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ORIGINAL RESEARCH ARTICLE

Sinapic Acid Attenuates High Blood Pressure, Oxidative Stress and Hyperlipidemia in L-NAME Induced Hypertensive Rats

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ABSTRACT

The present study was designed to evaluate the antihypertensive and antihyperlipidemic effect of sinapic acid (SA), a phenolic acid against N^{ω}-nitro-L arginine methyl ester hydrochloride (L-NAME) induced hypertension in male Wistar rats. Hypertension was induced by oral administration of L-NAME (40mg/kg) in drinking water for 4 weeks. Rats were treated with SA (10, 20 and 40 mg/kg body weight (bw)) for four weeks. L-NAME treated rats showed significant increase in diastolic blood pressure (DBP). A significant increase in the levels of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH), total cholesterol (TC), triglycerides (TC), and significant decrease in the levels of non-enzymatic antioxidants such as reduced glutathione (GSH), vitamin C and vitamin E in plasma were noticed in L-NAME induced hypertensive rats. Treatment with SA (10, 20 and 40 mg/kg bw) brings back all the above parameters to near normal level, in which 40 mg/kg showed the highest effect than that of other two doses. These results suggest that SA acts as an antihypertensive and antihyperlipidemic agent against L-NAME induced hypertension.

Key words: Hypertension, Hyperlipidemia, Antioxidant, Sinapic acid.

1. NTRODUCTION

Cardiovascular diseases (CVD) remain the leading cause of mortality worldwide [1]. The World Health Organisation (WHO) estimated that approximately 17.1 million people died from CVD and its complications in 2004, and the number of deaths will dramatically increase to almost 23.6 million by 2030 [2]. In humans, hypertension and hyperlipidemia are frequent causes of CVD and major risk factors for atherosclerosis; the presence of both conditions accelerates atherosclerosis [3]. Recent evidence indicates that oxidative stress as the main mechanism is responsible for cardiovascular complications such as hypertension and alteration in lipid metabolism [4,5].

Oxidative stress constitutes a unifying mechanism of injury in many types of vascular diseases. Reactive oxygen species plays an important role in several responses involved in vascular remodeling, including proliferation, migration, and hypertrophy [6,7]. It occurs when there is a serious imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense systems in the body [8]. The generated ROS induce lipid peroxidation, a type of oxidative deterioration in polyunsaturated fatty acids (PUFAs), which has been linked with altered membrane structure and enzyme inactivation [9]. Nitric oxide (NO) is a vital regulator of vascular endothelial function and blood pressure [10]. Chronic NO inhibition with L-NAME can increase regional vascular resistance, raise the blood pressure, oxidative stress, and renal damage in both *in vitro* and *in vivo* models [11].

Many modern drugs are effective in preventing CVD, but their use is often limited because of their adverse effects. Therefore, the screening and development of drugs for CVD are still in progress. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them [12]. Scientific interest in phenolic compounds in plants has been stimulated due to their anti-inflammatory,

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antimutagenic, and anticarcinogenic properties [13]. Sinapic acid is a cinnamic acid derivative which possesses 3,5-Dimethoxy-4-hydroxycinnamic acid is one of the phenolic acids widely distributed in edible plants such as cereals, nuts, oil seeds and berries [14]. Sinapic acid has already been pharmacologically evaluated for its potent antioxidant [15,16], antihyperglycemic [17], anti-inflammatory [18], peroxynitrite scavenging effects [19]. Extensive literature survey has shown that there are no scientific reports available on the effect of sinapic acid in L-NAME induced hypertensive rats. Hence in the present investigation, we are going to study the antihypertensive and antihyperlipidemic effect of sinapic acid in L-NAME induced hypertensive rats.

2. MATERIALS AND METHODS

2.1. Animals

Male albino Wistar rats, 8-10 weeks old weighing 180-220 g were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and they were housed (3 rats/cage) in polypropylene cages (47 × 34 × 20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22 °C. Food and water were provided ad libitum to all the animals. The rats were fed on a standard pellet diet (Kamadhenu Agencies, Bangalore, India). The whole experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA, approval number: 926) Annamalai University, Annamalainagar.

2.2. Chemicals

N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) and sinapic acid were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used in this study were of analytical grade obtained from Merck and Himedia, India.

2.3. Model of L-NAME-induced hypertension and experimental protocol

Animals were given L-NAME in drinking water at a dosage of 40 mg/kg for 4 weeks [20]. Each of the following groups consisted of six animals. SA was dissolved in corn oil and administered orally everyday using an intragastric tube throughout the experimental period.

Group I - Control
Group II - Control+SA (40 mg/kg bw)
Group III - Control animals received L-NAME - hypertensive control
Group IV - L-NAME+SA (10 mg/kg bw)
Group V - L-NAME+SA (20 mg/kg bw)
Group VI - L-NAME+SA (40 mg/kg bw)

The experimental duration was 30 days. During the experimental period, body weight gain was measured everyday. On 31st day, the rats were sacrificed by cervical dislocation. Blood was collected through orbital sinus in a heparinized tube and centrifuged at 1000 × *g* for 10 min and the plasma was separated by aspiration.

2.4. Blood pressure measurement

Before commencement of the experiment, animals were trained with instrument for measuring blood pressure. In all groups of animals, diastolic blood pressure (DBP) was measured every week during the entire period of the study noninvasively using a tail cuff method (IITC, model 31, USA) according to standard procedures. Values reported are the average of three sequential blood pressure measurements.

2.5. Estimation of lipid peroxidation products

The levels of thiobarbituric acid reactive substances (TBARS) in plasma was estimated by the method of Niehaus and Samuelson [21]. A total of 0.5 mL of plasma was diluted with 0.5 mL of double distilled water and mixed well, and then 2.0 mL of thiobarbituric acid (TBA)–trichloroacetic acid (TCA) - hydrochloric acid (HCL) reagent was added. The mixture was kept in boiling water bath for 15 min. After cooling, the tubes were centrifuged for 10 min and the supernatant was taken for measurement. The absorbance was read at 535 nm against reagent blank.

Estimation of plasma lipid hydroperoxides (LOOH) was done by the method of Jiang et al. [22]. Fox reagent (0.9 mL) was mixed with 0.1 mL of plasma and incubated for 30 min at room temperature. The color developed was read at 560 nm.

2.6. Determination of non-enzymatic antioxidants

Reduced glutathione (GSH) in the plasma was estimated by the method of Ellman [23]. 0.5 mL of plasma was pipetted out and precipitated with 2.0 mL of 5% TCA. A total of 2.0 mL of supernatant was taken after centrifugation and 1.0 mL of Ellman's reagent and 4.0 mL of 0.3 M disodium

hydrogen phosphate were added. The yellow color developed was read at 412 nm.

Vitamin C in the plasma was estimated by the method of Roe and Kuether [24]. To 0.5 mL of plasma, 1.5 mL of 6% TCA was added and allowed to stand for 5 min and centrifuged. To the supernatant, 0.3 g of acid washed Norit was added, shaken vigorously and filtered. A total of 0.5 mL of the filtrate was taken and 0.5 mL of dinitrophenylhydrazine (DNPH) was added, stoppered and placed in water bath at 37 °C for exactly 3 h, removed, placed in ice-cold water and added 2.5 mL of 85% sulphuric acid. The contents of the tubes were mixed well and allowed to stand at room temperature for 30 min. The color developed was read at 540 nm.

The level of Vitamin E in the plasma was estimated by the method of Baker et al. [25]. To 0.5 mL of plasma, 1.5 mL of ethanol was added, mixed and centrifuged. The supernatant was evaporated at 80 °C and to the precipitate, 3.0 mL of petroleum ether, 0.2 mL of 2,2′-dipyridyl solution and 0.2 mL of ferric chloride were added. Afterwards, all the tubes were mixed well and kept in dark for 5 min and 4.0 mL of n-butanol was added. The red color developed was read at 520 nm.

2.7. Extraction of lipids

Total lipids were extracted from plasma according to the method of Folch et al. [26] using chloroform: methanol mixture (2:1, v/v). Plasma was mixed with cold chloroform–methanol (2:1, v/v) and the contents were extracted after 24 hours. The extraction was repeated four times. The combined filtrate was washed with 0.7% of potassium chloride (0.1 N) and the aqueous layer was discarded. The organic layer was made up to a known volume with chloroform and used for the analysis of lipids.

2.8. Estimation of total cholesterol and triglycerides

The levels of total cholesterol (TC) was estimated by the method of Zlatkis et al. [27]. Lipid extract of 0.5 mL was evaporated to dryness. To this, 5.0 mL of ferric chloride-acetic acid reagent was added. The tubes were mixed well and 3.0 mL of concentrated sulphuric acid (H_2SO_4) was added. A series of standards containing cholesterol in the range 3–15µg were made up to 5.0 mL with the reagent and a blank containing 5.0 mL of the reagent was prepared. The absorbance was read after 20 minutes at 560 nm.

The content of triglycerides (TG) was estimated by the method of Fossati and Lorenzo [28]. Lipid extract of 0.5 mL was evaporated to dryness. To this, 0.1 mL of methanol was added followed by 4.0 mL of isopropanol. About 0.4 g of alumina was added to all the tubes and shaken well for 15 minutes. It was centrifuged and then accurately 2.0 mL of the supernatant was transferred to appropriately labeled tubes. The tubes were placed in a water bath at 65 °C for 60 minutes for saponification after adding 0.6 mL of the saponification reagent followed by 0.1 mL of sodium metaperiodate and 0.5 mL of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65 °C for an hour. A series of standards of concentration 8-40 µg triolein were treated similarly along with a blank containing only the reagents. All the tubes were cooled and read at 405 nm.

2.9. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using statistical package for the social science (SPSS) software version 14.0. Results were expressed as mean ± S.D. for six rats in each group. Values were considered significant when $P<0.05$.

3. RESULTS

3.1. Blood pressure measurements

(Fig 1) shows the effect of sinapic acid (SA) at three different doses (10, 20 and 40 mg/kg) on diastolic blood pressure in L-NAME induced hypertensive rats. The L-NAME rats showed significantly increased diastolic blood pressure while treatment with SA significantly reduced the diastolic blood pressure.

3.2. Body weight

(Fig 2) shows the effect of SA at three different doses (10, 20 and 40 mg/kg) on body weight in L-NAME induced hypertensive rats. The L-NAME rats showed significantly decreased body weight while treatment with SA significantly elevated the body weight. The 40 mg/kg dose showed better effect in reducing diastolic blood pressure and enhancing body weight than other two doses (10 and 20 mg/kg), so we have chosen 40 mg/kg dosage for further evaluation.

3.3. Lipid peroxidation products

(Table 1) shows the effect of SA on the levels of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide (LOOH) in the plasma of L-NAME induced hypertensive rats. The L-

NAME rats exhibited a significant increase in the levels of TBARS and LOOH. The administration of SA reduced the levels of TBARS and LOOH significantly.

3.4. Non-enzymatic antioxidants

Table 1 shows the effect of SA on reduced glutathione, vitamin C and E levels in the plasma of control and L-NAME induced hypertensive rats. The levels of reduced glutathione, vitamin-C and E decreased significantly in L-NAME rats, and administration of SA significantly increased these non-enzymatic antioxidants.

3.5. Plasma lipid level

Since the hypertension is associated with abnormalities in circulatory lipids, we measured the concentration of plasma lipids in hypertensive rats before and after treatment with SA **(Fig 3)**. The concentrations of plasma lipids (TC and TG) were increased in hypertensive rats as compared to the control rats. Treatment with SA significantly reduced the concentrations of plasma lipids (TC and TG respectively).

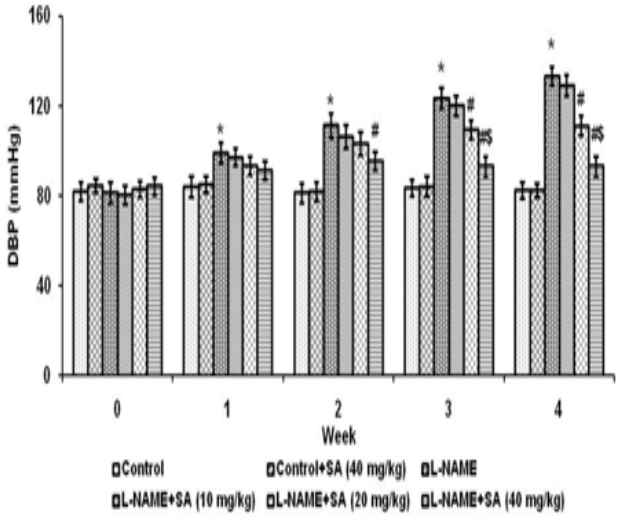

Fig 1: Effect of sinapic acid on diastolic blood pressure in control and L-NAME induced hypertensive rats

Values are mean ± S.D. for six rats in each group. * differs significantly at $P<0.05$ compared with control. # differs significantly at $P<0.05$ compared with L-NAME rats. $ differs significantly at $P<0.05$ compared with 20 mg/kg sinapic acid treated rats (DMRT).

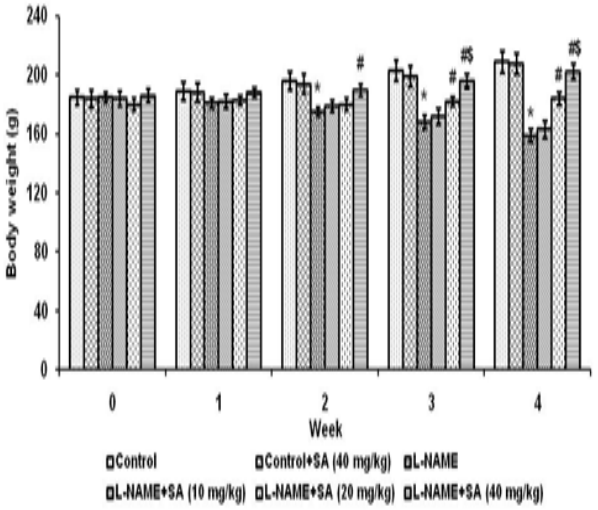

Fig 2: Effect of sinapic acid on body weight in control and L-NAME induced hypertensive rats

Values are mean ± S.D. for six rats in each group. * differs significantly at $P<0.05$ compared with control. # differs significantly at $P<0.05$ compared with L-NAME rats. $ differs significantly at $P<0.05$ compared with 20 mg/kg sinapic acid treated rats (DMRT)

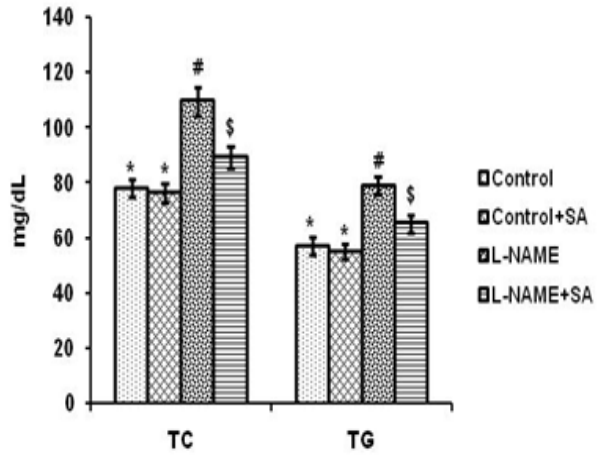

Fig 3: Effect of sinapic acid on total cholesterol and triglycerides in plasma of control and L-NAME induced hypertensive rats

Values are mean ± S.D. for six rats in each group. Values not sharing a common symbol differ significantly at $P<0.05$ (DMRT)

Table 1: Effect of sinapic acid on TBARS, LOOH, GSH, vitamin C, vitamin E, in plasma of control and L-NAME induced hypertensive rats

Parameters		Control	Control+SA	L-NAME	L-NAME+SA
TBARS	Plasma	0.15±0.01*	0.14±0.01*	0.42 ± 0.03$^{\#}$	0.23 ± 0.02^{s}
LOOH	(mmol/dL)	9.3± 0.57*	9.52±0.61*	21.21 ± 1.45$^{\#}$	13.3 ± 0.57^{s}
GSH	Plasma	34.1±1.6*	35.7± 1.81*	20.2 ± 1.83$^{\#}$	30.12 ± 1.68^{s}
Vitamin-C	(mmol/dL)	2.32±0.15*	2.45 ± 0.12	0.93 ± 0.06$^{\#}$	2.04 ± 0.11^{s}
Vitamin-E		2.14±0.14*	2.39 ± 0.1*	0.92 ± 0.07$^{\#}$	

Values are mean ± S.D. for six rats in each group. Values not sharing a common symbol differ significantly at $P<0.05$ (DMRT).

4. DISCUSSION

Nitric oxide (NO) synthesis and release by endothelial cells contribute to the modulation of vascular tone [29]. In addition, NO is important in other cellular events, such as vascular smooth muscle cell proliferation [30]. Besides, it is well established that chronic inhibition of NO

biosynthesis by *in vivo* administration of L-NAME, an L-arginine analog, leads to arterial hypertension and renal vasoconstriction [31]. Studies reported that antioxidants such as vitamins and superoxide dismutase normalize the endothelial dysfunction and improve vascular remodeling in experimental hypertension [32]. Sinapic acid has already been pharmacologically evaluated for its potent antioxidant efficacy [15,16]. In the present study, there is a significant increase in diastolic blood pressure of L-NAME induced hypertensive rats. Our finding shows that oral administration of sinapic acid (SA) resulted in a significant reduction in diastolic blood pressure. L-NAME rats showed significantly decreased body weight. After treatment with SA, the weight loss improved which might be as a result of its ability to reduce the loss or degradation of structural proteins [33].

Oxidative stress, characterized by increased bioavailability of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and lipid peroxides, plays an important role in the development and progression of cardiovascular dysfunction associated with hypertensive disease [34]. Lipid peroxidation is an important pathogenic event in hypertension and accumulation of LOOH reflects the various stages of this disease and its complications [35]. Our results showed that the lipid peroxidation end products, measured as TBARS and LOOH were increased in plasma of L-NAME-induced hypertensive rats. Thus, the antihypertensive effect of sinapic acid may be due to decreased oxidative stress.

The loss of the balance between oxidation and antioxidation may lead to promote the generation of $OH^{\cdot}$ which is a powerful oxidant for many compounds. Intracellular defense against active oxygen species is performed by antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidants such as reduced glutathione, vitamin C and vitamin E [36]. The non-enzymatic antioxidants scavenge the residual free radicals escaping from decomposition enzymes [37]. The major antioxidant of the aqueous phase is vitamin C, which acts as the first line of defense during oxidative stress. Vitamin E appears to be the most effective lipid soluble antioxidant in the biological system [38]. GSH, a tripeptide, is a powerful cellular antioxidant, which is directly involved in the removal of superoxide radicals, peroxyl radicals and singlet oxygen [39]. The lowered concentrations of vitamin C, vitamin E, and GSH observed in L-NAME-induced hypertensive rats might be due to neutralizing the production of free radicals. Treatment with SA enhanced the levels of non-enzymatic antioxidants in L-NAME-treated rats.

The presence of high blood pressure (BP) and hyperlipidemia is so common in hypertension that many have argued that the high BP itself may play a role in altering lipid metabolism, resulting in abnormalities [40]. High levels of circulating cholesterol and its accumulation in tissues are well associated with cardiovascular damage [41]. In our study, we observed increased levels of TC in plasma of hypertensive rats. SA supplementation decreased the levels of TC in hypertensive rats. Accumulation of TGs is one of the risk factors of CVD. The mechanism of observed increase in TGs after hypertension may be due to elevated flux of fatty acids and impaired removal of VLDL from the plasma. Treatment with SA decreased the levels of TGs in hypertensive rats.

Major findings from the present study demonstrated that SA at a dose of 40 mg/kg exhibited a greater antihypertensive and antihyperlipidemic effect than the other two doses (10 mg and 20 mg/kg) as evidenced by a considerable decrease in the blood pressure, oxidative stress and lipid levels in L-NAME induced hypertensive rats.

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