**Medicinal and Aromatic Plant Research Journal**
**Vol. 2(1), pp. 6-17, March 2014**
**Research Paper**

# Efficacy studies on *Mist Diodia,* a herbal preparation for the management of hypertension in rodents

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Accepted 24th February, 2014

***Abstract.*** The anti-hypertensive property of *Mist Diodia*, a herbal preparation for the management of hypertension, was assessed in two murine models of hypertension. Systolic and diastolic blood pressures were recorded using the Tail-cuff method under non-anaesthetized conditions. *In vitro* tissue chymase enzyme activity assay, serum and urine concentration of sodium and potassium ions and urine volumes were measured, to ascertain the possible involvement of the renin-angiotensin-aldosterone system (RAAS) in the anti-hypertensive activity. Lipid profiles and serum total antioxidant concentration were determined to find out if anti-hypertensive activity was due to improved lipidemia. The results indicated that *Mist Diodia* possesses significant ($p < 0.05$) anti-hypertensive activities in the two models used in this present study. *Mist Diodia* significantly ($p < 0.05$) increased urine output in all the models used. Sera from animal groups treated with *Mist Diodia* significantly ($p < 0.05$) inhibited tissue chymase enzyme activity *in vitro*. The antioxidant status and lipid profiles were significantly ($p < 0.05$) enhanced. Serum concentrations of sodium and potassium were significantly ($p < 0.05$) reduced while the corresponding urine concentrations of these two ions increased in all the animal models. It could therefore be concluded, that *Mist Diodia* has anti-hypertensive activity which may be mediated through the RAAS with accompanying diuresis. Improvement in lipidemia could be a possible mechanism for its action. These findings thus, support its ethno-medicinal use.

**Keywords:** *Mist Diodia, Diodia scandens, Aframomum melegueta,* SHRs, L-NAME, anti-hypertensive, SDRs.

## INTRODUCTION

Hypertension, a major risk factor for cardiovascular diseases (CVDs) (Ezzati et al., 2002) affects approximately 25% of the adult population worldwide and its prevalence is predicted to increase by 60% by 2025, when a total of 1.56 billion people may be affected by the condition (Kearney et al., 2005). Despite the availability of several classes of anti-hypertensive agents, the condition is poorly controlled worldwide. Some of the standard anti-hypertensive agents used in the management of hypertension do have serious side effects. For example, some diuretics may cause hypokaleamia and hypercalceamia (Keith, 2005). Sympatholytic anti-hypertensive agents, for example atenolol, may decrease high density lipoprotein cholesterol (HDL-C) concentration, or cause impotence and depression, while a combined therapy involving beta-blockers and vasodilators may cause severe bradycardia, heart blockade or pump dysfunction (Keith, 2005). The limitations of some of the standard anti-hypertensive agents together with other factors such as cost have necessitated the search for alternatives which includes herbal medicines. At the Centre for Plant Medicine Research (CPMR), Mampong, Ghana, *Mist Diodia* (MD) has been developed as an herbal alternative for the management of hypertension, based on ethno-pharmacological information obtained from local herbalists. Though MD

has been used for more than 25 years its anti-hypertensive activity has not been evaluated in preclinical studies. Hence, we sought to assess the anti-hypertensive property of MD in rodent hypertensive models.

The present study evaluates the potential anti-hypertensive effects of MD in two non-anaesthetized classical models of experimental hypertension: genetically modulated hypertension (spontaneously hypertensive rats; SHRs) and hypertension induced by N-nitro-L-arginine methyl ester (L-NAME); inhibitor of nitric oxide synthase (NOS), through the measurement of systolic, diastolic and mean arterial blood pressures. To understand potential mechanism of action we also evaluated the effect of MD preparation and its two component extracts; *Diodia scandens* (DS) and *Aframomum melegueta* (AM) on serum concentrations of nitric oxide (NO), cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP), prostaglandin $E_2$ ($PGE_2$), and total antioxidant concentration (TAC). The effect of the preparations on lipid profiles, serum concentrations of sodium, potassium, calcium and magnesium, urine concentrations of sodium, potassium and urine outputs were also determined. The effect on tissue chymase enzyme activity was evaluated *in vitro* to ascertain if the anti-hypertensive activity of the plant preparations involves the RAAS.

## MATERIALS AND METHODS

### Reagents and chemicals

Kits for total cholesterol (TC,) triacylglycerol (TAG), and high density lipoprotein cholesterol (HDL-C) were procured from Elitech Clinical Systems (Sees, France). N-nitro-L-arginine methyl ester (L-NAME) and the standard anti-hypertensive drug, chlorothiazide, together with kits for the estimation of chymase activity and total antioxidant concentration (TAC), were obtained from Sigma-Aldrich Chemical Company (MO, USA). The evaluation of cGMP, $PGE_2$ and cAMP concentrations was carried out with kits procured from Cayman Chemical Company (MI, USA). Estimation of NO concentration was carried out with kits obtained from BioAssay Systems (CA, USA). Standard anti-hypertensive drugs atenolol, captopril and nifedipine were all procured from Ernest Chemist, Accra, Ghana. All drugs were dissolved in distilled water.

### Plant material

*D. scandens* (aerial parts) were obtained from the Akwapim Ridge, Eastern Region, Ghana, and seeds of *A. melegueta*, from Praso, Central Region, Ghana, between the months of January to March. Both plant materials were authenticated by the staff of the Plant Development Department, CPMR, Mampong, Ghana and voucher specimen kept at the same Department. Aerial parts of *D. scandens* were shade-dried for a period of 4 weeks. *A. melegueta* seeds were also shade-dried for the same period of time.

### Animals

All experiments done with animals were reviewed and approved by the Scientific and Ethics Committee of the CPMR, Mampong, Ghana. Male Sprague-Dawley rats (SDRs) were procured from the animal experimentation facility of the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Ghana, and bred at the Animal Unit of CPMR, Mampong, Ghana. Spontaneously hypertensive rats (SHRs) of the Okamoto-Aoki strain were obtained from Taconic Farms Inc (NY, USA) and bred at the animal experimentation facility, of the NMIMR, University of Ghana, Legon. The animals were housed in aluminium cages with standard bedding of wood shavings. They were allowed to acclimatize for 14 days under standard environmental conditions made up of room temperature of 27°C, relative humidity not less than 30% and not more than 70% and 12 h each of light and darkness. The animals were provided with standard feed obtained from Ghana Agro Food Company (GAFCO), Tema, Ghana, and sterile water *ad libitum*.

### Plant extracts preparation

The preparation of the *Mist Diodia,* (MD) and aqueous extracts of *D. scandens* (DS) and *A. melegueta* (AM) were done in accordance with protocol provided by the production department of the CPMR, Mampong, Ghana. The DS extract was prepared by boiling 2.5 kg of crushed shade-dried aerial portions in 60 L of water for 25 min. The resulting solution was allowed to cool and filtered with Whatman No. 1 filter paper. The filtered preparation was freeze-dried (Heto Power Dry LL 3000, Jouan, Nordic, Denmark) and stored. The seeds of AM were extracted by boiling 25 g of shade-dried seeds for 25 min in 60 L of water and the resulting extract treated as described earlier for DS*. Mist Diodia* was prepared by boiling together 2.5 kg of DS and 25 g of AM in 60 L of water for 25 min and the resulting solution treated as described previously for DS and AM. Prior to the administration to experimental animals the freeze-dried plant preparations were reconstituted in sterilized water.

### Measurement of blood pressures

The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by the Tail-cuff method

(Rocha et al., 2008) in non-anaesthetized rats using a blood pressure recorder for rodents (UGO Basile 3500, Comerio-Varese, Italy). During the period of acclimatization of 14 days, the rats were trained to stay calm in a restrainer until the blood pressure (BP) of each rat was recorded with minimum restraint and stress. The first SBP and DBP measurements were discarded and a mean of 3 subsequent measurements was recorded. The mean arterial pressure (MAP) was calculated for each rat using the formula:

MAP = DBP + 1/3 (SBP – DBP) (Adeboye et al., 1999).

**Measurement of urine output**

Using metabolic cages, urine outputs of control groups and those treated with the standard anti-hypertensive agent, chlorothiazide and the three plant preparations (n = 5), were measured. Urine output of these animals were recorded before treatment (day 0) and then daily in the morning during the period of treatment. The mean urine output for days 0, 5, 10, 15, 20, 25 and 30, were calculated for each rat. Portions of the urine samples (3.0 ml) of each rat were pipetted into Eppendorf tubes on days 0 and 30 and stored at -20°C for elemental analysis.

**Experimental models**

***Spontaneously hypertensive rats***

A total of 80 male adult SHRs (300 to 350 g) were selected and randomly divided into 8 groups with 10 rats in each group. Groups 1 to 4 constituted the positive controls. The plant preparation-treated groups were designated as 5 to 7 and group 8 was the negative control. Baseline SBP, DBP and MAP of the rats were recorded after which groups 1 to 4 were treated orally with either atenolol, chlorothiazide, nifedipine or captopril, at previously determined median effective dose ($ED_{50}$) values of 30, 20, 30 and 100 mg/kg/day, respectively. The plant preparation-treatment groups received oral treatments of MD (30 mg/kg), DS (40 mg/kg) and AM (80 mg/kg). The negative control group received only distilled water. All the groups were treated daily for 30 days, during which SBPs and DBPs, of each rat were recorded at 5-day interval. The corresponding MAP values of each rat were also calculated.

***L-NAME hypertensive rats***

To induce L-NAME hypertension, a total of 100 male adult SDRs were selected and allowed to acclimatize for 14 days. The animals were next orally treated daily with L-NAME - 50 mg/kg/day (Rocha et al., 2008) for 30 days, after the baseline SBP, DBP and MAP of each rat have been recorded. During the period of treatment, SBP, DBP and MAP of each rat were recorded weekly. By the end of the $4^{th}$ week, a total of 80 rats weighing 300 to 350 g with SBP and DBP of more than 140 and 90 mmHg, respectively were selected and randomly divided into 8 groups of 10 rats each. The animals in the various groups were treated as described earlier for the SHRs.

**Serum nitric oxide (NO) concentration**

The sera of the negative control groups and those treated with the standard anti-hypertensive drug, nifedipine and the three plant preparations in the two experimental models were analyzed for NO content. A colorimetric method was used for this determination using the 96-well procedure with NO diagnostic kits (BioAssay Systems, CA, USA) according to the manufacturer's instructions.

**Serum cyclic guanosine monophospate (cGMP) concentration**

Serum concentration of cGMP was also measured for the groups used for the NO determination. The method involved a competitive enzyme immunoassay (EIA) (Pradelles and Grassi, 1989). The procedure followed was as per the protocol contained in the commercial assay kit (Cayman Chemical, MI, USA).

**Serum cyclic adenosine monophosphate (cAMP) concentration**

The concentration of cAMP was determined in sera of animals used as negative controls and those treated with atenolol and the plant preparations. The EIA method (Pradelles et al., 1989) was used for this assay following standard protocol contained in the cAMP ACE competitive EIA assay kit (Cayman Chemical, MI, USA).

**Serum prostaglandin $E_2$ ($PGE_2$) concentration**

This assay was performed using sera from animals treated and those not treated with the plant preparations. The competitive EIA method (Pradelles et al., 1985; Maclouf et al., 1987) was used following the protocol contained in the $PGE_2$ Express EIA monoclonal kit (Cayman Chemical, MI, USA).

***In vitro* tissue chymase enzyme activity**

*In vitro* chymase enzyme activity was determined per standard procedures contained in the Chymase assay kit

(Sigma, MO, USA). It was carried out on the sera from animals of the various models treated and not treated with the plant preparations.

### Serum total antioxidant concentration (TAC)

The serum TAC was determined for both treated and non-treated animals in all the experimental models. The determination involved the colorimetric method using commercial antioxidant diagnostic kit (Sigma, MO, USA) with Trolox (water-soluble vitamin E analog), as standard and in accordance with the manufacturer's instructions.

### Determination of serum lipid profile

Serum TC, TAG and HDL-C concentrations, were evaluated in male adult SHRs, and L-NAME hypertensive treated and non-treated rats. These estimations were done colorimetrically following the manufacturer's instructions, using EliTech Clinical Systems diagnostic kits (Sees, France). Low density lipoprotein cholesterol (LDL-C) content was calculated from the Friedewald formula:
LDL-C = TC ─ HDL-C ─ TAG/5 (Johnson et al., 1997).

### Elemental analyses

Sera samples of adult male rats in the various experimental groups treated and not treated with the standard anti-hypertensive agent, chlorothiazide and the plant preparations were analyzed for sodium, potassium, calcium and magnesium ions concentrations. Urine concentrations of sodium and potassium ions were also determined in the same experimental groups. These determinations were carried out in accordance with protocols established at the Ghana Research Reactor-1 Centre of Ghana Atomic Energy Commission (GAEC), Kwabenya, Accra.

### Statistical analyses

Statistical evaluation was performed using SPSS statistical software version 16.0 for Windows XP. All results are presented as means ± S.E.M. Data was analyzed using one-way analyses of variance (ANOVA) to determine statistical differences between groups and when necessary, Tukey post hoc analyses. Significant difference was set at $P < 0.05$.

## RESULTS

### Effect of treatments on SHRs

The time-course of the plant preparations on the SBP, DBP and MAP in SHRs over a 30-day period are presented in Figure 1. The SBP was reduced for all the treatment groups (Figure 1A). Compared with the negative control group, these reductions (22 to 53%) were statistically significant ($p < 0.05$). The degrees of reduction (44 to 53%) by the standard anti-hypertensive drugs atenolol, chlorothiazide, nifedipine and captopril and *Mist Dodia* (MD) were similar over the 30-day period. However, compared to the standard anti-hypertensive drugs and MD, the degree of reductions by *Diodia scandens* (DS) (31%) and *Aframomun melegueta* (AM) (22%) were lower and statistically significant ($p < 0.05$). Compared to values for AM, the degree of SBP reduction by DS was higher and statistically significant ($p < 0.05$).

Results for DBP are presented in Figure 1B. Baseline DBP did not show statistically significant differences ($p > 0.05$), between the groups. Between days 5 and 30 of treatment, the treatment groups had reductions in DBP of between 7 and 27% which were statistically significant ($p < 0.05$). The reduction in DS- and AM-treated SHRs could not be sustained after the initial five-day reduction. There was a gradual increase of about 13% for AM and 7% for DS by day 30. The difference between these two treatment groups was statistically significant ($p < 0.05$). The degrees of reduction by MD and the standard anti-hypertensive drugs (24 to 28%) over the treatment period were similar and there were no statistically significant differences between them ($p > 0.05$).

Figure 1C shows the effect of treatments on the MAP in SHRs. There were no statistically significant differences ($p > 0.05$) between the baseline MAP values for all the groups. By the $5^{th}$ day of treatment, all the treatment groups had significant drops in MAP values (24 to 29%), with the exception of the AM-treated groups. The degree of MAP reduction (29 to 30%) was comparable and statistically significant ($p < 0.05$) in SHRs treated with the standard anti-hypertensive drugs; atenolol, chlorothiazide, nifidipine, captopril and MD by day 30 of treatment. No statistically significant differences ($p > 0.05$) were observed between these groups treated with the standard anti-hypertensive drugs and MD. After the initial reduction in MAP by DS to about 24% below the baseline value on day 5, there was a gradual increase to about 21% below the baseline, by day 30. Throughout the treatment period, SHRs treated with AM had about 7% reduction in MAP. The difference between these two treatment groups was statistically significant ($p < 0.05$).

### Effect of treatment on L-NAME hypertensive rats

The SBP in L-NAME hypertensive rats during the 30-day treatment period are shown in Figure 2A. Baseline SBP was similar for all the groups ($p > 0.05$). All the treatment groups with the exception of AM had significant ($p < 0.05$) reduction in SBP (20 to 46%), by the $5^{th}$ day of treatment. The extent of SBP reductions was similar for all the treatment groups on day 10. However, from day 15, groups treated with DS and AM showed a gradual increase in

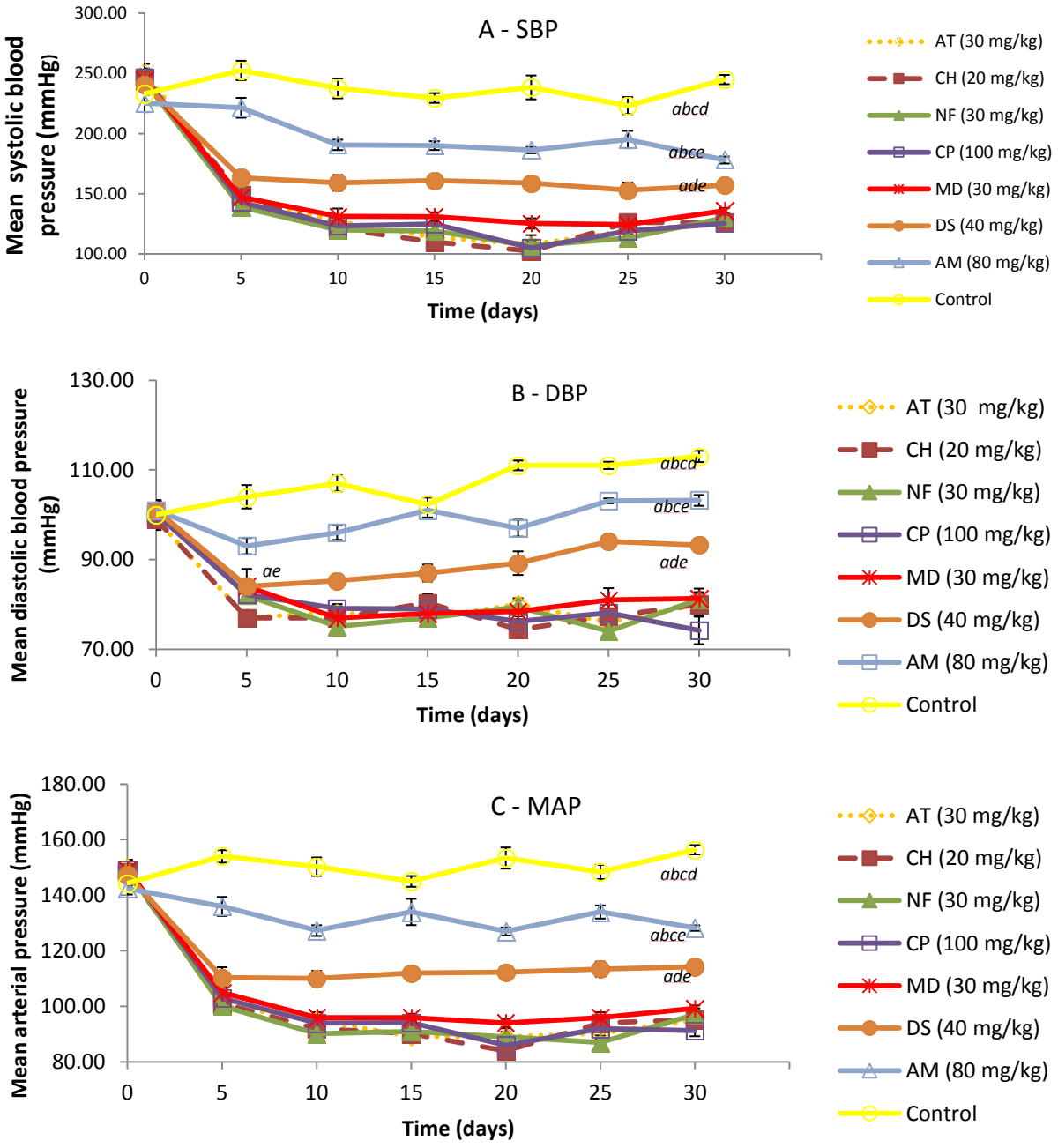


**Figure 1.** Effect of oral treatment with atenolol (AT), chlorothiazide (CH), nifedipine (NF), captopril, (CP) *Mist Diodia* (MD), *D. scandens* (DS) and *A. Melegueta* (AM) on SBP (A), DBP (B) and MAP (C), of male adult SHRs**.** Each point represents mean ± SEM. for n = 10. [a]Significantly different from control group ($p < 0.05$). [b]Significantly different from AT, CH, NF and CP ($p < 0.05$). [c]Significantly different from MD ($p < 0.05$). [d]Significantly different from DS ($p < 0.05$). [e]Significantly different from AM ($p < 0.05$).

SBP after the initial reductions in SBP. By day 30, the increase was about 7% for DS-treated rats and about 13% for AM-treated ones. The difference between these two treatments groups were statistically significant ($p < 0.05$). The degree of SBP reduction was similar and statistically significant ($p < 0.05$) in those treated with the standard anti-hypertensive drugs and MD (45 to 46%) by day 30 of treatment. The extent of SBP reductions was similar for the standard drugs; atenolol, chlorothiazide, nifedipine, captopril and the plant preparation, MD. No statistically significant differences ($p > 0.05$) were observed between these treatment groups.

Baseline DBP did not show significant differences ($p > 0.05$) between the groups (Figure 2B). By the 30$^{th}$ day of treatment there were reductions in the DBP in all the treatment groups, with the exception of AM-treated

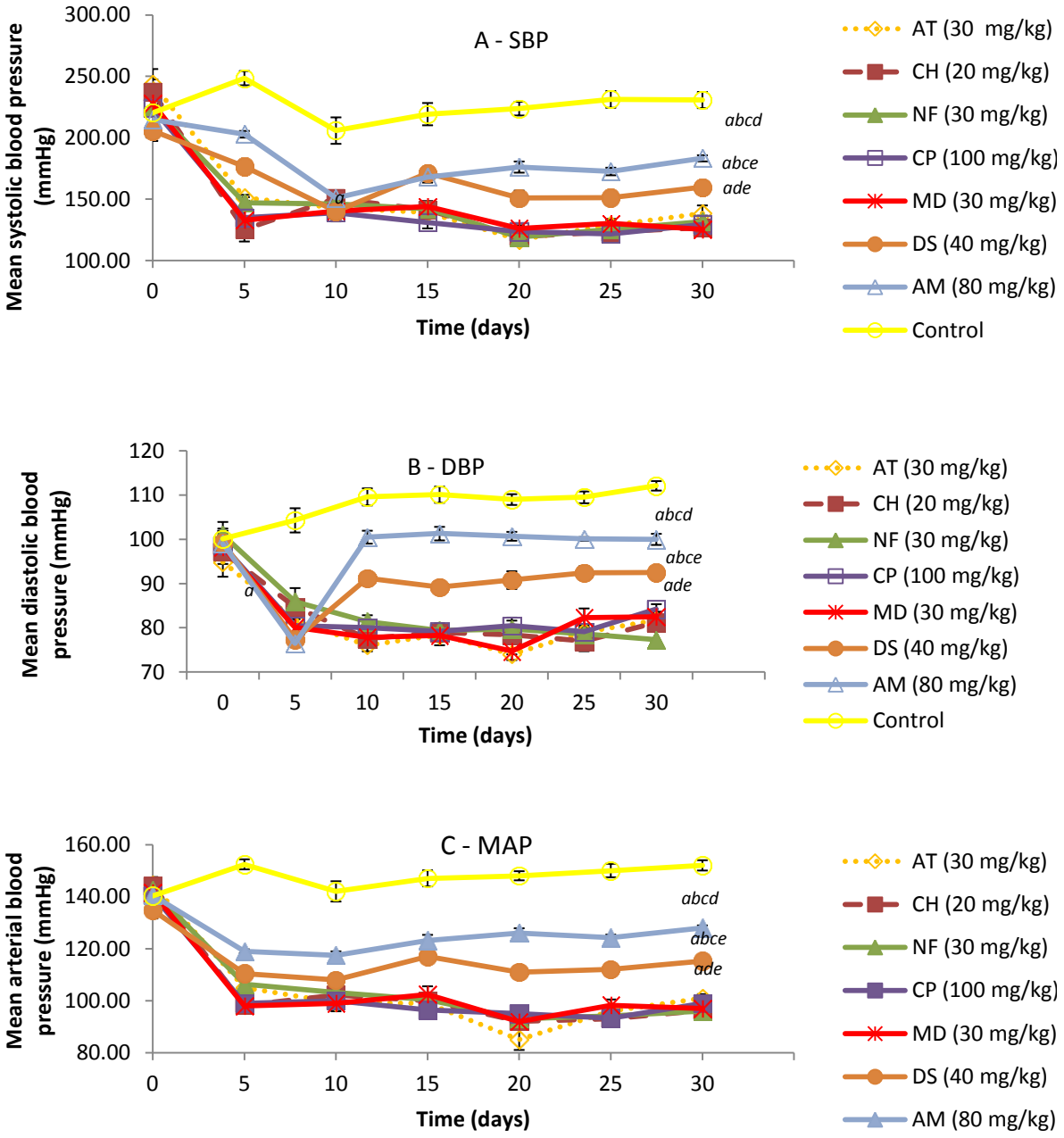


**Figure 2.** Effect of oral treatment with atenolol (AT), chlorothiazide (CH), nifedipine (NF), captopril (CP), *Mist Diodia* (MD), *D. scandens* (DS) and *A. melegueta* (AM), for a period of 30 days, on SBP (A), DBP (B) and MAP (C), of male adult L-NAME hypertensive rats. Each point represents mean ± SEM for = 10. [a]Significantly different from control group ($p < 0.05$). [b]Significantly different from AT, CH, NF and CP ($p < 0.05$). [c]Significantly different from MD ($p < 0.05$). [d]Significantly different from DS ($p < 0.05$). [e]Significantly different from AM ($p < 0.05$).

group, These reductions (6 to 23%) were statistically significant ($p < 0.05$) compared to the controls. Compared to values for rats treated with the anti-hypertensive drugs and MD, the extent of DBP drop in DS-treated group (5%) was lower but statistically significant ($p < 0.05$), by the end of treatment. The reduction in DBP produced by standard anti-hypertensive drugs was similar to that of MD (14 to 22%), by day 30. No statistically significant differences ($p > 0.05$) were observed between these treatment groups. After day 5, DBP of rats treated with DS and AM showed gradual elevation up to day 30, after an initial reduction on day 5. The difference between these two treatment groups was statistically significant ($p < 0.05$). The MAP values for day 0 did not indicate any statistically significant differences ($p > 0.05$) between the groups (Fig 2C). From days 5 to 30, all the treatment

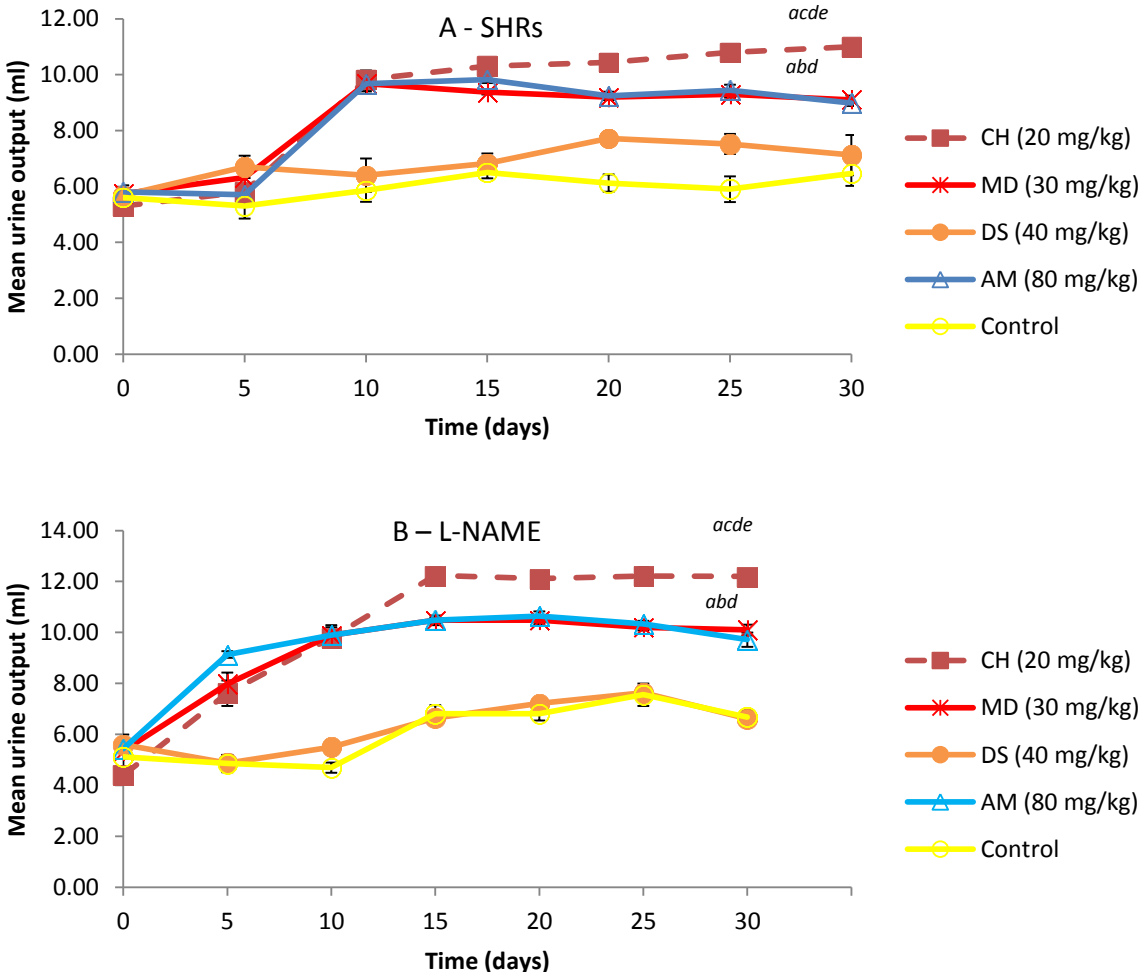


**Figure 3.** Urine output of male SHRs (A), and L-NAME hypertensive rats (B) orally treated with chlorothiazide (CH), *Mist Diodia* (MD), *D. scandens* (DS) and *A. melegueta* (AM). Each point represents mean ± SEM for n = 5. [a]Significantly different from control group ($p < 0.05$). [b]Significantly different from CH group ($p < 0.05$). [c]Significantly different from MD group ($p < 0.05$). [d]Significantly different from DS group ($p < 0.05$). [e]Significantly different from AM group ($p < 0.05$).

groups had reduced MAP values. Compared to the negative control group, these reductions (16 to 39%) were statistically significant ($p < 0.05$). Between days 15 and 30, the extent of reductions (13 to 21%) in MAP produced in DS and AM treated rats were lower compared to levels recorded for the standard anti-hypertensive drugs and MD (36 to 39%). Over the same period, the reductions by the standard anti-hypertensive drugs and MD appeared to be similar ($p > 0.05$). After the initial reduction in MAP values of groups treated with DS (23%) and AM (15%) on days 5 and 10, there were gradual increases (7 to 8%) up to day 30. However, compared to AM, DS-treated rats had significantly ($p < 0.05$) lower MAP values by day 30.

## Urine output

Urine output of the rats after treatment with standard diuretic, chlorothiazide (20 mg/kg) and the plant preparations, MD (30 mg/kg), DS (40 mg/kg) and AM (80 mg/kg) are shown in Figure 3. Urine output on days 0 and 5 in SHRs (Figure 3A), did not show significant differences ($p > 0.05$) among the groups. Groups treated with chlorothiazide (CH), MD and AM showed increased urine output from days 10 to 30. These increases (23 to 93%) were significant ($p < 0.05$). Compared with the increase recorded for CH-treated SHRs (93%) by day 30, values for MD (23%) and AM (23%) were significantly ($p < 0.05$) lower. Urine output was not significantly increased ($p > 0.05$) in DS-treated SHRs.

Urine outputs in L-NAME hypertensive rats are shown in Figure 3B. Baseline output appeared to be the same for all the groups ($p > 0.05$). With the exception of the DS group, urine output in the other treatment groups increased from days 5 to 30. Compared with the untreated group these increases (100 to 179%) were significant ($p < 0.05$). The increase in urine output (100%)

in groups on MD and AM was lower and significant ($p < 0.05$) compared with value for the CH-treated rats (179%). The extent of increase was similar for MD- and AM-treated rats, by the end of the experimental period.

**Modulation of serum NO, cGMP, cAMP and PGE production**

The plant preparations did not significantly alter the serum concentrations of NO, cGMP, cAMP and $PGE_2$ in all experimental models studied (results not shown).

**Inhibition of tissue chymase enzyme activity**

Inhibition of tissue chymase enzyme activity determined *in vitro* using sera of SHRs, and L-NAME hypertensive rats to establish possible mechanism for the anti-hypertensive action of the plant preparations, are presented in Figure 4. Results indicated that there were no significant differences ($p > 0.05$) in tissue chymase activity between the controls at baseline, and in all animal models. For the SHR and L-NAME models, the standard control, chymostatin caused significant ($p < 0.05$) inhibition of the chymase activity of 52 and 83%, respectively, compared to the sera of the controls. Also, sera of SHRs and L-NAME hypertensive rats treated with MD and DS caused significant ($p < 0.05$) inhibition of tissue chymase activity 29 and 48%, respectively. However, there was no significant ($p > 0.05$) reduction in tissue chymase activity by AM *in vitro* in all animal models.

**Serum total antioxidant and total cholesterol concentrations**

The effects of treatment of different animal models (SHRs, and L-NAME hypertensive rats) with MD, DS and AM on serum total antioxidant concentration (TAC) and total cholesterol (TC) at baseline and at the termination (not shown) indicated that baseline concentration of serum TAC and TC in all animal models was not significantly different ($p > 0.05$) from control and AM treatment groups. At the end of the treatment period MD and DS caused similar and significant elevations ($p < 0.05$) of TAC in SHRs (25%), and in L-NAME hypertensive rats (25%). The two preparations MD and DS caused significant reductions ($p < 0.05$) of serum TC concentrations in SHRs, and L-NAME hypertensive rats at termination of treatment.

**Serum HDL-cholesterol (HDL-C) concentration**

The results for serum HDL-C concentration of adult male SHRs and L-NAME hypertensive rats treated with MD, DS and AM (not shown), did not show significant differences ($p > 0.05$) between baseline concentration of serum HDL-C of controls and AM-treated rats and at termination of treatment, in all animal models used. However, there were significant ($p < 0.05$) increases in serum HDL-C concentration in MD- and DS-treated rats, in SHRs (20%) and L-NAME hypertensive rats (15%).

**Serum LDL-cholesterol (LDL-C) concentration**

Serum LDL-C concentration in male adult SHRs and L-NAME hypertensive rats animal models, at baseline and termination of treatment with MD, DS and AM (not shown), did not indicate significant differences ($p > 0.05$) between baseline values of controls and AM-treated rats and at termination of treatment. However, there were significant ($p < 0.05$) reductions in LDL-C concentration in MD and DS-treated rats, in SHRS (46%) and L-NAME hypertensive rats (85 to 91%) albeit differences between MD and DS-treated rats were not statistically significant ($p > 0.05$).

**Serum HDL-C/LDL-C ratio**

Results obtained for HDL-C/LDL-C ratios in SHRs and L-NAME hypertensive rats (not shown) showed significant ($p < 0.05$) increases in MD and DS-treated rats; in SHRs (43 to 71%) and L-NAME rat (160 to 200%). Although not significant ($p > 0.05$), the ratio for MD was higher than that obtained for DS. Also, at termination of treatment the controls had lower ratios compared to the baseline value in animal models used but these decreases were not significant ($p > 0.05$).

**Serum triacylglycerol (TAG) concentration**

There were no significant differences ($p > 0.05$) in serum TAG concentration between AM-treated rats and controls, at termination of treatment in all animal models (not shown). The concentration of TAG in MD and DS-treated rats was significantly ($p < 0.05$) reduced in SHRs (75 to 100%) and L-NAME hypertensive rats (17%) animal models, at termination of treatment.

**Serum sodium, potassium, magnesium and calcium ions concentrations**

Serum concentrations of sodium and potassium ions at the termination of treatment with standard drug, chlorothiazide (CH), MD, DS and AM in the various models (not shown), did not show significant ($p > 0.05$) difference in serum sodium and potassium ion concentrations, between baseline concentrations and those of controls and DS-treated rats. However, there

were similar and significant ($p < 0.05$) degrees of reduction in serum sodium ion concentration, by MD (30 to 42%) and AM (30 to 33%), compared to DS-treated rats and controls, in all animal models. Chlorothiazide produced the highest reduction (60 to 68%) in serum sodium ion concentration, in both animal models.

Serum potassium ion concentration reductions caused by CH, MD and AM were similar in SHRs (34%) animal model, at termination of treatment. However, the significant reduction ($p < 0.05$) in serum potassium ion concentration caused by MD and AM in L-NAME hypertensive rats were similar (63%), whilst that caused by CH was 84%. There were however, no significant differences ($p > 0.05$) in serum magnesium and calcium ion concentrations between all animal treatment groups, at termination of treatment and at the baseline in all animal models (results not shown).

### Urine sodium and potassium ion concentrations

Results of urine sodium and potassium ion concentrations in SHRs, L-NAME hypertensive rats animal models, after termination of treatment with CH, MD, DS and AM (not shown), did not indicate significant differences ($p > 0.05$) in urine sodium or potassium ion concentrations, between baseline values and controls and DS-treated rats. However, there were significant ($p < 0.05$) elevations in urine sodium ion concentration by CH, MD and AM in all animal models. The control drug, CH, caused the highest elevation (52 to 110%) whilst MD and AM produced similar degrees of elevations in SHRs (31 to 32%) and L-NAME hypertensive rats (80 to 84%), at termination of treatment. In the case of urine potassium ion concentration, there were similarly significant ($p < 0.05$) degrees of elevations in CH, MD and AM- treated rats in SHRs (103%) and L-NAME hypertensive rats (60%).

## DISCUSSION

This study shows for the first time a significant anti-hypertensive effect of *Mist Diodia* (MD) and the contribution of its component plants; *D. scandens* (DS) and *A. melegueta* (AM), to this effect in spontaneously hypertensive rats (SHRs) and N-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats.

Spontaneously hypertensive rats have previously been used by others as a model for essential or primary hypertension (Kundu and Rao, 2008). Similarly, rodents treated with L-NAME were used as a good model for secondary hypertension (Küng et al., 1995). The SBP, DBP and MAP are routinely measured to diagnose hypertension (Keith, 2005). The reduction of these blood pressures in both SHRs and L-NAME hypertensive rats by MD and its components, DS and AM suggested anti-hypertensive properties of these plant preparations. Rocha et al. (2008) had previously reported reductions in these pressures in male adult Wistar rats co-treated with L-NAME (50 mg/kg/day) and hydro-alcoholic preparation of *Euterpe oleracea* and suggested that the plant has anti-hypertensive properties. In all the results, the extent of reduction in the blood pressures by MD appeared to be similar to those of the standard anti-hypertensive agents (atenolol, nifedipine, chlorothiazdes and captopril), suggesting that the anti-hypertensive activity of MD at the dose used was comparable to these standard drugs. Also, the fact that individually the two components of MD, DS and AM, were not as efficient as MD in reducing SBP, DBP and MAP, showed that the anti-hypertensive effect of MD may be due to a combined action of the two components of the preparation.

The onset of hypertension is influenced by several vasodilators which when produced by vascular smooth muscles and endothelial cells are readily transported to the extracellular spaces and thus, enter the vascular lumen (Jackson and Mi, 2009). The serum concentration of some of these substances can therefore be used as an index of their levels in tissues (Shamaash et al., 2000). Vasodilators including acetylcholine are known to produce endothelium-dependent relaxations, through the synthesis and release of nitric oxide (NO) from L-arginine by nitric oxide synthase (NOS). The NO reacts with the heme group of soluble guanylate cyclase (sGC) to activate it (Hart, 1999). The activated sGC causes the production of cGMP which initiates vascular smooth muscle relaxation (Hart, 1999) reducing the elevated BP in the process. Results from this study did not show significant increases in NO and cGMP concentrations in SHRs and L-NAME hypertensive rats treated with MD (30 mg/kg), DS (40 mg/kg) and AM (80 mg/kg), suggesting that the anti-hypertensive activity of the three plant preparations may not involve the NO/cGMP pathway. A similar study by Manganelli et al. (2000) showed that vasorelaxation produced by the methanolic extract of *Gentiana kokiana* in male Wistar rats was not reduced, following treatment with 1H-[1, 2, 3]-oxadiazolo[4, 4-a] quinoxalin-L-one (ODQ), an inhibitor of soluble guanylate cyclase (sGC), indicating the absence of the NO/cGMP pathway in the anti-hypertensive activity of the plant.

Cyclic AMP, another chemical messenger, has been shown to be linked to the onset of hypertension by regulating renal vascular tone (Jackson and Mi, 2009), by altering cytosolic calcium ion concentration, through phospholipase C (PLC). Zhao et al. (2007) reported improved cardiac function and the inhibition of left ventricular remodeling in Sprague-Dawley rats (SDRs) with renovascular hypertension, following treatment with berberine. The investigators associated improvement in cardiovascular function to increased concentration of cAMP and linked the anti-hypertensive activity of the plant to it. In the present study, cAMP concentrations were not altered in any of the models following treatment with the plant preparations. This suggested that cAMP may not be

involved in the anti-hypertensive properties of the plants.

Prostaglandin $E_2$, ($PGE_2$) a major renal COX-2-derived eicosanoid (Imig, 2006), acts on $PGE_2$ receptors (EP receptors) to reduce renal vascular resistance (Breyer and Breyer, 2000a). The activation of either $EP_2$ or $EP_4$ receptors by $PGE_2$ increases renal vascular cAMP concentrations and subsequently, vascular relaxation (Imig, 2006), and hence, a drop in BP. Serum concentration of $PGE_2$ was neither altered by MD nor its component plant preparations, suggesting that $PGE_2$ may therefore, not be involved in the mechanisms leading to anti-hypertensive activity of MD and its components. In a previous experiment Owolabi et al. (2005) showed that relaxations produced by aqueous extract of *Persea Americana* were reduced by indomethacin (inhibitor of COX-2) and suggested that either $PGE_2$ or $PGI_2$ may be involved in the mechanism of action of the plant. The eicosanoid thromboxane $A_2$ ($TXA_2$) has been shown to activate the RAAS (Nasjletti, 1998) or PLC (Offermanns et al., 1994; Raychowdhury et al., 1994) to cause hypertension, it would therefore be interesting to determine the effect of MD and its components on $TXA_2$.

Chymases are a family of serine proteases found mainly in mast cells. They convert angiotensin-I (Ang-I) to Ang-II thus, playing an important role in hypertension through the RAAS (Caughey, 2007). Both sera of MD- and DS-treated experimental models inhibited tissue chymase activity *in vitro* suggesting that inhibition of RAAS might be involved in their mechanism of action. The RAAS involves the activation of the hormone renin, following a drop in the blood pressure of renal arterioles, resulting in an increase in the production of Ang-I from its precursor, angiotensinogen (Yan et al., 2003). Converting enzymes, including chymases, transform Ang-I to Ang-II (Caughey, 2007) which stimulates the production of aldosterone, leading to increased renal reabsorption of sodium and intravascular volume and subsequently, an elevated blood pressure. Through its AT-1 receptor, Ang-II can increase peripheral vascular resistance (PVR), and vasoconstriction via sympathetic nervous system (Faber and Brody, 1984), resulting in an elevated blood pressure. Increase in the production of reactive oxygen species (ROS) has been linked to hypertension (Welch et al., 2003) and its production has been shown to be increased by Ang-II, through the activation of NADPH oxidase and this can also cause hypertension (Paravicini and Touyz, 2006). Thus, by inhibiting chymase the plant preparation, MD, probably prevented the pro-hypertensive activities involving Ang-II and thereby reduced an elevated blood pressure.

We investigated the preparations' ability to modulate oxidative stress because ROS are known to contribute to the onset of some types of hypertension (Wu and Jourlink, 2002). Measurement of antioxidant status gives an indication of amount of ROS being produced (Redón et al., 2003). Antioxidants are known to lower TC, LDL-C, and TAG concentrations and increase that of HDL-C and the ratio of HDL-C/LDL-C (Ochani and D'Mello, 2009). There was a positive correlation between total antioxidant concentration (TAC) and HDL-C and HDL-C/LDL-C ration, in animal models treated with MD and DS. The negative correlation between TAC, LDL-C and TAG in animal models treated with MD and DS further lend support to the above observation. High concentrations of atherogenic lipids such as LDL-C, TC and TAGs, can cause hypertension by promoting the generation of foam cells which form plaques in blood vessels, resulting in atherosclerosis and impeding blood flow and consequently, hypertension (Vines, 1989; Gotto, 2005).

Abnormal lipid metabolism resulting in the production of some of these atherogenic lipids has been shown to cause an increase in Ang-I production (Nickenig and Harrison, 2002) and this can result in hypertension. Non-atherogenic lipids such as HDL-C prevents the onset of hypertension (Aviram, 1999; Gotto, 2005) by transferring cholesterol from peripheral tissues back to the liver for excretion (Mendel et al., 1988; Gurr and Janes, 1991), thus, preventing the onset of atherosclerosis and hypertension. Therefore, by reducing the concentrations of atherogenic lipids such as LDL-C, and increasing the concentration of non- atherogenic lipids like HDL-C, the plant preparations might be able to reduce the hypertensive condition in the animal models studied, possibly by preventing atherosclerosis and the production of Ang-I.

Diuretics are substances that eliminate water from the body through diuresis. Chlorothiazide, used as the standard diuretic at the therapeutic dose markedly increases the excretion of water, sodium, potassium, within an hour (Craig and Stitzel, 1990). In SHRs and L-NAME hypertensive rats, MD and AM induced increased urine output whilst DS was without effect. The possible involvement of diuresis in the anti-hypertensive effect of MD and AM was supported by the reduction of serum sodium ion and elevation of urine sodium ion concentration. Since the diuretic effect of MD and AM were similar in all animal experimental models, it is possible that the observed diuretic effect of MD may be due to AM.

The reduction in serum potassium ion concentration and an increase in the urine concentration, by MD and AM in all animal models, is an indication that like chlorothiazide, the plant preparation may not be potassium-sparing (Craig and Stizel, 1990). In certain types of hypertension the sensitivity of the pressure-natriuresis mechanism and, hence, the excretion of excess sodium is improved by increased concentration of potassium, calcium, and magnesium (Karppanen et al., 2005). There was no increase in serum magnesium and calcium ion concentrations in all animal models, suggesting that excretion of sodium ions and for that matter of water, may not be aided by magnesium and calcium ions.

## Conclusions

The current study has shown for the first time that *Mist*

*Diodia* (MD) preparation is effective in managing hypertensive state in spontaneously hypertensive rats (SHRs) and N-nitro-L-arginine methyl ester (L-NAME) hypertensive Sprague-Dawley rats (SDRs). This means that it might be able to manage both primary and secondary types of hypertension in a manner similar to standard anti-hypertensive agents, such as atenolol, nifedipine, chlorothiazide and captopril based on the dosages used for this study.

The mechanism of action of MD does not involve the nitric oxide/c guanosine monophosphate (NO/cGMP) system, or the beta-adrenergic pathways. It also does not involve the cyclooxygenase-2 (COX-2) pathway. A possible mechanism of action by MD may be by the renin-angiotensin-aldosterone-system (RAAS). The anti-hypertensive action of MD might also involve a non potassium-sparing diuretic action and improved lipidemia due to the presence of antioxidants. The *Diodia scandens* (DS) component of *Mist Diodia* might be responsible for the antioxidant properties, while the *Aframomum melegueta* (AM) component might contribute to its diuretic action.

## ACKNOWLEDGEMENT

We acknowledge funding from The Centre for Plant Medicine Research (CPMR), Mampong, Ghana.

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