

Antihypertensive and Cardioprotective Effects of *Ficus Exasperata* Leaf Extract in L-NAME- Induced Hypertensive Rats

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Abstract

In recent time, there has been a rising demand to investigate the antihypertensive and cardio-protective abilities of different therapeutic plants owing to their fewer undesirable side effects compared to the synthetic drugs. The aim of this study was to determine the effects of *Ficus exasperata* aqueous leaf extract (FLE) on blood pressure, heart, kidney and liver in L-NAME-induced hypertensive rats. Hypertension was induced by oral administration of 40 mg/kg/day No-nitro-L-arginine methyl ester hydrochloride (L-NAME) in distilled water for 28 days. The rats were concurrently treated with FLE in serial doses (200, 400 and 600 mg/kg/day) and Nifedipine 10 mg/kg/day in distilled water. Blood pressure was measured using non-invasive tail-cuff method at baseline, 14th and 28th days thereafter. Electrocardiograph (ECG) was used to assess the effects on the heart. Serum level of urea, creatine, alanine transaminase (ALT), aspartate transaminase and gamma-glutamyl transferase (GGT) were measured at the end of treatment. The results from this study showed that treatment with three doses level of FLE significantly ($p < 0.05$) reduced systolic blood pressure, diastolic blood pressure and mean arterial pressure in L-NAME-induced hypertensive rats in dose dependent manner. Treatment with FLE also ameliorated the derangement in ECG parameters, dyslipidemia, liver and renal injuries when compared with untreated L-NAME hypertensive rats' group. The findings provided evidence that FLE exhibited a considerable dose -dependent antihypertensive and organ protective effects. Thus, FLE may be considered as a complementary treatment in the management of hypertension and its complications.

Key words: Antihypertensive, L-NAME, *Ficus exasperata*, cardioprotective

Introduction

High blood pressure, the most common cardiovascular disorder affecting approximately one billion people worldwide, remains the leading single contributor to global burden of disease and death, accounting for approximately 9.4 million deaths yearly [1]. The effects of high of blood pressure if not well controlled are devastating, and may include stroke, myocardial infarction, cardiac failure, and renal failure among others[2]. Hypertension is diagnosed by physical history, laboratory tests, sphygmomanometer and digital blood pressure monitor[3]. The diagnosis of hypertension in adults is made when the average of two or more diastolic blood pressure measurements on at

least two subsequent visits is ≥ 90 mmHg, or when the average of multiple systolic blood pressure readings on two or more subsequent visits is ≥ 140 mmHg. Orthodox medicines provide several classes of antihypertensive drugs. However, controlled blood pressures are achieved in only a smaller number of patients in medical practice[4]. Also, there are greater barriers to the assessment of effective healthcare services by the most population in Sub-sahara Africa which resulted in a greater number of people opting for herbal remedy as considerable alternatives for the control of their hypertension and its complications[5].

Several techniques have been used to induce hypertension in an experimental animal model. No-

nitro-L-arginine methyl ester hydrochloride (L-NAME), an L-arginine analogue inhibits nitric oxide synthases (NOS) competitively. L-NAME-induced hypertension is a well-established experimental model that is commonly used in basic cardiovascular research[6]. Apart from inhibiting NO synthesis, L-NAME induces hypertension by causing oxidative stress. L-NAME is responsible to cause imbalance in renin-angiotensin system (RAS) whereby it increased the expression of angiotensin II and also caused renal dysfunction[7].

Several naturally existing medicinal plants have been reported for their blood pressure lowering effects[8]. However, more scientific studies are still required to elucidate the safety profile, efficacies, and action mechanisms of such herbal treatments. *Ficus exasperata* Vahl. (Moraceae), popularly referred to as “sandpaper leaf tree” owing to the rough surface of the leaves, is increasingly being used for a number of ailments including hypertension and hence, studies validating the traditional claims are on the increase[9]. Available reports indicate that leaves of *F. exasperata* exhibit anti-ulcer, hypotensive, hypoglycemic, hypolipidemic, anti-inflammatory, anxiolytic, oxytocin inhibiting, anticonvulsant, antinociceptive and antipyretic activities[10]. To date, relatively few studies have been done to explore blood pressure lowering effects of FE. To the best of our knowledge, this study is the first to examine the antihypertensive effect and to investigate the potential of FE in alleviating abnormalities in lipid profile, ECG parameters, and end-organ damage in L-NAME-induced hypertensive rats.

Materials and Methods

Chemical and reagents

All the chemicals and reagents used were of standard analytical grade.

Plant collection

The fresh leaves of *Ficus exasperata* was obtained from Ilorin, Kwara State, Nigeria North central in July, 2021. The plant material was identified and authenticated at Department of Plant Biology, University of Ilorin and voucher specimen (UIL/001/1568/2022) deposited in the herbarium.

Experimental animals

Male Wistar rats, aged 12-16 weeks and weighing 180-220 g were obtained and housed in the animal house of the College of Health Sciences, University of Ibadan, Nigeria in line with standard laboratory conditions of light, dark, temperature and humidity. The animals were fed with standard diets and water *ad libitum*, and allowed to acclimatize at least 7 days before the commencement of the study.

Ethical consideration

Experimental protocols used in this study were approved by the ethical committee, Faculty of Life Sciences,

University of Ilorin, Nigeria, according to the standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November[11].

Methods

Induction of hypertension

Hypertension was induced according to the previously described method[12] through oral administration of L-NAME (40 mg/kg/day) in distilled water.

Preparation of plant extract

The *Ficus exasperata* leaves were air dried and pulverized. The ground samples were extracted in distilled water for 48 hours[13]. The extract was filtered and the resulting filtrate lyophilized.

This was stored at 4°C prior to the study. The major phytochemical groups were determined using the Lieberman Buchard, ferric chloride, copo of magnesium and Vanillin-sulphuric acid tests. Polyphenolic compounds, flavonoids, alkaloids and saponins were identified in the extract.

Experimental design

Thirty already acclimatized rats were randomized into six groups with five animals in each group ($n=5$) and treated as follows: group A served as the normal control given distilled water only, group B (L-NAME-induced hypertensive rats) given 40 mg/L L-NAME in distilled water; groups C: LNAME-induced hypertensive rats co-treated with Nifedipine 10 mg/kg b.wts; groups D, E, F (L-NAME-induced hypertensive rat groups) co-treated with the serial doses of *Ficus exasperata* aqueous leaf extract (200, 400, 600 mg/kg. b.wts respectively).

Blood pressure measurement

Systolic blood pressure, diastolic blood pressure and mean arterial pressure were measured in a conscious rat by non-invasive tail-cuff CODA blood pressure system (Kent Scientific Corporation, USA). Measurements were taken at baseline, 14 and 28 days respectively[14]. At least eight readings were recorded during each measurement and the average values calculated[15].

Animal sacrifice and samples collection

At the end of the treatment period, animals were sacrificed under anesthesia and blood samples collected in plain tubes, centrifuged at 3000 rpm for 10 min to obtain the serum. After the separation, the serum was aspirated and used for biochemical analysis.

Determination of lipid profile

The concentrations of serum total cholesterol (TC), high density lipoprotein (HDL) and triglycerides (TG) in plasma were determined spectrophotometrically using a commercially available kit. Low density lipoprotein (LDL) content was calculated from the other lipid parameters according to the Friedewald *et al.*, [16] equation: $LDL = TC - (TG/5) - HDL$.

Determination of serum kidney markers

Creatine and Urea determinations

The serum levels of urea and creatine were assayed using the urease kinetic method and Jaff's reaction method respectively with the auto-analyser. The urease kinetic method is an enzymatic method based on two steps. The first step involves the enzyme urease hydrolyzing urea with the production of ammonium. The second step involves the quantitative measurement of ammonium kinetically in the auto-analyzer. The concentration of the ammonium is proportional to the urea present in the serum sample. The measurement of the creatine was based on the reaction of creatine with picric acid in an alkaline medium to produce an orange red complex that were measured spectrophotometrically [17].

Determination of serum liver markers

Spectrophotometric method was used for the assay of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and Gamma glutamyltransferase (GGT) using ELISA kits as previously described [18].

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.0. All results were expressed as means $\pm$ standard error of the mean. Data were analysed using one-way analysis of variance (ANOVA) followed by LSD Post Hoc test. Differences between means were considered to be significant when $p < 0.05$.

Results

Phytochemical composition of *Ficus exasperata* aqueous leaf extract

The preliminary phytochemical investigation of the aqueous crude extract of *Ficus exasperata* leaves revealed the presence of saponins, flavonoids, tannins, cardiac glycosides, alkaloids and phenol as shown in table 1.

Table 1: Phytochemical composition of *Ficus exasperata* aqueous leaf extract

S/N	Phytochemical composition	Extract
1	Cardiac glycoside	+
2	Phenol	+
3	Flavonoid	+
4	Saponins	+
5	Alkaloids	+
6	Tannis	+

+ indicate the presence of the compound

Effect of FE on systolic blood pressure (SBP) in L-NAME-Induced Hypertensive Rats.

At baseline, there was no significant difference in SBP among all the groups. After two weeks of treatment, L-NAME-induced rats had higher SBP (162.0 ± 1.4 mmHg) compared to control rats (108.4 ± 5.7 mmHg) ($p < 0.005$). Treatment of L-NAME-induced rats with all the three doses of FE (200, 400 and 600 mg/kg/day) (Fig 1) significantly reduced SBP to normal level (143.6 ± 3.7 , 142.8 ± 2.7 and 126.4 ± 9.8 mmHg, respectively) ($p < 0.005$). At the end of four weeks treatment, the SBP of L-NAME-induced rats were persistently high compared to control (183.4 ± 2.9 vs. 114.6 ± 5.2 mmHg, $p < 0.005$). Treatment with all three doses of FE significantly attenuated L-NAME-induced rise in SBP at the end of treatment (142.6 ± 6.27 , 113.2 ± 5.2 and 115.8 ± 3.4 mmHg, respectively) ($p < 0.005$). However, there was no significant difference in SBP between the three doses of FE following two and four weeks of treatment. In addition, administration of FE alone did not significantly alter the SBP compared to control.

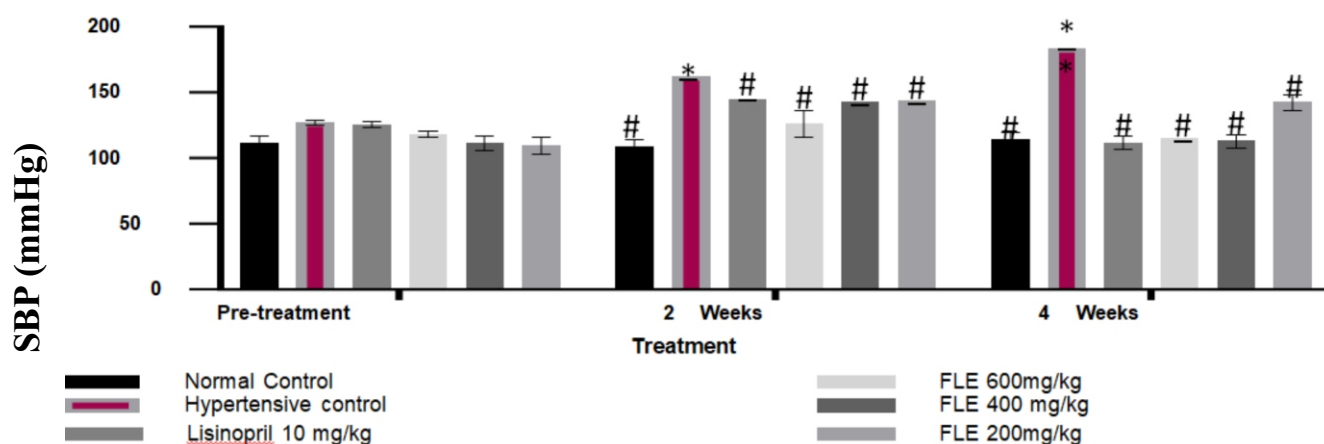


Figure 1 Effect of FE on DBP in L-NAME-Induced Hypertensive Rats. Data were expressed as Mean $\pm$ SE. of 5 experiments. Data were analyzed by one-way ANOVA followed by LSD for multiple comparison test, $*p < 0.05$ versus normal control and $\#p < 0.05$ versus hypertensive rats.

Effect of FE on DBP in L-NAME-Induced Hypertensive Rats

At baseline, there was no significant difference in DBP among the groups. Following two weeks of treatment, L-NAME-induced rats had higher DBP (106.0 ± 0.9 mmHg) compared to control rats (76.8 ± 4.6 mmHg) ($p < 0.05$). Treatment of L-NAME-induced rats with three doses of FE (200, 400 and 600 mg/kg/day) successfully reduced DBP (100.6 ± 5.8 , 95.8 ± 3.3 and 86.0 ± 7.5 mmHg) ($p < 0.05$). At the end of the four weeks treatment, the DBP of L-NAME-induced rats were persistently high compared to control (125.6 ± 7.3 vs. 71.6 ± 6.3 mmHg, $p < 0.05$). Treatment of L-NAME-induced rats with all three doses of FE significantly reduced DBP to normal level at the end of treatment (77.8 ± 7.8 , 78.0 ± 4.9 , and 69.2 ± 2.9 mmHg, respectively) ($p < 0.05$) with a comparable effect with the reference drug treated group (Fig. 2).

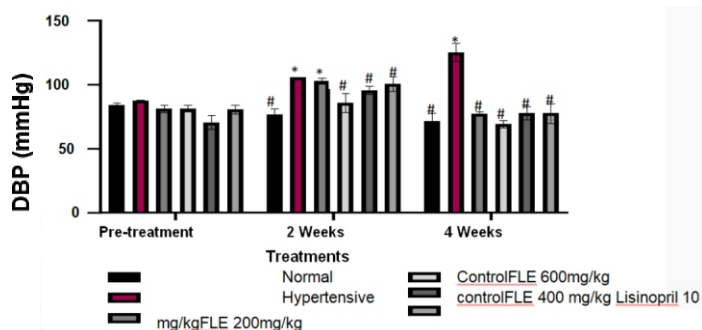


Figure 2: Effect of FE on DBP in L-NAME-Induced Hypertensive Rats. Data

Mean $\pm$ SE. of 5 experiments. Data were analyzed by one-way ANOVA followed by LSD for multiple comparison test, $*p < 0.05$ versus normal control and $\#p < 0.05$ versus hypertensive rats.

Effect of FE on MAP in L-NAME-Induced Hypertensive Rats

At baseline, there was no significant difference in MAP among all the groups. Following two weeks of treatment, L-NAME-induced rats had higher MAP (124.4 ± 1.0 mmHg) as compared to control rats (89.0 ± 3.9 mmHg) ($p < 0.05$). Treatment of L-NAME-induced rats with all three doses of FE successfully reduced MAP (114.4 ± 3.8 , 111.2 ± 2.2 and 99.2 ± 8.1 mmHg) ($p < 0.05$). At the end of the four weeks treatment, the MAP of L-NAME-induced rats were persistently high compared to control (144.4 ± 4.8 vs. 86.0 ± 4.5 mmHg, $p < 0.05$). Treatment of L-NAME-induced rats with all three doses of FE significantly reduced MAP at the end of treatment (99.6 ± 6.9 , 89.4 ± 3.3 and 84.4 ± 2.7 mmHg, respectively) ($p < 0.05$) with a comparable effect with the reference drug treated group (85.3 ± 1.9) (Fig3).

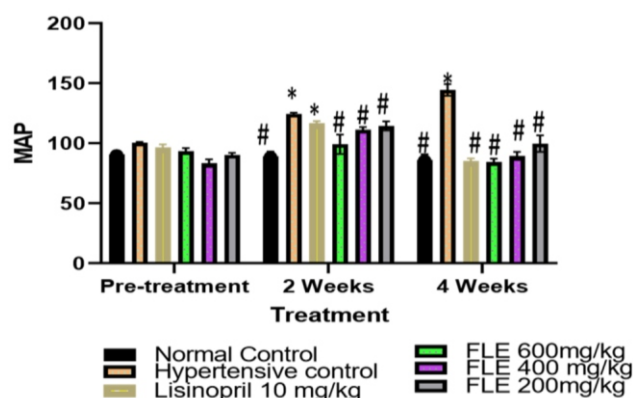
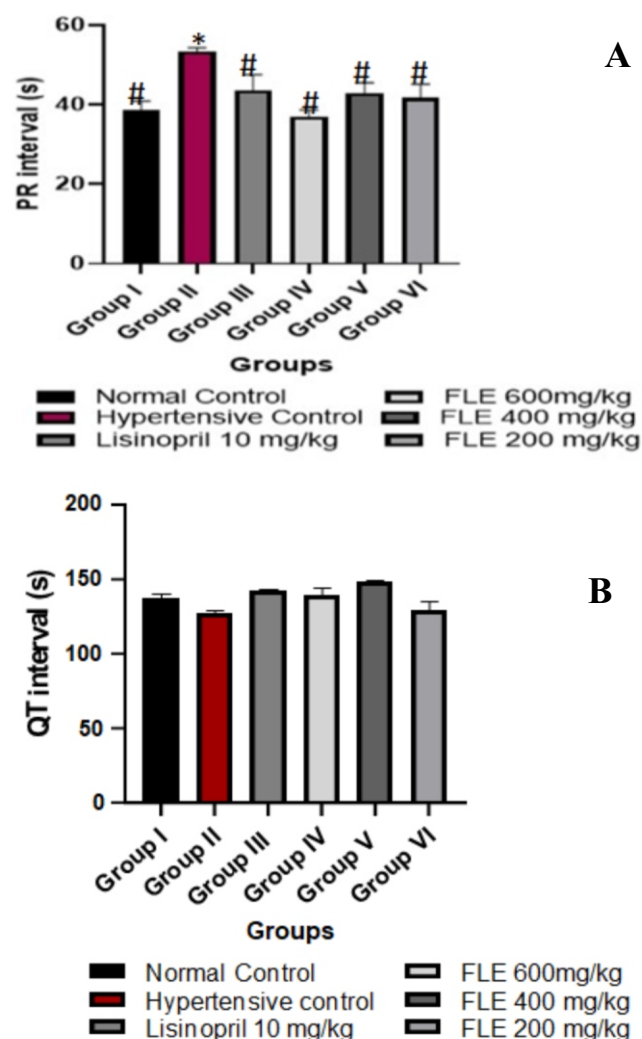


Figure 3: Effect of FE on Mean Arterial Pressure in L-NAME-Induced Hypertensive Rats.

Data were expressed as Mean $\pm$ SE. of 5 experiments. Data were analyzed by one-way ANOVA followed by LSD for multiple comparison test $*p < 0.05$ versus normal control and $\#p < 0.05$ versus hypertensive rats.

Effect of FE on electrocardiograph (ECG) parameters

L-NAME increased the heart rate and PR intervals. On the other hand, it decreased QRS, QT, QTc, and R-amplitude as compared to normal control rats. Remarkably, treatment with the serial doses of *Ficus exasperata* and lisinopril ameliorated the L-NAME-induced changes in ECG parameters after 28 days of treatment as shown in Figure 4 (A-F).



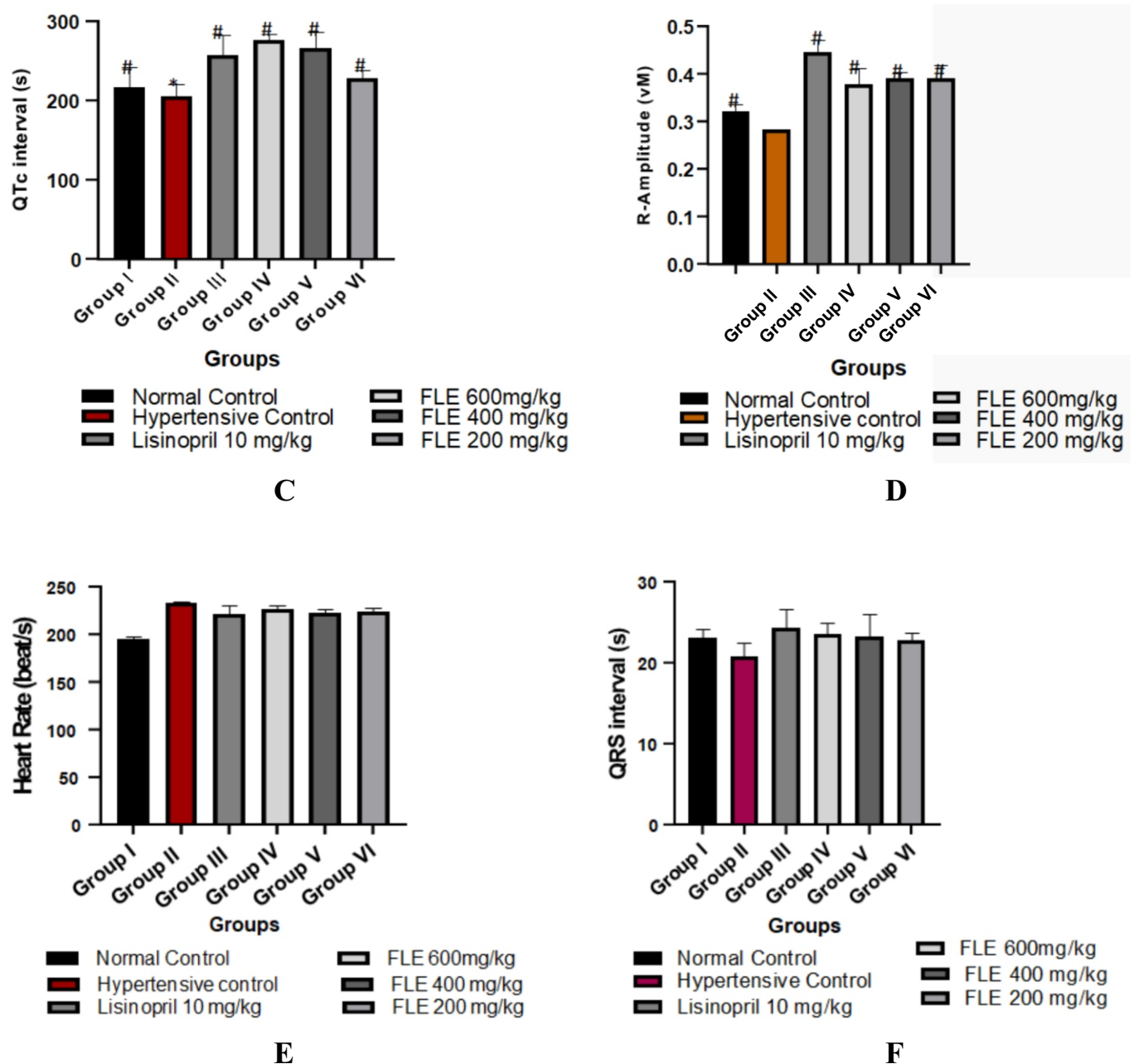


Figure 4: Effect of FE on ECG parameters (A) PR interval, (B) QT (C) QTc (D) R-amplitude (E) Heart rate (F) QRS L-NAME-induced hypertensive rats. Data were expressed as Mean $\pm$ SE. of 5 experiments. Data were analyzed by one-way ANOVA followed by LSD for multiple comparison test, $*p < 0.05$ versus normal control and $\#p < 0.05$ versus hypertensive rats.

Effect of FE on liver and kidney markers in L-NAME-induced hypertensive rats

As shown in Table 2. the activities of the liver marker enzymes alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyltransferase (γ -GT) were significantly ($p < 0.05$) elevated after L-NAME administration in comparison with normal controls. However, treatment with all the doses of the

extract significantly ($p < 0.05$) decreased AST, ALT and γ -GT activities to a near normal levels. Also, the creatinine and urea serum levels were observed to be significantly ($p < 0.05$) elevated in untreated L-NAME hypertensive rats. Meanwhile, treatment with the serial doses of FE significantly ($p < 0.05$) decreased both urea and creatinine levels in dose dependent manner.

Table 2: Effect of FE on liver and kidney markers in L-NAME-induced hypertensive rats

Groups	AST (U/L)	ALT (U/L)	GGT (U/L)	Urea (mg/)	Creatine (μmol/l)
Group I	37.4 ± 9.92 [#]	68.2 ± 7.84 [#]	8.1 ± 0.31 [#]	37.6 ± 3.42 [#]	137.9 ± 17.53 [#]
Group II	49.2 ± 7.02 [*]	117.6 ± 5.71 [*]	11.5 ± 0.45 [*]	55.8 ± 5.17 [*]	186.3 ± 11.40 [*]
Group III	32.2 ± 5.41 [#]	101.8 ± 6.02 ^{*#}	8.9 ± 0.54 [#]	49.8 ± 5.03 ^{*#}	136.0 ± 9.13 [#]
Group IV	31.8 ± 6.32 [#]	81.0 ± 8.38 [#]	7.6 ± 0.57 [#]	51.9 ± .99 ^{*#}	136.0 ± 17.57 [#]
Group V	41.5 ± 7.12 ^{*#}	81.3 ± 10.97 [#]	8.3 ± 0.71 [#]	41.9 ± 7.34 ^{*#}	101.1 ± 30.37 [#]
Group VI	44.3 ± 7.11 ^{*#}	77.3 ± 4.35 [#]	8.1 ± 0.42 [#]	44.5 ± 4.36 ^{*#}	146.8 ± 11.61 [#]

Data were expressed as mean ± SE. of 5 experiments. Data were analyzed by one-way ANOVA followed by LSD for multiple comparison test, * $p < 0.05$ versus normal control and # $p < 0.05$ versus hypertensive rats.

Effect of FE on lipid profile in L-NAME-induced hypertensive rats

As shown in Table 3, a significant ($p < 0.05$) rise in serum levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL) coupled with decreased level of high-density lipoprotein (HDL) were observed in untreated L-NAME-induced hypertensive rats when compared with the normal control group. However, concomitant administration of the serial doses of FE significantly ($p < 0.05$) decreased TC, TG, LDL and increased HDL to nearly normalized concentrations. However, no significant difference between all the three doses of the extract groups and the reference group.

Table 3: Effect of FE on lipid profile in L-NAME-induced hypertensive rats

Groups	Cholesterol (mg/dl)	Triglyceride (mg/d)	HDL (mg/dl)	LDL (mg/dl)
Group I	118.6 ± 3.35 ⁺	93.7 ± 4.02 ⁺	41.9 ± 0.79 ⁺	74.9 ± 3.00 ⁺
Group II	200.2 ± 3.93 [*]	130.7 ± 6.00 [*]	23.1 ± 0.21 [*]	147.2 ± 2.59 [*]
Group III	175.2 ± 1.43 [*]	109.7 ± 2.56 ⁺	30.5 ± 0.75 ⁺	106.4 ± 1.03 ^{†*}
Group IV	142.8 ± 0.557 ⁺	95.3 ± 0.92 ⁺	38.7 ± 0.63 ^{*†}	69.2 ± 0.69 ⁺
Group V	151.4 ± 1.46 ⁺	100.5 ± 0.41 ⁺	32.5 ± 0.94 ⁺	90.9 ± 1.58 ^{†*}
Group VI	171.3 ± 1.89 [*]	103.6 ± 3.48 ⁺	28.4 ± 0.39 ⁺	99.3 ± 2.25 ^{†*}

Data were expressed as mean ± SE. of 5 experiments. Data were analyzed by one-way ANOVA followed by LSD for multiple comparison test, * $p < 0.05$ versus normal control and # $p < 0.05$ versus hypertensive rats.

Discussion

L-NAME-induced high blood pressure is a well-established experimental model characterized by generalized nitric oxide deficiency and progressive increase in blood pressure in chronic administration. The hypertensive-induced effect is based on the fact that L-NAME, a structural analog of L-arginine, is metabolized by nonenzymatic hydrolysis into the active form, N omega-nitro-L-arginine, which competitively binds to endothelial nitric oxide synthase [19]. Nitric oxide produced by endothelial nitric oxide synthase (eNOS) is the major source of circulating nitric oxide,

thus inhibition of nitric oxide synthase attenuates both the synthesis and metabolism of nitric oxide, the smallest gaseous intercellular signaling molecule mediating the vascular relaxation. Ultimately, nitric oxide deficiency leads to systemic vasoconstriction and hypertension [20]. The main objective in this study was to evaluate whether the high blood pressure and cardiotoxicity in NO-deficient hypertensive rats induced by L-NAME, could be alleviated by *Ficus exasperata* administration. In this study, oral administration of L-NAME resulted in a significant rise in systolic blood pressure, diastolic blood pressure, and mean arterial

pressure compared with the normal control rats, validating the induction of hypertension as presented in Figures 1-3. These results are in agreement with previously described findings where L-NAME induced a prolonged increase in blood pressure[21]. Administration of the serial doses of *Ficus exasperata* extract (200, 400, and 600 mg/kg) and lisinopril (a reference drug) caused a significant decline of systolic blood pressure, diastolic blood pressure and mean arterial blood pressure in the hypertensive rats. This fall in blood pressure in FE treated rats may be attributed to the presence of phytochemical constituents like flavanoids and saponins in the plant extract. These bioactive phytochemicals are known to possess vasorelaxant, antihypertensive and antihyperlipidemic effects[22]. The derangement in ECG parameters caused by L-NAME were in line with the previous findings by Chaswal *et al* [23] and also could be attributed to the effect of the decreased nitric oxide production on vascular smooth tone[24]. Interestingly, treatment with the serial doses of FE and the reference drug ameliorated the L-NAME-induced changes in electrocardiograph parameters at the end of the treatment period (Figures 4A-F). Previous studies suggest that chronic inhibition of Nitric Oxide Synthase by L-NAME alters various biochemical indices[25]. In the current study, the activities of the liver marker enzymes (ALT, AST, and γ -GT) were significantly increased following L-NAME treatment when compared with normal control group, indicating liver injury (Table 2). These results agree with previous studies [26] that reported, nitric oxide maintains hepatic perfusion under physiological conditions, and so inhibition of NO synthesis by L-NAME leads to a marked increase in perfusion pressure. Protective effects of FE against hypertension-associated liver injury were confirmed by the reduction in ALT, AST and γ GT serum levels. The elevated urea and creatine levels in untreated L-NAME hypertensive group may be attributed to NO deficiency. NO acts as a key regulator of renal hemodynamics, the inhibited NO synthesis in hypertensive state leads to reduction in renal blood flow and impaired renal function markers. Ameliorative effects observed from the treatment with the serial doses of *Ficus exasperata* leave extract may be associated with its vasorelaxant effect through elevation of endothelial nitric oxide synthase activities. Results from this study reveals that FE exhibited antiatherogenic effects as evident by the significant reduction in triglyceride, total cholesterol and LDL-cholesterol coupled with increased HDL-cholesterol.

Conclusion

High blood pressure and dyslipidemia are considered as two concomitant cardiovascular risk factors. The drugs with additional effects other than the antihypertensive effect are preferable in the management of hypertension. The present study provide evidence that aqueous leaf extract of *Ficus exasperata* exhibited a considerable dose dependent antihypertensive effects at the three

examined dose levels (600, 400 and 200 mg/kg). In addition to the antihypertensive effects, *Ficus exasperata* leaf extract was observed to possess a beneficial cardioprotective effect as demonstrated by its ameliorative effects on vascular dysfunction (ECG derangements) and antiatherogenic effects.

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