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# Effect of Aqueous Extract of *Euphorbia hirta* (Euphorbiaceae) Leaves on Wistar Lactating Rat's Milk Density, Water and Fat Contents

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**Abstract** **Background and Objectives**: Breast milk is highly recommended due to its nutritional composition, which adapts to the needs of the child. Unfortunately, many mothers do not have access to synthetic galactagogues due to the high cost of medications. To avoid a decrease or interruption of breast milk production, they mostly resort to medicinal plants such as *Euphorbia hirta* (Euphorbiaceae). The objective of this work is to evaluate the effect of oral administration of the aqueous extract of the leaves of *E. hirta* (EHae) on the water and fat composition of breast milk. **Methods and Study Design**: 36 lactating rats divided into seven (7) experimental groups are treated regularly from the third to the seventeenth day of postpartum. Group I received only distilled water. Group II was treated only with Metoclopramide, Prokynil®. The experimental groups (III; IV; V et VI) were treated with EHea at doses of 100; 200; 400 and 600 mg/kg respectively. Each rat received orally 1 ml of the substance administered using a gavage tube. **Results:** At doses of 100; 200; 400 and 600 mg/kg, EHae induced a very significant increase in the water content of milk without, however, significantly modifying its composition in terms of fat and other compounds. **Conclusions:** The use of EHae at doses lower than 400 mg/kg could ensure that breastfeeding mothers have better breast milk production.

***Keywords:*** *Euphorbia hirta, leaves, breastfeeding, milk composition, lactating rats*

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## 1. Introduction

Breast milk is highly recommended because of its nutritional composition which adapts to the needs of the child [1]. In fact, breast milk undergoes significant changes in its composition in order to strengthen the infant's immune system and allow harmonious growth in height and weight. It is mainly composed of minerals, proteins, lactose, lipids and water which help quench the infant's thirst, raise their blood sugar levels and ensure satiety [2]. The better digestibility of milk fats is due to the presence of a bile acid-dependent lipase which compensates at the duodenal level for the lack of pancreatic lipase [3]. According to WHO [1], breastfeeding a child with breast milk ensures a good start to life while guaranteeing every chance of survival and development of intellectual potential.

Unfortunately, many mothers still fail to meet their need to exclusively breastfeed their infant for the first six months of life [4]. The main reason for this inability is hypogalactia and/or agalactia which occur early [5]. To treat hypogalactia and/or agalactia, there are synthetic galactogogues available in pharmacies which have undesirable side effects and, above all, high costs. So to avoid a drop or interruption in breast milk production, African mothers, especially those of the Brong and Koulango people (Tanda, Ivory Coast), have around fifteen medicinal plants at their disposal [6]. Among these plants, Euphorbia hirta (Euphorbiaceae) stood out as being the most used by these mothers from the commune of Tanda (Côte d'Ivoire). The evaluation of the galactogenic activity of the aqueous extract of *E. hirta* (EHae) confirmed the galactogenic activity of this plant [7]. According to the authors, EHae stimulates prolactin secretion and the development of the lobuloalveolar system of the mammary glands in rats. The objective of this work is to evaluate the effect of oral administration of the aqueous extract of E. hirta leaves (EHae) on the water and fat composition of breast milk.

# 2. Material and Methods

## 2.1. Preparation of *Euphorbia hirta* Aqueous Extract

Fresh leaves of *Euphorbia hirta* (Euphorbiaceae) were harvested in February 2016 in Béléouélé (village located at five kilometers from Tanda, Region of Gontougo, Côte d'Ivoire). The fresh leaves have been identified and authenticated by a Botany expert, Dr Dr Emma AKE-ASSI of the "Centre National de Floristique", UFR-Biosciences, Université Félix HOUPHOUËT-BOIGNY (Abidjan, Côte d'Ivoire). The plant extract was obtained according to the method used by Néné-Bi et al [8]. The fresh leaves were washed and dried at room temperature 28 ± 2°C. Dried leaves were pulverized to powder with the use of a laboratory blender. 100 g of powder were macerated during 24 h in distilled water (2 L), thereafter filtered. Rotary evaporator (BUCHI, France) at temperature of 50 ± 5°C was used to concentrate the filtrate and *Euphorbia hirta* aqueous extract (EHae) obtained was re-dissolved extemporaneously in normal saline (NaCl 0.9%) [9].

## 2.2. Experimental Animals

42 lactating rats (*Rattus norvegicus*) were used in this study. All mature female rats used were 3 months old, weighed 180-220 g and were purchased from the *Vivarium* of Ecole Nationale Supérieure (ENS, Abidjan, Côte d'Ivoire). They were kept in temperature- controlled environment (25 ± 2 °C) with a 12 h light-dark cycle and were allowed free access to water and feed *ad libitum* throughout the acclimation period. Experimental procedures and protocols used in this study were approved by Ethical Committee of Health Sciences of Université Felix HOUPHOUET-BOIGNY. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.

## 2.3. Chemicals Used

Metoclopramide, Prokynil® was purchased from Laboratoires TECHNI-PHARMA, (France). Oxytocin (Sandoz Products Ltda, Syntocinon®) was from France. EHae and Metocl) were dissolved and/or diluted in distilled water on each day of our experiments [10,11,12].

### 2.3.1. Experimental Protocols

#### 2.3.1.1. Experimental Groups And Substance Administration

36 lactating rats divided into seven (7) experimental groups are treated regularly from the third to the seventeenth day of *postpartum* [13]. Group I received only distilled water. Group II was treated only with Metoclopramide (Metocl). The experimental groups (III; IV; V and VI) were treated with EHea respectively at doses of EHae (100; 200; 400 and 600 mg/kg). Each rat received orally, 1 ml of the substance administered using a gavage tube.

#### 2.3.1.2. Equipment for Extracting Milk from Lactating Rats

A special comfort electric breast pump (PHILIPS AVENT, England), intended for human use, is modified for the extraction of milk from rats (Figure 1). To do this, the 125 ml bottle with a teat, was replaced by a empty small box (Doliprane® box, France) with a lid. The empty paracetamol box was connected to the pump (motor) of the breast pump through a tube connecting the pump to the box. The hole was properly sealed with glue to prevent air exchange between the inside of the box and the outside environment. The box also had a second hole drilled through the lid on top. This second hole allowed the empty paracetamol box to be connected to a syringe tip (2.5 mL) whose orifice corresponds perfectly to the size of the rat's mammary gland. Before each extraction session, the paracetamol box was opened and a 1.5 mL Eppendorff tube was inserted inside the box. The tube that enters the paracetamol box through the lid reached inside the Eppendorff tube in order to collect the milk directly into the Eppendorff tube. The breast pump was connected to a power outlet via a charger. The pump had a power button and three other buttons to adjust the suction strength of the electric breast pump.

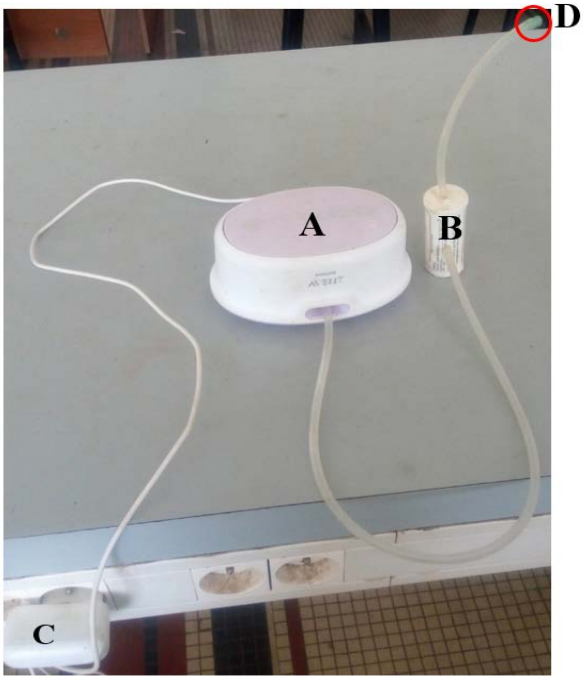



**Figure 1. Electric breast pump (Avent SCF332/01) modified for the extraction of milk from rats**. (A): pump (motor), (B): box containing the eppendorf tube receiving the milk, (C): Electric charger connected to the electricity, (D): syringe stopper

#### 2.3.1.3. Collection of Milk Samples

On day 15 of lactation, milk samples were collected from unanesthetized lactating rats [14]. Before each sample, the rats were separated from their pups for 4 hours. Then, they each received a dose of 5 units of oxytocin (Sandoz Products Ltda) intraperitoneally. After 15 minutes of oxytocin effect, the milk was directly expressed using a modified electric breast pump.

#### 2.3.1.4. Determination of the Density of Rat Milk

From the twelfth day of breastfeeding, milk samples were collected using an electric breast pump. The milk collected was aspirated using a 2.5 mL graduated syringe to estimate the volume of milk collected. To estimate the mass of milk collected, a 1.5 mL Eppendorff tube was weighed empty (P0). Then the same Eppendorff tube was reweighed when it contains the milk sample collected (P1). The difference in weight between P1 and P0 made it possible to determine the mass of milk collected. The density of each milk collected was calculated using the formula Below :

**Rat's milk density ($g/cm^3$) = [$P_1$ (g) – $P_0$ (g)] / [milk volume ($cm^3$)]**

#### 2.3.1.5. Determination of Rat's Milk Water Content

From the twelfth day of breastfeeding, milk samples were collected in an Eppendorf tube using an electric breast pump. Then the weight (P1) of the Eppendorff tube containing the fresh milk sample was measured. To obtain the weight of the dried milk sample, the Eppendorff tubes containing the fresh milk were placed in an oven at 63°C for 48 hours until a constant dry weight is obtained [15]. After drying, the weight (P2) of the Eppendorff tube containing the dried milk sample is measured. The water content of the milk is calculated according to the following formula :

**Rat's milk water content (%) = ([$P_1$ (g) – $P_2$ (g)] / [$P_1$ (g)]) x 100**

#### 2.3.1.6. Détermination of Rat's Milk Fat Content

After oven drying the fresh milk samples for 48 hours, one milliliter of hexane was added to each Eppendorff tube containing dried milk. The Eppendorff tubes were shaken manually and then left to stand at room temperature for 24 hours. Then, the dried milk immersed in hexane was centrifuged according to Yagil et al. [15] at 3000 rpm for 10 min. After centrifugation, the supernatent was collected using a 5 mL syringe. The Eppendorff tubes containing the dried milk were again placed in the oven at 63°C until a constant dry weight was obtained after one hour of drying. The weights (P3) of the tubes were again measured.

**Rat's milk fat content (%) = ([$P_2$ (g) – $P_3$ (g)] / [$P_2$ (g)]) x 100**

#### 2.3.2. Data Analysis

GraphPad Prism 7 software (Microsoft, San Diego California, USA) was used for statistical analyses and graphical representations. Values were expressed as the mean followed by the standard error of the mean (m ± sem). The significances of the treatments compared to the control group were assessed using the analysis of variances (ANOVA) of the Tukey-Kramer multiple comparison test contained in the GraphPad Prism 7 statistical software (Microsoft, San Diego California, USA). In this test, when :

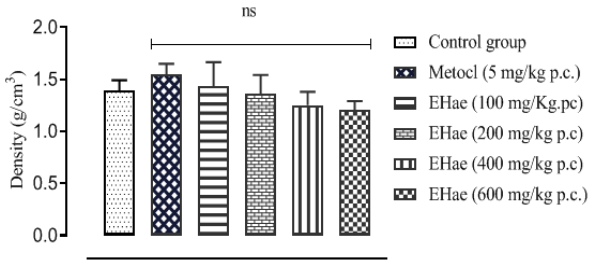
P > 0,05 the observed difference is not significant (ns),

P < 0,0001 the observed difference is highly significant (****).

# 3. Results

## 3.1. Rat's Milk Density

In order to evaluate the influence of different doses of the extract on the density of the milk of the rats in relation to water, the densities of the milk were calculated. The milk densities of rats in the control group and in the metoclopramide-treated group were 1.39 ± 0.07 and 1.55 ± 0.09 g/cm3, respectively. Compared to the experimental batches having received the doses of 100; 200; 400 and 600 mg/kg of the extract, the respective densities are 1.44 ± 0.17; 1.36 ± 0.14; 1.25 ± 0.10 and 1.21 ± 0.06 g/cm3 (Figure 2). Statistical comparison of these values indicates that there is no significant difference between the milk produced by each of the rats treated with metoclopramide, aqueous extract or distilled water (P > 0.05).

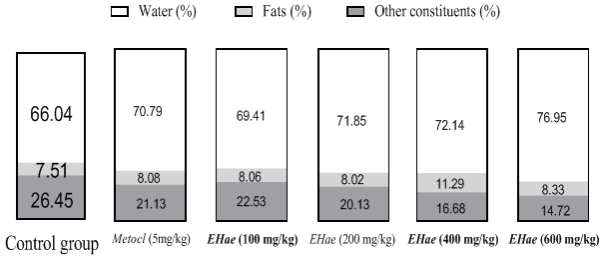



ns: not significant; Control group: group treated only with dislled water; Metocl: Metoclopramide; EHae: Aqueous extract of *Euphorbia hirta* (Euphorbiaceae)

**Figure 2.** Density of the rat's milk

## 3.3. Composition Fats of the Rat'S Milk

In rats treated with distilled water and metoclopramide, the respective milk fat contents were: 7.51 ± 0.02; 8.08 ± 0.2%. With the experimental batches treated at doses of 100; 200; 400 and 600 mg/kg, the respective percentages of milk fat are: 8.06 ± 0.34; 8.02 ± 0.21; 11.29 ± 1.27 and 8.33 ± 0.42%. Statistical analysis indicated that the milk produced by rats in the control group and that produced at doses of 100, 200, 400 and 600 mg/kg were statistically identical (P > 0.05) (Figure 3).




Control group: group treated only with dislled water; Metocl: Metoclopramide; EHae: Aqueous extract of Euphorbia hirta (Euphorbiaceae)

**Figure 3.** Nutrients composition of the rat's milk

# 4. Discussion

On day 15 of lactation, the density of milk samples was

determined. The results showed that all milk samples collected from control and treated rats had approximately the same density (P > 0,05). However, the density of milk from rats treated at doses of 400 and 600 mg/kg was closer to that of water (1 g/L). The density of a liquid corresponds to the mass per unit volume of this liquid. Therefore, EHae did not modify the density of milk produced by rats at the low doses of 100 and 200 mg. On the other hand, at high doses of 400 and 600 mg/kg, EHae appears to reduce the density of breast milk.

The density of milk varies depending on its fat and water composition. Skimming increases its density, while wetting (adding water) decreases it. Thus, milk with a high water content has a low density [16].

The influence of EHae on the dry matter content of rat milk was evaluated. The results showed that metoclopramide and doses of 100; 200; 400 and 600 mg/kg of EHae induced a very significant increase in milk water content compared to the control group (P < 0,001). This increase was even more pronounced at the 600 mg/kg dose than at other doses of EHae. Although the 600 mg/kg dose of EHae increased milk production by 22.63%, however, it did not lead to weight getting on suckling rats [7]. Consequently, milk produced at the dose of 600 mg/kg would be sufficiently diluted not to induce additional weight gain compared to pups in the control group.

According to Liu et al. [17], administration of *Euphorbia heterophylla* (Euphorbiaceae) extract to lactating rats significantly increases milk production and the expression of aquaporins (AQP) on the surface of mammary cells. AQPs are a class of membrane proteins that form pores permeable to water molecules in biological membranes. AQPs allow water to pass through the membrane and prevent ions from entering the cell [18]. The increase in the volume of water in the milk of rats treated with metoclopramide and at doses of 100, 200, 400 and 600 mg/kg could be explained by the capacities of the aqueous extract of E. hirta and metoclopramide to increase the expression of aquaporins on the surface of mammary cells.

According to the results in Figure 3, the milk of the rats in the control group is composed of 66.04% water, 7.5% lipid and 26.45% for the rest of the milk constituents (carbohydrate; protein and minerals). This distribution is close to the results of Keen et al. [14], which states that on the fifteenth day of lactation, the milk of the rats is composed of 73.83% water, 12.18% lipid, 11.10% protein and 2.89% carbohydrate. Compared to the control group, none of the administered substances significantly influenced the lipid composition of milk (P > 0.05). EHae would have no effect on the biosynthesis of lipids in breast milk.

## Conclusion

At doses of 100; 200; 400 and 600 mg/kg, EHae induces a very significant increase in the water content of milk without, however, significantly modifying its composition in terms of fat and other compounds. At low doses of 100 and 200 mg/kg, EHae does not alter the density of milk produced by rats. However, at high doses of 400 and 600 mg/kg, the density of milk becomes closer to that of water. The use of EHae at doses lower than 400 mg/kg may ensure better growth of the breastfed infant. However, the study of the effects on the hematological and biochemical parameters of suckling rats is necessary in order to verify the safety of EHae on the development of suckling rats.

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## Author's Contributions

All authors contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

## Consent

It is not applicable.

## Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. Also, this research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector. It was funded by personal efforts of the authors.

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