# Significant difference at $p < 0.05$ and ### at $p < 0.001$ between the CP treated group and CP treated group plus LEP.

Table 2. Effect of the administration of CP and LEP on heart lipid peroxidation and plasma biochemical parameters in the different experimental groups.

Parameters	Treatment (mg/kg)				
	Control	LEP	CP	LEP + CP	ATV + CP
MDA (nmol/mg protein)	1.17 ± 0.08	1.35 ± 0.10	3.82 ± 0.03***	1.88 ± 0.04###	1.84 ± 0.04###
CK-NAC (U/L)	188.74±3.53	180.79±2.66	294.19±5.33***	212.16±2.28###	201.06 ± 1.91###
CK-MB (U/L)	209.76±1.22	212.14±6.91	317.83±2.90***	247.56±5.47###	227.9 ± 3.25###
LDH (U/L)	582.71 ± 5.26	575.01 ± 10.82	832.66 ± 9.75***	609.87 ± 6.05###	595.1 ± 6.82###
TC (mmol/L)	2.13 ± 0.03	2.26 ± 0.08	3.12 ± 0.07**	2.44 ± 0.05###	2.24 ± 0.12###
TG (mmol/L)	0.93 ± 0.01	1.04 ± 0.05	1.67 ± 0.03***	1.18 ± 0.05##	1.12 ± 0.08###
LDL (mmol/L)	0.50 ± 0.01	0.49 ± 0.01	0.98 ± 0.02***	0.64 ± 0.03###	0.51 ± 0.03###
HDL (mmol/L)	1.50 ±0.04	1.46 ± 0.01	1.13 ± 0.05**	1.35 ± 0.02#	1.39 ± 0.03#
CI	1.42 ± 0.02	1.54 ± 0.05	2.75 ± 0.15**	1.81 ± 0.00###	1.61 ± 0.09###
AI	0.42 ± 0.02	0.54 ± 0.05	1.75 ± 0.15**	0.81 ± 0.00###	0.61 ± 0.09###
CAI	0.33 ± 0.02	0.33 ± 0.00	0.86 ± 0.01***	0.47 ± 0.03##	0.36 ± 0.03###

Data are expressed as means SD for six mice in each group.
** Significant difference at $p < 0.01$ and *** at $p < 0.001$ between control and CP treated group.
# Significant difference at $p < 0.05$; ## significant difference at $p < 0.01$ and ### at $p < 0.001$ between the CP treated group and CP treated group plus LEP.

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