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Research Article
Bio-Tolerance Evaluation of *Parkia biglobosa*-Based Dietary Supplement a Plant Species from Ivorian Medicinal Flora

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Abstract

Background and Objective: Broths have become an indispensable ingredient for over 80% of households. The aim of this study was to assess the bio-tolerance of a néré-based or *Parkia biglobosa*-based dietary supplement in broth form in rats. **Materials and Methods:** The bio-tolerance of the experimental broth was evaluated in rats by the oral route. The broth, containing a high percentage of néré fruit, was formulated at IPCI and the control broth was a culinary broth available on the Ivorian market. Rats with an average weight of 130 g received the different broths orally for 28 days. The bio-tolerance of the experimental broth was evaluated in rats by the oral route. Biochemical parameters were determined using ready-to-use kits and a Cobas C311 Hitachi and the AVL 9180 electrolyte analyzer. Hematological marker assays are carried out automatically using the Sysmex XN-1000 Kobe, Japan. **Results:** The results showed that the experimental broth did not excessively decrease or increase the weight of the rats during the course of the experiment. Similarly, the broth did not induce any toxicity on relative organ weights, nor did it cause any disturbance of biochemical parameters compared with the control broth. The concentrations of GGT, LDH and CPK obtained in rats fed experimental broth at the highest dose (6 g/kg body weight) were significantly lower than those obtained in rats fed control broth at a dose of 3 g/kg body weight. These values were respectively 4.66±0.3, 2587±13 and 2385.66±33 U/l in rats fed with experimental broth versus 12.3±0.8, 2678.67±36 and 2645.6±36 U/l in rats fed with control broth. **Conclusion:** The experimental broth showed good bio-tolerance in treated rats and an immunoregulatory effect. These results justify the numerous uses of néré fruits in African dishes.

Key words: Bio-tolerance, *Parkia biglobosa*, néré, medicinal flora, biotoxicity, dietary supplement

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Seasoning broths have become an indispensable ingredient for over 80% of households[1,2]. Culinary bouillon production is estimated at 75,000 tonnes per year. In 2016, more than 60 billion broths flooded the African market, which is dominated by a few large companies including Nestlé, Jumbo and KUB[3]. Food technology professionals are then attempting several formulations to meet the daily needs of consumers, who are growing in number due to population growth in developing countries. Several studies have reported the nutritional suitability of *Parkia biglobosa* seeds with a composition of 35% protein, 29% lipids, 16% carbohydrates, calcium and good organoleptic properties as well as a positive effect on intestinal flora[4,5]. Néré and spice grains were chosen, which are widely consumed by the African population.

Néré (*Parkia biglobosa*) is of great nutritional and pharmacological importance to mankind. For Appia *et al.*[6] it's a plant with a thousand and one virtues, as described by several communities on the dark continent, néré cures several illnesses and feeds more than 20 million people in 14 countries. Almeida *et al.*[7] and Kassi *et al.*[8] explain that néré flour and its fermented grains are prescribed by doctors in cases of both malnutrition and hypertension. "Néré yellow flour is rich in protein, calcium and iron[5]. The Food and Agriculture Organization of the United Nations (FAO) states in a study published in January 2016[9]: "Néré yellow flour is rich in protein, calcium and iron. The final goal of this project is to propose a culinary formulation based on néré (soumara) and other spices from African dietary habits, to help add value to our local products. This study aimed to assess the toxicological activities of this culinary formulation in Wistar rats.

MATERIALS AND METHODS

Study area: The experimental study was carried out from December, 2023 to January, 2024. The force-feeding the animals was carried out at the vivarium of the Félix Houphoüet Boigny University and the phases of pre-analytical, analytical and post-analytical of blood from the rats were carried out at the Biochemistry and Haematobiology Unit of Institut Pasteur of Côte d'Ivoire (IPCI).

Materials: Biological material consists of broth made from néré and other local spices (experimental broth X) and a commercially available industrial broth (control broth Y).

Laboratory animals including Wistar albino rats.

Technical equipment: It consists of:

- A biochemistry system, the COBAS C311 HITACHI from Roche Diagnostic France, was used for the determination of biochemical markers
- An Electrolyte Analyzer 9180 (Roche Diagnostics®, Germany)
- Sysmex XN-1000i Kobe, Japan for the determination of hematological markers
- Small items such as syringes, gavage cannulas for oral administration of substances, 0.01 g precision balances for various weighing operations, urine jars for preparation of various solutions and sampling tubes

Reagents:

- Specific reagent kits from Roche Diagnostics (France) were used for assays of classic biochemical parameters (urea, creatinine, ASAT, ALAT, GGT, LDH and CPK, etc.)
- SnapPak universal ready-to-use kit for blood ionograms

Ready-to-use blood count kits for determination of blood count and red blood cell count.

Methods

Formulation of the experimental broth: The experimental broth formulation is an organic food product consisting of 55% soumara flour, 10% garlic powder, 8% onion powder, 12% parsley powder, 1.5% ginger powder, 1% black pepper and 12.5% cooking salt. All ingredients were then blended using a Moulinex blender. This formulation was carried out under aseptic conditions.

Rat treatment: The experiments were carried out on 25 rats of the Albino Wistar strain, divided into batches of 5 in metabolic cages and acclimatized for 7 days before the start of the experiments. They were fed water and pellets. They were obtained from the animal house (vivarium) of the École Normale Supérieur within the Félix Houphouët University Boigny Cocody-Abidjan (Côte d'Ivoire) conducted by gavage for 28 days. The concentrations received by the different batches of rats are as follows:

- **Control batch:** Rats were given distilled water at 1 mL/100 g body weight orally
- **Batch 1 experimental broth:** Rats treated with broth at a dose of 0.25 g/kg body weight (Broth X1)
- **Batch 2 experimental broth:** Rats treated with broth at a dose of 3 g/kg body weight (Broth X2)
- **Batch 3 experimental broth:** Rats treated with broth at a dose of 6 g/kg body weight (Broth X3)
- **Control broth batch (Broth Y):** Rats treated with a commercially available broth at a dose of 3 g/kg body weight

Force-feeding and blood sampling: Gavage was performed every morning at 8 am for 28 days. Rats were fed daily with pellets. The animals were weighed twice a week. On days 14 (D14) and 28 (D28) of the experiment, the rats' blood was collected, after anesthesia, from the orbital sinus of the eye using pipettes' according to the technique described by Diehl *et al.*[10].

Blood from each animal is collected in tubes without anticoagulants for biochemical analysis (ionogram, ALAT, ASAT, urea, creatinine, GGT, LDH, CPK) and also in tubes containing EDTA for Complete Blood Count (CBC). These tubes, containing whole blood from the rats, were triple-packed and transported to the Biochemistry and Hematobiology Unit of the Institut Pasteur de Côte d'Ivoire (IPCI Cocody site) for the various pre-analytical, analytical and post-analytical phases.

Determination of biochemical parameters in plasma

Method for measuring electrolytes (Na^+, Cl^-, K^+): The determination of Na^+, Cl^- and K^+ ions was carried out with the 9180 electrolyte analyzer, based on the comparison of an unknown value with a known value to calculate the electrolyte values of the specimen.

Biochemical marker assay methods: The biochemical parameters were determined using ready-to-use kits and a Cobas C311 Hitachi from Roche Diagnostics, France.

Principle of the Hitachi Cobas C311: The Cobas C311 is a fully automated clinical biochemistry analyzer. Its principle is based on performing manual assays in a completely closed circuit without human intervention. It is designed for the qualitative and quantitative determination of a wide range of biochemical assays in various body fluids. It is an *in vitro* diagnostic medical device manufactured by Roche Diagnostics in Germany and distributed by Roche Diagnostic France. Biochemical parameters are determined by a variety of methods, following kit calibration and quality control.

Analytical method for biochemical parameters: Quantitative analysis of biochemical parameters was carried out on 500 µL of serum after kit calibration and validation of quality control results. Results are obtained directly in concentration after conversion of absorbances from the equations of the calibration lines incorporated in the automaton. Biochemical marker assays are performed according to the different principles and methods described in the instructions supplied with the reagent kits.

Hematological marker assay methods: The hematological marker assays are carried out automatically using the Sysmex XN-1000 Kobe, Japan combined independent variation and flow cytometry technology[11]. Its principle harnesses the power of fluorescent flow cytometry and hydrodynamic focusing technologies. Thanks to a photodiode laser worktable, Sysmex fluorescent flow cytometry offers the sensitivity needed to measure and differentiate cell types present in blood.

Ethical consideration: The approval of the ethical committee is not applicable because it is a food supplement but the study was performed according to OECD, 2008: 407 test guideline[12].

Statistical analysis: The statistical analysis of the results was carried out using GraphPad prism 8U software. The Newman-Keuls Student's t-test was used to compare means. A difference between two means is considered significant if $p<0.05$.

RESULTS

Assessment of the effect of daily broth consumption on physical parameters: The average body mass values obtained in all batches are shown in Fig. 1. At the end of the treatments, the average gains in animal body mass were 33±1.14, 26.33±0.33, 25.33±1, 21.3±1.13 and 31±1 g, respectively for the control batch, the experimental broth batches (0.25, 3 and 6 g) and the commercial control broth batch (3 g). The rate of change in body mass of rats treated with the control broth was not significantly different from that of rats in the control batch. The rates of change in body mass of rats treated with experimental broth were lower but not significant than those of control rats and rats fed control broth, with the exception of rats fed experimental broth at dose X3 (6 g/kg), at which there was a significant difference between rats fed control broth ($p<0.05$) and control rats ($p<0.01$). Overall, however, an increase in body mass was observed in all broth-treated and control rats (experimental and control).

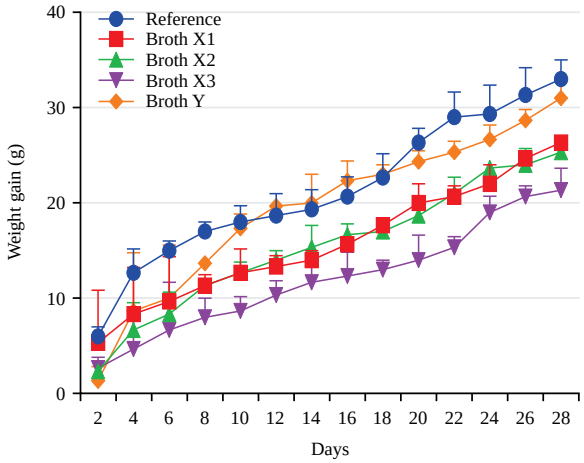

Fig. 1: Weight assessment of rats

Values are expressed as Means±Errors, n = 3. Body weights of broth batch X3 rats show significant differences from control (p<0.05)

Table 1: Relative weight of organs

Relative weight	Reference	Broth X1	Broth X2	Broth X3	Broth Y
Heart	0.36±0.04	0.42±0	0.39±0.01	0.42±0.1	0.47±0.03[a*]
Kidneys	0.608±0.03	0.67±0.01	0.64±0.05	0.7±0	0.66±0.01
Liver	5.18±0.2	4.98±0.04	5.30±0.15[b*]	4.42±0.03	3.78±0.03[a*]

Values are expressed as Means±Standard Errors, n = 3. [a*]: Relative weight of heart and liver in broth batch Y indicates significant differences from the control (p<0.05), [b*]: Relative liver weight of broth batch Y indicates significant differences from broth batch X (p 0.05), Bouillon X: Experimental broth and Broth Y: Control broth

Table 2: Blood electrolyte values in rats

Parameter	Date	Reference	Broth X1	Broth X2	Broth X3	Broth Y
Na (mEq/L)	14	133±0.57	134.3±0.33	133±1.15	133±1.5	135.33±2.6
	28	136±0.5	136.33±0.66	134±1	132.66±1	134±1
K (mEq/L)	14	7.066±0.28	7.53±0.59	9.93±0.9	8.23±1.39	9.46±1.08
	28	7.16±0.29	6.77±0.26	8.03±0.5	9.7±0.9	7.16±0.4
Cl (mEq/L)	14	105.67±0.33	106.33±0.33	105.66±0.33	104±1.5	105.33±2
	28	107±1.15	106±0.57	105.6±1.6	107.33±1.4	106

Values are expressed as Means±Errors, n = 3. Differences between batches are not significant (p>0.05)

Effects of broths on relative weight of organs: The relative weights of heart, kidney and liver of control rats were 0.36±0.04, 0.608±0.03 and 5.18±0.2 g, respectively. Significant differences (p<0.05) were observed in the relative masses of heart (0.47±0.03 g) and liver (3.78±0.03 g) compared to control rats. The relative organ masses of rats treated with experimental broth at the different doses ranged from 0.39±0.01 to 0.42±0.1 g (heart), 0.64±0.05 to 0.7 g (kidney) and 4.42±0.03 to 5.30±0.15 g (liver). There was no significant difference (p<0.05) between the relative organ weights of rats treated with experimental broth at the different doses compared with control rats, but with control broth on the relative weights of heart (increase) and liver (decrease), a significant difference (p<0.05) was observed compared with control rats. When comparing the two broths (experimental and control) at the same dose of 3 g/kg body weight, a significant difference was observed in relative liver weight (p<0.05) (Table 1).

Effects of broths on blood parameters

Effect of broth on electrolytes parameters

Sodium (Na) concentration: After 14 days of treatment, mean sodium concentration values were 133±0.57 mEq/L in control rats and 135.33±2.6 mEq/L in rats fed the control broth. These values ranged from 133±1.15 to 134.3±0.33 mEq/L in rats treated with experimental broth at different concentrations. After 28 days of treatment, the mean sodium concentration values were 136±0.5 mEq/L in control rats and 134±1 mEq/L in rats treated with control broth. For rats treated with experimental broth, values ranged from 132.66±1 to 136.33±0.66 mEq/L. No significant variation was observed in sodium concentrations in rats between 14th and 28th days of treatment. Furthermore, no significant differences were observed during this period between the sodium concentrations of control rats and those of rats treated with control broth and experimental broth (Table 2).

Table 3: Influence of broth on rat blood enzymes

Parameter	Date	Reference	Broth X1	Broth X2	Broth X3	Broth Y
ASAT (U/l)	14	181.87±1.23	192.53±20	190.17±3.01	196.6±19	162.06±12.62
	28	189.3±12	241.0±11	201.56±14	265.36±24	248.7±23
ALAT (U/l)	14	51.63±4.4	52.9±11.7	50.5±23.1	53.83±28	53.1±15.66
	28	50.53±5.9	57.93±5	59±2	68.86±11	65.23±0.7
GGTI (U/l)	14	4±1.5	2.67±0.3^{b*}	2.7±0.3^{b*}	3±1^{b*}	9.33±1.3^{a*}
	28	4.33±0.8	4±1^{b*}	3.67±1^{b*}	4.66±0,3^{b*}	12.3±0.8^{a*}
LDH (U/l)	14	1682.67±18	1673.33±50^{b*}	1640.67±20.6^{b*}	1485.33±28^{b**}	1990.33±29^{a*}
	28	2089.66±72	2087.67±36^{b**}	1992.33±21^{b**}	2587±13^{a**}	2678.67±36^{a**}
CPK (U/l)	14	1087.67±22	1038.33±29^{b***}	1042.66±64^{b***}	1216.66±17^{b**}	1600.67±14^{a***}
	28	1652.66±14	1656.6±30^{b***}	1730.66±30^{b***}	2385.66±33^{a,b**}	2645.6±36^{a***}

Values are expressed as Means±Errors , n = 3. Mean GGTI, LDH and CPK values of control broth batch rats indicate significant differences from control ($p<0.05$) near 14 and 28 days of treatment. Similarly, mean LDH and CPK values of experimental broth-treated rats indicate significant differences from control ($p<0.05$) near 28 days of treatment. ^{a*}: Weakly significant compared with control rats ($p<0.05$), ^{a**}: Low significance compared with control rats ($p<0.01$), ^{a***}: Compared with control rats ($p<0.001$); ^{b*}: Weakly significant compared with Y broth-treated rats ($p<0.05$), ^{b**}: Not significant compared with Y broth-treated rats ($p<0.01$), ^{b***}: Significant compared with Y broth-treated rats ($p<0.001$), ALAT: Alanine aminotransferase, ASAT: Aspartate aminotransferase, GGT: Gamma glutamyl transferase, LDH: Lactate dehydrogenase activity and CPK: Creatine phosphokinase activity

Potassium (K) concentration: Mean potassium concentrations after two weeks of treatment in control and control broth-treated rats were 7.066±0.28 and 9.46±1.08 mEq/L, respectively. In rats treated with experimental broth at different doses, concentrations ranged from 7.53±0.59 to 9.93±0.9 mEq/L. At the end of treatment, the potassium concentration was 7.16±0.29 mEq/L (control rats). In rats treated with control broth, potassium concentration showed a slight, non-significant decrease at the end of treatment. It fell from 9.46±1.08 to 7.16±0.4 mEq/L. Also, at the end of the treatments, potassium concentration in rats treated with experimental broth at doses X1 and X2 showed non-significant decreases ($p>0.05$). However, in rats treated with broth at dose X3, a slight non-significant increase from 8.23±1.39 to 9.7±0.9 mEq/L was reported.

Furthermore, no significant differences were observed during this period between the potassium concentrations of control rats and those of rats treated with control and experimental broth (Table 2).

Chloride (Cl) concentration: After 14 days of treatment, chloride concentrations in rats were 105.67±0.33, 106.33±0.33, 105.66±0.33, 104±1.5 and 105.33±2 mEq/L, respectively for the experimental broth batches (0.25, 3 and 6 g) and the commercial broth batch (3 g). After 28 days of treatment, mean chloride concentration values were 107±1.15 mEq/L in control rats and 106±0.57 mEq/L in rats fed the control broth. For rats treated with experimental broth, values ranged from 105.6±1.6 to 107.33±1.4 mEq/L. No significant variation was observed in the chloride concentrations of rats on day 14th and those on day 28th of treatment. Similarly, no significant difference was observed between the chlorine concentrations of control rats and those of rats treated with control and experimental broth (Table 2).

Effect of broth on enzyme activity

Effect on aspartate aminotransferase (ASAT): The mean ASAT activity values obtained from the rats are shown in Table 3. The ASAT values for control rats were 181.87±1.23 U/l (14 days) and 189.3±12 U/l (28 days). After 14 days of treatment, ASAT values in rats fed the experimental broth ranged from 190.17±3.01 to 196.6±19 U/l. The ASAT levels in rats fed control broth after 14 days of treatment were 162.06±12.62 U/l. There was a non-significant decrease ($p>0.05$) in the ASAT concentration of control broth-treated rats compared with the other batches. At the end of treatment, the ASAT concentration of rats treated with experimental broth showed significant increases of 48.47 (Broth X1), 11.39 (Broth X2) and 11.36 (Broth X3). Rats treated with control broth showed the strongest increase (86.64 U/l).

Effect on alanine aminotransferase (ALAT): The mean ALAT values obtained in the different batches are shown in Table 3. On day 14 of treatment, ALAT concentration values were 51.63±4.4, 52.9±11.7, 50.5±23.1, 53.83±28 and 53.1±15.66 U/l, respectively in the control, experimental broth at doses X1, X2 and X3 and control broth Y batches. Statistical analysis revealed no significant difference between ALAT concentration values in control and treated rats.

As for day 28th, ALAT concentrations were 50.53±5.9, 57.93±5, 59±2, 68.86±11 and 65.23±0.7 U/l, respectively for rats from control, Broth X1, X2, X3 and control broth batches. Statistical analysis showed no significant variation between control and treated rats.

Effect on Gamma Glutamyl Transferase (GGT): The mean GGT values after 14 days of treatment were 4±1.5, 2.67±0.3, 2.67±0.3 and 3±1 U/l for Control, Broth X1, Broth X2 and Broth X3 rats. The activity values of rats from this lot at the end of the experiment were 4.33±0.8, 4±1, 3.67±1 and 4.66±0.3 U/l. The GGT values of rats from the control broth lot were 9.33±1.3 U/l on day 14 and 12.3±0.8 U/l on day 28. Statistical analyses revealed a significant difference ($p<0.05$) in the GGT activity of rats treated with control broth compared with that of control rats. However, no significant difference ($p>0.05$) was observed between control and experimental broth-treated rats. In comparison of the two broths (experimental and control) a significant difference ($p<0.05$) was observed between the concentrations of the different doses from experimental broth 0.25, 3 and 6 g/kg to control broth 3 g/kg body weight (Table 3).

Effect on Lactate Dehydrogenase (LDH) activity: The mean LDH values of control and control broth-treated rats after 14 days of treatment were 1682.67±18 and 1990.33±29 U/l, respectively. Statistical analysis revealed a highly significant difference ($p<0.001$) in the LDH concentrations of rats from the experimental broth batch compared with those from control rats. The LDH concentrations ranged from 1485.33±28 to 1673.33±50 U/l. No significant differences were observed compared with controls. At the end of treatment, highly significant increases ($p<0.001$) in rat LDH concentrations were observed in all batches. These values were 2089.66±72, 2087.67±36, 1992.33±21, 2587±13 and 2678.67±36 U/l, respectively for control batches, experimental broth at concentrations of 0.25, 3 and 6 g/kg and control broth 3 g/kg body weight. Similarly, statistical analysis revealed a significant difference ($p<0.001$) in the LDH activity of rats treated with experimental broth and those treated with control broth compared with control rats on the 28th day. When comparing the two broths (experimental and control), a significant difference was observed between the concentrations of the different doses of experimental broth 0.25, 3 and 6 g/kg and control broth at 3 g/kg body weight at D14 and D28. This difference was particularly noticeable with the first two doses of experimental broth.

Effect on Creatine Phosphokinase (CPK) activity: The mean CPK values of control and experimental broth-treated rats after 14 days of treatment were 1087.67±22 and 1600.67±14 U/l, respectively. Statistical analysis revealed a significant difference ($p<0.001$) in CPK concentrations between experimental broth-treated and control rats. The CPK activity values in experimental broth-treated rats ranged from 1038.33±29 to 1216.66±17 U/l. At the end of treatment, a highly significant increase ($p<0.001$) in rat CPK activity was observed in all batches. These values were 1652.66±14, 1656.6±30, 1730.66±30, 2385.66±33 and 2645.6±36, respectively for control, Broth X1, X2, X3 and control broth Y batches. Similarly, statistical analysis revealed a significant difference ($p<0.001$) in CPK activity between experimental and control broth-treated rats compared with control rats on the 28th day of treatment. Values are expressed as Means±Standard Errors, n = 3. Mean GGT, LDH and CPK values of control broth batch rats show significant differences from control ($p<0.05$) at 14 and 28 days of treatment. Similarly, the mean LDH and CPK values of broth-treated rats indicated significant differences from control ($p<0.05$) near 28 days of treatment. In the comparison of the two broths (experimental and control), a significant difference ($p<0.05$) was observed between the concentrations of experimental broth at doses 0.25, 3 and 6 g/kg and control broth at 3 g/kg body weight (Table 3).

Effects of broth on renal function in rats

Effect on urea: The urea concentrations in rats from different batches after 14 days of treatment were 0.45±0.02, 0.39±0.02, 0.54±0.05, 0.39±0 and 0.43±0.08 mg/L, respectively for control, experimental broth at different doses 0.25, 3 and 6 g/kg and control broth at 3 g/kg body weight. Statistical analysis revealed no significant difference between control and treated rats.

After 28 days of treatment, urea concentrations were 0.45±0.03 mg/L in control rats and 0.31±0.04 mg/L in rats treated with control broth (Y). For rats treated with experimental broth (X), urea concentrations ranged from 0.42±0.03 to 0.58±0.07 mg/L. No significant variation was observed between the urea concentrations of rats on 14th and 28th days of treatment. Furthermore, no significant differences were observed in urea concentrations between control rats and those treated with broth X and Y (Table 4).

Effects on creatinine: Mean creatinine levels at D14 and D28 are shown in the table. After 14 days of rat treatment, creatinine levels were 4.33±0.3, 4.33±0.33, 5.33±0.88, 3.33±0.3 and 3±0.58 g/L. Significant analysis of rat creatinine levels after 14 days of treatment revealed no significant difference between treated and control rats. At the end of treatment, there was a non-significant change in creatinine levels in rats on the 28th day of treatment compared with creatinine levels in rats on the 14th day. Creatinine levels in control and broth-treated rats were 5±0.57 and 4.33±0.3 g/L, respectively. Those of control rats ranged from 5±0.57 to 6.33±0.8 g/L (Table 4).

Table 4: Influence of broths on renal function in rats

Parameter	Date	Reference	Broth X1	Broth X2	Broth X3	Broth Y
Urea (mg/L)	14	0.45±0.02	0.39±0.02	0.54±0.05	0.39±0	0.43±0.08
	28	0.45±0.03	0.42±0.03	0.49±0.02	0.58±0.07	0.31±0.04
Creatine (g/L)	14	4.33±0.3	4.33±0.33	5.33±0.88	3.33±0.3	3±0.58
	28	5±0.57	5±0.57	5.67±0.66	6.33±0.8	4.33±0.3

Values are expressed as Means±Errors, n = 3. Differences between batches are not significant (p>0.05)

Table 5: Influence of broth on erythrocyte parameters

Parameter	Date	Reference	Broth X1	Broth X2	Broth X3	Broth Y
RBC (10^6/µL)	14	7.15±0.07	7.25±0.05	7.22±0.09	7.11±0.12	7.49±0.24
	28	6.64±0.49	6.7±0.2	7.08±0.1	6.43±0.09	7.053±0.37
WBC (10^3 /µL)	14	11.80±1	15.86±1.65	14.28±0.99	14.75±2.6	15.94±0.94
	28	15.47±2	13.44±1	13.38±1	17.81±0.67	14.89±0.68
HGB (g/dL)	14	12.16±0.2	12.06±0.237	12.03±0.4	11.7±0.5	12.23±0.26
	28	11.76±0.29	12.53±0.1	11.8±0.18	12.66±0.08	12.93±0.4
PLT (10^3 /µL)	14	760.33±13	859±24	635±83.9	963±13.5	897.66±98
	28	952±11	1014±80	1195.33±28	1263.33±90	995.6±12

Values are expressed as Means±Errors, n = 3. Differences between batches are not significant (p>0.05), RBC: Red blood cells, WBC: White blood cells, HGB: Hemoglobin and PLT: Platelets

Effects of broth on haematological parameters

Effect on erythrocyte parameters: Mean red blood cell (RBC) levels in control rats were 7.15±0.07 10^6/µL (D14) and 6.64±0.49 10^6/µL (D28). Those of rats treated with control broth were 7.49±0.24 10^6/µL (D14) and 7.053±0.37 10^6/µL (D28). For rats treated with experimental broth, levels ranged from 7.11±0.12 to 7.25±0.05 10^6/µL (D14) and from 6.43±0.09 to 7.08±0.1 10^6/µL on 28th days of treatment. Statistical analysis showed no significant difference (p>0.05) between red blood cell levels in control and treated rats (Table 5).

Mean White Blood Cell (WBC) counts in control rats were 11.80±1.00 10^3/µL (D14) and 15.47±2.00 10^3/µL (D28). Those of control broth-treated rats were 15.94±0.94 10^3/µL (D14) and 14.89±0.68 10^3/µL (D28). For rats treated with experimental broth, levels ranged from 14.28±0.99 to 15.86±1.65 10^3/µL (D14) and from 13.38±1.00 to 17.81±0.67 10^3/µL on 28th days of treatment. Statistical analysis showed no significant difference (p>0.05) between white blood cell levels of control and treated rats.

Hemoglobin (HGB) concentrations in control rats were 12.16±0.2 g/dL (D14) and 11.76±0.29 g/dL (D28), while those in control broth were 12.23±0.26 g/dL (D14) and 12.93±0.4 g/dL (D28). The Hb concentrations in experimental broth rats ranged from 11.7±0.5 to 12.06±0.023 g/dL after 14 days of treatment and from 11.8±0.18 to 12.66±0.08 g/dL after 28 days of treatment. Statistical analysis revealed no significant difference (p-value) between the Hb concentrations of control and treated rats.

Platelet (PLT) levels in control rats were 760.33±13 10^3/µL (D14) and 952±11 10^3/µL (D28) and those in rats fed control broth were 897.66±98 and 995.6±12 10^3/µL.

Platelet levels in experimental broth-fed rats ranged from 635±83.9 to 963±13.5 10^3/µL, after 14 days of treatment and from 1014±80 to 1263.33±90 10^3/µL after 28 days of treatment. Significant increases (p<0.01) in platelet levels were observed in all batches, moreover, these increases were more marked in rats treated with experimental broth, for Broth X1, platelet levels increased by 155 and 560 10^3/µL (Broth X2) and 300 10^3/µL (Broth X3).

Effect of broth on leukocyte parameters

Effect on neutrophil percentage: The mean neutrophil values obtained during the different treatments after 14 days of treatment were 19.66±3.96, 24.16±4.4, 15.06±4.1, 15.03±3 and 18.13±3.16%, respectively for rats in the control, experimental broth 0.25, 3 and 6 g/kg and control broth 3 g/kg body weight batches. Statistical analysis revealed no significant difference (p>0.05) between rats in the treated and control batches. After 28 days of treatment, neutrophil percentages were 18.572.7±55, 19.1±0.9, 11.8±4.6, 15.6±1 and 21.47±3.2%. There was no significant variation in broth effects on the percentage of lymphocytes (Fig. 2-3).

Effect on lymphocyte percentage: The effects of broths (experimental and control) on lymphocyte levels were most marked during blood assays of rats in the 14 and 28 day batches. Lymphocyte percentages in control rats were 71.86±5.7% (D14) and 75.26±1.84% (D28). In rats treated with experimental broth, lymphocyte percentages ranged from 64.8±8 to 76.03±3.14% (day 14) and 67.1±3 to 76.03±3.14% (day 28). For rats fed with control broth, lymphocyte percentages were 76.03±3.14% (day 14) and 78.5±4% (day 28) (Fig. 2-3).

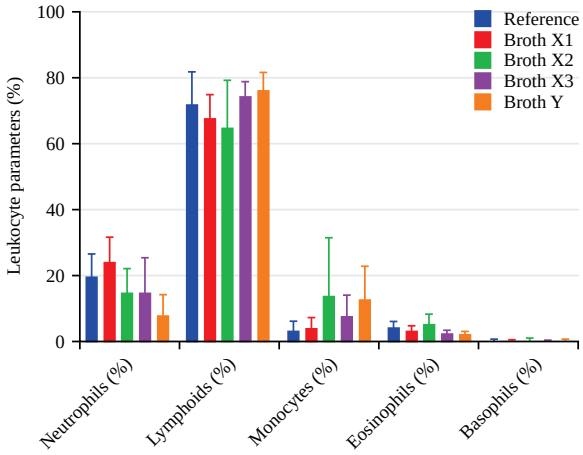

Fig. 2: Leukocyte parameters of rats after 14 days of treatment
Values are expressed as Means±Errors, n = 3. Differences between batches are not significant (p>0.05)

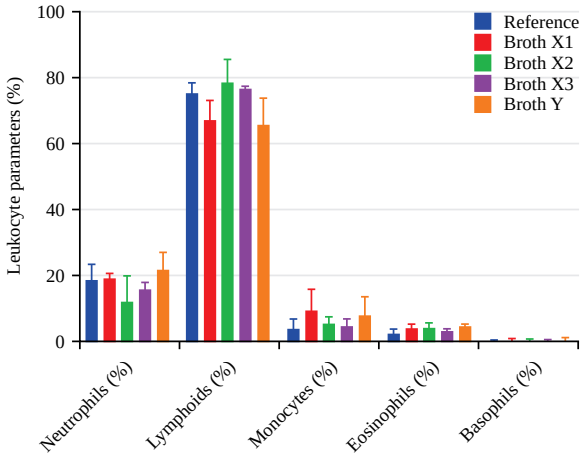

Fig. 3: Leukocyte parameters after 28 days of treatment
Values are expressed as Means±Errors, n = 3. Differences between batches are not significant (p>0.05)

Effect on basophil and eosinophil percentages: Basophils and eosinophils were the cells with the lowest percentages during the different assays. Eosinophil percentages were 0.53±0.18, 0.5±0.1, 0.73±0.3, 0.33±0.1 and 0.53±0.17% after 14 days of treatment and 0.23±0.03, 0.4±0.1, 0.43±0.1, 0,33±0.03 and 0.57±0.02% after 28 days of treatment respectively to control batches, treated with experimental broth 0.25, 3 and 6 g/kg to control broth 3 g/kg body weight. Eosinophil percentages were 2.13±1, 4.03±0.6, 3.97±0.9, 3±0.4 and 4.53±0.37% (Fig. 2-3).

DISCUSSION

This study assessed physical, biochemical and hematological parameters during daily gavage administration of experimental and control broth to rats. The results showed that there was no decrease in rat body weight over the course of the experiment in treated and control animals. Nevertheless, the increase in weight of rats treated with experimental broth was small compared with control rats, with a significant difference in rats treated with experimental broth at dose 3 (6 g/kg). This small variation in the weight of rats treated with experimental broth shows that experimental broth induces neither increased body growth nor decreased body mass. This normal body growth in animals that consumed the experimental broth would be due to the presence of nutritional molecules in the biological formulation such as vegetable proteins and fibers that promote intestinal transit (good digestion)[11]. In fact, changes in body mass are a reliable indicator of the physiological state of animals in general and rats in particular. It reflects the proper functioning or dysfunction of the organism.

Various organs such as the heart, liver and kidneys can be the target of xenobiotic substances. Alterations in the relative mass of these organs reflect the toxicity of a substance[12]. The experimental broth did not affect the relative mass of rat organs. The absence of toxicity of our broth, composed mainly of néré, justifies the use of néré flour in various African diets in the form of soumara[13].

The experimental broth induced no significant variation in ASAT and ALAT transaminase activities in treated animals compared with controls. This lack of variation shows that experimental broth did not affect the integrity of liver tissue. Indeed, any disturbance in these parameters would be due to tissue necrosis or destruction of the liver parenchyma[14]. On the other hand, the experimental broth did not cause any disturbance in GGT levels, an enzyme found particularly in the liver, bile ducts, kidney and bone[15]. However, the elevated GGT levels observed in rats fed the control broth indicate that this product may have disrupted GGT secretion or release. Indeed, GGTs are enzymes present in the liver and their increase in the circulation generally indicates hepatic insufficiency, cholestasis, biliary cirrhosis or disorganization of hepatic architecture[16].

In addition, significant differences were observed in enzymes characterizing biomarkers of heart tissue integrity such as CPK and LDH in rats treated with a high dose of experimental broth (6 g/kg body weight) after 28 days. On the other hand, in animals treated with control broth, the sharp increase in these enzymes from day 14th and at a lower dose (3 g/kg body weight) shows that control broth has considerable effects on heart architecture compared with experimental broth. The sharp increase in the concentrations of the enzymes CPK and LDH shows that experimental broth has a significant effect on the activities of these enzymes[17]. Soumara combined with spices could thus be a powerful antioxidant with anti-hypertensive properties and improve cardiac activity[13]. Regarding the influence of experimental and control broth on the kidneys, no changes were observed in kidney metabolites (creatinine and urea) or electrolytes (sodium, potassium and chloride). Indeed, any substance capable of modifying these various renal functions (glomerular filtration, tubular reabsorption and secretion) inevitably leads to changes in plasma concentrations of urea, creatinine, sodium, potassium and chloride[16,17]. In fact, soumara provides numerous high-quality nutrients (proteins, lipids, carbohydrates, iodine and various vitamins). It is a very important source of quality food and nutrition, with beneficial effects on people's health[12].

In this study, the effects of experimental and control broth on blood cells were assessed by means of hematological analyses. The analyses showed no significant differences in erythrocyte parameters (red blood cells, white blood cells, platelets and hemoglobins)[18]. Nevertheless, the increase in platelet and hemoglobin levels shows that the experimental broth is a good immunostimulant for vascular endothelial protection, since platelets are the protective cells of the vascular endothelium against free radical damage. The broths did not affect the rats' immune systems. In fact, reduced lymphocyte levels are an indicator of a weak immune system and predispose the body to opportunistic diseases. This reduction is thought to be due to the action of phytosterols and flavonoids[19]. Soumara also provides all the amino acids essential to the body, as well as iron (around 15.5 mg/100 g) and vitamin C to limit the risk of scurvy. This richness in iron indicates that cowpea flour is a powerful immunomodulator in cases of anemia[14]. It boosts immune defenses, particularly in cancer prevention. The therapeutic effects of the experimental broth are largely due to the nutritional and therapeutic value of soumara contained in the broth. Néré flour has anti-malarial, laxative and anti-hypertensive properties[20].

CONCLUSION

All in all, these results give grounds for optimism regarding the possible use of this culinary formulation in food. The absence of any difference in physiological anomalies throughout the experiment with the first two doses (0.25 and 3 g/kg body weight) explains the absence of harmful effects. The results showed that the new broth contains very good phytochemical and mineral values and satisfactory antioxidant properties. Confirmation of the biochemical and hematological parameters suggests that this food broth, whose composition is based on natural ingredients, could be recommended for consumption and, thanks to its properties, could play an important role in preventing and combating certain metabolic diseases.

SIGNIFICANCE STATEMENT

The aim of this study was to assess the bio-tolerance of a néré-based dietary supplement in broth form in rats. The toxicological analysis of our study broth containing a high percentage of néré fruit formulated at the IPCI was carried out in comparison to a control broth which is a culinary broth available on the Ivorian market. Thus, rats with an average weight received the different broths orally for twenty eight days. The blood of these rats was collected for toxicological biomarkers analysis. The experimental broth showed good bio-tolerance without toxicity in treated rats and good immunoregulatory activity. These results justify the numerous uses of néré fruits in African dishes.

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