doi.org/10.36721/PJPS.2020.33.2.SUP.745-749.1

Antihypertensive and vasoprotective effects of *Clausena lansium* fruits extract in L-NAME induced hypertensive rats

Wathita Phachonpai[1]* and Terdthai-Tongun[2]
[1]Division of Physiology, School of Medical Sciences, University of Phayao, Phayao, Thailand
[2]Division of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract: Oxidative stress is considered to play an important role in the pathophysiology of hypertension. The aim of this research was to find out whether *Clausena lansium* (Lour.) Skeels of Wampee (WP) fruits extract attenuate the progression of high blood pressure, endothelial dysfunction and preservation of antioxidant status with using a nitric oxide synthase (NOS) inhibitor, $N^{(G)}$-nitro-L-arginine methyl ester (L-NAME) induced hypertension and oxidative stress in rats. Healthy adult male rats were received L-NAME at dose of 50mg/kg/day in drinking water for 4 weeks and were orally administered 250 or 500mg/kg of an aqueous extract of WP fruits extract daily for 2 consecutive weeks. Quercetin (QC; 25 mg/kg) was served as a positive control. The results showed that arterial blood pressure, vascular superoxide production, and plasma malondialdehyde level were markedly induced in L-NAME treated rats. In addition, serum nitric oxide, and glutathione levels were also reduced after L-NAME administration. However, daily administration with the high dose of WP fruits extract significantly alleviated these deleterious effects by attenuated high blood pressure, reversed the L-NAME-induced suppression in serum levels of nitric oxide, mitigated endothelial dysfunction, reduced oxidative stress and restored antioxidant glutathione. This suggests that WP fruits extract is a potential candidate for the development as a novel antihypertensive agents in the future.

Keywords: Antioxidant, hypertension, L-NAME, Wampee, nitric oxide, *Clausena lansium.*

INTRODUCTION

Nowadays, trend in hypertension prevalence is increasing worldwide (Mills *et al.,* 2016). Several studies reported that oxidative stress and nitric oxide (NO) deficiency have a causal role in the pathogenesis of hypertension (Varadharaj *et al.,* 2015; Loperena and Harrison 2017). Novel anti-hypertensive drugs have been established with the aim to reduce blood pressure and prevent the associated risks (Massimo *et al.,* 2018).

Non-pharmacologic therapy such as lifestyle behaviors or exercise plays an essential role in the prevention and control of hypertension (Erejuwa *et al.,* 2019). However, many people use medicinal plants to prevent or treat high blood pressure, because they are derived from "natural" source, safe and effective (Kretchy *et al.,* 2014). In addition, the parts of medicinal plants are rich sources of bioactive components that have known therapeutic applications (Aye *et al.,* 2019).

Several *in vitro* and *in vivo* evidences have shown that herbs, fruits, vegetables and other plant products can have beneficial effects in reduction of hypertension, partly due to its antioxidant effect (Al Disi *et al.,* 2016, Borgi *et al.,* 2016, Jung *et al.,* 2018, Forni *et al.,* 2019). For example, daily consumption of green tea (*Camellia sinensis*) and oolong tea showed a positive effect to reduce high blood pressure (Peng *et al.,* 2014). Oral administration of *Lycopersicon esculentum* or tomato extract contains vitamin E, lycopene, and beta carotene, which are known as effective and powerful antioxidant, to reduce blood pressure in mild hypertension patients (Chen *et al.,* 2013). It had been already documented that oral administration of quercetin, a flavonoid with strong antioxidant properties and exert anti-hypertensive effects in women with rheumatoid arthritis (Javadi *et al.,* 2014). Therefore, there is still room for the researchers to development the new agents from natural sources to treat hypertension.

Clausena lansium (Lour.) Skeels or Wampee (WP), plant well known in the Southeast Asia region, known in Thai as "Mafai jean". The results of multiple studies have reported that several bioactive compounds for instance, coumarins, cyclic amides, sesquiterpene from WP isolate extract were found to reveal strong antioxidant, antifungal, anti-inflammation hepatoprotective, anticancer activities, cerebral protective, and nootropic properties (Ng *et al.,* 2003; Adebajo *et al.,* 2009; Prasad *et al.,* 2009). However, the effect of WP fruits extract to battle hypertension in L-NAME induced hypertensive rats was not observed until now.

Thus, the purpose of this study was to determine the potential antihypertensive effect of WP fruits extract and its possible mechanism to reduce high blood pressure in experimental hypertensive rats.

**Corresponding author:* e-mail: phachonpai@yahoo.com

MATERIALS AND METHODS

Plant materials and Aqueous extract preparation

The fresh fruits of WP at the mature stage were collected from Nan Province, Thailand. Fruits sample were washed immediately and carefully with tap water, then dried with hot air oven at 40°C, blend, ground into powder and macerate (WP fruits to water ration, 1:5) for 24 hours. Dried powder (10g) of WP was extracted with 200 ml of water at 30°C for 24 hours in a rotary shaker, and then filtered, concentrated using a rotary evaporator to obtain a crude extract. The residue of WP was dissolved in water and freeze dried approach, finally stored in a refrigerator until further study. The extraction yield was 15.87%.

Drug and chemicals

Quercetin (QC) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). L-NAME was purchased from Sigma Aldrich (Germany). Pentobarbital sodium was produced by China Medicine (Group) Shanghai Chemical Reagent, Co, Ltd. The chemicals 5,5-dithiobis-2-nitrobenzoic acid (DTNB), dihydrogen phosphate ($H_2PO_4^{-}$), trichloroacetic acid ((TCA), thiobarbituric acid ((TBA), Lucigenin and all other chemicals used in this experiment were of analytical grade and purchased from Sigma Chemical Company (St. Louis, MO, USA).

Animals and treatment protocols

Healthy adult male Sprague-Dawley rats weighing 180-250 g were conducted from the National Laboratory Animal Center, Salaya, Nakorn Pathom, Thailand. They were housed at 20±2°C on 12: 12 h dark/light cycle and fed a normal chow diet ad libitum. All animal procedures and experimental protocols were permitted by the Animal Ethic Committee of University of Phayao (5801040004).

After 1 week of adaption, all rats were randomly divided into various groups (n=6/group) as follows:

1) NT: normotensive control treated group.
2) LN: L-NAME treated group.
3) QC + LN: QC (Quercetin 25 mg/kg) + L-NAME (positive control) treated group
4) WP 250 + LN: wampee fruit extract (250 mg/kg) + L-NAME treated group
5) WP 500 + LN: wampee fruit extract (500 mg/kg) + L-NAME treated group

Except for the NT treated group that was received with normal saline only. whereas animals in the other groups were first treated with L-NAME (50 mg/kg) in drinking water for 4 weeks and then the LN, QC, WP 250 and WP 500 treated groups were co-received with normal saline were intragastrically administered for 2 consecutive weeks. The dose of quercetin is based on the report on its antihypertensive and antioxidant effects in hypertensive rats (Monteiro *et al.*, 2012).

Blood pressure measurement and biochemical determination

Blood pressure (BP) and heart rate (HR) were monitored at the beginning of the treatment by non–invasive technique, using a tail-cuff system (NIBP monitoring system, IIITC Inc, Woodland Hills, CA, USA). Rats with SBP ≥ 200 mmHg and DBP ≥ 160 mmHg were defined as hypertensive. At the end of the experimental period, rats were anesthetized by pentobarbital sodium (PB; 60 mg/kg i.p.). The right femoral artery of each rat was cannulated by polyethylene catheter and connected to a pressure transducer (iWorx 214 - BP -100 intravascular blood pressure probe) for continuous monitoring of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MABP), whereas the measurement of heart rate (HR) using program LabScribe2 analysis software (iWorx Systems Inc., Dover, NH, USA). Baseline values of BP and HR were recorded for 30 min and then all rats were sacrificed by an overdose with intraperitoneal PB injection. Blood samples were collected from vena cava transferred to anticoagulant (EDTA) containing tubes, centrifuged for 10 min and stored in the refrigerator immediately at -80 °C for used. Analysis of vascular superoxide (O2.-) production in carotid arteries using the Lucigenin-enhanced chemiluminescence method as previously described (Boonla *et al.*, 2014). Total serum nitric oxide (NO) levels were determined using colorimetric assay kit, the absorbance values of the solutions was measured at a wavelength 540 nm. Assay of malondialdehyde (MDA), a lipid peroxidation marker and blood glutathione (GSH) levels were carried out as previously described (Nakmareong *et al.*, 2012).

STATISTICAL ANALYSIS

All results were reported as mean ± Standard Error of Mean (SEM). The differences among various groups were determine by one-way analysis of variance (ANOVA) followed by Tukey's *posthoc* test for multiple comparisons using SPSS Version 11 software package. A probability level less than 0.05 were accepted as significance.

RESULTS

Effect of WP fruits extract on blood pressure in L-NAME induced hypertension

Oral administration of WP fruits extract did not produce any toxic signs or deaths in rats. In addition, there were no relative differences symptoms, observed in parameters such as behavioral pattern, appetite or body weights of all experimental groups.

At the start of the study, no significant differences in baseline values of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure

(MAP) in all experimental groups. However, SBP, DBP, and MAP were increased significantly ($p<0.05$) in all LN treated groups compared to NT group at the end of the study. Interestingly, statistically significant decreased of SBP, DBP, and MAP were observed in WP fruits extract at high dose (500 mg/kg) and QC when compared with LN hypertensive rats ($p<0.05$; table 1). On the other hand, there were no changes in these parameters in the WP fruits extract at the dose of 250 mg/kg group. Meanwhile, no significant differences in HR among of various groups.

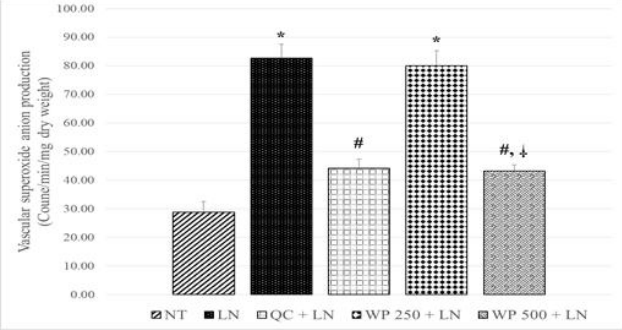

Fig. 1: Effect of WP fruits extract on vascular $O_2^{\cdot-}$ production in carotid arteries. Data are expressed as mean ± SEM. (n=6/group). *p<0.05 compared to NT control, #p <0.05 compared to LN group and ⁎p<0.05 compared to WP 250 + LN group.

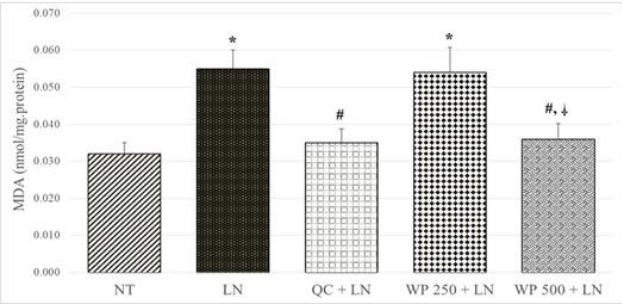

Fig. 2: Effect of WP fruits extract on plasma MDA levels. Data are expressed as mean ± SEM. (n=6/group). *p<0.05 compared to NT control, #p<0.05 compared to LN group, and *p<0.05 compared to WP 250 + LN group.

Effect of WP fruits extract on vascular superoxide ($O_2^{\cdot-}$) production and plasma MDA level in L-NAME induced hypertension

To assess whether the possible mechanisms of WP fruits extract to combat L-NAME induced high blood pressure via anti oxidative damage mechanism, the vascular $O_2^{\cdot-}$ production and plasma MDA level were evaluated (fig. 1 and 2). LN significantly increased the level of $O_2^{\cdot-}$ production in carotid arteries compared to the NT group ($p<0.05$). Increased oxidative stress was observed in LN hypertensive rats indicated by plasma MDA level enhancement compared to NT group ($p<0.05$). However, a significant reduction in $O_2^{\cdot-}$production and MDA levels were found in LN rats treated with the higher dose of WP fruits extract and QC ($p<0.05$).

Effect of WP fruits extract on serum NO levels in L-NAME induced hypertension

LN treatment significantly reduced the serum NO levels in all LN treated group compared to NT group. Again, WP fruits extract (500mg/kg) and QC significantly ($p<0.05$) attenuate LN induced decrease in serum NO levels (fig. 3).

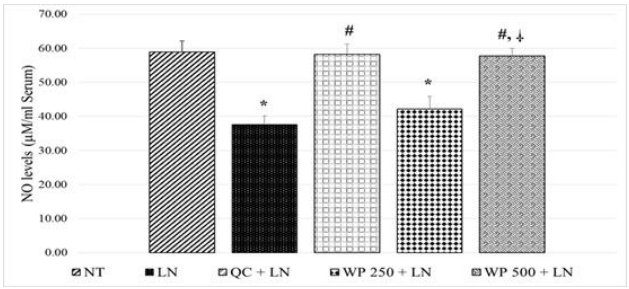

Fig. 3: Effect of WP fruits extract on serum nitric oxide (NO) levels. Data are expressed as mean ± SEM. (n=6/group). *p<0.05 compared to NT control, #p<0.05 compared to LN group and ⁎p<0.05 compared to WP 250 + LN group.

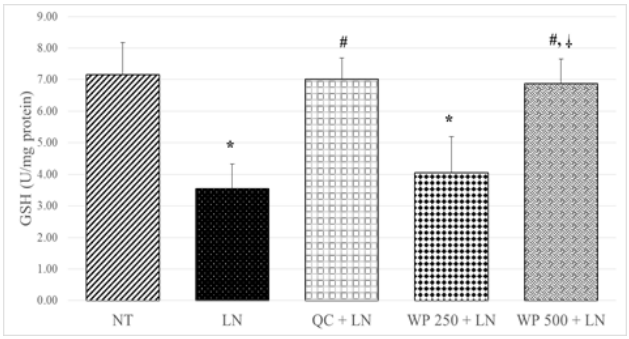

Fig. 4: Effect of WP fruits extract on plasma GSH levels. Data are expressed as mean ± SEM. (n=6/group). *p<0.05 compared to NT control, #p<0.05 compared to LN group and *p<0.05 compared to WP 250 + LN group.

Effect of WP fruits extract on antioxidant enzyme in L-NAME induced hypertension

LN hypertensive rats appeared to have lower GSH levels compared to NT group ($p<0.05$; fig. 4). In contrast, LN hypertensive rats treated with WP fruits extract (500 mg/kg) prevented the reduction of plasma GSH ($p<0.05$). Interestingly, induction of antioxidant status of LN hypertensive rats treated with a high dose of WP fruits extract appeared quite similar to the NT group.

DISCUSSION

Our current study has shown, for the first time the antihypertensive effect of aqueous extract from the fruits of WP in L-NAME induced hypertensive rats.

Important data has been previously declared by Robinson *et al.*, (2018) who reported that reactive oxygen species (ROS) may directly alter vascular tone or vascular

Table 1: Effect of WP fruits extract on arterial blood pressure of rats in LN induced hypertension.

Group	systolic blood pressure (SBP; mmHg)	diastolic blood pressure (DSP; mmHg)	mean arterial blood pressure (MAP; mmHg)
NT	123.7 ± 2.1	81.5 ± 2.2	95.57 ± 1.5
LN	185.3 ± 2.5*	135.2 ± 4.8*	155.24 ± 4.7*
QC + LN	154.7 ±3.2 #,	95.2 ± 2.2 #,	125.04 ± 2.6 #,
WP 250 + LN	184.5 ± 3.8*	134.4 ± 3.6*	151.1 ± 3.2*
WP 500 + LN	155.5 ± 2.1 #,	96.4 ± 2.0 #,	126.2 ± 2.5 #,

Data are expressed as mean ± SEM. (n=6/group). *$p < 0.05$ compared to NT control, #$p < 0.05$ compared to LN group, and □$p<0.05$ compared to WP 250 + LN group

function via several mechanisms such as decreased NO bioavailability. NO is the endothelium-derived relaxing factor, has been responsible for vasodilator tone regulation, and control systemic blood pressure both *in vitro* and *in vivo* (Touyz and Briones 2011; Konukoglu D and Uzun H 2017). Thus, there is an imbalance between of NO level or ROS production which result in endothelial dysfunction, leading to hypertension (Togliatto *et al.,* 2017).

It is widely accepted that increased O_2^- levels lead to the development of hypertension by decreasing the NO bioavailability (Conti *et al.,* 2013), since NO reacts with O_2^- to produce peroxynitrite ($ONOO^-$), a strongly cytotoxic reactive nitrogen species (Bartesaghi S and Radi R 2018). In addition, Tamás *et al.,* (2018) demonstrated that L-NAME administration could induce O_2^- generation, leading to increase $ONOO^-$ level and decrease the amount of NO. Similarly, in our study, L-NAME administration for 4 weeks produced a marked induction of NO, O_2^- level, blood pressure and oxidative stress phenomena.

Supplementation with exogenous antioxidants or boosting endogenous antioxidant could attenuate oxidative stress status and prevent progression to hypertension (Tan *et al.,* 2018). Correspondingly, our results of biochemical analysis presented that daily consumption of WP fruits extract at dose of 500 mg/kg for 2 weeks, attenuated the progression of high blood pressure in hypertensive rats. For the proper interpretation of the possible mechanisms of WP fruits extract to exert its blood pressure lowering effect may occur partly via the antioxidant properties and the vasoprotective effect. Our study provided evidence that oral administration with WP fruits extract was able to restore antioxidant GSH as well as decreased plasma MDA level, reduces O_2^- generation which in turn increases the amount of NO, improves endothelial dysfunction and induces vasodilation and hypotension which indicated by the decrease in SBP, DBP and MAP in LN hypertensive rats. Importantly, antihypertensive and antioxidant effects of WP fruits extract at dose of 500mg/kg were similar to that of the positive control, quercetin attenuates hypertension by reducing oxidative stress in L-NAME-induced hypertensive rats. Important data were published by Larson *et al.,* (2010) who demonstrated that oral administration of quercetin could attenuate hypertension, cardiac output and the functional vascular changes in hypertensive rats. These effects were related with a reduced oxidant status due to the antioxidant activity of the quercetin. Interestingly, our results revealed no difference in the heart rate (HR) between groups which indicated that WP fruits extract induced lowering of blood pressure results from a decline in peripheral vascular resistance (PVR) attributable to decreased venous and arterial resistance with no change in cardiac output.

However, one significant limitation of our study is identification the active components in WP fruits extract responsible for reduction in blood pressure and its possible mechanisms need to be discussed further.

CONCLUSION

To the best of our knowledge, this is the first scientific paper to indicate that WP fruits extract exhibits the peripheral antihypertensive and vasoprotective effects in L-NAME induced hypertensive rats. Therefore, the supplementation of WP fruits extract may enable development of future strategy for prevents the progression of hypertension. However, a clinical trial to examine the antihypertensive of this extract is still required.

ACNOWLEDGEMENT

All authors are grateful to the University of Phayao for providing the financial support this study.

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