Effects of Phikud Navakot Extract on Myocardial Ischemia/Reperfusion Injury in Rats

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Background: *Phikud Navakot (PN) is a set of nine medicinal plants and the main ingredient of "Yahom Navakot", a traditional Thai herbal formula for treatment of cardiovascular symptoms.*
Objective: *To investigate the cardioprotective effects of PN on myocardial ischemia/reperfusion (IR) in male Sprague Dawley rats.*
Material and Method: *Rats were randomly divided into 7 groups: sham, IR, and IR orally pretreated with PN (10, 50, 100, 200, and 400 mg/kg BW) for 7 days. After treatment, IR induction was performed by left coronary artery (LCA) ligation for 30 min, followed by reperfusion for 24 h. At the end of the experiment, blood was collected for hematological and biochemical parameters, and hearts were immediately removed for histopathological examination and Western blot analysis.*
Results: *IR induction caused ST elevation in the electrocardiogram and an increase in serum troponin I (TnI), confirming myocardial damage. In addition, histopathological changes of ischemic myocardium showed inflammation, infiltration, and edema. Oral administration of PN (10, 50, 100, 200, and 400 mg/kg BW) for 7 days prior to IR simulation showed no change on serum TnI and histopathology of cardiac tissues, when compared to IR group. However, Western blot analysis showed that IR rats pretreated with PN (10 mg/kg BW) significantly increased ($p<0.05$) pERK/ERK ratio, meanwhile pretreated with PN (50-200 mg/kg BW) up-regulated ($p<0.05$) the protein expression of HO-1, when compared with IR group.*
Conclusion: *The present study implied that 7-day pretreatment of PN failed to protect cardiac tissues against IR injury induced by LCA ligation. Investigation at molecular level found however that PN up-regulated the expression of protective proteins pERK/ERK ratio and HO-1 in cardiac tissues, suggesting molecular mechanism of PN in cardioprotection against IR injury.*

Keywords: *Phikud Navakot, Yahom, Left coronary artery ligation, Troponin, HO-1, ERK1/2*

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Phikud Navakot (PN) is a set of nine crude drugs namely, root of *Angelica dahurica* (Fisch.) Benth. & Hook. f., family Umbelliferae, rhizome of *Atractylodes lancea* (Thunb.) DC., family Compositae, rhizome of *Ligusticum chuanxiong* Hort., family Umbelliferae, root of *Angelica sinensis* (Oliv.) Diels, family Umbelliferae, aerial part of *Artemisia vulgaris* L., family Compositae, rhizome of *Saussurea costus* (Falc.) Lipsch., family Compositae, rhizome of *Picrorhiza kurrooa* Royle ex Benth., family Scrophulariaceae, gall of *Terminalia chebula* Retz., family Combretaceae and root and rhizome of *Nardostachys jatamansi* (D. Don) DC., family Valerianaceae. PN is the main ingredient of "Yahom Navakot", a traditional Thai herbal formula for treatment of cardiovascular symptoms such as dizziness and fainting[1]. Herbal medicines have become increasing popular worldwide as alternative medicines for treatment of cardiovascular diseases including myocardial ischemia, and atherosclerosis[2]. Recently, it has been shown that PN (50-1,000 microgram/mL) decreased intracellular production of reactive oxygen species (ROS) against H_2O_2-injured human umbilical vein endothelial ECV304 cells, and exhibited strong antioxidant activities against 2,2-diphenyl-1-picrylhydrazyl, superoxide anion, and hydroxyl

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radical[3], which is one of the major risk factors of myocardial infarction[4]. Some plants in PN have been shown to protect organs against ischemia/reperfusion (IR) injury, as follows. *N. jatamansi* decreased neuronal cell death induced by middle cerebral artery occlusion in Wistar rats[5]. The extract of herbal formula, containing *P. cocos*, *A. macrocephala* and *A. sinensis*, prevented brain injury and polymorphonuclear leukocytes infiltration induced by common carotid arteries occlusion in mice[6]. *T. chebula* extract attenuated isoproterenol (ISO) induced alterations mitochondrial ultrastucture and function of the heart in rats[7]. Thus, it is possible that PN might protect the heart against IR injury.

Myocardial infarction (MI) is one of the major causes of death and disability worldwide, and becomes one of the most challenging objects of clinical and basic science investigation[8]. Left anterior descending coronary artery (LCA) occlusion has been widely used as a model of myocardial infarction to mimic pathological progression of the disease in human[9].

There is no scientific evidence of cardio-protective effects of PN against IR injury. In the present study, the ethanolic extract of PN was tested for cardioprotection against IR-induced myocardial injury in vivo model of LCA ligation. The expression of survival proteins including ERK1/2 and HO-1 in cardiac tissues was also investigated.

Material and Method

Drugs and chemicals

RIPA, Halt™ protease and phosphatase inhibitor as well as endothelial nitric oxide synthase (eNOS) and primary antibody beta actin were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibodies specific for protein kinase B (Akt), pAkt, extracellular signal-regulated kinase (ERK) 1/2, pERK1/2, Bax, Bcl-2 and secondary goat anti-rabbit lgG (HRP conjugated) were purchased from Cell Signaling Technology (MA, USA). Heme oxygenase (HO)-1 was provided by Abcam, England. The enhanced chemiluminescence (ECL) Western blot detection reagent was obtained from Amersham Biosciences, USA. All other reagents were analytical grade.

Preparation of extracts

PN, obtained from an equal amount of nine crude drugs (as mentioned above), were purchased from a traditional Thai pharmacy in Bangkok, Thailand, on April 2011, and identified by Dr. Uthai Sotanaphun. Their voucher specimens (NVK01-09) have been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand. The PN coarse powder was soaked in 10 times by weight of 80% ethanol overnight and continuously extracted twice at 100°C for 3 hour. The two subsequent extracts were combined and concentrated under reduced pressure to give an oily extract. The total phenolic content of PN was 14.76±0.34% GAE (gallic acid equivalence) determined by Folin-Ciocalteau method[10]. For convenience in administration to the experimental animals, PN was turned into a dry powder by homogenization with N-lok (National Starch & Chemical, United Kingdom) and spray drying. The resulting powder (containing with 50% of PN) were suspended in water once a week and kept in a refrigerator for administration.

Surgical preparation of animals

Adult male Sprague Dawley rats (250-300 g) were supplied by the National Laboratory Animal Center, Mahidol University, Thailand. The rats were housed in a temperature-controlled room under 12-h light/dark cycle at 25±2°C, and 45-75% humidity throughout the experiment. They had free access to a commercial standard diet and chlorine-treated water (5-6 ppm). All procedures involving the use of animals for research were approved by the Animal Care and Use Committee of Mahidol University and the Animal Research Ethics Committee of the Faculty of Medicine, Srinakharinwirot University, Thailand (Approval No. 15/2553 and 12/2554).

Following seven days acclimatization, they were randomly divided into seven groups: sham group, IR group, and five groups of IR pretreated of PN (10, 50, 100, 200, or 400 mg/kg BW). All extracts were administrated via an oroesophageal needle once a day for 7 days before LCA ligation.

On the eighth day, acute MI was performed by the LCA ligation, as described previously[9]. In brief, rats were anesthetized with a mixture of xylazine (10 mg/kg BW) and ketamine (90 mg/kg BW) intraperitoneally, and then maintained with 1.5-1.8% isoflurane for surgical preparation. The rats were ventilated with a volume-regulated respirator via an endotracheal tube. After the skin was shaved and cleaned with betadine® solution and 70% alcohol, left thoracotomy approximately 2-cm in length was performed between a third and fourth-intercostal space. The pericardium was excised and the LCA was ligated

with a 7-0 silk suture. After 30-minute occlusion, the ligation was loosened to allow reperfusion for the additional 24 hour[11,12]. The sham group underwent the same surgical procedure except the LCA ligation. Ischemia was confirmed by a visible color change in the myocardial region. At the end of the reperfusion period, the rats were terminated by carbon dioxide euthanasia, and blood was collected from the inferior vena cava. The heart was removed for histopathological examination and Western blot analysis.

Hematological and biochemical parameters

Determination of cardiac markers in serum: troponin I (TnI) and lactate dehydrogenase (LDH) measured by electrochemiluminescence immunoassay, was carried out by the Roche on Modular E170 analyzer and the Cobas Integra 400, respectively. Plasma and serum concentrations of hematological and biochemical parameters, respectively, were measured by using an automatic biochemical analyzer (Cell Dyn 3700 system, Abbott Lab and Cobas c111, Roche USA).

Histopathological examination

For assessment of hematoxylin and eosin (H&E) staining, the heart tissues were fixed in 10% neutral buffer formalin for 8-16 hour. Specimens were washed and dehydrated in serial dilutions of absolute ethyl alcohol, cleared in xylene, embedded in paraffin, sectioned at 5 mm thickness, collected on glass slides, and assessed by H&E staining. Histopathological changes were examined and photographed under light microscope (400x magnification).

For assessment of an infarct size, the hearts were excised into 3 transverse sections. The sections were incubated with 2% 2, 3, 5-triphenyltetrazolium chloride (TTC) at 37°C for 20 minute, with agitation until tissue staining. Each section was photographed to identify unstained infarcted region and red stained for non-infarcted area[13].

Western blot analysis

The frozen heart tissues were homogenized on ice using a homogenizer (IKA, Germany) in RIPA buffer (150 mM NaCl, 50 mM Tris HCl, 1% Igepal, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate: SDS) (cardiac tissue: RIPA buffer = 1:10) with 1% protease and phosphatase inhibitor for 1.30 minute. The homogenates were centrifuged at 10,000 xg for 10 min at 4°C, and the supernatants were collected and stored at -80°C until use. Equal amounts of protein (50 mg) were boiled in loading buffer (225 mM Tris-HCl, pH 6.8, 6% SDS, 30% glycerol, 9% 2-mercaptoethanol, and 0.009% bromophenol blue), loaded on 10% SDS-PAGE and then transferred onto PVDF membrane. The membranes were blocked by 5% non-fat milk in Tris-buffered saline plus 0.1% Tween-20 (TBST) for 1 hour at room temperature, then incubated with specific primary antibodies, including anti-phospho Akt, anti-Akt, anti-phospho-ERK1/2, anti-ERK1/2, anti-HO-1, anti-eNOS, anti-Bax, anti-Bcl-2, and anti-beta actin at 4°C overnight. Membranes were subsequently incubated with specific secondary antibodies-HRP conjugated for 1 hour at room temperature, and the immuno-reactive proteins were visualized by ECL detection under gel documentation (GeneGnome5, Syngene, Cambridge, UK). Band intensities were quantified using densitometry (Image J).

Data and statistical analysis

All data were expressed as mean $\pm$ SEM. Data of blood parameters and band intensity of Western blot analysis, were statistically analyzed using one-way ANOVA followed by post hoc tests with Turkey. Values of $p<0.05$ were considered statistically significant.

Results

Left coronary artery ligation model in rats

To diagnose acute MI, serum cardiac biomarker troponin I and LDH combined with evidence of ischemia including ST-segment elevation was evaluated. In IR group, there was a significant increase ($p<0.05$) in the levels of troponin I when compared with sham group. Pre-treatment with PN (10-400 mg/kg BW) was not able to restore serum troponin I (Table 1). ST height showed a significant increase ($p<0.001$) from 0.019 ± 0.001 in sham rats (n = 18) to 0.057 ± 0.003 in IR rats (n = 92). Meanwhile, serum LDH did not change (Table 1). Our data implied that short-term administration (7 days) of PN could not protect cardiac damage induced by LCA ligation.

Hematological and biochemical parameters

IR rats and IR rats pretreated with PN (10-400 mg/kg BW) for 7 days showed no significant change in hematological and biochemical parameters when compared with sham rats (Table 2, 3).

Histopathological examination of the cardiac tissues

IR significantly increased % area fraction of infarction (23.60 ± 7.89) when compared with sham (0%). Pre-treatment of various doses of PN (10, 50, 100,

Table 1. The effect of Phikud Navakot (PN) on serum troponin I and LDH in Sprague Dawley rats-induced ischemia/reperfusion (IR) by left coronary artery ligation. Values are expressed as mean ± SEM. *$p<0.05$ vs. sham

Group	Troponin I (U/L)	LDH (U/L)
Sham (n = 3)	1.42±0.38	3,847.50±663.33
IR (n = 2)	17.89±3.96*	3,816.50±172.50
PN-pretreated rats (mg/kg BW)		
10 (n = 2-3)	14.83±4.24*	3,287.00±235.30
50 (n = 4)	41.21±10.31*	3,155.75±244.31
100 (n = 5)	21.29±4.06*	4,907.20±582.78
200 (n = 4)	30.48±6.97*	6,517.50±530.90
400 (n = 5)	49.83±10.77*	5,083.60±771.86

200 and 400 mg/kg BW) did not significantly attenuate infarct size (6.59±6.34, 30.60±5.11, 11.82±6.23, 22.36±7.64, 30.06±0.03, respectively). The representative of TTC-stained heart sections was shown in Fig. 1.

The effects of IR on the degree of histopathological changes in cardial tissues showed necrotic changes of myocardial fibers, white blood cells (neutrophil, lymphocyte, and plasma cells) accumulation, intramuscular edema, vascular congestion and hemorrhage, and perivascular edema when compared with control rats. Administration of various doses of PN (10-400 mg/kg BW) for 7 days was not able to decrease myocardial damage from transient occlusion of LCA as shown in Fig. 1.

Protein expression ratios in cardiac tissues

PN (10 mg/kg BW) significantly increased in the ratio of protective protein pERK/ERK (Fig. 2B). The expression of HO-1, but not those of eNOS and pAkt/Akt ratio, was significant increased after 7-days oral administration of PN at the concentrations of 50, 100, and 200 mg/kg BW when compared to the IR group (Fig. 2C). In contrast, pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2 ratio was not changed after ingestion of PN.

Discussion

Troponin I and ST-segment was significantly elevated after myocardial infarction induced by LCA ligation confirming myocardial injury. ST elevation, a hallmark of acute myocardial ischemia, is commonly used to confirm myocardial infarction during the first 24 hour[14]. Troponins, a universal biomarker of cardiac necrosis, are more rapid diagnosis of MI and more specific than creatine kinases[15]. Cardiac troponins are liberated after the onset of necrosis, reaching a peak between 18-36 hour, and slightly decline or remain up to 10-14 days after myocardial injury[15]. LDH, a marker of tissue breakdown to assess the extent cardiac injury, increased during ischemia (low oxygen supply) to catalyze the conversion of mitochondrial pyruvate to lactate in glycolytic pathway[16]. However, pretreatment of PN (10-400 mg/kg BW) before IR was unable to restore them to normal level. Thus, long-term administration of PN will be further investigated.

ERK1/2 and HO-1 played important roles in protection against cell damage in rat myocardial IR injury induced by coronary ligation[17]. The Nrf/HO-1 antioxidant pathway constitutes a therapeutic target for protection against IR-induced damage in various crucial organs included the heart[18]. In the present study, one week of PN treatment did not cause a significant reduction in % area fraction of infarction, when compared with IR group. However, pretreatment of PN for 7 days showed cardioprotective effect against induction of MI by LCA ligation in rats only at molecular level by up-regulation of pERK/ERK ratio and HO-1 expression, but not the pAkt/Akt ratio, in cardiac tissues. In addition, 7 days pretreatment of hydrogen sulfide inhibits IR injury by activating the binding of Nrf2 to HO-1 in an ERK-dependent manner[19]. Activation of ERK1/2 and up-regulation of HO-1 expression have been reported to protect H9c2 rat cardiac myoblast cell death induced by H_2O_2[20]. Induction of HO-1 might be activated by some compositions of PN, including *A. dahurica* extract on RAW264.7 cells[21], and *N. jatamansi* extract on pancreatic acinar cells of mice[22]. Moreover, the activation of ERK1/2 may be induced by some plants in PN as shown in previous study, included *L. chuanxiong* in rat neuronal-like pheochromocytoma cells[23] and *A. sinensis* on ECV304[24]. In contrast, it

Table 2. The effects of Phikud Navakot (PN) on the hematological parameters in serum of Sprague Dawley rats induced ischemia/reperfusion (IR) by LCA ligation. Values are expressed as mean ± SEM, n = 3-7

Treatment (mg/kg BW)	WBC (10^3 µl)	RBC (10^6 µl)	HBG (g/dL	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	PLT (10^5 µl)	RDW (%)	PDW (fl)	MPV (fl)	PCT (%)	Differential count (%)				
													NEU	LYMPH	EO	BASO	MONO
Sham	4.22±1.36	7.39±0.17	15.43±0.24	41.63±0.54	55.37±0.78	20.93±0.58	37.13±0.88	856.33±56.85	15.76±0.47	17.70±0.26	7.63±0.36	0.65±0.05	47.03±13.63	45.17±15.86	1.00±0.25	1.61±0.55	5.20±2.20
IR	4.61±0.83	7.75±0.26	15.13±0.59	41.98±1.21	54.20±0.70	19.55±0.44	36.05±0.53	791.75±126.84	15.75±0.29	17.55±0.22	7.55±0.26	0.60±0.09	71.00±11.91	25.47±11.71	0.90±0.17	0.55±0.29	2.10±0.73
10 + IR	5.18±0.96	7.67±0.17	15.00±0.28	43.39±0.92	56.52±0.31	19.55±0.25	34.60±0.29	879.00±30.95	16.38±0.26	17.63±0.20	7.34±0.26	0.65±0.04	56.80±7.06	30.48±7.71	0.39±0.17	1.80±0.82	10.52±2.43
50 + IR	4.47±1.02	7.46±0.14	15.00±0.26	42.49±0.91	56.99±0.27	20.11±0.18	35.33±0.44	855.86±22.75	15.79±0.17	17.86±0.24	7.22±0.19	0.62±0.02	61.64±9.08	26.06±7.33	0.43±0.16	2.61±1.51	9.24±1.75
100 + IR	4.60±1.19	7.55±0.26	14.77±0.55	42.08±1.29	55.77±0.32	19.57±0.21	35.08±0.38	807.50±45.15	15.72±0.08	17.55±0.22	7.25±0.14	0.59±0.04	57.27±8.92	33.32±9.23	0.68±0.08	0.88±0.28	7.85±1.46
200 + IR	4.96±1.07	7.32±0.04	14.87±0.30	41.23±0.50	56.32±0.54	20.27±0.38	36.02±0.49	968.17±70.92	15.78±0.19	17.67±0.12	7.41±0.18	0.72±0.06	58.50±12.51	33.01±11.80	0.64±0.10	1.29±0.54	6.55±2.22
400 + IR	5.00±2.48	7.23±0.19	14.56±0.50	40.51±1.07	56.14±0.42	20.14±0.23	35.97±0.60	839.00±86.02	16.20±0.24	17.46±0.34	7.27±0.20	0.61±0.07	61.40±11.05	32.83±10.54	0.59±0.17	1.05±0.45	4.14±1.89

WBC = white blood cell; RBC = red blood cell; HBG = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet; RDW = red cell distribution width; PDW = platelet distribution width; MPV = mean platelet volume; PCT = plateletcrit; NEU = neutrophil; LYMPH = lymphocyte; EO = eosinophil; BASO = basophil; MONO = monocyte

Table 3. The effects of Phikud Navakot (PN) on the biochemical parameters in serum of Sprague Dawley rats induced ischemia/reperfusion (IR) by LCA ligation. Values are expressed as mean ± SEM, n = 3-7

Treatment (mg/kg BW)	GLU (mg/dL)	BUN (mg/dL)	CREA (mg/dL)	CHOL (mg/dL)	TG (mg/dL)	URIC (mg/dL)	TP (g/dL)	ALB (g/dL)	GLOB (g/dL)	Bili-T (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
Sham	345.90±20.90	19.43±1.53	0.41±0.05	75.33±12.91	47.00±7.64	1.29±0.27	5.58±0.29	3.70±0.17	1.87±0.15	0.08±0.01	386.50±28.70	70.67±15.00	142.33±17.65
IR	227.73±47.48	18.65±6.02	0.36±0.04	77.50±11.69	82.50±18.49	1.16±0.10	5.41±0.57	3.78±0.21	1.63±0.45	0.08±0.01	849.43±246.19	109.23±31.71	122.25±12.87
10 + IR	326.94±48.96	28.50±2.48	0.57±0.08	92.60±14.85	93.20±15.72	4.60±1.21	6.99±0.60	4.10±0.58	2.90±0.41	0.06±0.01	638.88±51.96	148.06±13.50	118.40±27.30
50 + IR	354.10±56.62	28.66±1.83	0.49±0.04	115.14±3.33	77.71±3.71	4.57±0.95	7.10±0.16	4.46±0.09	2.64±0.08	0.09±0.01	982.96±194.22	151.64±12.65	145.71±19.64
100 + IR	300.42±25.89	24.70±1.45	0.44±0.03	103.40±7.82	75.60±12.34	1.20±0.14	6.54±0.30	4.06±0.20	2.48±0.12	0.09±0.01	484.80±66.05	101.44±17.29	137.80±15.78
200 + IR	278.18±22.06	24.58±1.83	0.40±0.06	92.75±10.66	55.50±3.23	1.29±0.12	5.96±0.57	3.68±0.23	2.28±0.38	0.10±0.02	575.00±143.21	80.20±14.86	118.00±9.70
400 + IR	305.53±32.02	25.02±3.16	0.46±0.04	117.00±7.66	107.29±17.98	1.05±0.09	6.65±0.45	4.19±0.26	2.47±0.19	0.08±0.01	705.93±209.79	107.29±22.54	139.57±19.09

GLU = glucose; BUN = blood urea nitrogen; CREA = creatinine; CHOL = cholesterol; TG = triglyceride; TP = total protein; ALB = albumin; GLOB = globulin; Bili-T = total bilirubin; AST = aspartate aminotransferase; ALT = alanine transaminase; ALP = alkaline phosphatase

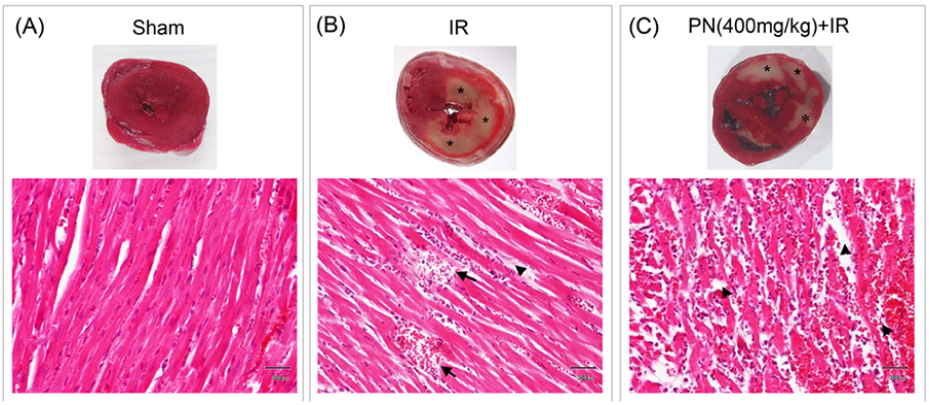

Fig. 1 Upper represented TTC stained heart and lower represented histopathological changes (H&E) of rats cardiac tissue (magnification, x400) in (A) sham (B) ischemia/reperfusion (IR) and (C) Phikud Navakot (PN) at doses of 400 mg/kg BW+IR. Asterisk: infarct area, arrow: infiltration of inflammatory cells, arrow head: intramuscular edema and perivascular edema. bar = 300 micron.

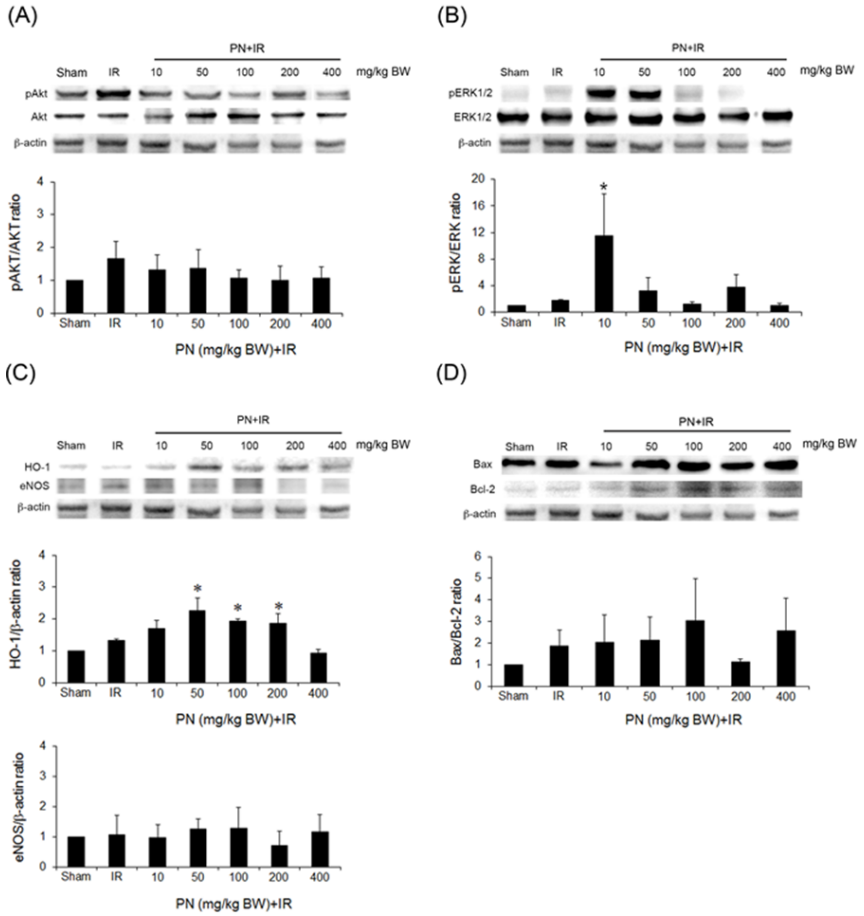

Fig. 2 The effect of Phikud Navakot (PN) at doses of 10-400 mg/kg BW on protein expression in the cardiac tissues. Representative protein bands of and their density: (A) pAkt/Akt ratio, (B) pERK/ERK ratio, (C) HO-1/beta-actin and eNOS/beta-actin ratios, and (D) Bax/Bcl-2 ratio. Data are presented as the mean ± SEM (n = 3). *p<0.05 vs. sham.

has been demonstrated that activation of protein kinase Akt was not sufficient to protect rat heart against IR induced by isolated heart[25].

To our knowledge, this is the first time showing that pre-treatment of PN for 7 days before induction of MI up-regulated the expression of survival proteins ERK1/2 and HO-1, even if PN was not able to prevent myocardial damage from LCA ligation. A longer treatment of PN prior to induction of MI heart should be further investigated.

What is already known on this topic ?

The present study is the first time to evaluate the effects of Phikud Navakot on myocardial ischemia/reperfusion injury induced by the left coronary ligation technique in rats.

What this study adds ?

Seven days pre-treatment of Phikud Navakot prior to induction of acute myocardial infarction up-regulates the protein expression of pERK and HO-1 in rat cardiac tissue.

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Potential conflict of interest

None.

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ผลของสารสกัดพิกัดนวโกฐต่อหัวใจของหนูแรทที่ชักนำให้เกิดภาวะหัวใจขาดเลือดแบบชั่วคราว

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ภูมิหลัง: พิกัดนวโกฐประกอบด้วยสมุนไพร 9 ชนิด เป็นส่วนประกอบหลักของยาหอมนวโกฐ ซึ่งเป็นยาแผนโบราณที่ใช้รักษากลุ่มอาการทางระบบหัวใจและหลอดเลือด

วัตถุประสงค์: เพื่อศึกษาฤทธิ์ของพิกัดนวโกฐในการปกป้องการตายของหัวใจหนูแรทเพศผู้สายพันธุ์ Sprague Dawley ที่ชักนำให้เกิดภาวะหัวใจขาดเลือดแบบชั่วคราว (IR)

วัสดุและวิธีการ: หนูแรทแบ่งออกเป็น 7 กลุ่ม ได้แก่ กลุ่มที่ได้รับการผ่าตัดแบบควบคุม กลุ่ม IR และกลุ่มที่ได้รับการป้อนสารสกัดพิกัดนวโกฐขนาด 10, 50, 100, 200 และ 400 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัมต่อวันทางปากเป็นเวลา 7 วัน ก่อนการชักนำให้เกิดภาวะ IR โดยวิธีการผูกหลอดเลือดแดงโคโรนารีข้างซ้ายเป็นเวลา 30 นาที จากนั้นคลายหลอดเลือดเป็นเวลา 24 ชั่วโมง เมื่อครบกำหนดเวลาเก็บตัวอย่างเลือดเพื่อตรวจวัดค่าทางเคมีและค่าทางโลหิตวิทยา และเก็บหัวใจเพื่อนำมาศึกษาทางจุลพยาธิวิทยาและศึกษาการแสดงออกของโปรตีนโดยวิธี Western blot analysis

ผลการศึกษา: หนูแรท IR เกิด ST elevation ในคลื่นไฟฟ้าหัวใจและระดับ troponin I (TnI) ในซีรัมเพิ่มขึ้น เป็นการยืนยันการเกิดกล้ามเนื้อหัวใจตาย และเมื่อนำหัวใจมาศึกษาทางจุลพยาธิวิทยาพบว่าเนื้อเยื่อหัวใจเกิดการอักเสบ มีการคั่งของเม็ดเลือดขาวและบวม หนูแรทที่ได้รับการป้อนสารสกัดพิกัดนวโกฐขนาด 10, 50, 100, 200 และ 400 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัมต่อวัน เป็นเวลา 7 วัน ก่อนการชักนำให้เกิดภาวะ IR พบว่าระดับ TnI ในซีรัม และพยาธิสภาพของหัวใจไม่มีการเปลี่ยนแปลงเมื่อเปรียบเทียบกับกลุ่ม IR อย่างไรก็ตามเมื่อศึกษาการแสดงออกของโปรตีนพบว่าหนูแรทที่ได้รับสารสกัดพิกัดนวโกฐขนาด 10 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัมต่อวัน มีอัตราส่วนของโปรตีน pERK/ERK เพิ่มขึ้น ($p<0.05$) ในขณะที่หนูแรทที่ได้รับสารสกัดพิกัดนวโกฐขนาด 50-200 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม มีการแสดงออกของโปรตีน HO-1 เพิ่มขึ้น ($p<0.05$) เมื่อเปรียบเทียบกับหนูแรท IR

สรุป: การได้รับสารสกัดพิกัดนวโกฐเป็นเวลา 7 วัน ก่อนการชักนำให้เกิดภาวะ IR โดยวิธีการผูกหลอดเลือดแดงโคโรนารีข้างซ้าย ไม่สามารถป้องกันการตายของเนื้อเยื่อหัวใจในระดับจุลพยาธิวิทยาได้ แต่พบการเปลี่ยนแปลงในระดับโมเลกุลของโปรตีนที่ทำหน้าที่ปกป้องการตายของเนื้อเยื่อหัวใจคือมีการเพิ่มของอัตราส่วน pERK/ERK และ HO-1 การศึกษาครั้งนี้ทำให้ทราบถึงกลไกในระดับโมเลกุลของพิกัดนวโกฐในการปกป้องหัวใจจากภาวะ IR