Curcumin Mitigates Hypertension, Endothelial Dysfunction and Oxidative Stress in Rats Chronically Exposed to Lead

เคอร์คูมินบรรเทาภาวะความดันเลือดสูง เอนโดทีเลียมทำงานผิดปกติ และภาวะเครียดออกซิเดชันในหนูแรทที่ได้รับตะกั่วเป็นเวลานาน

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ABSTRACT

The aim of this study is to evaluate whether curcumin (CUR) could alleviate hypertension, endothelial dysfunction and oxidative stress in rats chronically exposed to lead (Pb). Male Sprague-Dawley rats received lead acetate (100 mg/L) for 16 weeks. CUR (100 mg/kg/day) was administered for the last 4 weeks. Results showed that Pb exposure increased arterial blood pressure, elevated peripheral vascular resistance and blunted vascular response to acetylcholine. These alterations were associated with increased superoxide production, increased plasma malondialdehyde and decreased blood glutathione. CUR significantly improved hemodynamic status and vascular response when compared with untreated Pb controls ($p<0.05$). These improvements were associated with a suppression of oxidant formation and an increase of antioxidant glutathione.

บทคัดย่อ

วัตถุประสงค์ของการศึกษานี้เพื่อประเมินว่าเคอร์คูมินสามารถลดภาวะความดันเลือดสูง ภาวะเซลล์เอนโดทีเลียมทำงานผิดปกติ และภาวะเครียดออกซิเดชันได้หรือไม่ในหนูแรทเพศผู้ สายพันธุ์ Sprague-Dawley ที่ได้รับตะกั่วแอซิเตท (100 มก./ลิตร) เป็นเวลา 16 สัปดาห์ และใน 4 สัปดาห์สุดท้ายหนูแรทถูกป้อนด้วยเคอร์คูมิน (100 มก./กก./วัน) ผลการศึกษาพบว่าตะกั่วสามารถเพิ่มความดันเลือดแดง เพิ่มความต้านทานของหลอดเลือดส่วนปลาย และลดการตอบสนองของหลอดเลือดต่อแอซิทิลโคลีน การเปลี่ยนแปลงเหล่านี้สัมพันธ์กับการสร้างซูเปอร์ออกไซด์ที่เพิ่มขึ้น การเพิ่มระดับมาลอนไดอัลดีไฮด์ในพลาสมา และการลดลงของกลูต้าไธโอนในเลือด เคอร์คูมินสามารถปรับพลศาสตร์การไหลเวียนเลือดและเพิ่มการตอบสนองของหลอดเลือดได้ดีขึ้นเมื่อเปรียบเทียบกับกลุ่มที่ได้รับตะกั่วอย่างเดียว ($p<0.05$) ผลดีเหล่านี้สัมพันธ์กับการกดการสร้างออกซิแดนซ์และการเพิ่มสารต้านออกซิแดนซ์กลูต้าไธโอน

Keywords: Pb-induced hypertension, Curcumin, Vascular dysfunction
คำสำคัญ: ตะกั่วเหนี่ยวนำภาวะความดันเลือดสูง เคอร์คูมิน หลอดเลือดทำงานผิดปกติ

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Introduction

Lead (Pb) is widely used in various industries in particularly, battery, paint, electronics manufacturing, vehicle product and welding. In recent year there are various reports that Pb contaminates in various wears goods and food stuff in daily life such as cosmetics, plastic, sea and river food and in tap water (United Nations Environment Programme - World Health Organization [UNEP-WHO], 2012). Therefore, most of worldwide populations have unconsciously received low level of Pb in daily lifestyle. When Pb enters into the body it causes toxicity to various systems, especially cardiovascular system (Goch and Goch, 2005; Rizzi et al., 2009; Sica, 2004). Previous studies reported that Pb can cause hypertension, which is the most common cardiovascular risk (WHO, 2014). Oxidative stress is one of the major mechanisms of Pb toxicity (Flora et al., 2008). Pb induces excessive reactive oxygen species (ROS) production (Kasperczyk et al., 2005; Sangartit et al., 2014). In vasculature, the excessive ROS particularly superoxide ($O_2^{\bullet-}$) can quench nitric oxide (NO), via combination to form peroxynitrite ($ONOO^-$) (Pisoschi et al., 2009). Moreover excessive ROS also damage endothelial cell and blunt its response. The imbalance of vasoregulator molecule and endothelial damage can cause the vascular dysfunction leading to increased vascular resistance and finally increased blood pressure (Glasser et al., 1996).

Curcumin (CUR) is the richest isoform of curcuminoids which is extracted from the turmeric. CUR has been proven for the pharmacological advantages of antioxidant, anti-carcinogenic, anti-inflammatory and anti-hypertensive effects (Kukongviriyapan et al., 2014; Mukhopadhyay et al., 1982; Wang et al., 2009; Zheng et al., 2014). However, the effect of CUR on alleviating Pb-induced vascular dysfunction and oxidative stress in rats has not been explored. Therefore, the objective of this study is to investigate effect of CUR against oxidative stress and vascular dysfunction in rats chronically exposed to Pb.

Material and Methods

Male Sprague-Dawley rats (150-160g) were used in this study. All animals were maintained in a temperature controlled room (25±2 °C) with 12-h dark/light cycle at the Northeast Laboratory Animal Center, Khon Kaen University, Thailand. The animals were given free access to standard chow diet (Chareon Pokapan Co. Ltd., Thailand). The animals were carried out in accordance with recommendation in the Guide for the Care and Use of Laboratory Animal of the National Institutes of Health. All animals were randomly divided into four groups, 1) control group; 2) control+CUR; 3) Pb-treated group; and 4) Pb-treated group+CUR. In group 1 and 2, rats received deionized water for 16 weeks, and the last 4 weeks of experimental period rats in group 2 were intragastrically administered with CUR 100 mg/kg/day. In group 3 and 4, rats received Pb acetate in concentration of 100 mg/L as a drinking water for 16 weeks, and the last 4 weeks of experimental period rats in group 4 were intragastrically administered with CUR 100 mg/kg/day.

Hemodynamic and vascular responsiveness measurements

At the end of experiment, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). The surgical stage

of anesthetization, the tracheotomy was performed to facilitate respiration. The body temperature was kept between 37± 2°C throughout the study with the heating pad. The left femoral artery was cannulated with polyethylene tubing connected to a pressure transducer for continuously monitoring of arterial blood pressure. Left femoral vein was cannulated for infusion of vasoactive agent, acetylcholine (ACh) in concentration of 1, 3, 10 nM.

To test the vascular responsiveness the changing in mean arterial pressure (MAP) after infusion of bolus of ACh were recorded. The abdominal aorta was approached by minimal opening of intraperitoneal cavity for hind- limb blood flow (HBF) measurement. Hindlimb vascular resistance (HVR) was calculated from MAP and HBF as following equation; HVR = MAP/HBF (mmHg/min/100 g tissue/mL or Peripheral Resistance Unit: PRU).

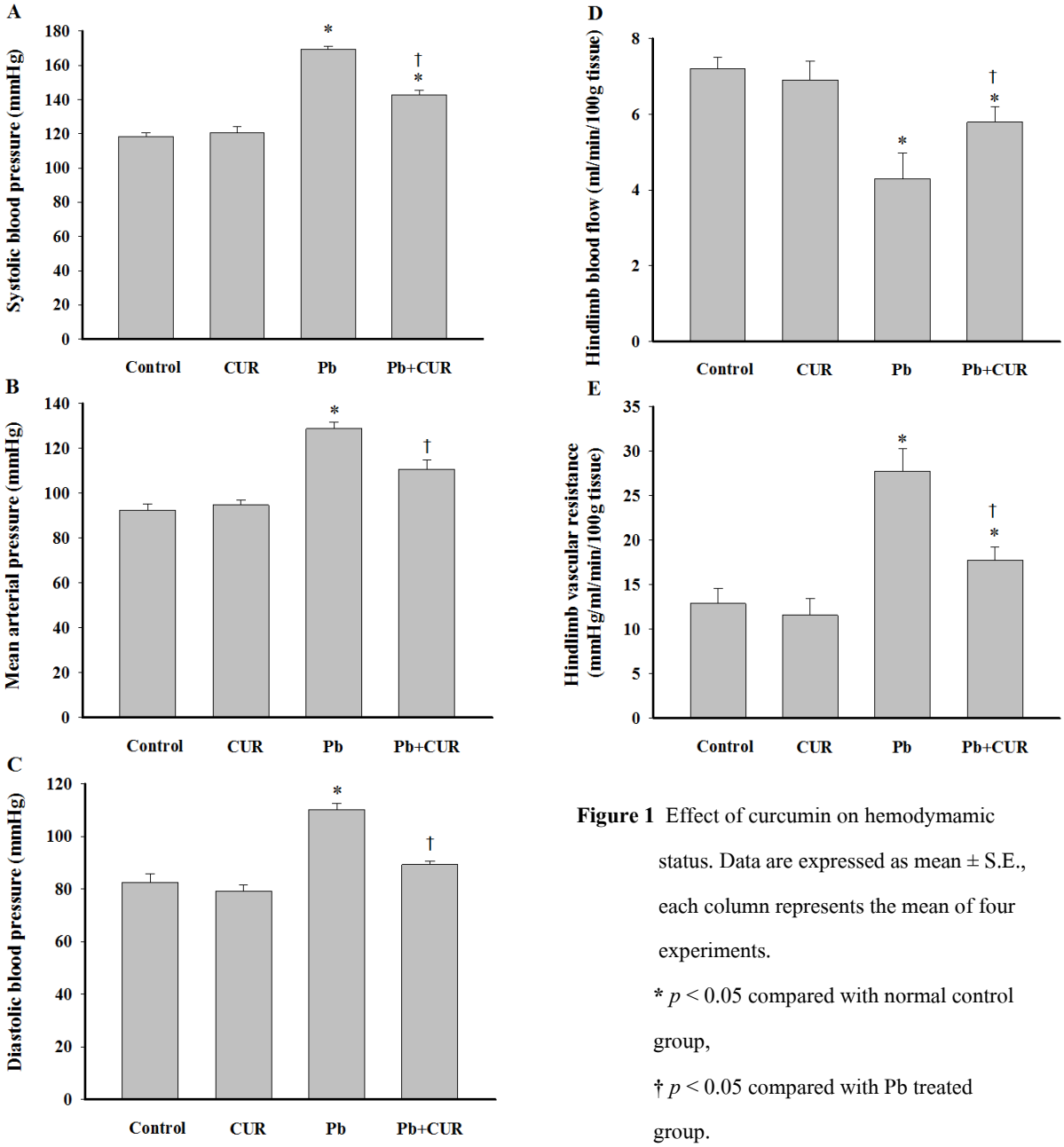

Figure 1 Effect of curcumin on hemodymamic status. Data are expressed as mean ± S.E., each column represents the mean of four experiments.

* $p < 0.05$ compared with normal control group,

† $p < 0.05$ compared with Pb treated group.

Biochemical assays

Assay of $O_2^{\bullet-}$ production

Vascular $O_2^{\bullet-}$ production in the carotid artery was determined by using luciginin-enhanced chemilu minescence as previously described (Kukongviriyapan et al., 2014). The carotid artery were rapidly excised and incubated in oxygenated Krebs-Ringer bicarbonate solution at 37°C for 30 min. The chemiluminescence signals were measured by added lucigenin and using luminometer (Turner Biosystems, 23 CA, USA). The photon counts are integrated every 15 s for 5 min. The data were expressed as relative light unit count/mg dry wt/min.

Assay of malondialdehyde (MDA)

The level of MDA in plasma was assayed following a previous method (Kukongviriyapan et al., 2014). The absorbance of the supernatant was measured at 532 nm by spectrophotometer, a standard curve was generated by using 1, 1, 3, 3-tetraethoxy propane.

Assay of glutathione (GSH)

Assay of redox status in whole blood were performed by previously described methods (Tietze, 1969) with some modifications (Nakmareong et al., 2011). Reaction and absorbance were monitored with UV/Visible spectrophoto- meter (Ultrospec 3600 *pro*. Biochom Ltd. UK). Optical density reading is set at 412 nm and read every 15 sec for 10 times.

Results

Effect of CUR on hemodynamic status and vascular responsiveness

Administration of CUR at dose of 100 mg/kg did not alter hemodynamic or vascular function in normal control rats. Daily intake of Pb acetate at 100 mg/L concentration caused a significant increase in systolic, diastolic, mean arterial blood pressure levels and vascular resistance when compared with the normal control group (Fig. 1).

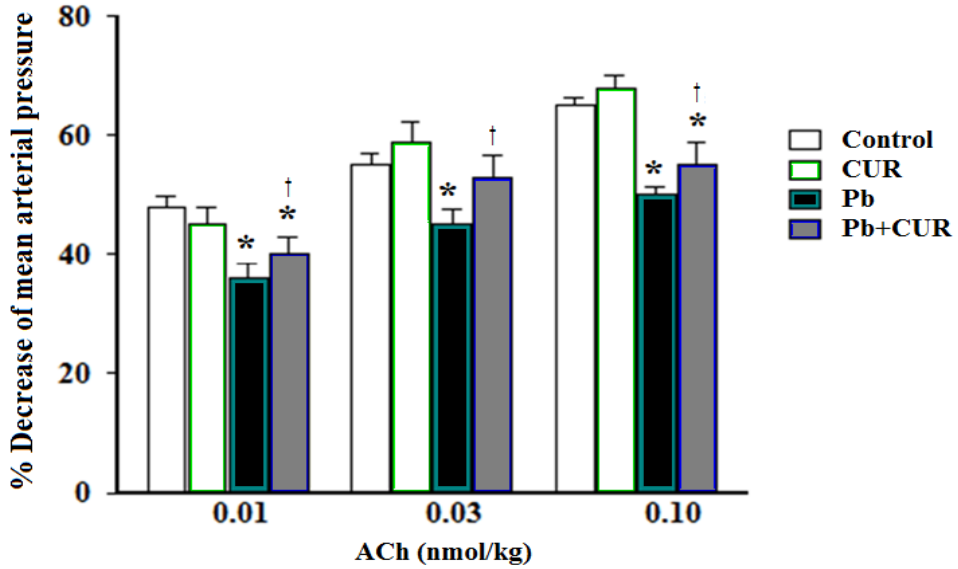

Figure 2 Effect of curcumin on vascular responses to acetylcholine (ACh). Results are expressed as mean ± S.E., each column represents the mean of four experiments.

* $p < 0.05$ compared with normal control group,

† $p < 0.05$ compared with Pb treated group.

CUR at tested dose significantly decreased mean arterial blood pressure of rats exposed to Pb ($p < 0.05$, Fig. 1). Importantly, administration with Pb impaired the vascular responses to ACh was shown in Fig. 2. These results indicate that Pb caused an impairment of vasorelaxation. CUR significantly restored the response of ACh 10.1% vs. Pb-treated controls ($p < 0.05$, Fig 2). Altogether, CUR protected against hypertension and prevented impairment of vascular responsiveness to ACh induced by Pb.

Effects of curcumin on oxidant and anti-oxidant status

To evaluate whether increase of blood pressure and blunt of vascular responsiveness in Pb-exposed rats was associated with oxidant formation and antioxidant effect of CUR, we measured the following parameters related with oxidative stress and antioxidant redox status, including vascular $O_2^{\cdot-}$ production, plasma MDA, and the blood level of GSH. CUR (100 mg/kg) did not change the normal levels of oxidant or antioxidant parameters in control rats (Fig 3). However, chronically exposed to Pb caused approximately 2.5-fold increase of $O_2^{\cdot-}$ production in the carotid artery and plasma MDA level when compared with normal controls ($p < 0.05$, Fig. 3). These data provide evidence of oxidative stress in rats exposed to Pb. CUR at tested dose significantly lowered the rate of $O_2^{\cdot-}$ production in carotid artery and decreased plasma MDA level in comparison to normal control values ($p < 0.05$, Fig. 3). These results indicate that CUR reduces oxidative stress and lipid peroxidation caused by Pb exposure. GSH plays an important role in regulating various redox-sensitive molecules. Therefore, alteration of cellular functions may be due to changes in redox status.

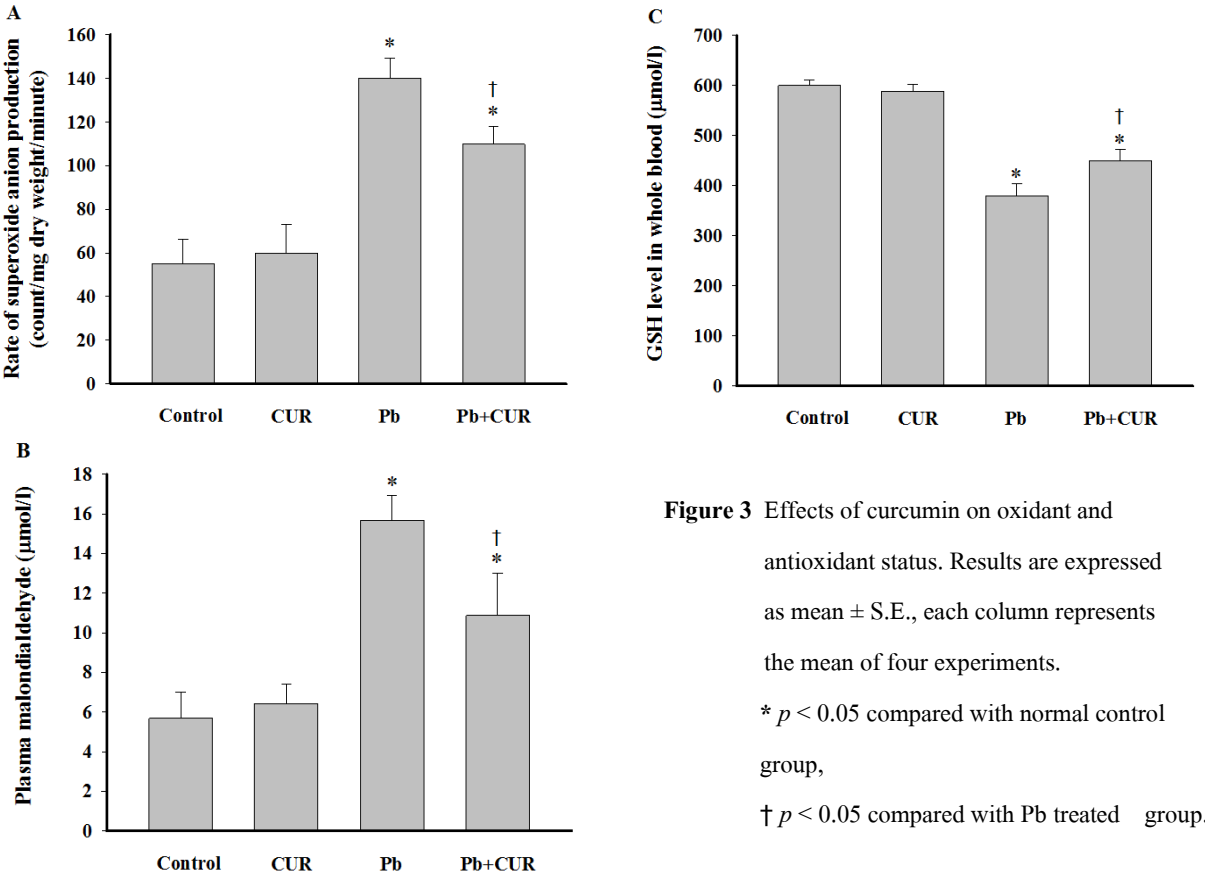

Figure 3 Effects of curcumin on oxidant and antioxidant status. Results are expressed as mean ± S.E., each column represents the mean of four experiments.
* $p < 0.05$ compared with normal control group,
† $p < 0.05$ compared with Pb treated group.

Rats-treated with Pb showed a reduction in blood GSH. Pb-treated rats that received CUR show significantly prevention of loss of GSH in the blood cells (Fig. 3).

Discussion

Considerable evidence suggests that the hypertensive effect of Pb exposure results from complex actions on the vascular endothelial cells and vascular smooth muscle cells (Goch et al., 2009). There is the evidence supported that Pb decreases the bioavailability of the potent vasodilator NO and leads to increase in blood pressure (Vaziri, 2008). Correlated with this study, experiment revealed a significant increase in blood pressure and blunt in vascular responsiveness to endothelial-dependent vasodilator, ACh, suggesting that Pb-induced blunted vascular response might be via NO-dependent partway. Therefore, the effect of CUR on reduction of blood pressure and increase endothelial function may be due to enhance NO bioaviability (Boonla et al., 2014).

Pb causes oxidative stress by inducing the generation of ROS through increase $O_2^{\cdot-}$ production and reducing the antioxidant defense systems by depleting GSH levels, increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition (Ozgocmen et al., 2007; Salehi et al., 2006) leading to increases of plasma MDA levels. CUR can decrease $O_2^{\cdot-}$ production, Plasma MDA level and increase plasma GSH level. The data indicate that CUR has protective effect to prevent biomolecules damage induced by Pb via improvement of antioxidant status and suppression of oxidative stress.

Conclusion

CUR improved vascular alteration leading to hypertension induced by Pb via its antioxidant properties in rats.

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