Effects of the aqueous extract of the rhizomes of Zingiber officinale (Ginger) on sexual parameters in female wistar rats

ABSTRACT

The rhizome of Zingiber officinal (Ginger) is widely consumed as juice and spices in Congo. It is also used in the treatment of different pathologies.The objective of this study was to evaluate the effects of ginger on the reproductive function of rats.Four groups of four rats each received orally the aqueous extract of the rhizomes of Zingiber officinal (Ginger) at doses of 300 and 600 mg/kg. then, 17β estradiol at a dose of 1 mg/kg and distilled waterfor 14 days.Before, acute toxicity was evaluated with mice and the results of the acute toxicity study at a dose of 5000 mg/kg of each extract showed no sign of toxicity in mice.Pharmacological tests with rats showed that the aqueous extract of ginger non-significantly increased and decreased the weight of rats at doses of 300 and 600 mg/kg respectively.The 600 mg/Kg dose blocks the sexual cycle at the estrus stage and lowers the plasma estradiol level.While the dose of 300 mg/kg increases the plasma estradiol level with a more or less regular sexual cycle.The chemical screening of this extract highlighted the presence of flavonoids, tannins, anthraquinones and steroids.

Keywords: Aqueous extracts, Ginger rhizomes, eosinophil indices, estradiol levels

1. INTRODUCTION

Preevention and treatment of diseases are community health priorities in humans (Crofford L. J., 2013). To treat injuries, a large diversity of medications from many chemical classes are generally used, including nonsteroidal anti-inflammatory drugs (NSAIDs), which prove anti-inflammatory and analgesic assets (Sekiguchi M. et al., 2008, Klaassen C. D. et al., 2013). In part to realize natural alternatives to toxic synthetic medicines, investigators have studied the potential of various plants and their extracts for treatments of various illnesses (Ogbera A., Dada O., Adeleye F. and Jewo P., 2010). For example, the vigorous components in various plants have been discovered as antioxidants that prove low toxicity and high efficiency. One of these plants is ginger (Zingiber officinale). Medicinal plants have been used to heal many infections since antiquity. Giving the World Health Organization, 80 percent of the world's people uses various plant parts and their active ingredients as traditional medicines (A. S. Alqahtani, R. Ullah, and A. A. Shahat, 2022; Ullah R. et al., 2020). The rhizomes of the ginger plant remain the greatest medicinally important part of the plant (N. Mascolo, R. Jain, S. C. Jain, and F. Capasso, 1989). It bears antiarthritic, antiplatelet, antitumor, antioxidant, anti-inflammatory, antiviral, and antihepatotoxic properties (Kamtchouing P. et al., 2002). Ginger has been recognized as defensive against many toxic agents, such as cisplatin and bromobenzene, accredited to its capability to intensify antioxidant enzyme activity. Researchers revealed a drop in plasma uric acid concentrations later giving ginger extract to broilers (Alsherbiny M. A., 2019). Besides, rats treated with 2%or 4% dietary ginger for one month proved lower gentamycin-induced nephrotoxicity and renal oxidative stress (T. A. Ajith, U. Hema, and M. S. Aswathy, 2007). Therefore, since antique times, Man has discovered his environment in pursuit of products to relieve ailments and treat wounds (plants, animals, stones, spirits).There Modern Western medicine has obscured most of these remedies by developing chemical drugs as well as sophisticated treatment techniques, while continuing to use certain herbal remedies (Fabert 2013). Numerous studies have been carried out to determine which food plants promote the appearance of diseases and even cancer, and therefore foods to avoid;but also studies using the opposite reasoning. That is to say, food plants which have a beneficial or even protective effect on health can be favored.These latter foods are generally considered as products having a beneficial effect on one or more target

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functions in the body. Beyond the usual nutritional effects, which can either improve the state of health and well-being of an individual, orreduce the risk of a disease (Roberfroid, 2000; Bouyahya 2016; Sekoura and Maya, 2021). The literature reports that different 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 mobilitysperm andPapaya grains lower sperm count. The high concentration of okra increases the secretions of the sexual glands in men and women (Chigumira Ngwerume, F., 2000; Febert 2013). In Congo, juice made from ginger rhizomes is regularly consumed as a drink, in addition it is prepared in the form of herbal tea and then consumed to relieve sore throats and also as an aphrodisiac.The decoction prepared from the rhizome is widely used to treat flu, cough, asthma, diabetes and relieve various illnesses.Temidayo and Oluwadare, (2023) reported that the aqueous extract of the rhizomes of Zingiber officinal (Ginger) alleviated hepatorenal disorders induced by carbon tetrachloride, and muscle pain as well as digestive disorders and poisoning.The antibacterial effects of ginger are reported by Bashige et al (2021) andFotsing et al, (2022). According to Khodaie al, (2015), Ginger promotes the production of testosterone in men, a male sex hormone involved in general well-being, which would explain its use as an aphrodisiac.In traditional Congolese medicine, ginger rhizomes are used in the treatment of infertility in women.It is in this context that this study aims to evaluate the effects of the aqueous extract of the rhizomes of zingiber officinale (Ginger) or Ginger on reproductive parameters in female wistar rats.

2. Material

2.1.Plant material

The plant material consisted of the rhizomes of Zingiber officinale (Ginger) or Ginger (Figure 1A), purchased in a market located at south of Brazzaville (Republic of Congo) in June 2022. These rhizomes cut into small pieces were dried forthree weeks at room temperature (25± 1°C). The experiment took place in the Pharmacodynamics and Experimental Pathophysiology laboratory of the Faculty of Sciences and Technology (FST) of Marien Ngouabi University.After drying, these rhizomes were pulverized using a wooden mortar to obtain the powder substance (Figure 1B).The collected powder was used as plant material for the extract preparation.

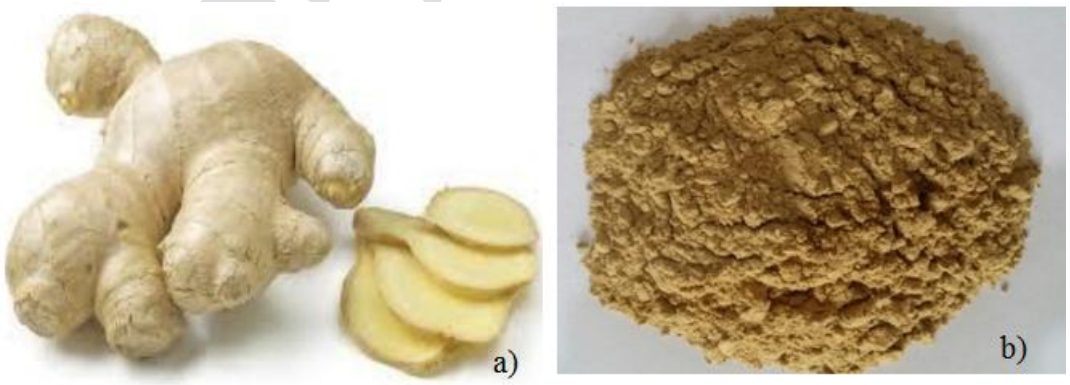

Figure 1: a) Ginger Rhizomes b) Ginger Powder

2.2.Animal material

The animals used were Swiss mice (Figure 2 a), aged 2 to 3 months, weighing between 17 g and 24 g;as well as female wistar rats (Figure 2b), virgins, aged 4 to 6 months and weighing between 120g to 145g.These animals from the animal house of the Laboratory of Pharmacology and Experimental Pharmacodynamics of the Faculty of Sciences and

Technology (Marien Ngouabi University), were housed under standard conditions. They were kept for 12 hours of light/dark cycle, at room temperature, with free accessto standard food and tap water.

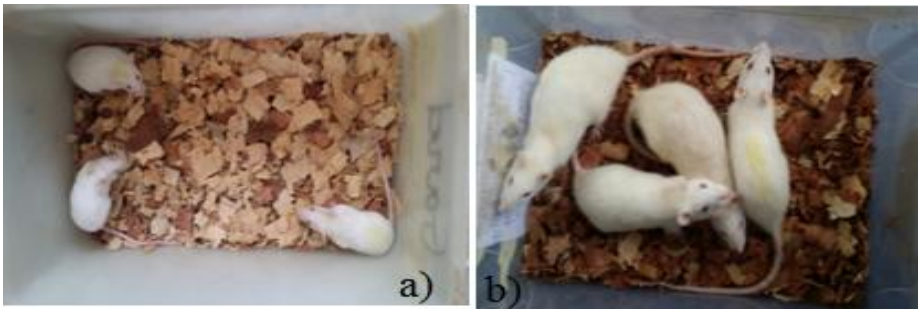

Figure 2: a) Swiss mice b) Wistar rats

2.3. Methods

2.3.1.Preparation of the aqueous extract of Ginger and the estradiol solution

The aqueous extract of Ginger rhizomes was obtained by maceration at 10% of the dried rhizome powder.Indeed, 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 hydrophilic cotton. Then, the filtrate was placed in theoven at 60°C for 48 hours to complete the solvent evaporation.The aqueous extract obtained was used for pharmacological tests. The solution of 17 β estradiol used as a reference molecule was obtained by diluting a 2 mg Oromone tablet in 10 ml of distilled water.

2.3.2.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 (2008).Two groups of three mice each belonging to the same sex were formed.Then, animals were treated as follows:

- ✓ The control group received 10 ml/kg of distilled water,
- ✓ The test group received 5000 mg/kg of aqueous extract of Ginger.

The products were administered orally using an esophageal tube.Macroscopic observations were made on the permanent lowering of the upper eyelid (ptosis), piloerection, urinary excretion and the reaction to external stimuli. The general conditions of the animals such as aggression, mobility vocalization, state of stools, convulsionswere carried out at ½, 1, 2, 3 and 4 hours after administration of each product.The mortality rate was assessed 48 hours after administration.The mice were left under observation for 14 days to observe the possible late appearance of toxicity signs. The body weight and food including water consumption were recorded every two days.

2.3.3. Pharmacological tests

The effect of the aqueous extract of Ginger rhizomes on the rat sexual parameters was carried out giving to the method reported by Bayala et al, (2006);Peneme (2017) andBafounguila (2021).The virgin rats divided into 4 groups of 5 animals each were treated daily and orally for two weeks as follows:

- ✓ Group1 or control group received distilled water at a dose of 10 ml/kg;
- ✓ Group 2 received 17 β estradiol and the reference molecule at a dose of 1 mg/kg;
- ✓ Groups 3 and 4 received the aqueous extract of Ginger at doses of 300 and 600 mg/kg respectively.

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

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2.3.3.1. Effect of the aqueous extract of Ginger rhizomes on weight change and food consumption.

The effect of the aqueous extract of Ginger at doses of 300 and 600 mg/kg was evaluated on the weight evolution and food consumption of the animals previously, during and after the administration of the products (6 days before, 6 days duringadministration and 4 days after).The weight of the animals was taken every 2 days, food and water consumption was monitored every 24 hours.

2.3.3.2. 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 the aqueous extract of Ginger on the variation of the vaginal meatus, the state of the cervical mucus and the sexual cycle of rats was evaluated from vaginal smears taken daily, according to the Harris Shor method, reported byPeneme (2017).The variation of the vaginal meatus of each rat was assessed during intromission of the cotton swab into the vagina for the collection of vaginal cells.The condition of the cervical mucus was assessed by the stringy state of the mucus when the sample is spread on the slide.Then, the stages of the sexual cycle were determined from the eosinophil index (rate of eosinophil cells in each vaginal smear).

2.3.3.3. 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 drawn from the ophthalmic vein using hematocrit tubes and collected in Vitex heparin tubes.Centrifugation of the blood was carried out at 3000 rpm for 30 min and the plasma collected was stored in the freezer at -4°C in 1 ml Eppendoff tubes for plasma estradiol determination.

3- Results

3.1. Results

3.1.1.Acute toxicity of the aqueous extract of the rhizomes of Zingiber officinale (Ginger)

3.1.1.1. General condition and behavior of the mice

Table I shows the results of the acute toxicity of the extract in mice.It is noticed that this extract at a single dose of 5000 mg/kg did not cause any change in the condition and general behavior of the mice compared to the control group.No animal mortality was occurred after 48 hours or after 14 days of observation.

Table I: 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

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Cardiac frequency	N	N

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

3.1.1.2. Weight evolution of mice

The weight evolution of mice can be seen in Figure 3, including the weight change of the mice under the effect of the extract administered at a single dose of 5000 mg/kg.From Figure 3, it is noticed that the high dose of the extract led to a non-significant variation in the weight of the animals compared to the control group after 14 days of observation.

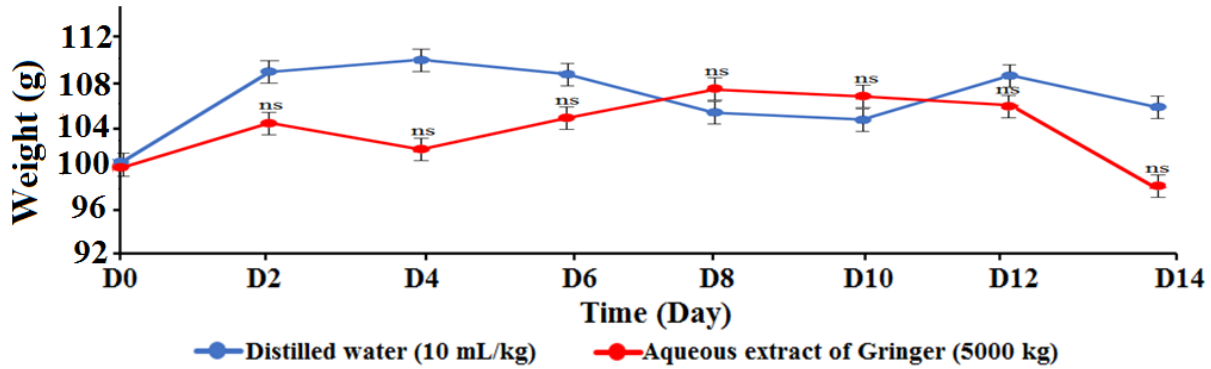

Figure 3: Weight change in mice under the effect of the aqueous extract of rhizomes of Zingiber officinale (Ginger) at a dose of 5000 mg/kg.

3.1.1.3. Food consumption and water intake

Figures 4 and 5 respectively show the variations in food and water consumption of mice treated with a single dose of 5000 mg of the aqueous extract of Ginger rhizomes, during the 14 days of observation.These Figures show that the extract led to a reduction in food consumptionand increased water intake in mice compared to the control group.But these variations are not significant.

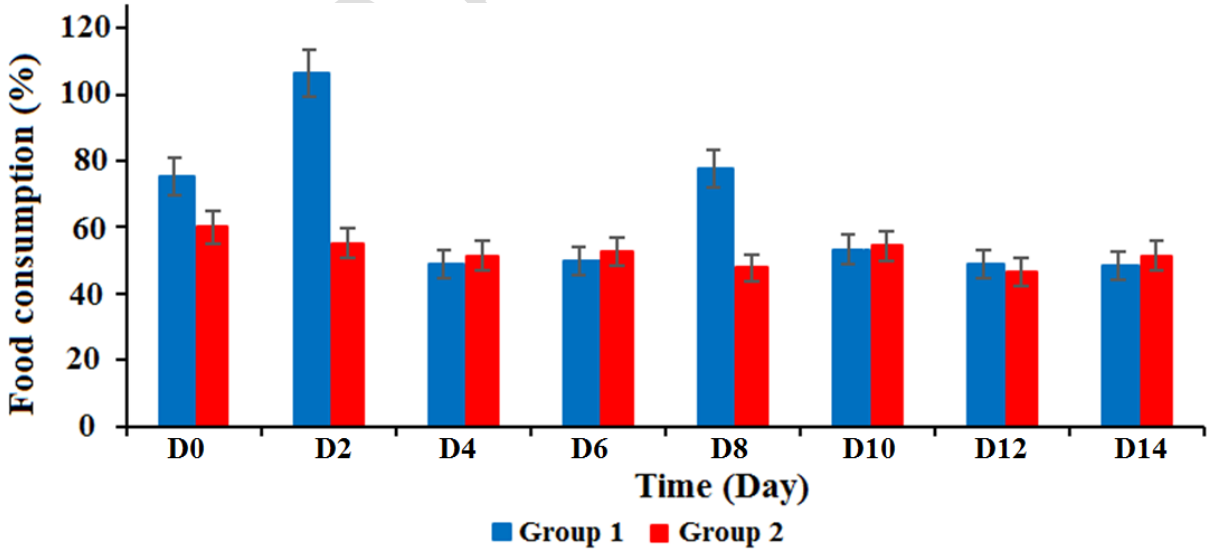

Figure 4: Variation in food consumption under the effect of the aqueous extract of rhizomes of Zingiber officinale (Ginger) at a dose of 5000 mg/kg

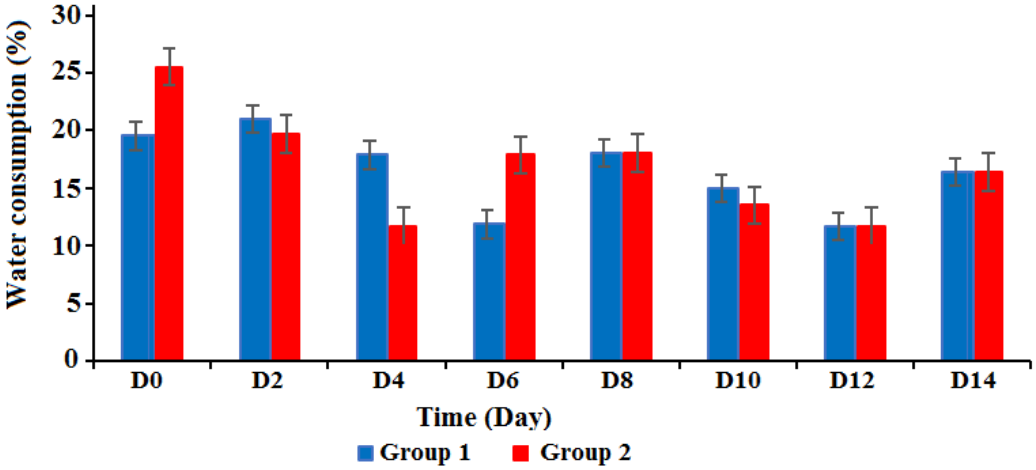

Figure 5: Variation in water consumption under the effect of the aqueous extract of the rhizomes of Zingiber officinale (Ginger) at a dose of 5000 mg/kg.

3.2.Pharmacological tests

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

Figure 6 shows the variation in body weight of rats treated with the aqueous extract of ginger rhizomes at doses of 300 and 600 mg/kg. This Figure shows that the extract at a dose of 300 mg/kg, 600 mg/kg and the reference molecule did not cause significant variations in the animals weight compared to the control group.

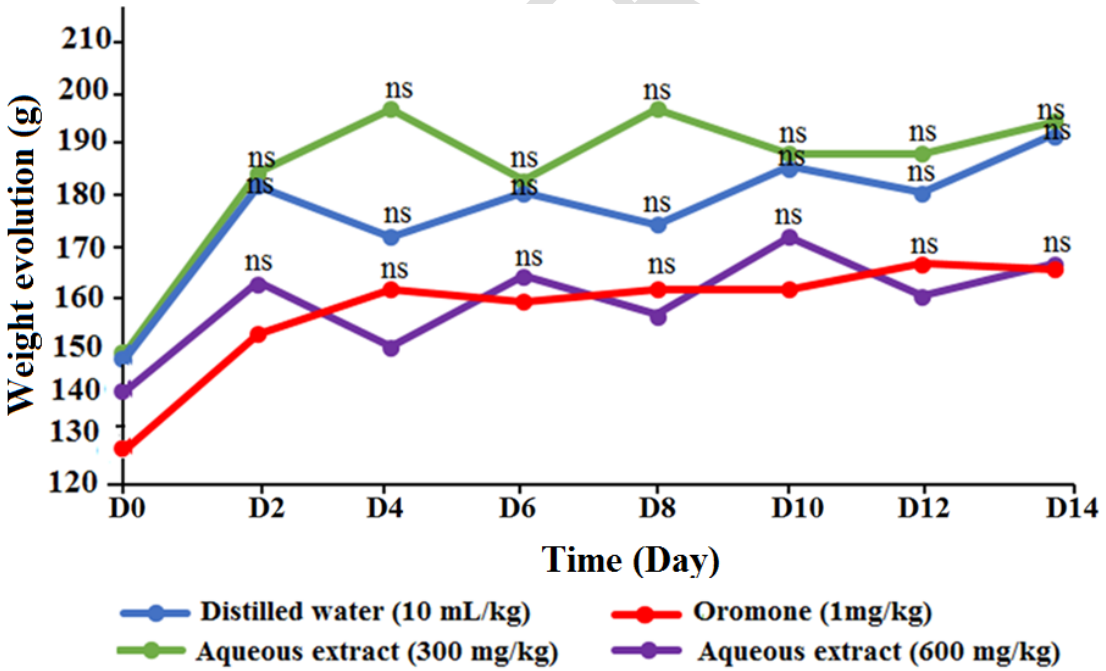

Figure 6: Effect of the aqueous extract of the rhizomes of Zingiber officinle (Ginger) on the weight evolution of rats

3.2.2.Effect of the aqueous extract of the rhizomes of zingiber officinale (Ginger) on the sexualcycle

Figure 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.

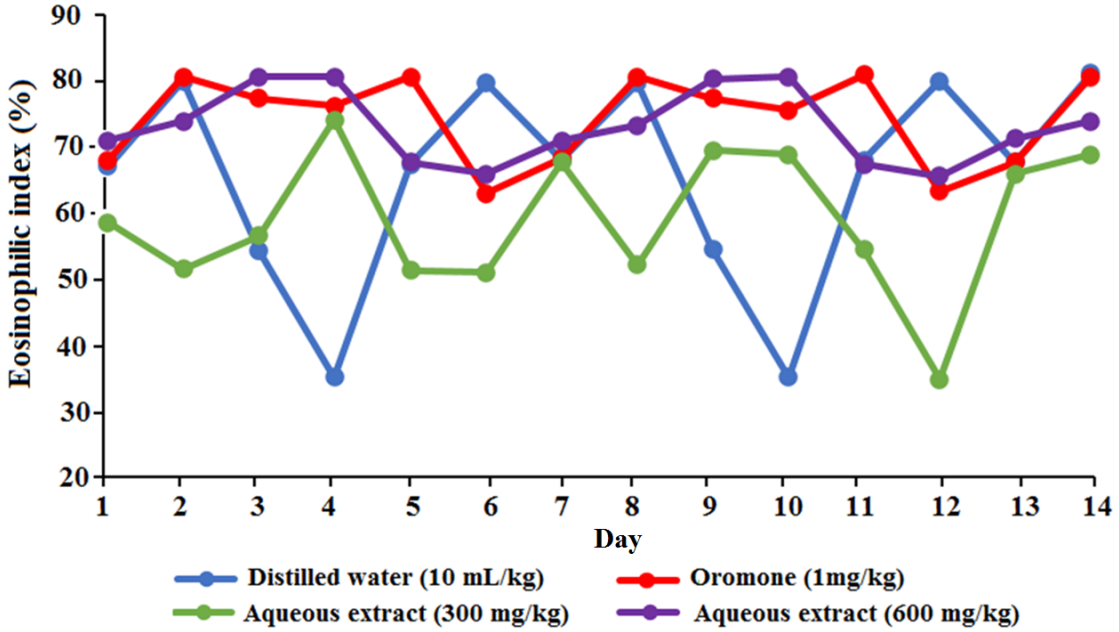

Figure 7: Variation in the sexual cycle of rats under the effect of the aqueous extract of the rhizomes of Zingiber officinale (Ginger).

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

Table II illustrates the variation of the meatus and the appearance of vaginal mucus in rats from different groups.It is noticed that the rats having received the extract at a dose of 600 mg/kg have an open vaginal meatus with abundant secretion of clean, stringy vaginal mucus during all stages of the sexual cycle compared to the rats in the control group.These variations are the same as in rats treated with the reference molecules.But in rats treated at a dose of 300 mg/kg, the variations are close to the control group.

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

3.2.4. Effect of the aqueous extract of the rhizomes of Zingiber officinale (Ginger) on the rate plasma estradiol

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Figure 8 shows the results of the estradiol dosage in animals from each group.It can be seen a significant increase in the estradiol level in the group treated with the aqueous extract of the rhizomes of Zingiber officinale (Ginger) at 300 mg/kg and a non-significant decrease at the dose of 600 mg/kg compared to the control group.The group of rats having received 17 β estradiol, the reference molecule at a dose of 1 mg/kg, also showed a drop in estradiol levels.

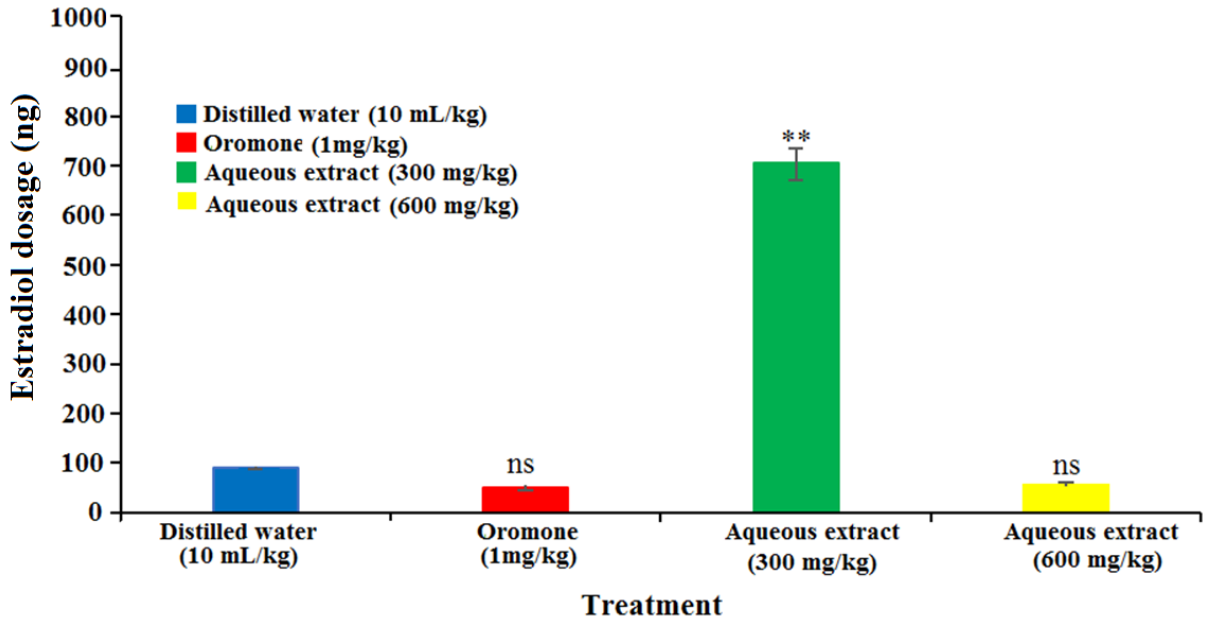

Figure 8: Effect of the aqueous extract of the rhizomes of Zingiber officinale (Ginger) on the plasma estradiol level of rats

3.3.Chemical screening

Table 3 presents the different chemical groups revealed by the phytochemical study of the aqueous extract of the rhizomes of Zingiber officinale (Ginger).

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

4.Discussion

This study is part of the promotion of Congolese food and culinary plants with pharmacological effects.It was initiated to evaluate the effect of the aqueous extract of the rhizomes of Zingiber officinale (Ginger) on the reproductive function in rats.The extract was therefore the subject of an acute toxicity study in mice, before assessing its effects on the

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reproductive function of the rat. The acute toxicity study showed that at a dose of 5000 mg/kg, the extract did not cause any perceptible signs of toxicity or any mortality after 48 hours.Thus, its LD50 would be at a dose greater than 5000 mg/kg according to the Lu scale (1992).Furthermore, the analysis of the weight change with the single dose of 5000 mg/kg of this extract did not cause significant variation in the body weight of the mice during the 14 days of observation.These results can 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 which leads to an inability in mice to eat properly and consequently to low food consumptionand therefore low weight gain.The aqueous extract of Ginger rhizomes being well tolerated by mice up to a dose of 5000 mg/kg can be considered as a good plant material for pharmacological studies. The results on the weight change of the rats having received the extract show a non-significant increase and decrease in the weight of the animals at doses of 300 and 600 mg/kg ,respectively.This result suggests that ginger at a dose of 300 mg/kg would have an anti-estrogen or perhaps progesteronic effectand at a dose of 600 mg/kg it would present an estrogenic effect.Indeed, according to Prakash (1975);Wade, (1993) and Peneme, (2017),the administration of extracts with progesteronic effects in adult rats caused an increase in weight and the administration of extracts with estrogenic effects caused a decrease in weight in rats.In addition, the rats treated with 17 β estradiol also showed a reduction in the weight of the animals as with the ginger extract at a dose of 600 mg/kg which seems to confirm the estrogenic effect observed with this dose.Indeed, Bringer et al. (2002) reported that 17 β estradiol causes weight loss in castrated rats and therefore a reduction in weight.Besides, the same authors indicated that estrogen deprivation causes obesity in women and estrogen therapy counters. Regarding the sexual cycle, the results show that the aqueous extract of the rhizomes of Zingiber officinal (Ginger) at a dose of 300 mg/kg presents a more or less regular sexual cycle. While the extract at a dose of 600 mg/kg as with the reference molecule (17 β estradiol) blocks the sexual cycle during the estrus phase.These results suggest that the extract at a dose of 600 mg/kg presents estrogenic properties like 17 β estradiol.These observations corroborate the results observed on weight change. The increase in mucus and the opening of the vaginal meatus with the dose of 600 mg/kg of ginger extract reflect a strong estrogenic impregnation of the vaginal mucosa caused by this plant.This result suggests that this extract promotes follicular maturation being responsible for significant production of estradiol (Thibaut and Levasseur, 2001). The results on the plasma dosage of estradiol in rats show that the administration of the aqueous extract of the rhizomes of Zingiber officinale (Ginger) at a dose of 300 mg/kg led to a significant increase in the plasma level of estradiol.Whereas, the dose of 600 mg/kg led to its reduction compared to control animals.The rats having received 17 β estradiol also showed a drop in the plasma level of estradiol like those having received the extract dose of 600 mg/kg.This drop in the plasma estradiol level of rats treated at a dose of 600 mg/kg and the reference molecule can be explained by a negative feedback mechanism induced by an additional intake of estrogens in rats with regular sexual cycles.This mode of action is reminiscent of oral contraceptives in women.Undeniably, the additional supply of ovarian hormones via the pill, numbs the regulatory system of the hypothalamic-pituitary complex by creating relatively low and constant levels of ovarian hormones, as if the woman were pregnant.This results in the development of ovarian follicles and therefore the absence of ovulation (Marieb and Hoehn 2015). The increase in plasma estradiol levels observed in animals treated at a dose of 300 mg/kg of ginger rhizome extract suggests that this extract would have a weak progesteronic or estrogenic effect which would act by positive feedback.Certainly, 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 (Lechat, 2006);Annabelle, 2018 and Emmanuelle, 2020).This is the

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paradoxical effect of hormones. The phytochemical study of the aqueous extract of the rhizomes of Zingiber officinal (Ginger) revealed the presence of flavonoids, tannins, alkaloids, anthraquinones, steroidsand the absence of osides and mucilages.The results obtained are comparable to those obtained by Amari, 2016;Ashraf et al., 2017;Meghezzi, 2018;Asamenew et al., 2019;Bashige et al., 2020;Sekoura and Meya, 2021;Fotsing et al., 2022. These authors have highlighted these different metabolites and established a link between them and the different therapeutic effects of Ginger rhizomes. Presently, ginger is the subject of numerous botanical, chemical and toxicological researches in order to prove its scientific effectiveness on a medical level as well as its safety.The medical and therapeutic as well as culinary use of ginger is developing strongly according to Foine (2017), cited by Sekoura and Maya (2021).

5-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.It appears that this aqueous extract is slightly toxic in mice, with an LD50 of 5000 mg/kg.

At a dose of 600 mg/kg in rats, it would present estrogenic properties which are manifested by blocking the sexual cycle in estrus and reduce the plasma level of estradiol with 17 β estradiol and the reference molecule.

At a dose of 300 mg/kg, the extract would exhibit progesteronic or weakly estrogenic properties which more or less disrupt the sexual cycle and significantly increase the plasma level of estradiol.

It would be desirable to evaluate subacute 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 improve traditional medicine in the treatment of reproductive disorders.

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