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Acute toxicity of seeds from *Sphenostylis stenocarpa* (Hochst ex A. Rich.) Harms

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ABSTRACT

The major fear bound to the consumption of leguminous plant is relative to the concentration of secondary metabolites accumulated in reserve organs such as seeds. These metabolites can induce an anti-nutritional or toxic effect to various degrees according to their nature and concentration. The present work aims to achieve a toxicity study of the seeds of *Sphenostylis stenocarpa* commonly called African Yam Bean. At the end of the phytochemical screening made on the flours of the seeds in the state and roasted, aiming to put in evidence the alkaloids, coumarins, tannins, flavonoids and saponosides, only the tannins, flavonoids and saponosides proved to be positive. The same analysis revealed the absence of these metabolites in boiled seed flour and the presence of tannins and flavonoids in the cooking water. Observation of the impact of the content of these present metabolites in the seeds on the biological and histological parameters of the experimental rats as compared to the control rats do not reveal any significant differences or particular remarks. It emerges from this study that *Sphenostylis stenocarpa* does not have a toxic effect on the health of the consumer, who should fully benefit from its nutritional potential.

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INTRODUCTION

Sphenostylis stenocarpa, commonly known as African Yam Bean (AYB), is a vegetable belonging to the family of Fabaceae, subfamily Papilionoideae, tribe Phaseoleae, sub-tribe Phaseolinae and genus of *Sphenostylis*. It's planted and distributed in West and Central Africa. Some varieties of this vegetable produce beyond seeds, the tubers very similar to sweet potato. Seeds can contain up to 21% protein of excellent nutritional quality and 24-68% carbohydrate (Ekanayake *et al.*, 2000). The tubers are richer in protein than sweet potato, cassava and potatoes. There are used to produce flours. AYB is rich in minerals such as potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), iron (Fe) and zinc (Zn) (Edem *et al.*, 1990). This vegetable adapts well to light and poor soils and can withstand various cropping systems. It grows on a wide range of soils, including acidic, leached and sandy soils at altitudes ranging from sea level to 1950 (Amoatey *et al.*, 2000).

Secondary metabolites participate in the life of the plant and play a wide variety function. They can allow communication between plants. They can also attract some species with beneficial roles (pollinators) or defend through bitter or toxic secretions (alkaloids) against predators (Duodu *et al.*, 2003). These secondary metabolites are sometimes accumulated in the reserve organs such as seeds of edible plants by humans and animals. This results in a selectivity in the choice of products of plant origin for use as feed for humans and animals.

Such products include vegetables. They occupy a prominent place in human and animal nutrition because of their nutritional potential coupled with their cost and then their great diversity.

The presence of antinutritional elements is observed among vegetables in general and more specifically of *S. stenocarpa* (Gilani *et al.*, 2005). Antinutritional elements are likely to reduce the bioavailability of nutrients or promote flatulence, diarrhea and other

digestive disorders. Among these anti-nutritional elements, we could cite antitrypsic factors, phytates, tannins, oxalate and alkaloids (Fasayiro *et al.*, 2006). Oligosaccharides such as raffinose, stachyose and α -galactosides are responsible for flatulence and diarrhea. Toxicity may be feared due to the consumption of seed which would be justified by the results of the work of Nyananyo *et al.* (2011) and Ghaniyah *et al.* (2016). They observed the presence of alkaloids in the seeds of *S. stenocarpa*. In Benin, for example, opinions suggest that cooking seeds at home would cause chicken pox (Ghaniyah *et al.*, 2016). All these arguments, sometimes justified or not, explain the reluctance observed in relation to the consumption of the seeds of *S. stenocarpa* in spite of the nutritional quality confirmed by several scientific studies. The AYB is therefore very little or poorly known and classified as neglected and under-exploited in Benin [16] and elsewhere.

Studies have shown the presence of secondary metabolites in *S. stenocarpa* seeds (Ghaniyah *et al.*, 2016). Azeke *et al.* (2007) showed that the preparation process can considerably influence the concentrations. Ndidi *et al.* (2014) estimated that transformation processes would reduce the concentration of anti-nutrients to a level tolerable to the human body. This work aims to carry out the acute toxicity study of *S. stenocarpa* seeds (as roasted and boiled) in order to make an objective contribution to the related statements. Phytochemical components were screened to investigate the presence of some secondary metabolites.

MATERIALS AND METHODS

Phytochemical analysis

The beans were purchased at Bantè, a town in north of Benin. Four samples were obtained from the seeds of beans. They were either ground into powder, roasted before grinding into powder or cooked before grinding and the boiled water was also recovered and tested for phytochemical group screening. All powdered samples were stored at room temperature until use. Phytochemical screening of the three samples was carried out basing on the method

described by Bladt and Wagner (2001). Different phytochemical groups were investigated including alkaloids, coumarins, flavonoids, tannins, triterpenes, anthracens and saponosides.

Preparation of animals

Twelve female Wistar albino rats, weighing 130 -150g body weight were used. They were housed in cages and maintained in a light controlled environment (12:12h light-dark cycle) and had unlimited access to food and water. They were assigned to four (4) lots of three rats (Lot1: Rats having received raw seeds extract, Lot2: Rats having received the roasted seeds extract, Lot3: Rats having received the cooked seeds extract). In the study, the animals were handled and cared for, in accordance with the internationally accepted standard guidelines for use of animals.

Acute oral toxicity test: Limit test

Acute oral toxicity of powder from *Sphenostylis stenocarpa* was evaluated in rats according to the Organization for Economic Co-operation and Development (OECD) guidelines for the Testing of Chemicals section4- 423- limit test, adopted in 2001. Rats were preliminarily acclimatized during five days in the laboratory. They had free access to water and standard diet. Rats were deprived of food but not water 12h prior to administration of the test substances. Four groups of three rats were made. A group of rats which received distilled water from day 1 to day 14 (control group). The three other groups of rats were administrated by gavage the differents extracts at a dose of 5000 mg / kg body weight. The boiled water was not tested for toxicity essay.

The animals were observed quietly and continuously for eight hours just after the administration and once daily up to 14 days. The monitoring was based on general behaviour changes, body weight evolution, mortality and any toxicity signs. At the end of the experience, 0.1 ml of Thiopental (100 mg/ml) was injected intraperitoneally to anesthetize the animals. Blood samples were collected in tubes with or without anticoagulant for respectively hematological and biochemical examinations.

About haematological analysis, hematocrit (HCT), Haemoglobin (Hb), Mean corpuscular haemoglobin concentration (MCHC), Erythrocytes, leukocyte count, mean corpuscular volume differential (MCV), mean corpuscular haemoglobin (MCH), platelet count (PLT), were performed on blood using Sysmex K x 21 automated haematology analyzer. The blood biochemical analyses including glucose, urea, cholesteroemia, creatinine, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), sodium, potassium and chloride were performed using an automate Cyan 130 expert (Belgium). The liver and kidneys of rats those have been fed with the cooked extracts were removed, immediately weighed and transferred into a buffered formalin solution for histological examinations.

Statistical analysis

Results were expressed as mean $\pm$ SD. Statistical significance was determined by one-way ANOVA using Minitab software and Bonferonni post hoc test. P-values less than 0.05 were considered significant.

RESULTS

Phytochemical screening

Phytochemical screening showed differences in the occurrence of secondary metabolites among the seed preparations (Table 1). Alkaloids and coumarins were absent from all four samples. Tannins and flavonoids were detected in the natural seed flour, roasted seed flour, and boiled water, but were absent from the boiled seed flour. Saponosides were present in the natural and roasted seed flours but were not detected in either the boiled seed flour or boiled water. Overall, the boiled seed flour showed negative results for all the phytochemical groups examined (Table 1).

Table 1. Results of phytochemical screening

Sample family	1	2	3	EC
Alkaloid	(-)	(-)	(-)	(-)
Coumarin	(-)	(-)	(-)	(-)
Tannins	(+)	(+)	(-)	(+)
Flavonoid	(+)	(+)	(-)	(+)
Saponoside	(+)	(+)	(-)	(-)

(-): Negative; (+): Positive; 1 = seed flour in its natural state; 2 = Roasted seed flour; 3 = boiled seed flour; EC= boiled water.

Body weight and relative organ weights

Body weight increased progressively across all groups during the 14-day observation period (Fig. 1). At day 1, body weights were approximately 130–145 g across the control and treatment groups and increased to approximately 155–175 g by day 14. The control group showed the greatest final body weight, whereas the groups receiving raw, roasted, and cooked seed preparations showed slightly lower final weights. No mortality or observable changes in general behavior were recorded following administration of the seed preparations.

The relative liver and kidney weights of the experimental rats were comparable with those of the control rats (Fig. 2). Relative liver weight was approximately 3.5 g in the control group and 3.2 g in the experimental group, while relative kidney weight remained below 0.5 g in both groups. No marked differences in organ weight were observed between the control and experimental rats.

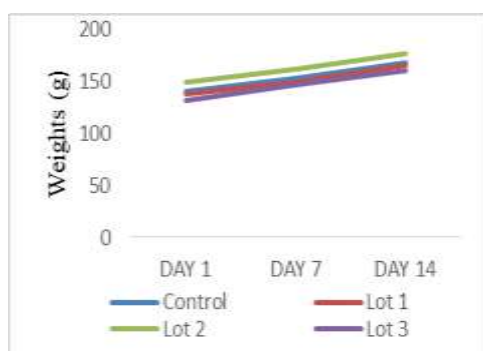


Fig. 1. Effect of seed flour of *S. stenocarpa* on weights of rats

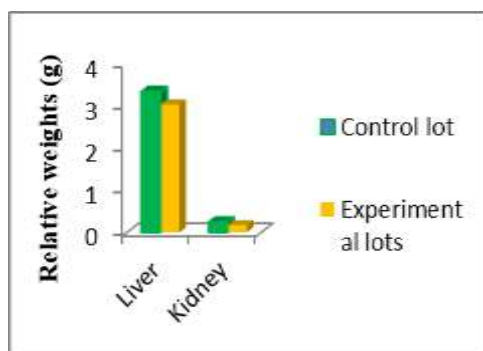


Fig. 2. Effect of seed flour of *S. Stenocarpa* on relative weights of rat's liver and kidney

Hematological parameters

The hematological profile varied among the treatment groups (Table 2). Erythrocyte counts were 7.68 ± 0.08 T/L in the control group and 7.61 ± 0.34 , 7.72 ± 0.26 , and 7.34 ± 0.53 T/L in Lots 1, 2, and 3, respectively. Hemoglobin concentration was significantly lower in Lot 1 (13.00 ± 0.30 g/dL) than in the control group (14.07 ± 0.25 g/dL), whereas values of 13.95 ± 0.15 and 14.03 ± 0.40 g/dL were recorded in Lots 2 and 3, respectively. Hematocrit was $43.33 \pm 0.58\%$ in the control group compared with $40.67 \pm 1.53\%$ in Lot 1, $40.00 \pm 2.65\%$ in Lot 2, and $37.00 \pm 2.00\%$ in Lot 3. The value for Lot 1 was significantly different from the control (Table 2).

Mean corpuscular volume (MGV) was 56.33 ± 0.58 fL in the control group and decreased significantly to 52.00 ± 1.00 fL in Lot 1; values of 53.33 ± 1.15 and 53.00 ± 1.00 fL were recorded in Lots 2 and 3, respectively. Mean corpuscular hemoglobin (MCH) was also significantly lower in Lot 1 (16.67 ± 0.58 pg) than in the control group (18.67 ± 0.58 pg), while Lots 2 and 3 recorded 18.00 ± 0.00 and 17.33 ± 0.58 pg, respectively. MCHC values ranged from $31.33 \pm 1.53\%$ to $32.67 \pm 0.58\%$ across the groups (Table 2).

Platelet counts were 1001 ± 61 G/L in the control group and 1016 ± 29 , 1013 ± 16 , and 1019 ± 21 G/L in Lots 1, 2, and 3, respectively, with the Lot 1 value indicated as significantly different from the control. Leukocyte count decreased significantly from 8.78 ± 0.14 G/L in the control group to 7.94 ± 0.14 G/L in Lot 1, while Lots 2 and 3 recorded 8.25 ± 0.49 and 7.46 ± 0.47 G/L, respectively. Lymphocyte counts were 3.85 ± 2.12 , 3.09 ± 0.70 , 3.81 ± 1.41 , and 3.89 ± 2.12 G/L in the control, Lot 1, Lot 2, and Lot 3 groups, respectively (Table 2).

Neutrophil counts were 1.42 ± 0.00 G/L in the control group, 1.23 ± 1.41 G/L in Lot 1, 1.31 ± 0.70 G/L in Lot 2, and 1.52 ± 0.70 G/L in Lot 3, with a significant difference indicated for Lot 1. Monocyte counts were 3.50 ± 2.12 G/L in the control group and 2.71 ± 0.70 , 3.10 ± 4.24 , and 3.25 ± 2.83 G/L in Lots 1, 2, and 3, respectively; all three treatment values were indicated as significantly different from the control (Table 2).

Biochemical parameters

The biochemical parameters are presented in Table 3. Urea concentrations were 0.84 ± 0.03 g/L in the control group and 0.83 ± 0.08 , 0.71 ± 0.04 , and 0.78 ± 0.08 g/L in Lots 1, 2, and 3, respectively. Creatinine concentrations were 6.00 ± 0.00 mg/L in the control group and 5.33 ± 0.58 , 5.33 ± 0.58 , and 5.60 ± 0.58 mg/L in Lots 1, 2, and 3, respectively. AST activity was

188.67 ± 3.21 UI/L in the control group and 173.00 ± 7.00 , 175.66 ± 6.02 , and 178.00 ± 2.50 UI/L in Lots 1, 2, and 3, respectively. Corresponding ALT activities were 100.00 ± 2.00 , 95.66 ± 2.08 , 92.33 ± 3.51 , and 92.67 ± 3.79 UI/L, respectively. Protide concentrations were 62.00 ± 2.65 g/L in the control group and 62.66 ± 0.58 , 61.00 ± 3.60 , and 64.67 ± 2.52 g/L in Lots 1, 2, and 3, respectively (Table 3).

Table 2. Result of hematological parameters

Parameters	Control	Lot 1	Lot 2	Lot3
Erythrocytes (T/L)	7.68 ± 0.08	7.61 ± 0.34	7.72 ± 0.26	7.34 ± 0.53
Haemoglobin(g/dL)	14.07 ± 0.25	$13 \pm 0.3^*$	13.95 ± 0.15	14.03 ± 0.40
Haematocrit (%)	43.33 ± 0.58	$40.67 \pm 1.53^*$	40 ± 2.65	37 ± 2
MGV (fL)	56.33 ± 0.58	$52 \pm 1^*$	53.33 ± 1.15	53 ± 1
MCH (pg)	18.67 ± 0.58	$16.67 \pm 0.58^*$	18 ± 0	17.33 ± 0.58
MCHC (%)	32.67 ± 0.58	32 ± 1	32.67 ± 0.58	31.33 ± 1.53
Platelets (G/L)	1001 ± 61	$1016 \pm 29^*$	1013 ± 16	1019 ± 21
Leukocytes(G/L)	8.78 ± 0.14	$7.94 \pm 0.14^*$	8.25 ± 0.49	7.46 ± 0.47
Lymphocytes (G/L)	3.85 ± 2.12	3.09 ± 0.7	3.81 ± 1.41	3.89 ± 2.12
Neutrophils (G/L)	1.42 ± 0	$1.23 \pm 1.41^*$	1.31 ± 0.7	1.52 ± 0.7
Monocytes (G/L)	3.5 ± 2.12	$2.71 \pm 0.7^*$	$3.1 \pm 4.24^*$	$3.25 \pm 2.83^*$

Asterik values are significantly different from controls

Table 3. Results of biochemical parameters

Parameters	Control	Lot 1	Lot 2	Lot 3
Urea (g/L)	0.84 ± 0.03	0.83 ± 0.08	0.71 ± 0.04	0.78 ± 0.08
Creatinin (mg/L)	6 ± 0.00	5.33 ± 0.58	5.33 ± 0.58	5.6 ± 0.58
AST (UI/L)	188.67 ± 3.21	173 ± 7	175.66 ± 6.02	178 ± 2.5
ALT (UI/L)	100 ± 2	95.66 ± 2.08	92.33 ± 3.51	92.67 ± 3.79
Protides (g/L)	62 ± 2.65	62.66 ± 0.58	61 ± 3.6	64.67 ± 2.52
Ionogram (mEq/L)	Na ⁺	114 ± 5	116 ± 3	118 ± 10
	K ⁺	7.33 ± 0.6	7.4 ± 0.26	7.46 ± 0.61
	Cl ⁻	85.33 ± 4.04	77 ± 4.58	$80.67 \pm 1.52^*$

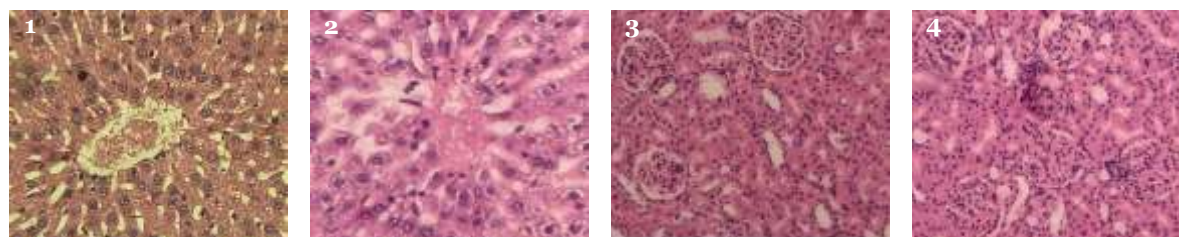


Fig. 3. Photographs of the histological sections of the liver and kidney of the control and experimental rats (x 40)
1 = control rat liver, 2 = experimental rat liver, 3 = kidney of control rat, 4 = kidney of experimental rat.

For the ionogram, sodium (Na⁺) concentrations were 114 ± 5 mEq/L in the control group and 116 ± 3 , 118 ± 10 , and 116 ± 4 mEq/L in Lots 1, 2, and 3, respectively, with the Lot 3 value indicated as significantly different from the control. Potassium (K⁺) concentrations were 7.33 ± 0.60 , 7.40 ± 0.26 , 7.46 ± 0.61 , and 6.97 ± 0.20 mEq/L in

the control, Lot 1, Lot 2, and Lot 3 groups, respectively. Chloride (Cl⁻) concentrations were 85.33 ± 4.04 mEq/L in the control group and 77.00 ± 4.58 , 80.67 ± 1.52 , and 84.00 ± 6.24 mEq/L in Lots 1, 2, and 3, respectively; significant differences were indicated for Lots 2 and 3 (Table 3).

Histological findings

Histological sections of the liver and kidney are shown in Fig. 3. The liver sections from the control and experimental rats showed comparable tissue architecture, with no visible abnormalities in the experimental section. Similarly, the kidney sections of the control and experimental rats showed no observable structural alterations. No differences in the gross appearance or coloration of the examined internal organs were recorded between the control and experimental groups (Fig. 3).

DISCUSSION

The phytochemical results revealed the presence of tannins, flavonoids and saponins crude and the roasted seeds' flours. The cooked extract was free from the metabolites while.

Many other studies have revealed the same results for different African yam beans (Onuoha *et al.*, 2017). Nyananyo and collaborators. (2011) have reported the presence of flavonoids and saponins in their study. In contrast to our results, alkaloids were detected by many authors (Onuoha *et al.*, 2017).

The variability of secondary metabolite concentrations from one variety to another is an intrinsic feature of each seed phenotype of the species. Nehad (1990) reported the relationship between the color of the pericarp and the tannin concentration of the seeds. This variability can also be considered as an influence of environmental factors, generating specific ecotypes. The phenotypic characteristics of the *S. stenocarpa* seeds, which are at the origin of the other the varieties of this vegetable, are thus closely related to intrinsic particularities.

The cooked extract of the seed was completely devoid of all secondary metabolites while the watery extract after cooking contains tannins and flavonoids and is devoid of saponins. We could say that the tannins and flavonoids firstly present in the seed would have been dissolved or drained in the cooking water due to the temperature. Absence of saponins could be

explained also as a evaporation or destruction due to the temperature during cooking. Similar observation was noted by Azeke *et al.* (2007).

The initial secondary metabolite contained in *S. stenocarpa* seeds could be to be a concern because of their effects sometimes on health, so, cooking in water would partially solve the problem. It would then be strongly recommended to change the seed cooking water at least once in order to rid the preparation of the dissolved anti-nutrients and ensure consumption without or reduced risk.

Moreover, this variability in phyto-elements content depending on the variety may be problematic since anti-nutritional factors originate from secondary metabolites. For example, many authors have reported the presence of phytates and anti-trypsin substances (Ghaniyat *et al.*, 2016). Phytates inhibit the bioavailability of nutrients, it is therefore important that phytochemical studies must be carried out on the different varieties of these vegetables for the safe use of consumers.

The present study found that single administrations of the different extracts of beans yam by the oral route up to a dose of 5000 mg/kg did not produce any mortality or alter the behavioral pattern of rats as compared with the control group. A decrease in food consumption could be considered as a negative effect of the plant extracts on the rats' development caused by alteration of glucides, lipids and proteins metabolism (Ezeonwumelu *et al.*, 2011).

Generally, the reductions in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to toxic substances (Teo *et al.*, 2002). The toxic effect is manifested by significant changes in the color, appearance and relative organ weight.

The rats body weight have slightly increased throughout the experiment. There was no significant difference between the organs relative weights (liver and kidney) of control and experimental of rats.

On the other hand, the biological parameters were not affected. However, significant differences were observed in rats treated by the crude powder of beans. Hematological studies easily reveal anomalies in body metabolic processes, and the blood profile usually provides vital information (Yakubu *et al.*, 2007) on the response of the body to injury, deprivation and/or stress.

Moreover, this analysis is relevant in risk assessment since changes in the hematopoietic system have a high predictive value for toxicity in humans when data are transferred from animal studies (olson *et al.*, 2000) In this study, throughout the analysis of the hematological parameters, the results suggest an normochromic microcytic anemia associated with leucopenia and thrombocytosis.

But all these differences didn't have a pathological impact. This could be explained by a lot concentration of substances in the crude powder of beans and these substances may deprive iron from the hemoglobin. The biochemical parameters showed no differences between control and experimental rats. It appears that the seeds of *S. stenocarpa* would have no adverse effect on renal, hepatic and metabolic functions.

These data are confirmed by the microscopy of the histological slides of the liver and kidneys of the experimental rats (Treated with the cooked extracts) and controls. The comparison revealed no particular abnormalities in the structure of the liver or kidneys. It can thus be said that the consumption of seeds is not detrimental to human organs.

After this study, we could conclude that the LD 50 of the different extracts from *S. stenocarpa*, were greater than 5000 mg/kg. Therefore, the safety of preparations based on this legume is guaranteed both for humans and for animals.

CONCLUSION

Seeds of *S. stenocarpa*, like all products of plant origin, contain secondary metabolites such as

tannins, flavonoids and saponosides. The concentration of these compounds is considerably affected by the treatments inherent in the preparation (soaking, fermentation, cooking). It is then able to induce no known harmful effects on the structure and functioning of the essential organs (liver and kidneys) of the consumer. It is desirable to change the cooking water of the seeds at least once, considering the solubility and drainage in the cooking water of certain secondary metabolites. A chronic toxicity study would be desirable to assess the health impact of long-term consumption of *S. stenocarpa* seeds. This study should extend to the different varieties of seeds and tubers of this legume.

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