Original Research Article

EVALUATION OF THE EFFECTS OF AQUEOUS EXTRACT OF *SARCOCEPHALUS LATIFOLIUS* ON SEXUAL BEHAVIOUR IN MALE RATS

ABSTRACT

Background: *Sarcocephalus latifolius,* a plant of the Rubiaceae family, is found exclusively in tropical Africa, more particularly in sub-Saharan West Africa, stretching from Senegal to Zaire, but is also found as far east as Sudan (Sudan-Guinean zone). The leaves of this plant are used in traditional African medicine to treat numerous pathologies such as infertility.

Aims/Objective: The present study aims to evaluate the effects of the aqueous extract of *Sarcocephalus latifolius* leaves on sexual parameters in male rats. .

Methods: The sexual parameters (latency of mating, latency of intromission, latency of ejaculation and mean copulation interval) were assessed by the classical method by administering distilled water, sildenafil citrate (reference molecule) called Viagra at the dose of 5 mg/kg of body weight and the aqueous extract of *Sarcocephalus latifolius* leaves at the dose of 1000 mg/kg of body weight per day for eight days to three batches of male rats respectively. These parameters were determined over a period of thirty minutes.

Results: The results obtained show that the aqueous extract of *Sarcocephalus latifolius* leaves at a dose of 1000 mg/kg of body weight significantly ($P < .001$) decreases the latency time of mating and the latency time of intromission, whereas this extract significantly ($P < .001$) increases the latency time of ejaculation and the average copulation interval.

Conclusion: Aqueous extract of *Sarcocephalus latifolius* leaves has aphrodisiac properties which are due to sterols, flavonoids and saponosides.

Key words: *Sarcocephalus latifolius,* aphrodisiac, sildenafil citrate.

1. INTRODUCTION

Reproduction ensures the continuity of the species through the transmission of life and the formation of a new living being. In the human species, reproduction is sexual and associated with the existence of the genitalia which ensure the production of gametes and their meeting during fertilisation. There are therefore male and female individuals capable of producing reproductive cells (spermatozoon in men and egg in women). Couples face many reproductive health problems, including the problem of infertility.

Infertility for a couple is defined as failure to conceive after at least 12 months of unprotected sex [1]. In men, it is estimated at 50% and is caused by various factors including: erectile or ejaculatory dysfunction, sperm abnormalities (azoospermia; oligospermia; teratozoospermia; asterospermia; necrospermia...), congenital, immunological, iatrogenic and endocrine causes [1, 2]. In the case of erectile dysfunction, many medicinal plants are prescribed alone or in combination by traditional therapists.

Erectile dysfunction is a public health problem that seriously affects the quality of life of patients and their partners (NIH Consensus Development Panel on Impotence, 1993). It often reflects a man's lack of sexual performance to meet his own expectations, which in turn are influenced by cultural and social factors [3].

More than 80% of the population in developing countries use herbal medicines for their health problems [4].
Despite this extensive use, very little work has been undertaken to verify the safety and efficacy of multiple plants consumed in herbal medicine; notably *Sarcocephalus latifolius* used in reproduction as an emmenagogue, abortifacient and aphrodisiac [5; 6; 7].
The present study aims to evaluate the properties of aqueous extract of *Sarcocephalus latifolius* leaves on sexual behaviour in male rats.

2. MATERIALS AND METHODS

2.1.Plant material

Fresh leaves of *Sarcocephalus latifolius* were collected in the Savannah Region, Department of Korhogo (Côte d'Ivoire). A sample of this plant was identified at the Centre National Floristique of the University Felix HOUPHOUËT-BOIGNY.

2.2. Animal material

Male rats and ovariectomised female rats of the species *Rattus norvegicus* and of the Wistar strain, weighing between 200 and 230 g, aged approximately 12 weeks, were used. The rats and mice were reared in the animal house of the Ecole Normale Supérieure d'Abidjan where the average temperature is 28 ± 3 °C with a relative humidity of 70%. The photoperiod was 12/24 hours. The animals had free access to water and food.

2.3. Preparation of the aqueous extract

The collected leaves of *Sarcocephalus latifolius* were rinsed with distilled water, dried in the shade (out of the sun). The dried leaves were pulverized with a Moulinex type electric grinder. The powder obtained was used for the preparation of the aqueous extract.
Thus 50 g of powder were macerated in 1L of distilled water and stirred for 3 minutes. After filtration on Hydrophilic cotton and on wattman paper, the filtrate obtained was evaporated at 55°C using an oven.

2.4. Phytochemical study method

Phytochemical screening is a set of methods and techniques for the preparation and analysis of natural plant organic substances.
It was carried out in order to highlight the presence or absence of certain chemical constituents of pharmacological interest via qualitative analysis techniques described in the literature [8].

2.5. Acute toxicity

Acute toxicity was performed in rats according to the Organisation for Economic Co-operation and Development (OECD) guideline 423 [9].

2.6. Ovariectomy of spleens

Ovariectomy was carried out following the technique of Cariton [10]. The animals were kept fasting for 12 hours and anaesthetised with ether. As soon as the effects of anaesthesia were effective, the dorsal lumbar area was bilaterally depilated and the skin cleaned with 95° ethyl alcohol. A dorsal incision of approximately ¾ of a cm penetrating the abdominal cavity was made. Peri-ovarian adipose tissue was identified and isolated. The externalised ovary was sectioned at the intersection with the uterine horns, the tip of which was cauterised with a soldering iron. The peritoneum and skin were then sutured, after the organs had been replaced. Penicillin ointment and liquid Betadine were used until complete healing, to disinfect the wounds and prevent infection. Ovariectomised females were used 21 days after surgery.

2.7. Induction of oestrus in ovariectomised females

Females were artificially induced into oestrus (heat) [11] using the method of Szechtman *et al.*[12]. A suspension of ethinyl estradiol (Lynoral tablets, Organon Pharma) was first administered orally at a dose of 100 µg per animal. Forty-eight (48) hours later progesterone (Dubaget tablets, Glenmark Pharma) was injected subcutaneously at a dose of 1 mg/animal.

2.8. Experimental protocol

The experimental batches were constituted according to the classical method described by Carro- Carro-Juarez *et al.*[13].

Three (3) batches of five (5) male rats were treated orally every 3 days for 7 days as follows

- batch 1 (control) received distilled water at a dose of 1ml/kg;
- batch 2 received sildenafil citrate (reference molecule) called Viagra at a dose of 5 mg/kg of body weight.
- batch 3 received the aqueous extract of *Sarcocephalus latifolius* leaves at a dose of 100 mg/kg of body weight. To improve the externalization of sexual manifestations to be observed, the rats were exposed to ovariectomized females in estrus (twenty 15 rats). This exposure was made six (6) hours after induction of estrus in these rats.

Six (6) hours after dosing, the rats were placed in the presence of the females to observe sexual behavioural parameters and possible mating.

Sexual parameters were observed on each rat for 30 minutes:

-The mating latency time which is the time interval between the introduction of a female into the cage and the first mating;

-The intromission latency time which is the time interval between the introduction of the female into the cage and the first intromission;

-The ejaculation latency time which is the time interval between the first intromission and the first ejaculation.

-The average copulation interval which is the time interval between the first intromission of a series and the ejaculation marking the end of the series.

The evaluation of the aphrodisiac properties of the aqueous extract of *Sarcocephalus latifolius* leaves on the sexual behaviour of rats was carried out following the protocol used by Mbongue [14]**.**

2.9. Statistical Analysis

The statistical analysis of the values and the graphical representation of the data were carried out using Graph Pad Prism 8 software (San Diego, California, USA). The statistical difference between the results was carried out using the analysis of variances (ANOVA), followed by the Tukey-Kramer multiple comparison test.

When $P < 0.05$ the difference is said to be significant. All values are presented as mean ± SEM (Standard Error on the Mean)

3. RESULTS

3.1. Phytochemical study

This study revealed polyphenols, flavonoids, catechic tannins, saponosides, alkaloids, sterols and polyterpenes in the aqueous extract of *Sarcocephalus latifolius* leaves. It did not reveal the presence of gall tannins.

3.2. Acute toxicity by OECD 423 in rats

The aqueous extract administered orally at a dose of 5000 mg/kg of body weight did not induce tremors, changes in respiratory rate, convulsions, salivation, diarrhoea, coma,

backward walking and self-mutilation in the animals. There were no deaths during the 14 days following administration of the extracts.

3.3. Effect of aqueous extract of *Sarcocephalus latifolius* leaves on mounting latency

Figure 1 shows the effect of aqueous extract of *Sarcocephalus latifolius* leaves on the latency of mounting on days 1 and 8 of the experiment.

At a dose of 1000 mg/kg of body weight, the aqueous extract of *Sarcocephalus latifolius* leaves caused a significant ($P < .001$) decrease in sexual mating latency on days 1 and 8, respectively, compared to distilled water. With the aqueous extract, the latency of sexual mating on days 1 and 8 was 93.2 ± 5.57 seconds and 263.4 ± 21.23 seconds, respectively, compared to control rats whose latency of sexual mating on days 1 and 8 was 430.6 ± 3.17 seconds and 539.40 ± 31.84 seconds, respectively. The rise latencies induced by *Sarcocephalus latifolius* aqueous extract on days 1 and 8 are lower than those induced by sildenafil citrate. These latency times induced by sildenafil citrate are 113.8 ± 2.08 and 297.6 ± 7.57 seconds on days 1 and 8 respectively.

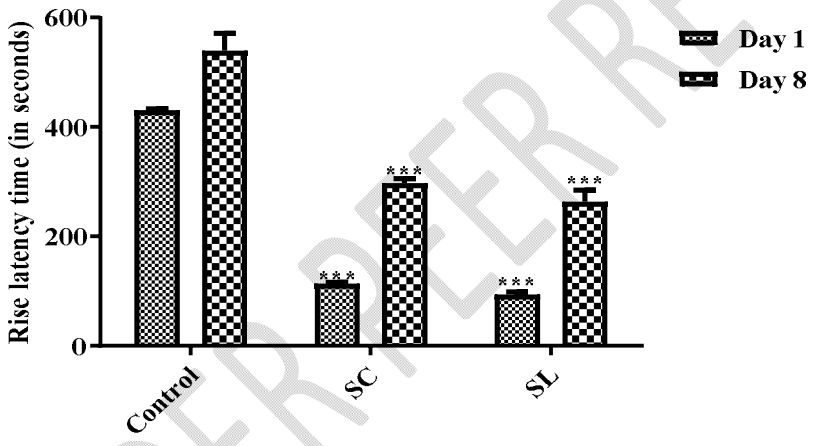

Figure 1: Effect of aqueous extract of *Sarcocephalus latifolius* leaves on mounting latency.
Values are means ± MSE, n = 5, *** $P < .001$, significant difference from control rats
SL: *Sarcocephalus latifolius*
SC: Sildenafil Citrate

3.4. Effect of aqueous leaf extract of *Sarcocephalus latifolius* on intromission latency

Aqueous extract of *Sarcocephalus latifolius* leaves at a dose of 1000 mg/kg of body weight caused a significant ($P < .001$) decrease in intromission latency. On days D1 and D8, it was 87.4 ± 4.75 seconds and 116 ± 3.20 seconds respectively compared to 329.00 ± 3.42 seconds and 371.6 ± 39.49 seconds in control rats.

A decrease in intromission latency was also observed with sildenafil citrate (5 mg/kg of body weight) with respective latencies of 33.2 ± 2.47 seconds and 48.2 ± 21.13 seconds on days D1 and D8 **(Figure 2).**

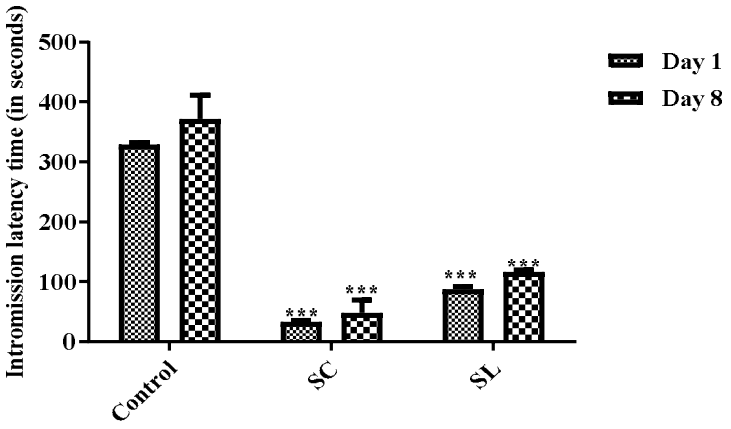

Figure 2: Effects of aqueous extracts of *Sarcocephalus latifolius* leaves on intromission latency
Values are means ± MSE, n = 5, *** $P < .001$, significant difference from control rats
SL: *Sarcocephalus latifolius*
SC: Sildenafil Citrate

3.5. Effect of aqueous extract of *Sarcocephalus latifolius* leaves on ejaculation latency time

Figure 3 shows the effect of aqueous extract of *Sarcocephalus latifolius* leaves on the ejaculation latency time on days 1 and 8.

Aqueous extract of *Sarcocephalus latifolius* leaves at a dose of 1000 mg/kg of body weight caused a significant ($P < .001$) increase in ejaculation latency time on days 1 and 8 compared to distilled water. These latency times for aqueous extract of *Sarcocephalus latifolius* leaves are 137.2 ± 6.24 and 247.6 ± 4.27 seconds at D1 and D8 respectively. With sildenafil citrate (5 mg/kg of body weight) the ejaculation latency was 138.2 ± 7.14 seconds and 271 ± 8.42 seconds ($P < .001$).

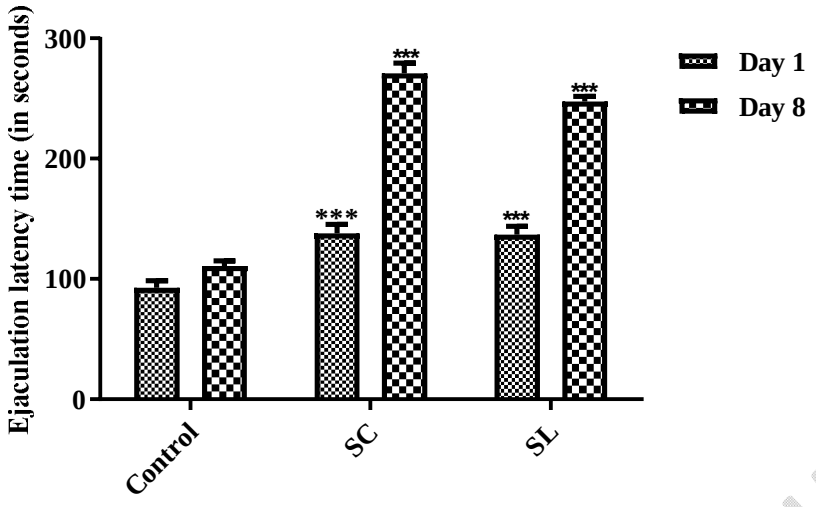

Figure 3: Effect of aqueous extract of *Sarcocephalus latifolius* leaves on ejaculation latency time

Values are mean ± MSE, n = 5, *** $P < .001$, significant difference from control rats

SL: *Sarcocephalus latifolius*

SC: Sildenafil Citrate

3.6. Effect of aqueous extract of *Sarcocephalus latifolius* leaves on the mean copulation interval

Figure 4 shows the effect of *Sarcocephalus latifolius* extract on the mean copulation interval on days 1 and 8.

The aqueous extract causes a significant increase ($P < .001$) in the mean copulation interval on days 1 and 8 compared to distilled water. It was 142 ± 3.20 and 126.8 ± 5.31 seconds for aqueous leaf extract compared to 61.2 ± 5.27 and 84.4 ± 6.38 seconds for distilled water at D1 and D8 respectively.

Sildenafil citrate (5 mg/kg of body weight) also caused a significant ($P < .001$) increase in the mean copulation interval (122.8 ± 1.65 seconds and 126.80 ± 2.7 seconds on days 1 and 8 respectively).

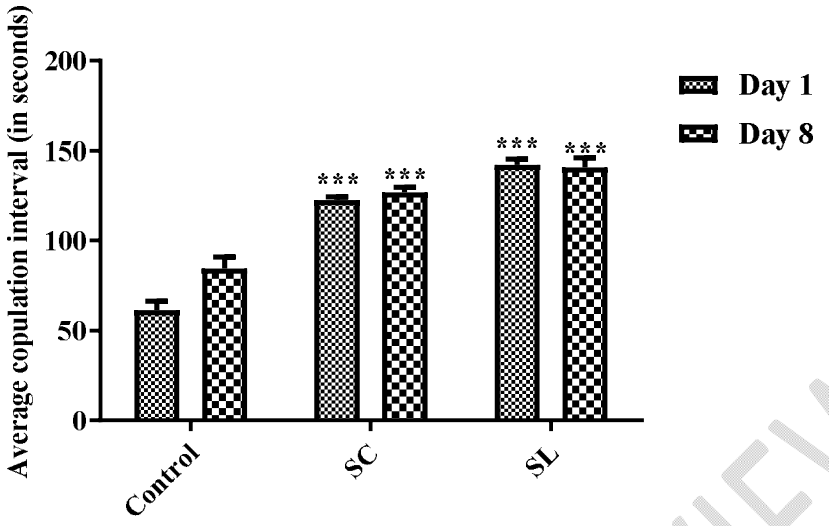

Figure 4: Effect of aqueous extract of *Sarcocephalus latifolius* leaves on mean copulation interval
Values are means ± MSE, n = 5, *** $P < .001$, significant difference to control rats
SL: *Sarcocephalus latifolius*
SC: Sildenafil Citrate

4. DISCUSSION

Phytochemical study of the aqueous extract of *Sarcocephalus latifolius* leaves revealed the presence of chemical compounds such as polyphenols, flavonoids, catechic tannins, saponosides, alkaloids, sterols and polyterpenes. These results are similar to those obtained by other authors [15; 16; 17]. On the other hand, some authors [18] have highlighted the same chemical compounds except for alkaloids.

No mortality and signs of toxicity were observed after administration of the dose limit (5000 mg/kg of body weight). These results on the acute toxicity of the aqueous extract of *Sarcocephalus latifolius* leaves have shown that the lethal dose of this plant is greater than the 5000 mg/kg of body weight limit dose.

The results obtained show that the aqueous extract of *Sarcocephalus latifolius* leaves decreases the latency time of sexual mounts and intromission but increases the latency time of ejaculation and the mean copulation interval. The decrease in the latency of sexual mounts and intromission shows that this extract stimulates sexual desire and has an aphrodisiac action [19]. These results are similar to those obtained by [20] with aqueous extract of *Bulbine natalensis* on the sexual behaviour of mature male rats.

The significant increase in the mean copulation interval in rats treated with the extract implies a sustained erection. Thus, the extract contains bioactive principles that maintain erection and increase sexual motivation. This is confirmed by the significant increase in ejaculation latency time in rats treated with the extract compared to the control. Indeed, it is generally accepted that in the normal, well-trained rat, mount and intromission latencies are copulatory parameters that are inversely related to sexual motivation, whereas erection is considered an indicator of sexual performance or libido [21]. Similar results were also obtained by [22] with

the aqueous extract of *Eurycoma longifolia* which resulted in a dose-dependent increase in mounts after a 10-day treatment.
On the other hand, it should be noted that there was no significant difference between the results of the copulation tests on day 1 and day 8. This would mean that the pro-sexual effects of *Sarcocephalus latifolius* would be established from the first day of treatment with an intensity that does not increase with the duration of the treatment. The aqueous extract of the leaves of *Sarcocephalus latifolius* would therefore be a non-androgenic aphrodisiac which would act directly on the erection:
- by stimulating the relaxation of penile cavernous tissues by various mechanisms, in particular a mechanism similar to that of sildenafil citrate. Indeed, sildenafil citrate, the reference molecule used in this study, is a non-androgenic aphrodisiac that acts directly on penile cavernous tissue. It inhibits the activity of phosphodiesterase-5, which prolongs the action of cGMP, the second messenger involved in the erection mechanism, and leads to muscle relaxation [23; 24].
-by acting directly on the central nervous system, in order to modulate the action of neurotransmitters and/or receptors involved in the sexual response in male rats; such a mechanism has been suggested to explain the effects of Yohimbine [25].
Furthermore, *Sarcocephalus latifolius* could, by stimulating the relaxation of penile tissues, lead to the local production of nitric oxide (NO), the main actor of erection [26].
These pro-sexual effects expressed in rats could be attributed to the presence of saponosides, flavonoids and alkaloids. Indeed, the steroidal nature of saponins could facilitate an intermediary role in the androgen production pathway [27]. Other studies have shown that flavonoids have antioxidant properties and are reported to elevate androgen levels in animals and may also contribute to the observed aphrodisiac effect [28, 29]. Another study had already shown that flavonoids are able to relax the corpora cavernosa, thus facilitating blood flow to these erectile bodies, which would promote erections [30]. Similarly, the presence of alkaloids, known for their ergogenic properties, can act either by inducing vasodilation through the production of NO and ultimately lead to erection; or by stimulating steroidogenesis in the testes of animals. Alkaloids may also act by relaxing the smooth muscles of the corpora cavernosa in the copulatory organ of male rats [30].

5. CONCLUSION

The study of aqueous extract of *Sarcocephalus latifolius* leaves does not cause any apparent toxicity when administered in a single dose. The aqueous extract of leaves of *Sarcocephalus latifolius* possesses, sexual stimulating activity or a potential aphrodisiac. The alkaloids, flavonoids, saponosides, terpenes and sterols contained in the aqueous extract of *Sarcocephalus latifolius* leaves could be responsible for these biological properties. These results confirm the traditional use of this plant.

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