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Original Research

Biochemical parameters as indicators of antihypertensive efficacy of leaf aqueous extract of *Tridax procumbens* (Linn.) in N-nitro-L-arginine methyl ester-induced hypertensive rats

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ABSTRACT

Background: Cardiovascular disease is a widespread public health problem and hypertension is one of the major risk factors of it. *Tridax procumbens* leaf extract is traditionally used in the treatment of hypertension. This study evaluates the effects of *T. procumbens* extract (TPE) on blood biochemical parameters of N-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats. **Materials and Methods:** Rats were divided randomly into four different treatment groups. Group I received 0.9% NaCl (control), Group II, III, and IV were given a nitric oxide synthase inhibitor (L-NAME) 40 mg/kg/day, orally for 6 weeks to induce hypertension. Groups III and IV were further treated with TPE (100 and 200 mg/kg/day), respectively, in the past 3 weeks of 6 weeks. Blood pressure, morphological variables, histopathology, plasma lipids, lipoproteins, cholesterol levels, hepatic enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other parameters were analyzed. **Results:** TPE markedly inhibited the sustenance of L-NAME-induced hypertension. This effect was accompanied by a partial or full amelioration of most of the adverse effects induced by L-NAME, such as: (1) Increases in blood pressure, organs weight indices; (2) hyperlipidemia; (3) increased plasma activity of glutamic-pyruvic transaminase (ALT) and AST; and (4) liver and cardiac histological lesions. **Conclusions:** This study provides evidence about the antihypertensive effects and end-organ protection of TPE in animal model of hypertension. These effects may be due to one or more constitution in this extract.

KEY WORDS: Hypertension, histopathology, lipid profile, *Tridax procumbens* extract

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of debility and premature death [1] and hence a major public health problem. The major risk factors include diabetes, smoking, and dyslipidemia [2]. Several studies acknowledged that hypertension is the most prevalent trigger for CVDs with other risk factors [1,3]. Hypertension is responsible for around 16.5% of annual deaths worldwide [1] and is the main cause of morbidity and mortality associated with CVDs [4]. By 2030, the annual death toll is predicted to reach 23.5 million people [1]. Hypertension frequently occurs in conjunction with metabolic disturbances and in particular, it occurs with hyperlipidemia and hypercholesterolemia [5]. Several reports have related the concentrations of lipids, cholesterol, triglycerides (TG), and their associated blood transporting lipoproteins (high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and very-low-density lipoprotein cholesterol [VLDL-C]) with the incidence of arteriosclerosis and coronary artery diseases [6,7]. The primary goal of antihypertensive therapy is to decrease risk of cardiovascular morbidity and mortality through the management of high blood pressure [8].

Therefore, drug development approaches are focused on discovering and designing molecules that could improve these pathophysiological conditions by targeting the chemicals that regulate vascular smooth muscle contraction and relaxation processes [9,10]. Natural product research remains a potential source in the production of novel candidates for the treatment of prevalent human diseases [11]. Despite great advances observed in orthodox medicines, plants still make an important contribution to health care.

In Africa, traditional healers have used herbal medicines to treat several ailments with relative success before the advent of orthodox medicines. This practice gradually waned with the development of synthetic drugs; however, despite the great advances observed in orthodox medicines, plants still make an important contribution to health care in most of the developing countries [12,13]. Although the mechanism of actions of these plants remedies is reportedly often lacking [13]. *Tridax procumbens* (Linn.), *Asteraceae*, belongs to one of the traditional plant remedy used for various disease conditions [14]. Vasodilatory activities of TPE have been reported in normotensive rat [15]. Previous studies in normotensive animal suggest that *T. procumbens* extract (TPE) caused vasodilatation through multiple mechanisms. This includes direct action on vascular smooth muscle [16]; endothelium-dependent and independent [17] and calcium-dependent pathways [18]. In an extensive search of literature, there is only one published study that evaluates the effects of *T. procumbens* on blood pressure in hypertensive model [19]. The biochemical basis of the use of this plant in the management of hypertension, as well as the biochemical impact of TPE administration in the hypertensive animal model has not been fully investigated. Therefore, the present study was designed to examine the efficacy of aqueous leaf extract of *T. procumbens* treatment on plasma electrolytes, liver enzymes, and lipid profile in N-nitro-L-arginine methyl ester (L-NAME) model hypertensive rats.

MATERIALS AND METHODS

Plant Material

T. procumbens leaves were obtained from the botanical garden of Lagos State University, Ojo, Lagos, Nigeria. The leaves were authenticated by a Taxonomist of the Forestry Research Institute (Mr K.A Adeniji) Ibadan, Nigeria. Following identification, a sample of the plant with reference number FHI 1008876 was deposited in the same institute.

Preparation of Extract

Aqueous extract of *T. procumbens* was obtained by extracting 1 kg air-dried and grounded leaves of *T. procumbens* with 100 ml distilled water at room temperature for 48 h. The procedure was repeated twice. The extract was filtered and dried in a freeze dryer at temperature of 26°C ± 1°C and solvent elimination of the resulting aqueous extract yielded 23.6% w/v of a brown powdery *T. procumbens* leaf crude aqueous extract. For administration, fresh solution was prepared daily using normal saline (vehicle).

Animals and Induction of Hypertension

Animal experimental procedures for this study were approved by the Animal Ethics Committee of Lagos State University College of Medicine. (No CM/THA. 16/174). The 1985 Guidelines for Laboratory Animal Care of the National Institute of Health (NIH) was also strictly complied with. Male Wistar rats weighing 150-200 g were used. The animals were housed in plastic cages with filter on top and kept in controlled laboratory conditions of room temperature, humidity, and light. Standard pellet diet (Live Stock Feeds Nig. Ikeja, Nigeria) and water were consumed by all the animals ad libilum. Hypertension was induced by the treatment with L-NAME (40 mg/kg body weight) through oral gavage daily for 6 weeks. Fresh solutions of L-NAME were prepared daily using normal saline (vehicle).

Experimental Design and Treatments

Animals were divided into four groups of 6 rats per group. Group I: Control received normal saline 0.9 ml/kg (vehicle); Group II, III, and IV were made hypertensive using L-NAME (40 mg/kg/body weight) for 6 weeks. However, Groups III and IV were further treated with TPE at 100 and 200 mg/kg/b.w, respectively, from 4th to 6th weeks. All treatments were through oral gavage.

Measurement of Body Weight

Body weights were measured with a Mettle weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The body weights were measured on the first day, 2nd and 6th week of the study. The weight difference between 2nd and 6th weeks, in reference to the initial weight, was calculated.

Measurement of Blood Pressure

The blood pressures were determined every week using a computerized non-invasive blood pressure system (Kent Scientific, Torrington, CT, USA). The animals were starved for 12 h before the commencement of blood pressure determination. At least five readings were recorded for each animal. The maximum and minimum values were discarded and the remaining three readings were averaged to obtain a mean blood pressure.

Biochemical Parameters and Lipid Profile

Blood samples were collected in heparinized tubes and the plasma was obtained by centrifugation at 1000 RPM for 15 min. Plasma obtained was used to assess the level of the following: Total cholesterol (TC) content of the plasma was estimated by the method of Zlatkis *et al.* [20] TG were estimated by the method of Foster and Dunn [21] HDL-C, LDL-C, and VLDL-C were determined according to the method of Meiathnin *et al.* [22]. Total protein was measured using biuret reaction [23]. Furthermore, albumin levels were measured by spectrophotometric estimation using the Sigma Diagnostic Kit (Sigma Diagnostics, UK). The atherogenic index was calculated as follows: Cardiac risk ratio (CRR) = TC/HDL-C; Artherogenic coefficient (AC) = (TC−HDL-C)/HDL-C; Atherogenic index of plasma = Log (TG/HDL-C) [24].

The activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by the method of Reitman and Frankel [25]. The plasma level of urea and creatinine were measured using spectrophotometric methods described by Coles [26]. The total bilirubin was determined as described by Balistreri and Shaw [27]. Plasma sodium and

potassium level were estimated using the reagent titrimetric method. Furthermore, plasma level of chloride was determined by the Schales and Schales Methods [28].

Organ Collection

The rats were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally) and sacrificed by cervical dislocations. The animals were then dissected and organs such as the heart, liver were removed and measured using an electronic weighing balance (Model AFP 110L, Adams equipment, Co Ltd, USA).

Histopathological Studies

Small sections of heart fixed in 10% buffered formalin were processed for embedding in paraffin. Sections (5-6 μm) were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The photomicrographs were taken using Moticam 1000 camera at 400 magnification.

Statistical Analysis

Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test using GraphPad Prism 5.1 model software. Results were presented as mean ± standard error of the mean. Values of $P < 0.05$ were regarded as statistically significant. For histological studies, random samples were selected for histopathological studies from each of the groups.

RESULTS

Effects of TPE on Body and Visceral Organ Weights

Table 1 shows the effect of TPE on body and visceral organs weight of L-NAME-induced hypertensive rats. The administration of L-NAME (40 mg/kg/body weight for 6 weeks) caused significant decrease ($P < 0.05$) in weight gain and a significant increase ($P < 0.05$) in the relative weight of heart of the hypertensive rats compared with the control group. Simultaneous administration of TPE with L-NAME (Group III and IV) significantly reversed ($P < 0.05$) the L-NAME-induced reduction in body weight and enlargement of the heart.

Effects of TPE on Blood Pressure

Basal values of the blood pressure were not significantly different between groups (97.2 ± 3.5). Administration of L-NAME resulted in significant elevation ($P < 0.001$) of mean arterial blood pressure (MABP) (153.8 ± 2.8). Co-treatment with TPE significantly decreased the level of MABP to 101.5 ± 2.6 and 119.7 ± 3.2 at doses of 100 and 200 mg/kg, respectively, with a maximum effect at a dose of 100 mg/kg. There was no significant ($P < 0.05$) difference in blood pressure between 100 and 200 mg/kg of TPE co-administrated groups [Figure 1].

Effects of TPE on Electrolytes

As shown in Table 2, sodium plasma level of the control animal (102.6 ± 0.6) was significantly ($P < 0.05$) lower than the hypertensive rats (145.7 ± 1.0). After co-treatment with TPE, the sodium plasma level of the hypertensive rat was significantly ($P < 0.05$) reduced to (116.8 ± 0.3 and 115.2 ± 0.3) for both 100 and 200 mg/kg of TPE, respectively. The potassium level of hypertensive animals (6.5 ± 1.0) was not significantly lower than control (6.9 ± 0.2), whereas the TPE co-treated animals (9.2 ± 0.4) and (9.4 ± 0.5) were significantly higher ($P < 0.05$) than both control and L-NAME only treated rats. The chloride and bicarbonate plasma levels of both L-NAME only treated and TPE co-treated groups were not significantly different from control.

Effects of TPE on Plasma Enzyme Levels

The plasma levels of the AST in hypertensive group (576.7 ± 90.2) were significantly higher ($P < 0.01$) when compared with the

Table 1: Effect of TPE on body weight and visceral organs in L-NAME-induced hypertensive rats

Treatment	Control	L-NAME (only)	L-NAME+TPE (100 mg/kg)	L-NAME+TPE (200 mg/kg)
Initial body weight (g)	154.3±6.1	159.5±8.7	154.9±8.1	151.8±6.5
Final body weight (g)	256.9±8.9	201.2±10.2*	251.7±6.1#	243.7±6.5#
Changes in weight (g)	102.6± 3.8	41.7±4.7*	96.8±3.2#	91.9±3.1#
Heart (g)	0.3±0.01	0.5±0.01*	0.3±0.02	0.29±0.02
Liver (g)	3.3±0.02	3.0±0.01	3.5±0.01	3.3±0.02

Data are presented as (mean±SEM value N=6 rats). *P<0.05 as compared with control group; #P<0.01 as compared to L-NAME hypertensive group. L-NAME: N-nitro-L-arginine methyl ester, TPE: *Tridax procumbens* extract, SEM: Standard error of the mean

Table 2: Effect of TPE on plasma electrolytes levels in L-NAME-induced hypertensive rats

Treatment	Control	L-NAME (only)	L-NAME+TPE (100 mg/kg)	L-NAME+TPE (200 mg/kg)
Na^+ (mmol/l)	112.16±0.6	145.7±1.0*	116.8±0.3	115.2±0.3
K^+ (mmol/l)	6.9±0.2	6.5±1.0	10.2±0.4	10.4±0.5
Cl^- (mmol/l)	105.2±1.6	103.5±2.5	105.6±0.8	107.5±2.1
HCO_3 (mmol/l)	20.8±1.7	17.3±2.4	19.8±2.1	14.6±1.8

Data are presented as (mean±SEM value N=6 rats). *P<0.05 as compared with control group. L-NAME: N-nitro-L-arginine methyl ester, TPE: *Tridax procumbens* extract, SEM: Standard error of the mean

control (313.4 ± 26.5). The co-administration of TPE at a dose of 100 and 200 mg/kg caused a significant decrease ($P < 0.05$) in plasma level of AST to 345.1 ± 20.1 and 442.1 ± 19.5 [Figure 2]. Similarly, the L-NAME only treated hypertensive group produced significant increase ($P < 0.05$) in plasma alanine aminotransferase (ALT) levels (9.2 ± 0.5) when compared with control group (6.6 ± 0.4). However, after co-treatment with TPE (100 and 200 mg/kg) the elevated ALT was reduced significantly to (6.7 ± 0.3 and 7.7 ± 0.2), respectively [Figure 3].

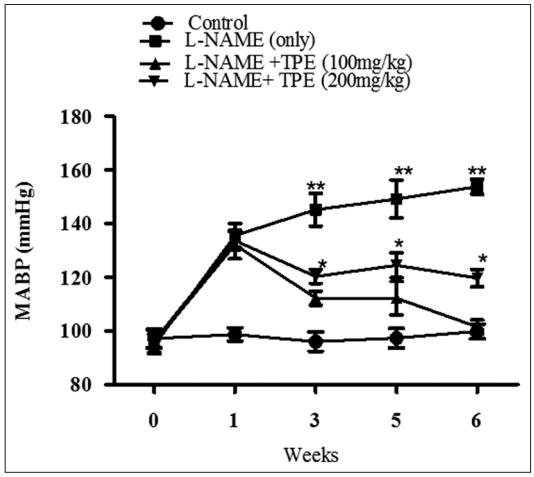

Figure 1: This is the effect of *Tridax procumbens* extract on the weekly mean arterial blood pressure in N-nitro-L-arginine methyl ester-induced hypertensive rats. Data are presented as (mean ± standard error of the mean value $N = 6$ rats) $^*P < 0.05$; $^{**}P < 0.01$ as compared with control group

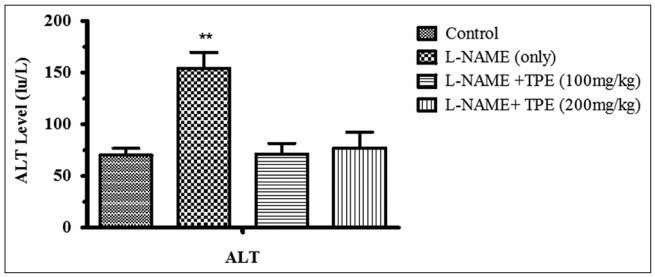

Figure 2: The effect of *Tridax procumbens* extract on plasma alanine aminotransferase levels in N-nitro-L-arginine methyl ester-induced hypertensive rats. Data are presented as (mean ± standard error of the mean value $N = 6$ rats) $^{**}P < 0.01$ as compared with control group

Effects of TPE on Plasma Lipid Profile and Phospholipids

The TC levels of the TPE co-treated group at 100 and 200 mg/kg (45.6 ±7.2 and 57.7 ± 1.7) were significantly decreased ($P < 0.05$) than the L-NAME only treated group (77.4 ± 2.9). There was no significant decrease in cholesterol level between the treated groups and control [Table 3]. L-NAME only treated hypertensive group had a significant increase ($P < 0.05$) in plasma LDL-C and VLDL-C (67.5± 0.1 and 7.3 ± 0.7) as compared to control group (18.3 ± 0.1 and 3.3± 0.3). While HDL significantly decreased to 16.5 ± 0.2 compared to control (24.8 ± 0.2). The hyperlipidemia induced by hypertension was significantly ameliorated ($P < 0.05$) by co-treatment with TPE (100 and 200 mg/kg). Similarly, the plasma TG level of L-NAME only treated hypertensive rats was significantly ($P < 0.05$) higher (96.5 ± 4.2). As shown in Table 3, co-treatment with TPE significantly decreased ($P < 0.05$) plasma level of TG to 72.5 ± 0.8 and 79.7 ± 0.2 at doses of 100 and 200 mg/kg.

Effects of TPE on Creatinine, Urea, Bilirubin, and Total Proteins

The L-NAME only treated hypertensive rats produced significant ($P < 0.05$) increases in plasma creatinine, urea, and bilirubin, when compared with the control. Co-treatment with TPE (100 and 200 mg/kg) significantly decreased these parameters ($P < 0.05$) toward control group [Table 4]. Furthermore, plasma levels of both albumin and total proteins in L-NAME only treated hypertensive rats were significantly lower ($P < 0.05$) compared with TPE co-treated and control rats [Table 4].

Effect of TPE on Histology of the Heart and Liver

As shown in Figure 4, histological section of the heart showed no visible lesion in the control rats. However, there was a moderate congestion of the coronary vessels of the L-NAME only group (40 mg/kg). There were no visible lesions seen in the TPE co-treated groups. As shown in Figure 5, histological section of the liver showed no visible lesion in the control rats. In L-NAME only treated group (40 mg/kg), there was a mild portal congestion and peritubular cellular infiltration. In TPE co-treated groups, there was also mild vascular degeneration of hepatocytes, mild portal congestion with peritubular cellular infiltration.

Table 3: Effect of TPE on plasma total cholesterol, triglycerides, HDL, LDL, and VLDL levels in L-NAME-induced hypertensive rats

Treatment	Control	L-NAME (only)	L-NAME+TPE (100 mg/kg)	L-NAME+TPE (200 mg/kg)
Cholesterol (mg/dl)	46.2±4.6	77.4±0.3	45.6±7.2$^{\#}$	57.7±1.7$^{\#}$
Triglyceride(mg/dl)	74.6±2.8	96.5±4.2*	72.5±0.8$^{\#}$	79.7±0.2
HDL (mg/dl)	24.8±0.2	16.5±0.0*	25.4±0.2	28.7±0.5
LDL (mg/dl)	18.3±0.1	67.5±0.1*	23.3±0.3$^{\#}$	25±0.3$^{\#}$
VLDL (mg/dl)	3.3±0.3	7.3±0.7*	3.8±0.5$^{\#}$	2.4±0.3$^{\#}$

Data are presented as (mean±SEM value $N=6$ rats) $^*P<0.05$ as compared with control group; $^{\#}P<0.01$ as compared to L-NAME hypertensive group. L-NAME: N-nitro-L-arginine methyl ester, TPE: *Tridax procumbens* extract, SEM: Standard error of the mean, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very-low-density lipoprotein

Table 4: Effect of TPE on plasma creatinine, urea, bilirubin, albumin, and total proteins levels in L-NAME-induced hypertensive rats

Treatment	Control	L-NAME (only)	L-NAME+TPE (100 mg/kg)	L-NAME+TPE (200 mg/kg)
Creatinine (mg/dl)	25.3±1.5	42.3±1.0*	27.3±0.3	26.8±0.2
Urea (mg/dl)	4.9±0.9	9.1±0.4*	4.6±0.04	4.8±0.3
Bilirubin (mg/dl)	0.2±0.01	0.7±0.1*	0.5±0.2	3.8±0.5
Albumin (mg/dl)	4.01±0.2	2.02±0.1*	4.2±0.2	3.7±0.3
T/proteins (mg/dl)	68.06±2.3	54.4±4.5*	65.17±3.6	67.4±6.2

Data are presented as (mean±SEM value N=6 rats) *P<0.05 as compared with control group. L-NAME: N-nitro-L-arginine methyl ester, TPE: *Tridax procumbens* extract, SEM: Standard error of the mean

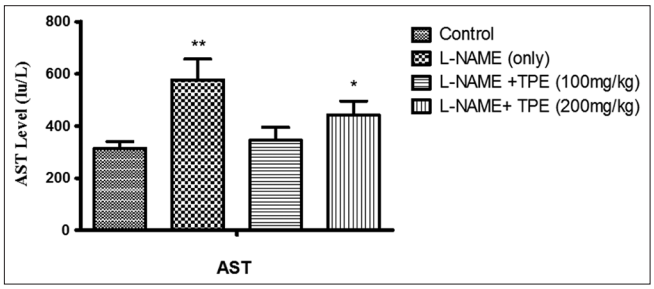

Figure 3: The effect of *Tridax procumbens* extract on plasma alanine aminotransferase levels in N-nitro-L-arginine methyl ester-induced hypertensive rats. Data are presented as (mean ± standard error of the mean value N = 6 rats) *P < 0.05; **P < 0.01 as compared with control group

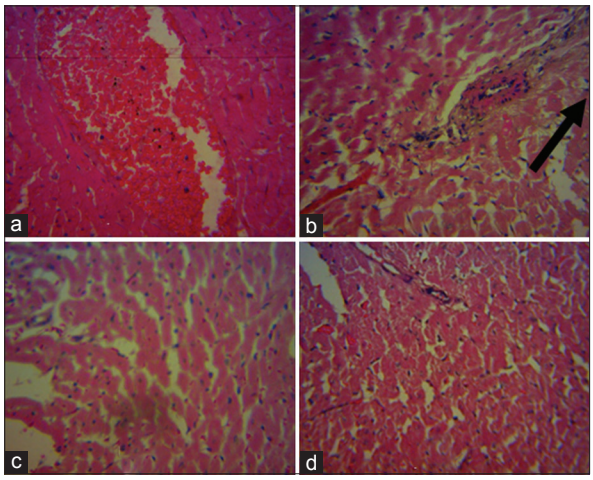

Figure 4: Photomicrographs showing the histological changes in heart in different groups (a) Group 1. Control rat liver showing normal histological appearance with no lesions. (b) Group II. N-nitro-L-arginine methyl ester (L-NAME) (40 mg/kg) hypertensive rats showing tubules appearance with moderate congestion of the coronary vessels. (c) Group III. L-NAME treated with *Tridax procumbens* extract (TPE) (100 mg/kg) showing no lesions. (d) Group IV. L-NAME treated with TPE (200 mg/kg) (H and E, ×400)

DISCUSSION

The present study examined the effects of TPE treatment on biochemical parameters in L-NAME hypertensive rats. The finding demonstrated that L-NAME-induced hypertension caused high blood pressure, low body weight, hypercholesterolemia, and hyperlipidemia, as well as increase in liver enzymes, plasma electrolytes, and cardiac histological lesions. Treatment with TPE reduced blood pressure, cholesterol, and lipid plasma levels.

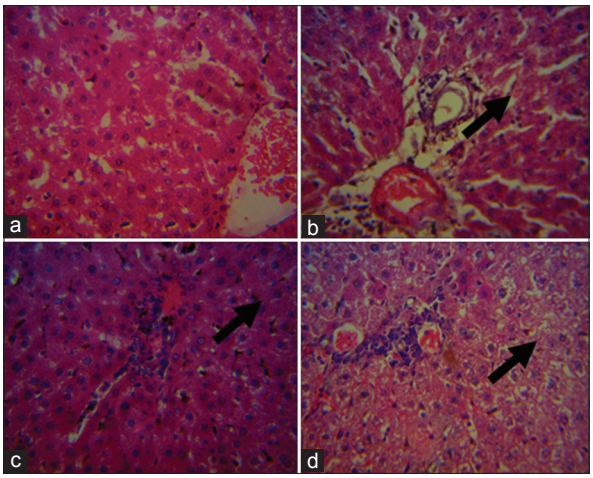

Figure 5: Photomicrographs showing the histological changes in liver in different groups (a) Group 1. Normal control rat liver showing normal histological appearance. (b) Group II. N-nitro-L-arginine methyl ester (L-NAME) (40 mg/kg) hypertensive rats showing hydropic degeneration and necrosis of hepatocytes. (c) Group III. L-NAME treated with *Tridax procumbens* extract (TPE) (100 mg/kg) showing vascular with mild portal congestion. (d) Group IV. L-NAME treated with TPE (200 mg/kg) showing diffuse vacuolar degeneration of hepatocytes (H and E, ×400)

We have previously reported that TPE reduced blood pressure and heart rate in normotensive rats [15,16]. In this study, we investigated the effect of biochemical parameters and other risk factors of CVDs using L-NAME-induced hypertensive rats.

Administration of L-NAME (40mg/kg) significantly increased the MABP while reducing the body weght gain in rats treated with L-NAME only. The decrease in body weight gains was significantly decreased in L-NAME hypertensive rats. This observation is in accordance with the previous studies [29,30]. Simultaneous administration of TPE at doses of 100 and 200 mg/kg/day with L-NAME (40 mg/kg/day) attenuated the elevated MABP and also returned the decreased body weight to control values in hypertensive rats. Although we did not monitor the food intake in this study since the animals were fed on the same standard feed throughout the experimental period. Reports indicate that changes in body weight may be used as an indicator of adverse effects of drugs or illness and the ability of an animal to gain or maintain weight may be considered a sensitive indicator of health [30]. The mechanism by which L-NAME-induced decrease in body weight is not known. However, it has been proposed that NO play a significant role in food intake and control of body weight. Deficiency of

NO was reported to decrease food intake and body weight in experimental animals [31,32].

In contrast, Khedara *et al.* [33] in their study observed that dietary supplement of L-NNA caused increase in body fat in rats [33]. The discrepancies in these studies may be due to experimental methods and the concentration of L-NNA used. In the former study, the concentration of L-NAME used was 60 mg/kg/day, whereas in the latter, 0.02% of L-NNA was added with diet. However, more work is needed to ascertain the particular effect of L-NAME at different concentration on lipids profiles and body weight.

It is well known that L-NAME model is usually characterized by high BP, hypertrophy, increased left ventricular (LV) myocardial thickness, stiffening, and the development of end-organ damage [34,35]. Our previous report showed that TPE had a favorable effect on blood pressure by dilating blood vessels, hence reducing peripheral resistance [16]. In this present study, TPE at doses of 100 and 200 mg/kg may possibly have served as a cardiotonic agent because it prevents L-NAME-induced enlargement of the heart. This is an indication that regular intake of TPE may protect the heart since the weight of the heart in the TPE co-treated groups as not significantly increased in this study.

This study demonstrates that treatment with a single daily dose of TPE over 3 weeks significantly reduced blood pressure in L-NAME-induced hypertensive rats. The TPE untreated rats continued to have sustained high pressure level. The mechanism by which TPE was able to reduce the blood pressure is likely to be a combination of several beneficial factors.

It is well known that plasma sodium and potassium play crucial role in the regulation of blood pressure and the interrelationship between sodium and potassium are very important in control of arterial resistance as previously reported [36,37]. We observed in this study that chronic inhibition of nitric oxide synthase (NOS) by L-NAME caused increase in sodium with decrease potassium levels. Administration of TPE brought back these parameters slightly comparable to control. This result is in agreement with Ikewuchi *et al.*, [38] who reported a significant decrease in plasma sodium and chloride levels, in salt-loaded rats treated with TPE. We have also reported in our previous study that TPE treatment can stimulate the opening of calcium-activated potassium channel in superior mesenteric artery of normotensive rats [39]. This may lead to hyperpolarization and relaxation of the smooth muscles to aid increase blood flow.

Our data showed that coadministration of TPE at doses of 100 and 200 mg/kg produced 41 and 25.4% reduction in plasma cholesterol level, respectively, compared with L-NAME group. Similarly, TPE at the same doses decreased plasma LDL by over 65 %. Lowering cholesterol and LDL levels have been linked to a lower risk of CVD [24]. It is also obvious from our result that the effect of TPE on lipid metabolism is not dose-dependent and that 100 mg/kg TPE elicited maximum effects on lipid metabolism in the animal studied. The results suggest that the administration of TPE to L-NAME hypertensive rats clearly does not alter L-NAME-induced hepatic TG biosynthesis and also activate redistribution of cholesterol among the different lipoprotein molecules.

The mechanism of the hypocholesterolemic action of TPE is presently not known. However, a study indicated that decrease in plasma nitric oxide (NO) level is one of the associated factors for the development of arteriosclerosis and CVDs [40]. Furthermore, other studies indicated that infusion or administration of L-arginine (a NO donor), decreased TG and cholesterol, and increased HDL and HDL/TC ratio [41,42]. Inhibition of NOS in rats caused elevation of the blood lipids confirming the earlier reports [43]. Therefore, the observed reduction in cholesterol and LDL levels in our study may be due to activation or stimulation of NOS by the constituents presents in TPE. Although we did not measure the NOS activity or NO in this study, our recent report indicated that TPE increases the production of nitrite in aortic ring isolated from normotensive rats in a dose-dependent manner [44]. Therefore, results of this study confirmed that TPE reduce the incidence of CVD through it effect on NOS activation.

Another observation drawn from this study is the significant increase in the AST, ALP, bilirubin, and urea in the L-NAME only treated hypertensive group. It is well known that plasma ALT and AST activities are the most reliable laboratory indicator of hepatotoxic effects. The increased serum levels of bilirubin, ALT after the administration of L-NAME in this study could be due to toxicity effects of L-NAME on hepatocytes. Damaged hepatocytes release their content including ALT and AST into the extracellular space [45]. The released enzymes ultimately enter into circulation and thereby increase the serum level of ALT and AST [45].

Repeated treatment with TPE significantly decreased the level of AST, ALT, and bilirubin. This was consistent with other reports which showed that TPE ameliorates hepatocellular injury and initiate liver parenchymal cell regeneration in hepatitis rat model [46,47]. Histopathological studies of the liver and heart also provide supportive evidence for observed biochemical changes.

Although TPE has been reported to ameliorate hepatocellular injury and parenchymal cell regeneration [46,47], co-treatment with TPE in this study showed that histopathological damage was not completely ameliorated. This we suggest may be due to limited duration for which the treatment was done in this study.

The observed significant decrease in urea and creatinine levels in the TPE co-treated groups accentuate its therapeutic advantage and possible ability at preventing hyperuricemia and creatininuria, a condition that can predispose to dysfunction and development of nephritis [48]. Epidemiologic studies showed that circulating high uric acid levels are associated with the prevalence of hypertension [49,50]. Increased uric acid levels have been linked with progression of chronic kidney diseases [51] and renal failure [51]. The mechanisms by which TPE reduced the plasma level of urea and creatinine are not

known; more studies will be needed to identify the active ingredient in TPE and their roles in regulating urea levels.

Phytochemical analysis of *T. procumbens* leaf revealed chemical constituents with potent anti-inflammation, antioxidant, and antihypercholesterolemia effects. Some of the compound identified includes lutein, vanillic acid, carotenoids, catechins, quercetin, linolenic, and alpha-linolenic acid [52,53].

Reports have shown that lutein reduced the risk of atherosclerosis by inhibiting autoxidation of cellular lipids and protect against oxidant-induced cell damage [54,55]. At higher doses lutein also restored the inhibited NO production to normal level in L-NAME-induced hypertensive rats [56]. Kumar *et al.* [57] in their study showed that vanillic acid a benzoic acid derivative ameliorate the increase in blood pressure through upregulating the endothelial NOS expression of L-NAME-induced hypertensive rats [57]. Quercetin propelled vasodilator effects had also been reported [58]. While the antihypertensive, anticholesterolemia, antilipidemia, cardiac protection, and hepatoprotection effects of linolenic acid and alpha-linolenic acid have been documented in experimental and epidemiological studies [59,60]. Therefore, these identified compounds singly or in combination with other constituents present in the extract may be responsible for the antiatherogenic and hypolipidemic effects observed in this study.

In conclusion, evidence from the present study confirmed the potential antihypertensive effects of TPE on blood pressure, lipid, and cholesterol levels in L-NAME-induced hypertensive rats. TPE exhibited hypocholesterolemic effects by reducing plasma levels of cholesterol and LDL in the animals investigated. Therefore, TPE shows therapeutic promise in the management of hypertension and possible related cardiovascular pathologies. However, more efforts are still required to elucidate the molecular mechanism responsible for the antihypertensive effect of TPE and CVDs.

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