ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Hydroethanolic Extract Of *Cassia alata* (Caesalpiniaceae) Leaves Can Protect Against L-NAME-Induced Hypertension In Rat.

Danielle Claude Bilanda[1], Adélaïde Marie-noël Tegah Kuissu[2], Paul Désiré Djomeni Dzeufiet[1], Jonas Kouamouo[2], Rosine Désirée Chougouo Kengne[2], Pierre Kamtchouing[1], and Théophile Dimo[1]*.

[1]Department of Animal Biology and Physiology, Laboratory of Animal Physiology, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon.
[2]High Institute of Health Sciences, Université des Montagnes, P.O. Box 812 Bagante, Cameroon.

ABSTRACT

Cassia alata is used in Cameroon by the population to manage high blood pressure. The present study was undertaken to evaluate the preventive activity of the hydroethanolic extract of *Cassia alata* leaves (HECA) on N-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rat. Male rats were used to induce hypertension by the administration of L-NAME (50 mg/kg/day). The others groups were receiving concomitantly L-NAME plus HECA (100 and 200 mg/kg/day) or captopril (20 mg/kg/day). All the treatments were given orally for 4 weeks. At the end of the treatment, the hemodynamic parameters were recorded. Kidney and liver functions as well as oxidative stress markers were evaluated by colorimetric method. The acute effects of *Cassia alata* were studied on blood pressure (BP) after intravenous administration in normotensive rats. Intravenous administration of HECA induced a significant hypotensive response. The hypotensive effect of the extract was unaffected by atropine but decreased by reserpine (5 mg/kg). Concurrent treatment with HECA (100 or 200 mg/kg) prevented high BP, oxidative stress as well as kidney and liver functions impairment without affecting heart rate (HR). HECA can prevent hypertension, its associated oxidative stress and kidney and liver functions impairment, passing at least in part through the catecholamine's inhibition and/or destruction.

Keywords: Hypertension, *Cassia alata*, L-NAME, oxidative stress.

https://doi.org/10.33887/rjpbcs/2019.10.4.2

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Hypertension remains a major health problem worldwide considering the prevalence of morbidity and mortality [1]. Chronic nitric oxide inhibition with N-nitro-L-arginine methyl ester (L-NAME) can increase regional vascular resistance, raise the blood pressure, and oxidative stress accompanied with renal and liver damages [2]. Vascular endothelium of hypertensive patients is already known to produce less nitric oxide (NO) [3]. The protective effects of antihypertensive drugs in the context of NO deficiency are then better understood with L-NAME-induced model of hypertension in rat. The use of Plant extracts in the treatment of many diseases has gained interest because plants remain a reliable source of effective and better tolerated drugs [4]. A large number of plant species which are important sources of traditional medicine are widely being used to treat hypertension and/or oxidative stress [5,6]. Many plants have already proved scientifically their effectiveness against chronical diseases, including hypertension [4,5]. Most of them contain natural antioxidants that can prevent ROS formation and its damages [7]. Moreover some flavonoid have proved to ameliorate hypertension, oxidative stress and lipid metabolism in L-NAME hypertensive rats [5,8].

Cassia alata (*C. alata*) used in the present study is a small tree of the family of Caesalpiniaceae widely used in the world to manage many ailments [9]. In Cameroon, the leaves are used in the treatment of skin infection, hepatitis and intestine helminthiases. It is also known by the populations for its diuretic activity and then it is used by them to manage hypertension. Moreover, its phytochemistry reveals a big among of flavonoids with antioxidant activity [10]. Its protective activity on cardiovascular system has already been demonstrated [11]. The present study was designed to give scientific bases to the traditional use of *C. alata*. It was performed to investigate the effects of hydroethanolic extract of *C. alata* in L-NAME-induced hypertension, the related oxidative stress on rats and some mechanisms.

SUBJECTS AND METHODS

Animals

Ten to twelve weeks old male albinos Wistar rats, weighting between 180 and 250 g were used. Animals were raised in the Animal House of the Faculty of Science, University of Yaounde I. they were maintained under standard light (12-hour day/night natural cycle) and temperature (25° C) with free access to a standard animal diet and tap water. The standard animal diet was made up with corn (40%), wheat (20%), fish flour (24%), palm oil (7%), groundnut (3%), bones flour (2%), edible salt (1%), cotton edible crab (2%) and vitamin complex (1%). All the procedures and protocols involving animals and their care were conducted in conformity with the institutional guidelines approved by the institutional animal ethics committee of Cameroon (Reg. No. FWA-IRB00001954).

Plant material

Fresh leaves of *C. alata* were collected in Jun 2016 at Yaounde (center region of Cameroun). The plant material was identified at the national herbarium in Yaounde, where voucher specimen N° 155/HNC has been deposited. The leaves were washed, dried in an oven at 45°C for 48 hours and ground into powder. The hydroethanolic extract was prepared according to Pieme *et al* [12]. Briefly, 185g of the powder were soaked in 930 mL of a water and ethanol mixture (30:70) for 72 hours, in a percolator supplied with a filter. The filtrate was concentrated by evaporation at 40°C under reduced pressure on a rotary evaporator (BUCHU R-100) and dried in an oven to afford 36.108 g of hydroethanolic extract of *C. alata*, yield of 20.06 %.

Effects of *C. alata* on blood pressure and heart rate

Acute effect of *C. alata*

This effect was evaluated with the direct method according to Bilanda *et al* [13]. Briefly, the rats were anaesthetized using an intraperitoneal injection of 15% urethane (1.5 g/kg). The trachea was exposed and cannulated to facilitate spontaneous respiration. A polyethylene catheter (PE 50) was inserted into the right femoral vein and an injection of 10% heparin (0.1 mL/100 g body weight) was immediately administered. Another catheter was inserted into the left carotid aorta and connected to a pressure transducer recording system (Biopac Student Lab MP35) for systolic blood pressure (SBP) and heart rate (HR) recording. The animals

were allowed to stabilize for at least 30 min before administration of any test substances (Dimo *et al*[14]. The test substances were injected through a cannula inserted into the femoral vein.

After equilibration for at least 30 min, the dose–response relationship of the *C. alata* (10, 20 and 40 mg/kg) extract was determined on normotensives rats (NTR). The vehicle (Mc Even) was also administered in a group of rats to ensure that the observed effects were not due to the vehicle. Cardiovascular parameters (SBP and HR) were recorded for 1 h after the extract administration. In another group of NTR, the effect of *C. alata* extract (40 mg/kg) was examined after administration of the muscarinic receptor antagonist atropine (1 mg/kg). Atropine was injected intravenously, 5 min before administration of the plant extract. An inhibitor of the vesicular momoamine transporter, reserpine (5 mg/kg) was given to another group of 5 NTR for 3 days; then the effect of *C. alata* extract (40 mg/kg) was assessed.

Subchronic effects of *C. alata*

The subchronic effect of *C. alata* was evaluated on L-NAME-induced hypertensive rat's model. The doses of *C. alata* were determined after a screening. Wistar NTR (25) were randomly divided into five groups of five animals. The first group (control) received distilled water (10 mL/kg), while the second one (L-NAME group) received L – NAME (50 mg/kg/day) plus the vehicle (water 10 mL/kg). The third group received at the same time L-NAME (50 mg/kg/day) and captopril (20 mg/kg/day). While the fourth and fifth groups received a combination of L-NAME (50 mg/kg/day) plus *C. alata* hydroethanolic extract (E 100 and E 200 mg/kg/day respectively). All the treatments were administered daily orally for 4 weeks at the corresponding volume of 1 mL/100 g. At the end of the respective treatments, arterial blood pressure and heart rate of all rats were recorded as previously described.

Blood and organs collection

Immediately after hemodynamic parameters recording, blood samples were collected from the abdominal artery, and centrifuged at 3000 rpm, 4°C for 15 minutes. The plasma obtained was kept at −20°C for biochemical analysis. Thereafter, the heart, the liver and the thoracic aorta were collected, washed in saline and weighed. Homogenates (20 %) were prepared in Mc Even solution for heart and aorta, in Tris–HCl 50 mM (pH 7.4) buffer for liver. Organs were crushed and then the mixture was centrifuged at 3000 g at 4 °C for 25 minutes. The supernatant was collected and stored at 20 °C for oxidative stress markers and nitrites evaluation.

Biochemical analysis

Tissue levels of reduced glutathione (GSH), malondylaldehyde (MDA) and superoxide dismutase activity (SOD) and catalase were assayed using colorimetric method [15,16,17§]. The tissue concentration of nitrites was evaluated using the Griess method [18]. Serum protein concentration was assayed according to Gornall *et al*[19] using the Biuret reagent and bovine serum albumin as a standard. The serum concentrations of creatinin and bilirubine, the activities of alanine and aspartate aminotransaminases (ALT & AST), glutamyl transferase gamma (γGT) were determined using commercial diagnostic kits (Fortress, UK). Serum concentrations of sodium and potassium were assessed using commercial diagnostic kits (Quimica Clinica Aplicada, Spain)

Statistical analysis

Results were expressed as the mean ± SEM. Data were analyzed with Graph pad prism 5.03 logiciel. The difference between the groups was compared using one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effect of acute injection of *Cassia alata* on blood pressure and some mechanisms

The intravenous administration of the vehicle had no effect on blood pressure (data not shown). The injection of the extract of *Cassia alata* in normotensive rats (NTR) resulted in a significant and rapid drop of

ISSN: 0975-8585

mean blood pressure (MBP). As shown in Figure 1 A, *Cassia alata* (10, 20 and 40 mg/kg) reduced the MBP in normotensive rats (NTR) by 17.91 % (p >0.05), 35% (p<0.001) and 27.44% (p<0.001) respectively. The first and rapid hypotensive response was followed by a relative rapid increase of MBP. Thereafter the MBP decreased gradually, and the reduction was significant (20%) at 40 mg/kg from the 25th minute after injection, until the end of observation period. The late decrease in MBP was dose-related.

The effect of two antagonists, atropine and reserpin on the hypotensive action of *Cassia alata* (40 mg/kg) was investigated (Figure 1 B). The pretreatment with atropine sulphate (200 µg/kg) did not significantly affect the immediate and rapid hypotensive effect of the plant extract. However, the late decrease was non significantly reduced (50 %) one hour after the injection of the plant extract. In contrast, the pretreatment with reserpine (5 mg/kg) significantly reduced (59.89 %) only the immediate hypotensive effect of the plant extract in NTR.

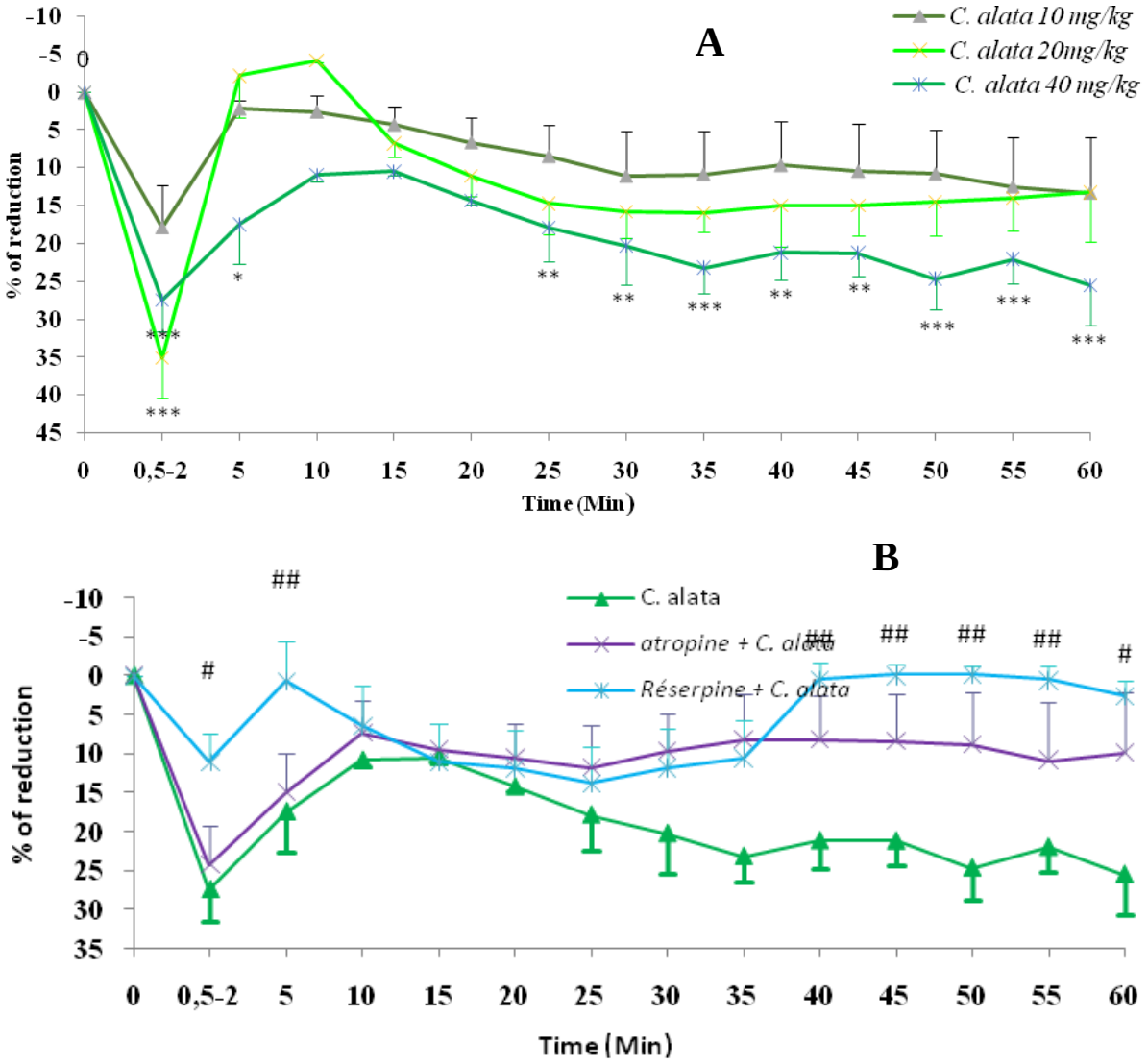

Figure 1: Effects of *C. alata* on blood pressure and some mechanisms.

Each point represents the mean ± S.E.M.; n=5; *P< 0.05, **P<0.01, ***P<0.001significantly different as compared to initial MBP.

ISSN: 0975-8585

Subchronic effects of *Cassia alata* on blood pressure, heart rate and body weight

The administration of L-NAME (50 mg/kg) during 28 days resulted to a significant ($p<0.001$) increase in systolic, diastolic and mean arterial blood pressure (SBP, DBP and MBP) (Table 1). The increase was of 89.32%, 116.43% and 102.98% respectively as compared to NTR. *Cassia alata* hydroethanolic extract (100 mg/kg, E100) prevented that increase by 41.81%, 45.33% and 43.30% respectively as compared to L-NAME hypertensive rats (HTR). At the dose of 200 mg/kg (E200), the prevention in the increase of blood pressure was by 35.39%, 37.09%, and 36.46% respectively for SBP, DBP and MBP as compared to L-NAME HTR. Captopril (20 mg/kg) acted in the same way and the reduction was of 48.16%, 48.40% and 48.31% respectively as compared to L-NAME HTR. No significant effect was observed on heart rate in all the groups.

The administration of L – NAME (50 mg/kg) during 28 days resulted in a significant drop in body weight gain as compared to NTR (Table 1). The concomitant treatment with L – NAME and *C. alata* (100 mg and 200 mg/kg) did not prevent the drop in body weight gain. However, captopril has succeeded to prevent the drop in body weight induce by L-NAME.

Table 1: Subchronic effects of *Cassia alata* on blood pressure and heart rate

Parameters	NTR	HTR	CAP 20	E 100	E 200
SBP (mmHg)	118.23 ± 7.86	188.65 ± 5.57 ^{c}	97.79 ± 5.93 $^{\gamma}$	109.77 ± 6.00 $^{\gamma}$	121.87 ± 10.07 $^{\gamma}$
MBP (mmHg)	83.47 ± 8.40	169.42 ± 5.88 ^{c}	87.57 ± 6.49 $^{\gamma}$	96.06 ± 8.09 $^{\gamma}$	107.64 ± 10.14 $^{\gamma}$
DBP (mmHg)	73.84 ± 10.18	159.81 ± 6.22 ^{c}	82.46 ± 6.78 $^{\gamma}$	87,36 ± 7.82 $^{\gamma}$	100.53 ± 10.22 $^{\gamma}$
HR (BPM)	359.22 ± 14.60	377,14 ± 10.36	395.83 ± 9.25	355.19 ± 5.64	344.75 ± 22.13
BWg (%)	11.88 ±3.10	-7.70 ±1.27 ^{c}	8.85 ±1.73 $^{\gamma}$	-4.49 ±0.80	-2.79 ±1.10

Each value represents a means ± S.E.M. of 5 rats; ^{c} $P < 0.001$, significantly different compared to normal rats. $^{\gamma}$ $P < 0.001$, significantly different compared to L-NAME hypertensive rats. NTR: normotensive rats receicing only distilled water (10 mL/kg); HTR: hypertensives Rats receiving L- NAME (50 mg/kg) and distilled water; CAP 20: Rats receiving L- NAME and Captopril (20mg/kg); E100: Rats receiving L- NAME and *C. alata* extract (100 mg/kg) ; E200: Rats receiving L- NAME and *C. alata* extract (200 mg/kg); DBP=diastolic blood pressure; SBP=systolic blood pressure; MBP=mean blood pressure; BPM=beat per minute; HR=heart rate; BWg= Body weight gain as comparerd to initial value at the begining of the treatment.

Effects of *Cassia Alata* on liver and kidney function parameters

As shown in Table 2, no significant difference in the level of serum protein was observed among all the treated groups. The administration of L – NAME (50 mg/kg) during 28 days resulted in a significant increase in the serum activity of AST, ALT, γGT and in bilirubin level. The increase was of 24.05%, 35.63%, 117.15%, and 137.60 for AST, ALT, bilirubin and γGT respectively as compare to NTR. The same increase was observed in the serum levels of creatinin, potassium and sodium in L-NAME-HTR. That increase was of 23.76%, 25.40% and 13.12% respectively as compare to NTR. The increase in the level of these parameters was significantly and non dose-dependently prevented in *C. alata*-treated animals as compared to HTR. The exception was observed for AST (E100) and sodium (E100 & E200) where though low, the differences in the values were not significant as compared to HTR. Captopril also significantly prevented the increase of all the above parameters except for AST and ALT.

Effects of *Cassia Alata* on oxidative stress marker variables

The effect of *C. alata* on oxidative stress marker parameters is summarized in Figure 2. The oxidative stress parameters evaluated in this study were superoxide dismutase SOD (A), catalase (B), reduced glutathione (C), malondialdehyde MDA (D) and nitrites (E) on the aorta, heart and liver. After 28 days of treatment with L-NAME, a slight and non significant decrease was observe in the levels of GSH in all the investigated organs. The concomitant treatment with the plant extract or captopril has successfully prevented that decrease, bringing the values far above those in NTR.

ISSN: 0975-8585

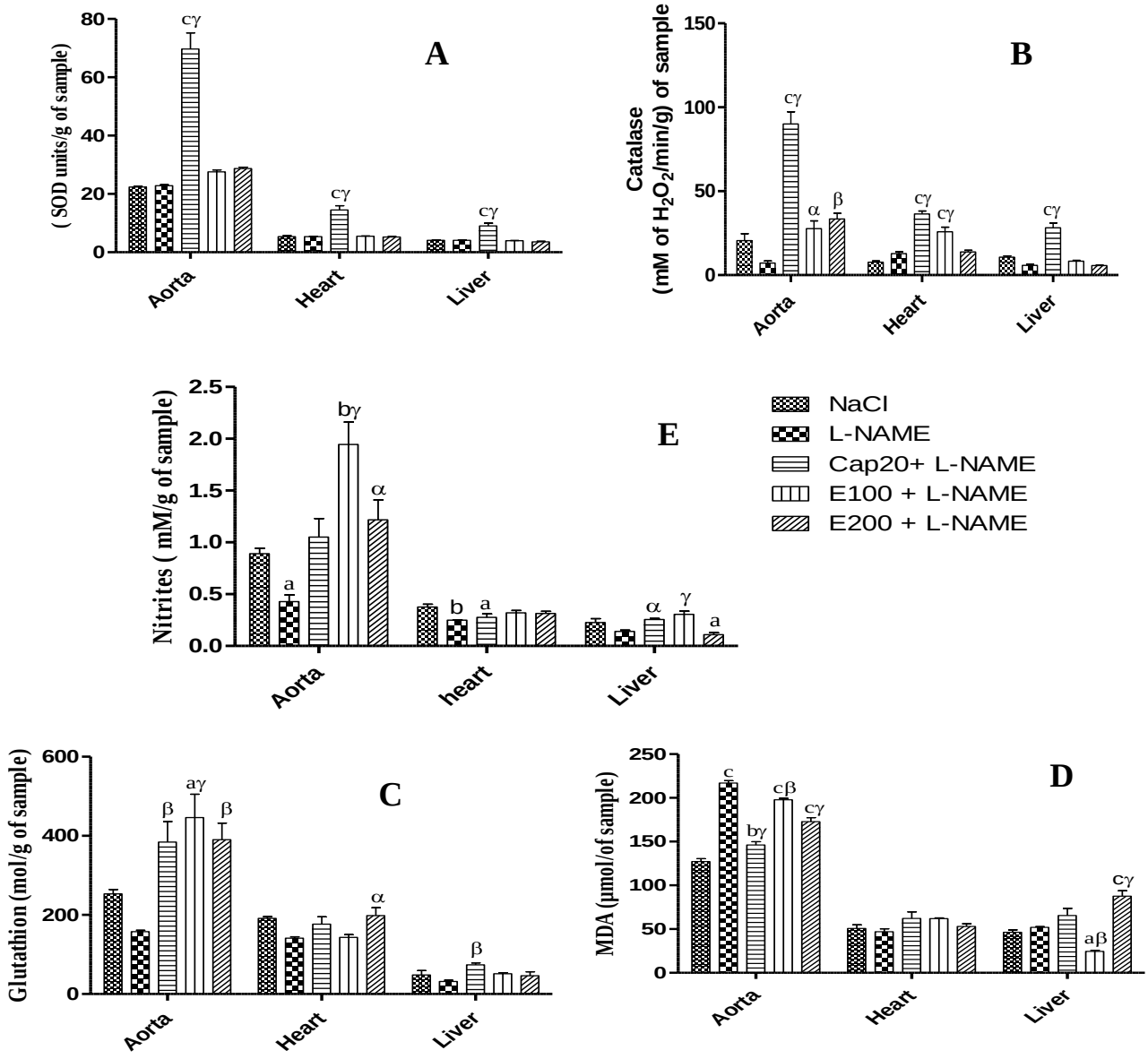

Figure 2: Effect of *C. alata* on oxidative stress marker parameters.

Each bar represents a means ± S.E.M. of 5 rats; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, significantly different compared to normal rats. $^{\alpha}P < 0.05$, $^{\beta}P < 0.01$, $^{\gamma}P < 0.001$, significantly different compared to L-NAME hypertensive rats. NTR: normotensive rats receicing only distilled water (10 mL/kg); HTR: hypertensives Rats receiving L- NAME (50 mg/kg) plus distilled water; CAP 20: Rats receiving L- NAME plus Captopril (20 mg/kg); E100: Rats receiving L- NAME plus *C. alata* extract (100 mg/kg) ; E200: Rats receiving L- NAME plus *C. alata* extract (200 mg/kg). Superoxide dismutase SOD (A), catalase (B), reduced glutathione (C), malondialdehyde MDA (D) and nitrites (E).

ISSN: 0975-8585

Table 2: Effects of *Cassia alata* on liver and kidney function parameters

Parameters	NTR	HTR	CAP 20	E 100	E 200
Protein (mg/dL)	82.78± 2.72	90.91 ± 7.02	87.71 ± 2.53	83.62± 2.58	88.14± 3.18
AST (U/L)	53.91± 2.91	66.88± 3.30 ^{a}	79.98± 4.38 $^{c\alpha}$	63.33± 2.12	49.72± 1.23 $^{\beta}$
ALT (U/L)	30.28± 0.93	41.07± 2.90 ^{b}	44.04± 2.27 ^{c}	18.82± 2.16 $^{b\gamma}$	16.41± 0.28 $^{c\gamma}$
Bilirubin (µmol/L)	3.44 ± 0.46	7.47 ± 0.15 ^{c}	2.78 ± 0.52 $^{a\gamma}$	1.40 ± 0.26 $^{c\gamma}$	1.49 ± 0.17 $^{c\gamma}$
γ GT(U/L)	1.25 ± 0.10	2.97 ± 0.08 ^{c}	1.38 ± 0.32 $^{\gamma}$	1.30 ± 0.22$^{\gamma}$	1.96 ± 0.17$^{\alpha}$
Creatinin (µmol/L)	170.89 ± 3.82	211.51± 5.47 ^{c}	128.49± 4.88 $^{c\gamma}$	139.10± 3.11$^{c\gamma}$	157.73± 2.02 $^{\gamma}$
Na^{+} (mmol/L)	93.83 ± 2.19	110.01 ± 0.67 ^{a}	57.67 ± 2.61 $^{c\gamma}$	106.13± 4.92	108.49 ± 4.30 ^{a}
K^{+} (mmol/L)	5.61 ± 0.46	7.03 ± 0.85	5.98± 0.36	4.42± 0.28 $^{\beta}$	4.86± 0.45 $^{\alpha}$

Each value represents a means ± S.E.M. of 5 rats; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, significantly different compared to normal rats. $^{\alpha}P < 0.05$, $^{\beta}P < 0.01$, $^{\gamma}P < 0.001$, significantly different compared to L-NAME hypertensive rats. NTR: normotensive rats receicing only distilled water (10 mL/kg); HTR: hypertensives Rats receiving L- NAME (50 mg/kg) plus distilled water; CAP 20: Rats receiving L- NAME plus Captopril (20 mg/kg); E100: Rats receiving L- NAME plus *C. alata* extract (100 mg/kg) ; E200: Rats receiving L- NAME plus *C. alata* extract (200 mg/kg).

The activity of SOD has not been affected by any treatment except the one involving captopril, where it was significantly increase. None of the treatment has affected the level of MDA in the heart. However, MDA was significantly increased with all the treatment in the aorta, though the concomitant treatment with the plant extract or captopril prevented significantly that increase, as compared to HTR. L-NAME in treatment alone has significantly dropped the level of nitrites in all the investigated organs except in the liver where only a slight decrease was observed. The concomitant treatment with plant extract has significantly prevented that drop in the aorta (E100 and E200) and in the liver (E100) as compared with HTR.

DISCUSSION

This study demonstrates for the first time that the hydroethanolic extract of *Cassia alata* induces an acute hypotensive effect on normotensive rats (NTR). Moreover, the antihypertensive effect of the extract is associated with antioxidant properties in hypertensive rats. The results of the present study demonstrated that the injection of the hydroethanolic extract of *Cassia alata* on NTR led to an acute decrease in blood pressure followed by a second decrease that persisted for least 1 hour. The first phase may be due to the effects on the cardiac pump activity while the late one could be the action on vessels [20]. This later phase, observed with all the doses (10, 20 and 40 mg/kg), was significant only at 40 mg/kg. The fact that atropine (a muscarinic antagonist) did not significantly affect the action of the plant extract may imply that the plant extract action does not pass through muscarinic receptors. In contrast, the decrease by reserpin of both the immediate and the late drop in blood pressure suggests that the action of the plant extract passed at least in part through the catecholamine's inhibition and/or destruction [21]. These findings revealed the hypotensive effect of hydroethanolic extract of *Cassia alata* and give an idea about its mechanism of action.

Subchronic oral treatment with the extract prevented the rise in blood pressure of rats subjected to L-NAME treatment. The effect was more significant at the dose of 100 mg/kg than at 200 mg/kg. This observation suggests that the plant extract has an antihypertensive activity passing through mechanisms leading to an increase in NO production and/or action. This could justify the rise in nitrites levels on aorta and heart. The mechanisms in this beneficial effect might involve the inhibition of the orthosympathetic activity as suggested by the results of the acute study. However, other mechanisms might also be involved, including the reduction of oxidative stress by *Cassia alata* extract. Indeed oxidative stress is enhanced in hypertension, atherosclerosis, and other forms of cardiovascular disease and participates in the mechanisms of vascular injury [22]. Moreover, in hypertensive patients, lower concentrations of antioxidants have been documented

[23]. Thus, drugs which improve the oxidative status would be able to lower arterial blood pressure. The results of the present study showed that several biomarkers of oxidative stress are impaired in L-NAME treated rats. Reduced glutathione (GSH) is the most abundant antioxidant endogenously produced in eukaryotic cells that interacts with activated oxygen species. An increase in organs' GSH levels in rats treated with plant extract may be attributable to the antioxidant effect of the extract. It may contain compounds capable to scavenge ROS, and therefore prevent GSH use to neutralize the ROS [24]. The association of *Cassia alata* or Captopril with L-NAME prevented the decrease of glutathione and SOD and the increase of MDA in the investigated organs. These results are in accordance with other authors [25] who demontrated that the Cardioprotective effects of *Vitex cienkowskii* methanol/methylene chloride extract passed through its antioxidant activity. Glutathione plays an excellent role in protecting cell from oxidative damages. Therefore *Cassia alata* may be acting as membranes protector to prevent the lipid peroxidation or/and as free radicals scavenger [26]. This was confirmed with the reduction of MDA by the plant extract. That action of the plant extract may in turn protect the organs against the injuries caused by free radicals. The antioxidant potential of *Cassia alata* might be due to the free radical scavenging properties of its known phytochemicals such as polyynes, flavonoids, phenylpropanoids and phenolics[27,28]. This can explain the protective effect of *Cassia alata* extract on liver and kidney. The evaluation of the toxicity of L-NAME on the functions of these organs revealed an increase in the levels of blood creatinin, sodium, potassium, bilirubine, γGT and transaminases (AST and ALT) activity. *Cassia alata* inhibited the elevation in serum levels of AST, ALT, creatinin, sodium,potassium, γGT and bilirubine induced by L-NAME; that is similar with the effect on oxidative stress markers. These results are consistent with those of some authors [5,29,30]. This may indicate that oxidative stress contributes in mechanism(s) of hepatotoxicity due to chronic consumption of L-NAME. The low level of serum enzyme activity following the concomitant treatment with *Cassia alata* as compared to untreated rats confirms the liver and kidney protective effects of *Cassia alata* [27] as well as its antioxidant activity [5]. In the present study, treatment with L-NAME increased the blood pressure in association with decreased in heart and aortic nitrite levels. The levels of heart and aortic nitrite were low with the co-administration of or captopril with L-NAME, showing the ability of the plant extract to stimulate the production or prevent the deletion of nitrites [5,25].

In summary, the present study provides evidences that the hydroethanolic extract may have hypotensive effects and is able to prevent hypertension with NO deficiency. It can improve endothelial function in L-NAME animal model of hypertension. These effects may be combined with its antioxidant as well as nephro and hepatho- protective activity. These findings point out the beneficial effects of *Cassia alata* leaves as a complementary treatment in the management of hypertension and its complications.

REFERENCES

[1]. Staessen JA. Hypertension: age-specificity of blood pressure- associated complications. Nature Rev Cardiol, 2014; 11(9): 499-501.

[2]. Abdel-Rahman RF, Hessin AF, Abdelbaset M, Ogaly HA, Abd-Elsalam RM, Hassan SM. Antihypertensive Effects of Roselle-Olive Combination in L-NAME-Induced Hypertensive Rats. Oxidat Med Cell Longev, 2017; 2017:1-24.

[3]. Moncada S, Higgs A. The L-arginine–nitric oxide pathway. N Engl J Med 1993; 329(27):2002-2012.

[4]. AL DISI SS, Anwar MA, Eld AH. anti-hypertensive herbs and their mechanism of action: part 1. Front Pharmacol 2016; 6:323. doi: 10.3389/fphar.2015.00323.

[5]. Bilanda DC, Dzeufiet DPD, Kouakep L, Aboubakar OBF, Tedong L, Kamtchouing P, Dimo T. Bidens pilosa Ethylene acetate extract can protect against L-NAME-induced hypertension on rats. BMC CAM 2017; 479(17): 1-7.

[6]. Ramanathan V, Thekkumalai M. Role of chrysin on hepatic and renal activities of Nω-nitro-l-arginine-methylester induced hypertensive rats. Int J Nutri Pharmacol Neurol Disea 2014; 4: 58-63.

[7]. El-Sherbiny GA, Taye A, Abdel-Raheem IT. Role of ursodeoxycholic acid in prevention of hepatotoxicity caused by amoxicillin-clavulanic acid in rats. An Hepatol 2009; 8:134-140.

[8]. .Rajendran P, Boobalan R. The flavonoid troxerutin ameliorates hypertension, oxidative stress and lipid metabolism in L-NAME hypertensive rats. Indo–Asian J Multidisciplin Res 2015; 1(3):280-289.

[9]. Sundaramoorthy S, Gunasekaran S, Arunachalam S, Sathiavelu M. A phytopharmacological review on Cassia specie. J Pharm Sci Res 2016; 8(5):260-264.

[10]. Qiang C, Pang L, Huang S, Lei W, Huang D. Effects of emodin and irbesartan on ventricular fibrosis in goldblatt hypertensive rats. Inter J Pharma Sci 2013; 69(5):374-378.

[11]. Neharkar V, Gautam G , Pandhare R. Effect of different fractions of methanolic extract of Cassia alata leaves against doxorubicin induced cardiotoxicity in rats. Int J Pha Pharm Res 2016; 7(3):451-460.
[12]. Pieme CA, Penlap VN, Nkegoum B, Taziebou PCL, Tekwu EM, Etoa FX. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of Senna alata (L.) Roxb (Caesalpiniaceae). Afr J Biotechnol 2006; 5(3):283-289.
[13]. Bilanda DC, Dimo T, Dzeufiet DPD, Bella NMT, Aboubakar OBF, Nguelefack BT, Tan VP, Kamtchouing P. Antihypertensive and antioxidant effects of Allanblackia floribunda Oliv. (Clusiaceae) aqueous in alcohol-and sucrose-induced hypertensive rats. J Ethnopharmacol 2010; 128: 634-640.
[14]. Dimo T, Nguelefack TB, Tan PV, Yewah MP, Dongo E, Rakotonirina SV, Kamany A, Bopelet M. Possible mechanisms of action of the neutral extract from Bidens pilosa L. Leaves on the cardiovascular system of anaesthetized rats. Phytother Res 2003; 17:1135-1139.
[15]. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82(1):70-77.
[16]. Wilbur KM, Bernheim F, Shapiro OW. Determination of lipid peroxidation. Arch Biochem Biophys 1949; 24:305-310.
[17]. Misra H, Fridovich I. The role of superoxide anion in the autoxidation epinephrine to adrenochrome and a simple assay for superoxide dismutase. J Biol Chem 1972; 247:3170-3175.
[18]. Grand F, Guitton J, Goudable J. Optimisation des paramètres du dosage des nitrites et nitrates sériques par la technique de Griess. Anal Bio Clin 2001; 59(5):559-565.
[19]. Gornall A, Bardawil C, Maxima D. Determination of serum proteins by means of the biuret reaction. J Biol Chem 1948; 77:167-182.
[20]. Imenshahidi M, Hosseinzadeh H, Javadpour Y. Hypotensive effect of aqueous saffron extract of Crocus sativus L. and its constituents, safranal and crocin, in normotensive and hypertensive rats. Phytother Res 2010; 24:990-994.
[21]. Mandela P, Chandley M, Xu Y-Y, Zhu M-Y, Ordway GA. Reserpine-induced reduction in norepinephrine transporter function requires catecholamine storage vesicles. Neurochem Int 2010; 56: 760-767.
[22]. Wojcik MH, Meenaghan E, Lawson EA, Misra M, Klibanski A, Miller KK. Reduced amylin levels are associated with low bone mineral density in women with anorexia nervosa. Bone 2010; 46(3):796-800.
[23]. Ramón R, Hernán Pt, Walter P, Julia A, Cristián G, Jean PB. Relationship between Oxidative Stress and Essential Hypertension. Hypertens Res 2007; 30: 159-1167.
[24]. Kukongviriyapan U, Luangaram S, Leekhaosoong K, Kukongviriyapan V, Preeprame S. Antioxidant and vascular protective activities of Cratoxylum formosum, Syzygium gratum and Limnophila aromatica. Biol Pharm Bull 2007; 30: 661-666.
[25]. Metchi FMD, Nguemfo E, Nana P, Temdié RJ, Ngo Lemba E, Nkeng-Efouet PA. Cardioprotective effects of methanol/methylene chloride extract of Vitex cienkowskii (Verbebaceae) in L-NAME induced hypertension in rats. Eur J Integra Med. 2013; 5: 519-526.
[26]. Abigail J, Larson J, David S, Thunder J. Therapeutic potential of quercetin to decrease blood pressure: review of efficacy and mechanisms. Adv Nutr. 2012; 3:39-46
[27]. Kviecinski MR, Felipe KB, Correia JFG, Ferreira EA, Rossi MH, Gatti FM, Filho DW, Pedrosa RC. Brazilian Bidens pilosa Linne´ yields fraction containing quercetin derived flavonoid with free radical scavenger activity and hepatoprotective effects. Libyan J Med 2011; 6:5651-5662.
[28]. Bartolome AP, Villaseñor IM, Yang WC. Bidens pilosa L. (Asteraceae): botanical properties, traditional uses, Phytochemistry, and pharmacology. E Based CAM. 2013; 2013:1–51.
[29]. Omaima M, Abd Allaha, Abeer AS. Renoprotective effect of saxagliptin and Hibiscus sabdariffa Linn extract in Nω-nitro-L-arginine methyl ester-induced hypertensive nephropathy in male albino rats: the role of proinflammatory and fibrogenic cytokines. Benha Med J 2017; 34:17-27.
[30]. Nakhoul GN, Huang H, Arrigain S, Jolly SE, Schold JD, Nally JrJV, Navaneethan SD. Serum potassium, end stage renal disease and mortality in chronic kidney disease. Am J Nephrol 2015; 41(6): 456-463.