

Methanol extract of *Allanblackia gabonensis* (Guttiferaceae) prevents hypertension and oxidative stress induced by chronic sucrose consumption in rat

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Abstract

This study was designed to evaluate the antihypertensive effect of *Allanblackia gabonensis* (*A. gabonensis*) methanol stem bark extract, and its antioxidative activity in sucrose-induced hypertensive rats. After 6-week treatment period, sucrose significantly increased systolic blood pressure as well as heart rate compare to control. *A. gabonensis* treatment (150 and 300 mg/kg) prevented the rise in blood pressure in rats with a significant effect on hearth rate. Sucrose induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), with no effect on total protein. In heart, liver and kidney, there was an increased in malondialdehyde (MDA) and superoxide dismutase (SOD) with a reduction of glutathione reduced (GSH). Treatment of rats with the plant extract significantly ($P < 0.05$) prevented the altered serum and antioxidant marker levels to near normal as compared to sucrose-treated rats. The activity of the extract was comparable to the standard drug, nifedipin (5 mg/kg). These findings suggest that the modulatory influence of the methanol stem bark extract of *A. gabonensis* on the oxidant status might be a relevant effect accounting for its antihypertensive properties against chronic sucrose-induced hypertensive rats.

Keywords: *Allanblackia gabonensis*, antioxidant, hypertension, sucrose

Introduction

Scientific and industrial progress led to the improvement of working conditions and change in life style, characterized by sedentarity and the consumption of hypercaloric diet. Therefore, the prevalence of obesity is increasing as well as the occurrence of cardiovascular diseases, including diabetes and hypertension.

Nowadays, studies on animal model are concordant on the fact that chronic carbohydrate consumption either as sucrose, fructose or glucose may lead to many physiological alteration and in long run, metabolic, cardiovascular and renal complications (1). In fact, increased in sucrose consumption, even in association with fat, protein and starch, directly increase systolic blood pressure and insulin resistance, both of which associated with oxidative stress (2). The involvement of oxidative phenomenon in the development of metabolic diseases like diabetes and hypertension has been demonstrated (3). Reactive oxygen species have a positive function in the body when they are in balance but when they are abundantly produced, they become harmful. Antioxidant, when preventing cells and organs from lipid peroxidation and other oxidative damages, can protect the organism from the development of many diseases. Hence antioxidant therapy is increasingly associated to the number of therapeutic protocols including diabetes and arterial hypertension (4; 5). Beyond the antihypertensive drugs, the prevention and the management of hypertension include alternative therapies like medicinal plants, vegetables, fruits, vitamins and minerals. Recently, more attention has been given to the use of herbal medicine. Many of these plants are reported to possess antioxidant activity, mainly related to their polyphenol contain (4; 6).

Allanblackia gabonensis (Guttiferaceae) is a species of the dense humid forest, widespread in Democratic Republic of and Cameroon (7). In Cameroon traditional medicine, the stem bark is used in the treatment of fertility, inflammation and hypertension.

Our main objective for this study was to investi-

gate the possible preventive effects of the methanol stem bark extract of *A. gabonensis* on sucrose-induced hypertension and oxidative stress in rat.

Material and method

Plant material

The stem bark of *A. gabonensis* was harvested in Yaounde locality, Centre province of Cameroon during the dry season (February) and authenticated at the National Herbarium, Yaounde where a voucher specimen was deposited (N° 13852/HNC). The sample was dried in the shade and ground into powdered form for extraction.

Preparation of crude extract

Seven hundred grams of dried powdered material was macerated in 3 L of methanol for 72 hours. The resulting liquid extract was filtered and then concentrated in a rotary evaporator under reduced pressure (60 °C). The extraction yield was 8.18%.

Animals and protocols

The experimental animals were albino Wistar rats weighing 180-220 g, raised in the Animal House of the Faculty of Science, University of Yaounde I. They were fed with a standard laboratory diet (Lanavet, Garoua, Cameroon) and given tap water *ad libitum*. They were kept at an ambient temperature under a 12 hour dark-light cycle. The study was approved by the institutional animal ethics committee (Reg. No. FWA-IRB 00001954).

Sucrose administration, treatment and measurement of blood pressure

Rats were randomly divided into five groups of five rats each and treated daily for six weeks. Normal control rats (Group 1) received tap water (10 mL/kg, *p.o.*). Rats from sucrose control group (Group 2) received 5% sucrose solution by gavage. Sucrose-treated animals (Groups 3, 4 and 5) were administered respectively nifedipin (5 mg/kg, *po*) or *A. gabonensis* extract (150 or 300 mg/kg, *p.o.*) and 5% sucrose by the same route. Six weeks later, the

body weight, arterial blood pressure and heart rate of all rats were measured as previously described (4; 8). Briefly, rats were anaesthetized with urethane (1 g/kg by *i.p* injection), and the right common carotid and left femoral vein were catheterized for the measurement of blood pressure and administration of heparin (0.1 mL/100 g body weight). The trachea was exposed and cannulated to facilitate spontaneous respiration. The systolic arterial pressure (SAP) and the heart rate (HR) were recorded using pressure transducer connected to a signal acquisition board (MP-35, BIOPAC) and computer processed.

Biochemical analysis

At the end of blood pressure measurement, the rats were killed by decapitation and the free-running blood was collected. Serum was separated for the determination of serum creatinin and bilirubin levels using commercial diagnostic kits, Randox Laboratories, San Diego-USA. The activities of alanine and aspartate transaminases were also determined using the method of Reitman and Frankel (9). The abdominal cavity was opened after collection of blood. Heart, aorta, liver and kidney were dissected out and homogenized in Tris-HCl 50 mM buffer solution to make a 20% homogenate. Serum and tissue protein concentration was assayed according to Gornall et al. (10) using Biuret reagent and bovine serum albumin as a standard. Glutathione reduced and superoxide dismutase were determined using the method of Ellman (11) and Misra et Fridovich (12), respectively. The end product of lipid peroxidation (malondylaldehyde, MDA) was determined using the procedure of Wilbur et al. (13). MDA concentration was expressed as n moles/mg protein.

Statistical analysis

Results were expressed as the mean \pm SEM. The difference between the groups was compared using one-way analysis of variance (ANOVA) followed by the Duncan's post hoc test. A value of $p < 0.05$ was considered statistically significant.

Results

Effect of *A. gabonensis* on systolic blood pressure, heart rate and body weight

As shown in table 1, the chronic administration of sucrose significantly increased systolic blood pressure with no significant effect on heart rate compared to control after 6 weeks. *A. gabonensis* treatment significantly prevented the rise in systolic blood pressure in chronic sucrose-treated rats. The decrease in blood pressure under *A. gabonensis* and nifedipin was even significant as compared to normal rats. The decrease in systolic blood pressure compared to chronic sucrose-treated rats was 24.84% ($p < 0.01$) and 22.61% ($p < 0.05$) at the respective doses of 150 and 300 mg/kg of the plant extract. The heart rate in control rats, measured simultaneously with the blood pressure was 412 ± 2 beats/min. Sucrose significantly increase the heart rate by 2.91%. Treatment with the plant extract significantly decreased the heart rate as compared to control and untreated chronic sucrose rats. As shown in table 1, the final body weights were significantly decreased in sucrose treated rats as compared to control. *A. gabonensis* at the dose of 150 mg/kg prevented the decrease of body weight.

Chronic administration of sucrose to rats during 6 weeks significantly increased heart and kidneys weight but had no effect on aorta and liver (Table 2). Concomitant administration of sucrose and plant extract significantly ($P < 0.05$) reduced heart and aorta weights as compared to normal and sucrose rats.

see Table 1.

see Table 2.

Effect of *A. gabonensis* on liver and kidney function

Table 3 shows the effect of chronic sucrose treatment on the level of serum protein, transaminases, bilirubin and creatinin. Sucrose-treatment did not affect serum protein. However, the activity of ALT and AST was significantly ($P < 0.001$). When rat

were concomitantly treated with the plant extract, there was a significant ($P < 0.001$) prevention of the increased of those parameters. There was a reduction of 43.24 and 43.83% for ALT at the respective doses of 150 and 300 mg/kg, and a reduction of 44.43 and 39.16% for AST at the same doses of the plant extract as compared to sucrose treated rats. Nifedipin also prevented the increased of ALT and AST. Administration of sucrose for 6 weeks increased the level of bilirubin by 79.17% as compared to vehicle (water). Simultaneous treatment with sucrose and the plant extract as well as nifedipin resulted in the prevention of bilirubin increase. The serum creatinin level was not significantly decreased in untreated sucrose-induced hypertensive animals as compared to the control rats.

Effect of *A. gabonensis* on oxidative stress

As shown in figure 1, the chronic sucrose administration caused a significant decrease in the tissue (heart, liver and kidney) SOD. In liver and kidney, the reduction of SOD was respectively 7.18 and 5.28% as compared to sucrose hypertensive rats. Concomitant treatment of rats with sucrose and the plant extract or nifedipin significantly prevented the decreased of SOD. The inhibition of SOD decreased by the plant extract was 7.01 and 7.18% in the liver and 5.26 and 2.28% in the kidney, respectively at the doses of 150 and 300 mg/kg as compared to sucrose hypertensive rats. In the same condition, the inhibitory effect of nifedipin was 6.97% for the liver and 5.18% for the kidney. There was a significant reduction in reduced glutathione (GSH) level in the liver, heart and kidney between normal and sucrose-treated rats. The reduction of GSH was 54.51% for the liver, 49.03% in the kidney and 52.42% in the heart. At the dose of 300 mg/kg, *A. gabonensis* significantly inhibited the reduction of GSH by 46.40% in the liver, 37.10% in the kidney and 36.72% in the heart as compared to sucrose hypertensive rats. Malondialdehyde (MDA) was significantly increased in the liver and the kidney of rat receiving only sucrose as compared to control rats. That increased was 15.93% in the liver and 6.66% in the kidney. Simultaneous administration of sucrose and *A. gabonensis* significantly prevented the increase of MDA in

the liver ($P < 0.05$) by 11.36% at the dose of 150 mg/kg and by 11.32% at the dose of 300 mg/kg as compared to sucrose hypertensive rats. In the kidney, the inhibition was 1.57 and 2.61% respectively.

see Table 3.

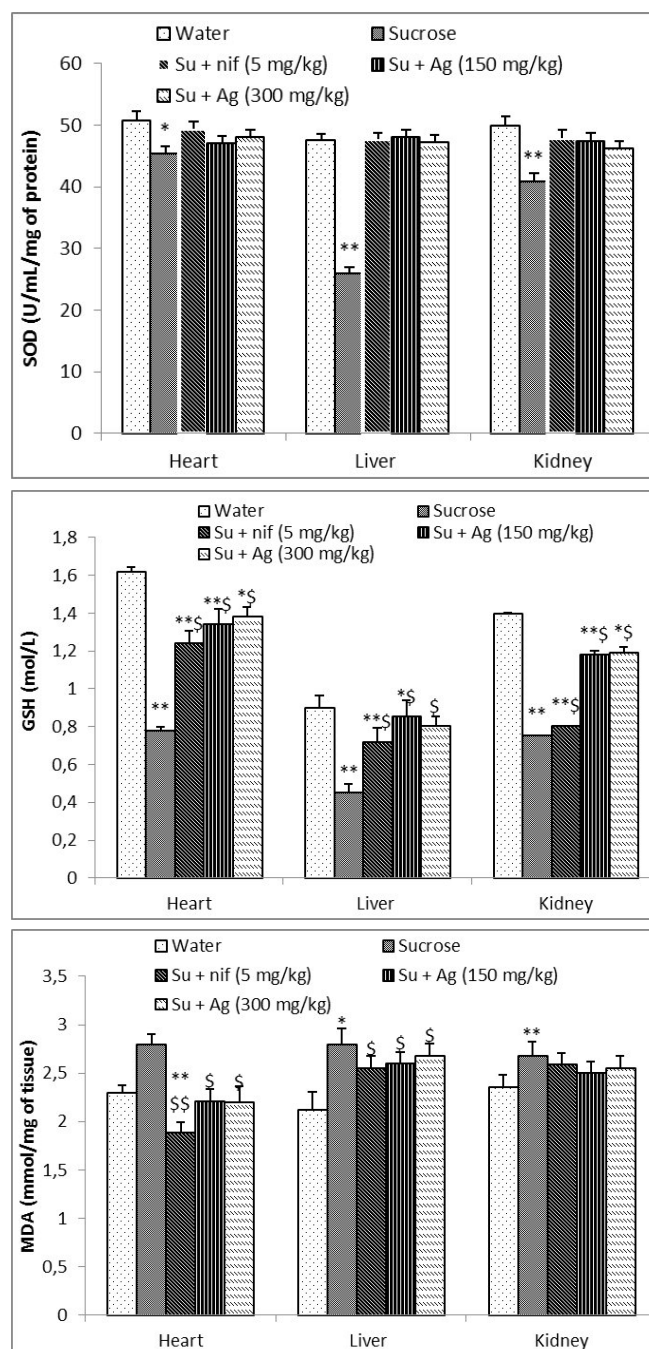


Figure 1: Effect of *Allanblackia gabonensis* on superoxide dismutase, reduced glutathione and malondialdehyde levels in sucrose-treated rats.

Values are means \pm SEM, $n = 5$. * $P < 0.05$, ** $P < 0.001$ compared to control; $^{\$}p < 0.05$, $^{\$\$}p < 0.001$ compared to sucrose treated animals. Su: Sucrose; Ag: *Allanblackia gabonensis*; Nif: nifedipin (5 mg/kg).

Discussion

The results obtained from the present study demonstrated that chronic sucrose administration in rats induced significant increase in systolic arterial pressure which was related by the elevation of the heart rate, serum alanine and aspartate transaminase activities, bilirubin level and heart and kidneys weight. In association with the high blood pressure, the chronic sucrose administration caused significant increases in lipid peroxidation and reduced superoxide dismutase and GSH levels in the heart, liver and kidney. These results were consistent with previous reports (4). In our study, we observed an increased in systolic arterial blood pressure.

As reported previously, the rise in blood pressure during chronic sucrose consumption can be attributed to several mechanisms including secretion of hormones and neurotransmitters, stimulation of the sympathetic nervous system, alteration of baroreceptor activity and volume overload (14). Another mechanism may involve a reduction of nitric oxide production or an increased of its utilization, leading to vasoconstriction and therefore arterial hypertension (15). In the present study, we observed a significant reduction of systolic arterial pressure levels when sucrose-treated rats were given *A. gabonensis* extract. Such an effect might be due, at least in part, by a decrease in arterial peripheral resistance. It has been reported that the increase in mean blood pressure in hypertension is due, for a given input flow, to an increase in vascular resistance, which is produced by a reduction in the caliber of small arteries (16). Phytochemistry of the extract used in the present study revealed the presence of some secondary metabolites like phytosterols, xanthons, biflavonoids and benzophenones (17). The blood pressure lowering effect of the plant extract could be due the presence of one of those compounds, especially biflavonoids (4). Our data also showed that sucrose consumption for six weeks affected resting heart rate, suggesting that heart rate changes may mediate the elevation of blood pressure in sucrose-treated rats. In this study, *A. gabonensis* extract at the dose of 150 mg/kg

significantly reduced heart rate of rats receiving sucrose and the plant extract compared to chronic sucrose treated and control rats, suggesting a direct action on cardiac activity provoking negative chronotropic effect.

Hypertension is associated with some damages in kidney and liver. Since ALT, AST, bilirubin and creatinin are parameters used to determined liver or kidney affection, those parameters was evaluated in the present study to asses liver and kidney functions in our hypertensive rats. The results revealed a significant increased in serum ALT and AST activities in untreated sucrose-induced hypertensive rats as compared to normal rats. The obvious sign of hepatic injury is the leakage of cellular enzymes into plasma (18). Chronic sucrose ingestion in our study caused significant changes in bilirubin levels, suggesting an effect on red blood cells and/or hemoglobin glycation. Those effects were prevented by the plant extract and nifedipin. Kidney is one of the important target organs in hypertension. Increase in creatinin has a positive correlation with kidney damages associated with hypertension. In the present study, the level of creatinin was not significantly increased in untreated hypertensive rats as compared to normal animals, showing that after six week sucrose consumption, the kidneys are not affected. Our results revealed that *A. gabonensis* may possess the capability to prevent at least in part, adverse effects of sucrose on liver.

Chronic sucrose consumption leads to the production of free radicals (4). In our study, sucrose enhanced the formation of MDA in the heart, liver and kidney indicating an increase in lipid peroxidation, a major end-point of oxidative damage, and caused drastic alterations in antioxidant defense systems. Particularly, the activities of tissue superoxide dismutase (SOD) and glutathione reduced (GSH) activity was significantly decreased. Concomitant administration of the plant extract and sucrose prevented oxidative stress. These results suggest that *A. gabonensis* extract was able to inhibit lipid peroxidation and the reduction of SOD and GSH. This may be attributed, at least in part to the flavonoid content of the extract, since they are

reported to be strong antioxidants.

In conclusion, the present study shows that the methanol stem bark extract of *A. gabonensis* possesses a preventive effect on sucrose-induced hypertensive rats. This activity may be related to its antioxidant activity, and therefore, justifies the empirical use of *A. gabonensis* for the management of arterial hypertension. Further investigation needs to be done in order to elucidate the exact mechanism of the antihypertensive effect of *A. gabonensis*.

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	Treatments				
	Water	Sucrose	Su+Nif (10mg/kg)	Su+Ag (150mg/kg)	Su+Ag (300mg/kg)
SAP (mmHg)	135.00 ± 1.05	159.20 ± 5.49*	126.80 ± 1.98* [§]	119.60 ± 2.11* ^{§§}	123.20 ± 2.65* [§]
HR (BPM)	412.00 ± 2.00	424.00 ± 2.00*	404.00 ± 4.00 [§]	401.00 ± 2.00* [§]	415.00 ± 3.00
Body weight (g)	210.14±2.03	202.12±3.25*	199.18±1.68*	203.38±2.06	200.05±2.59*

Table 1: Effect of *Allanblackia gabonensis* extract on blood pressure, heart rate and body weight in control and sucrose-hypertensive rats

Values are given as mean ± SEM, for 5 rats in each group. * P<0.05 compared to control; [§]P<0.05, ^{§§}P<0.001 compared to Sucrose; Su: Sucrose; Ag: *Allanblackia gabonensis*; Nif: nifedipin (5 mg/kg). SAP: systolic arterial pressure; HR: heart rate (beats per minute).

	Heart (mg/100g BW)	Kidneys (mg/100 g BW)	Aorta (mg/100g BW)	Liver (mg/100g BW)
Water	0.32±0.01	0.50±0.02	0.06±0.00	4.00±0.28
Sucrose	0.41±0.03*	0.58±0.03*	0.07±0.00	4.11±0.22
Su+Nif (5 mg/kg)	0.30±0.02 ^{§§}	0.55±0.02 ^{§§§}	0.05±0.01 [§]	3.10±0.20
Su+Ag (150 mg/kg)	0.31±0.01 [§]	0.58±0.02*	0.03±0.01*** ^{§§§}	3.02±0.45
Su+Ag (300 mg/kg)	0.30±0.01 [§]	0.62±0.02**	0.04±0.01*** ^{§§§}	3.59±0.32

Table 2: Effect of *A. gabonensis* on organ weights of sucrose hypertensive rats

Values are expressed as means ± SEM; n=5; *P< 0.05, **P< 0.01, ***P<0.001, significantly different compared to normal rats. [§]P<0.05, ^{§§}P<0.01, ^{§§§}P<0.001, significantly different compared to sucrose hypertensive rats. Su: Sucrose; Ag: *Allanblackia gabonensis*; Nif: nifedipin.

	Water	Sucrose	Su + Nif (5 mg/kg)	Su + Ag (150 mg/kg)	Su + Ag (300 mg/kg)
Protein (mg/dL)	50.75±5.99	41.48±6.02	42.65±3.89	50.10±6.12	51.66±4.01
ALAT (UI)	196.85±4.74	354.33±7.06***	295.19±6.25 ^{§§§}	201.09±5.87 ^{§§§}	199.00±6.41 ^{§§§}
ASAT (UI)	60.18±4.15	90.80±5.92***	72.64±4.09* ^{§§§}	50.45±3.08 ^{§§§}	55.24±5.34 ^{§§§}
Bilirubin (mg/dL)	4.13±0.85	8.66±1.06*	6.61±1.05	6.29±1.04	6.15±1.06
Creatinin (mg/dL)	42.30±6.95	44.55±5.96	33.25±6.33	37.79±5.23	34.18±5.00

Table 3: Effect of *A. gabonensis* on liver and kidney function parameters

Values are expressed as means ± SEM; n=5; *P< 0.05, ***P< 0.001, significantly different compared to normal rats. ^{§§§}P< 0.001, significantly different compared to sugar hypertensive rats.