นิพนธ์ต้นฉบับ ▪ Original Article

ผลของสารสกัดติ้วขาวต่อการเคลื่อนที่และความเข้มข้นของสเปิร์ม ในหนูขาวที่ถูกชักนำให้เกิดความดันเลือดสูงด้วยแอลเนม

อนุสรณ์ เปาะสะเกษ*[1,3], เทอดไทย ทองอุ่น[1], วรรณภา อิชิดะ[1], ปาริฉัตร ประจะเนย์[2,3], พัชรวิภา มณีไสย[1,3], ประภัสสร โพธิ์ตี๋[1,3], พวงรัตน์ ภักดีโชติ[1,3]

[1]ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อ.เมือง จ.ขอนแก่น

[2]ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อ.เมือง จ.ขอนแก่น

[3]กลุ่มวิจัยหัวใจและหลอดเลือด มหาวิทยาลัยขอนแก่น อ.เมือง จ.ขอนแก่น

Effect of Cratoxylum formosum Dyer Extract on Sperm Motility and Concentration in L-NAME Hypertensive Rats

Anuson Poasakate*[1,3], Terdthai Tong-un[1], Wannapa Ishida[1], Parichat Prachaney[2,3], Putcharawipa Maneesai[1,3], Prapassorn Potue[1,3], Poungrat Pakdeechote[1,3]

[1]Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[2]Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[3]Cardiovascular Research Group, Khon Kaen University, Thailand

หลักการและวัตถุประสงค์: *Cratoxylum formosum* (CF) หรือติ้วขาว มีรายงานว่ามีคุณสมบัติต้านอนุมูลอิสระและต้านการอักเสบ แต่มีข้อมูลเพียงเล็กน้อยที่แสดงให้เห็นถึงผลของติ้วขาวต่อความดันเลือดและคุณภาพของสเปิร์ม ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อประเมินผลของสารสกัดติ้วขาวต่อความดันเลือด คุณภาพของสเปิร์มและภาวะเครียดออกซิเดชันในหนูขาวที่ถูกชักนำให้เกิดความดันเลือดสูงด้วยแอลเนม (L-NAME)

วิธีการศึกษา: หนูขาวเพศผู้ถูกแบ่งออกเป็น 3 กลุ่ม (8 ตัว/กลุ่ม) ได้แก่ กลุ่มควบคุม กลุ่มความดันเลือดสูง (L-NAME) และกลุ่มความดันเลือดสูงที่ได้รับสารสกัดติ้วขาว 100 มก./กก. น้ำหนักตัว/วัน (CF100) กลุ่มความดันเลือดสูง หนูขาวถูกให้แอลเนมขนาด 40 มก./กก. น้ำหนักตัว ในน้ำดื่มเพื่อชักนำให้เกิดความดันเลือดสูง เป็นเวลา 5 สัปดาห์ ใน 2 สัปดาห์สุดท้าย หนูขาวในกลุ่ม CF100 ถูกป้อนด้วยสารสกัดติ้วขาวขนาด 100 มก./กก. น้ำหนักตัว เมื่อสิ้นสุดการทดลอง ความดันเลือดขณะหัวใจบีบตัว น้ำหนักตัว อัณฑะและท่อพักอสุจิถูกวัด การนับสเปิร์มถูกดำเนินการ

ผลการศึกษา: หนูที่ได้รับแอลเนม เพิ่มความดันเลือดขณะหัวใจบีบตัว และลดการเคลื่อนที่และความเข้มข้นของสเปิร์มอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) นอกจากนี้ยังพบว่ามีการเพิ่มขึ้นของการผลิตซูเปอร์ออกไซด์ในหลอดเลือดอย่างมีนัยสำคัญทาง

Background and Objective: *Cratoxylum formosum* (CF) or Taew Kaao has been reported to have strong antioxidant and anti-inflammatory properties. Little information regarding the effect of CF on blood pressure and sperm quality is demonstrated. This study proposed to evaluate the effect of CF extract on blood pressure, sperm quality and oxidative stress in L-NAME-induced hypertensive rats.

Methods: Male Sprague-Dawley rats were divided into 3 groups (8/group); control, L-NAME and hypertensive treated with CF extract 100 mg/kg BW/day (CF100) groups. Hypertensive group, rats were orally treated with L-NAME 40 mg/kg BW/day in their drinking water to induce hypertension for 5 weeks. In the last 2 weeks, rats in CF100 group were orally treated with CF extract at dose 100 mg/kg BW. At the end of study, systolic blood pressure (SBP), body, testes and epididymis weights were measured. Sperm counting was performed.

Results: L-NAME treated rats significantly increased SPB, and decreased sperm motility and concentration ($p<0.05$). Additionally, increased vascular superoxide

*Corresponding author : Anuson Poasakate, Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. E-mail:

สถิติ (p <0.05) ซึ่งบ่งชี้ว่ามีการเพิ่มขึ้นของภาวะเครียดออกซิเดชันในสัตว์ทดลองรูปแบบนี้ ในขณะที่ไม่มีความแตกต่างอย่างมีนัยสำคัญในน้ำหนักตัว น้ำหนักอัณฑะและท่อพักอสุจิระหว่างกลุ่มทดลอง สารสกัดติ้วขาว ช่วยบรรเทากาiเปลี่ยนแปลงของคุณภาพของสเปิร์มและภาวะเครียดออกซิเดชันในหนูที่มีความดันเลือดสูง

สรุป: การศึกษาครั้งนี้ชี้ให้เห็นว่าการชักนำความดันเลือดสูงด้วยแอลเนม ส่งผลต่อการเคลื่อนที่และความเข้มข้นของสเปิร์ม ซึ่งอาจเกี่ยวข้องกับการลดการไหลเวียนของเลือดไปยังอวัยวะสืบพันธุ์และการเพิ่มการผลิตอนุมูลอิสระในหลอดเลือด สารสกัดติ้วขาวสามารถบรรเทาการเปลี่ยนแปลงเหล่านี้ได้ด้วยคุณสมบัติต้านอนุมูลอิสระ

คำสำคัญ: ติ้วขาว, ต้านอนุมูลอิสระ, ความเข้มข้นและการเคลื่อนที่ของสเปิร์ม, ภาวะเครียดออกซิเดชัน

production was found in L-NAME treated rats (p<0.05), indicating increased oxidative stress in this animal model. There is no significant difference in body weight, testis and epididymis weight among experimental groups. CF extract alleviated the alterations of sperm quality and oxidative stress in hypertensive rats.

Conclusion: This study suggests that L-NAME induced hypertension affects sperm motility and sperm concentration. This might be related to decreased blood flow to the reproductive organs and increased vascular reactive oxygen species production. CF extract can alleviate these alterations via its antioxidant property.

Keywords: *Cratoxylum formosum*, Antioxidant, Sperm concentration and motility, Oxidative stress

ศรีนครินทร์เวชสาร 2562; 34(4): 312-317. ● Srinagarind Med J 2019; 34(4): 312-317.

Introduction

Hypertension is the most common chronic disorder which blood pressure in arteries is elevated. It is considered as a major public health problem that affects more than 25% of the general population around the world[1]. It is the main cause of morbidity and mortality of people associated with cardiovascular disorders and also affects to organ damage by increasing reactive oxygen species generation[2]. Additionally, hypertension associated with an impairment of male sexual function has been suggested since alterations in the testicular morphology and reproductive function in hypertensive patients has been revealed[4,5]. N^{ω}-nitro-l-arginine methyl ester hydrochloride (L-NAME, nitric oxide synthase inhibitor) induced-hypertension has been reported to associate with oxidative stress[6,7]. Akinyemi and coworkers showed a significant decrease in serum total testosterone and epididymal sperm progressive motility in L-NAME hypertensive rats that was consistent with local and systemic oxidative stress[8]. Recently, reductions of plasma testosterone, testicular sperm number, epididymal sperm number and sperm progressive motility, and an increase in oxidative stress have been demonstrated in the L-NAME hypertensive rats.

[*Cratoxylum formosum* (CF)(Jack) Dyer; Guttiferae] or Teaw kaao in Thai is widely consumed as dietary and herbal plant in Thailand, especially in the Northeast region . It has been used as Thai traditional medicine for the treatments of indigestion, bacterial infection, inflammation, asthma and blood disorders[9-11]. Furthermore, antioxidant and anti-cancer of CF have been demonstrated in both vivo and vitro studied[12]. Nowadays, there is no evidence regarding its effect on blood pressure and testicular function in L-NAME hypertensive rats. This study was designed to evaluate the effect of CF extract on blood pressure and sperm quality in L-NAME-induced hypertensive rats.

Methods

Chemicals

L-NAME was obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA).

Plant preparation

Fresh leaves of CF were collected from local agricultural fields in Khon Kaen province, Thailand. The leaves were weighed, chopped and boiled in distilled water for 30 minutes, then filtrated. The filtrates were dried into powder using a freeze dryer (Labconco, USA). The extraction process yielded residues of 11.5% per dry weight of CF[13]. The crude extract was kept in a light-protected container and stored at -20 °C until used. CF extract was received from Assoc. Prof. Dr. Laddawan Senggunprai, department of Pharmacology, faculty of Medicine, Khon Kaen university.

Animals

Male Sprague-Dawley rats weighing 180-220 g were purchased from Nomura Siam International Co., Ltd., Bangkok, Thailand. Rats were housed in the HVAC (Heating, Ventilation and Air-Conditioning) System (23±2 ℃) with a 12 h dark-light cycle, and free access to food and water at Northeast Laboratory Animal Center. All procedures were complied with the standards for the care and use of experimental animals and approved by Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (IACUC-KKU 48/2561).

Experimental designs

After one week of acclimatization, the animals were randomly divided into 3 groups (8/group). Control group, rats were orally received distilled water throughout the experiment. L-NAME group, rats were given L-NAME 40 mg/kg BW/day in their drinking water for 5 weeks and intragastrically administered with water (vehicle). L-NAME + CF extract group, rats were given L-NAME 40 mg/kg BW/day in their drinking water for 5 weeks and intragastrically administered with CF extract at dose 100 mg/kg BW/day for the last 2 weeks. At the end of experiment, systolic blood pressure of all groups was measured by tail-cuff plethysmography method (IITC/Life Science Instrument model 229 and model 179 amplifier; Woodland Hills, CA, USA). The animals were habituated with the apparatus before taking the measurements. The blood pressure value was detected and recorded. The mean value of three replications was expressed from each rat. Then, rats were anesthetized with thiopental sodium 40 mg/kg intraperitoneal injection. The testis and epididymis were rapidly removed and weighted for using in sperm measurements. Carotid artery was collected for detecting superoxide production.

Body and organ weight measurements

The body weight was measured in weekly throughout the experiment by electrical balance. After rats were sacrificed, the testis and epididymis were immediately removed and weighed to calculate absolute weight and an organo-somatic indices (OSI). OSI = 100 x organ weight (g)/body weight (g)

Sperm quality parameters

The sperm was collected immediately after a rat was sacrificed. Briefly, the right cauda epididymis was cut, and sperm was released onto a sterile clean glass slide. The sperm was diluted with 4 mL phosphate buffered saline (PBS) (pre-warm 37°C), mixed thoroughly and suck 10 µL of the solution drop onto hemocytometer after that covered with a 24 × 24 mm coverslip. Examination under a light microscope at 400× magnification to evaluate the sperm concentration and motility. The data were expressed as percentage of sperm motility.

Assay of vascular superoxide production

The carotid artery was rapidly removed and cleaned off connective tissue on normal saline. The vessel was cut into about 1 cm length and incubated with 940 µL oxygenated bicarbonate solution at 37 °C for 30 minutes. After 30 minutes of incubation, 60 µL lucigenin was added into sample tube and placed in a luminometer (Turner Biosystems, CA, USA). The vascular superoxide production was expressed as relative light unit count per minute per dried weight of vascular tissues as previously described [14].

Statistical analysis

Data were expressed as mean ± S.E.M. The differences between groups were analyzed by one-way ANOVA, followed by Fisher's least significant difference tests. The data have statistical significance when $p<0.05$

Results

Body and organ weights

There was no significant difference in rat body weight, absolute and an organo-somatic indices of the testes and epididymis in all experimental groups (Table 1).

Systolic blood pressure

Rats received L-NAME showed a significant increase in SBP when compared with control rats (191.8 ± 4.5 vs. 121.5 ± 0.7 mmHg) ($p<0.05$) (Figure 1). Oral administration with CF in L-NAME-treated rats significantly decreased SBP when compared with hypertensive rats (158 ± 2.0 vs. 191.8 ± 4.5 mmHg) ($p<0.05$).

Sperm quality parameters

L-NAME-induced hypertensive rats showed a significant decrease in both percentage of sperm

Table 1 Effect of CF extract on body weight, and absolute and an organo-somatic indices (OSI) of the testes and epididymis in L-NAME-induced hypertensive rats.

Groups	Body weight (g)	Absolute testis weight (g)	Absolute epididymis weight (g)	OSI of the testis (g)	OSI of the epididymis (g)
Control	466.8 ± 5.3	1.77 ± 0.03	0.55 ± 0.01	0.37 ± 0.004	0.12 ± 0.003
L-NAME	452.1 ± 6.0	1.78 ± 0.03	0.55 ± 0.009	0.39 ± 0.01	0.12 ± 0.002
CF100	452.3 ± 8.2	1.74 ± 0.03	0.53 ± 0.01	0.39 ± 0.006	0.12 ± 0.004

The results are presented as mean ± S.E.M. (n=5-7/group). OSI; organo-somatic indices.

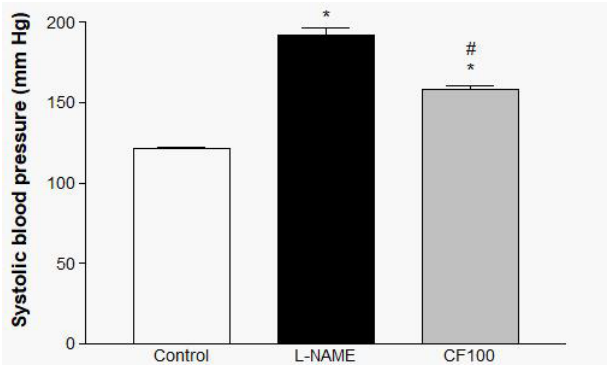

Figure 1 Effects of CF extract on the systolic blood pressure in L-NAME-induced hypertensive rats. CF100, 100 mg/kg CF extract. Data are presented as mean ± S.E.M. (n=5-7/group). *p<0.05 vs. control, #p<0.05 vs. L-NAME.

motility and sperm concentration comparing to control rats (42.7 ± 1.2 vs. 61.8 ± 1.1 % motility; 12.7 ± 0.3 vs. 17.2 ± 0.4 x 106/mL, respectively) (p<0.05). Treatment with CF extract significantly increased the sperm motility (57.3 ± 2.3%) and sperm concentration (15.1 ± 0.4 x 106/mL) when compared with untreated L-NAME-induced hypertensive rats (p<0.05).

Vascular superoxide production

There was a significant increase in vascular superoxide production in L-NAME-induced hypertensive rats when compared with control group (208.4 ± 27.7 vs. 50.6 ± 7.3 count/mg dry wt/min) (p<0.05). However, treatment with CF extract significantly decreased superoxide production when compared with untreated L-NAME-induced hypertensive rats (80.6 ± 11.8 vs 208.4 ± 27.7 count/mg dry wt/min) (p<0.05).

Discussion

In the present study, we found a high blood pressure, decreased sperm concentration and motility associated with increased oxidative stress in L-NAME treated rats. It is well established that inhibition of NO production by L-NAME caused systemic vasoconstriction, an increase in vascular resistance and hypertension in rats[15,16]. Recently, L-NAME induced hypertension has been characterized by increased superoxide production[17]. Superoxide reduces NO bioavailability since it rapidly reacts with NO to produced ONOO[18]. This study found excessive vascular superoxide production that was consistent with previous studies[17]. L-NAME-induced hypertension affected sperm quality observed in the present study.

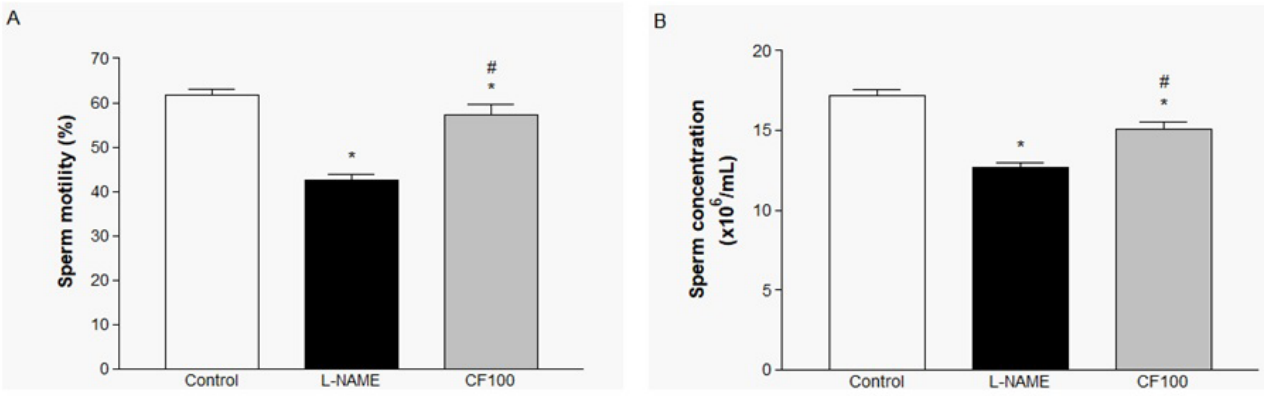

Figure 2 Effects of CF extract on sperm motility (A) and sperm concentration (B) in L-NAME-induced hypertensive rats. CF100, 100 mg/kg CF extract. Data are presented as mean ± S.E.M. (n=5-7/group). *p<0.05 vs. control, #p<0.05 vs. L-NAME.

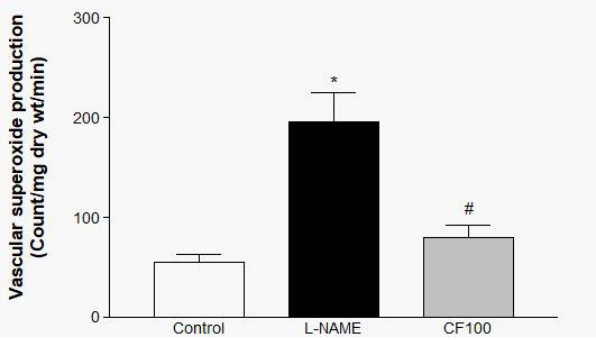

Figure 3 Effect of CF extract on vascular superoxide production in L-NAME-induced hypertensive rats. CF100, 100 mg/kg CF extract. Data are presented as mean ± S.E.M. (n=5-7/group). *p<0.05 vs. control, #p<0.05 vs. L-NAME.

There is substantial evidence to show that NO deficiency caused poor sperm quality[19]. Moreover, several previous studies have reported that reduction in blood flow to testis and vital organs might play a key role in male reproductive dysfunction in NO deficiency model[20]. In the present study, CF extract supplementation reduced blood pressure and improved sperm quality in L-NAME hypertensive rats. The underlying mechanism may involve its antioxidant property since CF extract reduced vascular superoxide production, resulting in increased NO bioavailability[12].

Conclusion

The finding of this study indicated that CF extract alleviated L-NAME induced hypertension and decreased sperm concentration and quality. The mechanism is likely to be associated with its antioxidant effect.

Acknowledgments

This study was supported by Invitation Research grant (IN62121), Faculty of Medicine, Khon Kaen University. Researcher holds the scholarship from Development and Promotion of Science and Technology Talents project.

References

1. Sowers JR, Epstein M. Diabetes mellitus and associated hypertension, vascular disease, and nephropathy. An update. Hypertension 1995; 26: 869-79.
2. Kizhakekuttu TJ, Widlansky ME. Natural antioxidants and hypertension: promise and challenges. Cardiovasc Ther 2010; 28: e20-32.
3. Dusing R. Effect of the angiotensin II antagonist valsartan on sexual function in hypertensive men. Blood Press Suppl 2003; 2: 29-34.
4. Fogari R, Zoppi A, Preti P, Rinaldi A, Marasi G, Vanasia A, et al. Sexual activity and plasma testosterone levels in hypertensive males. Am J Hypertens 2002; 15: 217-21.
5. Vlachopoulos C, Ioakeimidis N, Terentes-Printzios D, Aznaouridis K, Rokkas K, Aggelis A, et al. Plasma total testosterone and incident cardiovascular events in hypertensive patients. Am J Hypertens 2013; 26: 373-81.
6. Bunbupha S, Pakdeechote P, Kukongviriyapan U, Prachaney P, Kukongviriyapan V. Asiatic acid reduces blood pressure by enhancing nitric oxide bioavailability with modulation of eNOS and p47phox expression in L-NAME-induced hypertensive rats. Phytother Res 2014; 28: 1506-12.
7. Nakmareong S, Kukongviriyapan U, Pakdeechote P, Donpunha W, Kukongviriyapan V, Kongyingyoes B, et al. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. Naunyn Schmiedebergs Arch Pharmacol 2011; 383: 519-29.
8. Akinyemi AJ, Adedara IA, Thome GR, Morsch VM, Rovani MT, Mujica LKS, et al. Dietary supplementation of ginger and turmeric improves reproductive function in hypertensive male rats. Toxicol Rep 2015; 2: 1357-66.
9. Nakahara K, Trakoontivakorn G, Alzoreky NS, Ono H, Onishi-Kameyama M, Yoshida M. Antimutagenicity of some edible Thai plants, and a bioactive carbazole alkaloid, mahanine, isolated from Micromelum minutum. J Agric Food Chem 2002; 50: 4796-802.
10. Panthong A, Kanjanapothi D, Taesotikul T, Taylor WC. Ethnobotanical review of medicinal plants from Thai traditional books, Part II: Plants with antidiarrheal, laxative and carminative properties. J Ethnopharmacol 1991; 31: 121-56.
11. Trakoontivakorn G, Nakahara K, Shinmoto H, Takenaka M, Onishi-Kameyama M, Ono H, et al. Structural analysis of a novel antimutagenic compound, 4-Hydroxypanduratin A, and the antimutagenic activity of flavonoids in a Thai spice, fingerroot (Boesenbergia pandurata Schult.) against mutagenic heterocyclic amines. J Agric Food Chem 2001; 49: 3046-50.
12. Kukongviriyapan U, Luangaram S, Leekhaosoong K, Kukongviriyapan V, Preeprame S. Antioxidant and vascular protective activities of Cratoxylum formosum, Syzygium gratum and Limnophila aromatica. Biol Pharm Bull 2007; 30: 661-6.

13. Senggunprai L, Thammaniwit W, Kukongviriyapan V, Prawan A, Kaewseejan N, Siriamornpun S. Cratoxylum formosum extracts inhibit growth and metastasis of cholangiocarcinoma cells by modulating the NF-kappaB and STAT3 pathways. Nutr Cancer 2016; 68: 328-41.

14. Lu FJ, Lin JT, Wang HP, Huang WC. A simple, sensitive, non-stimulated photon counting system for detection of superoxide anion in whole blood. Experientia 1996; 52: 141-4.

15. Arnal JF, Warin L, Michel JB. Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhibition of nitric oxide synthase. J Clin Invest 1992; 90: 647-52.

16. Gardiner SM, Compton AM, Bennett T, Palmer RM, Moncada S. Control of regional blood flow by endothelium-derived nitric oxide. Hypertension 1990; 15: 486-92.

17. Maneesai P, Prasarttong P, Bunbupha S, Kukongviriyapan U, Kukongviriyapan V, Tangsucharit P, et al. Synergistic antihypertensive effect of Carthamus tinctorius L. extract and captopril in L-NAME-induced hypertensive rats via restoration of eNOS and AT(1)R expression. Nutrients 2016; 8: 122.

18. Xia Y, Zweier JL. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages. Proc Natl Acad Sci U S A 1997; 94: 6954-8.

19. Adams ML, Nock B, Truong R, Cicero TJ. Nitric oxide control of steroidogenesis: endocrine effects of NG-nitro-L-arginine and comparisons to alcohol. Life Sci 1992; 50: PL35-40.

20. Kim SW, Cuong TD, Hung TM, Ryoo S, Lee JH, Min BS. Arginase II inhibitory activity of flavonoid compounds from Scutellaria indica. Arch Pharm Res 2013; 36: 922-6.

SMJ