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Research Article

Antihypertensive and Cardio Protective Effects of the *Convolvulus arvensis* root in N^G-nitro-L-arginine methyl ester (L-NAME) Induced Hypertension in Swiss Albino Mice

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Abstract

Objective: To assess antihypertensive and cardio protective effects of the *Convolvulus arvensis* root powder in N^G-nitro-L-arginine methyl ester (L-NAME) induce hypertension in Female Swiss albino mice.

Methodology: Female Swiss albino mice were divided into four categories. Control, 2% gum acacia L-NAME (40 mg/kg orally), CA (500 mg/kg orally) + L-NAME (40 mg/kg orally), L-arginine (100 mg/kg orally) + L-NAME (40 mg/kg orally) were given for 1 month. On 29th day serum marker enzymes, cholesterol and hemodynamic parameters (systolic and diastolic BP) were measured and cardiac histology was performed. CA root powder was characterized by HPLC.

Result: Systolic blood pressure level was raised by L-NAME ($p<0.001$). In both of the drugs treated groups, systolic and diastolic blood pressure level reduced significantly ($p<0.001$) compared to L-NAME group. In the L-NAME group significantly ($p<0.01$) elevated cholesterol was reduced ($p<0.05$) by CA root powder treatment. In the L-NAME group, inflammation and necrosis (0-35%) were present in the heart, whereas there was no change in the myocardium of CA and L-arginine treated mice. Vitexin, Orientin and Isoorientin were identified in the Methanolic extract of CA powder.

Conclusion: The cardio-protective activity in CA and L-arginine treated mice, was evaluated by presence of the inflammation in the heart, absence of necrosis and remarkable depletion in Serum cholesterol parameters. Inhibition of oxidation activity by Orientin and Isoorientin appears to lessen the L-NAME induced damage. It is concluded that CA root extract possess antihypertensive and cardio protective activity.

Keywords: N^G-nitro-L-arginine methyl ester (L-NAME); *Convolvulus arvensis*; Isoorientin

Introduction

Cardiovascular disease (CVD) is the most significant foundation of mortality in Pakistan by the year 2015. It is meaningful to indicate that ischemic heart disease (IHD) remains to be the main cause of CVD. This disease is persisting as the main and the most common danger to human life. Hypertension is a major risk factor of IHD. NGnitro-L-arginine methyl ester (L-NAME) has been used to induce High Blood pressure in rats [1,2]. Therapeutic effect of many plant extracts and herbal formulation such as *Solanum torvum* Sw. (Solanaceae) fruits [3], *Crataegus tanacetifolia* (Lam.) Pers. (Rosaceae) leaf (Koçyildiz., *et al*. 2006), *Solanum anguivi* Lam. (Solanaceae) [4], *Fritillaria cirrhosa* D. Don (Liliaceae) [5], bark of *Mammea africana* Sabine (Guttiferae) [6] and herbal formulation Toki-shakuyaku-san [7] in reducing L-NAME induced hypertension have been previously reported.

In the traditional system *Convolvulus arvensis* root is used as a cardio tonic agent, in urinary infection, as an antihelmintic, hepato protective, diuretic, anti-hyperlipidemia, analgesic and anti-inflammatory [8,9]. Previous studies in the laboratory showed cardio-protective and antihypertensive effect of *Convolvulus arvensis* root powder in isoprenalin-induced cardiotoxicity and dexamethasone induced hypertension rats, respectively [10].

However, the cardio protective and antihypertensive effect of *Convolvulus arvensis* root in L-NAME induced hypertension model in albino mice have not been reported yet. Using chronic administration of L-NAME *in vivo*, we designed the present study with the following objectives. The first objective was to study the antihypertensive and cardio protective effect of *Convolvulus arvensis* root powder in L-NAME induced hypertensive mice. The second objective was to characterize the *Convolvulus arvensis* methanolic root extract by HPLC.

Materials and Methods

Preparation of samples

The roots of *Convolvulus arvensis* were collected in the month of April from the fields of Punjab, Pakistan and the plant was identified and authenticated Dr. Mubashar Niaz, Department of Botany, GC University Faisalabad Pakistan. A voucher specimen with no. 010410 was kept in the Herbarium of the College of Pharmacy, GC University, Faisalabad, Pakistan [9].

CA roots were treated at the manufacturing facilities of GC University Faisalabad (Punjab Pakistan). Whole roots were sliced into small flakes and dried in tray dryer at the temperature of 40°C. The withered flakes were pulverized to obtain the powder which was labeled as CA root powder.

Chemicals

N^{G} -Nitro-l-arginine methyl ester (L-NAME) manufactured by Sigma Aldrich, USA was procured for the induction of hypertension. L-arginine was procured from Central Drug House (P) Ltd., Islamabad, Pakistan [10].

Solution preparation

Weighed quantity of CA root powder (500 mg/kg, orally, CA suspended in 2% gum acacia), L-arginine (100 mg/kg) and L-NAME (40 mg/kg) were administered by oral route dissolved in distilled water using oral feeding gavages.

Experimental animals

Female Swiss albino mice weighing between 20 and 24g used in the study were obtained from National Institute of Biological Health, Faisalabad and kept in the animal house of the Department of Pharmacology. The animals were housed in an ambient temperature 25 ± 2°C and relative humidity 50 ± 2% and light and dark cycle (12 h light/dark). The animals have free access to pellet diet and water *ad libitum* [10].

Oral acute toxicity

The oral acute toxicity examination was accomplished by using OECD-423 guidelines. Female Swiss albino mice weighing 20-24 g were divided into two groups, each consisting of three animals. In the first group, CA root powder was administered orally was provided at the dose of 2,000 mg/kg, while the second group received 5,000 mg/kg. The animals were observed for toxic symptoms and mortality for 72 h after CA root extract administration [2].

Methodology

A total number of 24 animals were randomly distributed into four groups, each group comprising of six animals. Once a week, the mice's blood pressure was measured by tail-cuff (AD Instrument, Australia). Hypertension was induced in mice of Groups II, III and IV by the administration of L-NAME (40 mg/kg) p.o. [2,3];

- **Group I:** Control group - 2% gum acacia in distilled water administered as a vehicle for 4 weeks
- **Group II:** L-NAME group - L-NAME administered orally 40 mg/kg/day for 4 weeks
- **Group III:** L-arginine (100 mg/kg/day orally for 4 weeks) + L-NAME (40 mg/kg/day orally for 4 weeks)
- **Group IV:** (500 mg/kg, CA suspended in 2% gum acacia, orally for 4 weeks) + L-NAME (40 mg/kg/day orally for 4 weeks).

Serum parameters

On the day 29th the mice were anesthetized with anesthetic ether and blood was withdrawn from the retro orbital plexus of each mice. Serum was separated and lactate dehydrogenase (LDH), creatinine phosphokinase MB iso-enzyme (CK-MB), aspartate transaminase (AST) and alanine transaminase (ALT) were measured by using standard kits (Merck Specialties' Pvt. Ltd., Pakistan). The serum total cholesterol (CHOL), triglycerides (TG) was quantified using enzymatic kits (Accurex Biomedical limited Pvt. Ltd, Pakistan) as per manufacturer's instruction manual.

Hemodynamic parameters

The animals were anaesthetized with urethane (1.25 g/kg) at the end of experiment on the 29th day. The right carotid artery of each mice was cannulated for the measurement of heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MABP). The cannula was filled with heparinized saline and connected to a pressure transducer. After 30 min of stabilization the hemodynamic parameters were recorded

by eight channel Power labs recorder (AD Instruments, Australia) having LABCHART-6 pro Software. After recording hemodynamic parameters the animals were euthanized and heart from each animal was removed and placed in 10% formalin solution [2].

Histology

The organ specimens were subjected to dehydration for one hour with xylene and strength of alcohol of 70, 90 and 100%, for two hours respectively. Every time for one hour, the infiltration and impregnation was conceded by using paraffin wax twice, just to prepare paraffin L-molds. 3-5 mm thickness of the specimens was sliced and stained with hematoxylin and eosin (H & E). The diestrene phthalate xylene (D.P.X.) were used to mount these slices. The parameters of histopathology valuation of cardiology sections included inflammation, necrosis and congestion. The grading system used for evaluation of the parameter was [––, absence of alteration; +, 0-35% area shows alteration; ++, 35-70% area shows alteration; +++, 70-100 area shows alteration].

Characterization of CA methanol extract of root powder

The CA root powder was extracted with methanol (cold) and the filtrate was concentrated and dried below 40°C to get a paste of yield 33%. The obtained viscous material was dissolved in methanol and subjected to HPLC analysis under the following conditions:

- **Column:** Kromasil C-18 (5 micron RP) 250 mm length × 4.6 mm diameter
- **Detector:** UV 210 nm
- **Mobile phase:** Water: methanol starting at 75/25 and after 20 min 65/35 under linear gradient of 1 mL/min flow rate.
- **Instrument:** HPLC, JASCO - model binary-2000 series comprising of UV-2075 plus, PU-2080 plus, AS-2055 plus.

Reference standards (vitexin, orientin, isoorientin) from Chromadex were used and the flavonol C-glycosides were identified and estimated in the paste. The quantity present in the dried CA root powder was derived [2].

Statistical analysis

Data were expressed as the mean ± SEM. Statistical analysis was carried out by one-way ANOVA followed by post hoc Bonferroni test using Graph Pad Prism-5.

Result and Discussion

Acute toxicity

The oral acute toxicity test showed that the CA root powder at 2,000 and 5,000 mg/kg body weight were nontoxic to the mice.

Biochemical parameters

In L-NAME alone group there was a rise in serum level of CK-MB and LDH levels on the 29th day, whereas concomitant treatment with CA + L-NAME for 28 days showed reduction in serum CK-MB and LDH levels compared to control group. Concomitantly administration of L-arginine + L-NAME for 28 days showed reduced CK-MB and LDH that were elevated after L-NAME alone treatment.

L-NAME alone increased AST and ALT, whereas in CA + L-NAME treated animals, AST and ALT were not increased after 28 days. Similar concomitant treatment with L-arginine + L-NAME was also effective in reducing AST and ALT. In L-NAME alone group, there was a significant increase in serum cholesterol, but TG increased was not significant when compared to control group. Whereas concomitant treatment with CA + L-NAME for 28 days showed significant reduction in cholesterol and non-significant decrease in TG levels when compared to L-NAME alone (Table 1).

	CK-MB (IU/L)	LDH (U/L)	AST (U/L)	ALT (U/L)	CHOL (mg/dL)	TG (mg/dL)
Control	477.3 ± 74.73	1477 ± 269.5	197.2 ± 8.792	107.0 ± 14.76	41.27 ± 7.092	41.07 ± 1.950
L-NAME (40 mg/kg)	707.3 ± 118.2	1957 ± 162.1	247.0 ± 12.89	141.7 ± 11.46	130.7 ± 18.57**	57.33 ± 5.797
CA root extract (500 mg/kg, p.o.) + L-NAME (40mg/kg)	642.7 ± 45.99	1,907 ± 115.7	194.7 ± 2.907	87.67 ± 4.287	69.17 ± 8.403*	41.07 ± 3.274
L-arginine (100 mg/kg, p.o.) + L-NAME (40mg/kg)	571.2 ± 110.4	1,557 ± 208.7	211.0 ± 21.70	141.3 ± 12.71	87.41 ± 18.37	49.12 ± 3.671

Table 1: Effect of CA on serum biochemical parameters in mice after L-NAME induced hypertension.

Values are expressed as mean ± SEM. One-way ANOVA followed *post hoc* Bonferroni tests.

CK-MB: Creatinine Phosphokinase-MB Isoenzyme; LDH: Lactate Dehydrogenase; AST: Aspartate Transaminase; ALT: Alanine Transaminase; CHOL: Total Cholesterol; TG: Triglycerides; CA: *Convolvulus arvensis*; L-NAME: *N*^{G}-nitro-l-arginine methyl ester.

*p < 0.05 compared to L-NAME (40 mg/kg).

**p < 0.01 considered as significant comparison between control and L-NAME (40mg/kg).

Noninvasive blood pressure (Tail-cuff method)

L-NAME alone produced fundamental ($p < 0.001$) rise in the systolic BP (mmHg) compared to the control group on 2nd, 3rd and 4th week. In CA + L-NAME group the systolic BP significantly decreased compared to L-NAME alone on 3rd and 4th week.

The result indicated that CA pretreatment for 28 days is required to cause a reduction of L-NAME induced hypertension. The decrease was significant $p < 0.05$ after 3 weeks and $p < 0.001$ after 4 weeks treatment. These results are similar to that of L-arginine + L-NAME treated mice in which significant reduction of systolic blood pressure was observed compared to L-NAME alone (Table 2).

Groups	Systolic blood pressure mmHg			
	Week 1	Week 2	Week 3	Week 4
Control	117.3 ± 1.38	118.2 ± 1.34	118.2 ± 1.34	118.2 ± 1.34
L-NAME (40 mg/kg)	118.3 ± 1.044	134.5 ± 2.64***	144 ± 2.69***	163.7 ± 2.23***
CA root extract (500 mg/kg, p.o.) + L-NAME (40 mg/kg)	118.5 ± 1.87	127.2 ± 4.20	134 ± 4.78#	130.3 ± 1.39##
L-arginine (100 mg/kg, p.o.) + L-NAME (40 mg/kg)	119.2 ± 1.43	134.3 ± 5.77	131 ± 2.19##	122.7 ± 1.46##

Table 2: Effect of L-NAME on non-invasive blood pressure (Tail-cuff method) during 28 days treatment of CA (500 mg/kg).

Values are displayed as mean ± SEM. One-way ANOVA followed post hoc Bonferroni tests.

CA: *Convolvulus arvensis*; L-NAME: *N*^{G}-nitro-l-arginine methyl ester.

***p < 0.001 considered as significant comparison between control and L-NAME (40 mg/kg).

#p < 0.05 compared to L-NAME (40 mg/kg).

##p < 0.001 compared to L-NAME (40 mg/kg).

Invasive blood pressure

The control group of mice showed mean systolic blood pressure (SBP) 124.3 ± 1.25 mmHg, diastolic BP (DBP) 93.67 ± 2.72 mmHg on day 29th and the mean arterial blood pressure (MABP) of 119.2 ± 2.35 mmHg. Mice treated with L-NAME showed SBP 168.2 ± 0.85 mmHg, DBP 145.6 ± 0.81 mm Hg, MABP 152.9 ± 0.74 mmHg. L-NAME produced a fundamental increase in SBP, DBP and MABP in comparison to control. The results indicate the hypertensive effect of L-NAME. CA + L-NAME on the other hand showed significant reduction in blood in the parameters tested, with SBP 147.1 ± 3.35 mmHg, DBP 125.3 ± 2.30 mmHg and MABP 132.9 ± 1.03 mmHg in contrast to L-NAME alone group. Mice treated with L-NAME + L-arginine showed SBP 139.4 ± 1.68 mmHg, DBP 122.7 ± 1.11 mmHg and MABP 125.3 ± 1.20 mmHg. The results indicated further that, CA as well as L-arginine treatment produced significantly ($p < 0.001$) attenuation of the hypertensive effect of L-NAME (Table 3).

	SBP (mmHg)	DBP (mmHg)	MABP (mmHg)	BPM
Control	124.3 ± 1.25	93.67 ± 2.72	119.2 ± 2.35	307.8 ± 7.16
L-NAME (40 mg/kg)	168.2 ± 0.85***	145.6 ± 0.81***	152.9 ± 0.74***	397.1 ± 5.38***
CA root extract (500 mg/kg, p.o.) + L-NAME (40mg/kg)	147.1 ± 3.35##	125.3 ± 2.30##	132.9 ± 1.03##	366.5 ± 2.27##
L-arginine (100 mg/kg, p.o.) + L-NAME (40mg/kg)	139.4 ± 1.68##	122.7 ± 1.11##	125.3 ± 1.20##	337.7 ± 10.82##

Table 3: Effect of L-NAME alone and with CA and L-arginine on hemodynamic parameters recovered by invasive method in rats treated for 4 weeks.

Values are represented as mean ± S.E.M. one-way ANOVA followed post hoc Bonferroni tests.

CA: *Convolvulus arvensis*; L-NAME: N^{G}-nitro-l-arginine methyl ester.

***p < 0.001 considered as significant comparison between control and l-NAME (40 mg/kg).

p < 0.001 compared to l-NAME (40 mg/kg).

Histopathology

Histopathological examination of the heart of animals in the control group (A) showed deprivation of inflammation, necrosis and atrophy (graded as −: absence of alteration). L-NAME alone (40 mg/kg, p. o., 4 weeks) (B) administered group showed inflammation (+) and necrosis (+) which means that 0-35% region of the mice's heart was swollen and necrosis occurred in 0-35% area of the heart. Inflammation and necrosis in various areas of the heart showed cardio toxicity in mice. While inflammation and necrosis were absent in the heart of mice treated with CA and L-arginine. The outcome of histological study finally confirmed that the mice's heart was protected by concomitant administration of CA (500 mg/kg) or L-arginine (100 mg/kg) with L-NAME (Figure 1).

Figure 1: Photomicrographs of histological changes of mice heart.

(A) (40×) (−−, absence of change), Control group; (B) (40×) (+, 0–35% necrosis, inflammation); L-NAME (40 mg/kg, p.o.) group; (C) (40×) CA (500 mg/kg, p.o.) + L-NAME (40 mg/kg, p.o.) (−−, absence of change); (D) (40×) L-arginine (100 mg/kg p. o.) + L-NAME (40 mg/kg, p.o.)

U: Unremarkable; NM: Necrotic Myocardial Cells; I: Inflammatory Cells.

Characterization of CA methanol root extract by HPLC

The quantities of flavonol C-glycosides in 1mg/100 g of CA root powder were Vitexin: 28 mg/100 g (0.028%), Isoorientin: 95 mg/100 g (0.095%), Orientin: 18 mg/100 g (0.018%) and other constituents were identified as water soluble oligosaccharides (Figure 2).

Figure 2: HPLC Chromatographic analysis of the methanol extract of CA.

Long-term administration/exposure of NG-nitro-L-arginine methyl ester (L-NAME) introduces advancement of hypertension and hypertrophy of the left ventricle in rats associated with deficiency of nitric oxide (NO). It has been also reported that in L-NAME treated animals, cardiac hypertrophy is present, which is compensatory to chronic increase in blood pressure [11]. A number of reports have analyzed the reason for L-NAME induced arterial hypertension, which is said to be due to deficiency of NO reported to control coronary vascular tone [12].

This decrease in endothelium dependent NO arterial dilatation is related to the risk of coronary ischemia and infarction. The chronic elevation of NO synthase also caused myocardial infarction in rats. The blockade of NO synthase by L-NAME results in increased serum cholesterol level in mice [13]. In view of these reports, it is hypothesized that decreased NO levels may be a risk factor for coronary disease and myocardial infarction. In this study, chronic administration of L-NAME increased systolic BP in mice which was recorded by tail cuff method. Our results are thus in agreement with the earlier reports by Vogel and Vogel [14]. It has been reported that NO inhibition by L-NAME accelerates hypertension and induces perivascular inflammation [15]. L-NAME induced hypertension and cardiac damage were selected for evaluating the antihypertensive and cardio-protective activity of CA root extract in the current study.

It is well established that the biological markers like endogenous enzymes are organ specific and leak from the damaged organ during necrosis. The endogenous cardiac biomarkers such as LDH and CK-MB is released into the perfusate or serum during damage to the myocardium [16].

In the current research L-arginine and L-NAME were concomitantly administered. Nitric oxide (NO) is produced from L-arginine by a family of enzymes known as NO synthases (NOS). Numerous studies have shown that hypertension is accompanied by a reduced endothelial function, which can be rescued to varying degrees by

L-arginine supplementation [17]. L-Arginine is essentially dependent amino acid in the human diet. L-Arginine deficiency relates to a diversity of inflammatory and oxidative procedures in the vascular endot helium and may be critical in the formation of atherosclerosis. The most common/known dietary sources of L-arginine are meat, poultry, fish, dairy products and nuts. Minimum intake of L-arginine rich foods such as fish and nuts has been consistently shown to be associated with future cardiovascular risk [18].

The metabolic effects of anti-hypertensive are important because hypertension does not occur in isolation, but most often is accompanied by obesity, hyperlipidemia and hyperinsulinemia. This cluster is known as metabolic syndrome which leads to increased morbidity and mortality from cardiovascular disease [19]. The result of the present study showed attenuation of L-NAME induced hypertension by concomitant L-arginine treatment. Our recent study has shown that CA root possesses cardio protective activity.

This investigation confirmed the beneficial effect of CA root powder on regression of hypertension and the prevention of myocardial damages associated with L-NAME treatment. CA also showed antihyperlipidemic [10] and antihypertensive activity [9] in high fat diet induced hyperlipidemia and dexamethasone induced hypertension, respectively. Reduction in cholesterol in CA + L-NAME treated mice further reinforce the claims of antihyperlipidemic effect of CA in the present L-NAME model of hypertension.

Furthermore, in the latest study L-NAME produced inflammation and necrosis, which confirmed its cardiotoxicity. The histology of the heart of mice treated with CA or L-arginine did not show inflammation and necrosis. This observation correlated with the cardio protective effect of CA or l-arginine. In this investigation CA and L-arginine showed similarities in exhibiting antihypertensive, antihyperlipidemic and cardio protective effects.

It is well known that natural products show antioxidant activity. The correlation between antioxidant and cardio protective activities of phenolic extracts of fruits of *Aristotelia chilensis* (Molina) Stuntz (Elaeocarpaceae) have been demonstrated (Cespedes., *et al.* 2008). The antihypertensive activity of *Phyllanthus urinaria* L. (Euphorbiaceae) (Lin., *et al.* 2008), *Caesalpinia decapetala* (Caesalpinaceae) [20], *Opuntia monacantha* (Cactaceae) [21] and *Moringa oleifera* Lam. (Moringaceae) [22] has been reported.

Hypertension is a significant risk factor for coronary heart disease and stroke. Flavonoids have been considered as active principles of several antihypertensive plant extracts like leaves of *Tropaeolum majus* L. (Tropaeolaceae) [23], *Lagenaria siceraria* (Cucurbitacae) [2], *Pterospartum tridentatum* [24], *Holarrhena floribunda* (Apocynaceae) [25], *Hibiscus sabdariffa* (Malvaceae) [26] and *Crataegus oxyyacantha* L. (Rosaceae) [27].

The inflammatory process of the mammalian system is affected by flavonoids which possess *in vitro* and *in vivo* anti-inflammatory as well as immune-modulatory activities. As nitric oxide (NO) yielded by inducible nitric oxide synthase (iNOS) is one of the inflammatory mediators, the drawbacks of various naturally occurring flavonoids on NO production in LPS-activated RAW 264.7 cells were evaluated *in vitro* [28]. Flavonoid complexes occurring in the medicinal plants thus showed variation. Chromatographic analysis of the methanol extract of CA root powder showed presence of vitexin, isoorientin, and orientin Figure 1 [10].

The effects of plant flavonoids on mammalian cells have been of substantial recent interest with attention focused on the suggestions of these agents for cardiovascular disease and for cancer. Some epidemiological studies intended to examine a possible protective effect of flavonoids in cardiovascular disease have been reported [29].

Saleem., *et al.* [9] reported the separation of complex of flavone C-glycosides of CA. Vitexin was absent in flowering herbs, whereas saponarin, isoorientin and saponnarin 4-O-glucoside were present. Saleem., *et al.* [10] reported isolation of four new D: C-friedooleanane triterpenes from the methanol extract of the stems of CA and reported anticancer activity against lymphoblastic leukemia jurkat cell lines.

Conclusion

It is concluded that concomitant administration of CA root powder or L-arginine with L-NAME significantly reduced L-NAME induced hypertension in mice. The flavonol vitexin detected in CA appear to contribute to this antihypertensive activity. L-NAME damaged myocardium which was confirmed by the presence of inflammation and necrosis. The absence of myocardial necrosis and inflammation in CA treated group suggested its cardioprotective activity. The antioxidants orientin and iso-orientin detected in CA root powder could be responsible for the reduction myocardial in-

flammation and necrosis induced by L-NAME. It is thus concluded that CA root extract possess antihypertensive and cardio protective activity.

Conflict of Interest

The authors declared no conflict of interest.

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