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Antihypertensive effect of auraptene, a monoterpene coumarin from the genus *Citrus*, upon chronic administration

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ABSTRACT

Objective(s): Auraptene, a monoterpene coumarin from *Citrus* species, exhibits cardioprotective effects. In this study, the effects of auraptene administration were investigated on blood pressure of normotensive and desoxycorticosterone acetate (DOCA) salt induced hypertensive rats.
Materials and Methods: Five weeks administration of auraptene (2, 4, 8 and 16 mg/kg/day) and nifedipine (0.25, 0.5, 1, 2 and 4 mg/kg/day) in different groups of normotensive and hypertensive rats (at the end of 3 weeks treatment by DOCA salt) was carried out and their effects on mean systolic blood pressure (MSBP) and mean heart rate (MHR) were evaluated using tail cuff method.
Results: Our results indicated that chronic administration of auraptene (2, 4, 8 and 16 mg/kg/day) significantly reduced the MSBP in DOCA salt treated rats in a dose and time dependent manner. The percent of decreases in MSBP levels by the highest dose of auraptene (16 mg/kg) at the end of 4 th to 8 th weeks, were 7.00%, 10.78%, 16.07%, 21.28% and 27.54% respectively (P<0.001). Moreover the antihypertensive effect of auraptene was less than nifedipine (ED_{50} value of nifedipine = 0.7 mg/kg at 8th week and ED_{50} value of auraptene = 5.64 mg/kg at 8 week).
Conclusion: Auraptene considerably reduced MSBP in hypertensive rats, but not in normotensive (normal saline treated) rats. The results of MHR measurement showed that the increase in MHR was not significant in comparison with DOCA treated rats.

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Introduction

Hypertension is the most common cardiovascular disease with the highest epidemiological impact in the world and represents an important risk factor for developing other diseases such as endothelial dysfunction, metabolic syndrome, diabetes, renal dysfunction, congestive heart failure, coronary artery disease and stroke (1). Drugs currently used to lower blood pressure have some adverse effects including orthostatic hypotension, hypercholesterolemia, depression and impotency (2). So, it is necessary to search for drugs derived from nature that are more potent but have fewer negative side effects.

Recently, herbal medicines are being used for the treatment of a variety of disorders including cardiovascular diseases because of their safety, efficacy, cultural acceptability and lesser side effects (3).

Cardioprotective effects of *Citrus* spp. (Rutaceae) have been reported in several studies (4). It has been reported that frequent *Citrus* fruit consumption significantly reduced cardiovascular disease incidence (5). Moreover *Citrus* spp. could inhibit key molecules involved in the regulation of blood pressure and could decrease coronary vascular resistance and mean arterial pressure (6, 7).

Several mechanisms including improving lipid profile by decrease in the level of low density lipoprotein (LDL) and boosting high density lipoprotein (HDL) levels, antiinflammatory, antioxidant, anti-ischemic, antithrombotic and vasorelaxant effects are involved in cardioprotective activity of these plants (8, 9).

Auraptene (7-geranyloxycoumarin) is the most abundant prenyloxycoumarin which occurs in nature, particularly in the genus *Citrus* (Figure 1). It has also been found in many plants belonging to members of the *Citrus* genus such as orange, grapefruit, mandarin etc. (10).

Auraptene possesses valuable pharmacological properties including potent cancer chemopreventive, antibacterial, antiprotozoal, antifungal, antigenotoxic, antiinflammatory, antioxidant, immunomodulatory, hepatoprotective, and neuroprotective effects (11-13).

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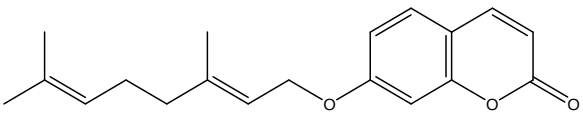

Figure 1. The chemical structure of auraptene

There are a few reports regarding the protective effects of auraptene on the cardiovascular system. The relaxatory effects on the smooth muscle cell**s** (14) as well as the inhibition of spontaneous heartbeat induced by calcium have been reported (15). Although it was indicated that auraptene had moderate hypotensive activity in our previous study (16), there has not been any study about the effect of auraptene on blood pressure of hypertensive rats upon chronic administration. Thus, the aim of the present study was to evaluate the effects of chronic administration of auraptene on blood pressure of normotensive and desoxycorticosterone acetate (DOCA) salt induced hypertensive rats, to explore whether long-term dietary supplementation with this phytochemical is of any benefit in terms of mono or adjunctive therapy for the management of hypertension. Furthermore as many *Citrus* plants esp. peels are used in Iranian folk medicine as hypotensive, in which this research would support that.

Materials and Methods

Animals and chemicals

Adult male Wistar rats (weight 250–300 g) were provided by animal center (School of Pharmacy, Mashhad University of Medical Sciences). They were maintained on a 12 hr light/dark cycle and at a temperature of 23 ± 1°C with free access to food and water. These conditions were maintained constant throughout the experiments. The experiments were performed under the Animals (scientific procedures) Act of 1986 and conform to the National Institutes of Health guidelines for the use of experimental animals. All animal experiments were carried out in accordance to Mashhad University of Medical Sciences, Ethical Committee Acts (Number of verification: 910166, the date of approval: 5/7/2012). Nifedipine (as positive control) and DOCA were obtained from Zahravi and Iran Hormone (Tehran, Iran). Auraptene and nifedipine were dissolved in a solvent mixture comprising dimethylsulfoxide and normal saline (1:3).

Preparation of auraptene

Auraptene (7-geranyloxycoumarin) was synthesized based on a previously described method (17). Briefly, 7-hydroxycoumarin and *trans*-geranyl bromide were reacted in acetone at room temperature, in the presence of DBU (1,8-diazabicyclo [5.4.0] undec-7-ene). Auraptene was purified from the concentrated reaction mixture as white crystals using column chromatography (petroleum ether/ethyl acetate 9:1 v/v) and its structure was confirmed by ^{1}H- and ^{13}C-NMR. The purity of auraptene was calculated using HPLC as 95%.

Induction of experimental hypertension

DOCA salt (20 mg/kg, twice weekly, for 3 weeks, SC) and NaCl (1%) in rat's drinking water were used for induction of hypertension (18). Rats were randomly divided into 15 groups: 1) Saline injected (0.5 ml/kg, twice weekly, SC for 3 weeks), this treatment was continued for another five weeks, 2) DOCA salt (20 mg/kg, twice weekly, for 3 weeks, SC), DOCA treatment was continued by IP injection of solvent (normal saline/DMSO, 1:3) for another five weeks, 3, 4, 5 and 6) (DOCA)-salt (20 mg/kg, twice weekly, for 3 weeks, SC), DOCA treatment was continued by IP injection of 2, 4, 8 and 16 mg/kg/day auraptene for another five weeks, 7, 8, 9 ,10 and 11) (DOCA)-salt (20 mg/kg, twice weekly, for 3 weeks, SC), DOCA treatment was continued by IP injection of 0/25, 0/5,1, 2 and 4 mg/kg/day nifedipine for another five weeks. All groups consisted of six rats. Table 1 describes the different groups which were selected in this study.

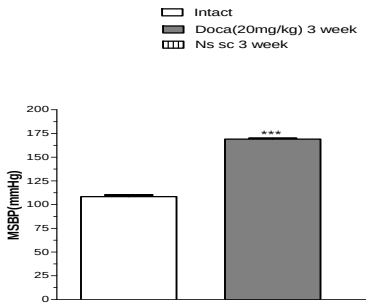

Figure 2. Hypertension induced by desoxycorticosterone acetate (DOCA) salt after 3 weeks. Each value is the mean± SEM of six experiments, *** $P < 0.001$ vs normal saline treated rats. One way ANOVA, Tukey Krumer test

Table 1. Summary of drug administration in different groups

Groups	DOCA (8 weeks)	Normal saline (8 weeks)	Normal saline plus DMSO (1:3) (4 th to 8 th weeks)	Auraptene (4 th to 8 th weeks)	Nifedipine (4 th to 8 th weeks)
1		*			
2	*		*		
3,4,5 and 6	*			*	
7,8,9, 10 and 11	*				*

DOCA: Desoxycorticosterone acetate

Blood pressure measurement

After saline or DOCA treatment, SBP was measured every week using tail cuff method in all groups as described by Lorenz (2002) (19). Briefly, three days before the last treatment, the training of rats in different groups for indirect SBP measurements was started. This training consisted of the regular handling of the animals and getting used to the restraining cage and the tail-cuff. Rats were heated for approximately 15 min at 30-32°C to increase blood flow to the tail. After that, animals were placed in small restraining cages with a cuff around the end of proximal of the tail. After placing of the cuff, a pulse transducer was used around the end of the tail. Then the tail cuff was inflated using the related button on the NIBP (Non-Invasive Blood Pressure) controller apparatus and Acquisition data were performed by a computerized system Power Lab (ADInstruments, v 5.4.2). The mean values of five BPs and HRs readings were used for each animal.

Statistical analysis

All results are expressed as mean±SEM. ANOVA Tukey Kramer tests were then performed to compare means. The software Pharm-PCS, was used to calculate of ED_{50} values. *P*-values less than 0.05 were considered as significant.

Results

Effect of DOCA on SBP

In DOCA treated rats, MSBP significantly increased in comparison with normal saline treated (normotensive) rats ($P < 0.001$) (Figure 2).

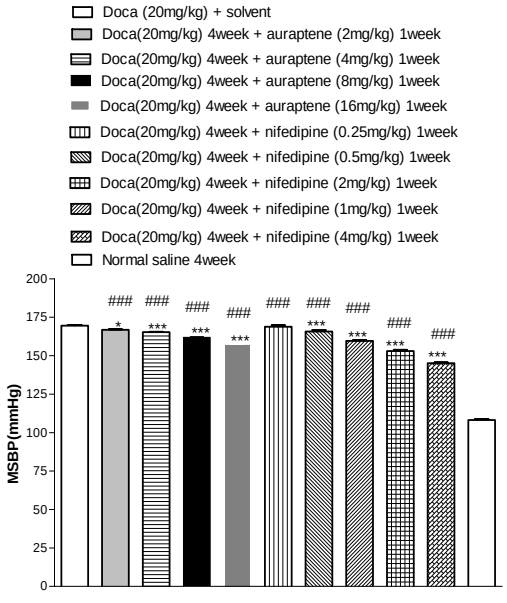

Figure 3. Mean systolic blood pressure (MSBP) in response to various doses of auraptene and nifedipine in normotensive and hypertensive rats at the end of four weeks. Each value is the mean±SEM of six experiments. One-way ANOVA, Tukey Kramer, $^{*}P<0.05$ and $^{***}P<0.001$ vs DOCA plus solvent treated rats, $^{###}P<0.001$ vs normal saline treated rats
DOCA: Desoxycorticosterone acetate

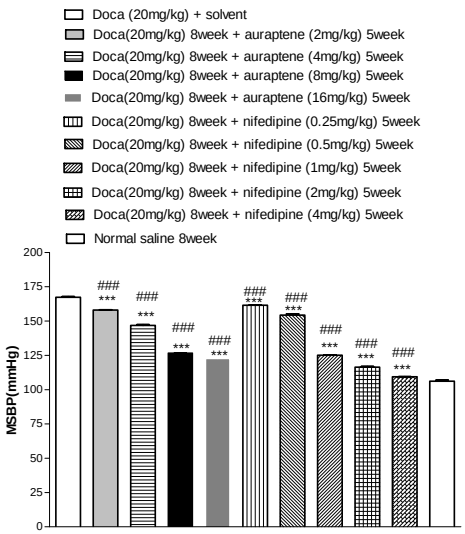

Figure 4. Mean systolic blood pressure (MSBP) in response to various doses of auraptene and nifedipine in normotensive and hypertensive rats at the end of eight weeks. Each value is the mean ± SEM of six experiments. One-way ANOVA, Tukey Kramer, $^{***}P<0.001$ vs DOCA plus solvent treated rats, $^{###}P<0.001$ vs normal saline treated rats
DOCA: Desoxycorticosterone acetate

Effects of chronic administration of auraptene and nifedipine on MSBP and MHR

As shown in Figures 3 and 4, the results of SBP measurement at the end of four and eight weeks, showed that auraptene (2, 4, 8 and 16 mg/kg) and nifedipine (0.25, 0.5,1, 2 and 4 mg/kg) reduced the MSBP in DOCA salt treated animals, dose dependently. Moreover, the antihypertensive effects of auraptene

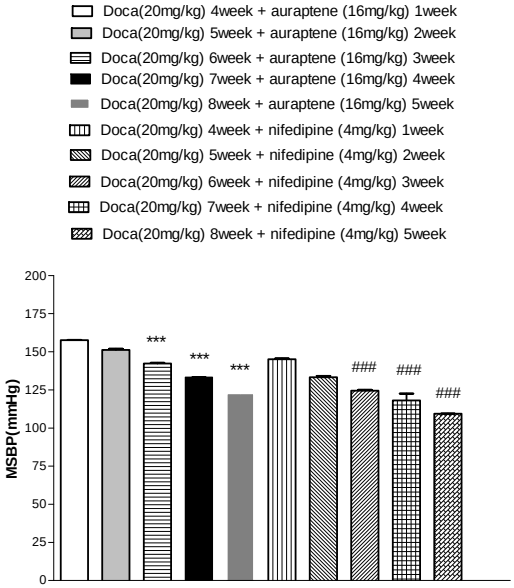

Figure 5. Time dependency of the antihypertensive effects of the highest doses of auraptene and nifedipine. Each value is the mean ± SEM of six experiments. Repeated measure, ***P< 0.001vs DOCA plus normal saline treated rats
DOCA: Desoxycorticosterone acetate

Table 2. The ED_{50} values of auraptene and nifedipine

	ED_{50} (mg/kg)(Nifedipine)	ED_{50} (mg/kg)(Auraptene)
4th week	4.65(95%CI = 3.62-5.98)	120.45(95%CI =72.27-225.64)
5th week	2.61(95%CI = 2.12-3.2)	44.37(95%CI = 37.23-67.29)
6th week	1.67(95%CI = 1.4-1.98)	17.47(95%CI = 13.22-23.09)
7th week	1.12(95%CI = 0.69-1.81)	9.76(95%CI = 7.76-12.28)
8th week	0.70(95%CI = 0.60-0.83)	5.65(95%CI = 4.78-6.68)

CI: Confidence Interval

and nifedipine were observed at the end of 5, 6 and 7 weeks (the results was not shown). The ED_{50} values showed that the hypotensive effect of auraptene was less than nifedipine. In addition, data demonstrated that auraptene and nifedipine (at the mentioned doses) did not reduce SBP in normotensive (normal saline treated) rats (Table 2). The results of mean heart rate (MHR) measurement in various doses of auraptene and nifedipine at the end of four to eight weeks showed that the increase in mean heart rate was not significant in comparison with DOCA treated rats. Time dependency of the antihypertensive effects of the highest doses of auraptene and nifedipine were shown in Figure 5. The increase of antihypertensive effect of auraptene and nifedipine was significant during time.

Discussion

In this study the chronic effect of auraptene on MSBP and MHR in DOCA salt treated rats was evaluated. DOCA salt significantly induced hypertension in comparison with saline group at the end of 3 weeks treatment. Chronic administration of auraptene and nifedipine (positive control) reduced the increase of MSBP induced by DOCA, but this hypotensive effect was not observed in normotensive rats. Our results also showed that antihypertensive effect of auraptene as well as nifedipine were dose and time dependently.

Recently, there has been increasing interest in the potential human health benefits of natural compounds (3). The genus *Citrus* is one of the most potential cardioprotective plants because of their various bioactive components including antioxidant vitamins, minerals, flavonoids and phenolic compounds (4, 14). In folklore, it was reported that *Citrus paradisi* Macfad. (grapefruit) consumption was generally used for the treatment of hypertension. The results of a study showed that *C. paradisi* peel extract decreased coronary vascular resistance and mean arterial pressure as compared with control. The coronary vasodilatory effect of *C. paradisi* was considered to be NO-dependent and the effect was blocked by L-NAME. Moreover, *C. paradisi* juice decreased diastolic and systolic arterial pressure both in normotensive and hypertensive human. A greater decrease in mean arterial pressure was observed with *Citrus paradisi* juice as comparison with *Citrus sinensis* Osbeck juice, cow milk and a vitamin C-supplemented beverage (7). Another study revealed that an aqueous extract of *Citrus limetta* Risso (sweet lemon) leaves reduced both systolic and diastolic blood pressure significantly, possibly due to the inhibition of angiotensin II (6). Furthermore, high flavonoid juice of sweetie fruit (a hybrid between grapefruit and pummel) was found to have a major beneficial effect in reducing diastolic blood pressure, in comparison with low flavonoid sweetie juice, in patients with stage I hypertension. Flavonoids naringin and narirutin were considered as active ingredients associated with the antihypertensive effect of sweetie juice (20).

In addition to the various phytonutrients and bioflavonoids which exist in *Citrus* spp., auraptene is of a special importance as it is mainly found in *Citrus* fruits. Auraptene, known as 7-geranyloxycoumarin, was first isolated in the 1930s by Komatsu *et al* (1930) (21). It is the most abundant naturally occurring prenyloxycoumarin.

Recent reports showed that auraptene possessed valuable pharmacological activities, including neuroprotective, antioxidant, antigenotoxic, anti-inflammatory, antibacterial, and anti-cancer properties (11-13). Auraptene has been also reported to have cardioprotective effects (12, 22). The results of a study showed that auraptene administration alleviated obesity and hepatic triglyceride accumulation partly due to the enhancement lipolysis in the livers of obese rats (23). Smooth muscle relaxant effects of auraptene and its analogues against some spasmogens including barium ion, acetylcholine and histamine, have been reported in another study (14). In our previous study a dose-dependent hypotensive effect was observed following auraptene injection. The mean arterial blood pressure (MABP) lowering effect of auraptene was found to be significantly lower than that of nifedipine (16). DOCA salt is a compound routinely used to induce hypertension in experimental animals (18). In the present study, DOCA salt significantly induced hypertension in comparison with saline group at the end of 3 weeks treatment.

There is evidence indicating that auraptene inhibits the spontaneous heart beating of cultured mouse myocardial cells via calcium channel antagonist activity which is comparable with that of verapamil (15). Moreover, it has been reported that hesperidin and rutin, two flavonoids present in *Citrus* peels, have hypotensive effects due to the suppression of NADPH oxidase, increase in NO bioavailability and alleviation of endothelial

dysfunction. Decrease in calcium influx and induction of NO together with vasorelaxation effect have been also observed (24-27). Considering the evidence, it might be concluded that hypotensive effect of auraptene in chronic treatment is related to the blocking of calcium channel or inhibition of sarcoplasmic reticulum calcium release into cytosol. Other mechanisms such as direct vasodilatory effect of auraptene and NO mediated relaxation might be involved in antihypertensive effect of auraptene.

The results of mean heart rate measurement in various doses of auraptene and nifedipine at the end of four to eight weeks showed that the increase in MHR was not significant in comparison with DOCA treated rats. Reflex tachycardia induced by auraptene and nifedipine might be suppressed due to the direct inhibitory effects of auraptene and nifedipine on cardiac muscles.

Conclusion

In summary, our results indicated that chronic administration of auraptene could reduce the MSBP in DOCA salt treated rats in a dose and time dependent manner. Although the hypotensive effect of auraptene was less than nifedipine, the current study suggested that long-term dietary supplementation with auraptene would be beneficial in terms of mono- or adjunctive therapy for the management of hypertension.

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References

1. Veeramani C, Al-Numair K, Chandramohan G, Alsaif MA, Alhamdan AA, Pugalendi KV. Antihypertensive effect of *Melothria maderaspatana* leaf fractions on DOCA-salt-induced hypertensive rats and identification of compounds by GC-MS analysis. J Nat Med 2012; 66:302-310.

2. Krum H, Pellizzer AM. New and emerging drug treatments for hypertension. Aust Fam Physician 1998; 27:235-*23*7.

3. Talha J, Priyanka MAA. Hypertension and herbal plants. Int Res J Pharm 2011; 2:26-30.

4. Mulero J, Bernabé J, Cerdá B, García-Viguera C, Moreno DA, Albaladejo MD, *et al*. Variations on cardiovascular risk factors in metabolic syndrome after consume of a Citrus-based juice. Clin Nutr 2012; 31:372-377.

5.Yamada T, Hayasaka S, Shibata Y, Ojima T, Saegusa T, Gotoh T, *et al.* Frequency of *citrus* fruit intake is associated with the incidence of cardiovascular disease: The Jichi medical school cohort study. J Epidemiol 2011; 21:169–175.

6. Perez YY, Jimenez-Ferrer E, Alonso D. Botello-Amaro CA, Zamilpa A. *Citrus limetta* leaves extract antagonizes the hypertensive effect of angiotensin II. J Ethnopharmacol 2010; 128:611-614.

7. Díaz-Juárez JA, Tenorio-López FA, Zarco-Olvera G, Valle-Mondragón LD, Torres-Narváez JC, Pastelín-Hernández G. Effect of *Citrus paradisi* extract and juice on arterial pressure both *in vitro* and *in vivo*. Phytother Res 2009; 23:948-954.

8. Gaziano JM. Antioxidant vitamins and cardiovascular disease. Proc Assoc Am Physicians 1999; 111:2-9.

9. Roza JM, Xian-Liu Z, Guthrie N. Effect of *citrus* flavonoids and tocotrienols on serum cholesterol levels in hypercholesterolemic subjects*.* Altern Ther Health Med 2007; 13:44-48.

10. Curini M, Cravotto G, Epifano F, Giannone G. Chemistry and biological activity of natural and synthetic prenyloxycoumarins. Curr Med Chem 2006; 13:199-222.

11. Soltani F, Mosaffa F, Iranshahi M, Karimi G, Malekaneh M, Haghighi F, *et al.* Auraptene from *Ferula szowitsiana* protects human peripheral lymphocytes against oxidative stress. Phytother Res 2010; 24:85-89.

12. Genovese S, Epifano F. Auraptene: A natural biologically active compound with multiple targets. Curr Drug Targets 2011; 12:381-386.

13. Curini M, Epifano F, Messina F, Genovese S. Antibacterial properties of auraptene and oxyprenylated naturally occurring benzoic and cinnamic acids. Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas 2012; 11:74–76.

14. Yamada Y, Okamoto M, Kikuzaki H, Nakatani N. Spasmolytic activity of auraptene analogs. Biosci Biotechnol Biochem 1997; 61:740-742.

15. Kakiuchi N, Senaratne LR, Huang SL, Yang XW, Hattori M, Pilapitiya U, Namba T. Effects of constituents of Beli (*Aegle marmelos*) on spontaneous beating and calcium-paradox of myocardial cells. Planta Med 1991; 57:43-46.

16. Imenshahidi M, Eghbal M, Sahebkar A, Iranshahi M. Hypotensive activity of auraptene, a monoterpene coumarin from *Citrus* spp. Pharm Biol 2013; 51:545-549.

17. Askari M, Sahebkar A, Iranshahi M. Synthesis and purification of 7-prenyloxycoumarins and herniarin as bioactive natural coumarins. Iran J Basic Med Sci 2009; 12: 63-69.

18. Imenshahidi M, Hosseinzadeh H, Javadpour Y. Hypotensive effect of aqueous saffron extract (*Crocus sativus* L.) and its constituents, safranal and crocin, in normotensive and hypertensive rats. Phytother Res 2010; 24:990-994.

19. Lorenz JN.A practical guide to evaluating cardiovascular, renal, and pulmonary function in mice. Am J Physiol Regul Integr Comp Physiol 2002; 282:R1565-1582.

20. Reshef N, Hayari Y, Goren C, Boaz M, Madar Z, Knobler H. Antihypertensive effect of sweetie fruit in patients with stage I hypertension. Am J Hypertens 2005; 18:1360-1363.

21. Komatsu S, Tanaka S, Ozawa S. Biochemical studies on grape fruits, *Citrus aurantium*. Nippon Kagaku Kaishi 1930; 51:478-498.

22. Epifano F, Genovese S, Curini M. Auraptene: Phytochemical and pharmacological properties. In: Matsumoto T, editor. Phytochemistry Research Progressed. New York: Nova Science Publishers Inc; 2008.p.145–162.
23. Nagao K, Yamano N, Shirouchi B, Inoue N, Murakami S, Sasaki T, *et al.* Effects of *Citrus auraptene* (7-geranyloxycoumarin) on hepatic lipid metabolism *in vitro* and *in vivo*. J Agric Food Chem 2010; 58:9028–9032.
24. Fusi F, Saponara S, Pessina F, Gorelli B, Sgaragli G. Effects of quercetin and rutin on vascular preparations: A comparison between mechanical and electrophysiological phenomena. Eur J Nutr 2003; 42:10–17.
25. Ari YN, Altan VM, Altinkurt O, Ozturk Y. Pharmacological effects of rutin. Phytother Res 2006; 5:19-23.
26. Xu YC, Leung SWS, Yeung DKY, Hu LH, Chen GH, Che CM, *et al*. Structure–activity relationships of flavonoids for vascular relaxation in porcine coronary artery. Phytochemistry 2007; 68:1179-1188.
27. Athiroh N, Permatasari N, Sargowo D, Widodo MA. Effect of *Scurrula atropurpurea* on nitric oxide, endothelial damage, and endothelial progenitor cells of DOCA-salt hypertensive rats. Iran J Basic Med Sci 2014; 17: 622-625.