

## Galactogenic effect of milk powder from nutsedge tubers (*Cyperus esculentus*) in lactating rats

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### Abstract

Early cessation of breastfeeding, often due to insufficient milk production, is a frequent problem for mothers. In order to provide a solution, this study assessed the galactogenic efficacy of nutsedge tubers. To do this, 72 nulliparous rats aged around three months were used. After parturition, each lactating rat was housed individually with six rat pups. For 17 days, the animals were divided into several groups receiving: a negative control diet (without galactagogue), a positive control diet (Galactogil with drinking water), and four experimental diets based on 10 %, 20 %, 30 % and 40 % nutsedge tuber milk powder. Milk production in lactating rats was assessed using the rat weighing method, while the histological structure of the mammary glands was observed. Serum prolactin concentrations were measured on days 0, 7 and 14. The results show that Tiger Nuts milk powder stimulates milk production and increases blood prolactin. Milk production reached  $36.58 \pm 0.41$  g for the MPNT 40 % diet, compared with  $27.52 \pm 0.65$  g for the control. The MPNT 40 % diet has the highest serum prolactin concentration and also promotes better lobule-alveolar organization of the mammary glands. The increase in milk production, accompanied by a significant improvement in the weight growth of the pups, confirms the galactogenic effect of yellow nutsedge tubers.

**Keywords:** Galactogenic effect; Milk powder; Prolactin; Milk production

### 1. Introduction

The World Health Organization recommends exclusive breastfeeding up to the age of 6 months because of the many recognized health benefits. Breastfeeding is beneficial in both the short and long term for the baby and also for the mother [1]. Breastfeeding is important because it provides essential and irreplaceable nourishment for the child's resistance and development. It is a child's first immunization, protecting against respiratory infections, diarrhoeal diseases and other potentially fatal conditions, as well as obesity [2].

For the mother, it can reduce the risk of breast cancer and promote better health after the birth of the child [3]. However, the prevalence of breastfeeding worldwide remains low. According to research carried out in 2021 in an urban community in Niamey, Niger, exclusive breastfeeding was practiced by 33.5 % of mothers [4]. In 2012, data from three health facilities in the city of Abidjan in Côte d'Ivoire showed that exclusive breastfeeding was practiced by only 33.51 % of mothers [5].

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There are many reasons for stopping breastfeeding early. One of the reasons most frequently cited by mothers is the feeling that they are not producing enough milk to ensure their baby's optimal growth. This affects around a third of early weaners [6, 7]. African women generally experience insufficient milk production. They use traditional plants to stimulate milk production or to increase milk yield. These plants are used either pure or in a mixture, in the form of a decoction or maceration. Previous studies have demonstrated the galactagogue activity of certain plants.

These include *Euphorbia Herta*, *Arachis hypogaea*, *Ocimum Canum* [8], *Calotropis proceri*, *Ficus sycomorus*, *Ficus platyphylline* [9], *Spondees mombin* L. [10] and Torbagun (*Coleus ambitious* L.) [11].

An ethnobotanical survey also mentions the use of nutsedge tubers (*Cyperus esculentus*) as a plant with galactogenic properties [12, 8].

In Côte d'Ivoire, tubers of nutsedge (*Cyperus esculentus*) are regularly used traditionally to increase milk production in breastfeeding women. The aim of this study is to develop the use of nutsedge tubers in the production of breast milk. More specifically, the aim is to quantify the milk produced, take histological sections of the mammary glands and measure serum prolactin in rats.

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## 2. Materials and methods

### 2.1. Plant material

The plant material used was dried brown tubers of Tiger Nut (*Cyperus esculentus*). These tubers were purchased from farmers in Tagbonon Bambarasso, a village located 3 km from Dabakala in the Humbol region of northern Côte d'Ivoire. The dried Tiger Nut tubers were then packed in airtight polythene bags and transported to the laboratory for analysis.

### 2.2. Animal material

72 nulliparous female rats (*Rattus norvegicus*) of the Wistar strain, aged approximately 03 months, weighing between 170 g and 200 g, were used with 432 rat pups at a rate of 06 rat pups per rat. These animals were reared in the vivarium of the Ecole Normale Supérieure (ENS) in Abidjan (Côte d'Ivoire). The temperature of the rearing room was around 25°C and the lighting was regulated by a cycle of 12 hours of light and 12 hours of darkness.

### 2.3. Production of the Tiger Nuts milk powder sample

Once the tubers were in the laboratory, they were further sorted to eliminate foreign bodies, as well as damaged and immature tubers. The tubers were then washed three (3) times with water and soaked for 72 hours for rehydration. Once the tubers had been removed from the soaking water, they were washed a second time. The rehydrated tubers were ground using a blender (iLUX). The crushed material was placed in a cloth (poplin) and the milk was extracted by pressing. The extraction required 200 ml of water for 100 g of tubers. The milk obtained after pressing was then freeze-dried and the milk powder obtained was stored in airtight jars.

### 2.4. Formulation of the diets

The various experimental diets were formulated according to the method of [13] with some modifications (Table 1). In total, six (6) diets were formulated during this experiment. A negative control diet (basal diet), a positive control diet (Gal D) consisting of the basal diet plus 3.2 g of galactogil per 150 ml of water (galactogil was added to the drinking water of the rats) and four (4) other diets composed of the negative control diet with an incorporation of 10 %, 20 %, 30 % and 40 % of milk powder from nutsedge tubers. All of these diets were enriched with a premix of vitamins and minerals with added water.

**Table 1** Composition of diets

Composition (g/kg)	Diets					
	C. negative	Gal D	MPNT 10%	MPNT 20 %	MPNT 30 %	MPNT 40 %
Maize starch	640	640	628.97	619.25	607.39	596.51
Sunflower oil	50	50	45	40	35	30
Tiger nut milk powder	-	-	16.03	31.75	47.61	63.49
Galactogil	-	3.2	-	-	-	-
Fish powder	250	250	250	250	250	250
Sugar	50	50	50	50	50	50
Premix	10	10	10	10	10	10

- C. negative: Control negative
- Gal D: Galactogil diet
- MPNT 10%: Control diet with 10% incorporation of milk powder from nutsedge tubers
- MPNT 20%: Control diet with 20% incorporation of milk powder from nutsedge tubers
- MPNT 30%: Control diet with 30% incorporation of milk powder from nutsedge tubers
- MPNT 40%: Control diet with 40% incorporation of milk powder from nutsedge tubers

## 2.5. Treatment of animals

Female rats are mated with rats at a rate of one rat for every three (3) female rats. When a rat was pregnant, it was isolated in an individual cage until parturition. After parturition, the rat pups were adjusted to six (6) rat pups for each lactating rat. The rats were fed a basal diet (control) for the first two (2) days postpartum. Then, in the evening of the second day until the seventeenth day, the rats were fed the different formulated diets.

## 2.6. Measurement of the quantity of milk produced by suckling rat pups and the mass of the rats

The quantities of milk produced by the rats in the different batches were determined by weighing them using the method described in [14].

During the experiment, the suckling rats were fed the portion of formulated feed intended for each group at 6 pm. The next day, the first weighing session (P1) was carried out at 8 am after the rat had spent the night with the rat. They were then isolated in cages for 4 hours. At 12 hours, the rat pups were weighed (P2), then returned to the suckling rats for a one-hour suckling period. The last weighing (P3) took place at 1 pm.

The quantity of milk produced nineteen (19) hours after consumption of the feed by the rats is estimated by P3-P2. A correction for weight loss due to basal metabolic processes in the rat pups estimated by (P2-P1)/4 was added. The gain in daily pup weight was calculated from P2. The amount of milk produced was estimated by the following formula:

$$\text{Quantity of milk (g)} = (P3-P2) + [(P2-P1) / 4]$$

With: P3-P2: Weight gain in rat pups after lactation

(P2-P1)/4: Correlation coefficient for weight loss due to basal metabolic processes

## 2.7. Histological study of the mammary glands of suckling rats

At the end of the experimental days, the rats were fasted for 16 hours. The rats were then anaesthetized with ethyl urethane and sacrificed [15]. The mammary glands were harvested and preserved in 10 % formaldehyde for histological sections using the paraffin embedding technique [16].

This technique is performed in several stages. Pieces of the organs were placed in cassettes and dehydrated in a rising alcohol bath. They were immersed in toluene baths and then the glands were embedded in paraffin in appropriate

moulds. The gland fragments embedded in the paraffin blocks were cut into 5  $\mu\text{m}$  thick slices. The sections were rehydrated, stained with haematoxylin-eosin, dehydrated and observed using a tri-ocular electron microscope.

## 2.8. Determination of serum prolactin content

Assessment of blood prolactin levels required the use of 36 rats grouped into 6 groups of 6 rats. For the first two days post-partum, the rats were all fed a basal diet (control). The rats were then fed the different diets formulated on the evening of the second day until the seventeenth day. The rats were fasted for sixteen (16) hours on days 0, 7 and 14 of the experimental periods. The animals were then anaesthetized with ethyl urethane and blood samples (2ml) were taken by caudal amputation. The blood samples were centrifuged at 3000 rpm for 10 minutes [15]. The serum obtained constituted the sample to be analyzed.

Prolactin levels were quantified by the standard method using a Mini Vidas autoanalyzer (BioMérieux, France). The enzyme-linked immunosorbent assay was used for this determination [17].

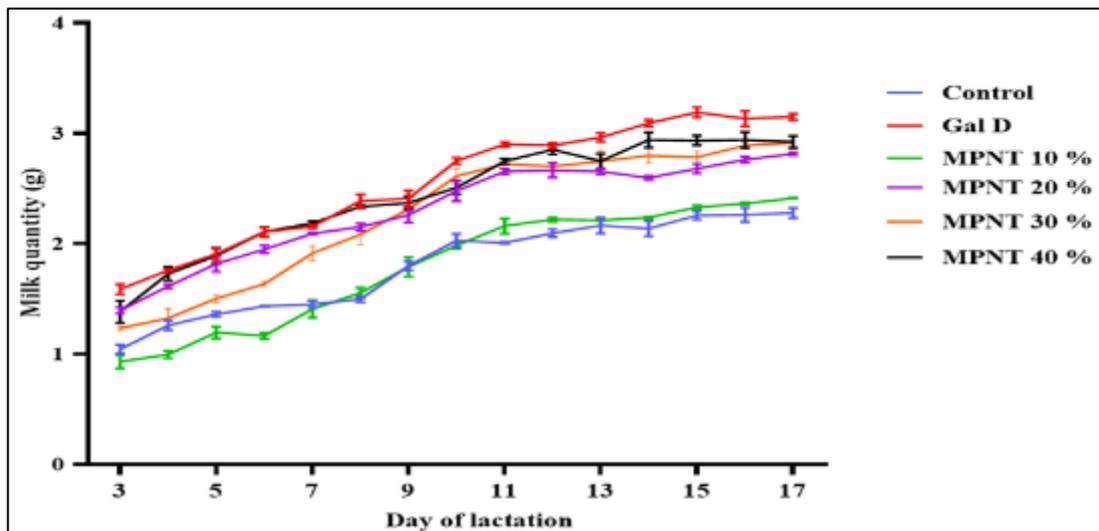
## 2.9. Statistical analysis

GraphPad Prism software version 8.0.1 was used for the statistical analysis of the results. All values are presented as the mean followed by the standard error of the mean ( $m \pm \text{SEM}$ ). Means were compared by analysis of variance (ANOVA) followed by Tukey's multiple comparison test at the 5 % threshold.

## 3. Results

### 3.1. Effect of incorporating milk powder from nutsedge tubers into the diet on the quantity of milk produced each day

Figure 1 shows the quantities of milk produced daily by suckling rats after consumption of the different diets. The figure shows an increase in milk production during lactation. Low milk production was observed from day three to day nine. Milk production increased from the tenth to the seventeenth day of lactation when the rats had consumed the different formulated diets.

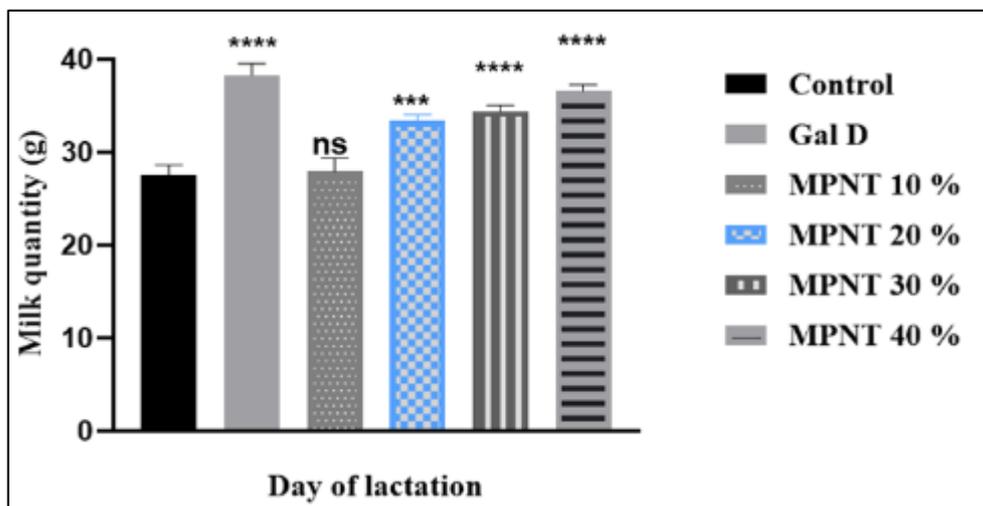


**Figure 1** Daily milk production from day 3 to day 17 of lactation after consumption of different formulated diets

- Gal D: Galactogil diet
- MPNT 10 %: Control diet with 10 % incorporation of milk powder from nutsedge tubers;
- MPNT 20 %: Control diet with 20 % incorporation of milk powder from nutsedge tubers;
- MPNT 30 %: Control diet with 30 % incorporation of milk powder from nutsedge tubers;
- MPNT 40 %: Control diet with 40 % incorporation of milk powder from nutsedge tubers.
- Mean  $\pm$  S.E.M; n = 6.

### 3.2. Effect of incorporating milk powder from nutsedge tubers into the diet on the amount of total milk produced

All the amounts of milk produced by the rats after consuming the different diets during the experimental period are shown in figure 2. Analysis of the results shows that the MPNT 20 %, MPNT 30 %, MPNT 40 % diets resulted in an increase in milk production from the MPNT 20 % diet, as well as the Gal D. Compared with rats on the control diet, which produced a total of  $27.52 \pm 0.65$  g of milk, the PLTS 20 % diet produced a highly significant increase in milk at the threshold ( $P < 0.001$ ), reaching  $33.40 \pm 0.37$  g. The MPNT 30 %, MPNT 40 % diets and Gal D showed highly significant increases in milk production at the threshold ( $P < 0.0001$ ), of  $34.42 \pm 0.36$  g,  $36.58 \pm 0.41$  g and  $38.33 \pm 0.70$  g respectively. The incorporation of MPNT 10 % resulted in milk production of  $27.97 \pm 0.81$  g. This quantity was statistically insignificant at the threshold ( $P > 0.05$ ) compared with that observed in rats on the control diet.



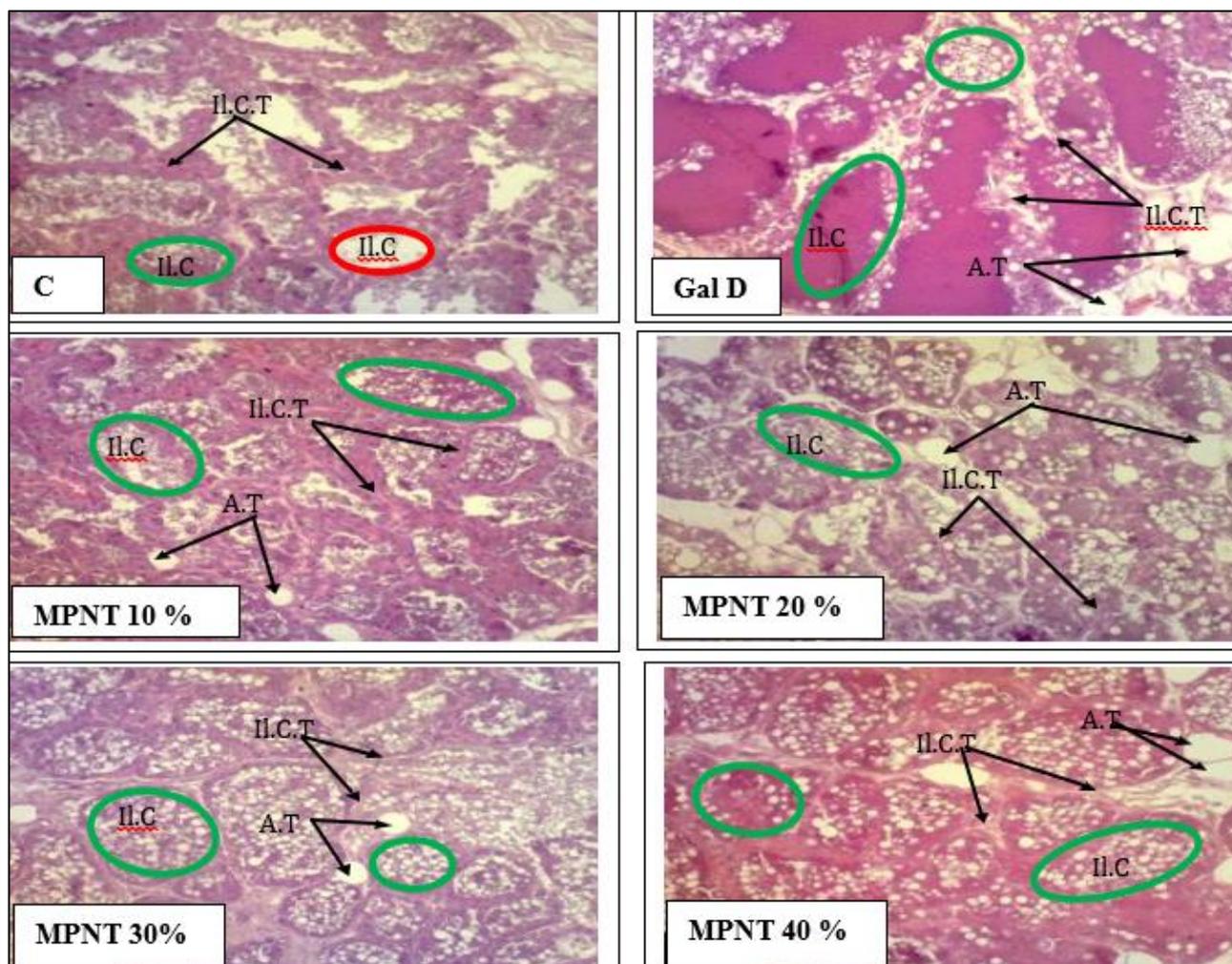
**Figure 2** Total milk production from day 3 to day 17 of lactation after consumption of the different formulated diets

Milk production is obtained by summing the quantities of milk produced daily during the experiment.

- Gal D: Galactogil diet
- MPNT 10 %: Control diet with 10 % incorporation of milk powder from nutsedge tubers;
- MPNT 20 %: Control diet with 20 % incorporation of milk powder from nutsedge tubers;
- MPNT 30 %: Control diet with 30 % incorporation of milk powder from nutsedge tubers;
- MPNT 40 %: Control diet with 40 % incorporation of milk powder from nutsedge tubers.
- ns' > 0.05: No significant difference; \*\*\*P < 0.001: Very significant difference;
- \*\*\*\*P < 0.0001: Highly significant difference; Mean  $\pm$  S.E.M; n = 6.

### 3.3. Effect of incorporating milk powder from nutsedge tubers into the diet on the histological structures of the mammary glands

Microscopic analysis of the mammary glands of suckling rats (Figure 3) revealed milk-containing alveoli. However, the alveoli in the mammary glands of rats fed the Gal D contained high concentrations of milk compared with the control. Similarly, the mammary gland alveoli of rats fed the MPNT 10 %, MPNT 20 %, MPNT 30 % and MPNT 40 % diets contained significant amounts of milk. The mammary glands of rats fed the MPNT 10 %, MPNT 20 %, MPNT 30 %, MPNT 40 % diets and Gal D contained fat cells.



**Figure 3** Histological section of rat mammary glands

- C: Control, Gal D: Galactogil diet;
- MPNT 10 %: Control diet with 10 % incorporation of milk powder from nutsedge tubers;
- MPNT 20 %: Control diet with 20 % incorporation of milk powder from nutsedge tubers;
- MPNT 30 %: Control diet with 30 % incorporation of milk powder from nutsedge tubers;
- MPNT 40 %: Control diet with 40 % incorporation of milk powder from nutsedge tubers;
- IL.C: Intra-lobular canal; A.T: Adipose tissue; IL.C. T: Intra-lobular connective tissue;
- ○: Intra-lobular duct containing a high concentration of milk;
- ○: Empty intra-lobular duct; Staining: Hematoxylin-Eosin; Magnification: 100.

### 3.4. Effect of incorporating milk powder from nutsedge tubers into the diet on prolactin secretion in lactating rats

Prolactin concentrations in the blood on day 0, day 7 and day 14 of lactation are shown in table 2. On day 0 of the experiment, baseline prolactin values in all rats were below 1 ng/ml. On day 7 of the experiment, prolactin levels in rats fed the MPNT 20 %, MPNT 30 %, MPNT 40 % diets and Gal D were  $16.07 \pm 0.12$  ng/ml,  $16.19 \pm 0.18$  ng/ml,  $17.76 \pm 3.05$  ng/ml and  $22.19 \pm 2.06$  ng/ml respectively. Compared with the control, these values are highly significant ( $P < 0.0001$ ). On day 14, for those who received the Gal D, MPNT 30 % and MPNT 40 % diets were significantly higher ( $P < 0.0001$ ) at

values of  $15.46 \pm 0.93$  ng/ml;  $5.97 \pm 0.95$  ng/ml; and  $9.53 \pm 0.91$  ng/ml respectively. There was no significant difference between the prolactin values of rats fed the MPNT 10 % and MPNT 20 % diets and those of rats fed the control diet.

**Table 2** Serum prolactin content in rats after consumption of different diets

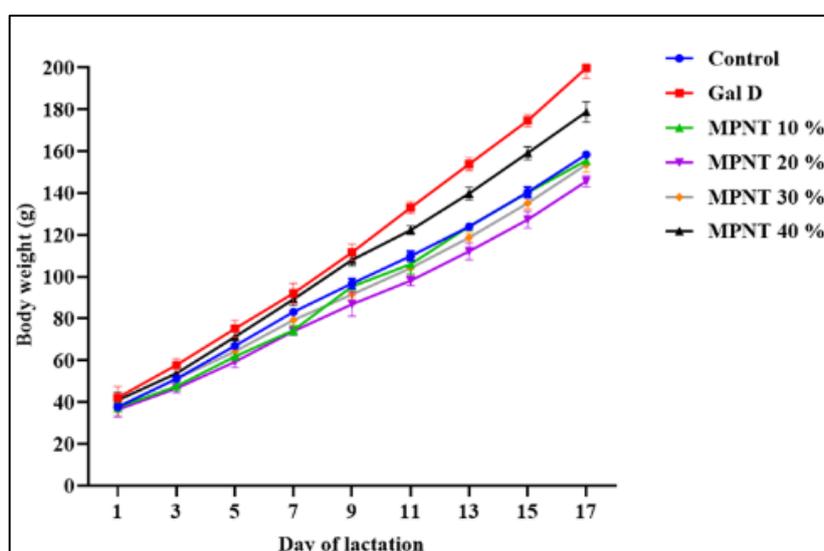
Diets	Prolactin concentrations (ng/ml)		
	Day 0	Day 7	Day14
Control	< 1	$1.46 \pm 0.80$	< 1.00
Gal D	< 1	$22.19 \pm 2.06^{****}$	$15.46 \pm 0.93^{****}$
MPNT 10 %	< 1	$1.85 \pm 0.24^{ns}$	$1.64 \pm 1.11^{ns}$
MPNT 20 %	< 1	$16.07 \pm 0.12^{****}$	$1.95 \pm 0.09^{ns}$
MPNT 30 %	< 1	$16.19 \pm 0.18^{****}$	$5.97 \pm 0.95^{****}$
MPNT 40 %	< 1	$17.76 \pm 3.05^{****}$	$9.53 \pm 0.91^{****}$

- Gal D: Galactogil diet;
- MPNT 10 %: Control diet with 10 % incorporation of milk powder from nutsedge tubers;
- MPNT 20 %: Control diet with 20 % incorporation of milk powder from nutsedge tubers;
- MPNT 30 %: Control diet with 30 % incorporation of milk powder from nutsedge tubers;
- MPNT 40 %: Control diet with 40 % incorporation of milk powder from nutsedge tubers.
- ns' > 0.05: Difference not significant; \*\*\*P < 0. 001: Very significant difference;
- \*\*\*\*P < 0.0001: Highly significant difference; Mean  $\pm$  S.E.M; n = 6.

### 3.5. Effect of the incorporation of milk powder from nutsedge tubers on the growth of rat pups

The curves showing the evolution of the body mass of the rat pups suckled during the experiment are illustrated in figure 4. All the rat pups increased their body mass during this experimental period. From day 03 to day 17 of lactation, compared with the control lot, the body mass of the pups whose mothers had received galactogil increased very significantly ( $P < 0.0001$ ).

The rat pups from the MPNT 40 % group had a mass that increased marginally ( $P < 0.05$ ) on day five and very significantly ( $P < 0.001$ ;  $P < 0.0001$ ) from day seven onwards. On the other hand, the body mass of the rat pups in the MPNT 20 % group fell very significantly ( $P < 0.0001$ ) from day five onwards.



**Figure 4** Growth curve for body mass of suckled rat pups

- Gal D: Galactogil diet;
- MPNT 10 %: Control diet with 10 % incorporation of milk powder from nutsedge tubers;
- MPNT 20 %: Control diet with 20 % incorporation of milk powder from nutsedge tubers;
- MPNT 30 %: Control diet with 30 % incorporation of milk powder from nutsedge tubers;
- MPNT 40 %: Control diet with 40 % incorporation of milk powder from nutsedge tubers.
- Mean  $\pm$  S.E.M; n = 6.

#### 4. Discussion

In this study, various diets were administered daily to lactating female rats in order to evaluate the galactogenic activity of milk powder derived from nutsedge in this study, various diets were administered daily to lactating female rats to assess the galactogenic activity of milk powder derived from nutsedge tubers. The results obtained indicate that the MPNT 20 %, MPNT 30 %, MPNT 40 % diets and Gal D induced milk production of  $33.40 \pm 0.37$  g,  $34.42 \pm 0.36$  g and  $36.58 \pm 0.41$  g and  $38.33 \pm 0.70$  g respectively. These values, in particular that obtained with the diet containing 40 % nutsedge, are comparable to those reported by [18], who observed a production of  $39.38 \pm 1.5$  g in rats treated with an aqueous extract of *Euphorbia Hirta* leaves at a dose of 200 mg/kg body weight. These results suggest a significant galactogenic effect of nutsedge, similar to that of plants traditionally known for their lactogenic activity.

There is growing interest in the use of galactogenic products based on foods or medicinal plants, both in traditional medicine and in functional nutrition [19]. One of the main reasons for this interest is the presence, in certain plant species, of bioactive compounds capable of stimulating milk secretion by acting on the physiological mechanisms involved in milk production [20]. The work of [21] on the chemical composition of milk powder from nutsedge tubers highlighted the presence of phenolic compounds, flavonoids and tannins, whose bioactive activity could explain the galactogenic effect observed.

These results are in line with those reported by [22] in Senegal, who also highlighted the galactogenic potential associated with the consumption of nutsedge tubers. Galactogil, on the other hand, contains extracts of fennel (*Foeniculum vulgare*), a plant widely recognized for its galactagogic properties. The presence of these bioactive compounds helps to stimulate the production of breast milk [23].

In addition to quantifying the milk produced, the galactogenic activity of the different diets was assessed by measuring serum concentrations of prolactin, a key hormone in the initiation and maintenance of lactation after parturition [24, 25]. This assay was carried out on day 0, day 7 and day 14 of the experiment. The results showed a significant increase in prolactin levels on day 7 in rats fed the MPNT 20 %, MPNT 30 %, MPNT 40 % diets and Gal D indicating significant galactogenic activity.

These observations are in agreement with the work of [20], which showed that administration of 100 and 200 mg/kg body weight of the ethyl acetate fraction of *Hibiscus sabdariffa* Linné induced an increase in prolactin levels, reaching  $31.45 \pm 2.39$  ng/ml and  $34.03 \pm 1.67$  ng/ml respectively on the eighteenth day post-partum in lactating rats. Furthermore, a study by [26] reported that *Trigonella foenum-graecum* (fenugreek) extract promoted increased milk production in the immediate postpartum period, with a beneficial effect on weight recovery in newborns. Similarly, [18] demonstrated a significant increase in prolactin secretion in rats treated with an aqueous extract of *Euphorbia hirta* leaves at a dose of 200 mg/kg, supporting the hypothesis of a galactogenic effect of certain medicinal plants.

The observed increase in milk production and serum prolactin levels in rats fed the MPNT 20 %, MPNT 30 % and MPNT 40 % diets highlights the galactogenic potential of *Cyperus esculentus* tuber milk powder.

Galactogil, a galactogenic supplement frequently used in clinical phytotherapy, contains extracts of fennel (*Foeniculum vulgare*) and aniseed (*Pimpinella anisum*), substances known for their ability to stimulate prolactin secretion. Their mechanism of action is thought to involve direct action on the mammary glands and modulation of the hypothalamo-hypophyseal axis, thereby helping to improve milk production [27, 23].

Histological analysis of the mammary glands of suckling rats revealed significant morphological changes following consumption of milk powder derived from *Cyperus esculentus* tubers (yellow nutsedge). Compared with the control group, whose connective tissue was relatively loose, the rats fed diets containing Galactogil and 10 %, 20 %, 30 % and 40 % Tiger Nuts showed increased infiltration of adipose cells and tissue organization suggesting intense secretory activity.

The observation of the different regions of the mammary gland indicates a significant stimulation of galactogenic activity, probably induced by the experimental diet. This galactogenic potential could be attributed to Nutsedge's ability to promote the development of the lobular system. Indeed, all diets enriched with yellow nutsedge powder resulted in a visible expansion of the lobular system of the mammary glands. These results are consistent with those of [28], which showed that *Acacia nilotica ssp. adansonii* leaves stimulated the development of the lobuloalveolar system in suckling rats. These observations therefore support the hypothesis that nutsedge tubers have significant galactogenic properties, as also suggested by the work of [12]. As a result, regular consumption of nutsedge tubers would promote milk production in breastfeeding mothers, thus helping to improve the nutritional status of infants during the first six months of life, and contribute to the fight against infant malnutrition.

In addition, the results obtained from the weight masses of the rat pups indicate that the diets administered to the rats had a positive influence on the weight growth of the rat pups via lactation. The weight gain observed in the newborns was closely linked to the quality and quantity of milk produced by the mothers. In particular, rat pups born to rats fed the Galactogil and MPNT 40 % diets showed significantly greater weight development than the other groups, including the control. This difference could be attributed to more abundant milk production in the rat pups in these groups. These observations are consistent with the work of [29], which showed that the nutritional status of the lactating mother directly influences the composition and nutritional value of the mother's milk. These results are consistent with those reported by [30], who examined the effect of fenugreek on milk production in rabbits and the weight growth of young rabbits before weaning. These authors highlighted a correlation between the quantity of milk produced and that actually consumed by the young, significantly influencing their weight at weaning. Similarly, the data from our experiment suggest that the consumption of milk powder from nutsedge tubers helps to increase milk production, with beneficial effects on the growth of newborns.

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## 5. Conclusion

Milk powder from nutsedge tubers promotes milk production, prolactin secretion, the development of the lobule-alveolar system of the mammary glands and the growth of rat pups that have consumed diets with milk powder incorporated. The increase in these various parameters effectively shows the galactogenic activity after consumption of these tubers in lactating rats.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of ethical approval*

The animals were treated in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes. Every effort was made to minimize animal suffering and reduce the number of animals used.

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