JJBS
Jordan Journal of Biological Sciences

Volume 2, Number 2, June 2009
ISSN 1995-6673
Pages 69 - 76

Effects of Trans-Resveratrol, Isolated from *Smilax Aspera*, on Smooth Muscle, Blood Pressure, and Inflammation in Rats and Nociception in Mice

Abeer H. Harb[a], Musa abu Zarga[b], Shtaywy Abdalla[a,*]

[a]*Deptartment of Biological Sciences* ,[b]*Dept. of Chemistry, University of Jordan, Amman 11942, Jordan*

Abstract

Smilax aspera (Liliaceae) has been used in herbal medicine for the treatment of many disorders. Chemical analysis of this plant resulted in the isolation of the stilbene trans-resveratrol. The effects of this compound on rat isolated ileum and urinary bladder, blood pressure and inflammation, and nociception in mice have been examined. Resveratrol ($3x10^{-5}$ -$1x10^{-1}$ mg/ml; or $1.3x10^{-7}$ - $4.4x10^{-4}$ M) caused a concentration-dependent relaxation in both ileal longitudinal segments and urinary bladder rings. Intravenous doses of resveratrol (0.028-8.5 mg/kg) caused a dose-dependent fall of systolic and diastolic blood pressure of anesthetized rats. Oral administration of this compound (1.3, 4.0. 13 and 40 mg/kg) decreased carrageenan-induced paw edema in rats. Furthermore, oral administration of resveratrol (3, 10, 30 and 100 mg/kg) decreased the number of writhes induced by i.p. injection of 0.8% acetic-acid in mice. These effects of resveratrol and its potential as a medicinal source were discussed in the light of the reported effects of resveratrol on nitric oxide and prostaglandin synthesis.

الملخص

استخدمت نبتة العليق (*Smilax aspera*)، التي تنتمي للعائلة الزنبقية، في طب الأعشاب لمعالجة العديد من الأمراض. وقد أدى التحليل الكيميائي لهذه النبتة إلى عزل مركب ترانس-رزفيراترول. في هذا البحث، درست تأثيرات رزفيراترول على اللفائفي والمثانة البولية وعلى ضغط الدم والالتهاب في الجرذ، وعلى الألم في الفأر. أحدث رزفيراترول بتراكيز تتراوح من 3×10^{-5} إلى 1×10^{-1} ملغم/مل (تعادل 1.3×10^{-7} إلى 4.4×10^{-4} جزيئي) انبساطاً معتمداً على التركيز في قطع اللفائفي الطولية وفي حلقات المثانة البولية المعزولتين من الجرذ. وأحدثت جرعات من رزفيراترول حقنت بالوريد وتتراوح بين 0.028 إلى 8.5 ملغم/كغم انخفاضاً معتمداً على التركيز في الضغط الانقباضي والانبساطي في الجرذان المخدرة. كذلك، فإن جرعات عن طريق الفم للجرذان مقدارها 1.3، 4، 13، 40 ملغم /كغم من رزفيراترول قللت من حجم الاستسقاء الناتج عن حقن مادة كراجينين في كف الجرذ. فضلاً عن ذلك، فإن جرعات من رزفيراترول أعطيت عن طريق الفم للفأر مقدارها 3، 10، 30، 100 ملغم/كغم قللت من عدد مرات التلوي بسبب الألم الناتج عن حقن 0.8 % من حامض الخليك داخل الصفاق. تم مناقشة تأثيرات رزفيراترول هذه وآفاق استخدامه كمادة علاجية في ضوء المعلومات المدونة عنه في الأدب العلمي والمتعلقة بتحرر حامض النتريك وتحرر البروستاغلانديناتز.

Keywords: Resveratrol; *Smilax Aspera*; Smooth Muscle; Blood Pressure; Inflammation; Analgesia.

1. Introduction

Smilax aspera is an evergreen, creeping, extremely tough shrub that belongs to the Liliaceae family (Longo and Vasapollo, 2006) and has been used in herbal medicine for muscle relaxation, skin ailment, rheumatic pain, depurative, diuretic, diaphoretic, antigout, dropsy, stimulant and for its tonic properties (Longo and Vasapollo, 2006; Aburjai *et al*., 2007). *S. aspera* has also been used traditionally for the treatment of syphilis (Vermani and Garg, 2002), diabetes (Fukunaga *et al*., 1997), rheumatism (Ageel *et al*., 1989), as an antioxidant (Demo *et al*., 1998) and to treat symptoms of menopause in women (Weil *et al*., 2000). In fact, several species of the genus *Smilax* are well-known Chinese traditional medicines, and are used as anti-inflammatory, anticancer and analgesic agents (Shu *et al*., 2006). Chemical and pharmacological studies were carried out on this species (e.g. Ageel *et al.*, 1989; Ruan *et al*., 2005; Selenu *et al*., 2005; Xu *et al*., 2005; Longo and Vasapollo, 2006). In an attempt to isolate chemical ingredients from the local *Smilax aspera*, we have been able to isolate the known stilbene trans-resveratrol.

Resveratrol is a non flavonoid polyphenolic compound found in a large number of plant species (at least 72 species), a number of which are human diet components, including mulberries, peanuts, grapes, and red wines (Lastra and Villegas, 2005). Resveratrol has been proposed as an effective agent in the prevention of pathologic processes such as inflammation, atherosclerosis, oxidative stress and carcinogenesis (Miatello *et al*., 2005; Baur and Sinclair, 2006). It has also been reported as neuroprotective, cardioprotective and to modulate lipoprotein metabolism (Martin *et al*., 2004; Baur *et al*., 2006). The interest in resveratrol was rekindled after reports that it extends life span in the yeast *Saccharomyces cervisiae* (Howitz *et al*., 2003) and the worm *Caenorhabditis elegans* and the fruit fly *Drosophila*

* Corresponding author. Shtaywy@ju.edu.jo.

melanogaster (Wood *et al*., 2004). In spite of reports to the contrary (Bass *et al*., 2007; Pearson *et al*., 2008), the debate on the biological effects of resveratrol continued to generate scientific interest (Valenzano *et al*., 2006). This communication is a contribution to evaluate other potential effects of resveratrol such as its effects on isolated smooth muscle preparations, blood pressure, and inflammation in rats and on pain in mice.

2. Materials and Methods

2.1. Effect of Resveratrol on Isolated Ileum and Urinary Bladder

Male and female albino rats weighing 250-300gm were used. Animals were lightly anesthetized with ether, and then sacrificed in compliance with the university adopted guidelines for animals' use in research. A mid piece of the ileum was excised, flushed with aerated physiological salt solution (PSS) and cut into several pieces (1-2cm long each) (Hammad and Abdalla, 1997). Urinary bladder was also isolated, and cut into 2-3mm rings taken from the middle region of the bladder (Khattab and Al-Hrasen, 2006). The preparations were placed in 10-ml water-jacketed glass tissue baths containing aerated PSS at 37^{o}C and gassed with a mixture of 95% O_2- 5% CO_2 and mounted for isometric recording using force transducers (Grass FTO3C) connected to a physiograph (Graphtec Thermal Arraycorder, WR5000) under a tension of 1g (Abdalla *et al*., 1994).

After an equilibration period, cumulative concentration-response curves of resveratrol were established by addition of 3-fold increasing concentration of resveratrol. When the maximum response to resveratrol was achieved, a strong non-specific relaxant agent (10^{-3} M papaverine HCl) was added to the tissue bath. The response to resveratrol was expressed as percent of the maximum effect of papaverine (Onwukaeme *et al*., 1999).

2.2. Effect of Resveratrol on Blood Pressure

Male albino rats (250-350 gm body weight) were anesthetized with sodium thiopental (50mg/kg body weight; i.p.). The right common carotid artery was exposed and a catheter was introduced for the recording of blood pressure using P23AA Statham pressure transducer situated at the level of the heart and connected to a Gilson polygraph (Abdalla *et al*., 1994). The right femoral vein was also catheterized for intravenous injection of resveratrol. After a steady baseline of blood pressure was obtained (about 15 minutes), resveratrol was injected intravenously in doses of 0.028, 0.085, 0.28, 0.85, 2.8, and 8.5 mg/kg body weight. The changes in systolic and diastolic blood pressure were observed and expressed as percent of their respective control values obtained before resveratrol injection.

2.3. Anti-Inflammatory Effect of Resveratrol

Male albino rats, weighing 130-200 gm were used. Animals were fasted for 24 hours before the beginning of the experiment with free access to water (Amanlou *et al*., 2005). Animals were divided into 6 groups each of eight animals as follow:

1. Control group: Received 0.9% NaCl.
2. Four treated groups: Received 1.3, 4, 13, and 40 mg/kg of resveratrol.
3. Positive control group: Received 10 mg/kg of indomethacin, a reference anti-inflammatory drug.

All doses were administered orally at a dose volume of 0.5ml/100 g animal. One hour later, acute paw edema was induced by plantar injection of 0.1ml of 1% freshly prepared carrageenan suspension in normal saline into the right hind paw of each rat. The paw volume was measured before (0 h) and at intervals of 1, 2, 3, 4, 5, and 6 hrs after carrageenan using a plethysmometer (Type 7140 Ugo Basile, Italy) (Wu *et al*., 2006).

2.4. Antinociceptive Effect of Resveratrol

Male and female albino mice, weighing 20-30g were used. Animals were deprived of food, but with free access to drinking water for 12 hours prior to the experiments (Costa-Lotufo *et al*., 2004). The animals were divided into 6 groups each of eight animals as follows:

1. *Control group:* received 0.9% NaCl.
2. *Four treated groups:* received 3, 10, 30, and 100 mg/kg of resveratrol.
3. *Positive control group:* received 10 mg/kg of indomethacin, a reference analgesic drug.

Animals were pretreated orally with resveratrol 60 minutes prior to i.p. injection with 0.1ml/10g body weight of 0.8% (v/v) acetic acid solution in 0.9% NaCl (Rabelo *et al*., 2003). The number of writhes was counted during a 30-min period following the injection of acetic acid. A writhe was defined as a contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body (Choi *et al*., 2005).

2.5. Solutions

The composition of PSS in mM was: NaCl 118.0; KCl 4.7; $CaCl_2.2H_2O$ 2.5; $MgCl_2$ 0.5; NaH_2PO_4 1.0; $NaHCO_3$ 24.0; and glucose 11.1. Trans-resveratrol was isolated from *Smilax aspera* and was identified by comparison of its spectral data with literature (Vitrac *et al*., 2005). Stock solution of resveratrol was prepared daily by dissolving resveratrol in minimal amount of 1N NaOH, the volume was then completed to 1ml with 0.9% NaCl (final concentration of NaOH was 0.075%). Papaverine HCl (Acros Organics, New Jersey) solution (10^{-1}M) was prepared by dissolving papaverine hydrochloride in distilled water. Indomethacin (Sigma-Aldrich Chemie, Germany) was prepared by dissolving in 0.5% carboxymethylcellulose sodium. Carrageenan (Fluka Biochemika, Switzerland) was prepared by dissolving 0.1 g of carrageenan in 10 ml 0.9%NaCl.

2.6. Statistical Analysis

Data are presented as means ± the standard error of means (SEM). One way-analysis of variance (ANOVA) and Student *t*-test for independent samples were used to screen for differences between the means of the samples. The differences were considered significant if $P < 0.05$. The median effective concentration producing 50% of the maximum response (EC50) was calculated from the plot. Experimental data were analyzed by a computer fitting treatment using GraphPad Prism 5.0 software.

3. Results

3.1. Effect of Resveratrol on Isolated Smooth Muscle Preparations

Figure 1 shows that $3x10^{-5}$ -$1x10^{-1}$ mg/ml resveratrol (=$1.3x10^{-7}$ - $4.4x10^{-4}$ M), caused concentration-dependent relaxation in the rat isolated ileal longitudinal segments. The EC50 of resveratrol for relaxation of ileal longitudinal segments was (8.0 ± 2.3) x 10^{-3} mg/ml (n=4).

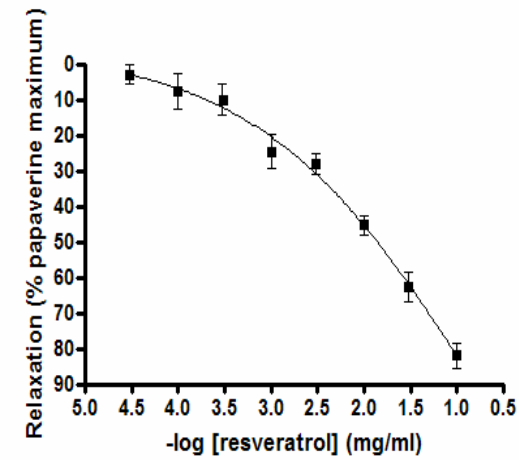

Figure 1. Cumulative concentration- response curves of resveratrol on rat isolated ileal longitudinal segments.

Figure 2 shows that resveratrol ($3x10^{-5}$ -$1x10^{-1}$ mg/ml) caused a concentration-dependent relaxation of the urinary bladder rings. The maximum relaxation induced by resveratrol reached (82.0 ± 4.1) % and (43.0 ± 6.5) % for ileal longitudinal segments and urinary bladder rings, respectively.

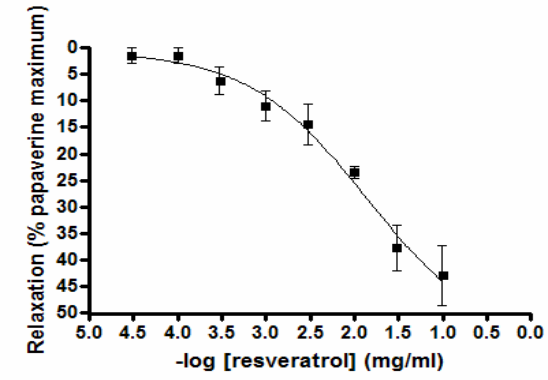

Figure 2. Cumulative concentration-response curves of resveratrol on rat isolated urinary bladder rings.

3.2. Hypotensive Effect of Resveratrol

Intravenous injection of resveratrol at doses ranging from 0.028 to 8.5 mg/kg induced dose-dependent fall of both systolic and diastolic blood pressure of the anesthetized rats (Figs. 3 and 4).

3.3. Anti-Inflammatory Effect of Resveratrol

Table 1 shows that resveratrol in doses of 1.3 and 4.0 mg/kg caused an inhibition in the hind paw volume over time although the decrease in the volume at these two

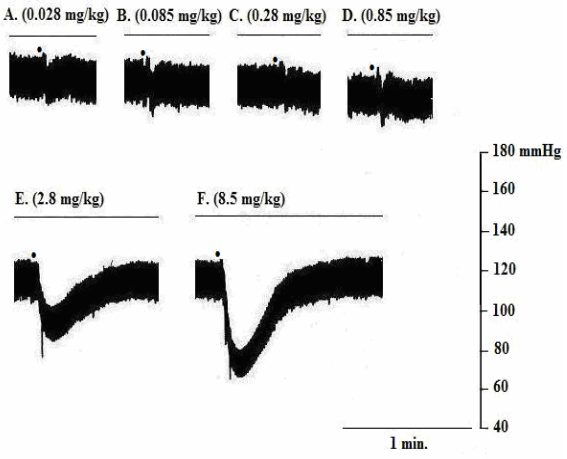

Figure 3. Typical responses of blood pressure to intravenous injections of resveratrol in the doses indicated (A-F). A-F were obtained from the same preparation. The solid line above the traces is a reference baseline.

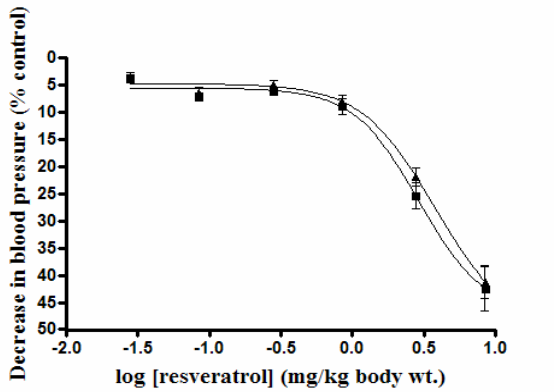

Figure 4. Dose-response curves of resveratrol on systolic (solid squares) and diastolic (solid triangles) blood pressure of anesthetized rats.

doses did not reach the level of statistical significance when compared to the control group. At concentrations of 13 and 40 mg/kg, there was a significant decrease in the hind paw volume, when compared to the control group. The positive control group also showed reduction of the volume of the hind paw and this inhibition reached the level of statistical significance 4, 5 and 6 hours after carrageenan administration.

3.4. Analgesic Effect of Resveratrol

Figure 5 shows that resveratrol-treated groups showed a dose-dependent decrease in the number of writhes counted in 30 minutes after injection of acetic acid. The highest reduction was obtained at a dose of 100 mg/kg (76.6 % of inhibition). This effect is comparable to that induced by indomethacin (71.3 % of inhibition) albeit a higher dose of resveratrol. Other resveratrol-treated groups (3, 10, and 30 mg/kg) showed intermediate inhibition values of 39.3 %, 54.0 %, and 56.6 %; respectively. This inhibition in the number of writhes reached the level of statistical significance at all doses of resveratrol-treated groups.

Table 1. Hind paw volumes in male rats observed over a period of six hours after oral administration of 0.9% NaCl, 1.3, 4, 13, and 40 mg/kg resveratrol, and indomethacin [a].

Experimental group	Zero time	1h	2h	3h	4h	5h	6h
Control (0.9% NaCl)	1.01±0.05	1.24±0.02	1.29±0.03	1.35±0.04	1.37±0.09	1.37±0.06	1.40±0.06
Resveratrol (1.3 mg/kg)	1.10±0.05	1.26±0.05	1.30±0.06	1.32±0.05	1.35±0.04	1.36±0.05	1.35±0.05
Resveratrol (4 mg/kg)	1.09±0.04	1.21±0.05	1.26±0.05	1.30±0.04	1.25±0.05	1.27±0.05	1.25±0.05
Resveratrol (13 mg/kg)	0.90±0.04	1.10±0.03	1.14±0.04	1.14±0.03*	1.12±0.02*	1.13±0.03*	1.06±0.03*
Resveratrol (40 mg/kg)	0.94± 0.03	1.03±0.03*	1.01±0.03*	1.03±0.03*	1.09±0.02*	1.07±0.02*	1.06±0.03*
Indomethacin (10 mg/kg)	1.06±0.04	1.12±0.04	1.13±0.02	1.18±0.02	1.15±0.03*	1.14±0.03*	1.13±0.01*

[a]All values represent means of the volume ± SEM for 8experiments.
*P < 0.05 compared to the control group.

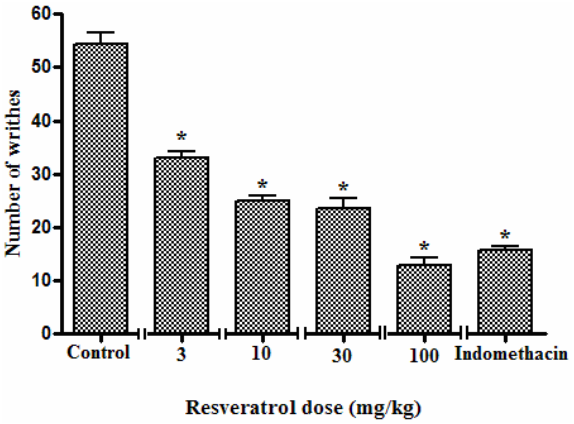

Figure 5: Number of writhes induced by acetic acid in mice treated with: 0.9% NaCl (control), 3, 10, 30, and 100 mg/kg resveratrol, and with 10 mg/kg indomethacin. Number of writhes is given as the means ± SEM of 8 animals. Asterisks indicate that P < 0.05 compared to the control group (0.9% NaCl).

4. Discussion

The growing interest in the use of herbs and their bioactive compounds is attributed largely to the fact that most herbs are relatively inexpensive and are easily available, yet have fewer adverse effects (Kim *et al*., 2006). *Smilax aspera,* a member of the Liliaceae family, is well known for its medicinal uses and has been used for many disorders. Many constituents have been isolated from this plant including flavonoids, tannins, asperoside, sapogenins, β-sitosterol and essential oils (Ayengar and Rangaswami, 1967; Petricic and Radosevic, 1969; Tschesche *et al*., 1974; Bruno *et al*., 1985; Long and Vasapollo, 2006). The current study on the whole plant material of *Smilax aspera* yielded resveratrol (0.0067% yield) which has been isolated from the genus *Smilax* previously (Zhang *et al*., 2006).

Resveratrol has been proposed as an effective agent in the prevention of pathologic processes such as inflammation, atherosclerosis, oxidative stress, and carcinogenesis (Miatello *et al*., 2005). Resveratrol, a polyphenolic compound found in many edible plant species, is satisfactorily absorbed from the intestine and distributed in the blood stream and can be detected in significant concentrations in the blood and a number of organs. Little is known about the transport and the distribution of resveratrol through the body but it must be bound to proteins and/or be conjugated to remain at high concentration in the serum as a consequence of its relatively low water solubility (Lastra and Villegas, 2005). Therefore, its pharmacological effects and its pharmacokinetics are of paramount importance to human health.

4.1. Resveratrol Spasmolytic Effects

In the current study, resveratrol has been found to cause concentration-dependent relaxation in both rat ileal longitudinal segments and urinary bladder rings. This observation is consistent with other studies which showed relaxant effects for resveratrol on isolated smooth muscles preparations. The relaxant effect of this compound was documented in many vascular beds including rat aorta, porcine coronary arteries, guinea pig mesenteric and uterine arteries and sheep coronary arteries (Naderali *et al*., 2000; El-Mowafy, 2002; Slater *et al.*, 2003; Campos-Toimil *et al.*, 2005; Buluc and Demirel-Yelmaz, 2006; King *et al*., 2006).

The mechanism of resveratrol relaxation cannot be deduced from the current experiments but observations made by other workers are suggestive of such mechanism. In one hand, several studies showed that nitric oxide relaxes ileum and urinary bladder via increasing cGMP synthesis (Hedlund, 2005; Gharib-Naseri *et al*., 2007) and that resveratrol relaxed endothelium-intact rat aortic rings constricted by phenylepherine and KCl via NO release (King *et al*., 2006). On the other hand, resveratrol stimulates the endothelium-dependent vasorelaxation through the stimulation of the NO/cGMP cascade in rat aortic rings (Fitzpatrick *et al*., 1993; Slater *et al.*, 2003), and it was shown that resveratrol upregulates the GC/cGMP system in coronary artery smooth muscle and to

trigger a vasorelaxant response (El-Mowafy, 2002). The GC/cGMP system plays a key role in spasmolytic signaling mechanisms (Song *et al*., 2006). NO, synthesized by the enzyme NO synthase, is released by peripheral nerves and mediates relaxant effect on vascular and non-vascular smooth muscles (Capasso *et al*., 2004). NO produces smooth muscle relaxation by activating the soluble guanylate cyclase (sGC) and thereby increasing tissue levels of cGMP, which in turn interacts with various intracellular components that regulate activities of the contractile proteins (Hedlund, 2005). Other effects of resveratrol pertains to variable effects on Ca in various tissues and cells are also possible and may play a role in the mechanism of action of resveratrol (Buluc and Demirel-Yilmaz, 2006; Wang and Scherer, 2008).

4.2. Resveratrol Effect on Blood Pressure

In this study, resveratrol was found to reduce arterial blood pressure of anesthetized normotensive rats when administered intravenously, indicating that it has a hypotensive effect. Although one component of the effect of resveratrol on both systolic and diastolic pressure was transient, blood pressure, in general, did not return to the original baseline. The present work is consistent with the recent studies that demonstrated the effects of resveratrol on blood pressure in various *hypertensive* rat models. It has been shown that resveratrol decreased systolic and diastolic blood pressure in ovariectomized rats (Li *et al*., 2006), and in the partially nephroctomized rats (Liu *et al*., 2005). It is likely that the hypotensive effect of resveratrol in the present study is due to an increase in NO production from the vascular endothelium. This suggestion stems from the observations that resveratrol administration prevented blood pressure elevation and reduced both vascular and myocardial endothelial NO synthase (eNOS) activity in fructose-fed rats (Miatello *et al*., 2005). Moreover, resveratrol was shown to restore the eNOS activity in the rat aorta and to normalize blood pressure of rats with modeled arterial hypertension (Gumanova *et al*., 2007). NO generated in endothelial cells by eNOS is known to play a major role in blood pressure control (Minuz, 2006).

4.3. Resveratrol Effect on Inflammation

Among the several models of acute inflammation, carrageenan-induced inflammation has been well established as a valid model to study free radicals generation in paw tissue after inflammatory states. The cellular and molecular mechanism of carrageenan-induced inflammation is well characterized, and this model of inflammation is a standard model to screen the anti-inflammatory activity of various experimental compounds (Bilici *et al*., 2002). Carrageenan-induced edema is caused by the release of a number of inflammatory mediators and is initiated by dilation of arterioles and an eventual increase in permeability of postcapillary venules resulting in exudation of inflammatory cells and fluids at the site of injury. Inhibition of this acute phase of inflammation will therefore ultimately abort the inflammatory process (Duwiejua *et al*., 2002). In this study, the edema induced in the rat hind paw by carrageenan was maximal at the 6th hour of its plantar injection. This time course of edema is consistent with that reported by Zakaria *et al*., (2006) but longer than that observed by Koo *et al*., (2006) who showed progressive paw volume reaching a maximum at 4 hrs, or that reported by Vasudevan *et al*., (2006) who showed maximum swelling after 3 hrs. Our results showed that resveratrol caused a dose-dependent decrease in the rat hind paw volume and this inhibition was statistically significant after 3h and 1h for doses of resveratrol of 13 and 40 mg/kg respectively. This observation is consistent with the finding that resveratrol isolated from *Smilax china* in a dose of 0.2 mg/kg/day inhibited the cotton pellet-induced granuloma in rats (Ruan *et al*., 2005). Resveratrol has also a significant suppressive effect on hind paw edema induced by egg albumin in bilateral adrenalectomized rats. The anti-inflammatory effects of resveratrol was found independent of adrenal gland but might be related to inhibition of inflammatory mediators and free radicals (Liu *et al*., 2006). Contrary to our observation, another study failed to demonstrate any anti-inflammatory effect of resveratrol using the carrageenan-induced paw edema model in which the administration of resveratrol at doses of 0.4, 2, 10, and 50 mg/kg did not reverse the swelling and edema, although they reversed the hyperalgesia induced by local tissue injury provoked by carrageenan (Gentilli *et al*., 2001). This contradiction might be attributed to differences in the route of resveratrol administration (oral vs. i.p.), the time between injection of resveratrol and injection of carrageenan (1hr vs. 30 min), differences in the instrument used for measurements (plethysmometer vs. analgesiometer) and the parameter measured (paw volume vs. paw circumference).

Several possibilities could account for the anti-inflammatory effects of resveratrol: i) inhibition of cyclooxygenase activity, since resveratrol has been shown to inhibit the induced production of PGE_2 in a dose-dependent manner in human peripheral blood leukocytes (Richard *et al*., 2005), and it decreased significantly the elevated levels of rat PGD_2 *in vivo*. In the *in vitro* and *in vivo* models, resveratrol decreased the expression of cyclooxygenase-2 (COX-2) (King *et al.,* 2006). Because COX-2 is the key enzyme catalyzing the rate-limiting step in PG biosynthesis, it is likely that at least part of the anti-inflammatory activity of resveratrol can be attributed to inhibition of COX-2 expression, ii) inhibition of the NO-generating pathway, since resveratrol has been shown to inhibit both the generation of NO and the expression of inducible nitric oxide synthase (iNOS) in activated macrophages and in cultured cells (Tsai *et al*., 1999). The expression of inducible isoform of NO synthase has been proposed as an important mediator of inflammation (Bilici *et al*., 2002). Resveratrol is known to inhibit the synthesis and release of pro-inflammatory mediators, inducible nitric oxide synthase (iNOS) and COX-2 via its inhibitory effects on nuclear factor – (kappa)B (NF- κB) (Sharma *et al*., 2007a). Tsai *et al*. (1999) showed that resveratrol protected against endotoxin-induced inflammation by preventing the NF- κB activation. In addition, resveratrol has been shown to be a potent and specific inhibitor of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) and consequently the induced NF-κB activation (Fulgenzi *et al*., 2001).

4.4. Resveratrol Analgesic Effects

In this study, resveratrol was also shown to decrease the number of writhes in a dose-dependent manner. We used the acetic acid-induced mice abdominal writhing model to study the antinociceptive effect. Contortions induced by intraperitoneal injections of acetic acid originate from the pain of inflammation mediated by prostaglandins (Oliveira *et al*., 2001). When the tissues and cells suffer harmful stimulation, they release compounds such as H^+, prostaglandin E_2 (PGE_2) or 5-hydroxytryptamine (5-HT) which cause pain at the location. Acetic acid H^+ itself may cause pain; at the same time it can stimulate the tissue to produce PGE_2 which causes further pain. Thus, acetic acid is widely used to screen compounds for antinociceptive activity and is accepted as a suitable model for antinociception (Shu *et al*., 2006). Levels of prostaglandins were reported to be high during the first 30 min after intraperitoneal injection of acetic acid (Neto *et al*. 2005). Our observations are consistent with those by Torres-Lopez *et al.* (2002) which showed that resveratrol has peripheral antinociceptive effect since it reduced nociception in a dose-dependent manner in the second phase of formalin test which is dependent on many mediators including prostaglandins (Sayyah et al., 2002). In addition, resveratrol has been shown to prevent streptozotocin-induced thermal hyperalgesia in diabetic mice (Sharma *et al*., 2007b). Although we have not investigated the mechanism of action of resveratrol as antinociceptive in the present work, we postulate that resveratrol causes this effect through inhibition of prostaglandin synthesis through an action on COX enzyme since there is evidence that resveratrol produces antinociception through the selective inhibition of COX-1 and COX-2 (Grandos-Soto *et al*., 2002). Other workers showed that resveratrol has the ability to disrupt arachidonic acid metabolism by inhibiting COX-1 and hyperperoxidase activity of COX-1. Furthermore, resveratrol has been reported to inhibit COX-2 expression induced by carcinogens in human breast and oral epithelial cells (Tang *et al*., 2006) and to inhibit potently PGE_2 production and free radical scavenging in activated microglial cells (Candelario-Jalil *et al*., 2007). Other mechanisms of analgesia include the reduction of prostaglandin production at the primary afferent neurons by resveratrol thus diminishing the depolarization of nerve terminals otherwise observed during the nociception process. Alternatively, the opening of Ca^{2+}-activated K^+ channels by resveratrol would further reduce the depolarization leading to antinociception (Grandos-Soto *et al*., 2002). Also, resveratrol in doses of 5, 10, 20, and 40 mg/kg i.p. caused dose-dependent analgesia in the hot plate test and this effect was suggested to be mediated via an opioidergic mechanism (Gupta *et al*., 2004).

In conclusion, our results demonstrated that trans-resveratrol has spasmolytic, hypotensive, anti-inflammatory effects on rats and analgesic effects when tested in mice, and this compound could represent a source for the development of plant-based therapy useful in the control of arterial blood pressure, inflammation and pain. Further work is needed to elucidate the mechanism of action of this compound.

References

[1] Abdalla S, Abu Zarga M, Sabri S. 1994. Effects of flavone luteolin, isolated from *Colchicum ritchii*, on guinea pig smooth muscle and heart and on blood pressure and blood flow. Phytother. Res. 8(5): 265-270.

[2] Aburjai T, Hudaib M, Tayyem R, Yousef M, and Qishawi M. 2007. Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. J. Ethnopharmacol. 110(2), 294-304.

[3] Ageel AM, Mossa JS, Al-Yahya MA, Al-Said MS, and Tariq M. 1989. Experimental studies on antirheumatic crude drugs used in Saudi traditional medicine. Drugs. Exp. and Clin. Res. 15(8): 369-372.

[4] Amanlou M, Dadkhah F, Salehnia A, and Farsam H. 2005. An anti-inflammatory and anti-nociceptive effects of hydroalcoholic extract of *Satureja khuzistanica* Jamzad extract. J. Pharm. Pharm. Sci. 8(1): 102-106.

[5] Ayengar KNN, and Rangaswami S. 1967. Asperagenin, a rare type of steroidal sapogenin with 25-hydroxyl group. Curr. Sci. 36(24): 653-655.

[6] Bass TM, Weinkove D, Houthoofd K, Gems D, Partridge L. 2007. Effects of resveratrol on life span in *Drosophila melanogaster* and *Caenorhabditis elegans*. Mech. Ageing. Dev. 128(10): 546-552.

[7] Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Couteur DL, Shaw RJ, Navas P, Puigserver P, Ingram DK, Cabo RD, and Sinclair DA. 2006. Resveratrol improves health and of mice on a high-calorie diet. Nature. 444(7117): 337-342.

[8] Baur JA, and Sinclair DA. 2006. Therapeutic potential of resveratrol: in vivo evidence. Nat. Rev. Drug. Discov. 5(6): 493-506.

[9] Bilici D, Akpinar E, and Kiziltunc A. 2002. Protective effect of melatonin in carrageenan-induced acute local inflammation. Pharmacol. Res. 46(2): 133-139.

[10] Bruno S, De Laurentis N, Amico A, and Stefanizzi L. 1985. Flourescence spectra of some steroidal sapogenin fluorophors. Fitoterapia. 56(1): 39-41.

[11] Buluc M, and Demirel-Yilmaz E. 2006. Resveratrol decreases calcium sensitivity of vascular smooth muscle and enhances cytosolic calcium increase in endothelium. Vasc. Pharmacol. 44(4): 231-237.

[12] Campos-Toimil M, Elies J, and Orallo F. 2005. Trans- and cis- resveratrol increase cytoplasmic calcium levels in A7r5 vascular smooth muscle cells. Mol. Nutr. Food Res. 49(5): 396-404.

[13] Candelario-Jalil E, De Oliveira ACP, Gräf S, Bhatia HS, Hüll M, Muñoz E, and Fiebich BL. 2007. Resveratrol potently reduces prostaglandin E_2 production and free radical formation in lipopolysaccharide-activated primary rat microglia. J. Neuroinflammation. 4:25.

[14] Capasso R, Izzo AA, Romussi G, Capasso F, Tommasi ND, Bisio A, and Mascolo N. 2004. A secoisopimarane diterpenoid from *Salvia cinnabarina* inhibits rat urinary bladder contractility *in vitro*. Planta Med. 70(2): 185-188.

[15] Choi J, Lee KT, Choi MY, Nam JH, Jung HJ, Park SK, and Park HJ. 2005. Antinociceptive anti-inflammatory effect of

monotropein isolated from the root of *Morinda officinalis*. Biol. Pharm. Bull. 28(10): 1915-1918.

[16] Costa-Lotufo LV, De Lucena DF, Andrade-Neto M, Bezerra JNS, Leal LKAM, De Sousa FCF, and Viana GS. 2004. Analgesic, antiinflammatory and central depressor effects of the hydroalcoholic extract and fractions from *Aeolanthus suaveolens*. Biol. Pharm. Bull. 27(6): 821-824.

[17] Demo A, Petrakis C, Kefalas P, and Boskou D. 1998. Nutrient antioxidants in some herbs and mediterranean plant leaves. Food Res. Intl., 31(5), 351-354.

[18] Duwiejua M, Woode E, and Obiri D. 2002. Pseudo-akuammigine, an alkaloid from *Picralima nitida* seeds, has anti-inflammatory and analgesic actions in rats. J. Ethnopharmacol. 81(1): 73-79.

[19] El-Mowafy AM. 2002. Resveratrol activates membrane-bound guanylyl cyclase in coronary arterial smooth muscle: a novel signaling mechanism in support of coronary protection. Biochem. Biophys. Res. Comm. 291(5): 1218-1224.

[20] Fitzpatrick DF, Hirschfield SL, and Coffey RG. 1993. Endothelium-dependent vasorelaxing activity of wine and other grape products. Am. J. Physiol. 265(2): H774-H778.

[21] Fukunaga T, Miura T, Furuta K, and Kato A. 1997. Hypoglycemic effect of the rhizome of *Smilax glabra* in normal and diabetic mice. Biol. Pharm. Bull. 20(1): 44-46.

[22] Fulgenzi A, Bertelli AAE, Magni E, Ferrero E, and Ferrero ME. 2001. *In vivo* inhibition of TNF α-induced vascular permeability by resveratrol. Transplant. Proc. 33, 2341-2343.

[23] Gentilli M, Mazoit JX, Bouaziz H, Fletcher D, Casper RF, Benhamou D, and Savouret JF. 2001. Resveratrol decreases hyperalgesia induced by carrageenan in the rat hind paw. Life Sci. 68(11): 1317-1321.

[24] Gharib-Naseri MK, Asadi-Moghaddam M, and Bahadoram S. 2007. Antispasmodic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileum. DARU. 15(3): 123-128.

[25] Granados-Soto V, Argüelles CF, and Ortiz MI. 2002. The peripheral antinociceotive effect of resveratrol is associated with activation of potassium channels. Neuropharmacol. 43(5): 917-923.

[26] Gumanova N, Artyushkova E, Metel'skaya V, Kochkarov V, Pokrovskaya T, Danilenko L, Korneev M, Pokrovskii M, and Pashin E. 2007. Effect of antioxidants pQ510 and resveratrol on regulatory function of the endothelium in rats with modeled arterial hypertension. Bull. Exp. Biol. Med. 143(6): 619-622.

[27] Gupta YK, Sharma M, and Briyal S. 2004. Antinociceptive effect of trans-resveratrol in rats: involvement of an opioidergic mechanism. Methods Find. Exp. Clin. Pharmacol. 26(9): 667.

[28] Hammad HM, and Abdalla SS. 1997. Pharmacological effects of selected flavonoids on isolated ileum: structure-activity relationship. Gen. Pharmacol. 28(5): 767-771.

[29] Hedlund P. 2005. Nitric oxide/ cGMP-mediated effects in the outflow region of the lower urinary tract: is there a basis for pharmacological targeting of cGMP. World J. Urol. 23(6): 362-367.

[30] Howitz KT, Bitterman KJ, Cohen HY,et al. 2003. Small molecule activators of sirtuins extend *Saccharomyces cervisiae* lifespan. Nature. 425(6954): 191-196.

[31] Khattab MM, Al-Hrasen MN. 2006. Contractile activity ATP and diadenosine tetraphosphate on urinary bladder in the rats: role of superoxide anion and urothelium. Auton. Autacoid Pharmacol. 26(2): 149-156.

[32] Kim MJ, Lee HJ, Wiryowidagdo S, and Kim HK. 2006. Antihypertensive effects of *Gynura procumbens* extract in spontaneously hypertensive rats. J. Med. Food 9(4): 587-590.

[33] King RE, Bomser JA, and Min DB. 2006. Bioactivity of resveratrol. CRFSFS, 5(3): 65-70.

[34] Lastra CA, and Villegas I. 2005. Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications. Mol. Nutr. Food Res. 49(5): 405-430.

[35] Li H, Yao X, Zheng T, Li W, and Jia Z. 2006. Effects of resveratrol on arterial blood pressure and pressor response in ovariectomized rats. J. Lanzhou Univ. 42(6), 50-54.

[36] Liu F, Qu JB, Li H, and Yang SJ. 2006. The anti-inflammatory mechanisms of resveratrol. Zhongguo Zhongyao Zazhi. 41(15): 1138-1141.

[37] Liu Z, Song Y, Zhang X, Liu Z, Zhang W, Mao W, Wang W, Cui W, Zhang X, Jia X, Li N, Han C, and Liu C. 2005. Effects of *trans*-resveratrol on hypertension-induced cardiac hypertrophy using the partially nephrectomized rat model. Clin. Exp. Pharmacol. Physiol. 32(12): 1049-1054.

[38] Longo L, and Vasapollo G. 2006. Extraction and identification of anthocyanins from *Smilax aspera* L. berries. Food Chem. 94(2): 226-231.

[39] Martin AR, Villegas I, La Casa C, Alarcon DL, and Lastra C. 2004. Resveratrol, a polyphenol found in grapes, suppresses oxidative damage and stimulates apoptosis during early colonic inflammation in rats. Biochem. Pharmacol. 67(7): 1399-1410.

[40] Miatello R, Vázquez M, Renna N, Cruzado M, Zumino A, and Risler N. 2005. Chronic administration of resveratrol prevents biochemical cardiovascular changes in fructose-fed rats. Am. J. Hypertension. 18(6): 864-870.

[41] Minuz P. 2006. Antagonistic modulation of vascular tone by Rho-kinase and nitric oxide: new insights into the mechanisms of blood pressure control. J. Hypertension. 24(7): 1253-1255.

[42] Naderali EK, Doyle PJ, and Williams G. 2000. Resveratrol induces vasorelaxation of mesenteric and uterine arteries from female guinea-pigs. Clin. Sci. 98: 537-543.

[43] Neto A, Costa J, Belati C, Vinhólis A, Possebom L, Filho ADS, Cunha W, Carvalho J, Bastos J, and Silva M. (2005). Analgesic and anti-inflammatory activity of a crude root extract of *Pfaffia glomerata* (Spreng) Pedersen. J. Ethnopharmacol. 96(1-2): 87-91.

[44] Oliveira AC, Leal-Cardoso JH, Santos CF, Morais SM, and Coelho-de-Souza AN. 2001. Antinociceptive effects of the essential oil of *Croton zehntneri* in mice. Braz. J. Med. Biol. Res. 34(11): 1471-1474.

[45] Onwukaeme ND, Lot TY, and Udoh FV. 1999. Effects of *Daniellia oliveri* bark on isolated rat bladder. Phytother. Res. 13(5): 416-418.

[46] Pearson KJ, Baur, JA, Lewis, K et al. 2008. Resveratrol delays age- related deterioration and mimics transcriptional aspects of dietary restriction without extending lifespan. Cell Metab. 8(2): 157-168.

[47] Petricic J, and Radosevic A. 1969. Asperoside, a new bisdesmosine 22-hydroxyfurostanol saponin from *Smilax aspera*. Farmaceutski Glasnik 25(3): 91-95.

[48] Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA, Criddle DN. 2003. Antinociceptive properties of the essential

oil of *Ocimum gratissimum* L. (Labiatae) in mice. Braz. J. Med. Biol. Res. 36(4): 521-524.

[49] Richard N, Porath D, Radspieler A, and Schwager J. 2005. Effects of resveratrol, piceatannol, tri-acetoxystilbene, and genistein on the inflammatory response of human peripheral blood leukocytes. Mol. Nutr. Food Res. 49(5): 431-442.

[50] Ruan J, Zou J, and Cai Y. 2005. Anti-inflammatory components of *Smilax china.* Yivao Daobao. 24(8): 670-672.

[51] Sayyah M, Peirovi A, and Kamalinejad M. 2002. Anti-nociceptive effect of the fruit essential oil of *Cuminum cyminum L.* in rat. Iran. Biomed. J. 6(4): 141-145.

[52] Selenu MB, Carrus F, and Bonsignore L. 2005. Ethnopharmacobotany of east-central Sardinian: chemical and biological study on *Smilax aspera*. Boll. Chim. Farm. 144(1): 79-92.

[53] Sharma S, Kulkarni S, and Chopra K. 2007a. Effect of resveratrol, a polyphenolic phytoalexin, on thermal hyperalgesia in a mouse model of diabetic neuropathic pain. Fundam. Clin. Pharmacol. 21(1): 89-94.

[54] Sharma S, Chopra K, and Kulkarni SK. 2007b. Effect of insulin and its combination with resveratrol or curcumin in attenuation of diabetic neuropathic pain: participation of nitric oxide and TNF-alpha. Phytother. Res. 21(3): 278-283.

[55] Shu XS, Gao ZH, and Yang XL. 2006. Anti-inflammatory and anti-nociceptive activities of *Smilax china* L. aqueous extract. J. Ethnopharmacol. 103(3): 327-332.

[56] Slater SJ, Seiz JL, Cook AC, Stagliano BA, and Buzas CJ. 2003. Inhibition of protein kinase C by resveratrol. Biochim. Biophys. Acta.1637(1): 59-69.

[57] Song LH, Pan W, Yu YH, Quarles LD, Zhou HH, and Xiao ZS. 2006. Resveratrol prevents CsA inhibition of proliferation and osteoblastic differentiation of mouse bone marrow-derived mesenchymal stem cells through an ER/NO/cGMP pathway. Toxicol. In Vitro 20: 915–922.

[58] Tang HY, Shih A, Cao HJ, Davis FB, Davis PJ, and Lin HY. 2006. Research articles: therapeutics: resveratrol-induced cyclooxygenase-2 facilitates p53-dependent apoptosis in human breast cancer cells. Mol. Cancer Ther. 5(8): 2034-2042.

[59] Torres-López JE, Ortiz MI, Castañeda-Hernădez G, Alonso-López R, Asomoza-Espinosa R, and Granados-Soto V. (2002). Comparison of the antinociceptive effect of celecoxib, diclofenac and resveratrol in the formalin test. Life Sci. 70(14): 1669-1676.

[60] Tsai SH, Lin-Shiau SY, and Lin JK. 1999. Suppression of nitric oxide synthase and down-regulation of the activation of NFκB in macrophages by resveratrol. Br. J. Pharmacol. 126(3): 673-680.

[61] Tschesche R, Harz A, and Petricic J. 1974. Steroid saponins with more than one sugar chain. VIII. asperoside, a bisdesmosidic 22-hydroxyfurostanol glycoside of *Smilax aspera*. Chem. Ber. 107(1): 53-61.

[62] Valenzeno DR, Terzibasi E, Genade T, Cattaneo A, Domenici L, Cellerino A. 2006. Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr. Biol. 16(3): 296-300.

[63] Vasudevan M, Gunnam KK, and Parle M. 2006. Antinociceptive and anti-inflammatory properties of *Daucus carota* seeds extract. J. Health Sci. 52(5), 598-606.

[64] Vermani K, and Garg S. 2002. Herbal medicines for sexually transmitted diseases and AIDs. J. Ethnopharmacol. 80(1): 49-66.

[65] Vitrac X, Bornet A, Vanderlinde R, Valls J, Richard T, Delaunay JC, Merillon JM, and Teissedre PL. 2005. Determination of stilbenes (δ-viniferin, trans-astringin, trans-piceid, cis- and trans-resveratrol, ε-viniferin) in Brazilian wines. J. Agric. Food Chem. 53(14): 5664-5669.

[66] Wang ZV, and Scherer PE. 2008. Adiponectin, cardiovascular function, and hypertension. Hypertension, 51(12): 8-14.

[67] Weil V, Cirigliano M, and Battistini M. 2000. Herbal treatments for symptoms of menopause. Clin. Pers. Complem. Med. 35-44.

[68] Wood JG, Rogina B, Lavul S, Helfand SL, Tatar M, Sinclair D. 2004. Sirtuin activators mimic calorie restriction and delay aging in metazoans. Nature, 430(7000): 686-689.

[69] Wu Y, Zhou C, Li X, Song L, Wu X, Lin W, Chen H, Bai H, Zhao J, Zhang R, Sun H, and Zhao Y. 2006. Evaluation of antiinflammatory activity of the total flavonoids of *Laggera pterodonta* on acute and chronic inflammation models. Phytother. Res. 20(7): 585-590.

[70] Xu J, Xian L, Zhang P, Li ZL, and Wang Y.2005. Antiinflammatory constituents from the roots of *Smilax bockii* warb. Arch. Pharm. Res. 28(4): 395-399.

[71] Zakaria ZA, Sulaiman MR, Jais AMM, Somchit MN, Jayaraman KV, Balakhrisnan G, and Abdulla FC. 2006). The antinociceptive activity of *Muntingia calabura* aqueous extract and the involvement of L-arginine/nitric oxide/cyclic guanosine monophosphate pathway in its observed activity in mice. Fund. Clin. Pharmacol. 20(4): 365-372.

[72] Zhang CL, Wang DM, Zhu W, Zhang HI. 2006. Study on chemical constituents from stem of *Smilax scobinicaulis*. Zhongguo Zhongyao Zazhi. 41(20): 1540-1542.