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(RESEARCH ARTICLE)

EFFECT OF CASHEW APPLE PREPARATION ON CIRCULATING PROLACTIN CONCENTRATIONS IN ADULT FEMALE WISTAR RATS

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ABSTRACT

Background: Exclusive breastfeeding remains insufficiently practiced in Côte d'Ivoire, and perceived insufficient milk production is one reason for early cessation. Cashew apple (*Anacardium occidentale L.*) contains potentially bioactive compounds.

Objectives: To characterize the physicochemical and phytochemical properties of cashew apple and assess the effect of an aqueous cashew apple preparation on circulating prolactin concentrations in adult female Wistar rats.

Methods: The physicochemical composition and phytochemical profile of cashew apple were assessed. Circulating prolactin was measured in adult female Wistar rats administered aqueous cashew apple powder preparations at different concentrations, with distilled water as the negative control and Galactogil as the reference product.

Results: Carbohydrates, ash, dietary fiber, sterols and polyterpenes, flavonoids, polyphenols, saponins, and tannins were identified. No significant difference in prolactin concentrations was observed between the 100 mg/mL group and the distilled-water control. The 200 and 300 mg/mL preparations were associated with significant increases in circulating prolactin compared with the negative control. The response remained statistically lower than that of Galactogil, which had the highest mean prolactin concentration (14.82 ± 0.33 ng/mL).

Conclusion: The findings indicate an endocrine response under the experimental conditions, but do not establish a galactagogue effect. The animals were non-lactating, and milk production and mammary gland function were not assessed.

Keywords: Cashew apple, Wistar rats, Prolactin, Exclusive breastfeeding.

1 INTRODUCTION

Infant and young child nutrition is critical to their survival, development, and growth. The World Health Organization (WHO) therefore recommends exclusive breastfeeding for the first six months of life [1]. Physiologically, the production and composition of breast milk are closely regulated by the mother's neuroendocrine state and the infant's demand [2,3]. In Côte d'Ivoire, however, exclusive breastfeeding remains underutilized, with estimates rising only from 12% in 2012 to 23.5% in 2016 [4]. Insufficient breast milk whether actual or perceived by the mother is one of the main reasons for early weaning [5,3]. To address this challenge, galactagogues, substances intended to stimulate milk production are frequently used. Among these remedies, medicinal plants derived from traditional pharmacopoeias play a prominent role, although their mechanisms of action and clinical efficacy require rigorous validation [6,7,8]. It should also be noted that while prolactin is a key hormone in lactation, an increase in its plasma concentration alone does not necessarily indicate an actual increase in milk production. The cashew apple (*Anacardium occidentale L.*), a major but often underutilized byproduct of cashew cultivation, contains significant amounts of carbohydrates, fiber, and phenolic compounds [9,10]. While this biochemical profile justifies further study, any potential galactagogue properties cannot be directly inferred from it. The objectives of this study were therefore to characterize the physicochemical and phytochemical properties of the cashew apple and to evaluate the impact of oral administration of its aqueous preparation on circulating prolactin levels in adult Wistar rats. This study focuses on two main areas: identifying its main chemical constituents and determining whether its administration via gavage modulates serum prolactin concentrations in adult animals.

2 MATERIAL AND METHODS

2.1 Animal Material

The experiment involved 30 sexually mature albino Wistar rats (*Rattus norvegicus*) (20 weeks old, 160 ± 5 g) (Fig. 1), divided into groups of 6 and housed in metabolic cages at the École Normale Supérieure vivarium. The animals had free access to water and a standardized pellet diet. The use of non-lactating females was intended to specifically evaluate the endocrine response, independent of mammary function. The estrous cycle was not synchronized, constituting a potential source of biological variability. Housing conditions strictly complied with the standards of European Directive 2010/63/EU on the protection of animals used for scientific purposes.



Figure 1: ***Rattus norvegicus***

2.2 Plant Material and processing

The fruits of Anacardium occidentale L. (Fig. 2) were harvested on April 4, 2023, in Lôgnion (Korhogo, Poro Region) and transported to the laboratory in airtight containers. After careful selection of healthy fruits, the apples were disinfected by immersion in a diluted bleach solution (25 to 30 mL of 2% bleach in 25 L of water), rinsed under running water, cut into 4 to 6 sections, dried in an oven at 105 °C for 2 hours, and then finely ground (Fig. 3).



Figure 2: *Anacardium occidentale* L



Figure 3: Cashew apple powder

2.3 Determination of Physico-Chemical Parameters and Phytochemical Compounds of the Cashew Apple

2.3.1 Determination of Physico-Chemical Parameter Content

2.3.1.1 Moisture Content (MC)

Determined according to the official AOAC method [11] by drying in an oven (MEMMERT 854, SCHWABACH, Germany) at 105 °C until the mass stabilizes. The calculation is defined by THM (%) = [(Fresh weight − Dry weight) / Fresh weight] × 100

2.3.1.2 Protein Content

Determined using the Kjeldahl method [11]. A 1-g sample was digested at 420 °C for 1 hour (Buchi Digest System K-437, Switzerland) with 20 mL of H_2SO_4 (96%), potassium sulfate, and a catalyst. After dilution to 100 mL, the ammonia was distilled (Buchi Distillation Unit K-355, Switzerland) for 10 minutes, collected in 5% boric acid, and titrated with hydrochloric acid: Protein (%) = [(Ve − Vt) × N × F] / M

Where: (M = sample mass; N = normality of hydrochloric acid; Ve = volume of HCl used for the sample; Vt = volume of HCl used for the blank; F = conversion factor (6.25).

2.3.1.3 Lipid Content

Quantified by Soxhlet extraction [11]. A 5-g sample was extracted for 5 hours at 175 °C using pure hexane. The solvent was removed under reduced pressure (Buchi ROTAVAPOR R-3, Switzerland), and the extract was dried at 50 °C before weighing:

Lipids (%) = [(Mass of flask + oil) − Mass of empty flask] / Mass of sample × 100

2.3.1.4 Ash Content

The total ash content was determined by weighing the mineral residue obtained after complete calcination of the plant material at 550 °C, following the AOAC protocol [11]. A 5-g sample was placed in a pre-tared porcelain crucible and then incinerated in a muffle furnace (Nabertherm B 180, Germany) for 5 hours. After cooling in a desiccator, the mass was measured: Ash content (%) = [(Mass of crucible + ash − mass of crucible) / mass of sample] × 100.

2.3.1.5 Dietary Fiber Content

The crude fiber fraction (cellulose, hemicelluloses, and lignin) was quantified using the Van Soest method [12]. Two grams of cashew apple powder (m) were subjected to successive boiling, first in 50 mL of sulfuric acid (1.25 N), then in 50 mL of sodium hydroxide (1.25 N), for 30 minutes at each stage. The extraction residue was dried at 150 °C for 8 hours (m_1), then ashed at 550 °C for 3 hours (m_2). The crude fiber content was expressed per 100 g of dry matter (DM) using the following formula:

Fiber content (% MS) = $(m_1 - m_2) / (m \times MS)$.

2.4 Digestible Carbohydrates (DC) and Energy Value

The digestible carbohydrate (DC) content was calculated by difference using the FAO method [13]: GD = 100 − [protein (% DM) + fat (% DM) + fiber (% DM) + ash (% DM)].

The total energy value (E) was estimated using the Atwater factors recommended by the FAO [13]: E (kcal/100 g MS) = (4 × P) + (9 × L) + (4 × G).

2.5 Phytochemical Study

2.5.1 Identification of Some Secondary Metabolites of the Aqueous Cashew Apple Extract

Qualitative identification of the major families of secondary metabolites was performed using a stock solution (prepared by dissolving 5 g of powder in 50 mL of distilled water). The coloration and precipitation tests were performed according to the chemical principles described by Wagner and Bladt [14] and based on the methodological protocols of Lazureskii et al. [15], Evans [16], Edeoga et al. [17], and Mea et al. [18]:

2.5.2 Sterols and Polyterpenes (Liebermann reaction)

A 5 mL volume of the solution was evaporated to dryness on a sand bath. The residue was immediately dissolved in 1 mL of acetic anhydride while still hot. This solution was then gently added to 0.5 mL of concentrated sulfuric acid along the wall of the tube. The development of a purple or violet ring that changes to blue and then to green confirms the test.

2.5.3 Polyphenols

One drop of an alcoholic solution of ferric chloride (2% $FeCl_3$) was added to 2 mL of the extract. The appearance of a more or less intense bluish-black or green color indicates their presence.

2.5.4 Flavonoids (Cyanidin reaction)

A 2 mL volume of the solution was evaporated to dryness, and the residue was dissolved in 5 mL of hydrochloric acid. After transferring the mixture to a tube containing 2 to 3 magnesium shavings, the appearance of a pink-orange or purpurin color was recorded as a positive result.

2.5.5 Tannins

Catechinic (non-hydrolyzable) tannins were characterized by treating the residue from 5 mL of extract with Stiasny's reagent in a water bath at 80 °C for 30 minutes; the formation of a coarse flocculent precipitate confirms their presence. Gallic tannins (hydrolyzable) were identified in 5 mL of filtrate saturated with sodium acetate by adding three drops of 2% $FeCl_3$, which produced an intense blue-black color.

2.5.6 Quinone compounds (Borntraeger reaction)

The evaporation residue from 2 mL of extract was treated with 1/5 diluted hydrochloric acid. After heating in a boiling water bath for 30 minutes and cooling, the organic phase was extracted with 20 mL of chloroform. The addition of 0.5 mL of diluted ammonia to this phase reveals a red to violet color if the test is positive. Alkaloids: The residue from 6 mL of evaporated extract was dissolved in 6 mL of 60° alcohol. The solution was divided between two test tubes to undergo the Dragendorff (formation of a reddish-brown precipitate) and Bouchardat tests.

2.5.7 Saponins

A 15 mL volume of the solution was added to a test tube and shaken vigorously for 10 seconds. The formation of a persistent column of foam more than 1 cm high after a 10 minute rest period indicates the presence of saponins.

2.6 Quantification of Polyphenols and Flavonoids

2.6.1 Total polyphenols

The total phenolic content was determined using the Folin-Ciocalteu method, as described by Singleton et *al*. [19]. A volume of 1 mL of Folin-Ciocalteu reagent was added to 1 mL of aqueous extract. After a 3-minute reaction, 1 mL of sodium carbonate (20%) was added, and the final volume was adjusted to 10 mL with distilled water. After a 30-minute incubation in the dark, the absorbance was measured at 745 nm against a blank. Concentrations were extrapolated from a calibration curve established using gallic acid.

2.6.2 Flavonoids

Flavonoid quantification was based on the method of Meda et *al*. [20]. A 0.5 mL volume of extract was successively mixed with 0.5 mL of distilled water, 0.5 mL of aluminum chloride, 0.5 mL of potassium acetate, and 2 mL of distilled water. After homogenization and incubation in the dark for 30 minutes, the optical density was measured at 415 nm against a blank. The flavonoid concentration was calculated from a calibration curve prepared using quercetin.

2.7 Assessment of Circulating Prolactin Levels

2.7.1 Administration Protocol (Gavage)

The animals were divided into homogeneous groups in accordance with the nutritional guidelines of Reeves et al. [21]. Housed in metabolic cages, the rats were fed a diet consisting of bread, corn, soybeans, and dried fish, supplemented with free access to water. The experimental force-feeding phase lasted 4 days, with two daily administrations scheduled for 8:00 a.m. and 4:00 p.m. The 30 animals were divided into 5 distinct groups (n = 6 female rats per group):

- Negative control: Administration of 5 mL of distilled water per animal.
- Experimental groups: Administration of the aqueous cashew apple preparation at concentrations of 100, 200, and 300 mg/mL (equivalent to 0.5, 1, and 1.5 g of powder dissolved in a constant volume of 5 mL).
- Positive control: Administration of the reference galactagogue, Galactogil.
- Daily monitoring revealed no deaths or abnormal clinical or behavioral signs, indicating good tolerance of the force-feeding protocol. This 4 day protocol was chosen for the purpose of conducting an exploratory analysis of the early endocrine response, without aiming to evaluate a sustained lactogenic effect.

2.7.2 Blood Sampling and Hormone Assay

At the end of the 4-day treatment period, blood samples were collected from fasting animals under general ether anesthesia. Serum was isolated by centrifugation to measure prolactin levels. Serum concentrations were measured using the VIDAS Prolactin (PRL) automated system (bioMérieux SA, Marcy-l'Étoile, France; catalog number 30410). This method is based on a sandwich-type fluorescence immunoassay and requires a serum volume of 200 µL per test. The analytical measurement range of this instrument extends from 0.5 to 200 ng/mL, with a technical detection limit set at 0.5 ng/mL. The intra-assay coefficients of variation (CV) range from 3.2% to 4.1%, while the inter-assay CVs range from 4.7% to 6.6%, in accordance with the manufacturer's specifications.

2.8 Statistical Analyses

The obtained values were subjected to an analysis of variance (ANOVA) followed by the Newman-Keuls post-hoc test for multiple comparisons at a 5% significance level. Statistical analyses and graphical representations were performed using GraphPad Prism 8.4 software. The results are presented as means with their standard errors (Mean ± SEM). The significance levels used were $p < 0.05$ (significant), $p < 0.01$ (very significant), and $p < 0.001$ (highly significant). p-values greater than 0.05 were considered nonsignificant.

3 RESULTS

3.1 Physicochemical Parameters

The physicochemical characteristics of the cashew apple are presented in Table I. The analyses indicate that the fresh fruit has a high moisture content, amounting to 82.7 ± 0.11 g/100 g, along with a distinctly acidic nature (pH = 4). Expressed on a dry matter (DM) basis, the results reveal a predominance of digestible carbohydrates, which amount to 84.93 ± 5.03 g/100 g DM, followed by dietary fiber (9.33 ± 4.66 g/100 g DM). The protein (2.61 ± 1.3 g/100 g DM), ash (1.73 ± 0.86 g/100 g DM), and fat (1.40 ± 0.72 g/100 g DM) contents are minor. The calculated energy value is 362.76 ± 22.2 kcal/100 g DM.

Table 1: Physicochemical parameters of *Anacardium occidentale* (Cashew apple)

Parameters	Mean content
pH	4
Moisture (g/100g)	82.7 ± 0.11
Ash (g/100g DM)	1.73 ± 0.86
Carbohydrates (g/100g DM)	84.93 ± 5.03
Proteins (g/100g DM)	2.61 ± 1.3
Lipids (g/100g DM)	1.40 ± 0.72
Dietary Fibers (g/100g DM)	9.33 ± 4.66
Energy Value (kcal/100g DM)	362.76 ± 22.2

The values in the table are averages of three trials, assigned with their standard deviations.

3.2 Phytochemical Parameters

Qualitative phytochemical screening (Table 2) revealed the presence of sterols, polyterpenes, phenolic compounds, flavonoids, saponosides, and tannins (catechin and gallic acid fractions). Conversely, no quinone compounds or alkaloids were detected. Quantitative analyses (Table 3) confirm this high content of phenolic antioxidants, revealing levels of 112 ± 3.52 mg QE/g DM for flavonoids and 191 ± 3.46 mg GAE/g DM for total polyphenols.

Table 2: Phytochemical screening results of the aqueous solution of the *Anacardium occidentale* L. apple.

Target Compounds	Test or Reagents	Results
Sterols and polyterpenes	Liebermann	+
Polyphenols	Ferric chloride	+
Flavonoids	Cyanidin	+
Saponosides	Vigorous shaking	+
Quinone compounds	Borntraeger	-
Alkaloids	Dragendorff / Bouchardat	- / -
Tannins (Catechutic / Gallic)	Stiasny / Hydrochloric acid	+ / +

(+) : Presence of the compound (–) : Absence of the compound

Table 3: Phytochemical parameters of *Anacardium occidentale* (Cashew Apple)

Parameters	Mean Values
Flavonoids (mg QE/ g DM)	112 ± 3.52
Total Polyphenols (mg GAE/ g DM)	191 ± 3.46

The values in the table are averages, assigned with their standard deviations from three trials.

3.3 Determination of serum prolactin Concentration

Analysis of serum prolactin levels revealed contrasting patterns depending on the doses administered (Table 4). Administration of the minimum dose of 100 mg/mL resulted in a concentration of 5.00 ± 0.03 ng/mL, a value that remains statistically comparable to that recorded in the negative control group receiving distilled water (5.21 ± 0.07 ng/mL). Conversely, the use of concentrations of 200 mg/mL and 300 mg/mL induced a significant, progressive, and dose-dependent increase in circulating prolactin levels, reaching 9.23 ± 0.30 ng/mL and 13.59 ± 0.08 ng/mL, respectively ($p < 0.05$). Post-hoc analysis shows, however, that these endocrine responses remain statistically lower than that induced by the reference product, Galactogil, which had the highest mean concentration in the study (14.82 ± 0.33 ng/mL).

Table 4: Numerical serum prolactin concentrations

Treatment group	Treatment concentration	serum prolactin (ng/mL), mean ± SEM
Distilled water	Control	5.21 ± 0.07 D
Cashew apple preparation	100 mg/mL	5.00 ± 0.03 D
Cashew apple preparation	200 mg/mL	9.23 ± 0.30 C
Cashew apple preparation	300 mg/mL	13.59 ± 0.08 B
Galactogil	Positive control	14.82 ± 0.33 A

Values are expressed as mean ± SEM (n = 6). Different letters indicate statistically significant differences according to the Newman–Keuls post-hoc test ($p < 0.05$); groups sharing the same letter are not significantly different.

4 DISCUSSION

The moisture content identified in this study (82.7 ± 0.11%) is in perfect agreement with the findings of Dakuyo et al. [9], whose reported values range from 83.64% to 86.27%. This high moisture content confirms the intrinsic fragility of fresh cashew apples, highlighting their susceptibility to rapid postharvest deterioration and spontaneous fermentation [10]. The measured protein (2.61 ± 1.3 g/100 g DM) and total carbohydrate (84.93 ± 5.03 g/100 g DM) contents indicate that these are the major macromolecules in the fruit, which validates the general profiles described in the literature [9,10]. The minor quantitative variations observed compared to previous studies are typically explained by the influence of the cultivar, geographic origin, stage of fruit ripeness, or the drying conditions applied. The estimated energy value (362.76 kcal/100 g DM) stems directly from this predominance of carbohydrates. The dietary fiber content (9.33 ± 4.66 g/100 g DM) reflects a substantial fiber intake, although this parameter remains subject to variation due to genotypic factors [22,10]. At the same time, the ash content (1.73 ± 0.86 g/100 g DM) confirms the presence of a significant mineral fraction, consistent with the nutritional assessment data for this byproduct [9,10]. From a phytochemical perspective, the concentrations of polyphenols (191 mg GAE/g DM) and flavonoids (112 ± 3.52 mg QE/g DM) are consistent with the literature regarding the high phenolic content of this plant material, which is known to contain various flavonol derivatives related to myricetin and quercetin [23,22]. The screening confirmed the simultaneous presence of sterols, polyterpenes, flavonoids, saponosides, and tannins. The detection of these secondary metabolites attests to the fruit's chemical diversity and supports the hypothesis of lactotrophic activity. Indeed, several studies have documented the presence of these chemical families in plant taxa with recognized lactogenic properties, such as *Euphorbia balsamifera* [24], *Arachis hypogaea* [25], and *Vigna unguiculata* [26]. According to Salifou et al. [8], this activity can be explained by a synergistic interaction between nutrients and secondary metabolites, which are capable of stimulating prolactin secretion by the lactotrophic cells of the anterior pituitary. Saponins, in particular, have been highlighted in various formulations intended to promote lactation [27], while flavonoids and tannins have been described as potential contributors to the modulation of lactation-related functions [28,29]. However, the specific mechanistic contributions of each of these compounds within the extract under study remain to be elucidated. From an endocrine perspective, while the 100 mg/mL concentration proved insufficient to alter prolactin levels, the 200 and 300 mg/mL doses induced a rise followed by a highly significant, dose-dependent difference in serum prolactin concentrations compared with the negative control group. Although this endocrine response reflects clear stimulation of the lactotrophic axis, it remains statistically lower than that generated by the reference product, Galactogil (14.82 ± 0.33 ng/mL). These preliminary results highlight a clear neuroendocrine effect of the preparation but do not allow for a conclusion of strict bioequivalence with the positive control or for the characterization of a complete functional galactagogue effect. Indeed, direct evaluation of the histological structure of the mammary glands and measurement of milk yield were not performed in this model. Our observations are nevertheless consistent with the conclusions of Adepo et al. [30] regarding the ability of certain plant extracts to significantly increase circulating prolactin levels.

List of abbreviations

- **ANOVA** : Analysis of Variance (Analyse de variance, test statistique).
- **AOAC** : Association of Official Analytical Chemists (Association officielle des chimistes analytiques).
- **CV** : Coefficient of Variation (Coefficient de variation, précision du dosage).
- **DC** : Digestible Carbohydrates (Glucides digestibles).
- **DM** : Dry Matter (Matière sèche).
- **FAO** : Food and Agriculture Organization (Organisation des Nations unies pour l'alimentation et l'agriculture).
- **mg GAE/g DM** : milligrams of Gallic Acid Equivalents per gram of Dry Matter (milligrammes d'équivalent acide gallique par gramme de matière sèche).

- **mg QE/g DM** : milligrams of Quercetin Equivalents per gram of Dry Matter (milligrammes d'équivalent quercétine par gramme de matière sèche).
- **n** : sample size (taille de l'échantillon / nombre de rats par lot).
- **p** : probability value / p-value (valeur de probabilité statistique pour la significativité).
- **PRL** : Prolactin (Prolactine).
- **SEM** : Standard Error of the Mean (Erreur standard sur la moyenne).
- **WHO** : World Health Organization (Organisation mondiale de la santé - OMS).

5 CONCLUSION

Oral administration of a cashew apple (*Anacardium occidentale L.*) preparation is associated with a significant, dose-dependent increase in serum prolactin levels in adult Wistar female rats at concentrations of 200 and 300 mg/mL. The 100 mg/mL dose remains inactive, and the magnitude of the overall response is lower than that of the reference product (Galactogil). Given the use of a non-lactating animal model, an exposure duration limited to 4 days, and the absence of a direct assessment of milk production or mammary physiology, these results do not allow for a formal establishment of a galactagogue effect. Further investigations using lactating physiological models are necessary to quantify milk yield, evaluate the persistence of the hormonal response, ensure the biological safety of the product, and identify the active ingredients responsible for this endocrine activity.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest and they are alone responsible for the accuracy and integrity of the paper content.

Ethics Approval Statement

All experimental procedures involving animals were conducted in accordance with the provisions of European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Key points

- Cashew apple preparation contained several phytochemical classes.
- The 200 and 300 mg/mL preparations increased circulating prolactin relative to the negative control.
- Prolactin response was lower than that observed with Galactogil.
- Milk production was not measured; a galactagogue effect cannot be concluded.

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