RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2015.371.376

Antihypertensive Effects of Apple Peel Extract on Spontaneously Hypertensive Rats

[1]Nileeka Balasuriya, [1]H.P. Vasantha Rupasinghe, [2]Marva Sweeney, [2]Shelby McCarron and [2]Katherine Gottschall-Pass

[1]Department of Environmental Sciences, Faculty of Agriculture, Dalhousie University, Truro, Nova Scotia B2N 5E3, Canada

[2]University of Prince Edward Island, Charlottetown, C1A 4P3, Canada

ABSTRACT

Background and Objectives: Apple peel is a rich source of biological active phytochemicals such as flavonoids. The present study investigated the antihypertensive effect of flavonoid-rich Apple Peel Extract (APE) on Spontaneously Hypertensive Rats (SHR). **Methodology:** Three groups of animals: Control, captopril (20 mg kg^{-1} of body weight/day) and APE (25 mg kg^{-1} of body weight/day) were fed standard rat chow and their corresponding treatment in sugar-free gelatin, daily for a period of eight weeks. Blood Pressure (BP) was monitored weekly using the tail cuff method. Blood and tissue samples were collected after the eighth week. **Results:** As expected, treatment with captopril consistently reduced BP ($p<0.05$). APE treatment reduced both systolic and diastolic BP by 15 and 11 mg Hg, respectively, after 5 weeks of treatment, However, statistical significance was only achieved in systolic BP after eight weeks when compared with control ($p<0.05$). There were no significant differences in serum and lung ACE activity at week eight. Treatment with APE increased liver superoxide dismutase (SOD) activity by 78% and total reduced Glutathione (GSH) concentrations by 42% when compared to control ($p<0.05$) but had no effect on the activity of glutathione reductase or peroxidase. **Conclusion:** Long term intake of APE reduces high blood pressure in SHR possibly through endogenous antioxidant pathways. This preclinical trial suggests that APE as a dietary supplement could be effective in managing early stages of hypertension.

Key words: Flavonoids, blood pressure, antioxidants, superoxide dismutase, glutathione, dietary supplement

Pharmacologia 6 (8): 371-376, 2015

INTRODUCTION

Hypertension is a progressive disorder which consists of persistent high blood pressure (>140/90 mm Hg). It is strongly associated with increased risk of myocardial infarction, stroke and development of renal disease. Blood pressure regulation is a complex process which involved neurogenic control, renin angiotensin system, kallikrein-kinin system, endothelial function and sodium and water excretion. Malfunction in these internal systems could lead to blood pressure changes[1]. Current antihypertensive drugs in use are designed to address different aspects of pathogenesis of hypertension. Some examples are Angiotensin Converting Enzyme (ACE) inhibitors, calcium channel blockers, β blockers, natriuretic peptides, adrenergic inhibitors and direct vasodilators. The long term intake of drugs could decrease the cardiac output and peripheral vascular resistance to protect the internal organs from damage. However, life style modifications are still considered as the first step in management of hypertension. Moderate sodium intake, reduced weight in obese individuals, low consumption of alcohol, increased intake of fruits and vegetables are among the key factors[1].

Diet plays a significant role in reducing blood pressure[2,3]. Fruits are a major component in the human diet. Current research findings emphasize the importance of phytochemicals found in fruits as disease combating agents. Flavonoids are one category of phytochemicals with proven health benefits[4]. There are several proposed mechanisms of actions by which flavonoids may reduce high blood pressure. Dysregulation of renin angiotensin system is one of the causative factors for development of hypertension. One

Corresponding Author: H.P. Vasantha Rupasinghe, Department of Environmental Sciences, Faculty of Agriculture, Dalhousie University, Truro, Nova Scotia B2N 5E3, Canada Tel: (902) 893-6623 Fax: (902) 893-1404

such example is over expression of ACE producing genes leading to high levels of ACE. Flavonoids were reported as ACE inhibitors both *in vitro*[5] and *in vivo*[6]. It is become evident that oxidative stress in the arterial walls and kidneys also plays a role in the development and progression of hypertension, with antioxidant systems such as superoxide dismutase (SOD) and glutathione being down-regulated[7]. Since flavonoids act as antioxidants, it could be postulated that flavonoids could support to enhance the activity of down-regulated antioxidant system at cellular level.

Apples are among the most frequently consumed fruits in temperate regions. A fresh apple contains 14-36 mg of flavonoids/100 g of fresh weight[8]. Apple peel is a particularly rich source of flavonoids and contain 3-6 fold more flavonoids than the flesh[9]. Apple peel extracts are known to act as antioxidants[10], antiviral[11], neuroprotective[12], antiproliferative and anticancer agents[4,13]. Since apple peel is a waste product of apple processing industry, there is potential to convert this bioresource into a natural health product to treat hypertension[14]. In this research study, an Apple Peel Extract (APE) rich in flavonoids was investigated on its effectiveness in reducing high blood pressure in Spontaneously Hypertensive Rats (SHR).

MATERIALS AND METHODS

Animals: Eight weeks old male SHR were obtained from Charles River Laboratories, Quebec, Canada. Animals were housed individually, maintained at a temperature of 22°C with 12 h light/dark cycle and acclimated for one week before starting the treatments. All protocols were approved by the Animal Care Committee of the University of Prince Edward Island, Charlottetown, PE, Canada according to the guidelines set by the Canadian Council on Animal Care.

Treatments: Animals were randomly assigned to one of three treatment groups: control, APE (25 mg kg^{-1} of body weight/day), or captopril (20 mg kg^{-1} of body weight/day), calculated weekly according to each rat's body weight. Captopril is a known ACE inhibitor used to treat hypertension and was used as a positive control. APE was prepared as described previously[15]. Sugar free raspberry flavoured gelatin was used to deliver the treatments to rats. Gelatin blobs were prepared incorporating calculated amounts of APE and Captopril (Sigma Aldrich Canada Ltd, Oakville, ON, Canada) according to body weights of animals. The volume of one gelatin blob was 9 mL. Control animals received only gelatin. In addition, all animals received free access to standard rat chow and water. Food intakes were measured daily and body weights assessed weekly.

Blood pressure measurements: Weekly blood pressure measurements were taken using the CODA rat and mice tail cuff blood pressure measuring system (Kent Scientific, Torrington, Connecticut, USA). Animals were placed inside the weight matched cylindrical compartments and acclimated for 10 min on a heated (37°C) platform. Tail temperatures were monitored continuously using an infrared thermometer (Kent Scientific, Torrington, Connecticut, USA). Tail cuffs were fitted onto the animal after the tail temperature reached >28°C. Blood pressure was measured in 20 cycles which included 5 cycles for acclimation. Measures were taken to minimize stress induced changes in blood pressure.

Blood and tissue collection: At week 10, animals were fasted overnight for 14-16 h and the anesthetized deeply with sodium phenobarbital (65 mg kg^{-1}). A heparinized blood sample was collected by cardiac puncture, placed on ice and later centrifuged at 10,000×g at 4°C to produce plasma which was stored at -80°C. Rats were then euthanized, lung and liver were collected, flash frozen in liquid nitrogen and stored at -80°C until further analysis.

Determination of ACE activity: The ACE activity of lung and plasma samples was determined as described previously[5,16]. Heparin, histidine-L-hippuryl-L-leucine-chloride (HHL), NaOH, HCl, ethanol anhydrous and o-phthalaldehyde were purchased from Sigma Aldrich Canada Ltd. (Oakville, ON, Canada).

Determination of endogenous antioxidants: Liver samples (100 mg) were first homogenized in the respective buffers needed for assay and then antioxidant concentrations or enzyme activities were determined using commercial assay kits (Cayman Chemical Company, Ann Arbor, MI, USA) and following the manufacturer protocols. Briefly, levels of total glutathione, an endogenous antioxidant were assayed by applying an enzymatic recycling method, using Glutathione Reductase (GR), to quantify glutathione concentrations. Total glutathione concentrations were measured at five-minute intervals for 30 min using a multilevel reader set at an absorbance of 405 nm.

Glutathione Peroxidase (GPX), GR and SOD concentrations in liver homogenates were determined using Cayman® Chemical Company SOD assay kit

protocol. GR activity is determined through the rate of NADPH oxidation while GPX activity is estimated indirectly by its coupled reaction with GR. The SOD catalyzes the dismutation of superoxides to molecular oxygen and hydrogen peroxide and so its activity was determined by removal of superoxides. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Statistical analysis: Means and standard errors were calculated for each treatment and data sets were analyzed using one-way analysis of variance (ANOVA) with Tukey's *post hoc* test using Graph Pad Prism from Graph Pad Software, Inc. (La Jolla, CA). In all analysis, p-values of less than 0.05 were considered significant.

RESULTS

All rats weighted the same at the beginning of the study and gained similar amounts of weight over the 8 week study (Fig. 1). However, captopril-fed SHR weighted 4.3 and 4.7% less than control-fed rats at week 3 and 5 (Fig. 1). As expected, treatment of SHR with captopril significantly lowered BP throughout the study when compared with both control and APE treated animals (Fig. 2a, b). APE treatment reduced both systolic and diastolic BP by approximately 10-15 mm Hg after 5 weeks of treatment, However, statistical significance was only achieved in lowering systolic BP after 8 weeks when compared with control-treated rats ($p<0.05$) (Fig. 2). However, there was no significant ($p<0.05$) difference existed between the ACE activity of the lung and plasma samples within each group.

APE treatment increased total GSH in liver from 5.4±0.5-7.7±0.8 mM mg^{-1}, representing a 43% increase (Fig. 3a, p = 0.046), while captopril had no effect. However, the enzymes involved in glutathione metabolism, namely GR and GPX, were unaffected by APE and captopril. The SOD activity was almost doubled by APE treatment, although captopril also had no effect (Fig. 3b).

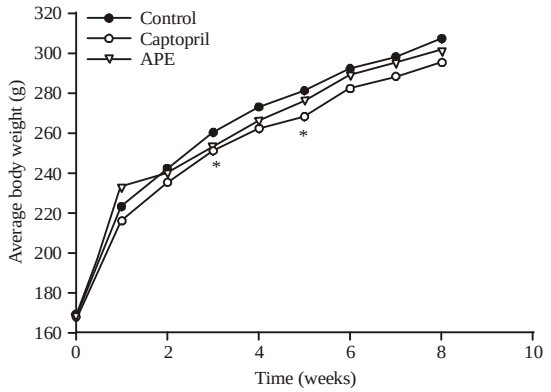

Fig. 1: Weight gain of animals over the 10 week period. Asterick (*) represent significantly ($p<0.05$) different groups (n = 12) APE, flavonoid-rich apple peel extract

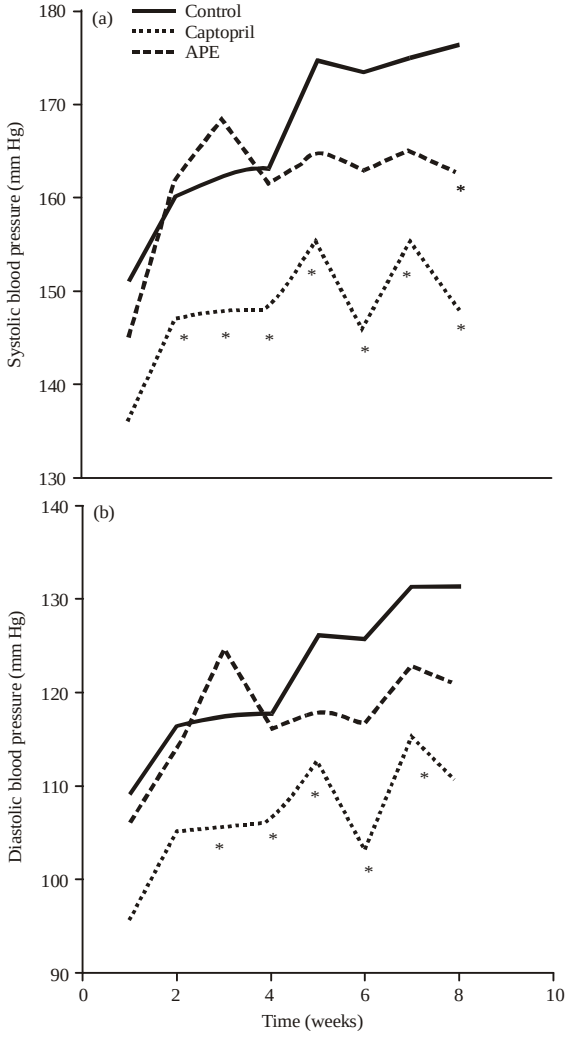

Fig. 2(a-b): Mean (a) Systolic and (b) Diastolic blood pressure values of SHR. Asterick (*) represent significantly ($p<0.05$) different groups (n = 12) APE, flavonoid-rich apple peel extract

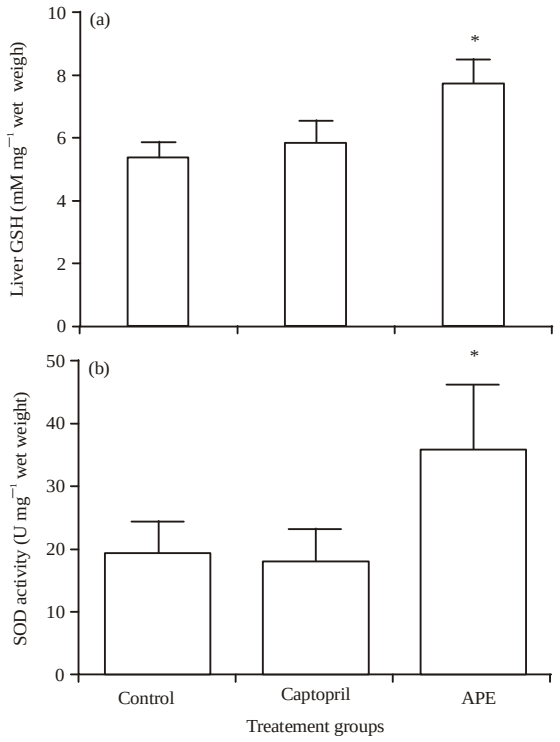

Fig. 3(a-b): (a) Liver GSH activity and (b) SOD activity APE, flavonoid-rich apple peel extract

DISCUSSION

There is growing evidence of the impact of dietary factors in reducing the risks associated with onset of chronic diseases. The objective of the current preclinical study was to evaluate the potential to use APE as a natural antihypertensive agent using a rodent model. APE is a rich source of polyphenols, primarily flavanols, flavan-3-ols, dihydrochalcones and chlorogenic acid[5]. These groups of phytochemicals are well documented for their function as antioxidants, enzyme inhibitors and cell signaling pathway modulators[17]. Many studies have evaluated their antihypertensive effects under *in vitro* conditions. The mechanisms of actions of flavonoids include functioning as ACE inhibitors[18], antioxidant activity and vasorelaxation[19].

In the current study, 0.5 g of APE kg^{-1} of body weight, was incorporated as the daily dose which contained 25 mg of total polyphenols where the majority (around 20 mg) were flavonoids. These values were selected to provide an effective biologically active metabolite concentration. In most studies the incorporated levels of plant extracts vary in between 0.1-1 g kg^{-1} body weight of animals[20,21]. Consumption of APE had no effect on body weight gain and feeding behavior of animals.

After five weeks of treating APE daily, the increasing trend of blood pressure of animals started to retard showing a significant reduction of systolic blood pressure at the eighth week (Fig. 1). There are different plant extracts examined on the hypotensive effects. Traditional herbal extracts[18], blueberry[22], cocoa[20], purple corn, purple sweet potato and red radish[19] are some examples. A Brazilian folk medicine *Cecropia glaziovii* Sneth has reduced the mean systolic blood pressure by 20 mm Hg after 2 weeks of treatments where the single dose treatments of the extracts had not exerted any difference[20]. Radish leaf extracts rich in polyphenols have shown the same trend of reducing blood pressure after 3 weeks of oral administration[23]. In all these studies, the blood pressure lowering ability is found to be associated with the presence of phytochemicals.

In accordance with the previous studies, after 5 weeks of APE treatment systolic blood pressure increments were reduced by 10 mm Hg and after eight weeks, it was reduced by 15 mm Hg. Long term consumption of APE rich in flavonoids showed hypotensive effect on SH rats. Repeated treatments are found to be effective since it could enhance the accumulation of metabolites in target tissues which is beneficial on the bioactivity[24].

Captopril treated group showed significant reduction of blood pressure (systolic and diastolic) starting from the fourth week of study and continued till the end of the study week 8 (Fig. 2).

APE was found to be an effective ACE inhibitor under *in vitro* conditions[5]. Therefore, the effect of APE on the ACE activity of blood plasma and lung extracts on SHR was determined using a fluorimetric method. There were no significant difference exist on enzyme activity between groups (Data not shown).

Polyphenols including flavonoids are reported on their ability to improve endothelial function and antioxidant activity thereby provide cardio-protective effects[25]. Reactive oxygen species are highly generated in the vasculature and kidney tissues during hypertension. GSH and associated GPX and GR enzymes play a key role in mediating oxidative stress in cells. A high ratio between the reduced GSH to oxidized glutathione (GSSH) is essential to protect the cells from oxidative stress[7]. The APE treated had significantly increased the reduced form of GSH levels. Increased levels of GSH indicate a protective environment from free radical damage. However, the enzymes associated with the GPX system i.e., GPX and GR activity did not show any significant difference when compared to the control group. The SOD catalyzes the transformation of ROS to hydrogen peroxide (H_2O_2) which is the initial step of

deactivation of ROS[26]. In APE treated mice liver, SOD activity had almost doubled in amount when compared with the control group. An increased concentration of SOD indicates a higher capacity to scavenge free radicals[26]. Captopril had not shown any significant difference on these parameters when compared with the control.

This is the first pre-clinical study to demonstrate that APE reduces blood pressure in an *in vivo* model. This study supports recommendations that adding more fruit or fruit-based products to the diet improves risk factors for disease and provides insight to the development of apple-based natural health products.

CONCLUSION

Long term intake of APE reduced the expected increase in blood pressure in SHR. Though APE is a promising ACE inhibitor *in vitro*, *in vivo* studies did not show significant ACE inhibition. APE produced a significant increase in liver SOD and total GSH activity suggesting a mechanism of action through mediation of oxidative stress. Further studies are necessary to clarify the mode of action of the APE in reducing high blood pressure. The results of this study suggest that APE has a potential to be used as a dietary supplement in managing early stages of hypertension.

REFERENCES

1. Foex, P. and J.W. Sear, 2013. Hypertension: Pathophysiology and treatment. Continu. Educ. Aneaesthesia Crit. Care Pain, 4: 71-75.
2. Karanja, N., T.P. Erlinger, L. Pao-Hwa, E.R. Miller and G.A. Bray, 2004. The DASH diet for high blood pressure: From clinical trial to dinner table. Cleveland Clin. J. Med., 71: 745-753.
3. Frisoli, T.M., R.E. Schmieder, T. Grodzicki and F.H. Messerli, 2011. Beyond salt: Lifestyle modifications and blood pressure. Eur. Heart J., 32: 3081-3087.
4. He, X. and R.H. Liu, 2007. Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple's anticancer activity. J. Agric. Food Chem., 55: 4366-4370.
5. Balasuriya, N. and H.P.V. Rupasinghe, 2012. Antihypertensive properties of flavonoid-rich apple peel extract. Food Chem., 135: 2320-2325.
6. Hackl, L.P., G. Cuttle, S.S. Dovichi, M.T. Lima-Landman and M. Nicolau, 2002. Inhibition of angiotensin converting enzyme by quercetin alters the vascular response to brandykinin and angiotensin I. Pharmacology, 65: 182-186.
7. Slemmer, J.E., J.M. Livingston-Thomas, K.T. Gottschall-Pass and M.I. Sweeney, 2013. Cranberries and wild blueberries treated with gastrointestinal enzymes positively modify glutathione mechanisms in Caco-2 cells *in vitro*. J. Food Sci., 78: H943-H947.
8. Arabbi, P.R., M.I. Genovese and F.M. Lajolo, 2004. Flavonoids in vegetable foods commonly consumed in Brazil and estimated ingestion by the Brazilian population. J. Agric. Food Chem., 52: 1124-1131.
9. Wolfe, K.L. and R.H. Liu, 2003. Apple peels as a value-added food ingredient. J. Agric. Food Chem., 51: 1676-1683.
10. Huber, G.M. and H.P. Rupasinghe, 2009. Phenolic profiles and antioxidant properties of apple skin extracts. J. Food Sci., 74: C693-C700.
11. Suarez, B., A.L. Alvarez, Y.D. Garcia, G. del Barrio, A.P. Lobo and F. Parra, 2010. Phenolic profiles, antioxidant activity and *in vitro* antiviral properties of apple pomace. Food Chem., 120: 339-342.
12. Keddy, P.G., K. Dunlop, J. Warford, M.L. Samson, Q.R.D. Jones, H.P.V. Rupasinghe and G.S. Robertson, 2012. Neuroprotective and anti-inflammatory effects of the flavonoid-enriched fraction AF4 in a mouse model of hypoxic-ischemic brain injury. PLoS One, Vol. 7 10.1371/journal.pone.0051324
13. He, X. and R.H. Liu, 2008. Phytochemicals of apple peels: Isolation, structure elucidation and their antiproliferative and antioxidant activities. J. Agric. Food Chem., 56: 9905-9910.
14. Rupasinghe, H.P.V., L. Wang, G.M. Huber and N.L. Pitts, 2008. Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder. Food Chem., 107: 1217-1224.
15. Rupasinghe, H.P.V., N. Erkan and A. Yasmin, 2010. Antioxidant protection of eicosapentaenoic acid and fish oil oxidation by polyphenolic-enriched apple skin extract. J. Agric. Food Chem., 58: 1233-1239.
16. Santos, M.R., M.J. Rodriguez-Gomez, G.C. Justino, N. Charro, M.H. Florencio and L. Mira, 2008. Influence of the metabolic profile on the *in vivo* antioxidant activity of quercetin under a low dosage oral regimen in rats. Br. J. Pharmacol., 153: 1750-1761.
17. Rodrigo, R., D. Gil, A. Miranda-Merchak and G. Kalantzidis, 2012. Antihypertensive role of polyphenols. Adv. Clin. Chem., 58: 225-254.

18. Gasparotto, Jr. A., F.M. Gasparotto, E.L.B. Lourenco, S. Crestani and M.E.A. Stefanello *et al*., 2011. Antihypertensive effects of isoquercitrin and extracts from *Tropaeolum majus* L.: Evidence for the inhibition of angiotensin converting enzyme. J. Ethnopharmacol., 134: 363-372.
19. Shindo, M., T. Kasai, A. Abe and Y. Kondo, 2007. Effects of dietary administration of plant-derived anthocyanin-rich colors to spontaneously hypertensive rats. J. Nutr. Vitaminol., 53: 90-93.
20. Quinones, M., M. Miguel, B. Muguerza and A. Aleixandre, 2011. Effect of a cocoa polyphenol extract in spontaneously hypertensive rats. Food Funct., 2: 649-653.
21. Ninahuaman, M.F.M.L., C. Souccar, A.J. Lapa and M.T.R. Lima-Landman, 2007. ACE activity during the hypotension produced by standardized aqueous extract of *Cecropia glaziovii* Sneth: A comparative study to captopril effects in rats. Phytomedicine, 14: 321-327.
22. Wiseman, W., J.M. Egan, J.E. Slemmer, K.S. Shaughnessy, K. Ballem, K.T. Gottschall-Pass and M.I. Sweeney, 2011. Feeding blueberry diets inhibits angiotensin II-converting enzyme (ACE) activity in spontaneously hypertensive stroke-prone rats. Can. J. Physiol. Pharmacol., 89: 67-71.
23. Chung, D.H., S.H. Kim, N. Myung, K.J. Cho and M.J. Chang, 2012. The antihypertensive effect of ethyl acetate extract of radish leaves in spontaneously hypertensive rats. Nutr. Res. Pract., 6: 308-314.
24. D'Archivio, M., C. Filesi, R. Vari, B. Scazzocchio and R. Masella, 2010. Bioavailability of the polyphenols: Status and controversies. Int. J. Mol. Sci., 11: 1321-1342.
25. Pandey, K.B. and S.I. Rizvi, 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med. Cell. Longevity, 2: 270-278.
26. Jearapong, N., W. Chatuphonprasert and K. Jarukamjorn, 2013. Miroestrol, a phytoestrogen from *Pueraria mirifica*, improves the antioxidation state in the livers and uteri of β-naphthoflavone-treated mice. J. Nat. Med., 68: 173-180.