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Antioxidant, anti-inflammatory and antiapoptotic effects of Naringin on cardiac damage induced by cisplatin

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Cisplatin (CP), an anticarcinogenic agent, is declared to have side effects including cardiotoxicity. Naringin (NA), a flavonoid, was shown to have strong antioxidant structure and anti-inflammatory characteristics. Pupose of our study was to investigate the protective effects of NA on Caisplatin-induced cardiotoxicity. Sprague-Dawley male rats in the range of 220-250 g were exerted in this experimental studies. The rats used in experiment separated to 5 groups (10 in each one): Control, CP, NA100+CP, NA200+CP and NA200. The groups received drugs for 14 days. The rats were decapitated on 15th day. Cardiac tissue and blood samples was taken. When the cardiac tissue was assessed for oxidative stress, a prominent increase was found in CP group than other groups ($p<0.05$). It was assigned that serum CK, CK-MB, LDH, AST and ALT activities and levels of cytokines (TNF-alpha, IL-8, IL-1β and IL-6) in the cardiac tissue, a significant increase was found in CP group than control group ($p<0.05$). In both control and NA200+CP groups, similar values obtained for comparative values. Caspase 3 and iNOS activity were significantly raised for CP group, compared to NA100+CP and NA200+CP, control groups. NA has a protective effect on CP-induced heart injury. It has been determined that NA prevents CP-induced apoptosis in the heart.

Keywords: Cardiotoxicity, Caspase 3, Cisplatin, Ciytokine, iNOS, Naringin, Rat

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CP which is made of platinum agents, is an efficacious member of chemotherapeutic drugs. CP is used on oncological cases. It has a wide usage in the treatment of various cancer types like ovarian, pulmonary, cervical, stomach tissues and cranial[1,2]. And also side effects such as cardiovascular complications, hepatotoxicity and nephrotoxicity are observed when CP is used[3,4]. Many studies have reported cardiotoxicity due to the CP usage. Congestive heart failure, arrhythmia, electrocardiographic changes and cardiomyopathy characterize CP. Although CP, an anticancer agent, has side effects, it is widely used because of its effectiveness. Despite of having side effects, CP remains a powerful, commonly used anticancer agent[5]. Nowadays, there is interest in medicinal plants having protective effects against cardiotoxicity. Food products obtained from these medicinal plants are of great interest. Flavonoids are polyphenolic compounds and have a variety of chemical structures. They are commonly found in plant-derived foods like fruits, vegetables, teas and wines. Flavonoids are antioxidants that formed inherently in biological membranes and prevent lipid peroxidation. A flavonoid generally found in grapes and other citrus species is NA[6]. β-glucosidase and α-ramnosidase enzymes do the conversion to NA[7]. Antioxidants or cardiomyocytes with antioxidant enzymes are used in the pre-treatment stage. At this stage, injury of ischemic reperfusion is prevented with reduction of the free radicals formation[8]. Several studies have shown that NA exhibits antimutagenic, antimicrobial, anti-inflammatory, anticancer, antioxidant pharmaceutical effects and free radical scavenging[9-13].

In this study, the objective was analyse the preventive effects of NA on cardiotoxicity, CP-induced, apoptosis and inflamation by commentating the biochemical results. When previous studies were examined, it was found that this study is the first one which investigates the protective effects of NA against myocardial damage caused by CP-induced in rats.

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Material and methods

Animals

Animal Experiments Local Ethics Committee (HADYEK-No: 230/2018) of Atatürk University approved this study. Fifty Sprague Dawley rats which are adult and male, weighing approximately 220-250 g, were used in this study. The animals were kept at appropriate light, room temperature and humidity until the day of the experiment and fed with water and feed.

Experimental protocol

Rats were separated into 5 groups as 4 experimental groups and 1 control group.

Group I (Control) The control group received solely intragastric (i.g.) saline during 14 days.

Group II (CP) The group received i.g. saline during 14 days (placebo), but however, on the 12th[15] day of the study, this group was given only one dose of intraperitoneal (i.p.) CP (15 mg/kg)[14]

Group III and IV (NA100+CP and NA200+CP) The groups received 100 mg/kg and 200 mg/kg[16] of NA dissolved in saline, respectively. These groups were applied i.g. during 14 days. On the 12th day only, this group was given only one dose of intraperitoneal (i.p.) CP (15 mg / kg).

Group V (NA200) The group received a 200 mg/kg dose of NA along with i.g. during 14 days.

On the 15th day, all rats were anesthetized. All the animals were killed after samples of intracardiac blood obtained. The collected cardiac tissue and blood samples were used for biochemical examination of cytokine levels, oxidative stress, iNOS and Caspase-3 activity.

Analysis of cardiac function markers

The blood samples which were collected, were centrifuged at 1500 g for 10 min within 1 h to obtain sera samples. The sera were stored in the freezer at −80°C before analyzing. The CK, CK-MB, LDH, AST and ALT in the serum samples were measured.

Analysis of lipid peroxidation and antioxidant enzyme activities

The weight of cardiac tissues was measured. Phosphate buffered saline pH 7.4 homogenized this measured tissue. The homogenates were centrifuged at 10,000 g at 4^0C during 20 min.

Then, supernatants were obtained and the superoxide dismutase (SOD) activity as well as level of nitric oxide (NO), thiobarbituric acid reactive substances (TBARS) and the glutathione (GSH) were identified as described previously[17-20].

Analysis of cytokines levels and iNOS activity

The rat-specific cytokines levels were identified for interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), interleukin-1β (IL-1β) concentrations and interleukin-8 (IL-8). The iNOS activity was identified using immunoassay kits (Cayman, USA), which were conducted based the protocols of manufacturer. The resulted outcomes are expressed as the mean±SD of the concentration of each factor in the tissue.

Analysis of cardiac apoptosis

The rat-specific caspase-3 activity was identified using immunoassay ELISA kits (Elabnscience, USA), which were conducted based on the protocols of manufacturer. Outcomes were expressed as the mean±SD of the activity of each factor in the tissue.

Statistical analysis

All data was analyzed by SPSS 20.00. One-way ANOVA was used. Duncan post hoc test was evaluated statistically. $p<0.05$ taken into account statistically significant and data was indicated as mean±SD.

Results

Naringin effects on the cardiac function tests in CP-treated rats

Some of the experimental group animals' 15th-day cardiac parameters (CK, CK-MB, LDH, AST and ALT) are shown in Table 1. The CP-treated rats' serum CK, CK-MB, LDH, AST and ALT activities demonstrated a prominent rise when other groups are considered ($p<0.05$), but statistically considerable variety was not detected amongst the other groups ($p>0.05$).

Naringin effects on oxidative stress in CP-treated rats

Activity levels of SOD in cardiac homogenates from rats which treated by CP were considerably lower than in the control group ($p<0.05$). Additionally, in the group treated with 200 mg NA, the SOD activity levels were elevated than in the CP group ($p<0.05$). Fig. 1 presents the activities of SOD in the cardiac homogenates of rat.

The GSH levels of the animals which were treated by CP were considerably lower than the control group ($p<0.05$). Otherwise, the 100–200 mg NA-treated

Table 1 Serum cardiac parameters for all groups. The letters indicate the statistical differences among groups ($p<0.05$, n=10), the results were expressed as mean±SD.

Groups	CK ± SD	CK-MB ± SD	LDH ± SD	AST ± SD	ALT ± SD
Control	369 ± 27^{a}	249 ± 34^{a}	488 ± 62^{a}	253 ± 56^{a}	62 ± 11^{a}
CP	671 ± 5^{b}	536 ± 77^{b}	722 ± 27^{b}	487 ± 22^{b}	94 ± 18^{b}
NA100+CP	643 ± 80^{b}	329 ± 24^{a}	689 ± 88^{b}	223 ± 25^{a}	58 ± 7^{a}
NA200+CP	426 ± 49^{a}	216 ± 20^{a}	502 ± 69^{a}	200 ± 15^{a}	64 ± 9^{a}
NA200	371 ± 63^{a}	247 ± 31^{a}	452 ± 54^{a}	231 ± 33^{a}	69 ± 13^{a}

Note: The letters were indicate the statistical differences among groups, p value was considered as 0.05 (n=10).

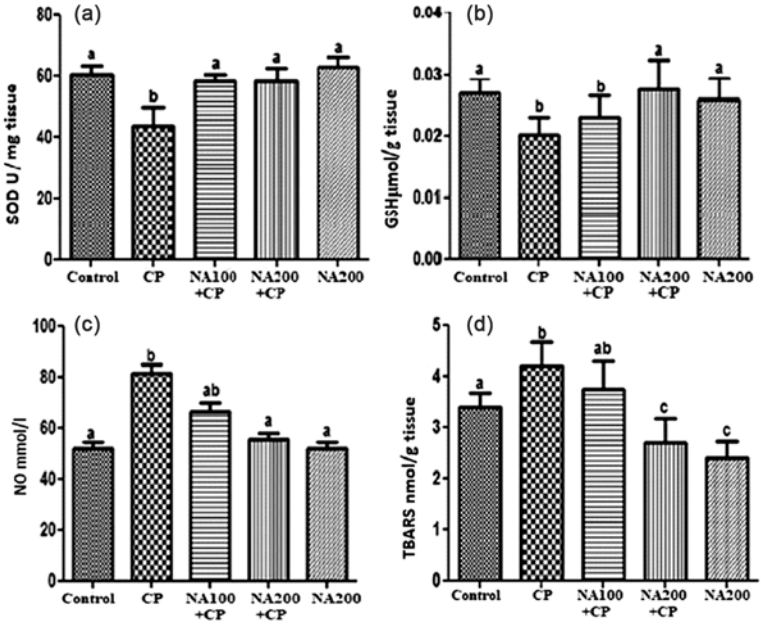

Fig. 1 — Illustration of levels of oxidative parameters (SOD, GSH, NO and TBARS) for all groups in the cardiac tissues. A; SOD activity, B; GSH level, C; NO Level and D; TBARS levels, the letters indicate the statistical differences among groups ($p<0.05$, n=10), the results were expressed as mean+SD

groups' levels of GSH were considerably elevated than the CP group ($p<0.05$). Fig. 1 presents statistics and levels of GSH for all groups.

The CP group NO level was considerably higher than the control, NA200+CP, and NA200 groups ($p<0.05$). Treatment with the 200 mg dose of NA considerably prevented an increase in rats' NO levels ($p<0.05$).

The study also assessed the TBARS levels to identify oxidative stress. The CP group's TBARS level was considerably higher than the other groups. But, treatment with a 200 mg dose of NA significantly prevented an increase in the rats' TBARS level ($p<0.05$).

Effects of naringin on levels of cardiac cytokines (IL-6, TNF-alpha, IL-1β and IL-8) in CP-treated rats

The cytokines revealed that the IL-8, IL-6, and IL-1β levels were considerably high in the CP group comparison to other groups ($p<0.05$). In addition, the CP and NA100+CP groups' TNF alpha levels were considerably high comparison the other groups ($p<0.05$). NA treatment group had significantly ameliorated cytokine levels ($p< 005$). Fig. 2 presents the levels of cytokines in the cardiac homogenates of rat.

Effects of naringin on iNOS activity in CP-treated rats

The iNOS activities were considerably high in the CP group comparison to other groups ($p<0.05$). NA administration, especially at high doses, prevented increases in the iNOS activity by CP induced. Fig. 3 presents the activity of iNOS in the cardiac homogenates of rat.

Effects of naringin on apoptosis in CP-treated rats

The caspase 3 activity was considerably high in the CP group comparison to other groups ($p<0.05$). Both

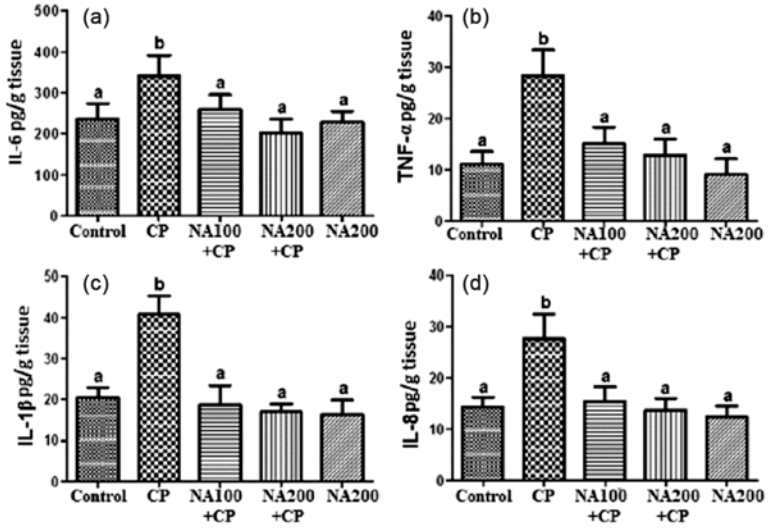

Fig. 2 — Biochemical cytokines levels in the cardiac tissues for all groups. A;IL-6, B; TNF-α, C; IL-1β, D; IL-8, the letters indicate the statistical differences among groups (p<0.05, n=10), the results were expressed as mean±SD

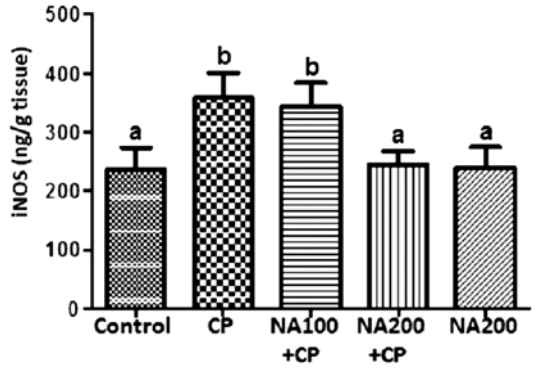

Fig. 3 — iNOS activity (ng/g tissue) in the cardiac tissues for all groups. The letters indicate the statistical differences among groups (p<0.05, n=10), the results were expressed as mean±SD

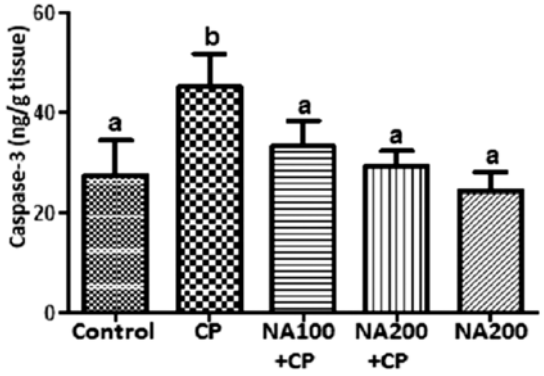

Fig. 4 — Caspase-3 activity in the ardiac tissues for all groups. The letters indicate the statistical differences among groups (p<0.05, n=10), the results were expressed as mean±SD

doses (100 mg/kg and 200 mg/kg) of NA prevented apoptosis caused by CP. Fig. 4 presents the activity of caspase 3 in the cardiac homogenates of rat.

Discussion

CP therapy has been reported to conduce to varied arrhythmias like heart failure and ischemic heart disease[21]. The cardiac side effects of CP alone and with other chemotherapeutic agents are associated with CP-induced oxidative damage[22]. The objective of our study was to analyze protective effects of NA CP-induced cardiotoxicity on apoptosis in rats. CP which leads to myocardial cells' destruction and myocardial endothelial damage, is a cardiotoxic agent. Consequently, CK, CK-MB, LDH, AST and ALT are given into bloodstream and used as diagnostic markers for myocardial tissue damage[23]. High levels of the enzymes listed are related with some heart damages like heart failure, myocardial infarction and myocarditis. When NA was administered, a dose-dependent decrease in high biomarkers caused by CP-induced heart damage was observed. This kind of effect demonstrates that NA is liable for limiting the leakage of biochemical markers because of membrane stabilizing properties.

In the studies, an increased lipid peroxidation, a reduced antioxidant capacity in cardiac tissue and a significant oxidative stress have reported following CP treatment[24]. CP may cause transient cardiotoxicity, contribute to a variety of permanent cardiac complications, and even cause congestive heart failure[25]. For these reasons, it is important to note drug reactions to CP in the clinic. It is also very important to investigate the cardioprotective agent in

CP-induced cardiotoxicity. The body has endogenous enzymatic antioxidants, like glutathione peroxidase (GSH-Px), SOD and catalase (CAT) which supply cellular defense against reactive oxygen species (ROS)[26]. In this study, it was observed that levels of NO in the heart increased considerably after CP administration. Excessive NO production is associated with direct cardiac damage in cardiotoxicity models linked to other chemotherapeutic agents such as CP[27]. Myocardial performance impairment and a negative inotropic effect resulting in myocardial injury induction[28] were referred to the overproduction of NO. In addition, NO was reported to cause cellular damage as the end result of reducing intracellular GSH levels. NO causes oxidative stress-induced cell damage by the peroxynitrite anions creation, a cytotoxic intermediate which lead to tissue damage and protein degradation[29]. In this study, an increase for NO levels and TBARS and a reduction for GSH level and SOD activities were observed. NA treatment avoided oxidative stress-mediated cellular damage in CP-induced cardiac damages.

The proinflammatory cytokines role in the cardiotoxicity pathogenesis according to cellular signaling pathways is currently under investigation. Secretion of cytokine is the inflammation mediator and conduces to pathogenesis of tissue injury[30,31]. After CP treatment in rats, a significant connected increase have been reported between the proinflammatory cytokines and serum IL-6 and TNF-α levels[32].

The flavonoids inhibitory effects in the chemical mediators release, includingIL-6, IL-1α, and TNF-α have been reported[33]. Rectrictive effects of NA on IL-6, IL-1α and TNF-α[34] were declared in the previous studies. In this study, it was detected that CP treatment considerably increased IL-6, IL-1β, IL-8 and TNF-α levels[35-38]. Inversely, NA treatment led toa prominent reduce in levels of TNF-α, IL-8, IL-1β and IL-6 in CP-induced experimental rats. This is probably due to its anti-inflammatory features. With these findings, it can be concluded that NA cardiac inflammation is caused by CP.

The excessive production of nitric oxide due to superoxide anion causes peroxynitrite, which is a strong oxidant that causes oxidative damage in the cell. Studies show that the cardiomyocytes in mice due to the formation of peroxinitrite iNOS resulted in heart block and sudden death[39]. In addition, the nitric oxide produced by iNOS induced cardiotoxicity, suppressed myocardial contractility and caused apoptosis in myocytes[40]. This study found a prominent rise in the nitric oxide level and iNOS activities in the cardiac tissue of CP-induced cardio toxic rats. But, NA administration had a cardio protective effect as it brought the NO and iNOS closer to their normal levels.

A free radical is a production of the body's metabolism that affects cell metabolism and leads to apoptosis. ROS which is an important cause of secondary heart lesions caused by chemotherapeutic drugs, is a product of CP metabolism. ROS production can cause cardiomyocyte apoptosis by causing lipid peroxidation and cardiomyocyte membrane damage[41]. CP treatment induced significant accumulation of ROS and superoxide anion in cardiac tissue, suggesting that oxidative stress and CP toxicity have close relation which is supported by previous studies[42]. This study shows the therapeutic benefit of NA treatment on cardiac apoptosis in the CP-induced group. Cardiac caspase-3 activity, which was increased in CP group, decreased after NA treatment.

Conclusion

NA treatment can mitigate cardiac damage and apoptosis caused by CP-induced cardiotoxicity in rats. Since NA also has anti-inflammatory, antioxidant and anti-apoptotic properties, restoration increases oxidative stress. Much more studies are needed to research its future clinical applications.

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