

Original Research Article

Effects of *Ficus capensis* leaves aqueous extracts (lactogenic medicinal plants) on NMR mice milk secretion

ABSTRACT

Introduction

Ficus capensis is a plant used in traditional medicine to stimulate lactation in women and animals in Africa. However, the effects of their extracts on the mammary gland are poorly documented. The objective of the study was to evaluate the effects of *Ficus capensis* leaves aqueous extracts on NMR mice milk secretion.

Methodology

This was an experimental animal study using virgin female NMR mice aged eight to ten weeks. The mice were grouped into four groups of eight mice each. Each group received one of the following products: distilled water, Galactogil™, sulphiride, aqueous extracts (AE) of *capensis* leaves. Data were analysed and processed using Microsoft Excel 2016 and Stata MP16 with $P \leq 0.05$ as the significance threshold.

Results

Arborescence of the galactophorous ducts was more developed in the sulphiride lot. Galactogil™, and *F.*

capensis leaves extract treated groups showed almost equivalent arborescence with a higher tendency than the distilled water. With histological haematoxylin-eosin staining, the ratio of galactophorous ducts containing secretions to total ducts was higher in the groups of *Ficus capensis* AE treated group than distilled water ($P = .0001$). Galactogil™, sulphiride and the group of *Ficus capensis* extracts each had higher levels of beta-casein in mammary tissue and average prolactinemia than distilled water ($P < .01$). Mammary tissue stained by immunohistochemistry with anti-prolactin receptor antibodies showed more intensely labelled mammary glands in the sulphiride and *Ficus capensis* extracts groups. There was no statistically significant difference between average prolactinemia among the different groups.

Conclusion

F. capensis leaves AE administered to virgin female NMR mice showed lactogenic and mammogenic effects.

Keywords: mammary gland, histology, Ficus capensis, milk secretion, mouse

INTRODUCTION

For the first six months after birth, the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend that mothers exclusively breastfeed their babies [1]. In Africa, 80% of children are breastfed. However, in Burkina Faso, only 40% to 50% of children are exclusively breastfed, despite the recommended practice of exclusive breastfeeding [2]. Conventional medicine uses sulphiride and Galactogil™ to stimulate lactation. However, sulphiride has side effects for both mothers and breast-fed babies [3]. In Burkina Faso, dairy products and conventional medicines are not readily available to everyone

due to their limited availability and high cost. For this reason, some African people turn to traditional medicine, using plant extracts to boost lactation. Many ethnopharmacological studies have reported that extracts from various plants are used throughout Africa as lactogens [4–11]. Among the plants used to stimulate lactation, *Ficus capensis* is listed [12–14]. *Ficus capensis* Thunb. is a member of the Moraceae family and is known as «womsèèga» in the Mooré language (Burkina Faso). This study was initiated to contribute to the promotion of breastfeeding and, at the same time, to the promotion of traditional medicine. The World Health Organization (WHO) recommends the inclusion of traditional medicine in the health policies of its Member States [15]. The aim of this study was to evaluate the effects of *capensis* extracts on milk secretion in mice. *Ficus*

MATERIAL AND METHODS

Ficus capensis leaves were collected at Sapouy, 102 km south of Ouagadougou (Burkina Faso) and were identified as such in the national herbarium under number 8763. Aqueous was carried out after extraction maceration. The amount of total flavonoids in the extracts was determined by the Dowd method adapted by Arvouet-Grand and al [16]. Total polyphenols were estimated by the method of Singleton and al [17]. The reference products were sulphiride, Galactogil™ and distilled water. The study of the effects of the extracts on the mammary gland was carried out on pubescent and virgin NMRI (Naval Medical Research Institute) mice, aged eight to ten weeks and weighed 27 g to 35 g. The NMRI mice were obtained from Joseph KI-ZERBO University's animal house at Ouagadougou. The university's animal experimentation ethics committee approved the experimental protocol under number CE-UJKZ/2023-11. Each product was administered to a group of eight mice for eight days, following Table 1. Gavage was initiated after applying the Whitene effect to synchronize the mice's estrous cycles [18, 19].

Table 1: dosages of gavaged products

Products	Dosages
Distilled water	0,3 ml at 8AM, 1PM and 6PM
Galactogil™	50mg/ Kg body weight (BW) at 8AM, 1PM and 6PM
Sulpiride	2mg/kg BW at 8AM and 6PM
AE of <i>Ficus capensis</i> leaves	500mg/kg BW at 8AM, 1PM and 6PM

At the end of the treatment, the mice were euthanised under general anaesthesia using ketamine/lidocaine for tissue and organ removal. Mice were euthanised one hour after the last gavage. One abdominal mammary gland was used for the "whole mount" technique [20–22].

The second mammary gland was fixed in 10% formalin for standard histological examination and immunohistochemistry. Immunohistochemistry focused on prolactin receptors in mammary tissue using a Biorbyt antibody anti-PRLR kit orb 389311 lot-RB1054. The two thoracic mammary glands were used for ELISA determination of beta-casein levels in breast tissue using a Biorbyt orb 565466-lot FT6316 Mouse CSN2 ELISA kit. Prolactinemia levels were determined using a mouse prolactin ELISA kit Biorbyt orb 409008-lot T6793. Progesterone level was determined using a mouse progesterone ELISA kit Biorbyt orb 1669464-lot I2524.

Statistical analysis

Data were processed using Microsoft Word, Microsoft Excel and Stata 16 MP. Comparisons of means were made using Student's t-test. The significance level was set at $P \leq 0.05$.

RESULTS

Phytochemical studies

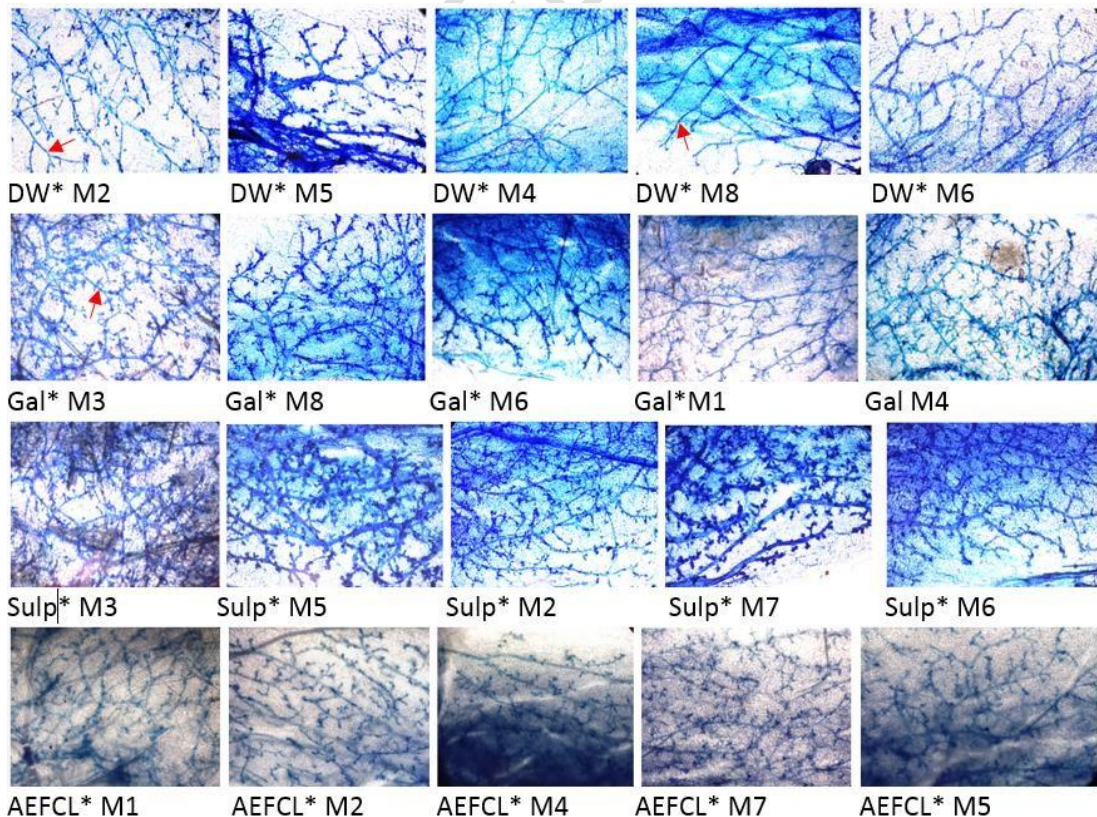
The phytochemical compounds found in the extracts were flavonoids, tanins, polyphenols and quinones. The total flavonoid content was 1.16 ± 0.05 mg quercetinequivalent (QE)/g extract. The total polyphenol content was 0.51 ± 0.05 mg ascorbic acid equivalent/g extract. The antioxidant power of the aqueous extract of *Ficus capensis* leaves was close to that of quercetin. The 50% inhibition concentration of α -diphenyl β -picrylhydrazyl (DPPH) was 6.17 ± 0.05 μ g/ml for quercetin and 10.32 ± 0.21 μ g/ml for aqueous extract of leaves. *Ficus capensis*

Evaluation of the effects of plant extracts on the mammary gland

The average relative weight of the abdominal mammary gland (g/100 g PC) was 0.78 ± 0.11 , 0.86 ± 0.13 , 0.9 ± 0.3 and 0.83 ± 0.05 for the distilled water, Galactogil™, sulphiride and *Ficus capensis* EA groups, respectively. There were no statistically significant differences between the groups ($P > .27$).

Ductal tree arborescence

Figure 1 illustrates the most common types of ductal tree in each group. Mice from the sulphiride group showed the most developed arborescence with more appendages and branches. The Galactogil™ and aqueous extracts of *capensis* leaves groups showed almost equivalent arborescence with a higher density than the distilled water group.



*DW:distilledwater;Gal:Galactogil™;Sulp
:sulpiride;AEFCF:aqueousextractof *Ficuscapensis*leaves;redarrow:galactophorous duct

Figure 1:galactophorusductaltreearborescenceofmiceinthefourgroups

Ratioofductswithsecretionscomparedtototalducts

Figure2showstheaverageratioofductswithsecretionsinrelationtothetotalnumberofductsperlot. Thefourhighestratio,indescendingorder,werethoseofthesulpiride, *capensis* *Ficus* fruitandleafEHEandGalactogil™groups.

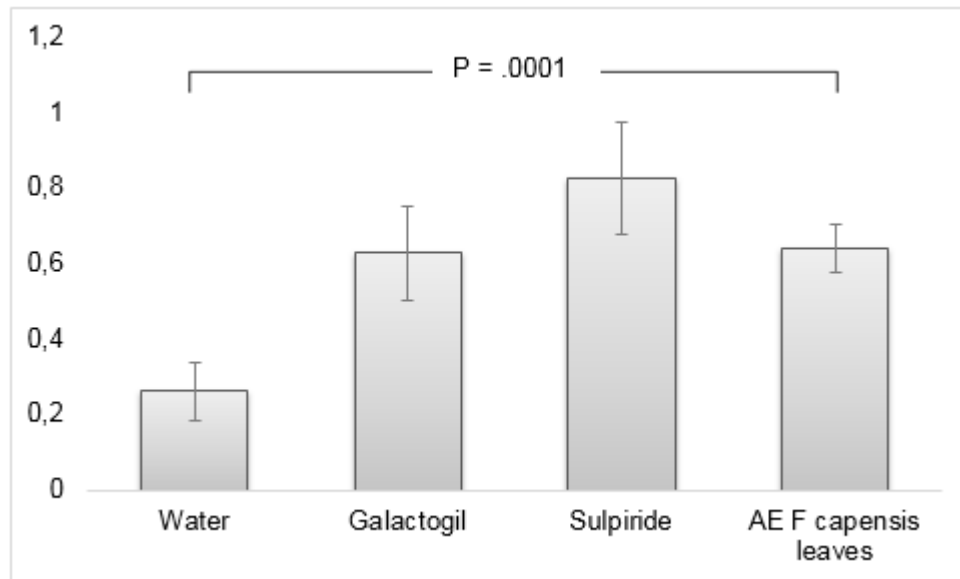
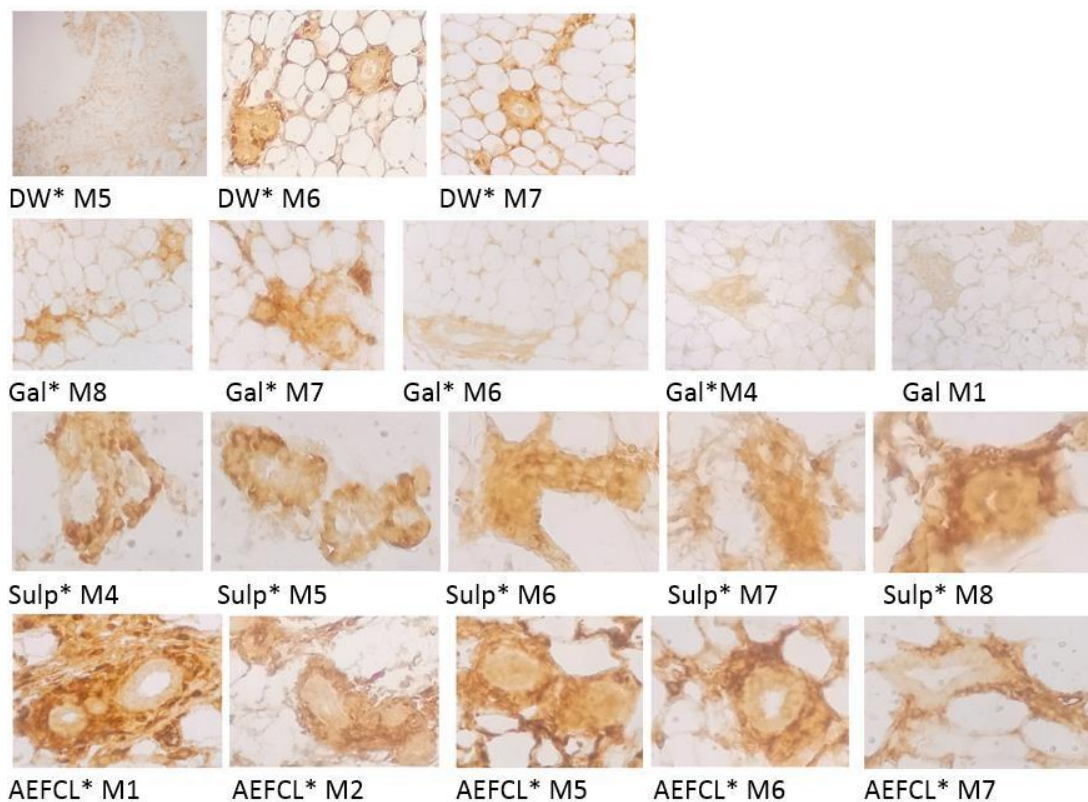


Figure 2:averageratio ofductswithsecretiontototalductspergroup

Immunohistochemical marking of breast tissue with anti-prolactin receptor antibody(antiPRLR)

Figure 3 shows immunohistochemical marking of breast tissue with anti-prolactin receptorantibody. The figures reported here are those of the most labelled mammary glands in thedifferent groups. The groups in which the glands were most intensely labelled were those ofsulpiride andaqueous extracts of *Ficuscapensis*leaves.



*DW:distilledwater;Gal:Galactogil™;Sulp :sulpiride;AEFCF:aqueousextractofFicuscapensisleaves

Figure3:immunohistochemicalmarkingofbreasttissuewithanti-prolactinreceptorantibody

Betacasein concentrationinbreasttissue

Theaverageconcentrationofbeta-caseininbreasttissueisshowninFigure4.The distilledwatergroup hadalowerconcentration thantheotherthreegroups($P < .02$).

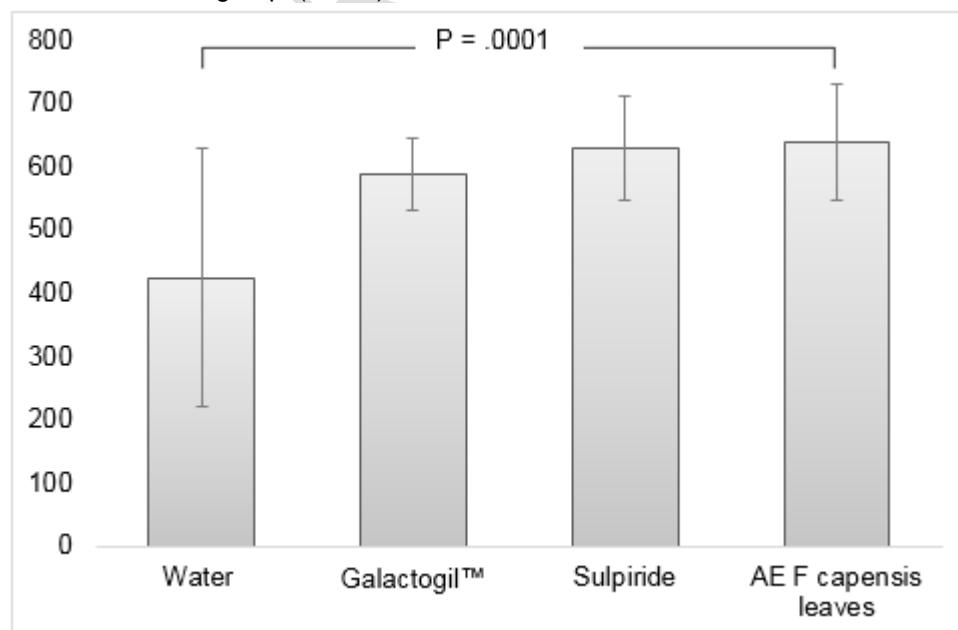


Figure 4:averageconcentrationofbeta-caseininmammarytissueofdifferentgroupsofmice

Prolactinemia

Figure 5 shows the average prolactinemia for each group. The groups of mice fed with the different extracts and the positive control group had higher average prolactinemia levels than the negative control group.

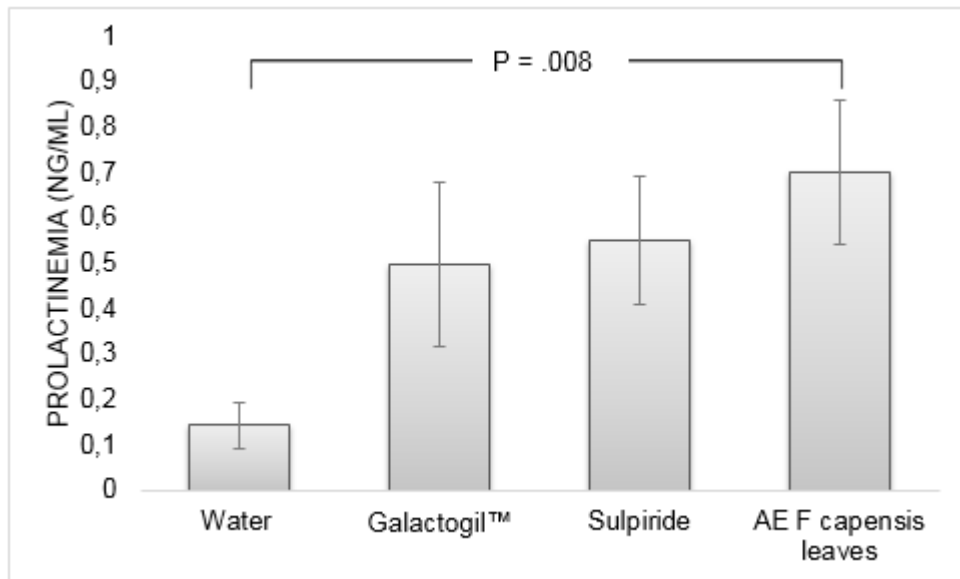


Figure 5: average prolactinemia in different groups of mice

Progesteronemia

The average progesteronemia was $334.028 \pm 96,89$ pg/ml, $277.69 \pm 99,69$ pg/ml, $294.55 \pm 131,09$ pg/ml and $304.04 \pm 81,1$ pg/ml for the distilled water, galactogil™, sulpiride and *Ficus capensis* EA groups, respectively. There were no statistically significant differences between groups ($P > .2$ in all cases).

Discussion

Arborisation of the galactophorous ducts

When examining the arborisation of the galactophorous ducts, the mice in the sulpiride group had the most developed arborisation with more appendages and ramifications. These appendages were alveoli, alveolar buds and/or terminal buds. Sulpiride is known to be mammogenic, possibly due to prolactin [23–25]. Sulpiride was also given to our mice at a lactogenic dose. This may favour this mammogenic effect more than the *Ficus capensis* extracts. It has been reported that in the post-pubertal period, the entire fat pad is occupied by galactophorous ducts, even tertiary ones, with only a few terminal end buds [26,27]. Most of the mice gavaged with distilled water showed fewer appendages on whole mount images. Aqueous extracts of *Ficus capensis* had more mammogenic effects than distilled water, and therefore more than the hormones of the simple oestrous cycle in mice. Considering the hormone levels measured in this study, the progesteronemia of the different groups of mice did not show any statistically significant differences. This could be due to the synchronisation of oestrous cycles by the Whitener effect. The effects observed on the mammary glands of the group of *Ficus capensis* aqueous extracts might be attributed to this effect. The aqueous acts of *Ficus capensis* might also mediate prolactin for their mammogenic effects, as the mice in their group have higher prolactinemia levels than those in the distilled water group. (Fig. 5).

Mammary secretion

Some of the ducts observed on the histological sections contained eosinophilic material, one of the characteristics of a secretory product. These secretion products were found in varying proportions in all histological sections. It is accepted that secretions are present in the galactophorous at all stages of the oestrous cycle [28–30]. This basal secretion is due to hormonal stimulation of the cycle, but remains low. The ratio of ducts containing secretion to the total ducts gives a better indication of the intensity of secretion in the gland histologically. In this study, the positive control mice showed higher proportions than the neutral control, confirming the lactogenic properties of sulpiride and Galactogil™. In this sense, our aqueous extracts, which also showed higher proportions than the distilled water group, would then show histological evidence of prosecretory effects. The histological assessment of secretion was combined in our study with assays of beta-casein in breast tissue. These assays make it possible to verify and confirm the histological results in the context of whether or not there is an increase in milk secretion [12]. Both the extract and positive control groups showed more beta-casein in mammary tissue than the distilled water group. Combining the observations on histological sections and the results of the biochemical assays, the extracts increased the secretion of milk proteins in mouse mammary tissue. At doses of 500 mg/kg body weight, this increase in milk secretion was significant (Fig. 4). Concentrations might be considered low and this might be related to the fact that stimuli to significantly increase the amount of milk produced would require a physiological state similar to that of lactation and/or pregnancy [26, 31]. Also, the doses used for positive controls were those used in humans, whereas sulpiride and Galactogil™ pharmacological parameters may differ from those observed in mice. Humans differ from animals in the expression and activity of drug-metabolizing enzymes [32–34]. It is also accepted that milky secretions present in the galactophorous ducts may be phagocytosed or reabsorbed into epithelial and/or connective tissue [31, 35, 36]. In the present study, the increase in secretion was associated with an overexpression of prolactin receptors in certain groups of mice analyzed immunohistochemically in the mammary glands. These were essentially groups of aqueous extracts of *Ficus capensis* and sulpiride (Fig. 3). The increase in milk secretion was also associated with increased prolactin concentrations in the blood samples. The increase in tissue concentrations of mammary gland beta-casein suggests that the extracts tested are not only lactogenic but may also modulate the quality of milk secretion. Milk protein concentration is an important parameter in neonatal nutrition. In this study, the group of mice gavaged with aqueous extracts of *Ficus capensis* leaves showed an increase in the density of the galactophorous duct network and an increase in the secretion of beta-caseins. These effects could be due to direct effects of the extracts on the mammary gland and/or via pituitary and ovarian hormones. The fact that aqueous extracts of *Ficus capensis* leaves and fruit overexpressed prolactin receptors leads us to at least conclude that these extracts pass via prolactin to ensure their lactogenic effects [24, 37–39]. However, the *Ficus capensis* extracts used contained bioactive compounds such as polyphenols, flavonoids and tannins. These compounds are thought to act on the secretory function of the mammary gland, either by increasing prolactinemia, blood levels of ovarian hormones, or by increasing blood flow in the mammary gland [40]. Some flavonoids are thought to increase prolactin secretion and possess anti-inflammatory and antioxidative properties, which are also known to improve lactation [41–43]. Some tannins have lactogenic properties [44–48]. Lactogenic properties are also attributed to certain polyphenols [46, 49–51]. Prolactin synthesis has been reported to be increased by some polyphenols [42].

CONCLUSION

This study was conducted with the aim to contribute to the promotion of breastfeeding by testing the lactogenic properties of *Ficus capensis* extracts. By measuring beta-casein in mammary tissue, groups of mice treated with these extracts showed higher levels than those treated with distilled water. The increase in protein secretion is evidence that the extracts are able to improve the nutritional quality of the milk produced, although this does not rule out the possibility of phytochemicals passing into the milk. The phytochemicals found in the extracts might explain the lactogenic and mammary effects.

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