Journal of Food Research; Vol. 9, No. 5; 2020
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Nutritional Value of *Raphia hookeri* Fruit, Hematological Properties of Its Powder and Aqueous Extract in A Model of Aluminum Chloride Inducing Neurotoxicity by Using Rats

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Received: April 11, 2020 Accepted: September 1, 2020 Online Published: September 25, 2020

doi:10.5539/jfr.v9n5p113 URL: https://doi.org/10.5539/jfr.v9n5p113

Abstract

This study was aimed at evaluating the nutritional value of the mesocarp of *Raphia hookeri* (Rh) fruit and the effect of powder and aqueous extract of the fruit on hematological parameters in rats which have undergone neurotoxicity by aluminum chloride. The nutritional content was evaluated with the standard method. Seven groups of six *Wistar* rats were used, neurotoxicity was induced by 4.2 mg/kg of body weight of aluminum chloride 3 times a week intraperitonealy with treatment. Rat treatment was as follow: aqueous extract at 200 and 400 mg/kg body weight, 5 and 10% of formulation, negative, positive and normal groups. The experiment lasted 28 days. The data obtained from the nutritive value showed that Rh mesocarp is a good source of lipids (48.97%), fibers (25.82%), calcium (3183.3mg/100g of dry matter), potassium (1218.3 mg/100g of DM), zinc (0.88 mg/100g of DM) and selenium (8.6 mg/100g of DM). Nevertheless, it contains a little amount of phytic acid and hydrocyanic acid which is acceptable for human consumption. It can then be used in many formulations as a source of these nutrients. Aluminum administration indicates the reduction in food intake, a low weight gain and Hematological alteration in the Positive control group. However, consumption of *Rh* mesocarp indicated an increase in food intake, weight gain and a restoration of hematological parameters to the normal level with the best values in groups that were administered Rh powders (*Rh*5% and *Rh*10%).

Raphia hookeri mesocarp is a rich source of nutrients involved in the prevention of hematological disorder.

Keywords: *Raphia hookeri*, nutritional value, hematological parameters, aluminum chloride

1. Introduction

Aluminum (Al) is a metal which is widely distributed in the environment. It is found in the soil, water and air. This metal is extensively used in daily life as medicine, food additive, vaccines adjuvants, phosphate binders, dialysis, total parenteral nutrition solutions and foods, hence increasing exposure of human being to it (Newairy et al., 2009). The increasing use in preparation and storage of food in aluminum utensils may increase one’s exposure to aluminum, particularly when used with salty, acidic or alkaline foods (Lukyanenko et al., 2013). Furthermore, excessive consumption of food baked with aluminum foil may bring a serious health risk (Sahin et al., 1994). Biological effects of aluminum (Al) are linked to the development of many diseases. Many studies have shown that $AlCl_3$ induces toxic effects on the brain, bone, immune and hematopoietic system (Gu et al., 2009). A chronic exposure to aluminum diminishes affinity of transferrin for aluminum due to the fact that the binding sites of transferring available for aluminum are mostly occupied by iron. (Azadeh & Mohammad, 2011).

However, there is certainly growing evidence that several fruits possess interesting pharmacological effects. This is probably the case of *Rh* fruit. It is the largest palm tree in Africa and is commonly found in the tropical rainforest (Ndon, 2003). Rh is one of the most economically useful plants in Africa. Leaves are used for shelter and stem produces palm sap which is drunk as beverage. The fruits are oblong to ovoid and covered with glossy golden brown scales. They can be eaten once boiled, or used in traditional medicine for its laxative and stomachal properties in the treatment of dysentery and hemorrhage (Leung et al., 1968; Ukwubile et al., 2013). The phytochemical evaluation of the mesocarp and peel of *Raphia* palm fruits showed that the mesocarp is rich in bioactive compounds such as vitamin E, niacin, alkaloid, saponins, flavonoid and phenols (Edem et al., 1984; Ogbuagu, 2008). The effects of this fruit have been shown on exogenous testosterone and eostradiol induced benign prostatic hyperplasia (Mbaka et al., 2013) and it leaf to modulate carbohydrate hydrolyzing enzymes linked to type-2 diabetes (Dada et al., 2017). However, the effect of Rh fruit on hematological disorders is not well known. Bioactive compounds contained in Rh fruit may also have some benefit effects on hematological parameters. This leads us to evaluate nutritional value of *Raphia hookeri* fruit and hematological properties of its powder and aqueous extract in a model of Aluminum chloride inducing neurotoxicity by using rats.

2. Materials and Methods

2.1 Plant Material

Fresh mature *Raphia hookeri* fruits were harvested from swampy field of the West region of Cameroon from April to June 2018.

2.2 Methods

2.2.1 Preparation of Powder, Different Formulation and Extraction of Aqueous Extract of Mesocarp

The fresh fruits were cleaned and peeled. The mesocarp was cut into small pieces using a rustproof knife and dried in an electric air-dried oven at 45 ℃ for 48 hours. The dried mesocarp was grinded in a blender machine (*Moulinex*) and sieved (Diameter of pore: 1 mm) for further analysis. The formulation (Rh5% et Rh10%) was done as follows: Rh 5% was prepared using 95g of food staple + 5g of *Rh* powder and *Rh* 10% was prepared using 90g of food staple + 10g of *Rh* powder. The food staple was composed as follows: corn flour (77.8%), fish flour (20%), bone flour (0.1%), palm olein (1%), vitamins (0.1%), and salt (1%). 20g of Rh powder was extracted into 200ml of water and the mixture was regularly shaken during the extraction. After 48 hours of maceration, the mixture was filtered with a Wathman N°1 filter paper and the filtrates were subjected to evaporation at 45 ℃ under reduced pressure for the removal of the solvent. Dry extracts were stored at 4 ℃ for further analysis.

2.2.2 Evaluation of the Nutritive Value of *Raphia hookeri* Mesocarp, Food Staple and Different Formulation

- **Determination of macronutrient content in *Raphia hookeri* mesocarp, food staple and different formulation**

Determination of moisture content, carbohydrate, protein according to the Association of Official Analitical Chemists (AOAC., 1980), fat content with soxhlet method according to the International Union of Pure and Applied Chemistry (UIPAC) and the determination of fiber content was done according to (Pauwels et al., 2007).

- **Determination of micronutrient content of *Raphia hookeri* mesocarp, food staple and different formulation**

Determination of ash content (AOAC., 1980), phosphorus, Iron, Calcium, Magnesium, Sodium, Potassium, zinc, Manganese and selenium (Pauwels E., Erba P. and Kostkiewicz M. 2007).

- **Determination of anti-nutritional content (hydrocyanic acid and phytic acid) of *Raphia hookeri* mesocarp, food staple and different formulation**

Hydrocyanic acid was determined according to (Makkar et al., 2007) and the phytic acid according to (Edem et al., 1984)

2.3 Experimental Animals: Induction of Neurotoxicity with Aluminum Chloride and Administration of Aqueous Extract and Different Formulation of Rh Mesocarp

Seven groups of six animals each weighing between 200 and 230g were obtained from the Animal Centre Department and allowed to be accustomed to their new environment for 1 week according to the guidelines of the Organization for Economic Cooperation and Development (OECD, 2008). Animals were individually housed under room temperature (25 ℃), (12-h light/12-h dark cycle) and had free access to water and diet. $AlCl_3$ (4.2mg/kg/intraperitonial) was administered three time a week to all groups except the vehicle group for 28 successive days (Bhatia et al., 2018). *Rh aqueous extract* (200 and 400 mg/kg body weight) and powder (5% and 10%) were administered daily to different groups of rats orally for 28 consecutive days. Otherwise the negative control group received 200 mg vitamin C per Kg body weight and positive control group was given only aluminum chloride without any treatment. All experiments were carried out according to the regulations and ethical approval of Experimental Animal Welfare and Ethics Committee of the Institution (No. 2017/056).

2.4 Evaluation of the Amount of Food Intake by the Animals During the Treatment with Aluminum Chloride, Aqueous Extract and Different Formulation of Rh Mesocarp

The amount of food intake was determined everyday by calculating the difference between the amount of food given the previous day and the food left the next day.

2.5 Evaluation of the Weight Gain by Animals during Treatment with Aluminum Chloride, Aqueous Extract of Rh Mesocarp and Different Formulation

The weight gain was evaluated every day and the percentage was determined as follows:

$$\boldsymbol{p}(\%) = \frac{\mathbf{wf-w0}}{\boldsymbol{wf}} \ *100$$

P (%) = percentage of weight gain on day x

wf = body weight of animal on day x

wo= initial body weight of animal (after the beginning of treatment)

2.6 Evaluation of the Hematological Parameters of Rats during Treatment with Aluminum Chloride, Aqueous Extract and Different Formulation of Rh Mesocarp

Blood samples collected in Ethylene DiamineTetraacetic Acid (EDTA) tubes were used to perform a blood count using an impedance hematology automated system. The counting principle is based on impedance variation and flow cytometry to determine the size and type of blood cells. Flow cytometry measures on a suspension of particles (cells, bacteria, parasites, beads). Individual characteristic of each particle such as the size, shape and complexity of any component or function that can be detected by a fluorescent compound. The cells in suspension passed one by one in front of one or more laser beams (X) and detectors pick up signals emitted by each cell. Each passage of a cell through the opening then causes an increase in electrical resistance. This increase is translated into electrical pulses whose height is directly proportional to the cell volume. The cells emit naturally or after treatment signals which are analyzed by the computer linked to the cytometer making it possible to establish for the leucocytes the leucocyte formula giving the percentages of the different types of leucocytes. The number of red blood cells is determined by the total number of pulses recorded. The hematocrit level is then deduced by the formula:

$$\text{Hematocrit} = \text{red blood cell} \times \text{average cell volume} / 10$$

Hemoglobin is assayed by the spectrophotometric method (525 to 550 nm) after lysis of red blood cells (Driscoll et al., 1980).

2.7 Statistical Analysis

Results obtained in the present study were subjected to one-way analysis of variance (ANOVA) with turkey test using Minitab version 17.0 to evaluate the statistical significance of the data. A probability value at $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Results

3.1.1 Nutritive Value of *Raphia hookeri* Mesocarp, Food Staple and Different Formulation

3.1.1.1 Chemical Composition of Macronutrients of *Raphia hookeri* Mesocarp, Food Staple and Different Formulation

Table 1 presents the approximate chemical composition of *Raphia hookeri* mesocarp fruit, the staple food and the different formulations (Rh5% and Rh10%). It shows that there is a significant difference ($P< 0.05$) between the water content of the different samples. The highest content was noted in the staple food (19.52 ± 0.32%), followed by formulations of Rh5% (18.27 ± 1.37%) and Rh10% (17.61 ± 0.71%) respectively. The staple food also had the highest protein content (12.83 ± 0.63%) followed by the different formulations Rh10% (10.27 ± 1.37%) and Rh5% (8.61 ± 0.71%). In terms of lipid content, the highest content was found in *Rh* fruit mesocarp powder (48.97 ± 1.02%), followed by the different formulations: Rh10% (22.98 ± 0.08%) and Rh5% (21.55 ±

2.01%). There is also a significant difference between the carbohydrate content of the different samples with the highest content in the staple food (42.78 ± 1.20%). The highest fiber content was noted in *Rh* fruit mesocarp powder (25.82 ± 0.27%), followed by the formulation of Rh5% (23.21 ± 1.36%) and then Rh10% (22.41 ± 0.61%). For the ash content, no significant difference (P <0.05) was observed between the different samples. *Rh* fruit mesocarp powder had the highest energy value (585.85 ± 54.80%) followed by the formulation Rh10% (426.02 ± 40.10%).

Table 1. Evaluation of macronutrients present in *Raphia hookeri* mesocarp, food staple and different formulation

Paramètres	***Rh***	**Al**	***Rh*5%**	***Rh10*%**
Moisture(%)	9.54 ±3.54^{c}	19.52 ± 0.32^{a}	18.87 ±1.37^{a}	18.06 ± 0.71^{ab}
Protein(%DM)	1.93 ± 0.51^{b}	12.83 ± 0.63^{a}	12.18 ± 0.423^{a}	11.54 ± 1.27^{a}
Lipid (%DM)	48.97 ± 1.02^{a}	18.75 ± 1.77^{c}	21.55 ±2.01^{bc}	22.98 ± 0.08^{b}
Carbohydrate(%DM)	32.66 ± 4.74^{b}	42.78 ± 1.20^{a}	40.76 ± 3.15^{a}	40.92 ± 1.12^{a}
Fiber (%DM)	25.82 ± 0.27^{a}	21.04 ± 0.11^{b}	22.41 ± 1.36^{ab}	23.21 ± 0.61^{a}
Ash(%DM)	6.90 ± 0.26^{a}	6.12 ± 0.26^{a}	6.64 ± 0.36^{a}	6.50 ± 0.61^{a}
Energy(Kcal/100g)	585.31 ±54.80^{a}	401.2 ± 21.40^{b}	415.24 ± 45.4^{b}	426.1 ± 40.10^{b}

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05; Al : food staple; Rh: powder of *Raphia hookeri mesocarp*; Rh 5%: formulation food with 5% of *Rh mesocarp* powder; Rh 10%: formulation food with 10% of *Rh mesocarp* powder; DM: dry matter

3.1.1.2 Composition of Micronutrients Present in *Raphia hookeri* Mesocarp, Food Staple and Different Formulation

Table 2 shows the mineral composition of the different samples. Calcium content differed significantly in the different samples and ranges between 2433.79 ± 47.62 mg and 3183.30 ± 199.80 to / 100g of DM. The highest value is found in Rh mesocarp powder fruit and the lowest in the staple food. The magnesium content varied significantly between 1102.30 ± 19.80 and 1220.00 ± 60.80 mg / 100g of DM. The highest content was observed in the staple food and the lowest in the Rh mesocarp. The potassium content was also evaluated and the values differed significantly between the different samples ranging between 689.03 ± 4.10and 1218.3 ± 182.70 mg / 100g of DM. The highest value was observed in the fruit powder. The sodium and phosphorus contents varied significantly between 122.00 ± 18.70 and 291.3 ± 7.9 mg/100g of DM and 198.00 ± 8.66 and 226.87 ± 10.79 mg / 100g of DM respectively. Zinc content ranged from 0.88 ± 0.03 to 0.41 ± 0.03mg/100g of DM, the highest value being found in *Rh* fruit and the lowest in the staple food. Iron content ranged from 22.12 ± 2.50to 32.76 ± 2.06 mg / 100g of DM, with the highest value being found in the staple and the lower in fruit powder. Selenium content also varied significantly and ranged from 0.88 ± 0.03 to 2.04 ± 0,11µg/100g.

Table 2. Composition of micronutrients present in Raphia hookeri mesocarp, food staple and different formulation

Component	***Rh***	**Al**	***Rh*5%**	***Rh10*%**
Ca (mg/100g)	3183.30 ± 199.80^{a}	2433.79 ± 47.62^{d}	2630.00 ± 60.80^{c}	2865.43 ±242.50^{b}
Mg (mg/100g)	1102.30 ± 19.80^{b}	1220.00 ± 60.80^{a}	1197.33 ± 4.62^{a}	1145.33 ± 12.50^{ab}
k (mg/100g)	1218.3 ± 182.70^{a}	689.03 ± 4.10^{b}	695.13 ± 85.28^{b}	701.21 ± 37.9^{b}
Na (mg/100g)	122.00 ± 18.70^{c}	291.3 ± 37.9^{a}	230.33 ± 15.28^{b}	247.33 ± 4.10^{b}
P (mg/100g)	198.00 ± 8.66^{b}	226.87 ± 10.79^{a}	225.90 ± 10.02^{a}	210.90 ±23.29^{ab}
Zn (mg/100g)	0.88 ±0.03^{a}	0.41 ± 0.03^{b}	0.45 ±0.13^{b}	0.57 ±0.08^{b}
Fe (mg/100g)	22.12 ± 2.50^{c}	32.76 ± 2.06^{a}	26.40 ± 5.01^{b}	29.84 ±1.2^{a}
Se (µg/100g)	8.600 ± 0.27^{a}	2.04 ±0.11^{b}	2.93 ± 0.30^{b}	3.51 ± 0.31^{b}

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05. Al : food staple; Rh: powder of *Raphia hookeri mesocarp*; Rh 5%: formulation food with 5% of *Rh mesocarp* powder; Rh 10%: formulation food with 10% of *Rh mesocarp* powder

3.1.1.3 Anti-nutrient Composition of Mesocarp Powder of *Raphia hookeri* Fruit, Different Formulations and Food Staple of Animals

The table 3 below shows the amount of phytic acid and hydrocyanic acid present in mesocarp powder of *Raphia* *hookeri* fruit, the different formulations and the food staple. We observed that the amount of phytic acid differ significantly between the mesocarp powder of *Rh* fruit and food staple. The highest value which is 91.81 ± 8.76mg/100g of DM is found in food staple and the lowest value which is 40.16 ± 3.31mg/100g of DM is in mesocarp powder of *Raphia hookeri* fruit. However there is no significant value between the different formulation and the staple food. The amount of hydrocyanic acid was also evaluated and significantly differs between the mesocarp powder of *Rh* fruit, different formulation and food staple. The *Rh mesocarp* fruit present the highest value which is 0.36 ± 0.06 mg/100g of DM and the lowest value which is 0.18 ± 0.03mg/100g of DM was found in staple food.

Table 3. Phytic acid and hydrocyanic acid composition of mesocarp powder of *Raphia hookeri* fruit, the different formulations and the food staple of animals

Sample	%Phytic acid(mg/100g of DM)	% HCN (mg/100g of DM)
Al	91.81 ± 8.76[a]	0.18 ± 0.03[c]
Rh	40.16 ±3.31[b]	0.36 ± 0.06[a]
Rh5%	86.24 ±5.73[a]	0.22 ± 0.06[b]
Rh10%	63.12 ±8.76[a]	0.26 ± 0.08[b]

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05; Al : food staple; Rh: powder of *Raphia hookeri mesocarp*; Rh 5%: formulation food with 5% of *Rh mesocarp* powder; Rh 10%: formulation food with 10% of *Rh mesocarp* powder; HCN: hydrocyanic acid

3.1.2 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on the Amount of Food Intake and the Weight of Animals

3.1.2.1 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on the Amount of Food Intake

The following figure shows the effect of the aqueous extract of the mesocarp of *Raphia hookeri* fruit and the different formulations on the amount of food intake.

It appears from this figure that the food intake of animals varies during treatment and is between 20 and 32g. During the first two weeks, except for the VC200 and the Rh5% groups, food intake decreased in the other groups. However, during the third week of treatment, there was an increase in food intake in all animals except the VC200 group which remained constant. The highest amount of food intake is observed in ARh200 group follows by Rh5% and Rh10%.

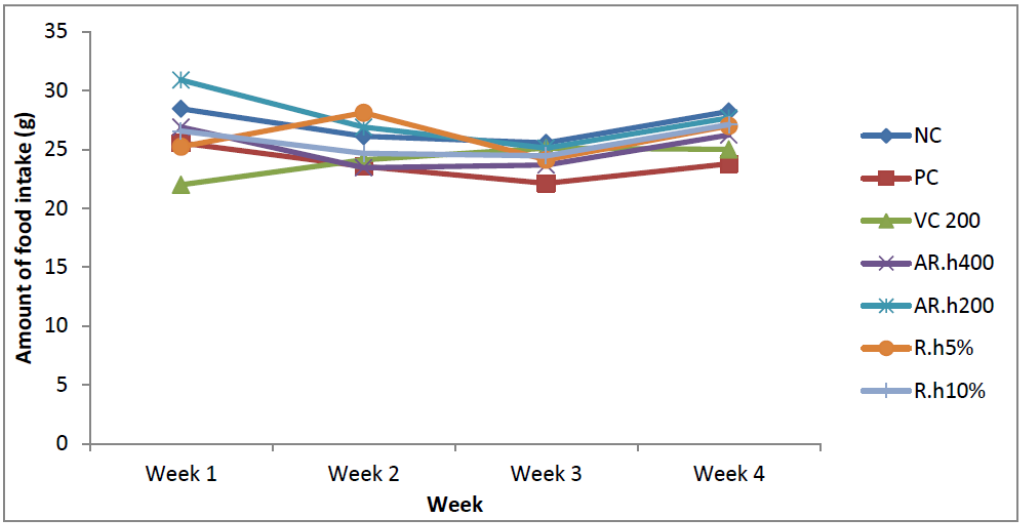

Figure 1. Effect of the aqueous extract and the different formulations of *Raphia hookeri mesocarp* on the amount of food intake

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05; NC: vehicle group received water; PC: induced rats (positive control rats) received water; VC200: induced rats received 200 mg/kg bw of vitamin C(negative control rats) ; A.Rh 200: induced rats received 200 mg/kg bw aqueous extract of *Rh* mesocarp; A.Rh 400: induced rats received 400 mg/kg bw aqueous extract of *Rh* mesocarp; Rh 5%: induced rats received formulation food with 5% of *Rh mesocarp* powder; Rh 10%: induced rats received formulation food with 10% of *Rh mesocarp* powder.

3.1.2.2 Effect of the Aqueous Extract and the Different Formulations of the Mesocarp of *Raphia hookeri* on the Weight of Animals

Figure 2 shows the effect of the aqueous extract of *Raphia hookeri* fruit mesocarp and the different formulations on the weight gained of animals. A weight increase between 5 and 10% was observed during the first week except for the ARh200 group, which showed a weight gain less than 5%. This weight gain increased to the end of treatment except in the Rh5% group who presented a constant weight gain from the second week to the fourth week. The highest weight gain (between 20 and 25%) is observed in the ARh400 group and the lowest in the PC group with a weight gain between 10 and 15% throughout the treatment.

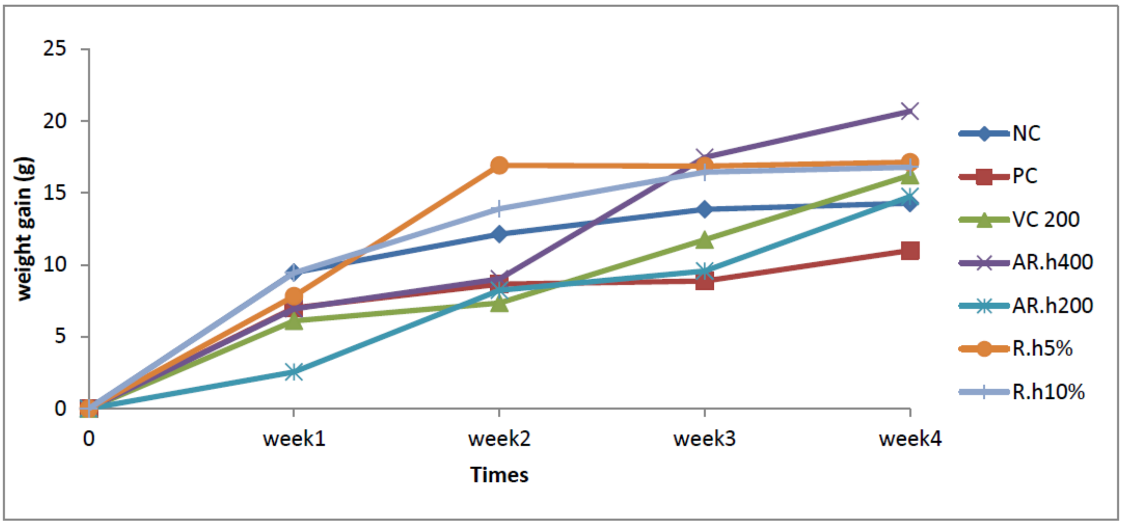

Figure 2. Evolution of weight gain of different group during the treatment

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at $P< 0.05$; NC: vehicle group received water; PC: induced rats (positive control rats) received water; VC200: induced rats received 200 mg/kg bw of vitamin C (negative control rats); A.Rh 200: induced rats received 200 mg/kg bw aqueous extract of *Rh* mesocarp; A.Rh 400: induced rats received 400 mg/kg bw aqueous extract of *Rh* mesocarp; Rh 5%: induced rats received formulation food with 5% of *Rh mesocarp* powder; Rh 10%: induced rats received formulation food with 10% of *Rh mesocarp* powder.

3.1.3 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on the Hematological Parameters

3.1.3.1 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on Red Blood Cell Counts

The following table 4 shows the effect of the different treatments on the count of red blood cells and the figured elements of the blood. It is noted that the induction of stress has led to a significant decrease in red blood cell count and hematocrit in induced group with the value of 5.4210^6 /µl and 11.65% respectively. On the other hand, the administration of the aqueous extract increased significantly in the red blood cell count and the hematocrit level in the latter. It is also observed that the intake of the various formulations leads to an increase in the red blood cell count in the animals. The high level of RBC, HGB and HCT is observed in groups Rh5% with the value of $8.86\ 10^6$ /µl, 17.2g/dl and 13.21% respectively.

Table 4. Effect of the aqueous extract and the different formulations of *Raphia hookeri* on the count of red blood cells and the figurate elements of blood

Groups	RBC (10^6 /µl)	HGB (g/ dl)	HCT (%)	MCV (fl)	MCHC (g/dl)
NC	6.96 ± 0.96^b	14.36 ±0.96^b	13.25 ±4.85^a	53.13 ±2.99^a	22.5 ±0.96^a
PC	5.42 ± 0.99^c	14.43 ±0.99^b	11.65 ±4.59^b	54.16 ±3.5^a	22.5 ±1.22^a
VC 200	6.81 ±0.37^b	14.53 ±0.31^b	13.87 ±1.44^a	54.23 ±0.92^a	21.26 ±0.5^a
ARh200	6.46 ±1.54^b	14.5 ±1.54^b	13.43 ±8.91^a	54.63 ±1.7^a	22.3 ±0.55^a
ARh400	6.82 ±1.55^b	14.73 ±1.55^b	12.78 ±5.72^{ab}	5 3.7 ±4.34^a	21.7 ±1.03^a
Rh5%	8.86 ±0.76^a	17.2 ±3.93^a	13.21 ±5.01^a	53.6 ±1.34^a	21.8 ±0.72^a
Rh10%	6.39 ±0.19^b	13.6 ±0.19^b	10.92 ±2.67^b	52.96 ±2.97^a	21.23 ±0.2^a

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05 ; NC: vehicle group received water; PC: induced rats (positive control rats) received water; A.Rh 200: induced rats received 200 mg/kg bw aqueous extract of *Rh* mesocarp; A.Rh 400: induced rats received 400 mg/kg bw aqueous extract of *Rh* mesocarp; Rh 5%: induced rats received formulation food with 5% of *Rh mesocarp* powder; Rh 10%: induced rats received formulation food with 10% of *Rh mesocarp* powder; VC200: induced rats received 200 mg/kg bw of vitamin C (negative control rats); RBC: red blood cell count; HGB:hemoglobin; HCT: hematocrit; MCV: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

3.1.3.2 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on the Leucocyte Formula

The following table 5 shows the effect of the different treatments on the leucocyte formula. It shows that the induction of stress has led to a significant increase in white blood cell, monocyte and granulocyte levels with the value of 18.06$10^3$/µl, 8.23% and 16.56% respectively. However, the administration of the aqueous extract and the various formulations has led to a significant decrease in these parameters in the animals. The best reduction of WBC is observed with the formulation in group Rh10% with the value of 10.66 10^3/µl and the best reduction of monocyte and granulocyte in the formulation 5% with the values of 4.5% and 8.76% respectively.

Table 5. Effect of the aqueous extract and the different formulations of *Raphia hookeri* mesocarp on the leucocyte formula

Groups	WBC(10^3/µl)	LYM (%)	MID(%)	GRAN (%)	PLT (10^3/µl)	MPV (fl)
NC	9.63 ±1.1^c	79.83 ±6.56^{ab}	6.6 ±0.86^{ab}	13.16 ±0.28^b	333 ±23.6^b	8.4 ±0.52^a
PC	18.06 ±1.2^a	76.03 ±8.52^b	8.23 ±2.54^a	16.56 ±0.8^a	290.33 ±8.54^c	8.86 ±0.15^a
VC 200	13.62 ±2.03^b	77.53 ±1.1^b	7.66 ±2.63^a	14.8 ±17.6^{ab}	353.33 ±69.24^b	8.86 ±1.09^a
ARh200	21.8 ±0.0^a	79.9 ±0.95^b	7.03 ±0.57^a	13.16 ±1.48^b	293.66 ±15.01^c	8 ±0.2^a
ARh400	11.8 ±1.82^b	82.53 ±1^a	5.1 ±0.81^b	13.03 ±0.92^b	286.33 ±78.51^c	8.46 ±0.85^a
Rh5%	11.16 ±0.28^b	85.86 ±1.3^a	4.5 ±0.6^b	8.76 ±1.41^b	439 ±20.78^a	9.3 ±0.97^a
Rh10%	10.66 ±2.02^{bc}	80.96 ±1.46^a	6.56 ±1.45^{ab}	14.13 ±0.63^{ab}	412.33 ±73.32^a	8.7 ±0.34^a

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05 ; NC: vehicle group received water; PC: induced rats (positive control rats) received water; A.Rh 200: induced rats received 200 mg/kg bw aqueous extract of *Rh* mesocarp; A.Rh 400: induced rats received 400 mg/kg bw aqueous extract of *Rh* mesocarp; Rh 5%: induced rats received formulation food with 5% of *Rh mesocarp* powder; Rh 10%: induced rats received formulation food with 10% of *Rh mesocarp* powder; VC200: induced rats received 200 mg/kg bw of vitamin C (negative control rats);WBC: white blood cell count ;LYM: lymphocyte; MID: monocyte; GRAN: granulocyte; PLT: platelet count

3.2 Discussion

The characterization of different samples was done. Water content is a parameter used in the treatment and organoleptic properties of foods. The water contents of our samples were all low and the lowest water content noted in *Rh mesocarp* (9.54 ± 3.54). This would justify the fact that this product can be preserved for a long time without enzymatic browning process being initiated. These results are in agreement with those of (Esiegbuya et al., 2013) which showed that the mesocarp of the *Rh* had a water content of 9.87%.

Proteins play a major role in the body as they build up all the structures of an organism. They are mostly contained in foods from animal sources but also in vegetables. The highest protein content was observed in the staple food. This is surely due to the fact that the staple food contained the highest quantity of fish, meat and soya beans compared to other food samples. The mesocarp of *Rh* showed the lowest protein content (1.93 ± 0.51%). This value is lower than that obtained by (Leung et al., 1968), who found that the core of *Rh* fruit had a protein content of 8% and slightly lower than that of (Akpabio et al., 2012) who found a protein content of 2.5% on the exudates of *Rh*. This difference can be attributed to several factors such as the genetic constitution of the plant, the climatic conditions, the stage of maturity of the fruits and the part of the plant studied (Morris et al., 2004).

Lipids are one of the essential components of our diet. This study revealed that the *Rh* fruit had a lipid content of 48.97%.This result does not corroborate with that of (Esiegbuya et al., 2013), who found a lipid content of 8% in fresh *Rh* mesocarp and of 20.88% in the dry one. The difference observed may be due to fruit maturity, weather conditions under which the plant grew up and the fruit moisture content (dry or fresh) (Morris et al., 2004).

Carbohydrates content was also determined as they represent the main macronutrient in human diet with an energy requirement ranging between 50 and 55% a day. The highest carbohydrates content was observed in the food staple. This may be explained by the fact that the food staple highly contained corn flour which is known as a very good source of carbohydrate. As the results show, the *Rh* mesocarp presented the lowest carbohydrate content. It can therefore be used for this beneficial property in the treatment of metabolic disorders due to high carbohydrate intake including diabetes. The studies of (Dada et al., 2017) have shown that phenolic compounds of *Rh* leaf extract have a modulatory effect on starch hydrolysis by activating enzymes like α-amylase and α-glucosidase. *Rh* fruit presented the highest energy value (485.74Kcal/100g). It depends on the macronutrient content with the highest energy intake in lipids. The difference observed is due to a difference in the proportion of macronutrient present in different samples. Our results are showed higher value than those of (Leung et al., 1968) and (Bhatia et al., 2018), which obtained an energy value of 326 and 375Kcal/100g respectively. The higher fiber content of *Rh* mesocarp (25.82%) makes it a food of choice in the prevention of certain diseases such as: cancer of the colon, diabetes, cardiovascular diseases and others (Igboh et al., 2009). The results obtained in this study are lower than those of (Esiegbuya et al., 2013), which had a fiber content of 56.15%, which can be attributed to the degree of maturity of the fruit, the geographic conditions and the condition of the fruit. Indeed, fresh fruits have a higher fiber content compared to dried fruits (Esiegbuya et al., 2013).

Ash is an inorganic residue remaining after water and organic matter are removed by calcination. It indicates the amount of minerals contained in a food (Mcclements, 2003). Minerals are important components of diet because of their physiological role and metabolic function in the body. In this study, the main minerals present in the different samples were: calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and phosphorus (P). *Rh mesocarp* powder sample had the highest calcium content. Indeed, humans need a significant amount of calcium for the construction and maintenance of bones, blood clotting, transmission of nerve impulses, formation of teeth and bones. It is also an important factor in enzymatic metabolic processes (Senga et al., 2013). The amount of calcium obtained was 3183.30 mg /100g of dry matter. This value is highly above the one recommended daily by WHO (800 mg). This result showed that *Rh* fruit is an important source of calcium that can be used as a supplement in calcium deficient people (Murray et al., 2000). This result obtained is higher than that of (Esiegbuya et al., 2013) who showed that *Rh* fresh mesocarp contained 789.1mg / 100g of fresh matter. This difference is probably due to the fact that in this study, a dry matter was used to determine the calcium content contrary to other studies where a fresh matter was used.

Phosphorus is jointly absorbed with calcium. It is essential for the fortification of bones and teeth as well as children and nursing mothers. Phosphorus is vital for the flow of metabolic reactions especially those involving buffers in body fluids (Andzouana & Mombouli, 2012). Energy production within living cells involves the formation or breakdown of high-energy bonds that bind oxygen from phosphorus to carbon or carbon-nitrogen compounds. Phosphorus is also an essential element for plants (Abugre, 2011). The daily dose recommended in adults and children is 800mg / day (Pillai & Nair, 2013). The highest phosphorus content noted in the staple food may be due to the fact that phosphorus is present in large quantities in foods like meat and fish because these ingredients are mostly found in the staple food than the other samples.

The magnesium content was also evaluated and the highest content was observed in the staple food. In fact, magnesium plays an important role in the body by activating enzymes involved in protein synthesis. The recommended daily dose is 420mg for men and 320mg for women. However, magnesium deficiency leads to growth retardation, behavioral disorders, body weakness and muscle contractions (Murray et al., 2000).

Potassium is also an important mineral for the body because it maintains the acid-base balance, the osmotic pressure and the conduction of nerve impulses. The recommended quantity is 2.5mg / day and its deficit leads to muscle weakness and paralexia. It is found in foods such as milk, meat and fruits. The results showed that the *Rh* fruit had the highest content. The amount obtained was 1218.3mg/100g of dry matter. This fruit represents an important source of exploitable potassium for food formulations. These results do not line with those of (Akpabio et al., 2012) who found very lower potassium content from *Rh* exudates (20.95mg/100g). This difference could be due to the plant part used which is different in the two studies.

Sodium content was determined and the highest amount was observed in the staple food (291.3mg/100g). It has numerous functions in the body including the maintenance of acid-base balance and osmotic pressure between cells and interstitial fluid. The recommended daily dose is 115-75000mg/kg for adolescents, 324-975mg/kg for children and 1100-3300mg/kg for adults (Lawal & Fagbohun, 2012).

Zinc plays a vital role in human growth and development (Divrikli et al., 2006). Highest zinc content was observed in *Rh* fruit (0.88 mg/100 g DM). The recommended daily dose is between 0.3 and 1 mg/kg in adults (FAO / WHO, 2014). This result is not similar to that of (Esiegbuya et al., 2013) who obtained 10.45 Ppm/100g fresh matter from the *Rh* fresh mesocarp. The difference is certainly linked to the maturity of the fruit.

The present study showed that the highest iron content was observed in the staple food. Nevertheless, the amount of iron present in mesocarp of *Rh* fruit was also higher 22.12mg/100g dry matter than the recommended daily dose which is 10mg/100g dry matter(FAO / WHO, 2014). Iron is a component involved in numerous metabolic pathways including proteins (Andzouana & Mombouli, 2012). It is important for a normal functioning of the central nervous system and proteins transportation. Iron is also essential in the diets of pregnant women, nursing mothers, infants and elderly so as to prevent anemia and other related diseases (Alinor & Oze, 2011). So, this fruit can be recommended to people constantly exposed to anemia in order to prevent hemolysis caused by oxidative stress, inflammation, erythrocyte deformability and mechanical rupture from foot strike (FAO / WHO, 2014).

However, food usually contains anti-nutritional substances that prevent the absorption of some nutrients. This is why some anti-nutrients such as hydrocyanic acid and phytic acid were evaluated in this study. Hydrocyanic acid content was evaluated and the highest value (0.36mg/100 dry matter) was obtained in the *Rh* powder. This value is much lower than the lethal dose (35mg/100 g of weight body). This result is very lower than that of (Akpabio et al., 2012) who obtained a value of 23.76mg/100 of fresh matter with Rh exudates. This difference could be due to the plant matrix that was used and also the fruit moisture content (dry or fresh fruit). Storage and drying may significantly reduce hydrocyanic acid content.

Phytic acid content was also determined. The highest value (91.81mg/100g dry matter) was observed in the staple food and the smallest value in *Rh* mesocarp (40.16 mg/100g dry matter). This value is extremely higher than that of (Akpabio et al., 2012) who obtained 7.82mg/100g DM. Low anti-nutrients contents obtained in Rh mesocarp fruit could be exploited to formulate some foods (Nwokolo & Bragg, 1977).

Induction of stress by aluminum chloride resulted in a low percentage of weight growth and a decrease in food intake in untreated induced rats. This is due to the fact that aluminum has a direct effect on the appetitive pathways and /or malabsorption of nutrients induced by $AlCl_3$ effects on the gastro-intestinal tract and/or inhibition of protein synthesis. And therefore a low food intake would be the cause of low weight gain (Lukyanenko et al., 2013). Concomitant food intake with increased weight gain would be due to the fact that the mesocarp powders and aqueous extract of *Rh* fruit would stimulate appetite, as the work of (Mann & Wendl, 1864), reveal that the mesocarp powder of this fruit and used in certain food formulations to enhance the flavor.

Induction of stress with aluminum chloride resulted in a significant decrease in red blood cell count and hematocrit levels in animals. It can be explain by the fact that the oxidative stress caused by $AlCl_3$ increase production of free radicals, decrease catalase activity and the erythrocyte ATP concentration (Newairy et al., 2009; Rim, 2007; Al-Hashem, 2009). All or some of these deleterious effects of $AlCl_3$ on RBCs membrane caused increased membrane fragility, increased RBCs destructions. The decrease of hematocrit would be due to an increase in the rate of destruction or a reduction in the rate of formation of red blood cells. Indeed, aluminum can disrupt erythropoiesis through its combined effect on mature erythrocytes and delayed cellular metabolism of progenitor erythroids (Bouasla et al., 2014). Aluminum can be accumulating in the liver and decrease or inhibing the erythropoese activitie (Ojiako et al., 2018). However, administration of aqueous extract and differents formulations of the mesocarp of *Rh* least to an increase of theses parameters. It is due to the present of some metabolites which stimulate hematopoietic activity in the bone marrow. (Bouasla et al., 2014) showed the antioxidant effect of alpha lipoic acid which stimulate hematopoietic activity in the bone marrow of the hepatotoxicity rat induced by aluminum chloride. In the same line, (Domingo et al., 2011) showed that malic acid can act as chelating agent, reduce the concentration of $AlCl_3$ levels in the brain by fecal and urinary excretion. Malic acid act as powerful antioxidant by decreasing the oxidative stress in comparison with group that was received $AlCl_3$ alone Sadhana, 2011). The different formulation also contain high amount of iron which in addition to vitamin B12, vitamin A, folate, riboflavin, and copper are required for the proper production of hemoglobin (De Benoist et al., 2008).

Induction of stress with aluminum chloride also led to a significant increase in the level of white blood cells, monocytes and granulocytes in the plasma of positive control group of rats. Oxidative stress usually leads to activation of the white blood cells that are lymphocytes and neutrophils (Vignais, 2002) which indicated the activation of defence and immune system (Mahdy & Farrag, 2009). These results are similar to those of (Turkman et al., 2005), which following induction of fibrosis by carbon tetrachloride showed a significant increase in plasma white blood cell level of rats. Also, to those of (Bouasla et al., 2014) which observe the decrease of theses parameters in rats which has induced hepatotoxicity with aluminum chloride. Moreover, the administration of the aqueous extract of the *Rh mesocarp* and the different formulations resulted in a significant decrease in the rate of these parameters. This drop is due to the presence of metabolites (minerals and phenolic compound) present in our extract and formulations. In addition, the work of (De Benoist et al., 2008) showed that antioxidants in plants reduce white blood cell levels by inhibiting pro-inflammatory pathways associated with acute or chronic toxicity in rats. These results are similar to those of (Ojiako et al., 2018) who observed a decrease in white blood cell count after inducing hepatic stress with carbon tetrachloride and administering crude and infused herbs in *wistar* rat.

4. Conclusion

The evaluation of the nutritional value of the different samples showed that Rh mesocarp fruit is rich in macronutrients namely lipids and fibers and micronutrients including calcium, potassium, zinc, iron and selenium. However anti-nutrients such as phytic acid and hydrocyanic acid were also present but under the toxic concentration prescribed by WHO. The evaluation of hematological parameters showed that the formulation made with Rh mesocarp powders presented the best result by increasing the concentration of red blood cells, hemoglobin and hematocrit and reducing white blood cell concentration.

Acknowledgement

The authors thank gratefully Professor Kuiate Jules Roger and Professor Zambou Ngoufack Francois of the Department of Biochemistry, University of Dschang, Cameroon.

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