

Protective effects of curcumin and beta-carotene on cisplatin-induced cardiotoxicity: An experimental rat model

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ABSTRACT

Objective: Cisplatin (CDDP) has been known to be an effective antineoplastic drug; however, it has a cardiotoxic effect. Curcumin (CMN) and beta-carotene (BC) have been suggested to protect biological systems against CDDP-induced damage. The current study was conducted to evaluate the possible protective roles of CMN and BC on CDDP-induced cardiotoxicity in rat cardiac tissues.

Methods: A total of 49 adult female Wistar albino rats were equally divided into seven groups as follows: control (no medication), sesame oil (1 mg/kg), CDDP (single dose injection two times as once a week, 5 mg/kg/week), BC (100 mg/kg), CDDP+BC (pretreated BC for 30 min before CDDP injection), CMN (200 mg/kg), and CDDP+CMN (pretreated CMN for 30 min before CDDP injection). These treatments were applied intraperitoneally for CDDP and with gavage for CMN and BC. The oxidative/antioxidant indicators, inflammatory cytokines, and histopathological alterations were examined.

Results: These alterations included a marked increase in malondialdehyde (MDA) level, significant decrease in catalase (CAT) and superoxide dismutase (SOD) activities, and significant elevation of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , interleukin (IL)-6 in the CDDP group compared with the other groups. Histopathologically, CDDP-induced severe myocardial degenerative changes were observed. However, the CDDP-induced disturbances in the above-mentioned parameters significantly improved by treatment with BC and particularly CMN.

Conclusion: This study indicated that CDDP treatment markedly caused cardiotoxicity; however, treatment with CMN or BC ameliorated this cardiotoxicity in rats. Furthermore, these findings revealed that treatment with CMN has a higher cardioprotective effect than that with BC against CDDP-induced cardiotoxicity in rat cardiac tissues. (*Anatol J Cardiol* 2018; 19: 213-21)

Keywords: cisplatin, cardiotoxicity, curcumin, beta-carotene, rat heart tissue

Introduction

Chemotherapy, particularly using cisplatin [cis-diamminedichloroplatinum (II); CDDP], declines the normal homeostasis of the body (1). CDDP is a platinum-based effective chemotherapeutic drug that widely used in oncological applications. Owing to its potential activity on carcinoma cells, CDDP has been used to treat various types of malignancies such as bladder, cervical, ovarian, testicular, breast, lung, and head and neck cancer; however, it has frequent adverse effects (2), which include hepatotoxicity, nephrotoxicity, cardiotoxicity and so on (3-5). Although the underlying mechanisms concerning the antitumor effects of CDDP are relatively well known, the cellular and molecular mechanisms concerning its cardiotoxic effects are still unclear (6).

CDDP-induced cardiotoxic effects have been observed through serious changes in electrocardiography and arrhyth-

mias, which include ventricular arrhythmias, supraventricular tachycardia, atrial fibrillation, occasional sinus bradycardia, and occasional complete atrioventricular block (6-10). These cardiotoxic (and other potentially unknown) problems eventually lead to congestive heart failure and sudden cardiac death (11). Clinical approaches to improve CDDP-induced cardiac toxicity have also met little achievement (12, 13). Therefore, it has become necessary to develop a therapy to limit these CDDP-induced toxicities as they still remain as a major challenge despite several clinical interventions (11).

Curcumin (diferuloylmethane, CMN) is a biologically active and natural dietary polyphenol component extracted from turmeric rhizomes, *Curcuma longa* Linn, a plant species found in Asian subcontinents. It is now used in several countries including the United States, India, Japan, Korea, Thailand, China, Tur-

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key, South Africa, Nepal, and Pakistan as a dietary supplement and in traditional herbal medicine (14, 15). The protective effects of CMN against various diseases such as cancer and autoimmune, neurological, metabolic, lung, liver, and cardiovascular diseases (CVDs) have been attributed to its antioxidant, antimicrobial, anti-inflammatory, anticancer, and antiangiogenic activities (14-17). It inhibits cancer cell survival and proliferation and induces apoptosis without promoting the development of side effects (14, 18). Although, the molecular basis for the CMN effects is not fully understood, this polyphenol has been suggested to have various promising therapeutic properties, particularly anti-tumor activity through multiple mechanisms (18).

Carotenoids belong to the teraterpene family, which is found in plants, algae, photosynthetic bacteria, fungi, and animals (19). They are a group of naturally occurring lipid-soluble compounds that have strong antioxidant effects in humans and animals. Over 700 naturally occurring carotenoids have been identified so far. Beta-carotene (BC) is one of the isoprenoid compounds named carotenoids. BC, a well-known natural antioxidant and precursor of vitamin A, is responsible for the dark green or yellow-orange color of fruits and vegetables and plays a key role in photosynthesis (19, 20). BC is used in the prevention and treatment of many diseases that develop with the participation of oxidative stress (mainly cancer and cardiovascular abnormalities) because of its free radical scavenging properties (21). However, it may also act as a tissue-damaging pro-oxidant depending on the physiologic environment (22). However, *in vitro* studies have shown the potent antioxidant activity of BC; thus, it protects biological systems against CDDP-induced damage (20).

The aim of the present study was to examine the protective role of CMN and BC on CDDP-induced cardiotoxicity by investigating the effects of CDDP, CMN, and BC on (a) antioxidant and oxidative stress indicators such as malondialdehyde (MDA) and superoxide dismutase (SOD) antioxidant enzyme namely catalase (CAT), (b) inflammatory cytokines like interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α), and (c) histopathological and immunohistochemical alterations in rat cardiac tissues.

Methods

Chemicals and drugs

CDDP, CMN, and BC were purchased from Sigma-Aldrich (Saint. Louis, MO, USA). Cisplatin was administered intraperitoneally (ip); CMN and BC were dissolved in sesame oil and given by gavage. All the solutions were prepared freshly before the experiment.

Animals

This study was performed at the Experimental and Clinical Research Centre of Erciyes University. All applications were performed under veterinarian control in accordance with the Uni-

versal Declaration of International Animal Rights after approval was received from Erciyes University, Clinical Research Ethics Committee (Approval protocol no. EUHADYEK-16/124).

A total of 49 8-week-old healthy adult female Wistar albino rats weighing between 190 and 250 gm were included. The rats were housed in separate clean plastic cages at a constant temperature of 24°C-26°C and humidity of 40%-60% with a constant 12 h light/dark cycle. Food and water were provided *ad libitum*. All treatments were started after almost 1 week of stabilization from arrival. The use of animals and the experimental protocol were approved by the Institutional Animal Care and Use Committee, and animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals of Research Council.

Treatment and experimental design

CDDP was dissolved in normal saline and injected at single doses of 5 mg/kg/week, which is well documented to induce cardiotoxicity in rats (23, 24). CMN was dissolved in sesame oil and was administered as 200 mg/kg BW (25). BC was dissolved in sesame oil and was administered as 100 mg/kg BW (26).

The rats were equally and randomly divided into seven groups with seven rats in each group as follows:

Group I (Control): rats did not receive any treatment until the end of the study.

Group II (sesame oil): rats received only sesame oil (1 mg/kg BW)

Group III (CDDP): rats received a single dose injection of CDDP two times as once a week (5 mg/kg BW/week, ip)

Group IV (BC): rats treated with BC (100 mg/kg BW) orally.

Group V (CDDP+BC): rats pretreated with BC for 30 min before CDDP injection, then received CDDP (5 mg/kg/week, ip)

Group VI (CMN): rats orally treated with CMN (200 mg/kg BW)

Group VII (CDDP+CMN): rats pretreated with CMN (200 mg/kg) for 30 min before CDDP injection, then received CDDP (5 mg/kg/week, ip).

All treatments were applied at the same time of day. The second administration was done 1 week after the first administration and the same injection and ip and/or gavage procedures as in the first administration were applied to the experimental groups for the second time.

Sampling

After 5 days from the second administration, rats were intraperitoneally anesthetized with a mixture of 50 mg/kg BW of ketamine hydrochloride 10% (Ketalar; Eczacıbaşı İlaç Sanayi ve Ticaret AŞ, Lüleburgaz, Turkey) and 10 mg/kg BW of xylazine hydrochloride 2% (Alfazyne; Alfasan International BV, Woerden, Netherlands). A middle-line incision was made in the chest, and the cardiac tissues were quickly removed and separated from the surrounding tissues and washed twice with an ice-cold phosphate-buffered saline solution (pH: 7.4). Samples from the left ventricle were collected and fixed in 10% buffered neutral

formalin solution for histopathological and immunohistochemical examinations. The other cardiac tissues were stored at -80°C to determine the level of MDA, CAT, and SOD. Then, all the rats were immediately sacrificed by exsanguination.

ELISA analysis

Cardiac tissues collected for enzyme-linked immunosorbent assay (ELISA) analyses were stored at -80°C until analyses were performed, at which time, the cardiac tissues were removed to room temperature and left to warm for 15 min and homogenized on ice-cold according to the procedures of the ELISA kit. After homogenization, the cardiac tissues were centrifuged at $+4^{\circ}\text{C}$ for 30 min at 12,000 rpm; the supernatants were then aliquoted and prepared for ELISA procedures.

Determination of MDA content

To investigate the cardiac oxidative damage caused by the induction of CDDP, the levels of MDA, a marker of lipid peroxidation were measured in the heart using a commercially available ELISA kit (MDA Elabscience® ELISA Kit Catalog no. E-EL-0060, USA) according to the manufacturer's instructions. The MDA level was assessed by measuring thiobarbituric-acid reactive substances at 540 nm and was expressed as nmol/g tissue for cardiac tissue homogenates.

Determination of CAT activity

The CAT activity was assayed using a commercially available assay kit (CAT Elabscience® ELISA Kit Catalog no. E-EL-R2456, USA) according to the manufacturer's instructions. This kit uses the peroxidase function of catalase to determine the enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2 . Absorbance was read at 540 nm using a plate reader. The CAT activity was expressed as U/g for cardiac tissue homogenates.

Determination of SOD activity

The SOD activity was measured in accordance with the protocol supplied by commercially available SOD assay kit (SOD Elabscience® ELISA Kit Catalog no. E-EL-R1424, USA). The assay used a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical, and the absorbance was read at 450 nm using an ELISA plate reader. The SOD activity was expressed as U/g protein for cardiac tissue homogenates.

Quantitative immunohistochemistry

The quantification of the IR intensity in both control and treated groups was included in the analysis. The cardiac tissues were taken from random ten of the visual fields for each section at an original magnification $\times 40$. The IL- 1β , IL-6, and TNF- α IR intensities for each cardiac sections were calculated with the mean of IR intensities using Image J software.

Histopathological evaluation

Tissue samples were fixed in 10% buffered neutral formalin solution and placed in fixation solution for 48 h and in water for one night. Next, tissue specimens were dehydrated by passing through increasing concentrations of alcohol, cleared by passing through xylol, and finally embedded in paraffin blocks. Paraffin sections of 5- μm slice thickness were cut from each specimen and placed on poly-lysine coated slides. Slides were deparaffinized, rehydrated through a graded series of ethanol, and rinsed in distilled water. The specimens were stained with haematoxylin-eosin (H&E) stain to evaluate the tissue morphology and integrity.

Immunohistochemistry

Immunohistochemical staining was performed using the avidin-biotin method to determine the differences in immunoreactivity (IR) intensity of IL- 1β , IL-6, and TNF- α . Briefly, 5- μm thick sections were rinsed in deionized water, and antigen retrieval was performed by incubation in 0.01 M sodium citrate buffer (pH: 6.0) at 300 W for 10 min and cooling to room temperature for 20 min. The sections were incubated in 3% H_2O_2 for 10 min and rinsed in phosphate-buffered saline (PBS). Anti-Polyvalent HRP kit (Thermo Scientific, USA) was used for the following steps. To reduce nonspecific staining, sections were pretreated with normal block serum for 20 min. After sections were incubated with the primary antibodies IL-1 (1: 100, NBP1-19-775, Littleton, USA), IL-6 (1: 100, EPP14346, Hubei, China), and TNF- α antibody (1: 50, sc.25280; Santa Cruz Biotechnology, Santa Cruz, CA) were incubated overnight at 4°C in a humidified chamber. After washing thrice for 5 min in PBS, sections were incubated with the biotinylated secondary antibodies for 15 min. After washing in PBS, 3, 3'-diaminobenzidine tetrahydrochloride (DAB) (Lab vision, UltraVision detection system Large volume DAB substrate system, TA-125-HD) was added as chromogen for 3-5 min at room temperature. After washing with deionized water, sections were counterstained with Gill's hematoxylin and mounted using a clear mounting medium (00-8010; Zymed Laboratories Inc. South San Francisco, CA). The specificity of the immunoreaction was confirmed by incubation with PBS instead of the primary antibodies.

Statistical analysis

Data were expressed as either median (min-max) values or mean standard deviation (SD) depending upon the overall variable distribution. A computer program SPSS Version 15.0 (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis. Normally distributed data were analyzed by one-way analysis of variance (ANOVA) test followed by Tukey post-hoc test. Non-normally distributed data were compared by nonparametric Kruskal-Wallis H test followed by Dunn-Bonferroni test among the groups. Differences were considered to be significant at $p < 0.05$.

Table 1. Comparison of the oxidative/antioxidant status in cardiac tissue of each experimental group

Parameters	Experimental Groups							P value
	Control mean±SD	Sesame oil mean±SD	CDDP mean±SD	BC mean±SD	CDDP+BC mean±SD	CMN mean±SD	CDDP+CMN mean±SD	
MDA (nmol/g tissue)	29.239±0.91 ^a	28.562±1.09 ^a	33.274±1.35 ^b	30.383±2.13 ^{a,b}	31.528±1.24 ^{a,b}	27.701±1.60 ^a	28.748±1.12 ^{a,b}	0.187
SOD (U/g tissue)	51.815±1.39 ^a	51.021±3.02 ^a	27.197±2.56 ^c	45.441±3.05 ^{a,b}	30.335±3.42 ^{c,d}	43.263±3.46 ^{a,b,d}	36.460±3.21 ^{b,d}	0.000
CAT (U/g tissue)	62.089±3.46 ^a	53.849±1.65 ^a	28.018±4.82 ^c	48.550±2.17 ^b	35.771±3.50 ^{c,d,e,f}	45.258±3.45 ^{b,d}	42.575±4.72 ^{b,d,e}	0.000

CDDP - Cisplatin; BC - beta-carotene; CMN - curcumin; MDA - malondialdehyde; CAT - catalase; SOD - superoxide dismutase
Values are expressed as mean±SD for seven rats in each group. One-way ANOVA test was also used for comparisons. Tukey test was applied for multiple comparisons. Similar letters on the same line indicate similarity between groups, whereas different letters indicate differences between groups. $P < 0.05$ was considered significant

Results

Biochemical findings

Table 1 summarizes the results of the biochemical parameters of oxidative stress, namely MDA, SOD activity, and antioxidant enzymes namely SOD and CAT activity in rat cardiac tissues in each experimental group.

The MDA levels were significantly higher in the CDDP group than in the control ($p=0.025$), sesame oil ($p=0.018$) and CMN ($p=0.035$) groups. Those levels were found to be much lower in the CDDP+CMN and CDDP+BC groups than in the CDDP group, which was not statistically significant ($p>0.05$) (Table 1).

The SOD activity in the CDDP group was significantly lower than that in the control ($p=0.04$), sesame oil ($p=0.004$), BC ($p=0.004$), CMN ($p=0.004$), and CDDP+CMN ($p=0.025$) groups, but this decrease was not statistically significant in the CDDP+BC group ($p>0.05$). Also, the SOD levels in the CDDP+BC group were significantly lower than those in the control ($p=0.004$), sesame oil ($p=0.004$), and BC ($p=0.025$) groups. As for the CDDP+CMN group, the SOD levels were significantly lower than those in the control ($p=0.004$), sesame oil ($p=0.016$), and BC ($p=0.025$) groups. However, the cardiac SOD levels in the groups treated with only BC or CMN did not significantly differ with those in the control and sesame oil groups ($p>0.05$) (Table 1).

The CAT activity in the cardiac tissue was significantly impaired by the induction of CDDP. The CAT activity in the CDDP group was significantly lower than that in the control ($p=0.004$), sesame oil ($p=0.004$), CMN ($p=0.006$), BC ($p=0.006$), and CDDP+CMN ($p=0.006$) groups, but this decrease was not statistically significant except in the CDDP+BC group ($p>0.05$). Also, the CAT levels in the CDDP+BC group were significantly lower than those in the control ($p=0.004$), sesame oil ($p=0.004$), and BC ($p=0.016$) groups. As for the CDDP+CMN group, the CAT levels were significantly lower than those in the control ($p=0.010$), sesame oil ($p=0.016$), and CDDP ($p=0.006$) groups. Meanwhile, in the control and sesame oil groups, the CAT activities were sig-

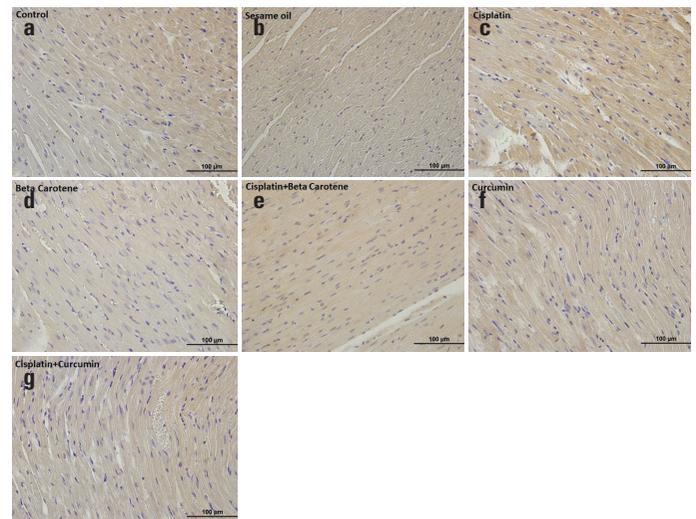


Figure 1. Representative photomicrographs of cardiac immunostaining for IL-1 β in experimental groups. (a) Control group (b) Sesame oil group demonstrated weak IL-1 β immunoreaction (c) Cisplatin group showing dense IL-1 β expression (d) Only β -carotene treated group (e) cisplatin+ β -carotene group showing mild IL-1 β expression (f) Only curcumin-treated group (g) cisplatin+curcumin group treated group showing mild staining of IL-1 β in myocardial fibers. IL-1 β immunohistochemical staining of heart samples from the experimental groups (Original magnification=40x, Scale bar=100 μ m)

nificantly higher than those in the BC and CMN groups ($p=0.016$ for each). Antioxidant treatment with BC ($p=0.006$) and especially with CMN ($p=0.004$) demonstrated a tendency to increase the CAT activity and this increase was statistically significant in the CDDP+CMN group (Table 1).

Immunohistochemical findings

IL-1 β expression

Sections stained with IL-1 β primary antibody are represented in Table 2 and Figure 1. Diffuse and cytoplasmic stainings were assessed in the myocardial tissue slides. Some of the myocardial fibers have demonstrated moderate expressions of IL-1 β in the

Table 2. Comparison of the TNF- α , IL-1 β , and IL-6 levels in cardiac tissue of each experimental group

Parameters	Experimental Groups							P value
	Control median (25%-75%)	Sesame oil median (25%-75%)	CDDP median (25%-75%)	BC median (25%-75%)	CDDP+BC median (25%-75%)	CMN median (25%-75%)	CDDP+CMN median (25%-75%)	
IL-1 β	79.31 (68.10-90.72) ^a	81.95 (72.43-88.51) ^a	89.26 (84.86-99.52) ^b	81.51 (68.17-89.61) ^{a,c}	88.54 (76.94-98.71) ^b	80.50 (73.61-95.28) ^a	85.02 (69.60-93.67) ^c	0.000
IL-6	59.65 (47.60-68.60) ^a	64.40 (47.40-76.19) ^a	74.98 (52.35-99.63) ^b	59.74 (50.32-69.51) ^c	62.90 (56.57-67.00) ^d	62.57 (47.85-78.25) ^c	58.92 (50.77-69.80) ^d	0.000
TNF- α	61.29 (48.59-73.68) ^a	61.00 (50.34-77.04) ^a	70.59 (55.00-79.99) ^b	68.49 (52.33-83.30) ^b	70.23 (61.02-78.82) ^b	66.70 (59.43-76.91) ^{a,b}	59.55 (53.18-78.37) ^a	0.000

CDDP - cisplatin; BC - beta-carotene; CMN - curcumin; TNF- α - tumor necrosis factor-alpha; IL-1 β - interleukin-1 beta; IL-6 - interleukin-6
 Data are expressed as median (min.-max.) for seven rats in each group. Kruskal-Wallis analysis was used to compare the groups. Dunn-Bonferroni test was applied for multiple comparisons. Similar letters on the same line indicate similarity between groups, whereas different letters indicate differences between groups. $P < 0.05$ was considered significant.

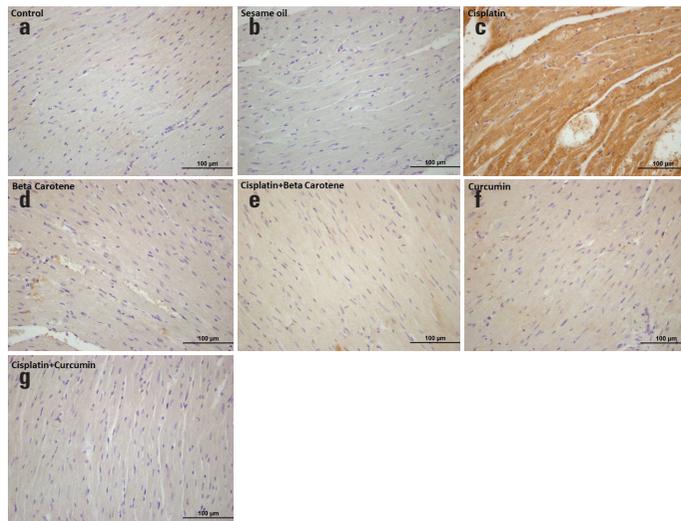


Figure 2. Representative photomicrographs of cardiac immunostaining for IL-6 in experimental groups. (a) Control group (b) Sesame oil group demonstrated weak IL-6 immunoreaction (c) Cisplatin group showing more denser IL-6 expression (d) Only β -carotene treated group (e) cisplatin+ β -carotene group showing mild IL-6 expression like (f) Only curcumin-treated group and (g) cisplatin+ curcumin group. IL-6 immunohistochemical staining of heart samples from the experimental groups (Original magnification=40x, Scale bar=100 μ m)

control (Fig. 1a) and sesame oil (Fig. 1a) groups. In only the CDDP group (Fig. 1c), the expression of antiapoptotic protein IL-1 β was higher than the control (Fig. 1a), sesame oil (Fig. 1b), BC (Fig. 1d) and CMN (Fig. 1f) groups ($p=0.001$ for each). The IL-1 β IR intensity in the CDDP+BC group (Fig. 1e) was similar to that in the CDDP group (Fig. 1c), but was significantly higher than in the control (Fig. 1a), sesame oil (Fig. 1b), CMN (Fig. 1f), and BC (Fig. 1d) groups ($p=0.001$ for each). Meanwhile, in the CDDP+CMN group (Fig. 1g), the IL-1 β IR intensity was higher than that in both control (Fig. 1a) and sesame oil (Fig. 1b) groups ($p=0.05$ for each). However, tissue samples of the CDDP+CMN group (Fig. 1g) exhibited lower IR intensity than both the CDDP+BC (Fig. 1e) ($p=0.017$) and CDDP

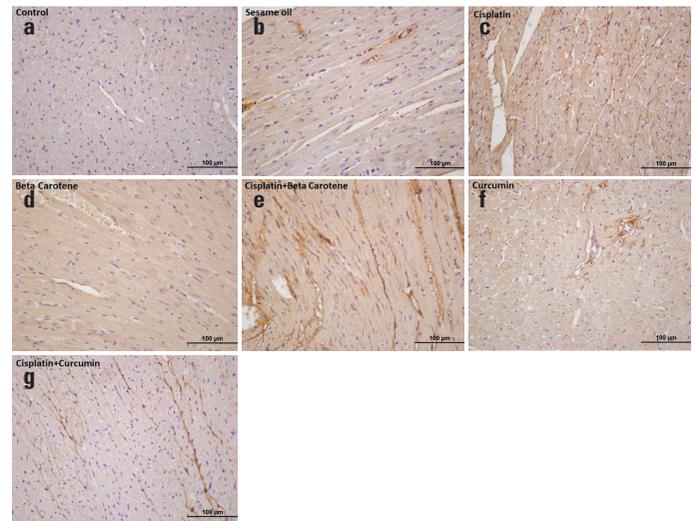


Figure 3. Representative photomicrographs of cardiac immunostaining for TNF- α in experimental groups. (a) TNF- α immunoreactivity was weak in the control group (b) Sesame oil group. (c) TNF- α immunoreactivity intensity of myocardial fibers in cisplatin group was higher than the control group (d) TNF- α immunoreactivity of only β -carotene treated group (e) cisplatin+ β -carotene group was lower when the compared to cisplatin group (f) Only curcumin-treated group (g) cisplatin+ curcumin group showed TNF- α immunoreactivity decreased in the cardiac tissues compared to cisplatin group. TNF- α immunohistochemical staining of heart samples from the experimental groups (Original magnification=40x, Scale bar=100 μ m)

(Fig. 1c) ($p=0.001$) groups. Furthermore, in the samples from only BC (Fig. 1d) and CMN (Fig. 1f) administered groups, there were moderate expressions of IL-1 β ($p > 0.05$) (Table 2).

IL-6 expression

We confirmed the expression of IL-6 by immunohistochemistry (Table 2 and Fig. 2). Immunostaining of IL-6 expressions demonstrated moderate staining in the control (Fig. 2a) and sesame oil (Fig. 2b) groups compared with the CDDP group (Fig. 2c). The

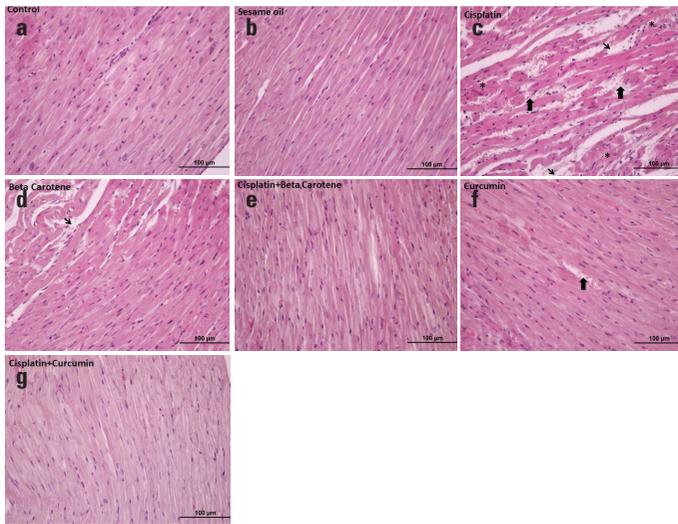


Figure 4. The morphology of the heart tissue. The images were representatives the H&E-stained sections of heart from experimental groups (a) Sagittal sections of the rat cardiac tissue showing the normal structure in control group (b) Only sesame oil treated group (c) Cisplatin-injured cardiac tissue obtained from cisplatin group (d) Only β -carotene treated group (e) The cardiac tissue obtained from the cisplatin+ β -carotene group treated with β -carotene (f) Only curcumin-treated group (g) The cardiac tissue obtained from cisplatin+ curcumin group treated with curcumin (Original magnification=40x, Scale bar=100 μ m) Thick arrow, hemorrhage Arrow, interstitial edema Star, muscle fiber damage and cytoplasmic vacuolization

IR intensity of IL-6 in the CDDP group was significantly higher (Fig. 2c) than that in the control (Fig. 2a), sesame oil (Fig. 2b), BC (Fig. 2d), CDDP+BC (Fig. 2e) and CMN (Fig. 2f), CDDP+CMN (Fig. 2g) groups ($p=0.001$ for control, sesame oil, BC, CMN, and CDDP+CMN groups; $p=0.006$ for CDDP+BC group). In other words, the IR intensity of IL-6 was significantly decreased in the CDDP+CMN (Fig. 2g) and CDDP+BC (Fig. 2e) groups compared with the CDDP group ($p<0.01$). However, IL-6 IR showed no significant change between the BC (Fig. 2d) and CMN (Fig. 2f) groups ($p>0.05$). Also, the IL-6 expression was reduced in the CDDP+CMN group (Fig. 2g) compared with the CDDP+BC group (Fig. 2e), but the result was not statistically significant ($p>0.05$) (Table 2).

TNF- α expression

Regarding TNF- α IR, the values were significantly lower in the control (Fig. 3a) and sesame oil (Fig. 3b) groups than in the CDDP group (Fig. 3c) ($p=0.001$ for each) Also, the IR intensity of TNF- α in the CDDP+CMN group (Fig. 3g) was significantly lower than that in the CDDP group (Fig. 3c) ($p=0.001$); the IR intensity of TNF- α in the CDDP+BC group (Fig. 3e) was significantly higher than that in the control group (Fig. 3a) ($p=0.001$). In the CDDP+CMN group (Fig. 3g), the TNF- α IR was significantly lower than that in both CDDP+BC (Fig. 3e) ($p=0.001$) and BC (Fig. 3d) ($p=0.005$ for each) groups. However, in the BC group (Fig. 3d) TNF- α IR was significantly higher than that in both control (Fig. 3a) ($p=0.008$) sesame oil (Fig. 3b) groups ($p=0.015$ for each). Also,

in the CDDP+BC group (Fig. 3e), TNF- α IR was significantly higher than that in the sesame oil (Fig. 3b) group ($p=0.001$). TNF- α IR did not manifest increased immunostaining patterns only in the BC (Fig. 3d) and CMN (Fig. 3f) groups ($p>0.05$). The expression intensity of TNF- α was almost never reduced in the CDDP+BC group (Fig. 3e), whereas it showed a statistically significant decrease in the CDDP+CMN group (Fig. 3g) compared with the CDDP+BC group (Fig. 3e) ($p=0.001$) (Table 2).

Histological findings

The general architecture of the cardiac tissues from animals in the control group appeared as regularly arranged cardiac myofibers that had several myocytes consisting mainly of single ovoid and centrally located nuclei (Fig. 4a). The histology of the heart of the sesame oil group was similar to that of the control group (Fig. 4b). In the CDDP groups, normal cardiac architecture was not observed. Microscopic examination of the heart after CDDP administration showed several instances of muscle fiber damage and cytoplasmic vacuolization, hemorrhage, and interstitial edema on section profiles compared with control group (Fig. 4c). However, similar histologic findings such as cytoplasmic vacuolization, hemorrhage, and edema at a more moderate level were observed in only the BC (Fig. 4d) and CMN-treated (Fig. 4f) groups. Interestingly, both CDDP+BC (Fig. 4e) and CDDP+CMN (Fig. 4g) groups showed an improved histological appearance compared with CDDP group. Histopathological changes in the CDDP group were not significantly obvious according to these groups.

Discussion

Cellular protection against the toxic effects of chemotherapeutic agents is considered as an essential target and is the objective of several studies on dietary antioxidants supplementation. Several medicinal plants and/or essential oils may be used as chemopreventive agents because they contain natural antioxidant active constituents and also prevent the generation of free radicals that may influence the progression of oxidative damage induced by chemotherapeutic agents (3, 4, 18, 27). The present study was conducted to assess the effect of the chronic supplementation of CMN and BC on CDDP-induced cardiotoxicity, to evaluate oxidative stress markers and antioxidant molecules, including MDA, SOD, and CAT, and proinflammatory cytokines like IL-1 β , IL-6, and TNF- α in rat cardiac tissues. Additionally, we evaluated the histological and immunohistochemical alterations as well as biochemical and proinflammatory parameters. To the best of our knowledge, there is no report showing that both CMN and BC ameliorated CDDP-induced cardiotoxicity in rats.

Some studies have been indicated that CDDP decreased antioxidant enzymes activities (CAT, SOD, glutathione S-transferase; GST, glutathione peroxidase; GSH-Px,) and increased MDA levels (1, 3, 5, 7). However, it has been demonstrated that CMN increased the reduced level of CAT, SOD, GST, and GSH and

decreased the elevated level of MDA and lipid peroxidase in rat cardiac tissues against experimental-induced different cardiotoxicity models, except for CDDP-induced cardiotoxicity model in rats (16, 28). Regarding the CDDP-induced cardiotoxicity model, Uday Kiran et al. (29) documented that the elevated levels of MDA clearly indicate the myocardial damage with a significant reduction in the GSH and CAT levels in the CDDP-treated rat groups. However, BC-treated rats showed a significant decrease in lipid peroxidation in both prophylactic and curative groups compared with the CDDP group. Thus, they suggested that BC has a protective role against CDDP-induced cardiotoxicity because of its antioxidant and radical scavenging properties. Other studies have also reported a decrease in activities of GSH-Px and CAT and an increase in MDA levels in the cardiac tissues of CDDP-treated rats (1, 7, 27). In the current study, a significant increase in MDA level was observed in the cardiac tissues in the CDDP-treated group compared with the control, sesame, and CMN groups as adherence to previous studies (27, 30). A possible explanation for the enhancement of MDA concentration may be decreased formation of antioxidants in the CDDP-induced tissues, which in view of the augmented activity of reactive oxygen species allowed a consequent increase in MDA production (27). However, we noted that CDDP-induced elevation of MDA level was reversed by CMN treatment compared with the CDDP+CMN group, this reduction was not found statistically different. This decrease was also observed in the BC group, but was not significant. Our results are in line with the results of the previous studies (27, 30). Thus, our study confirmed the CDDP-induced oxidative stress by causing a marked increase in the MDA level in the cardiac tissue.

In terms of the SOD activity, it was marginally decreased in the cardiac tissues of CDDP-treated animals compared with other groups, except for the CDDP+BC groups, in agreement with the literature (1, 5). On the other hand, CMN and BC significantly improved CDDP-induced reduction of the SOD activity in heart consistent with the results of Oz et al.'s (30) study in brain tissues.

Additionally, the CAT activities in the CDDP-treated group rats were lower than those in the control, sesame oil, CMN, BC, and CDDP+CMN groups. When SOD and CAT levels were compared between the CDDP and control groups, significant differences were observed. Our study showed that both CMN and BC were effective in increasing SOD and CAT levels. All these findings indicated the preventive effects of these antioxidants on the CDDP-induced cardiac injury.

Tumor cells exhibit an elevation in the constitutive production of several proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6. They are important mediators of acute inflammation and play decisive roles at different stages of tumor development, including initiation, promotion, malignant conversion, invasion, and metastasis (31). It has also been demonstrated that myocyte necrosis and apoptosis can be linked to elevated proinflammatory cytokine levels, such as IL-1 β , TNF- α , and interferon- γ (32). The CDDP treatment also enhances the TNF- α and IL-6 cytokine levels (3, 7, 17). Additionally, many studies reported that stimu-

lation of the inflammatory response in vivo lead to exacerbated CDDP-induced cardiotoxicity (33), and the inhibition of TNF- α activity and deficiency of TNF- α have demonstrated protection from CDDP toxicity (34). Thus, the inhibition or suppression of proinflammatory cytokines during CDDP treatment could prevent the influx of macrophages and neutrophils and could limit the inflammatory process, thus protecting the heart against CDDP toxicity (11).

However, CMN inhibits the expression of various inflammatory cytokines such as TNF- α , ILs-1, 2, 6, 8, and 12, interferon γ , monocyte chemoattractant protein (MCP), and migration of inhibitory protein, oxidative stress markers, some other chemokines by multiple genes/pathways involved in apoptosis, cell invasion, and adhesion (35). Moreover, it has been postulated that CMN is a TNF- α expression blocker due to its direct union to TNF- α (36). In addition, CMN showed noticeable resistance to cardiac hypertrophy caused by banding of the aorta, and the progression of heart failure was decreased by NF- κ B activation and monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-1 β , and TNF- α mRNA and protein expression induced by aortic banding (37). Also, Song et al. (38) demonstrated that CMN reduces the systemic and local expressions of NF- κ B, IL-6, and IL-1 β in the myocardium as evidenced by ameliorated symptoms of myocarditis. In a different study by Wang et al. (39), CMN downregulated the levels of proinflammatory cytokines in the ischemic heart, which is concomitant with reduced TNF- α and IL-6 levels, infarct size, and ameliorated ischemic injury. These studies suggest that CMN can restrain the expression or activity of proinflammatory cytokines to protect the heart (16). Besides, there are numerous studies demonstrating the association of IL-6 downregulation and/or of IL-6 inhibition signaling with therapeutic effects of CMN suggesting a role for modulation of IL-6 in anti-inflammatory effects of CMN. So, CMN can be considered as a potential therapy against IL-6 involved pathologic status (40). In agreement with these results, we found that the TNF- α , IL-1 β , and IL-6 expressions were significantly increased in the cardiac tissues of the CDDP-treated rats. Also, CMN significantly reduced the intensities of CDDP-induced increased TNF- α , IL-1 β , and IL-6 immunoreactivity in accord with the results of Kumar et al. (17) in kidney tissues. Thus, our results suggested that CMN has a higher cardioprotective effect than BC in terms of TNF- α , IL-1 β , and IL-6 expression intensities.

According to the histopathological findings, the CDDP-treated group showed prominent histopathological changes including muscle fiber damage, cytoplasmic vacuolization, hemorrhage, and interstitial edema in cardiac tissues in this study. Similar findings including severe myocardial degenerative changes associated with dark stained pyknotic nuclei and cardiac hypertrophy were previously observed after CDDP administration (3, 5, 7, 41).

CMN may effectively prevent tissue damage to the heart by decreasing the oxidative stress and restoring the antioxidant status (42). Kim et al. (43) reported that the cardioprotective effects of CMN on myocardial ischemia-reperfusion injury rat

model could exhibit anti-inflammatory activities and inhibition of apoptosis that occurred in the cardiomyocytes. Also, there are several pieces of evidence suggesting that an increased intake of antioxidants, vitamins, ascorbic acid (vitamins C), tocopherols (vitamin E), and beta-carotene (vitamin A), has some protective role in coronary heart disease and CVDs (29, 44). Although previous studies have indicated that BC was able to inhibit the growth of cancer cells (45, 46), it has been reported that lung cancer risk is increased in smokers and breast cancer risk is increased in women by high-dose BC consumption. Accordingly, it has adverse effects on the risk of death from CVDs mainly among smokers (45, 47). Thus, BC has both beneficial and potentially harmful effects in the cardiovascular system because of its antioxidant property. Several clinical studies investigated the cardiovascular effects of BC, but all these results are rather controversial (21). In the present study, it was found that CMN or BC exhibited counteraction against the mentioned changes associated with CDDP administration. Besides, normal myocardial morphology structure was observed in rats pretreated with CMN. According to our histopathological findings, BC and especially CMN have been found to possess cardioprotective activities.

In the current study, CDDP-induced obvious cardiac injuries were demonstrated by biochemical, histopathological, and immunohistochemical alterations. These alterations included decreased CAT and SOD activities, increased MDA level, and elevated immunoreactivity intensity of IL-1 β , IL-6 and TNF- α . Histopathologically, CDDP-induced severe myocardial degenerative changes were observed. Consequently, results of this study confirmed certain cardioprotective effects of CMN and BC on CDDP-induced cardiotoxicity in isolated rats. The antioxidative properties of CMN and BC have shown beneficial influence on ameliorating effects of chronic CDDP treatment, as the consequence of oxidative damage, by means of improvement of oxidative status and attenuation of morphological changes in isolated rat tissue. These results could recommend CMN and BC supplementation as a potential protection against CDDP-induced cardiotoxicity.

Study limitations

Our study had some limitations. Cardiac injury biomarkers such as creatine kinase (CK), creatine kinase isoenzyme MB (CK-MB), lactate dehydrogenase (LDH) activities, plasma cardiac troponin I (cTnI) concentration, and glutathione (GSH) contents could not be evaluated due to the limited facilities in our study. In addition, we could not estimate other oxidative stress parameters such as tissue antioxidant capacity, total oxidative status, and oxidative stress index levels.

Conclusion

The results of the current study highlight the significant impact of histopathological, biochemical, and cytokine changes in cardiotoxicity induced by CDDP treatment. Furthermore, our find-

ings show that the use of CMN or BC before CDDP may be effective for protection against cardiac damage in CDDP-treated animals. However, CMN has a more remarkable ameliorative effect against the CDDP-induced cardiotoxicity than BC. Hence, CMN may be an excellent choice for cardioprotection against CDDP-induced cardiotoxicity.

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