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Effects of a recipe based on three leafy vegetables consumed in Brazzaville on the reproductive function of the wistar rat

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ABSTRACT

Spinacia oleracea, *Amaranthus hybridus* of the Chenopodiaceae family and *Brassica campestris* of the Brassicaceae family are vegetables consumed in Brazzaville for their nutritional virtues. The aim of this study is to evaluate the effects of the recipe based on these three leafy vegetables on reproductive functions. The recipe at doses of 250, 500 and 1000 mg/Kg was administered to male and female rats. The aphrodisiac effects of the recipe in males were evaluated on the numbers of sexual mounts, erections, ejaculations, and latency time; and the estrogenic activities in females, based on the variation of the sexual cycle, the state of the vaginal meatus and the cervical mucus. On acute toxicity, the results show that the aqueous extract (EA) of the recipe does not alter the general condition and behaviour of mice, and no mortality was recorded. In male rats the 500 mg/Kg recipe extract significantly increased the number of sexual mounts, erections, and ejaculations; and decreased the latency time. In females, the 500 mg/kg dose of the extract caused blocking of the cycle in oestrus and pro-oestrus, with increased cervical mucus and vaginal opening. Estradiol levels were also increased. The flavonoids, saponosides and steroids observed in this extract could be responsible for the effects observed on the sexual parameters studied in male and female rats.

Keywords: *Amaranthus hybridus*, *Brassica campestris*, *Spinacea oleacea*, Plant extract, Sexual Parameters.

INTRODUCTION

The food factor considerably influences the different functions of the organism among which, the cardiac frequency, the regulation of the transit and the reproductive performances. Siransy-Balaysac, ^[1] has shown that the quantity of flavanols contained in cocoa can influence the blood pressure of some people. It is known that in pregnant women, the level of isoflavones contained in soy flour can disturb the reproductive function of the mother and child.

Numerous studies now show that fertility is closely linked to the nutritional status of the body ^[2]. Indeed, an imbalance in the quality or quantity of nutrients in a food can lead to disorders of the reproductive function. According to Niangaly *et al.* ^[3]; disorders such as: erectile dysfunction, sexual impotence and infertility can influence human and animal sexual life and activity from time to time. These are multifactorial disorders that are related to age, cardiovascular disease, smoking, hormonal factors, certain drugs and diet.

Thus, toxicological, phytochemical and pharmacological studies of the food consumed are becoming more and more necessary in order to investigate possible links between certain disorders of the body and the food ingested. However, vegetables consumed in the human diet have not been studied at all in the laboratory. It is in this context that this study is being carried out to evaluate the effects of leafy vegetables regularly consumed in Brazzaville on the reproductive function in men and women.

The aim of the present work is to evaluate the effects of the recipe based on three leafy vegetables composed of *Amaranthus hybridus* L., *Spinacia oleracea* and *Brassica campestris* L. on the reproductive function in male and female rats.

MATERIALS AND METHODS

Collection and identification of vegetable leaves

The leaves of *Amaranthus hybridus* L, *Brassica campestris* L and *Spinacea oleacea* were purchased in the "Total de Brazzaville" state market after the ethnobotanical survey. They were identified and compared with the reference sample of the national herbarium at the Institute National de Recherche en Sciences Exactes et Naturelles (IRSEN) in Brazzaville. These leaves were dried in the shade and then ground with a mortar.

Preparation of the extract of the recipe

The aqueous extract of the leaves of *Spinacea oleacea*, *Amaranthus hybridus* and *Brassica campestris* was prepared by decoction at 10%. 50 g of the mixture of the three vegetables (i.e., 16.666 g of each powder) were boiled for 15 minutes. After cooling, the resulting mixture was filtered through cotton wool and evaporated at a temperature of 50-60°C. The dry extract obtained is used for toxicological, aphrodisiac and oestrogenic tests.

Animal material

Mice with a body weight between 20 and 25 g and Wistar rats, weighing 130 and 220 g were used in the tests. All these animals were provided by the animal house of the Faculty of Science and Technology of the Marien Ngouabi University of Brazzaville. These animals were kept under standard conditions (12 hours of light and 12 hours of darkness). They all had free access to food and tap water.

Assessment of acute toxicity

Acute toxicity was carried out according to the Organisation for Economic Co-operation and Development (OECD) guideline N° 423.

Two batches of three (3) mice each were made up. They receive orally respectively:

- ❖ Batch 1: 0.5 mL/100 g distilled water;
- ❖ Batch 2: 5000 mg/kg of aqueous extract of the recipe.

The general condition and behaviour of each animal was assessed during the first 4 h. The rate after 48 h. The weight evolution of each animal, the water and food consumption are noted for 14 days.

Effect of the aqueous extract of the three-leaf vegetable recipe on sexual parameters in rats

The method reported by Akassa et al, [4] was used to evaluate the aphrodisiac effects of the food recipe. Rats were divided into 5 batches of 4 rats each and given for seven days:

- ❖ Batch 1 control, distilled water (1 ml/100 g per day);
- ❖ Batch 2, the reference molecule, Viagra (10 mg /kg per day);
- ❖ Batches 3, 4 and 5, the aqueous extract of the recipe at doses of 250, 500 and 1000 mg/kg per day respectively.

The females were previously treated before mating for 72 hours with oestradiol valerate at a dose of 600 µg/kg per day to make them receptive.

Six hours after the last administration of the products, each rat was placed in a mating cage with a treated rat for one hour to assess male sexual parameters on the second, fifth and seventh day of treatment. The parameters assessed were: numbers of sexual mounts, erections and ejaculations and latency time.

Effect of the aqueous extract of the three-leaf vegetable recipe on sexual parameters in female rats

The method reported by Peneme et al, [5] was used to evaluate the effect of the recipe on female sexual parameters. Indeed, 4 batches consisting of 4 rats each were given the products orally for seven (7) days according to the following protocol:

- ❖ Batch 1 control, distilled water at the dose of 1 mL/100g;
- ❖ Batch 2, the reference molecule, Estradiol Valerate 2 mg at the dose of 600 µg/kg;

- ❖ Batches 3 and 4, the extract from the recipe, at doses of 250 and 500 mg/kg, respectively.

Vaginal smears were taken each morning in female rats before administration of the products. The effect of the aqueous extract was evaluated on the estrous cycle, mucus and cervical mucus status, and plasma estradiol levels.

Phytochemical screening of the recipe

The phytochemical screening of the recipe was carried out to reveal the presence of chemical groups contained in the aqueous extract of the leaves of *Spinacea oleacea*, *Amaranthus hybridus* L and *Brassica campestris* L. For this purpose, the tube staining and precipitation reactions were used [6].

RESULTS

Acute toxicity of the aqueous extract of the three leafy vegetable recipes

No change in the general condition and behavior of the mice compared to the control batch. No mortality was observed after 72 hours.

Figures 1 and 2 show the changes in food and water consumption for 14 days following acute toxicity, respectively. Mice treated with a single dose of 5000 mg/kg showed low food and water consumption compared to control mice.

Effects of the aqueous extract of the recipe on the number of erections

Figure 4 shows that at the dose of 500 mg/kg the recipe causes a significant increase in the number of erections like the reference molecule. While at the doses of 250 and 1000 mg/Kg, the recipe causes a more or less significant decrease in the number of erections ($p < 0.01$) compared to the control batch.

Effects of the aqueous extract of the recipe on the number of ejaculations

Figure 5 shows that at the dose of 500 mg/Kg, the recipe causes an increase in the number of ejaculations like the reference molecule. While at the doses of 250 and 1000 mg/Kg the extract causes a more or less significant decrease in the number of ejaculations.

Effect of the aqueous extract of the three leafy vegetables recipe on sexual parameters of the rat

Effect of the aqueous extract of the recipe on the sexual cycle

Daily vaginal smears taken during the 7 days of administration of the products were used to monitor the evolution of the oestrous cycle of the rats based on the eosinophilic index of each rat. Figure 7 shows the variation curves of the eosinophilic index of the rats of each treated batch. It can be seen from this figure that the animals treated with the aqueous extract of the recipe at a dose of 500 mg/kg show a blocked sexual cycle at the oestrus and pro-oestrus stage as in the rats treated with oestradiol valerate, the reference product. In contrast, the batch treated with the 1000 mg/kg extract showed a normal cycle like those treated with distilled water.

Effect of the aqueous extract of the recipe on the vaginal meatus and the cervical mucus

Table 1 shows the variation of the vaginal meatus and the state of the cervical mucus during the administration of the products. It shows that the extract at dose of 500 mg/kg leads to an increase in vaginal opening with stringy and clean mucus as with the reference molecule.

The dose of 1000 mg/kg of the recipe does not cause any variation in the sexual cycle.

Effect of the aqueous extract of the three leafy vegetable recipes on plasma estradiol levels

Figure 8 shows the results of the estradiol determination of the rats of each batch. It shows a non-significant increase in estradiol levels in rats treated with the 500 mg/kg recipe and a decrease with the 1000 mg/Kg recipe compared to the control.

Phytochemical profile of the recipe based on three leafy vegetables

The major chemical families present in the recipe were determined by the tube staining and precipitation reactions with specific chemical reagents [6]. The phytochemical screening results of the recipe of *Amaranthus hybridus* L, *Spinacia oleracea* L and *Brassica campestris* L. leaves are presented in Table 2.

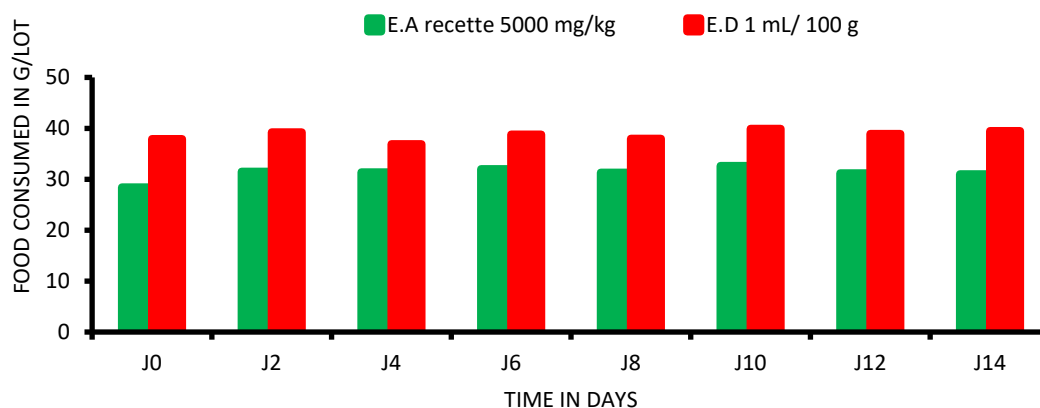


Figure 1: Effect of the aqueous extract of the recipe on the food consumption of mice

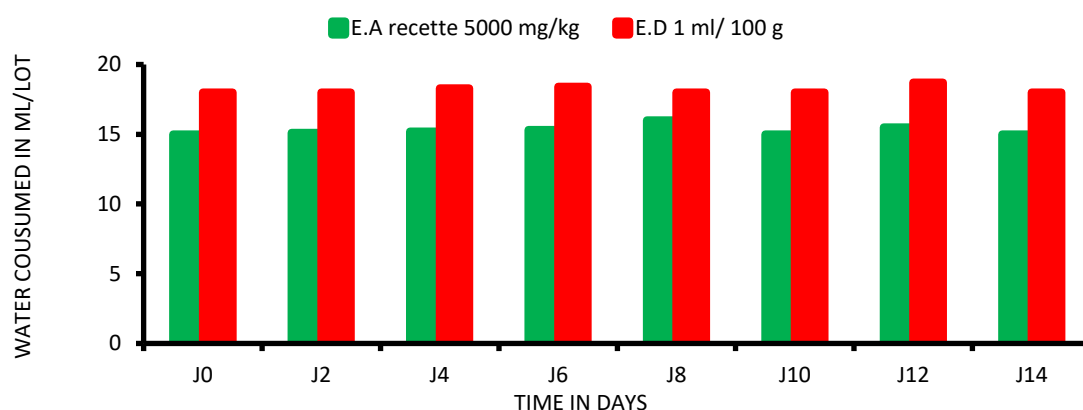


Figure 2: Effect of the aqueous extract of the recipe on the water consumption of mice

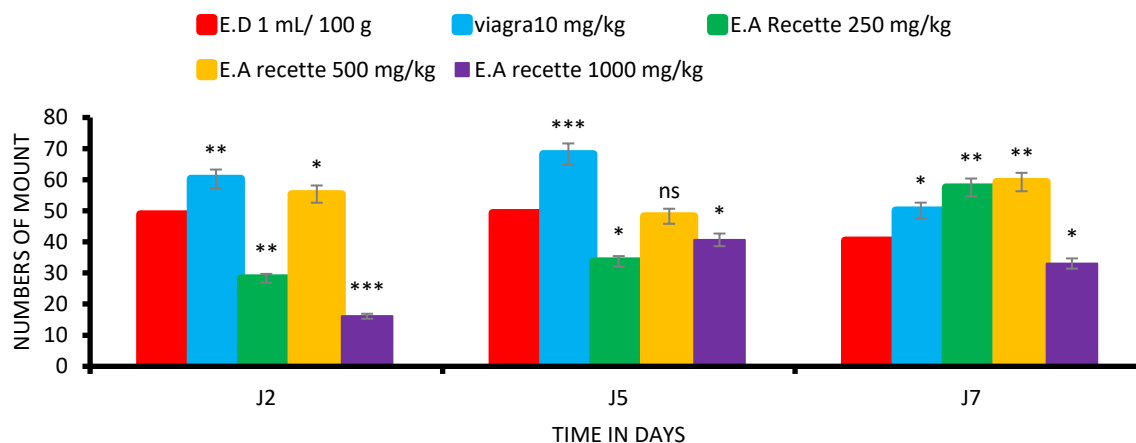


Figure 3: Effect of aqueous extract of the recipe on the number of mounts in rats

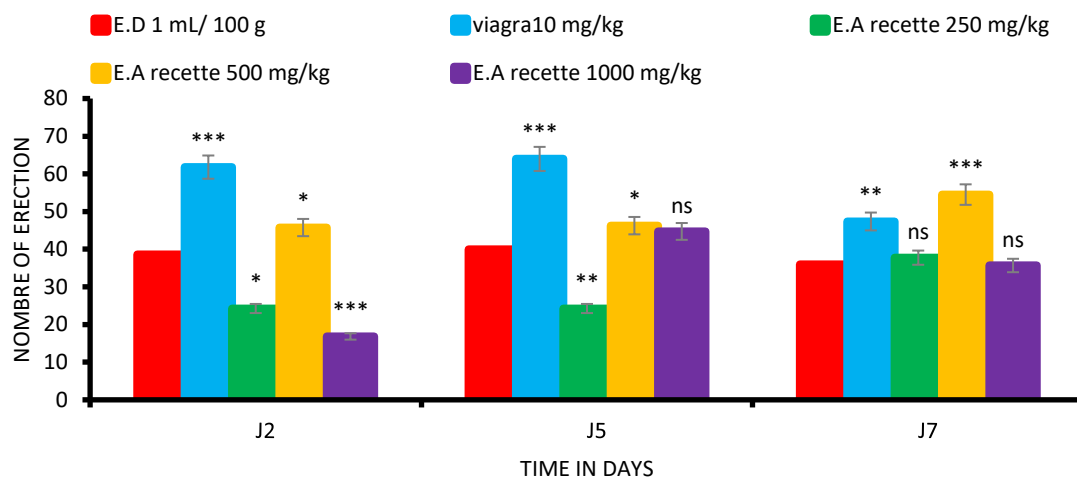


Figure 4: Effect of the aqueous extract of the recipe on the number of erections in rats

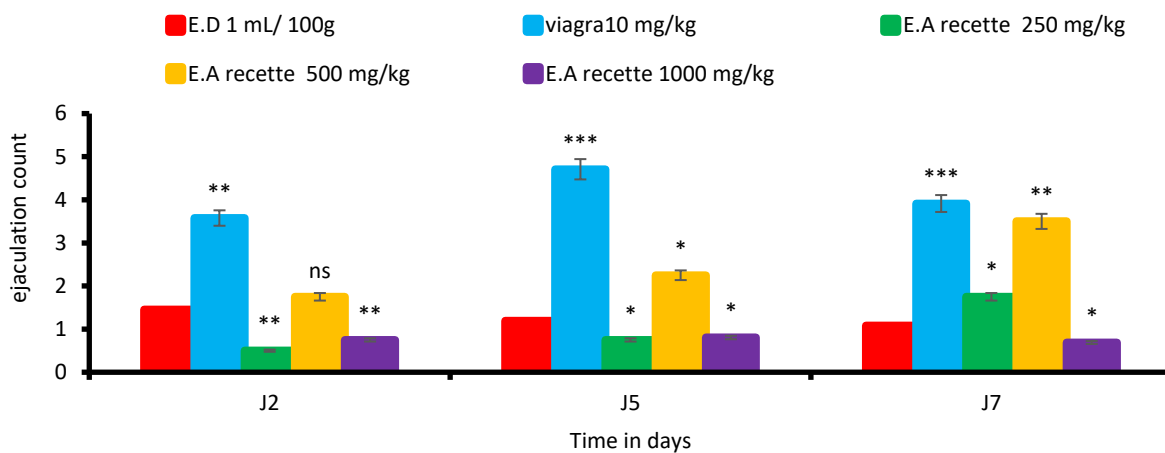


Figure 5: Effect of the aqueous extract of the recipe on the number of ejaculations of the rat

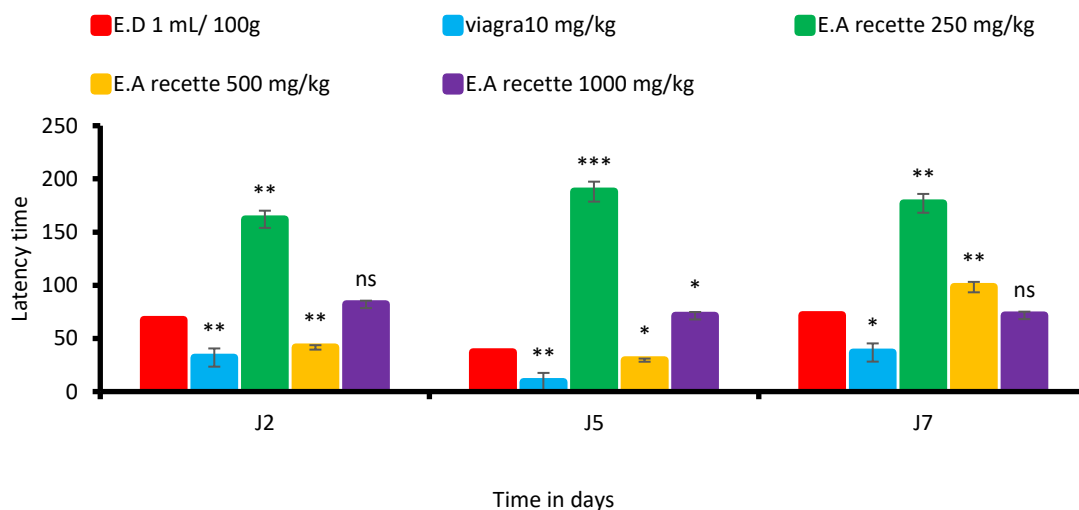


Figure 6: Effect of the aqueous extract of the recipe on the latency time in rats

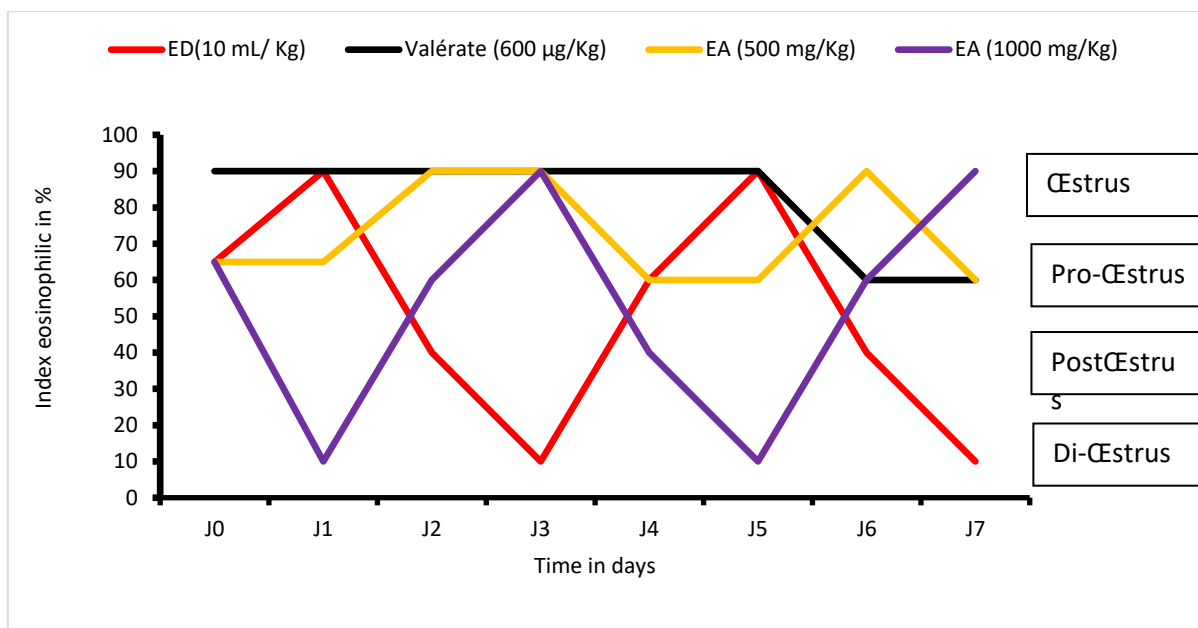


Figure 7: Variation in the eosinophilic index of rats under the effect of the aqueous extract of the recipe based on three leafy vegetables

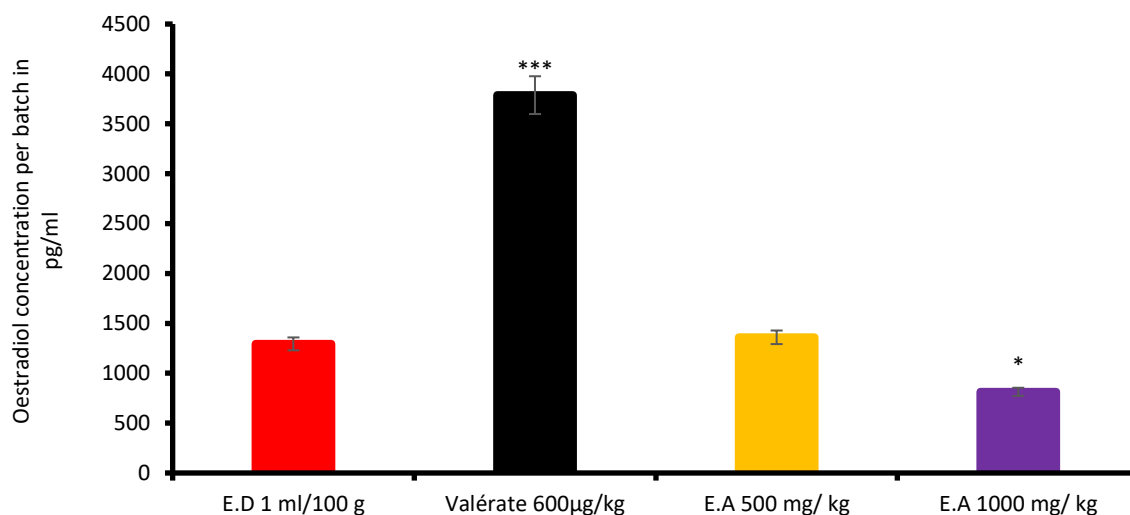


Figure 8: Effect of aqueous extract of the recipe on plasma estradiol levels

Table 1: Effect of food recipe on the vaginal meatus and the appearance of cervical mucus

Batch	Condition of mucus and vaginal meatus			
	Pro-oestrus	Oestrus	Post- oestrus	Di- oestrus
Distilled water 1 mL/100 g	CM m± open	Stringy CM m open	DM±viscous m± tight	DM visqueux m tight
Estradiol valerate 600 µg/kg	Stringy CM m open	Stringy CM m open	CM m±open	CM m±open
EA of the recipe 500 mg/kg	Stringy CM m open	Stringy CM m open	CM m open	CM m open
EA of the recipe 1000 mg/kg	CM m±open	Stringy CM m open	DM ± viscous m± tight	DM viscous m tight

M: Mucus; CM: Clean mucus; DM: Dirty mucus; m: meatus; ±: more or less

Table 2: Phytochemical profile of the recipe based on the three leafy vegetables

Chemical composition	Result
Tannin	++
Alkaloids	+
Flavonoids	++
Oses-holosides	++
Mucilage	++
Free anthraquinone	++
Triterpenoid steroid	++
Saponins	+

(+): Presence; (++) : High presence

DISCUSSION

This work was carried out with the aim of evaluating the effects of a recipe based on three leafy vegetables consumed in Brazzaville on the reproductive function of the wistar rat. The acute toxicity study of the recipe in mice was done to determine the safety margin of the recipe and the pharmacological doses used during the tests. The extract, at a dose of 5000 mg/kg, showed no discernible or visible signs of toxicity after 4 hours and no mortality after 72 hours. Thus, its LD₅₀ would be above 5000 mg/kg according to the Lu scale. The decrease in water and food consumption in mice is probably related to the high dose of toxicity that caused anorexia in the treated animals. The administration of the recipe at the dose of 500 mg/kg caused, like Viagra at the dose of 10 mg/kg, an increase in the number of sexual mounts, which indicates that this recipe reinforces the physical contact and attachment between the male and the female; this suggests that this extract stimulates sexual desire in the male rat, a state in which an individual wishes to experience a sexual act. This recipe could have an aphrodisiac effect at the dose of 500 mg/Kg. However, at doses of 250 and 1000 mg/Kg the number of sexual mounts was reduced. This result can be explained by the fact that sexual desire is dependent on testosterone, also called the libido hormone. A significant change in the level of this hormone can have the opposite effect. The aqueous extract of the recipe at a dose of 500 mg/kg caused an increase in erections in rats. This result suggests that the extract increases libido and has a positive effect on the blood flow in the penis, which is responsible for the erection. Furthermore, it is known that flavonoids and alkaloids may also act by relaxing the smooth muscles of the corpora cavernosa in the copulatory organ of male rats, thereby facilitating blood flow to the erectile bodies that promote erections [7]. Indeed, chemical screening of the aqueous extract of this recipe revealed the presence of alkaloids, known for their ergogenic properties, inducing vasodilation through sexual stimulation, causing the release of carbon monoxide which is one of the main mediators of erection. Thus, the presence of alkaloids in this extract corroborates the observations of this author. The decrease in erection with the doses of 250 and 1000 mg/Kg indicates that these doses were probably too low and too high to allow normal erection in the treated rats. The number of ejaculations was also increased with the extract at the 500 mg/Kg dose. These results suggest that the aqueous extract of the recipe promotes orgasm in rats. Indeed, ejaculation is controlled by the same nerve centres as erection. When the same nerve impulses that triggered the erection continue to increase in intensity to a certain critical threshold; they will lead to ejaculation [8]. Fouche *et al.*, [9] showed that the aqueous extract of the aerial parts of *Monsonia angustifolia* increases the number of ejaculations in rats. This extract is composed of flavonoids, alkaloids, saponosides, sterols and terpenes, tannins and anthraquinones. The chemical screening of the three vegetables recipe also revealed the presence of alkaloids, flavonoids and steroids, chemical compounds comparable to those obtained by the above-mentioned authors. The number of ejaculations is probably related to the chemical groups contained in the consumed vegetable recipe [10]. The aqueous extract of the recipe at the dose of 500 mg/kg decreased the latency time like Viagra during the first two

phases of observation of the mounts. This result indicates that this recipe would have a positive effect on the sexual performance and resistance of the rat. These results are like those obtained by Akassa *et al* [4] and Morabandza *et al* [11] who respectively showed that *Pausinystalia yohimbe* bark and aqueous and hydroethanol extract of *strychnos camptoneura* decreased the latency time between two consecutive mounts. In females, the aqueous extract of the three leafy vegetable recipes at a dose of 500 mg/kg blocked the sexual cycle of rats in oestrus and pro-oestrus, as did the reference molecule Viagra. There was also an increase in mucus and vaginal meatus opening. These results reflect a strong estrogenic impregnation of the vaginal mucosa induced by the aqueous extract of the leaves of *Amaranthus hybridus* L, *Spinacia oleracea* and *Brassica campestris* L at a dose of 500 mg/kg. This result suggests that this extract promotes follicular maturation, which is responsible for high oestradiol production. The maintenance of high oestradiol production throughout the period of administration of the aqueous extract of the three-vegetable recipe therefore does not allow for the normal ovulatory discharge of LH [12]. It is therefore possible that this extract causes anovulation by inhibiting the secretion of peak LH. Concerning the plasma estradiol level, the results obtained show that the administration of the aqueous extract of the three leafy vegetable recipes at a dose of 1000 mg/Kg resulted in a significant decrease of the plasma estradiol level in the treated animals compared to the animals that received distilled water. With the 500 mg/Kg dose there was a non-significant increase in estradiol levels whereas the reference molecule caused a significant increase in plasma estradiol levels. The decrease in plasma estradiol levels observed in animals given the 1000 mg/Kg dose may be explained by a negative feedback mechanism induced by an additional estrogen supply. The dose of 500 mg/Kg caused a non-significant increase in blood estradiol levels, while at this dose the sexual cycle of the rats is blocked in the estrus and pro-estrus stage, with opening of the vaginal meatus. This result shows that the estrogenic effect of the recipe does not disturb the hormonal level of the rats. The phytochemical profile of the aqueous extract of the recipe revealed the presence of flavonoids, steroids, mucilages, saponins and other chemical families. These results are comparable to those of Peneme *et al.* [13] who observed estrogenic effects with aqueous extracts of *B. coriacea*, possessing almost the same chemical groups. It is known that steroids promote the aromatisation of testosterone to estrogen, in addition flavonoids promote the aphrodisiac effect [14]. Indeed, the estrogenic and aphrodisiac effects observed with this recipe are probably related to the presence of steroids and flavonoids contained in the recipe. In addition, the saponins by their leaching power increase the use of cholesterol in the synthesis of sex hormones [15].

CONCLUSION

The acute toxicity study shows that the extract at a dose of 5000 mg/kg does not modify the general behaviour of mice, nor does it lead to their death. This extract at 500 mg/Kg has aphrodisiac and oestrogenic effects in rats. It causes a non-significant increase in estradiol levels. Chemical screening revealed the presence of flavonoids, steroids, saponins, which could be responsible for the

observed effects. A study of the aqueous extract of this recipe on female rat parturition deserves to be carried out.

Conflict of Interest

The authors declare that they have no conflicts of interest related to the publication of this study.

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