

Research Article***In Vivo* Assessment of the Nutritional and Subchronic Toxicity of *Tacca leontopetaloides* (L.) Tubers**Ndouyang CJ^{1,3}, Nguimbou RM^{1,3}, Njintang YN^{1,2*}, Scher J³, Facho B⁴, Mbofung CMF¹¹Department des Sciences Alimentaires et Nutrition, ENSAI, Université de Ngaoundéré, B.P. 455 Ngaoundéré, Cameroun²Faculté des Sciences, Université de Ngaoundéré, BP 454, Ngaoundéré, Cameroun³Laboratoire d'Ingénierie des Biomolécules (LIBios), Nancy-University, 2 avenue de la Forêt de Haye B.P. 172 F-54505 Vandoeuvre-lès-Nancy, France⁴Département de Biologie, Facultés des Sciences Exactes et Appliquées, Université de N'Djaména, B.P. 1027 N'Djaména, Tchad***Corresponding author**

Njintang YN

Email: njintang@yahoo.fr

Abstract: *Tacca leontopetaloides* tubers are largely used as food in sahelian arid zone during the periods of cereal shortages. The tubers are traditionally subject to post harvest treatments in order to eliminate the bitterness and toxicity. The present study evaluated the subchronic toxicity of processed and unprocessed tacca in rat models (*Rattus norvegicus* L.) one month old. Rats were fed for 7x7 days on aperitif (1.5 g) in the morning and standard food (no restriction) in the evening. The basic composition of aperitifs was 20% fish and 80% cassava. In the experimental design, the groups were formed by substitution of cassava with processed (100%) and unprocessed tacca (100%, 66% and 33%). The change in body weight and plasmatic biochemical parameters of rats groups were determined every week. The results revealed that while the consumption of processed tacca was 100%, the aperitif intake increasingly diminished as the level of unprocessed tacca increased. Rats fed unprocessed tacca excreted more lipids in feces as the intake level increased. Generally no significant difference was observed on the organ weight while rats groups fed unprocessed tacca presented high levels of plasmatic transaminases and high level of cholesterol. Beside no significant difference was observed on the hematological parameters.

Keywords: *Tacca leontopetaloides*, subchronic toxicity, food intake, biochemical parameters, hematological parameters.

INTRODUCTION

Food deficiency is a permanent problem in the soudano-sahelian countries of the world where efforts are continuously made by national and international authorities to assist the most stripped one. Food deficiency is generally associated with health problems and risks of food poisoning [19, 30, 33]. In fact during the long period of cereals shortage, populations often turn to nonconventional crop products in order to meet their daily food intake. This is more crucial in the soudano-sahelian dry zone where rainfall lasts less than 3 months. In this respect, *Tacca leontopetaloides* has been shown to play an important role in Central Africa, and particularly in Chad as a source of dietary starch [19, 26, 32].

Tacca is an annual herb, which grows upright to a height of 30 cm and is widespread in the savanna zone of Central Africa including Chad, Nigeria and Cameroun [24]. It produces an underground tuber which is subject to commercialization in local market for human consumption. As many non conventional tubers, the consumption of tacca is limited by the presence of toxic components [31]. In this respect, populations have developed traditional strategies to

detoxify *T. leontopetaloides* tubers and nowadays, the detoxified tubers are not only consumed in households but are also commercialized in local market [19, 25, 31]. The traditional method of detoxification consists of diluting in water the crushed fresh tubers followed by a step of decantation. The operation is repeated a number of time depending on the manufacturer [19, 25]. The fundamental question concerning the nutritional value and the toxicology of manufactured tacca needs to be answered. This question is pertinent in the way that multiple soakings are needed to remove the toxic while this significantly reduces the nutritional quality of the tubers.

The toxicity of the *Tacca* genus has been widely evocated [20, 22, 37, 39]. Two alkaloids, taccalonolide E and A of *Tacca chantrieri*, have been identified and their activity on isolated cells in mitosis was determined. In this respect they possess a stabilizing effect of microtubules in interphase and an initiating activity of apoptosis [37]. Long time ago and more recently [35, 40], studies revealed that the bitterness of *T. leontopetaloides* is due to taccalin (3,5,7,4'-tetrahydroxy-flavylum-3-xyloside), a phenolic compound [40]. The question of the effect of

consumption of processed and unprocessed tacca on the hematological and biochemical parameters of rat's blood is still to be answered since these parameters constitute the determinant variables conditioning their safety and health benefit. To our knowledge, very few if not such studies have been conducted.

In an effort to investigate this issue, this work was carried out with the objective to study the nutritional and sub chronic toxicity of processed and unprocessed tacca flour in rat models.

MATERIAL AND METHODS

Production of tacca flours

The plant materials, the fresh tubers of *T. leontopetaloides* were collected in the village of Binder, at the West of the region of Mayo-Kebbi in Chad. The sample was taken to the Laboratory of Biophysics, Food Biochemistry and Nutrition of ENSAI/University of Ngaoundere Cameroon for the production of flour according to Kunle *et al.* [21] and Ukpabi *et al.* [40]. The tubers were separated into two batches for the production of processed and unprocessed tacca flour. For the unprocessed tacca flour, the tubers were peeled, cleaned, grated, dried for 24h in an electrical convection dryer set at 50 °C. In the procedure of production of processed tacca, the second batch of samples was chopped in a Moulinex machine (France), mixed with water (1:3 w/v) for 30 min, left for 10 min for suspension to settle and the water discarded. Water was again added to the precipitate and the procedure redone twice. The precipitate presumably free of toxic was then spread out on aluminum clay and allowed to dry as described above. The dried processed and unprocessed dried chips were then finely milled to pass a 500 µm diameter sieve using an electrical harmer grinder (Cullati, France). The flours were packed in polyethylene bags and stored at 4°C for further studies.

Animal sampling

The animal material consisted of male rats (*Rattus norvegicus*) of 30 days old obtained by coupling 6 groups of 4 couples of rat male and female in the animal unit of the School of Agro-Industrial Sciences (ENSAI), Cameroon. The resulting rats 30 days after birth weighting 50 to 55 g were used for the experiment. They were housed in plastic cages, kept at room temperature (18-25°C), allowed free access to water and fed standard diet substituted or not with tacca powder.

Experimental design

Rats were randomized and divided into 6 groups of 6 animals each receiving specific diet according to Traore and Nebout [38] and Dzeufiet [9]. The diets in all groups were composed of an aperitif diet of 1-1.5 g/rat served each morning in the form of paste to the animals, and in the afternoon an isoenergetic food served and left at their disposal all the day and withdrawn at the end of

the day. Water was given to the animals in the morning and in the evening without restriction. The basic composition of aperitifs was 20% fish and 80% cassava. In the experiment, the groups were formed by substitution of cassava with tacca as followed: 0% substitution constituted group 1, group 2 was 100% substituted with processed tacca, 33% substitution with unprocessed tacca formed group 3, group 4 was fed diet 67% substituted with unprocessed tacca, group 5 was 100 substituted with unprocessed tacca, and finally group 0 received no aperitif. The isoenergetic food composition was as followed: 55% corn starch, 10% Fish meal, 10% corn flour, 20% groundnut paste, 0.5% cooking salt and 4.5% dried milk.

Food and rat weight measurements

When experiencing, the rats were weighed on the first day at the beginning, then weekly up to the end 6 weeks of the study. The initial and the final weights of rats were recorded and the daily food intake (aperitif and isoenergetic food) quantified weekly. The food intake was calculated as mass of food consumed daily per rat while the food efficiency ratio (FER) was expressed as the ratio of total food intake to total increase in rat weight during the 6 weeks experiment. The specific growth rate (SGR) was calculated by the following formula [8]:

$$\text{SGR (\% . day}^{-1}\text{)} = \frac{\ln(W_f) - \ln(W_i)}{t} * 100$$

Where, W_f and W_i are respectively body weight of rats at the final and initial steps, t represents the time of experimentation.

Hematologic and biochemical analyses of blood samples

At the end of the experimentation lasting 7x7 days, blood samples were collected in heparin tubes from rats by jugular vein puncture under chloroform anesthesia and used for the determination of serum hematological and biochemical parameters. Feces were also sampled at the end of the experiment.

Hematological analyses were performed on blood samples using an automated hematology analyzer (Hemocount; Human, Weisbaden, Germany). The recorded parameters were white blood cells, lymphocytes, granulocytes, red blood cells, hemoglobin, hematocrit and platelets.

Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT) and Gamma-glutamyltransferase (GGT or γ -GT) of blood samples expressed in international unit per liter (IU/L) were assayed using kit on a programmed spectrophotometer (Spectrophotometer 3000 revolution, International Business Technology Medical IBT) following Henry [18].

Glycemia expressed as mg glucose dL⁻¹ was evaluated using a glucometer (One Touch Ultra 2)

provided with a reader of glycemia and strips. Total cholesterol and triglycerides of blood samples were proportioned using a kit of enzymatic colorimetric quantification by the method of Allain *et al.* [3] and Ter Welle *et al.* [36] respectively. The HDL cholesterol level was determined after quantitative precipitation of the low density lipoproteins (VLDL and LDL) and the fractions of chylomicrons by addition of buffer PEG6000 [5]. The reading wavelength of the optical densities was 510 nm. The fraction of LDL cholesterol (LDL-C) was proportioned by difference between total Cholesterol (TC) and HDL Cholesterol (HDL-C) and triglycerides (TG) using Friedewald *et al.* formula [14]: $LDL-C = TC - (HDL-C + TG/5)$. The Atherogenic Index was calculated based on the classical method, AI1 (ratio of total cholesterol to HDL cholesterol), and the new approach, AI2 (ratio of the LDL cholesterol to HDL cholesterol) according to Orekhov *et al.* [27].

The plasmatic proteins were quantified according to the method of Gornall *et al.* [16]. The fecal proteins were first extracted according to the method of Bottomley *et al.* [4] and proportioned according to the method of Gornall *et al.* [16]. The fecal lipids were quantified by the method of Folch *et al.* [12].

Statistical analysis

Means and standard deviations (\pm SD) were calculated from 6 individual values. One way analysis of variance was tested to detect the effect of diet on the blood and feces parameters. When effect was significant ($p < 0.05$), the duncan multiple test range was used to compare two means. The software Statgraphics Plus 5.0 was used for the statistical analysis.

RESULTS

Food intake and nutritional properties of the diets

The effect of tacca served as aperitif on the food intake and the growth parameters of rats are given in table 1 and fig 1. Generally no significant difference was observed on the initial and final weights of the rats. This suggested similar food intake as confirmed by the non significant variation of the isoenergetic food intake. Consequently the weight gain and feed efficiency ratio did not vary significantly with diet regime. However the aperitif intake significantly ($p < 0.01$) varied from one diet regime to another. In fact the groups fed cassava and processed tacca completely consumed their aperitif while aperitifs made of unprocessed tacca were less consumed and the level of consumption increasingly diminished as the substitution level of cassava by unprocessed tacca increased. In this respect aperitif 100% cassava substituted with unprocessed tacca was consumed at 24%, while 67% and 33% substituted cassava were consumed at 31% and 60% respectively. This clearly demonstrated that the unprocessed tacca was rejected by rats, as a consequence of toxic compound as suggested in literature. Some toxic components were determined in tacca and in this respect the unprocessed tacca flours were significantly higher in phytates and saponins. One could have expected to observe a decrease in weight gain at the end of the experiment as the aperitif intake differed from one group of rats to another. But this was not the case (fig 1), and might reflect the low contribution of the aperitif to the overall food intake (isoenergetic + aperitif). In fact the ratio of isoenergetic food intake to tacca intake varied from 1.05 to 4.56. This could be explained by the compensation with the isoenergetic food intake.

Table 1: Effect of tacca served as aperitif on the nutritional properties of rats

Parameters	Level of substitution of cassava (%)						Level of significance P*
	No aperitif	0% substituted	100% PT	33% UT	66% UT	100% UT	
Isoenergetic food (g/rat/day)	13.4 \pm 0.69 ^a	15.2 \pm 0.61 ^a	13.8 \pm 0.71 ^a	13.3 \pm 0.68 ^a	13.4 \pm 0.53 ^a	13.4 \pm 0.38 ^a	0.23
Aperitif (g.DW/rat/day)		0.93 \pm 0.021 ^c	0.8 \pm 0.02 ^c	0.5 \pm 0.07 ^b	0.28 \pm 0.071 ^a	0.20 \pm 0.061 ^a	0.01
Aperitif (%)		100 ^c	100 ^c	60.1 \pm 7.91 ^b	30.6 \pm 6.69 ^a	23.9 \pm 7.54 ^a	0.01
Tacca (g.DW/rat/day)			0.6 \pm 0.01 ^b	0.14 \pm 0.022 ^a	0.15 \pm 0.042 ^a	0.16 \pm 0.050 ^a	0.01
Initial weight(g)	49.5 \pm 3.60 ^a	56.0 \pm 4.41 ^{ab}	61.5 \pm 3.89 ^b	56.5 \pm 3.58 ^{ab}	57.5 \pm 3.29 ^{ab}	57.0 \pm 2.20 ^{ab}	0.33
Final weight (g)	171.0 \pm 10.38 ^a	178.0 \pm 11.28 ^a	174.0 \pm 8.42 ^a	153.8 \pm 12.37 ^a	167.8 \pm 7.69 ^a	162.0 \pm 7.75 ^a	0.52
Weight gain (g)	121.5 \pm 10.98 ^a	122.0 \pm 9.80 ^a	112.5 \pm 7.66 ^a	97.2 \pm 11.76 ^a	110.2 \pm 6.72 ^a	105.0 \pm 6.82 ^a	0.36
Feed efficiency ratio	0.11 \pm 0.011 ^a	0.13 \pm 0.012 ^{ab}	0.12 \pm 0.013 ^a	0.15 \pm 0.021 ^{ab}	0.13 \pm 0.011 ^{ab}	0.13 \pm 0.012 ^{ab}	0.24
Tacca/isoenergetic (%)	0	0	4.56	1.05	1.12	1.20	-

*P is level of significance following the ANOVA test; Means \pm SD followed by different letters (a–c) in the same line are significantly different ($p < 0.05$). PT processed tacca; UT unprocessed tacca

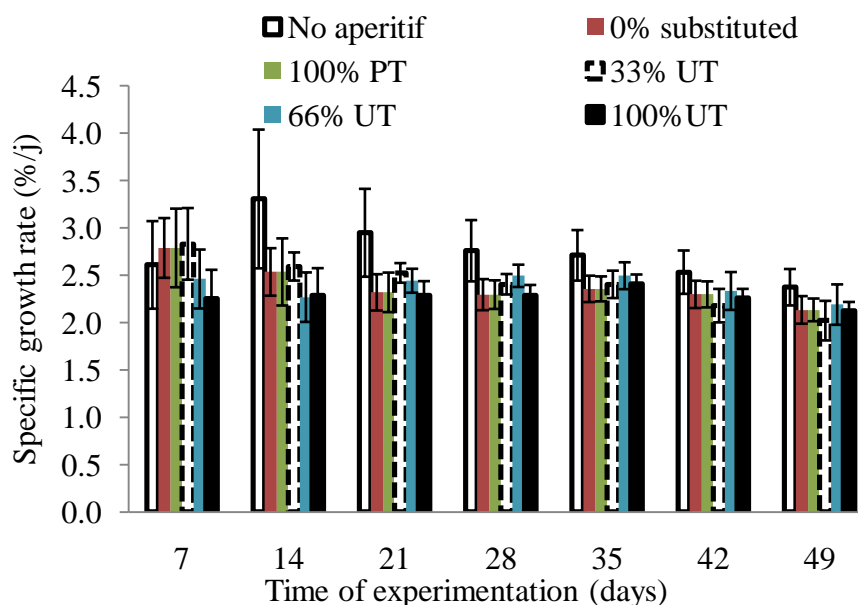


Fig 1: Rats specific growth rate as affected by aperitif and the level of tacca in aperitif. PT processed tacca; UT unprocessed tacca

Fecal losses and Organs weight

The variation in fecal proteins, ashes and lipids, plasmatic proteins are presented in table 2. The consumption of tacca as aperitif had no significant effect on fecal proteins while significant variation ($p < 0.01$) was observed on the fecal ashes which were much higher in group fed cassava and processed tacca aperitif. In addition the fecal lipids level was significantly ($p < 0.05$) affected by introduction of tacca in the food. In this respect rats group fed tacca excreted

more lipids in feces than group having no aperitif and rats fed cassava as aperitif. Rats fed unprocessed tacca excreted more lipids as the intake level increased. The result suggested a disturbance of the metabolism of rats which might reflect the changes in metabolism of organs (table 2). The weights of organs as affected by the feed regime are shown in table 3. Generally no significant difference was observed on the organ weight.

Table 2: Fecal and plasmatic biochemical parameters and WG/IW ratio

Group	Fecal proteins (%)	Fecal ashes (%)	Fecal Lipids (%)	Plasmatic proteins (g/L)	WG/IW
No aperitif	25.14±0.72 ^{ab}	15.46±0.41 ^a	7.37±0.40 ^{ab}	82.28±1.88 ^c	2.57±0.3 ^b
0% Substituted	23.45±2.26 ^a	17.75±0.67 ^b	6.61±0.55 ^a	85.48±7.17 ^c	2.26±0.2 ^{ab}
100% PT	24.80±1.04 ^{ab}	17.38±0.16 ^b	8.46±0.73 ^{bc}	73.31±3.64 ^c	1.88±0.2 ^a
33% UT	31.11±1.98 ^c	15.57±0.43 ^a	7.49±0.59 ^{ab}	60.11±2.20 ^a	1.79±0.3 ^a
66% UT	30.13±2.00 ^{bc}	15.74±0.57 ^a	9.57±0.49 ^c	64.40±5.31 ^{ab}	1.96±0.2 ^{ab}
100% UT	25.26±1.94 ^c	16.61±0.27 ^{ab}	9.62±0.54 ^c	75.56±1.70 ^{bc}	1.85±0.1 ^a

*P is level of significance following the ANOVA test; Means ± SD followed by different letters (a–c) in the same column are significantly different at P ($p < 0.05$). PT processed tacca; UT unprocessed tacca; WG/IW Weight Gain/Initial Weight

Table 3: Weight of the organs of the rats having received various aperitifs

Groups	Weight of organs of experienced rats (in grams, g)					
	Heart	Lungs	Stomach	Liver	Kidneys	Brain
No aperitif	0.61±0.04 ^a	1.52±0.14 ^a	1.12±0.05 ^a	6.64±0.40 ^a	1.62±0.12 ^a	1.51±0.06 ^a
0% substituted	0.66±0.04 ^a	1.51±0.07 ^a	1.27±0.07 ^a	6.78±0.52 ^a	1.63±0.08 ^a	1.52±0.09 ^a
100% PT	0.63±0.04 ^a	1.57±0.08 ^a	1.17±0.05 ^a	6.62±0.35 ^a	1.50±0.09 ^a	1.52±0.04 ^a
33% UT	0.55±0.03 ^a	1.60±0.09 ^a	1.20±0.05 ^a	6.56±0.43 ^a	1.53±0.09 ^a	1.53±0.04 ^a
66% UT	0.66±0.03 ^a	1.42±0.08 ^a	1.08±0.06 ^a	6.49±0.35 ^a	1.49±0.03 ^a	1.59±0.04 ^a
100% UT	0.63±0.03 ^a	1.39±0.06 ^a	1.00±0.01 ^a	6.64±0.42 ^a	1.50±0.09 ^a	1.48±0.03 ^a

*P is level of significance following the ANOVA test; Means ± SD followed by different letters (a–c) in the same column are significantly different at P ($p < 0.05$). PT processed tacca; UT unprocessed tacca

Plasmatic concentrations in transaminases

The variations in some plasmatic enzyme ALAT, ASAT, GGT, as well as glycemia on empty stomach are presented in table 4. Compared to rats groups without aperitif and those fed cassava and processed tacca, the level of transaminases were significantly ($p < 0.05$)

higher in group fed unprocessed tacca. The high levels of plasmatic transaminases generally reflect liver damage [10, 11, 28] and in this respect suggests a toxicity of unprocessed tacca. Besides, glycemia did not varied significantly with diet regime.

Table 4: Plasmatic concentrations in transaminases and glycemia of rats groups fed different tacca regimes

Level of substitution of cassava (%)	ALAT (UI/L)	ASAT (UI/L)	GGT (UI/L)	Glycemia (g/L)
No aperitif	38.0±1.73 ^a	33.4±1.55 ^a	37.0±3.45 ^a	0.98±0.05 ^a
0% substituted	50.2±1.91 ^b	47.9±3.03 ^b	37.3±1.45 ^a	0.81±0.08 ^a
100% PT	60.5±3.31 ^{bc}	48.9±4.43 ^b	38.0±1.73 ^a	0.85±0.04 ^a
33% UT	62.3±4.94 ^{bc}	80.3±3.50 ^c	51.4±3.33 ^b	0.89±0.03 ^a
66% UT	62.1±3.81 ^c	78.3±5.52 ^c	66.5±2.75 ^c	0.88±0.04 ^a
100% UT	61.3±4.27 ^c	86.8±2.97 ^c	70.1±0.49 ^c	0.85±0.05 ^a
Level of significance P*	<0.01	<0.01	<0.01	<0.676

ALAT Amino Transferase Alanine; ASAT Aspartate Amino Transferase; GGT Transpeptidase Gamma-Glutamyl; PT processed tacca; UT unprocessed tacca; values are expressed as mean ± SD, n= 6 in each group. Means followed by different letters (a–b) in the same raw are significantly different ($\alpha < 0.05$).

Blood cholesterol content (Total, HDL and LDL-Cholesterol)

The cholesterol levels (table 5) varied significantly ($p < 0.05$) with diet. Generally groups fed unprocessed tacca had high level of cholesterol, and this increased as the level of substitution increased. In addition the rat

groups fed tacca and no aperitif exhibited high level of HDL-cholesterol and low level of LDL compared to rats fed cassava. As consequence the atherogenic indices of rat groups fed unprocessed tacca were systematically lower compared to others.

Table 5: Lipids profile (Triglycérides; total, HDL & LDL- Cholesterol) of rats groups fed tacca different tacca regimes

Groups	Tot Chol (mg/L)	HDL Chol (mg/L)	Triglycérides (mg/L)	LDL Chol (mg/L)	IA1 (%)	IA2 (%)
No aperitif	560±40 ^a	270±24 ^{abc}	939±49 ^a	102±20 ^a	213±15 ^{ab}	113±15 ^{ab}
0% substituted	607±44 ^a	234±15 ^a	1055±67 ^a	162±28 ^{ab}	264±21 ^c	164±21 ^c
100% PT	573±34 ^a	241±19 ^{ab}	993±57 ^a	134±26 ^{ab}	246±21 ^{bc}	146±21 ^{bc}
33% UT	567±49 ^a	284±24 ^{bc}	933±64 ^a	96±25 ^a	204±16 ^{ab}	104±16 ^{ab}
66% UT	639±37 ^a	322±22 ^c	977±53 ^a	104±21 ^a	200±9 ^{ab}	100±9 ^{ab}
100% UT	633±55 ^a	335±15 ^c	980±81 ^a	102±34 ^a	189±14 ^a	89±14 ^a

*P is level of significance following the ANOVA test; Means ± SD followed by different letters (a–c) in the same column are significantly different at P ($p < 0.05$). PT processed tacca; UT unprocessed tacca

Effect of tacca served as aperitif on the hematological parameters of rats

The results of tacca served as aperitif on the hematological parameters of rats are presented in table 6. No significant difference was observed between the

groups. The amount of processed tacca (PT) and unprocessed tacca (UT) consumed as aperitif was without effect on white blood cell count (WBC), lymphocytes, granulocytes, red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht) or platelets (Plt).

Table 6: Effect of tacca served as aperitif on the hematological parameters of rats

Parameters	Level of substitution of cassava (%)						Probability P ($\alpha=0.05$)
	No aperitif	0%Substituted	100% PT	33% UT	66% UT	100% UT	
WBC (*10 ⁹ /L)	3.8±0.57 ^a	5.1±1.99 ^a	3.9±0.30 ^a	4.5±0.56 ^a	4.0±0.79 ^a	4.3±0.36 ^a	0.94
RBC (*10 ¹² /L)	7.7±0.79 ^b	6.9±0.33 ^{ab}	6.8±0.30 ^{ab}	6.9±0.23 ^{ab}	5.0±0.83 ^a	6.4±0.26 ^{ab}	0.22
Hb (g/dL)	14.9±1.80 ^b	13.1±0.74 ^{ab}	13.0±0.67 ^{ab}	13.2±0.35 ^{ab}	9.2±1.82 ^a	12.0±0.54 ^{ab}	0.31
Ht (%)	37.9±0.93 ^{ab}	37.2±1.88 ^{ab}	37.1±1.85 ^{ab}	38.2±0.90 ^b	23.9±5.66 ^a	34.8±1.53 ^{ab}	0.24
Plt (*10 ⁹ /L)	197.6±21.88 ^a	258.7±56.06 ^a	155.3±20.67 ^a	186.2±78.68 ^a	242.9±114.0 ^a	185.9±24.25 ^a	0.70

WBC: white blood cell count; Lym: lymphocytes; Gra: granulocytes; RBC: red blood cells; Hb: hemoglobin; Ht: hematocrit; Plt: platelets; values are expressed as mean ± SD, n= 6 in each group. Means followed by different letters (a–b) in the same line are significantly different. PT processed tacca; UT unprocessed tacca

DISCUSSION

Our previous studies revealed that tacca is essentially composed of carbohydrate (90-92%) with low level of proteins (2-3%), ash (2.5%) and lipids (2.1%) [26]. This makes tacca an energy source for populations producing them. However the tubers also contained appreciable amount of antinutrients among which saponins, phytic acid and polyphenols were most represented [40, 41]. These antinutrients also called antimetabolic/antiphysiological substances naturally occur in plants and interfere with utilization of nutrients. It has been shown that polyphenols and tannins inhibited the hydrolytic action of enzymes in the intestine, complexed dietary proteins and reduced their hydrolysis by enzymes [3]. In addition, phytic acid lowers the bioavailability of minerals and inhibits proteases and amylases actions in the intestine. Saponins occur widely in plants and are the best-known toxic constituents in fishing poisons. The toxicity of saponin has been associated to its structure similar to that of cholesterol which allowed its insertion in the cell membrane [13]. They induce bitterness to food [43] and they would also lower cardiovascular diseases [25]. On one hand, positive nutritional effects of specific saponins such as hypocholesterolemic effects and improvement of growth in various animal species have also been reported [23]. On the other hand, various studies also demonstrated the toxicity of unprocessed tacca related to saponins [1, 30, 44].

The present study revealed high levels of saponins, alkaloids and phytates in fresh tacca tubers, but the post harvest treatment generally applied to them seem to lower their concentrations to a non toxic level. In the present study we limited the consumption of tacca to 1-1.5 g/rat/day in order to give a choice to the animal to either reject it or not. As expected the rats fed cassava and processed tacca as aperitif consumed totally their food while those fed unprocessed did not. As the level of unprocessed tacca increased in the aperitif from 33% to 100% substitution, the consumed quantity of aperitif diminished from 24% to 60% respectively. The rats then ate the quantity of food according to their preference. As a result the levels of tacca ingested in the present study were low enough to induce metabolic disorders, but rather biological activities. In fact phytates represented 0.004 % to 0.008 % dry food while saponins varied from 0.003 to 0.05 % dry food. Although the levels of phytates and saponins were low in the isoenergetic food, the rats did not accept them either due to the bitterness or the toxicity they induced. Whatever the case, all the rats groups exhibited similar increase in weight, similar organs weights, synonym of no acute toxicity. According to Francis G [13], levels of saponins lower than 1% do not cause cellular lesions. We equally observed no significant change in the hematological parameters.

ASAT and ALAT are two biomarkers associated with myocardial infarction and hepatic lesions [29]. GGT is

an enzyme abundant in liver, kidneys, pancreas and lung which catalyses the transfer of γ -glutamyl groups from one peptide to another [7]. The high levels of GGT are associated to hepatic, pancreatic and bile disorders or cardiac dysfunction [7]. Generally consumption of drugs or ingested chemical compounds induce lipid peroxidation and damage the membranes of hepatic cells and organelles, and consequently cause inflammation and necroses in hepatocytes from where cytosolic transaminases such as ASAT, ALAT, GGT, alkaline phosphatase are released in the blood circulation [10, 42]. In the present study, the levels of ASAT, ALAT and GGT in the blood of animal groups fed aperitif either made of cassava or tacca were not significantly different to the group which did not consume aperitif, although those fed unprocessed tacca were a little high.

Saponins have been shown to affect cholesterolemia. The primary mechanism of action of saponin is interference with absorption of nutrients such as lipids especially cholesterol, and minerals [6, 34]. This consequently increases the quantity of fecal lipids. In concordance with Gorinstein *et al.* [15], the present study revealed no significant change in the total cholesterol which could have diminished with the increase of tacca in the feed. These authors reported that in groups of rats fed cholesterol-free diets, the rats synthesized more total cholesterol, and the level of HDL-cholesterol stayed higher than the LDL. So, the phenomenon of complexation of lipid in digestive gut confers to the diet a lipid-free diet character. The hypocholesterolemic effect of saponins has been demonstrated elsewhere [25].

Unprocessed tacca seems then to possess medical values if used in adequate conditions while processed tacca may be used as food. The levels of tacca ingested in groups fed unprocessed tacca were similar with a mean value of 0.15g/rat/day. This quantity is probably the quantity than can induce a benefit to consumers. Taken in account the mean weight of a rat equal to 104 g, the quantity with benefit biological effect was calculated to 1.3 g unprocessed tacca per per day and kg of rat.

CONCLUSION

Although *T. leontopetaloides* is recognized toxic, its toxicity depends on the quantity ingested. At low levels in the diet, ingestion of unprocessed tacca reduced the level of LDL-cholesterol and limit digestion of lipids by increasing the fecal lipids. No abnormality in biochemical markers of toxicity was observed in rats fed unprocessed tacca. Unprocessed tacca can then be recommended for ingestion a day at a quantity not higher than 1.34 g unprocessed tacca per kg body weight of rat and per day. Ingestion of unprocessed tacca in low quantities seems to have positive effect on the lipid metabolism, but this need to be investigated.

ACKNOWLEDGEMENTS

This study was carried out under the financed project CORUS reference N° 6052-2, a collaborative research between ENSAI of the University of Ngaoundere, LIBIO of INPL of Nancy-University, France and the Faculty of Sciences of the University of N'Djamena, Tchad.

REFERENCES

1. Abdel-Aziz A, Brain K, Bashir AK; Screening of Sudanese plants for molluscicidal activity and identification of leaves of *Tacca leontopetaloides* (L.) O. Ktze (Taccaceae) as a potential new exploitable resource. *Phytotherapy Research*, 1990; 4(2): 62-65.
2. Adebowale YA, Adeyemi IA, Oshodi AA; Functional and physicochemical properties of flours of six mucuna species. *Afr J Biotechnol.*, 2005; 4: 416-468.
3. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC; Enzymatic determination of total serum cholesterol. *Clin Chem.*, 1974; 20: 470-475.
4. Bottomley G, Popineau Y; Séparation et dosage des protéines et protéases. In *Guide pratique d'analyses dans les industries des céréales* (Bottomley G, Loisel V). Technique et Documentation, Lavoisier, Paris, 1984: 230.
5. Burstein M, Scholnick HR, Morfin R; Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *Scand J Clin Lab Invest.*, 1980; 40: 583-595.
6. Chunmei L, Zhifeng L, Bing R, Ke L; Effect of saponins from *Anemarrhena asphodeloides bunge* on blood lipid concentration of hyperliperua quail. *Journal of Molecular and Cellular Cardiology*, 2006; 40: 884.
7. Connell MD; Serum D-glutamyl transferase following myocardial infarction. *J clin Path.*, 1973; 26: 684-686.
8. Dada AA; Effects of herbal growth promoter feed additive in fish meal on the performance of Nile Tilapia (*Oreochromis niloticus* (L.)). *Egypt Acad J Biolog Sci.*, 2012; 4(1): 111-117.
9. Dzeufiet DPD, Tedong L., Asongalem EA, Dimo T, Sonkeng SD, Kamtchouing P. (2006). Hypoglycaemic effect of methylene chloride/methanol root extract of *Ceiba pentandra* in normal and diabetic rats. *Indian Journal of Pharmacology* 38 (3) : 194 -197.
10. Etuk EU, Agaie BM, Ladan MJ, Garba I (b); The modulatory effect of *Cochlospermum tinctorium* A. Rich. aqueous root extract on liver damage induced by carbon tetrachloride in rats. *African Journal of Pharmacy and Pharmacology*, 2009; 3(4): 151-157.
11. Etuk EU, Francis UU, Garba I (a); Regenerative action of *Cochlospermum tinctorium* aqueous root extract on experimentally induced hepatic damage in rats.

- African Journal of Biochemistry Research*, 2009; 3(1): 1-4.
12. Folch J, Lees M, Stanley GHS; A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 1957; 226: 497-509.
 13. Francis G, Kerem Z, Makkar HPS, Becker K; The biological action of saponins in animal systems: a review. *British Journal of Nutrition*, 2002; 88: 587-605.
 14. Friedewald WT, Levy RI, Fredrickson DS; Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.*, 1972; 18: 499-502.
 15. Gorinstein S, Leontowicz H, Leontowicz M, Drzewiecki J, Najman K, Katrich E *et al.*; S. Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats. *Life Sciences*, 2006; 78: 655-663.
 16. Gornall AG, Bardwill RH, David MM; Determination of serum proteins by means of the Biuret reactions. *J Biol Chem.*, 1949; 177: 167-182.
 17. Harborne JB; Phenolic compounds. In: *Phytochemical methods. A guide to modern techniques of plant analysis*. Chapman and Hall Ltd London, England, 1973: 33-88.
 18. Henry RJ; *Clinical Chemistry, Principles and Techniques*. 2nd edition, 1974: 543.
 19. de Garine I; *Nourriture de brousse chez les Muzey et les Masa du Nord Cameroun*. Méga-Tchad 2002, CNRS, Paris, 2002: 13.
 20. Kitjaroennirut N, Jansakul C, Sawangchote P; Cardiovascular effects of *Tacca integrifolia* Ker-Gawl extract in rats. *Songklanakarin J Sci Technol.*, 2005; 27(2): 281-289.
 21. Kunle OO, Ibrahim YE, Emeje MO, Shaba S, Kunle Y; Extraction, physicochemical and compaction properties of *tacca* starch – a potential pharmaceutical excipient. *Starch/Stärke*, 2003; 55: 319-325.
 22. Liu H-Y, Ni W, Xie B-B, Zhou L-Y, Hao X-J, Wang X *et al.*; Five new withanolides from *Tacca plantaginea*. *Chem Pharm Bull.*, 2006; 54(7): 992-995.
 23. Makkar HPS, Siddhuraju P, Becker K; *Plant Secondary Metabolites*. *Methods in Molecular Biology*, 2007; 393: 93-100.
 24. Manek RV, Kunle OO, Emeje MO, Builders P, Rao GVR, Lopez GP *et al.*; Physical, Thermal and Sorption Profile of Starch Obtained from *Tacca leontopetaloides*. *Starch/Strärke*, 2005; 57 (2): 55-61.
 25. Matsuura H; Saponins in garlic as modifiers of the risk of cardiovascular disease. *Journal of Nutrition*, 2001; 131: 1000-1005.
 26. Ndouyang CJ, Ejoh AR, Aboubakar, Facho B, Njintang YN, Mohammadou BA *et al.*; Valeur

- nutritionnelle de *Tacca leontopetaloides* (L.) Kuntze, tubercule non conventionnel. Revue de génie industriel., 2009; 3 : 24-32.
27. Orekhov AN, Tertov VV, Mukhin DN; Desialyated low density lipoprotein- naturally occurring modified lipoprotein with atherogenic potency. *Atherosclerosis*, 1991; 86(2-3): 153-161.
 28. Otimenyin S, Sabo SY; Pharmacological basis for the continual use of the aqueous leave extract of *Sterculia setigera* Del (Sterculiaceae) in Folk medicine. *IJPI'S Journal of Pharmacology and Toxicology*, 2011; 1(3): 1-15.
 29. Quevauvilliers J, Somogyi A, Fingerhut A; Dictionnaire Médical, 5th edition, 2007 : 1093.
 30. Risinger AL, Mooberry SL; Taccalonolides: Novel Microtubule Stabilizers with Clinical Potential. *Cancer Lett.*, 2010; 291(1): 14-19.
 31. Rouers B ; L'eau, agent de détoxication alimentaire Étude de deux techniques de détoxication des plantes alimentaires utilisées par les Aborigènes Australiens. *Altérités, Revue des doctorants en anthropologie du Québec*, 1996 ; Volume 1, numéro 1. Available from <http://www.anthro.umontreal.ca/varia/alterites/vol1no1/vol1no1.html>.
 32. Ruffo CK, Birnie A, Tengnäs B; Edible wild plants of Tanzania. Regional Land Management Unit-Technical book, 2002; 27: 642-643.
 33. Seignobos C ; La parade à la Razzia dans la zone soudanienne au XIX^e siècle: la domestication de la cueillette. In: M. ELDON and MILLEVILLE (éds) *Le risque en Agriculture*. ORSTOM, Paris, 1989 : 355-374.
 34. Shi J, Arunasalam K, Yeung D, Kakadu Y, Mittal G, Jiang Y; Saponins from edible legumes: Chemistry, processing, and health benefits. *Journal of Medicinal Food*, 2004; 7: 67-78.
 35. Swanholm CE; A chemical study of the bitter principle of Pia (*Tacca leontopetaloides* (L.) Kuntze). A thesis submitted to the graduated school of the University of Hawaii in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1959: 91.
 36. Ter Welle HF, Baartscheer T, Fiolet JWT; Influence of free glycerol on enzymatic evaluation of triglycerides. *Clin Chem.*, 1984; 30: 1102–1103.
 37. Tinley TL, Randall-Hlubek DDA, Leal RM, Jackson EM, Cessac JW, Quada JC *et al.*; Taccalonolides E and A: Plant-derived Steroids with Microtubulestabilizing Activity. *Cancer Research*, 2003; 63, 3211–3220.
 38. Traoré I et Nébout Max ; Formules et techniques à partir des matières premières locales concernant la nourriture des souris d'expérimentation en pays tropicaux. *Acta Leprologica*, 1983; 1: 3.
 39. Tripathi RD, Tiwari KP; Phytochemical investigation of the roots of *Tacca aspera*. *Planta Medica*, 1981; 41(4): 414-415.
 40. Ukpabi UJ, Ukenye E, Olojede AO; Raw-material potentials of Nigeria wild polynesian arrowroot (*Tacca leontopetaloides*) tubers and starch. *Journal of Food Technology*, 2009; 7(4): 135-138.
 41. Ubwa ST, Anhwange BA, Chia JT; Chemical Analysis of *Tacea leontopetaloides* Peels. *American Journal of Food Technology*, 2011; 6(10): 932-938.
 42. Venukumar MR, Latha MS; Hepatoprotective effect of the methanolic extract of *Curculigo orchioides* in CCl₄ – treated male rats. *Int J Pharmacol.*, 2002; 34: 269-275.
 43. Wszelaki AL, J Delwiche F, Walker SD, Liggett RE, Miller SA, Kleinhenz MD; Consumer liking and descriptive analysis of six varieties of organically grown edamame-type soybean. *Food Quality and Preference*, 2005; 16: 651–658.
 44. Yokosuka A, Mimaki Y, Sashida Y; Spirostanol saponins from the rhizomes of *Tacca chantrieri* and their cytotoxic activity. *Phytochemistry*, 2002; 61(1): 73-78.
-