Effects of Morellofavone from *Garcinia dulcis* on the Relaxation Response, Malondialdehyde Levels and Histology of Cisplatin-Treated Rat Aorta

Adha Sumalee, Wilawan Mahabusarakam**†, Pornpimol Kiriratǂ , Siriphun Hiranyachattada**

Abstract

This study aimed to investigate an acute and protective effect of morelloflavone isolated from *Garcinia dulcis* on the relaxation and structural damage of thoracic aorta, and the oxidative status of cisplatin-treated rat. Male Wistar rats were used and either cisplatin (7.5mg/kg) or vehicle (0.9% NaCl) was given intraperitoneally. An acute vasorelaxing effect of morelloflavone (10^{-12} -10^{-5} M) and its mechanism of action were tested in isolated thoracic aortic rings of control (intact endothelium and denuded) and of cisplatin-treated rats on day 7 after the injection. To study the protective role of morelloflavone in cisplatin-treated rat, the animals were further divided into three groups, vehicle control, cisplatin- and cisplatin+morelloflavone-treated group. Morelloflavone (0.1, 1, 10 mg/kg, i.p.) were given twice, 1 day and 10 minutes before cisplatin injection. Seven days after the treatment, the contractile response of isolated thoracic aorta to 10^{-10} -10^{-5} M phenylephrine (PE) and the vasorelaxing responses to 10^{-12}-10^{-5} M acetylcholine (ACh) and sodium nitroprusside (SNP) were investigated along with plasma malondialdehyde (MDA) level determination and histological study. It was found that the vasorelaxing effect of morelloflavone cannot be observed in denuded control aortic rings and found less in cisplatin-treated groups than in the endothelium-intact control group. The contraction response to PE was significantly higher in cisplatin-treated group, whereas the relaxation response to ACh was found significantly lower when compared to control. Morelloflavone (1 mg/kg) treatment was able to improve the contractile responses to PE, and the vasorelaxing responses to ACh. Histological examination revealed that the tunica media proliferation in cisplatin group was restored by morelloflavone treatment. Plasma MDA level which increased significantly in cisplatin group was also supressed by morelloflavone treatment. It is concluded that morelloflavone possesses free radical scavenging property *in vivo* and can act as a vasodilator in which its mechanism of action is likely to involve endothelium-dependent nitric oxide signaling pathway.

J Physiol Biomed Sci. 2017; 30(1): 23-30.

Keywords: *Cisplatin, endothelium, morelloflavone, thoracic aortic ring*

Introduction

Cisplatin, or *cis*-diaminedichloroplatinum (II), is a platinum coordinate compound consisting of two molecules of ammonia and two groups of chlorides.[1] It is one of the most widely used drugs for cancer chemotherapy. However, cisplatin has been reported to cause a variety of cardiovascular side effects, such as stroke,[2] myocardial infarction,[3] arterial and venous thromboembolism,[4] enhancement of carotid artery intima media thickness,[5] coronary artery dissection,[6] and hypertension.[7] One of the molecular mechanisms of cisplatin action that causes the cardiovascular adverse effects has been shown to involve the generation of reactive oxygen and nitrogen species.[8]

The use of plant compounds as the scavengers of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been widely researched both *in vivo* and *in vitro*. Morelloflavone, a biflavonoid compound extracted from *Garcinia dulcis* (Kurz), has been shown to possess several biological activities, such as anti-HIV activity by inhibiting both HIV-1 reverse transcriptase (HIV-1RT*) in vitro* and HIV-1 (strain LAV-1) in human lymphocytes,[9] anti-inflammation by inhibiting secretory phospholipase A_2 in mouse ear homogenate.[10] Morelloflavone was reported to exhibit strong antioxidation effects in both Fe^{2+}-mediated and non-metal-induced human low-density lipoprotein (LDL) oxidations in rat.[11] Other effects of morelloflavone included an ability to reduce plasma lipid levels and prevent the formation of atherosclerosis plague in hypercholesterolemia rabbit.[12] Morelloflavone can block the injury-induced neointimal formation by inhibiting vascular smooth muscle cell migration via a decrease in phosphorylation of ERK, FAK, c-Src and Rho activity.[13] It is also reported that this plant compound can inhibit

*Department of Physiology, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla 90112, Thailand;
**Department of Chemistry Faculty of Science, Prince of Songkla University, Hatyai, Songkhla 90112, Thailand;
†Natural Product Research Center of Excellence, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla 90112, Thailand;
ǂDepartment of Anatomy, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla 90112, Thailand.

Corresponding author: Siriphun Hiranyachattada, PhD
E-mail: siriphun.h@psu.ac.th

Received 5 June 2017

angiogenesis, the pivotal step in tumor growth by targeting Rho GTPases and ERK signaling pathways.[14] In addition, morelloflavone can inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) *in vitro*, suggesting its possible activity in lowering plasma cholesterol.[15]

According to morelloflavone antioxidant property, this study aimed to investigate, 1) an acute effect of morelloflavone on the contraction of isolated rat thoracic aorta of cisplatin-treated rats and compared with control (endothelium intact and denuded), and 2) the protective effect of morelloflavone against cisplatin-induced impairment of rat thoracic aorta. The dose of cisplatin (7.5 mg/kg BW i.p.) was chosen based on its minimal dose that induced nephrotoxicity.[16] The acute effects of morelloflavone on the relaxation responses of isolated thoracic aortic rings were evaluated and compared between control and cisplatin-treated rats by cumulative addition of 10^{-12}-10^{-5} M morelloflavone using organ bath technique. To study the protective role of morelloflavone on aortic ring contraction of cisplatin-treated rat, three doses of morelloflavone (0.1, 1 and 10 mg/kg body weight) were given twice, 24 hour and 10 minutes before cisplatin injection. Then, the contraction and relaxation responses of isolated thoracic rings were performed on day 7 after cisplatin injection by cumulative addition of vasoconstrictor (10^{-10}-10^{-5} M phenylephrine, PE) or vasodilators (10^{-10}-10^{-5} M acetylcholine and sodium nitroprusside) and compared with respective vehicle control groups. The structural damage of the aorta was examined by histopathological study. The oxidative status of the animals was evaluated by measuring plasma malondialdehyde (MDA) levels.

Materials and Methods

Extraction of morelloflavone from *Garcinia dulcis*

The fresh ripe fruits (3 kg) of *G. dulcis* were collected from Songkhla province, Thailand. The voucher specimen (Collection. No. 02, Herbarium No. 0012652) has been placed at Prince of Songkla University Herbarium, Department of Biology, Faculty of Science, Prince of Songkla University, Thailand. The fruits were washed, chopped and then immersed in acetone (Me_2CO) for 5 days at room temperature. Acetone was removed by evaporation to give a liquid extract that was partitioned with hexane followed by ethyl acetate (EtOAc) to give solid extract. Then, the solid was further fractioned by dissolving in dichloromethane (CH_2Cl_2) to give CH_2Cl_2 soluble. The soluble was subjected to chromatography using CH_2Cl_2-methanol (MeOH) to generated morelloflavone (35.2 mg), a biflavonoids consisting of apigenin and luteolin (molecular weight 556) as described previously.[17]

Reagents

Phenylephrine (PE), acetylcholine (ACh), sodium nitroprusside (SNP), and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Cisplatin and thiopental sodium were obtained from Pfizer Pty Limited, Bentley, Australia and Scott-Edil Pharmacia Ltd., Soltan (H.P.), India, respectively.

Animals

Male Wistar rats (body weight, BW, 250-300 g) were supplied by Laboratory Animal Facility Unit, Prince of Songkla University, Hatyai, Thailand. Rats were housed under controlled condition (temperature 23-25ºC, relative humidity 50-55% and 12 hr light-dark cycle). They were given a commercial animal feed (S.W.T., Thailand) and free access to reverse osmosis water ad-libitum. All experiments were approved by the Prince of Songkla University Animal Ethics Committee (Reference No. 20/2015).

Experimental design

Acute vasorelaxation effects of morelloflavone were determined by cumulative addition of 10^{-12} -10^{-5} M morelloflavone in the isolated thoracic aortic rings of control (intact endothelium and denuded) and cisplatin-treated groups (n = 6 each). The dose of cisplatin for intraperitoneal injection was 7.5 mg/kg BW. The experiments were performed on day 7 after cisplatin (7.5 mg/kg BW) intraperitoneal injection. 0.9% NaCl was used as cisplatin solvent and as vehicle control.

To study the protective role of morelloflavone on cisplatin-induced vascular toxicity, rats were divided into three main groups: 1) vehicle control (Con); 2) cisplatin (Cis); and 3) cisplatin + morelloflavone (Cis+Mor) group (n = 6-8 each). The dose of cisplatin for intraperitoneal injection was 7.5 mg/kg BW. Morelloflavone, either 0.1, 1 or 10 mg/kg BW was given twice, at 24 hours and 10 minutes before cisplatin injection. DMSO and 0.9% NaCl were used as morelloflavone and cisplatin solvent, respectively. The contraction and relaxation responses to PE, ACh, and SNP were investigated in isolated thoracic aortic rings along with the determination of plasma MDA level and histopathological study.

Preparation of thoracic aortic rings

Seven days after cisplatin injection, rats were anaesthetized by i.p. injection of thiopental sodium at the dose of 60 mg/kg. Then, blood samples (4-5 ml) were collected by heart puncture and plasma were separated by centrifugation at 4,000 rpm for 10 minutes and stored at -70°C until MDA analysis. The animals were sacrificed by decapitation and thoracic aorta was dissected and placed in a 4°C Krebs-Henseleit solution which was composed of (mM): KCl, 4.6; $MgSO_4.7H_2O$, 1.12; KH_2PO_4, 1.18; $NaHCO_3$, 25.0; D-glucose, 11.66; NaCl, 118.41; $CaCl_2$, 1.90. The pH of the Krebs-Henseleit solution was maintained at 7.4 by continuous bubbling in the bath with 95% O_2 and 5% CO_2. The aortas were then cut into ring pieces of 4-5 mm in length for

contractile response measurement (4 pieces) and 2 mm for histopathological study (2 pieces).

Measurement of the relaxation responses of thoracic aortic rings to morelloflavone and vasoactive agents

The aortic rings were mounted in 20-ml organ baths containing the Krebs-Henseleit solution. The bath solution was maintained at 37.0°C and bubbled continuously with a carbogen gas mixture of 95% O_2 and 5% CO_2. The rings were equilibrated for 45 minutes at a resting tension of 1 g. Isometric tension was measured with force isometric transducers FT03 (Grass Instruments, Astro-Med, Rhode Island, USA) connected to the Labchart 7 system (AD Instruments, Colorado, USA). In the control group, the rings were pre-constricted by the addition of PE (10^{-7} M) then tested for endothelial presence with 10^{-5} M ACh and was accepted at 80% relaxation. In some aortic rings, the endothelium was mechanically removed. The removal of endothelial cells was confirmed by the loss of 10^{-5} M ACh-induced relaxation.

To investigate the effect of morelloflavone on the contraction of aortic rings of cisplatin-treated rats, precontraction of aortic rings from control (intact endothelium and denuded) and cisplatin rats were performed by addition of 10^{-7} M PE into the bath, relaxation was evaluated by cumulative addition of 10^{-12}-10^{-5} M morelloflavone. After each addition, the steady-state phase (3-5 minutes) were obtained before reading the tension.

To test the contractile response to vasoactive reagent, 10^{-10}-10^{-5} M PE were added cumulatively. After each addition, the steady-state phases were obtained before reading the tension. After a 30-minute washout with Krebs-Henseleit buffer, aortic rings were returned to and stabilized at a resting tension of 1 g for 30 minutes for the next experiment. To test the responses to vasodilating agents, 10^{-12}-10^{-5} M ACh and SNP were added cumulatively after the rings were precontracted with 10^{-7} PE. After each addition, the steady-state phases were again obtained before reading the tension.

Measurement of plasma MDA

Lipid peroxidation was determined in plasma by measuring the levels of thiobarbituric acid reactive substances (TBARS) measured as malondialdehyde (MDA), according to the method of Ohkawa (1979). Plasma were mixed with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid then pH was adjusted to 3.5 with NaOH and 1.5 ml of a 0.8% thiobarbituric acid were added. The mixture was heated in 95°C water bath for 60 minutes. Then it was cooled in an ice bath, and 1.0 ml of DW and 5.0 ml of n-butanol solution were added and was shaken vigorously. After centrifugation at 4,000 rpm for 10 minutes, the absorbance was measured at 532 nm with the spectrophotometer (model Spectro SC, Labomed, Culver City, California, USA). MDA levels were determined from standard curve generated by acid hydrolysis of 1,1,3,4-tetramethoxy-propane. Plasma MDA was expressed as μmol/l.

Histological examination

The two thoracic aortic rings (2 mm each) were fixed in Bouin's solution for 48 hours and embedded in paraffin and serially cut into 6 μm thickness slices. Each tissue slide was stained with Mayer's hematoxylin-eosin (H&E). The tissue slides were then examined under light microscopy using Olympus DP 73 microscope (Olympus Optical Co, Ltd, Tokyo, Japan). The thickness of the tunica media of the aortic wall was measured under 40X objective magnification.

Statistical analyses

Statistical analysis was accomplished using the Sigma plot 11.0 program (Systat Software, Inc., San Jose, CA, USA). All data were expressed as mean ± SEM. Multiple comparisons were performed using *t* test and one-way ANOVA follow by Student-Newman-Keul *post hoc* test. Statistical significance of the mean difference were considered at $P < 0.05$.

Results

Acute effect of morelloflavone on thoracic aortic ring relaxation

As shown in the Figure 1, it is found that morello-flavone relaxed PE precontracted aortic rings with the endothelium intact in a concentration dependent manner. When 10^{-11}-10^{-5} M morelloflavone were added, the percentage of aortic ring relaxation of cisplatin-treated group was significantly lower than the control endothelium intact group at each particular dose of morelloflavone *($P < 0.05$).* The maximal relaxation when 10^{-5} M morelloflavone was added in control group (intact endothelium) and cisplatin-treated group were 75.44 ± 0.95% and 57.54

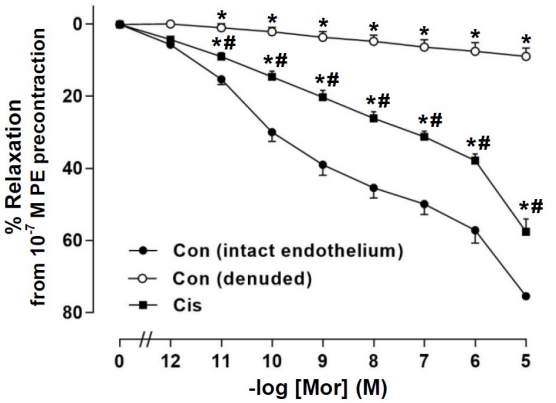

Figure 1. Effect of morelloflavone on the relaxation of rat thoracic aorta isolated from control (Con), with intact endothelium or denuded, and from cisplatin-treated rats (Cis). Each value shows mean ± SEM in percentage relaxation from 10^{-7} M PE precontraction. * and # $P < 0.05$ compared with control (intact endothelium) and control (denuded), respectively (n = 6 each).

Table 1. Half maximal effective concentration (EC_{50}), and maximal effective response (E_{max}) of phenylephrine (PE), acetylcholine (ACh) and sodium nitroprusside (SNP) in isolated rat thoracic aorta of control (Con), cisplatin (Cis) and cisplatin + morelloflavone (Cis + Mor) groups.

Treatment	PE		ACh		SNP	
	EC_{50} (-log M)	E_{max} (% contraction from resting tension)	EC_{50} (-log M)	E_{max} (% relaxation from PE precontraction)	EC_{50} (-log M)	E_{max} (% relaxation from PE precontraction)
Con (n=6)	7.10 ± 0.04	144.8 ± 2.7	7.54 ± 0.08	107.6 ± 1.3	8.99 ± 0.21	109.2 ± 2.0
Cis (n=8)	7.30 ± 0.04*	170.4 ± 2.3*	7.02 ± 0.07*	95.1 ± 1.2*	8.81 ± 0.14	108.5 ± 1.5
Cis + Mor (0.1 mg/kg) (n=6)	7.28 ± 0.03*	165.6 ± 2.6*	7.15 ± 0.03*	94.3 ± 1.3*	8.93 ± 0.19	107.3 ± 1.4
Cis + Mor (1 mg/kg) (n=8)	7.16 ± 0.02#	154.4 ± 1.3*#	7.26 ± 0.03*#	101.2 ± 1.4*#	8.95 ± 0.08	106.2 ± 2.0
Cis + Mor (10 mg/kg) (n=6)	7.27 ± 0.05*	178.8 ± 3.0*	7.02 ± 0.11*	94.4 ± 1.1*	8.59 ± 0.16	104.3 ± 1.4

Values are mean ± SEM. *and # indicates $p < 0.05$ compared to control and cisplatin group, respectively.

± 3.53%, respectively. Denuded endothelium of the aortic rings of control group completely abolished morelloflavone-induced relaxation suggesting that the mechanism of morelloflavone action is endothelium-dependent and indicating the impairment of endothelial function of cisplatin-treated rats.

Contraction response of aortic rings to PE and relaxation responses to ACh and SNP

The protective role of morelloflavone on the contractile function of aortic rings was studied by using cumulative addition either of PE, ACh or SNP, as shown in Table 1 and Figure 2. The half maximal effective concentration (EC_{50}) of PE on the contractile response of aortic rings was significantly lower in cisplatin group when compared to control group (-log [M], 7.30 ± 0.04 *vs.* 7.10 ± 0.04). The maximal effective response (E_{max}), as shown in % increase from resting tension of PE on the contractile response of aortic rings, was significantly higher in cisplatin group when compared to control group (170.4 ± 2.3 *vs.* 144.8 ± 2.7%). However, when the three doses of morelloflavone (0.1, 1 and 10 mg/kg) were given along with cisplatin, only one dose of morelloflavone (1 mg/kg) showed a significant alteration in both EC_{50} and E_{max} in response to PE (-log [M],

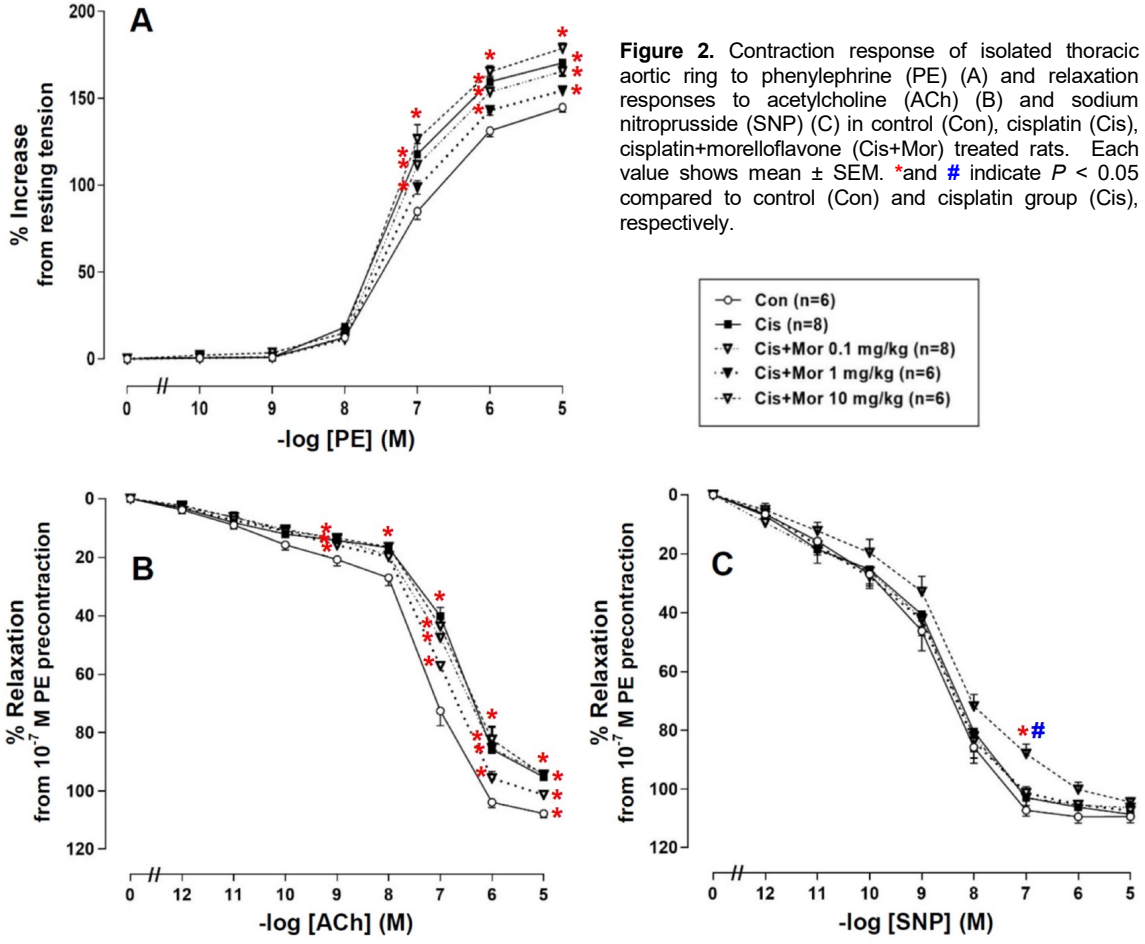

Figure 2. Contraction response of isolated thoracic aortic ring to phenylephrine (PE) (A) and relaxation responses to acetylcholine (ACh) (B) and sodium nitroprusside (SNP) (C) in control (Con), cisplatin (Cis), cisplatin+morelloflavone (Cis+Mor) treated rats. Each value shows mean ± SEM. *and # indicate *P* < 0.05 compared to control (Con) and cisplatin group (Cis), respectively.

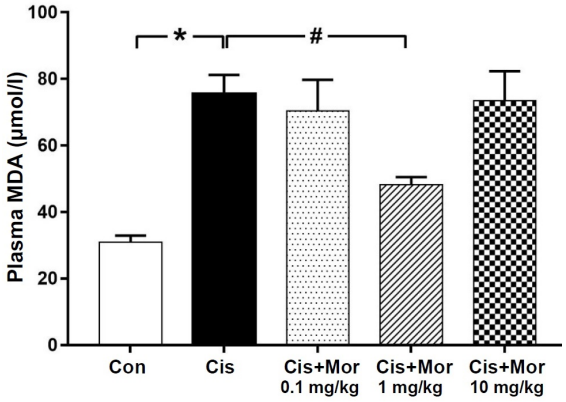

Figure 3 Effect of cisplatin (7.5 mg/kg, i.p.) (Cis) and cisplatin with morelloflavone co-administration (Cis+Mor) on plasma MDA concentration. Values are mean ± SEM. * and # indicate $P < 0.05$ compared to control (Con) and cisplatin group (Cis), respectively. (n = 6 each).

7.16 ± 0.02 and 154.4 ± 1.3%), respectively.

Alternatively, the EC_{50} value of ACh on the relaxation response of aortic rings were significantly higher and lower in cisplatin group when compared to control group (-log [M], 7.02 ± 0.07 *vs.* 7.54 ± 0.08) while the E_{max} was significant lower (94.1 ± 1.2 *vs.* 107.6 ± 1.3%). However, only one dose of morelloflavone (1 mg/kg) showed a significant change in both EC_{50} and E_{max} on the vasorelaxing response to ACh (-log [M], 7.26 ± 0.03 and 101.2 ± 1.4%), respectively.

The relaxation response of aortic rings to the cumulative addition of 10^{-12}-10^{-5} M SNP of cisplatin-treated group showed a similar degree of concentration-dependent relaxation as the control group (Figure 2C). The EC_{50} and E_{max} of SNP showed no significant changes among the control, cisplatin and cisplatin with morelloflavone treated groups.

Effect of morelloflavone treatment on Plasma MDA concentration

As shown in Figure 3, plasma MDA levels were significantly higher in cisplatin group when compared to control group (75.52 ± 5.70 *vs.* 30.83 ± 2.04 µmol/l, $P < 0.05$). When 1 mg/kg morelloflavone was given along with cisplatin, the plasma MDA levels were significantly lower when compared to cisplatin group (48.32 ± 2.16 *vs.* 75.52 ± 5.70 µmol/l, $P < 0.05$).

Histopathological examination

As shown in Figure 4, the H&E stain of aortic rings of control, cisplatin and cisplatin and morelloflavone (0.1, 1 and 10 mg/kg) groups demonstrated non-significant difference in the tunica intima (TI) appearance among these five groups. As shown in Table 2, the tunica media (TM) of cisplatin group was found significantly thicker than the control group (112.9 ± 4.1 *vs.* 83.5 ± 0.8 µm) and this made the vessel diameter and hence the ratio between TM and vessel diameter increased. In addition, the disorganized arrangement of elastic laminae in tunica media was observed along with the less clearly defined smooth muscle cell layer. Morelloflavone (1 mg/kg) treatment significantly decreased the thickening of tunica media to 92.5 ± 2.3 µm and made the smooth muscle cell layer clearly seen. However, the lower dose of morelloflavone (1 mg/kg) did not affect the thickening of this increased TM caused by cisplatin. In contrast, the higher dose of morelloflavone (10 mg/kg) treatment significantly strengthen an increase in the thickening of tunica media to 1.7 folds in control and 1.3 folds in cisplatin group.

Discussion

The first part of this study (Figure 1) showed that the

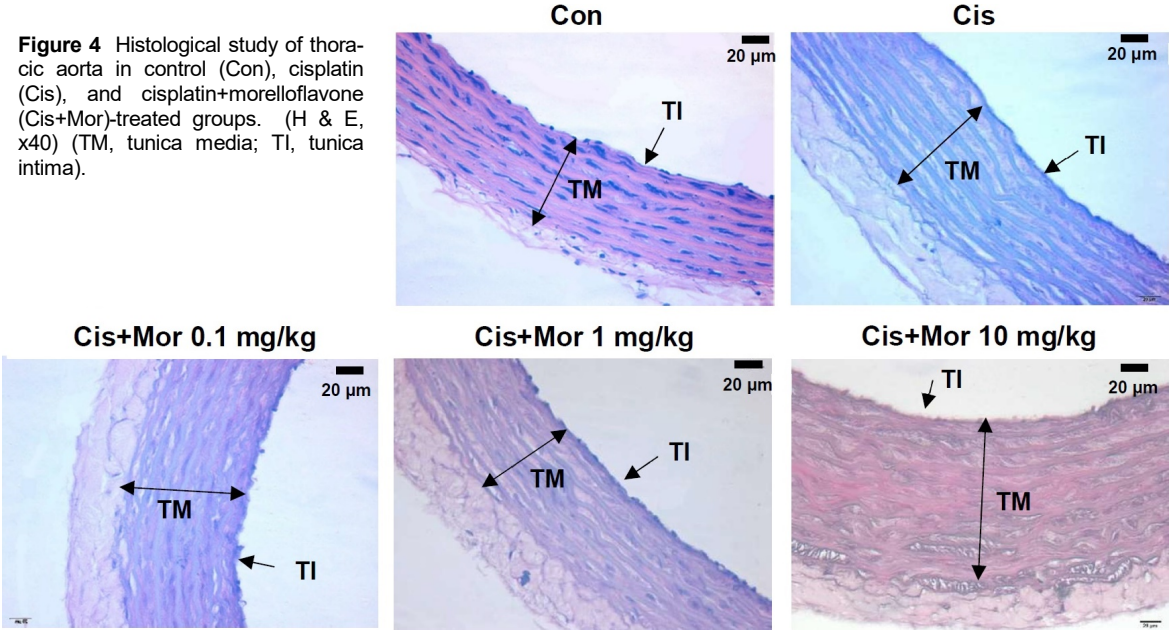

Figure 4 Histological study of thoracic aorta in control (Con), cisplatin (Cis), and cisplatin+morelloflavone (Cis+Mor)-treated groups. (H & E, x40) (TM, tunica media; TI, tunica intima).

Table 2 Tunica media thickness and vessel diameter of thoracic aorta in control (Con), cisplatin (Cis), and cisplatin+ morelloflavone (Cis+Mor)-treated groups.

Treatment	Tunica Media (TM) thickness (μm)	Vessel diameter (μm)	TM thickness / Vessel diameter
Con (n=5)	83.5 ± 0.8	118.8 ± 2.1	0.70 ± 0.01
Cis (n=4)	112.9± 4.1*	150.4 ± 6.8*	0.75 ± 0.01*
Cis + Mor (0.1 mg/kg) (n=5)	112.2 ± 2.4*	149.5 ± 4.4*	0.75 ± 0.01*
Cis + Mor (1 mg/kg) (n=5)	92.5 ± 2.3#	130.2 ± 3.7#	0.71 ± 0.00#
Cis + Mor (10 mg/kg) (n=5)	146.0 ± 9.5*#	174.5 ± 10.8*#	0.84 ± 0.01*#

Values are mean ± SEM; n, number of rats; * and # indicate $P < 0.05$ compared to control and cisplatin group, respectively.

relaxation of thoracic aortic ring induced by morelloflavone was endothelium-dependent similar to previously reported.[18] The mechanism of morelloflavone action on vasorelaxation was reported to involve mainly nitric oxide signaling pathway. However, seven days after cisplatin injection, the thoracic aorta isolated from this group of rats had a prominently less vasorelaxation response to morelloflavone when compared to vehicle control. Thus it is likely that an impairment of endothelial function occurred in cisplatin-treated rat.

The protective role of morelloflavone in cisplatin-induced impairment of vascular smooth muscle contraction and relaxation was investigated in the second part of this study (Table 1 and Figure 2). The three selected doses of morelloflavone were chosen to co-administrate with cisplatin. It is found that the thoracic aortic rings of cisplatin-treated rat showed a significantly higher force of contraction in response to PE when compared to control group, as indicated by the EC_{50} and E_{max} values. Besides an impairment of endothelium that might occur in cisplatin-treated rats, an increase in PE receptor sensitivity is also likely to be responsible for an increase force of contraction. The cumulative addition of PE to the aortic rings in the organ bath resulted in the contraction response of vascular smooth muscle via binding to α1-adrenergic receptor, even the mechanism of increased sensitivity to PE remained unclear.[19] It is possible that upregulation of signal transduction of this receptor signaling pathway may occur in cisplatin-treated group and responsible for an increase in force of contraction, as earlier reported that the pretreatment with cisplatin resulted in an increase in the sensitivity to PE and KCl of the isolated aortic rings.[20] This study indicated that morelloflavone (1 mg/kg) administration along with cisplatin can restore the higher response of aortic force of contraction to PE, whereas the lower and higher dose (0.1 and 10 mg/kg) cannot improve this. The effect of morelloflavone, perhaps like other drugs, requires a minimal effective dose and the exaggerated dose would result in an altered response of the receptor signaling pathway.

The protective role of morelloflavone against endothelium damage caused by cisplatin was also investigated. This study indicated the partial damage of aortic ring endothelium of cisplatin-treated rats, since the vasorelaxation response to ACh was found significantly lower than control rats (Figure 2B), and as indicated by both EC_{50} and E_{max} values (Table 1). The vascular endothelium function has been shown to involve the release of various vasodilators, including nitric oxide (NO), prostaglandin (PGI_2), and the endothelium-derived hyperpolarizing factor (EDHF).[21] The impaired endothelial modulation of vascular relaxation induced by ACh in this study may be related to decreased NO production, increased NO inactivation and/or altered NO-cyclic guanosine monophosphate (cGMP) signaling. Our experiment using SNP, a nitric oxide donor, resulted in a similar degree of aortic ring relaxation between cisplatin and control group (Figure 2C and Table 1). These data suggested that the impaired vasorelaxation of cisplatin rats may be due to endothelium damage, however, the vascular smooth muscle functional alteration caused by cisplatin could not be excluded. Administration of morelloflavone in cisplatin-treated rat improved aortic ring relaxation and the effective dose in this study was 1 mg/kg.

The histological study of aortic ring tunica intima cannot confirm the functional damage of endothelial layer caused by cisplatin, since the structural damage could not be clearly seen with H&E staining (Figure 4). However, the tunica media layer of cisplatin-treated rat was found thicker when compared to control group (Table 2), suggesting an increase in the proliferation of smooth muscle cells (SMCs) in the aortic wall as previously described.[5,22] One of the important functions of NO is as negative regulator of smooth muscle proliferation. Thus, a reduction in the bioavailability of NO may lead to the development of vascular smooth muscle proliferation.[23] Morelloflavone (1 mg/kg)-treated group showed more reduction of tunica media thickening caused by cisplatin, when compared to the lower dose (0.1 mg/kg). This may be due to the effect of Morelloflavone that can decrease the aortic proliferation at the optimal low dose.[12] In contrast, at the higher dose of morelloflavone treatment (10 mg/kg), the significantly thickest tunica media was observed. It is possible that high dose of morelloflavone can cause vascular smooth muscle hypertrophy by suppressing the growth inhibitory factor(s). However, further study needs to be done to clarify the toxicity effect of morelloflavone.

The measurement of lipid peroxidation has been used to support the oxidative status of experimental animal. MDA is a well-known biomarker of plasma and tissue lipid peroxidation and can represent the

overall oxidative damage to cellular constituents.[24] In this study, the plasma MDA level of cisplatin-treated group was significantly higher when compared to control, suggesting that the oxidative stress critically occurred. Many research reports indicate that oxidative stress plays an important role in the pathogenesis and leads to various cardiovascular diseases including atherosclerosis, hypertension, myocardial infarction, and heart failure. Oxidative stress caused by an increase in ROS can induce cellular damage and apoptosis. Cisplatin treatment was shown to induce apoptosis in patients and experimental animals.[25] Morelloflavone (1 mg/kg) provided the protective effect against the oxidative damage associated with cisplatin in this study, whereas the lower dose (0.1 mg/kg) and the higher dose (10 mg/kg) did not. It is likely that the potentiality of morelloflavone to prevent plasma lipid peroxidation which resulted in a decrease in plasma MDA, may be mainly attributed to its antioxidant property as previously reported.[11,12,17]

It is noted that the highest dose of morelloflavone (10 mg/kg) in this study did not show the protective role in cisplatin-treated rats as indicated by its EC_{50} and E_{max} values of vasorelaxing responses (Table 1), or lowering the plasma MDA levels (Figure 3) and even suppressing the thickening of TM (Table 2). Moreover, the high dose of this morelloflavone treatment, in turn, strengthened its activity as a pro-oxidant in which it could exert the particular reducing property which leads to the generation of free radicals through certain reactions. The toxicity of high dose morelloflavone and its activity as pro-oxidant should be further investigated.

Conclusion

Cisplatin treatment resulted in an impairment of endothelial cells, which was confirmed by the lesser vasorelaxation induced by ACh and the stronger vasoconstriction induced by PE when compared to vehicle control. Endothelial impairment resulted in a reduction in vasorelaxant derived from the endothelium which exerts its effect on vascular smooth muscle growth and contraction. Co-administration of morelloflavone with cisplatin would be able to improve the impaired vascular contractile function both physiologically and anatomically. The mechanism of morelloflavone action on vascular smooth muscle may occur via its antioxidant property and may involve endothelial NO signaling pathway.

Acknowledgments

The authors thank Faculty of Science and Graduated School Research Support Funding for Thesis, Prince of Songkla University, Hatyai, Songkhla, Thailand, The Royal Bangkok Sports Club, and The Foundation of Islamic Culture for Education and Development, for their funding support.

Conflict of Interest

None to declare.

References

1. Jamieson E, Lippard S. Structure, recognition, and processing of cisplatin − DNA adducts. Chem Rev. 1999; 99(9): 2467–98.
2. Huddart RA, Norman A, Shahidi M, Horwich A, Coward D, Nicholls J, et al. Cardiovascular disease as a long-term complication of treatment for testicular cancer. J Clin Oncol. 2003; 21(8): 1513–23.
3. Takashi F, Hirofumi S, Koichi S, Sunao K, Seigo S. Acute myocardial infarction induced by cisplatin-based combination chemotherapy for malignant melanoma: A case report. J Cardiol. 2006; 47: 191–5.
4. Dieckmann K-P, Gehrckens R. Thrombosis of abdominal aorta during cisplatin-based chemotherapy of testicular seminoma - a case report. BMC Cancer. 2009; 9: 459.
5. Olszewski PK, Klockars A, Olszewska AM, Fredriksson R, Schiöth HB, Levine AS. Molecular and Immunohistochemical Evidences for Cardiovascular Damage in Addition to Renal Toxicity of Cisplatin. Endocrinology. 2010; 151(10): 4736–44.
6. Ghosh N, Chow C-M, Korley V, Chisholm R. An unusual case of chronic coronary artery dissection: Did cisplatin play a role?. Can J Cardiol. 2008; 24: 795–7.
7. Sagstuen H, Aass N, Fosså SD, Dahl O, Klepp O, Wist EA, et al. Blood pressure and body mass index in long-term survivors of testicular cancer. J Clin Oncol. 2005; 23(22): 4980–90.
8. Norbury CJ, Hickson ID. Cellular Responses to DNA Damage. Annu Rev Pharmacol Toxicol. 2001; 41: 367–401.
9. Lin Y-M, Anderson H, Flavin MT, Pai Y-HS, Mata-Greenwood E, Pengsuparp T, et al. In Vitro Anti-HIV Activity of Biflavonoids Isolated from *Rhus succedanea* and *Garcinia multiflora*. J Nat Prod. 1997; 60(9): 884–8.
10. Gil B, Sanz MJ, Terencio MC, Gunasegaran R, Payá M, Alcaraz MJ. Morelloflavone, a novel biflavonoid inhibitor of human secretory phospholipase A2 with anti-inflammatory activity. Biochem Pharmacol. 1997; 53(5): 733–40.
11. Hutadilok-Towatana N, Kongkachuay S, Mahabusarakam W. Inhibition of human lipoprotein oxidation by morelloflavone and camboginol from *Garcinia dulcis*. Nat Prod Res. 2007; 21(7): 655–62.
12. Decha-Dier U, Hutadilok-Towatana N, Mahabusarakam W, Sawangjaroen K, Pinkaew D. Anti-atherogenic effects of morelloflavone from *Garcinia dulcis* leaves in cholesterol fed rabbits. J Nat Remedies. 2008; 8(2): 151–9.
13. Pinkaew D, Cho SG, Hui DY, et al. Morelloflavone blocks injury-induced neointimal formation by inhibiting vascular smooth muscle cell migration. Biochim Biophys Acta - Gen Subj. 2009; 1790(1): 31–9.
14. Pang X, Yi T, Yi Z, Cho SG, Qu W, Pinkaew D, et al. Morelloflavone, a biflavonoid, inhibits tumor angiogenesis by targeting Rho GTPases and

extracellular signal-regulated kinase signaling pathways. Cancer Res. 2009; 69(2): 518–25.
15. Tuansulong KA, Hutadilok-Towatana N, Mahabusarakam W, Pinkaew D, Fujise K. Morelloflavone from *Garcinia dulcis* as a novel biflavonoid inhibitor of HMG-CoA reductase. Phyther Res. 2011; 25(3): 424–8.
16. Kunworarath N, Muangnil P, Itharat A, Hiranyachattada S. Acute and subchronic treatment of Hibiscus sabdariffa Linn. extract on renal function and lipid peroxidation in cisplatin-induced acute renal failure rats. J Physiol Biomed Sci. 2014; 27(1): 5–12.
17. Deachathai S, Mahabusarakam W, Phongpaichit S, Taylor WC. Phenolic compounds from the fruit of *Garcinia dulcis*. Phytochemistry. 2005; 66(19): 2368–75.
18. Lamai J, Mahabusarakam W, Ratithammatorn T, Hiranyachattada S. Effects of morelloflavone from *Garcinia dulcis* on vasorelaxation of isolated rat thoracic aorta. J Physiol Biomed Sci. 2013; 26:13–7.
19. Russell A, Watts S. Vascular reactivity of isolated thoracic aorta of the C57BL/6J mouse 1. J Pharmacol Exp Ther. 2000; 294: 598–604.
20. Atalik KE, Keleş B, Uyar Y, Dündar MA, Öz M, Esen HH. Vasoprotection by melatonin and quercetin in rats treated with cisplatin. Indian J Exp Biol. 2010; 48(12): 1188–93.
21. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. Circ J. 2009; 73: 411–8.
22. Saleh RM, Awadin WF, Elseady YY, Waheish FE. Renal and cardiovascular damage induced by cisplatin in rats. Life Sci J. 2014; 11(2): 191–203.
23. Tsutsui M, Shimokawa H, Otsuji Y, Yanagihara N. Pathophysiological relevance of NO signaling in the cardiovascular system: Novel insight from mice lacking all NO synthases. Pharmacol Ther. 2010; 128(3): 499–508.
24. Ho E, Karimi Galougahi K, Liu C-C, Bhindi R, Figtree GA. Biological markers of oxidative stress: Applications to cardiovascular research and practice. Redox Biol. 2013; 1(1): 483–91.
25. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol. 2012; 2012: 1–13.