



Effects of the Aqueous Extract of the Rhizomes of *Zingiber officinale* (Ginger) on Sexual Parameters in Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The rhizome of *Zingiber officinale* (Ginger) is widely consumed as a juice and spice in the Congo, and is also used in the treatment of various pathologies. The aim of this study was to evaluate the effects of Ginger on the reproductive function of the rat. Four batches of four female rats each received the aqueous rhizome extract of *Zingiber officinale* (Ginger) at doses of 300 and 600 mg/kg, 17 β -estradiol at a dose of 1 mg/kg and distilled water, orally for 14 days. Acute toxicity was previously assessed in mice. The results of the acute toxicity study at a dose of 5000 mg/kg of each extract showed no signs of toxicity in mice. Pharmacological tests with rats showed that

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aqueous ginger extract non-significantly increased and decreased rat body weight at 300 and 600 mg/kg respectively. The 600 mg/Kg dose blocked the sexual cycle at the estrus stage and lowered plasma estradiol levels. Whereas the 300 mg/kg dose increases plasma estradiol levels with a more or less regular sexual cycle. Chemical screening of this extract revealed the presence of flavonoids, tannins, anthraquinones and steroids.

Keywords: Aqueous extracts; ginger rhizomes; eosinophil indices; estradiol levels.

1. INTRODUCTION

Since time immemorial, man has scoured his environment in search of products to relieve their ailments and treat their wounds (plants, animals, stones, spirits). Modern Western medicine has ignored most of these resources by developing chemical drugs and sophisticated treatment techniques, while continuing to use certain plant remedies [1]. Numerous studies have been carried out to determine which food plants promote the onset of disease or even cancer, and are therefore foods to be avoided; but there are also studies that make the opposite argument, that means, food plants that have a beneficial or even protective effect on health, and are therefore to be favored. The latter foods are generally considered to be products with a beneficial effect on one or more target functions in the body, over and above the usual nutritional effects, which can either improve an individual's state of health and well-being, or reduce the risk of a disease [2-4]. The literature reports that various food and culinary plants such as Squash, Okra, Papaya and Ginger have beneficial effects on reproductive function in men and women: squash seeds improve sperm quality and motility; Papaya seeds lower sperm count; the high concentration of Okra increases sex gland secretions in men and women [5,1]. In Republic of Congo, juice made from ginger rhizomes is regularly consumed as a beverage, and is also prepared as an herbal tea and consumed to relieve sore throats and as an aphrodisiac [6]. The decoction prepared from the rhizome is widely used to treat flu, coughs, asthma, diabetes and other illnesses. Temidayo and Oluwadare [7] reported that the aqueous extract of *Zingiber officinale* (Ginger) rhizomes alleviated carbon tetrachloride-induced hepatorenal disorders, muscular pains, digestive disorders and intoxications. The antibacterial effects of Ginger are reported by Bashige et al. [8]; Fotsing et al. [9]. According to Khodaie al. [10], Ginger promotes the production of testosterone in men, the male sex hormone involved in general well-being, which explains its use as an aphrodisiac.

In traditional Congolese medicine, ginger rhizomes are used to treat infertility in women. It is in this context that this study aims to evaluate the effects of the aqueous extract of *Zingiber officinale* (Ginger) rhizomes on reproductive parameters in female wistar rats. Ginger is said to promote the production of testosterone in men, the male sex hormone involved in general well-being, which explains its use as an aphrodisiac. In traditional Congolese medicine, ginger rhizomes are used to treat infertility in women. It is in this context that this study aims to evaluate the effects of the aqueous extract of *Zingiber officinale* (Ginger) rhizomes on reproductive parameters in female wistar rats.

2. MATERIALS

2.1 Plant Materials

The plant material consisted of rhizomes of *Zingiber officinale* (Ginger) or Ginger (Fig. 1A), purchased in a market south of Brazzaville (Republic of Congo) in June 2022. The plant was authenticated by comparison with Voucher herbarium reference number PBT 01 [11]. These rhizomes, cut into small pieces, were dried for three weeks in the Pharmacodynamics and Experimental Physiopathology laboratory of the Faculty of Science and Technology (FST) at room temperature ($25 \pm 1^\circ\text{C}$). After drying, these rhizomes were pulverized with a wooden mortar to obtain the powder (Fig. 1B). The powder collected served as plant material for the preparation of the extract.

2.2 Animal Materials

The animals used were swiss mice (Fig. 2 A), aged 2 to 3 months and weighing between 17 g and 24 g; and virgin female wistar rats (Fig. 2B), aged 4 to 6 months and weighing between 120 g and 145 g. These animals were housed in the animal house of the Laboratoire de Pharmacologie et Pharmacodynamie Expérimentale of the Faculté des Sciences et Technique (Université Marien Ngouabi), under

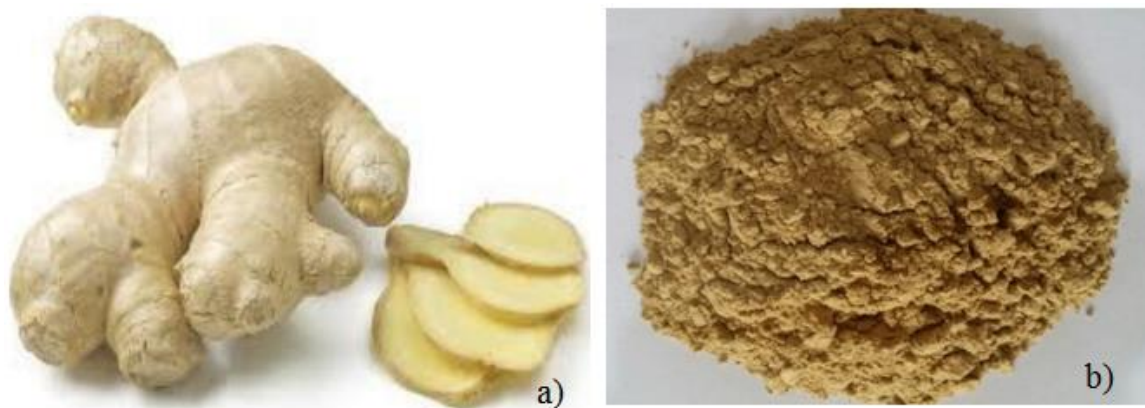


Fig. 1. a) Ginger Rhizomes; b) Ginger Powder

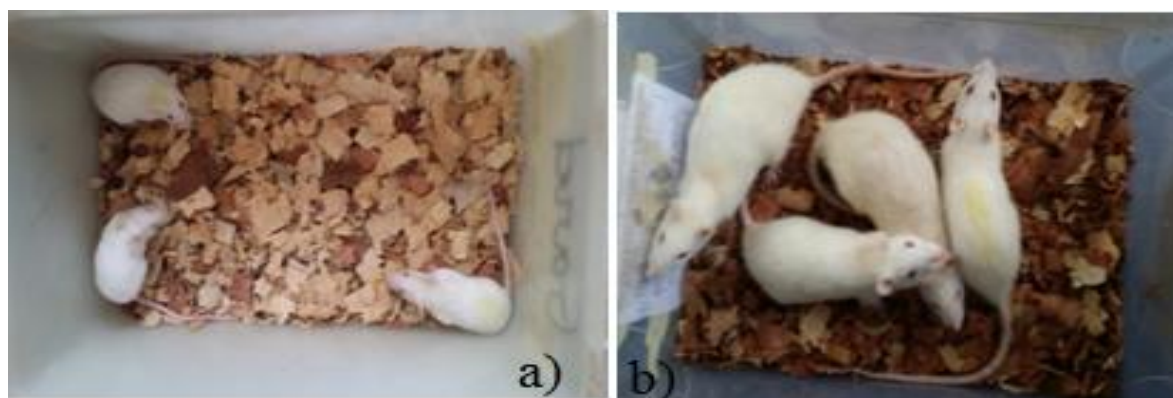


Fig. 2. a) Swiss mice; b) Wistar rats

standard conditions: 12h light/dark cycle, at room temperature, with free access to standard food and tap water.

3. METHODS

3.1 Preparation of the Aqueous Extract of Ginger and the Estradiol Solution

The aqueous extract of Ginger rhizomes was obtained by 10% maceration of dried rhizome powder. In fact, 50 g of powder was mixed with 500 ml of distilled water in a beaker for 24 hours under a magnetic stirrer, followed by filtration (3 times) on absorbent cotton. The filtrate was placed in an oven at 60°C for 48 hours to allow complete evaporation of the solvent. The aqueous extract obtained was used for pharmacological tests.

The solution of 17 β -estradiol used as a reference molecule was obtained by diluting one tablet of Oromone 2 mg in 10 ml of distilled water.

3.2 Evaluation of the Acute Toxicity of the Aqueous Extract of Ginger

The acute toxicity of the aqueous extract of ginger rhizomes was assessed in accordance with OECD guideline no. 423 [12]. Two batches of three mice each, of the same sex, were set up. The animals were treated as follows:

- The control batch received 10 ml/kg of distilled water,
- The test batch received 5000 mg/kg aqueous ginger extract.

The products were administered orally via an esophageal tube. Macroscopic observations of permanent lowering of the upper eyelid (ptosis), piloerection, urinary excretion, reaction to external stimuli and general condition of the animals (aggressiveness, vocalization mobility, stool condition, convulsions, etc.) were made at ½, 1, 2, 3 and 4 hours after administration of each product. Mortality was assessed at 48 h post-administration. Mice were left under

observation for 14 days to observe the possible late onset of signs of toxicity. Body weight, food and water consumption were recorded every two days.

3.3 Pharmacological Tests

The effect of the aqueous extract of Ginger rhizomes on the sexual parameters of the rat was done according to the method reported by Bayala et al [13]; Peneme [14]; Bafounguila [15]. Virgin rats divided into 4 batches of 5 animals each were treated daily for two weeks per os as follows:

- batch 1 (control) received distilled water at a dose of 10 ml/kg;
- batch 2 received 17 β -estradiol, the reference molecule, at a dose of 1 mg/kg;
- batches 3 and 4 received aqueous ginger extract at doses of 300 and 600 mg/kg respectively.

The effect of the extract was evaluated on food and water intake, body weight, sexual cycle, vaginal meatus variation, cervical mucus status and plasma estradiol levels.

3.3.1 Effect of the aqueous extract of ginger rhizomes on weight change and food consumption

The effect of aqueous ginger extract at doses of 300 and 600 mg/kg was evaluated on weight trends and food consumption before, during and after administration of the products (6 days before, 6 days during administration and 4 days after). Animal weights were taken every 2 days, and food and water consumption were monitored every 24 hours.

3.3.2 Effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger) on the sexual cycle and the state of cervical mucus

The effect of aqueous Ginger extract on vaginal meatus variation, cervical mucus status and sexual cycle of rat cows was assessed from daily vaginal smears, using the Harris Shor method, reported by Peneme [14]. Variation in the vaginal meatus of each rat was assessed when the cotton swab was inserted into the vagina to collect vaginal cells. The state of the cervical mucus is assessed by the stringy state of the mucus when the sample is spread out on the slide. And the phases of the sexual cycle are

determined by the eosinophilic index (eosinophilic cell count in each vaginal smear).

3.3.3 Effect of the aqueous extract of ginger rhizomes on plasma estradiol levels

24 h after the last administration, the animals were anesthetized with diethyl ether. Blood from each rat was gently drawn from the ophthalmic vein using hematocrit tubes and collected in Vitex heparin tubes. The blood was centrifuged at 3,000 rpm for 30 min, and the plasma collected was stored in a freezer at -4°C in 1 ml Eppendorf tubes for plasma estradiol assay.

4. RESULTS

4.1 Acute Toxicity of the Aqueous Extract of the Rhizomes of *Zingiber officinale* (Ginger)

4.1.1 General condition and behavior of the mice

Table I shows the acute toxicity results of the extract in mice. It shows that the extract, at a single dose of 5000 mg/kg, caused no change in the general condition or behavior of the mice compared with the control batch. No animal mortality was observed after 48 hours or 14 days of observation.

Table 1. General condition of animals after administration of products

Parameters	Distilled water (10 ml/kg)	Ginger (5000 mg/kg)
Number of animals	3	3
Mobility	N	N
Aggressiveness	N	N
Condition of stools	C	C
Tremor	A	A
Sleep	A	A
Pain sensitivity	N	N
Vomiting	A	A
Vocalization	A	A
Pilo-erection	A	A
Ptosis	A	A
Vigilance	N	N
Number of deaths	0	0
Cardiac frequency	N	N

DW: Distilled water; A: Absent; N: Normal; C: Compact; P: Present

4.1.2 Weight evolution of mice

Fig. 3 shows the weight evolution of the mice under the effect of the extract administered at a single dose of 5000 mg/kg. It shows that the high dose of the extract resulted in a non-significant variation in the weight of the animals compared with the control batch after 14 days of observation.

4.1.3 Food consumption and water intake

Figs. 4 and 5 respectively show variations in food and water intake in mice treated with a single 5000 mg dose of the aqueous extract of ginger rhizomes, over 14 days of observation. They show that the extract reduced food consumption and increased water intake in mice compared with the control group. However, these variations were not significant.

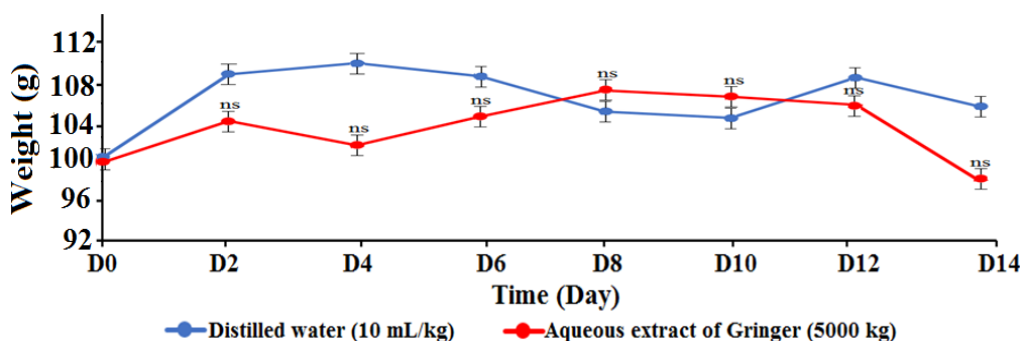


Fig. 3. Weight change in mice under the effect of the aqueous extract of rhizomes of *Zingiber officinale* (Ginger) at a dose of 5000 mg/kg

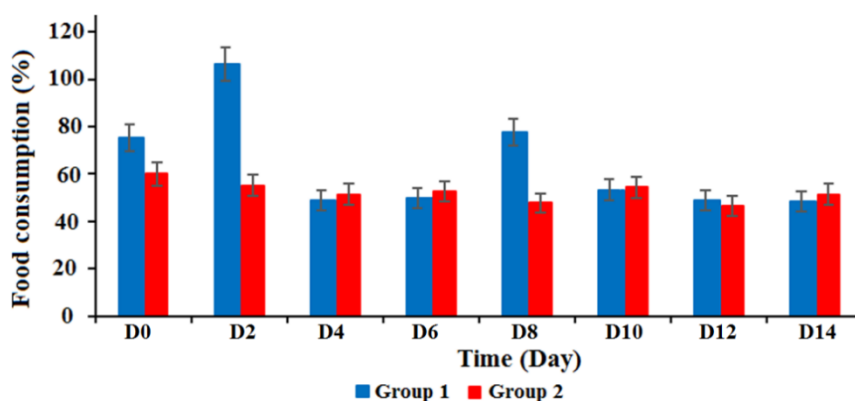


Fig. 4. Variation in food consumption under the effect of the aqueous extract of rhizomes of *Zingiber officinale* (Ginger) at a dose of 5000 mg/kg

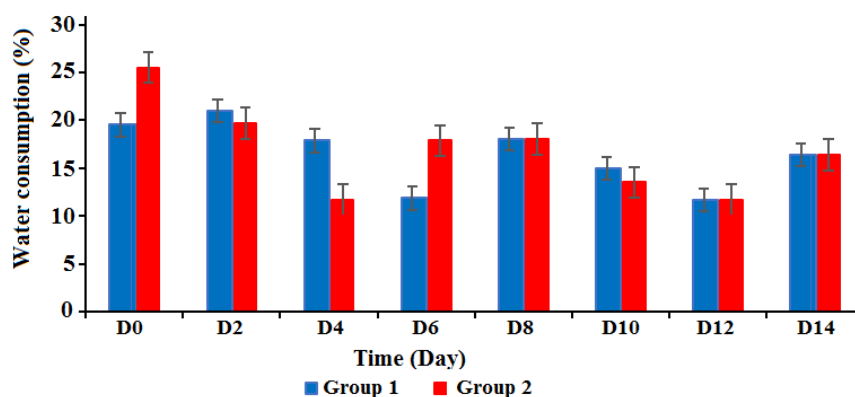


Fig. 5. Variation in water consumption under the effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger) at a dose of 5000 mg/kg

4.2 Pharmacological Tests

4.2.1 Effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger) on the weight evolution of rats

Fig. 6 shows the variation in body weight of rats treated with aqueous extract of ginger rhizomes at doses of 300 and 600 mg/kg over the course of the trial. It shows that the extract at 300 mg/kg, 600 mg/kg and the reference molecule did not cause significant variations in animal body weight compared with the control batch.

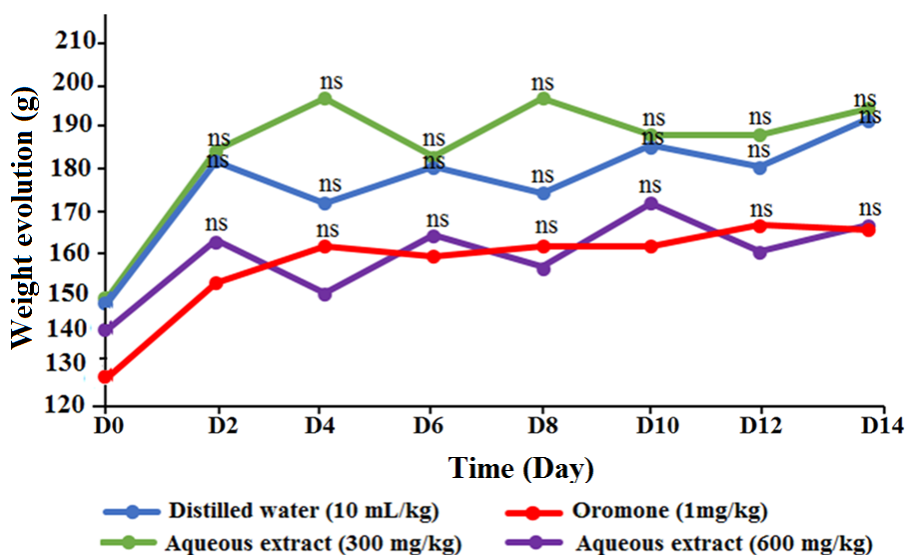


Fig. 6. Effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger) on the weight evolution of rats

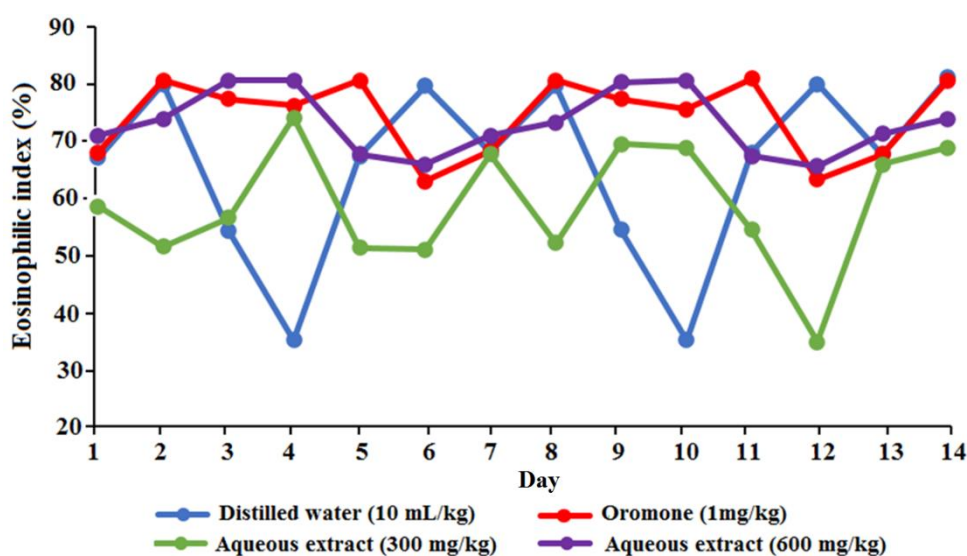


Fig. 7. Variation in the sexual cycle of rats under the effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger)

4.2.2 Effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger) on the sexual cycle

Fig. 7 illustrates the variations in the sexual cycle of rats obtained from the eosinophil indices of the vaginal smear from each group. It shows that the animals treated with the extract at a dose of 300 mg/kg have a more or less regular sexual cycle close to that of the rats in the control group. While the extract at a dose of 600 mg/kg blocked the cycle at the estrus stage as in rats having received the reference molecule.

4.2.3 Effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger) on the vaginal meatus and vaginal mucus

Table 2 shows meatus variation and vaginal mucus appearance in rats from different batches. It shows that rats treated with the 600 mg/kg extract have an open vaginal meatus with abundant secretion of clean, stringy vaginal mucus during all phases of the sexual cycle, compared with control rats. These variations are the same as in rats treated with the reference molecule. However, in rats treated with 300 mg/kg, the variations were close to those of the control batch.

4.2.4 Effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger) on the rate plasma estradiol

Fig. 8 shows the results of estradiol assays in animals from each batch. It shows a significant increase in estradiol levels in the batch treated with the aqueous extract of *Zingiber officinale* (Ginger) rhizomes at 300 mg/kg, and a non-significant decrease at 600 mg/kg compared with the control batch. The rat batch treated with the reference molecule 17 β -estradiol at 1mg/kg also showed a drop in estradiol levels.

4.3 Chemical Screening

Table 3 shows the different chemical groups revealed by the phytochemical study of the

aqueous extract of *Zingiber officinale* (Ginger) rhizomes.

5. DISCUSSION

This study is part of the valorization of Congolese food and culinary plants with pharmacological effects. It was initiated to evaluate the effect of the aqueous extract of *Zingiber officinale* (Ginger) rhizomes on the reproductive function of the rat. The extract was subjected to an acute toxicity study in mice, before its effects on rat reproductive function were assessed.

The acute toxicity study showed that, at a dose of 5000 mg/kg, the extract caused no perceptible signs of toxicity and no mortality after 48 hours. Thus, its LD50 would be above 5000 mg/kg according to the Lu scale [16]. Furthermore, analysis of body weight changes with a single dose of 5000 mg/kg of this extract did not result in any significant variation in body weight in mice over the 14-day observation period. This may be explained by the fact that the variation in food and water consumption was not significant.

However, some authors have shown that, at high doses, certain plant extracts are responsible for a loss of appetite in mice, leading to an inability to feed properly and consequently to low food consumption and weight gain. The aqueous extract of ginger rhizomes is well tolerated by mice up to a dose of 5000 mg/kg and can be considered as a good plant material for pharmacological studies.

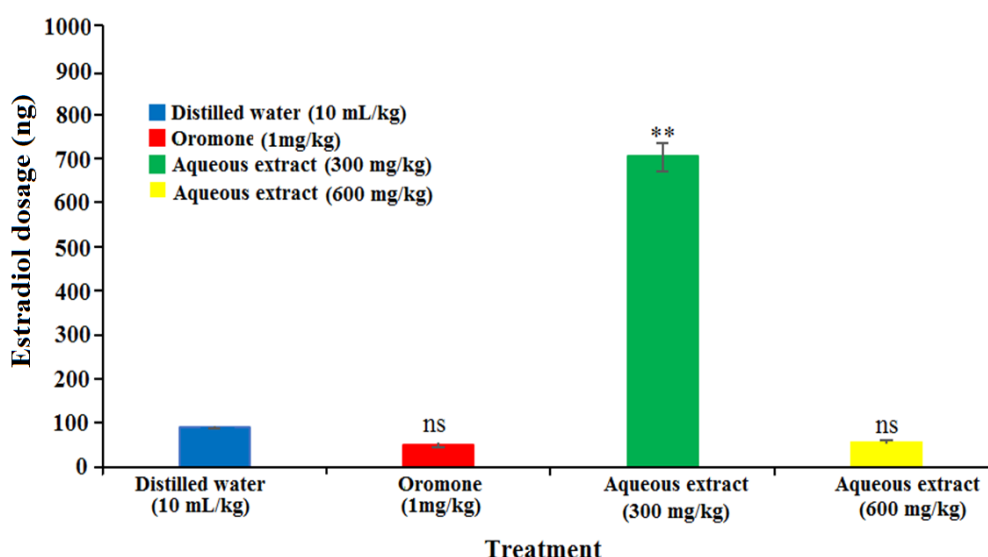


Fig. 8. Effect of the aqueous extract of the rhizomes of *Zingiber officinale* (Ginger) on the plasma estradiol level of rats

Table 2. Appearance of cervical mucus and opening of the vaginal meatus

Treatment	Conditions of mucus and vaginal meatus			
	Pro-estrus	Oestrus	Post-estrus	Di-estrus
Distilled water	PM m ± open	MP and running m open	MS ± viscous m ± tight	MS m tight
Oromone (1 mg/kg) or 17 β estradiol	PM m open	MP m open	MP spinning m ± open	MP m open
Zingiber O rhizome (600mg/kg)	Shooting PM m open	MP m ± open	MP m open	MP m ± tight
Rhizome of Zingiber O (300 mg/kg)	PM m± open	MP m open	MS m ±tight	MS M tight

M: Mucus; MP: Clean mucus; MS: Dirty mucus; m: meatus; ±: more or less

Table 3. Result of phytochemical screening

Chemical compounds	Observation	Results
Alkaloids	/	+
Anthraquinones	Red coloring	++
Flavonoids	Purplish pink coloring	++
Osides	Precipitate	-
Steroids	Red coloring	++
Tannins	Green coloring	+
Mucilages	Flaky precipitate	-

“+”: presence “++”: strong presence

Results on the weight development of rats fed the extract showed a non-significant increase and decrease in body weight at doses of 300 and 600 mg/kg respectively. This suggests that ginger at 300 mg/kg has an anti-estrogenic or perhaps progesterone effect, and at 600 mg/kg an estrogenic effect. According to Prakash, [17]; Wade [18] and Peneme [14]; administration of progesterone extracts in adult rats caused an increase in weight, and administration of estrogenic extracts caused a decrease in weight. In addition, rats treated with 17 β-estradiol also showed a reduction in body weight, as with the Ginger extract at 600 mg/kg, which seems to confirm the estrogenic effect observed with this dose. Indeed, Bringer et al [19] reported that 17 β-estradiol causes weight loss in castrated rats. The same authors also reported that estrogen deprivation causes obesity in women, which estrogen therapy counteracts.

With regard to the sexual cycle, the results show that the aqueous extract of *Zingiber officinale* rhizomes (Ginger) at a dose of 300 mg/kg presents a more or less regular sexual cycle; whereas the extract at a dose of 600 mg/kg as with the reference molecule (17 β estradiol) blocks the sexual cycle at the estrus phase. These results suggest that the 600 mg/kg extract has the estrogenic properties of 17 β-estradiol.

These observations corroborate the results observed on weight development.

The increase in mucus and opening of the vaginal meatus with the 600 mg/kg dose of Ginger extract reflects the strong estrogenic impregnation of the vaginal mucosa caused by this plant. This suggests that the extract promotes follicular maturation, responsible for high estradiol production [20].

Results on estradiol plasma levels in rats show that administration of the aqueous extract of *Zingiber officinale* (Ginger) rhizomes at a dose of 300 mg/kg resulted in a significant increase in plasma estradiol levels, whereas the 600 mg/kg dose resulted in a decrease compared with control animals. Rats given 17 β-estradiol also showed a decrease in plasma estradiol levels, as did those given 600 mg/kg of the extract. This drop in estradiol plasma levels in rats treated with the 600 mg/kg dose and the reference molecule may be explained by a negative feedback mechanism induced by an additional estrogen supply in rats with regular sexual cycles. This mode of action is reminiscent of that of oral contraceptives in women. Indeed, the additional supply of ovarian hormones via the pill numbs the regulatory system of the hypothalamo-hypophyseal complex, creating relatively low and constant levels of ovarian

hormones as if the woman were pregnant. This results in the normal development of ovarian follicles and thus the absence of ovulation [21].

The increase in plasma estradiol levels observed in animals treated with 300 mg/kg of ginger rhizome extract suggests that this extract has a weak progesterone or estrogenic effect, acting through positive feedback. In fact, it is known in hormonology that the administration of low doses of a hormone in mammals can increase its plasma level, and the administration of high doses can lower this level: [22], Annabelle, [23] and Emmanuelle, (2020). This is the paradoxical effect of hormones.

The phytochemical study of the aqueous extract of *Zingiber officinal* (Ginger) rhizomes revealed the presence of flavonoids, tannins, alkaloids, anthraquinones, steroids; and the absence of osides and mucilages. The results obtained are comparable to those obtained by Amari [24]; Ashraf et al. [25]; Meghezzi [26]; Asamenew et al. [27]; Bashige et al., [8]; Sekoura and Meya [4]; Fotsing et al. [9]. These authors have highlighted these different metabolites and established a link between them and the various therapeutic effects of Ginger rhizomes.

Ginger is currently the subject of numerous botanical, chemical and toxicological studies to prove its scientific efficacy and safety. The medical, therapeutic and culinary use of ginger is developing. Foine, (2017), quoted by Sekoura and Maya [4]. Bakwaye and al. [28] reported on the importance of the use of food plants in traditional medicine in relation to their safety; to this end they speak of a food-drug continuum [29-31]. Consumption of these nutraceuticals could be a low-cost alternative for the population to treat themselves by enriching foods with these bioactive plant species [32] [33,34].

6. CONCLUSION

The aim of this study was to evaluate the effect of the aqueous extract of *Zingiber officinal* (Ginger) rhizomes on reproductive function in rats. The results show that this aqueous extract is weakly toxic in mice, with an LD50 > 5000 mg/kg.

At a dose of 600 mg/kg in rats, it is said to have estrogenic properties, manifested by the blocking of the sexual cycle in estrus and the lowering of plasma estradiol levels, as with 17 β -estradiol,

the reference molecule. At a dose of 300 mg/kg, the extract is said to have progesterone or weakly estrogenic properties, which more or less disrupt the sexual cycle and significantly increase plasma estradiol levels.

It would be desirable to evaluate sub-acute and chronic toxicity studies of the aqueous extract of Ginger rhizomes and the effects of this extract on sexual parameters in male rats. Also, to develop an improved traditional medicine for the treatment of reproductive disorders.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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