

Impact of *Gnetum africanum* and *Amaranthus retroflexus* Plant Food on Urea, Creatinine and Plasma Electrolytes

Guy Stéphane Padzys¹, Joseph Privat Ondo² & Linda Priscilla Omouendze¹

¹Département de Biologie, Faculté des Sciences, Université des Sciences et Technique de Masuku, Franceville, Gabon

²Département de Chimie, Faculté des Sciences, Université des Sciences et Technique de Masuku, Franceville, Gabon

Correspondence: Guy Stéphane Padzys, Département de Biologie, Faculté des Sciences, Université des Sciences et Technique de Masuku, Franceville 943, Gabon. E-mail: padzys@gmail.com

Received: October 19, 2014 Accepted: December 30, 2014 Online Published: January 15, 2015

doi:10.5539/jas.v7n2p174

URL: <http://dx.doi.org/10.5539/jas.v7n2p174>

Abstract

Amaranthus retroflexus (*AmE*) and *Gnetum africanum* (*GnA*) is the most popular green leafy vegetable in Gabon and is gaining equal popularity as a delicious food leaf in other African countries such as Cameroon, Nigeria, Congo and Angola. The purpose of this study was to assess the impact of these plants in modulating urea, creatinine and plasma electrolytes. 50 mg/kg of aqueous extract were given to rats in the experimental groups for two week, while rats in the control group received an appropriate volume of water. Urea creatinine, and Plasma electrolytes were measured by spectrophotometric method. Chronic oral administration for two week of 50 mg/kg of *AmE* and *GnA* aqueous extracts significantly decreased plasma sodium concentration benefit to potassium ($P < 0.05$). No significant harmful changes ($P > 0.05$) in weight, blood glucose, creatinine and urea plasma concentration.

Keywords: *Amaranthus retroflexus*, *Gnetum africanum*, electrolytes, urea, creatinine

1. Introduction

Plants, particularly green leafy vegetables, serve as a major dietary reservoir of the essential PUFAs, dietary fiber, antioxidants and other bioactive compounds that exert cardioprotective biological mechanisms (Veer et al., 2000; Kris-Etherton et al., 2000; Hu, 2003; Slavin, 2003). Several cross-sectional and prospective studies have found that consumption of legumes and legume-based diets are inversely associated with the prevalence and incidence of obesity, cardiovascular disease, type 2 diabetes (Trinidad et al., 2010; Villegas et al., 2008; Venn et al., 2004; Kolonel et al., 2000; Flight et al., 2006).

Amaranthus extrafolius (*AmE*) and *Gnetum africanum* (*GnA*) are the most popularity plant food in Gabon and is gaining equal popularity in other African countries such as Cameroon, Nigeria, Congo and Angola (Eyo & Abel, 1983). *AmE*, is an erect, annual herb reaching a maximum height near 3 m. The leaves are nearly 15 cm long on large individuals, the ones higher on the stem having a lance shape and those lower on the plant diamond or oval in shape (Figure 1A). The seed of *GnA* is oval in shape and small in size, about 0.5 cm in diameter. They are greenish in color when and randomly from a thick bush around the rain unripe and reddish when ripe (Figure 1B).

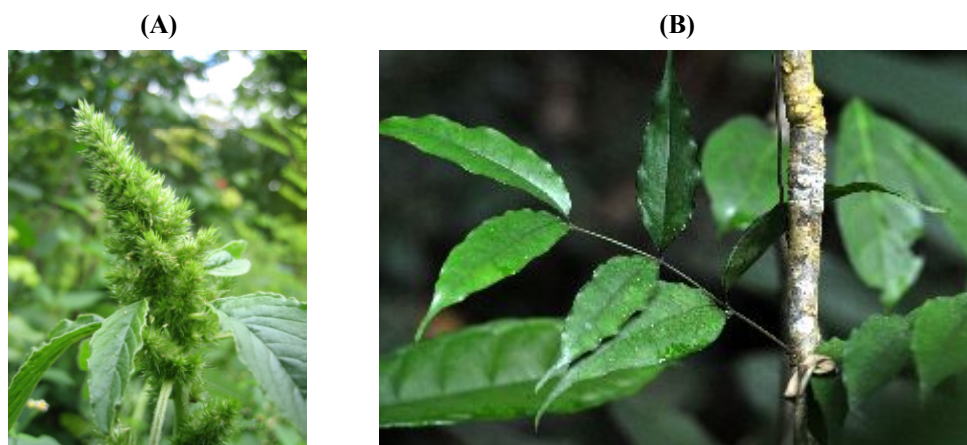


Figure 1. Photo of *Gnetum africanum* (A) and *Amaranthus retroflexus* (B)

Little or no information is available on the impact of *AmE* and *GnA* chronic consumption on physiological parameters. Topics will show firstly the impact of regular consumption of *GnA* and *AmE* on body weight, blood glucose, urea and creatinine. Finally study the impact of *GnA* and *AmE* consumption on plasma concentration of Chloride (Cl), Potassium (K) and Sodium (Na).

2. Materials and Methods

2.1 Animals

Twenty eight males Wistar rats weighing 255-279 g (IFFA-CREDO, France) were used for this experiment. The animals were born in the laboratory from twenty litters, culled to 7 pups per litter to ensure normal body growth. The animals were housed in standard cages under controlled temperature conditions (22 ± 1 °C). Food and water were available ad libitum throughout the experiment. From birth, the rats were kept on a reversed 12:12 light–dark cycle (dark period 08:00–20:00).

2.2 Preparation of *AmE* and *GnA* Aqueous Extracts

Fresh plant samples of, *AmE* and *GnA* were obtained from market. The preparation of plant materials was as previously reported (Olatunji et al., 2006). The extract was then filtered through Whatman No.1 filter paper and the residue was discarded. The resultant filtrate was then evaporated to dryness and stored in capped bottles at 4 °C until use. The desired amount of extract was dissolved in water to make 50 mg/ml of stock solutions for lower and higher doses respectively.

2.3 Chronic Consumption of *AmE* and *GnA* Aqueous Extracts

All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (no. 85-23, revised 1996), the recommendations of the European Community Council for the Ethical Treatment of Animals (no. 86/609/EEC) - and the regulations of the University of Masuku. All efforts were made to minimise animal suffering.

Rats in the control group received an appropriate volume of water per day as vehicle by gavage. The experimental rats received 50 mg/kg of the *AmE* and *GnA* aqueous extract by gavage. The control group or of *Ame* and *GnA* aqueous extract administration was stopped 24 h before the animals were killed. Weighed and an intracardiac blood sample (500–1000 ml) was taken between 11h and noon for electrolytes, urea and creatinine measurements. Blood was collected over 1–2 min into tube heparin or EDTA.

2.4 Biochemical Assays

Plasma concentrations of urea, creatinine, and electrolytes were performed by spectrophotometric method. These studies were made respectively using automata Bs 400 mindray chemistry analyzer (Biorad, France) for urea and creatinine. Electrolytes plasma were analysed by Spotlyte Na K Cl analyzer (Medica Corporation, USA).

Concentration of blood glucose was determined using method described by (Padzys et al., 2011). This approach using glucometer (Glucose-test, CarenSensN Sud Korea)

2.5 Preparation of Extracts for Phytochemical Screening

Air-dried powdered leaves (10 g) of *AmE* and *GnA* were separately extracted with 100 ml of water (Aq), by

maceration for 24 h. Extracts were filtered and the filtrate was used for photochemical screening.

2.6 Phytochemical Screening

The extract, were screened for their classes of bioactive compounds using standard procedures (Culei, 1982; Harbone, 1984; Adegunloye, 1993; Sofowora, 1983; Trease & Evans, 2002) The extracts were tested qualitatively for the presence of chemical constituents such as tannins, terpenes, saponins, flavonoids, alkaloids, anthraquinones etc.

For gallic tannins, 2 ml of 1% ferric chloride solution was added to 2 ml of the filtrate (Stiasny's test). Dark-greenish coloration indicated their presence. Tannins catechic, 2 ml of a solution of hydrochloric n-butanol are added to 2 ml of filtrate, and then heating in a water bath for 5 to 10 minutes (Bate-Smith's test). Intense red coloration indicated the presence of the catechin tannins. For total flavonoids and anthocyanes, 1 ml of the sulfuric acid was added to 2 ml of the filtrate, then 1 ml NaOH. There shown a dark color after adding acid, indicating the presence of flavonoids, the color changes to purple after addition of NaOH, indicating the presence of anthocyanins. 2 ml of the filtrate were added magnesium strips followed by hydrochloric alcoholic (cyanidine test). A rose-orange effervescence showed the presence of flavones, rose-purplish indicated of flavanones and red denoted of flavonols. We had applied the Folin's test to determine polyphenols contents. 1 ml of the Folin reagent was added to 2 ml of the filtrate, then 1 ml NaOH. Dark green coloration indicated the presence of polyphenols.

For coumarins, 2 ml of filtrate combined with 2 ml of NH_4OH , then, lookin at UV lamp (366 nm). The fluorescence presence indicated the presence of coumarins. 2 ml NH_4OH solution was added to 2 ml of the filtrate (Borntrager's test). A rose pink colour in the ammonia layer indicated the presence of anthraquinones.

For alkaloids, some drops of sulfuric Dragendorff's reagent were added to 2 ml of the filtrate. Orange precipitate formed had showed the presence of alkaloids.

To determine terpenes, test such as Salkowski's and Lieberman's test were applied. 2 ml of concentrated H_2SO_4 were added to 2 ml of filtrate, a reddish-brown ring indicated the presence of steroid, an aglycone part of the cardiac glycoside (Salkowski's test). Another part of the filtrate (2 ml) was added with 2 ml of acetic anhydride and cooled well in ice and concentrated H_2SO_4 (2 ml) was carefully added. A colour change from blue to green indicated the presence of terpenes (Lieberman's test).

Saponins were determined through frothing test. The filtrate was vigorously shaken. Frothing which persisted on warming for about 15 min indicated the presence of saponins.

2.7 Statistical Analyses

The results were expressed as group means \pm SEM. Student's t-test was used to establish the comparison between control and treatment animals since all data were normally distributed. Specific mean comparisons were then made using t-test with the Bonferroni correction. Differences were considered significant at $P < 0.05$.

3. Results and Discussion

3.1 Effects of *GnA* and *AmE* Aqueous Extract on Body Weight and Blood Glucose

Administration of *GnA* and *AmE* aqueous extract did not produce any significant effects on the body weight ($P > 0.05$). To determine if the administration of *GnA* and *AmE* aqueous extract was associated with change in glycemia, blood glucose levels were obtained (Table 1). No difference was observed in blood glucose level between control and experimental group rats: ($P > 0.05$). These results to be explain by the legumes are low-glycemic-index low-energy-dense foods that contain high amounts of dietary fiber, vegetable proteins, oligosaccharides, and phenolic and other bioactive compounds (Kromhout et al., 1996; Messina et al., 1999; Anchana, 2004).

Table 1. Effects of *AmE* and *GnA* aqueous extracts on body weight and blood glucose

Group	Body weight (g)	Blood glucose (mg/dl)
Control	255 \pm 15,64	131.25 \pm 12.8
<i>AmE</i>	279 \pm 5,73	132 \pm 11.5
<i>GnA</i>	272 \pm 6,23	132.6 \pm 10.1

Values are means \pm SEM (n = 7/group).

3.2 Effects of *GnA* and *AmE* Aqueous Extract on Urea and Creatinine

We also assessed the possible effects of *GnA* and *AmE* on the kidney by urea and creatinine measurement. No significant changes were seen in these indices after *GnA* and *AmE* administration Table 2 shows that no significant change ($P > 0.05$).

Table 2. Effects of *AmE* and *GnA* aqueous extracts on plasma urea and creatinine

Group	Urea (mmol/l)	Creatinine (μ mol/l)
Control	11.1 \pm 2.1	102 \pm 5
<i>AmE</i>	12 \pm 1.1	104 \pm 4.5
<i>GnA</i>	11 \pm 1.2	106 \pm 3.2

Values are means \pm SEM (n = 7/group).

3.3 Effects of *AmE* and *GnA* Aqueous Extract on Plasma Electrolytes

We also measured Na and K to assess any electrolyte disturbance; analyses showed significant changes in Na and K after plant administration. Chronic administration of *GnA* and *AmE* aqueous extract produced a significant decrease ($P < 0.05$) of plasma sodium concentration (Table 3). These results show that a decrease plasma sodium concentration was associated with an increase plasma potassium concentration ($P < 0.05$). No significant change was observed in level of plasma chloride concentration after consumption of *GnA* and *AmE* extract aqueous ($P > 0.005$). These findings support the previous findings on the effect of legumes on plasma electrolytes in rats (Chen et al., 2003). Vegetable consumption was increased with a consequent increase in potassium intake from 37 mmol/day to 71 mmol/day, showed a large fall in blood pressure despite sodium intake being fixed at a low intake of 130 mmol/day (Appel et al., 1997). Published study by the same group clearly showed an additive effect of increasing potassium and reducing sodium intake (Sacks et al., 2001).

Table 3. Effects of *AmE* and *GnA* aqueous extracts on plasma electrolytes

Group	Sodium	Potassium	Chloride
Control	137.66 \pm 0.982	9.48 \pm 0.3	112 \pm 2.1
<i>AmE</i>	133.1 \pm 0.294*	12.02 \pm 0.2*	113 \pm 0.8
<i>GnA</i>	133.02 \pm 1.3*	13.44 \pm 0.86*	113.5 \pm 1.3

Values are means \pm SEM (n = 7/group). *Significantly different from control group at $P < 0.05$.

The phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts. The results of this screening were shown in Table 4. The qualitative analyze of extracts had shown the presence of triterpenoids, polyphenol and more coumarins in *AmE* and *GnA* in the phytochemical constituent of the aqueous extracts.

Table 4. Phytochemical screening of *AmE* and *GnA* water extracts

Chemical constituents	<i>AmE</i>	<i>GnA</i>
Saponins	+++	-
Tannins	<i>Gallic</i>	-
	<i>Catechin</i>	-
Alkaloids	+++	-
Triterpenoids	+	++
Polyphenols		+
	<i>Flavonols</i>	-
Flavonoids	<i>Flavones</i>	-
	<i>Flavanones</i>	-
Free anthraquinones	+	-
Coumarine	++	+++
Proanthocyanane		-
	<i>Digitoxine</i>	++
Cardiac glycosides	<i>Digitoxigenine</i>	-
	<i>Gitoxine</i>	-
	<i>gitoxigenine</i>	-
Reducing sugars	-	-

Legend: -: Not detected; +: Rare; ++: Abundant; +++: Very abundant.

4. Conclusions

The objective of this study was to demonstrate if the intake of *Amaranthus retroflexus* and *Gnetum africanum*, is associated with modulation of urea, creatinine and plasma electrolytes in rat. We have demonstrated for the first time that, intake of *Amaranthus retroflexus* and *Gnetum africanum* induces modulation of potassium and sodium plasma concentration in rat. This modulation is necessary for the prevention of cardiovascular disease (Appel et al., 1997; Sacks et al., 2001; Chang et al., 2006; Cook et al., 2007). The present diet may serve as a model of human alternative therapy of cardiovascular disease.

Acknowledgements

Funding provided by grants to Dr Isaac Mouaragadja Institut of Biotechnology, University of Masuku for assistance.

References

- Anchana, C., Aphiwat, T., & Nuansri, R. (2005). Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chemistry*, 92, 491-497. <http://dx.doi.org/10.1016/j.foodchem.2004.07.035>
- Appel, L. J., Moore, T. J., Obarzanek, E., Vollmer, W. M., Svetkey, L., & Sacks, F. M. (1997). A clinical trial of the effects of dietary patterns on blood pressure. *N. Engl. J. Med.*, 336, 1117-24. <http://dx.doi.org/10.1056/NEJM199704173361601>
- Chang, H. Y., Hu, Y. W., & Yue, C. S. J. (2006). Effect of potassium-enriched salt on cardiovascular mortality and medical expenses of elderly men. *Am. J. Clin. Nutr.*, 83, 1289-1296.
- Chen, C. C., Hsu, J. D., & Wang, S. F. (2003). Hibiscus Sabdariffa extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Journal of Agricultural and Food Chemistry*, 51, 5472-5477. <http://dx.doi.org/10.1021/jf030065w>
- Cook, N. R., Cutler, J. A., & Obarzanek, E. (2007). The long-term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the Trials of Hypertension Prevention. *Br Med J*, 334, 885-888. <http://dx.doi.org/10.1136/bmj.39147.604896.55>
- Eyo, E., & Abel, U. (1983). Chemical composition of amino-acid content of *G. africanum* leaves. *Nig J Nutr Sci*,

- 4, 52-5.
- Flight, I., & Clifton, P. (2006). Cereal grains and legume in the prevention of coronary heart disease and stroke: a review of the literature. *Eur J Clin Nutr*, *60*, 1145-59. <http://dx.doi.org/10.1038/sj.ejcn.1602435>
- Hu, F. (2003). Plant-based foods and prevention of cardiovascular disease: an overview. *Am J Clin Nutr*, *78*, 544S-551S.
- Kolonel, L. N., Hankin, J. H., Whittemore, A. S., Wu, A. H., Gallagher, R. P., Wilkens, L. R., ... West, D. W. (2000). Vegetables, fruits, legume and prostate cancer: a multiethnic casecontrol study. *Cancer Epidemiol Biomarkers Prev*, *9*, 795-804.
- Kris-Etherton, P. M., Taylor, D. S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V., ... Etherton, T. D. (2000). Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr*, *71*, 179S-188S.
- Kromhout, D., Bloemberg, B. P., Feskens, E. J., Hertog, M. G., Menotti, A., & Blackburn, H. (1996). Alcohol, fish, fibre and antioxidant vitamins intake do not explain population differences in coronary heart disease mortality. *Int J Epidemiol*, *25*, 753-759. <http://dx.doi.org/10.1093/ije/25.4.753>
- Mirmiran, P., Noori, N., Zavareh, M., & Azizi, F. (2009). Fruit and vegetable consumption and risk factors for cardiovascular disease. *Metabolism*, *58*, 460-468. <http://dx.doi.org/10.1016/j.metabol.2008.11.002>
- Olatunji, L. A., Adebayo, J. O., Adesokan, A. A., Olatunji, V. A., & Soladoye, A. O. (2006). Chronic administration of aqueous extract of *Hibiscus sabdariffa* enhances Na⁺-K⁺-ATPase and Ca²⁺-Mg²⁺-ATPase activities of rat heart. *Pharm Biol*, *44*, 213-216. <http://dx.doi.org/10.1080/13880200600686129>
- Padzys, G. S., Thornton, S. N., Martrette, J. M., & Trabalon, M. (2011). Effects of short term forced oral breathing in rat pups on weight gain, hydration and stress. *Physiology & Behavior*, *102*, 175-180. <http://dx.doi.org/10.1016/j.physbeh.2010.10.018>
- Culei, I. (1982). Methodology for the analysis of vegetable drugs. *Practical manual on the industrial utilization of medicinal and aromatic plants* (pp. 67-81). Center Building, Romania.
- Harbone, J. B. (1984). *Phytochemical methods* (2nd ed., pp. 84-196). London: Champion and Hall Publishers. <http://dx.doi.org/10.1007/978-94-009-5570-7>
- Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa* (2nd ed., pp. 1-153). Spectrum Books limited, Ibadan, Nigeria.
- Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed., pp. 214-393). Saunders.
- Adegunloye, B. J., Omoniyi, J. O., Owolabi, O. A., Ajagbonna, O. P., Sofola, O. A., & Coker, H. A. B. (1993). Petals extract of *Hibiscus sabdariffa* elicits relaxation of rat aorta by stabilizing the membrane and stimulating Na⁺-K⁺-ATPase. *Nig J Physiol Sci.*, *9*, 74-79.
- Slavin, J. (2003). Why whole grains are protective: biological mechanisms. *Proc Nutr Soc*, *62*, 129-134. <http://dx.doi.org/10.1079/PNS2002221>
- Trinidad, T. P., Mallillin, A. C., Loyola, A. S., Sagum, R. S., & Encabo, R. R. (2010). The potential health benefits of legume as a good source of dietary fibre. *Br J Nutr*, *103*, 569-74. <http://dx.doi.org/10.1017/S0007114509992157>
- Veer, P., Jansen, M., Klerk, M., & Kok, F. (2000). Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutr*, *3*, 103-107.
- Venn, B. J., & Mann, J. I. (2004). Cereal grains, legume and diabetes. *Eur J Clin Nutr*, *58*, 1443-61. <http://dx.doi.org/10.1038/sj.ejcn.1601995>
- Villegas, R., Gao, Y. T., Yang, G., Li, H. L., Elasy, T. A., Zheng, W., & Shu, X. O. (2008). Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women's Health Study. *Am J Clin Nutr*, *87*, 162-7.

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).