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Effects of the aqueous extract of *Sarcocephalus latifolius* (smith) sheets on the reproduction parameters in male rats

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Abstract
The purpose of this study is to evaluate the effects of the aqueous leaf extract of *Sarcocephalus latifolius* (Rubiaceae), a medicinal plant much appreciated by african populations in general and ivoirians in particular, on the reproductive parameters of male Wistar rats. To achieve this work, forty adult male rats were divided into four groups of ten. The first group (control) received distilled water. The second, third and fourth groups were daily gauged with 1 ml of aqueous leaves extract, at the respective doses of 250; 500 and 1000 mg/kg body weight (bw), for 60 days. On day thirty-one of the treatment, five (5) rats of each lot were sacrificed and the rest, on day sixty-one. Parameters such as body weight, weight of androgen dependent organs, sperm number, motility and morphology, LH, FSH and testosterone levels of these rats were investigated. The results showed a significant increase in fresh weight ($p<0.001$) and dry weight ($p<0.01$) of the Cowper glands, fresh weight ($p<0.05$) and dry weight ($p<0.01$) of Levator Ani Bulbo Cavanous muscle (LABC) and dry weight ($p<0.05$) of the glans, at 1000 mg/kg bw, the number and normal spermatozoa with doses of 500 and 1000 mg/kg bw of extract, sperm motility rate and serum testosterone level at the three doses of extract compared to the controls at the end of treatment of 30 days. In addition, after treatment of 60 days, the results showed a significant increase of the prostate fresh weight ($p<0.05$), epididymis fresh ($p<0.05$) and dry weight ($p<0.01$) at 1000 mg/kg bw dose of extract and dry weight ($p<0.05$) of the epididymis with the 500 mg/kg bw aqueous extract, as well as a significantly increased of sperm count and sperm motility at a dose of 500 mg/kg bw of extract, normality at three doses of extract and testosterone level at 1000 mg/kg bw of extract compared to controls. All these results show that the aqueous extract of *Sarcocephalus latifolius* leaves would contain androgenic compounds or would have androgenic-like effects, with direct action on the testis.

Keywords: Androgeno-dependent, spermatozoa, testosterone, *Sarcocephalus latifolius*, androgen-like effects

1. Introduction
In all developing countries, such as Côte d'Ivoire, medicinal plants are the most widely used, especially in rural areas, as a means of solving public health problems [1]. According to WHO (2002) [2], 80% of people in african regions use traditional medicine. They are within reach of all stock exchanges and easily accessible. In all this rich african pharmacopoeia, one can quote *Sarcocephalus latifolius* (Smith). This plant belongs to the Rubiaceae family. It is a tree or a shrub with several trunks up to 9 to 12 meters. It is commonly referred to as "pêcher africain" in French, "African Peach" in English, "bâti" in Malinke, "tôlè" in Baoulé or "dohou-tou" in Guéré. It is widely used in traditional african medicine for the treatment of a variety of diseases.

All parts of this plant are used. In West Africa, it is used as an antimalarial, analgesic, antidiabetic, anti-abortif, treatment of sterility [3, 4]. In Côte d'Ivoire, infusions and decoctions of stems and roots of *Sarcocephalus latifolius* are used against malaria by traditional healers [5]. In Nigeria *Sarcocephalus latifolius* is used as a toothpick and as a remedy for stomach aches and tuberculosis [6]. In Benin and Nigeria, its roots are recognized by some practitioners of traditional medicine as having an antihypertensive effect. It is therefore widely used by traditional healers [7]. It has been studied for its androgenic effects [8], its antimalarial activities [9], antidiabetics in association with *Daniella oliveri* [10] and its analgesic effect due to the important amount of tramadol contained therein [11].

Most studies of male reproductive parameters have focused on the root extract of this plant. Indeed, the infusion of the whole roots of *Sarcocephalus latifolius* improves the fertility of the male rat [12], as well as the lipid extract of the whole roots [13]. The objective of this study is to

evaluate the androgenic activity of the aqueous extract of *Sarcocephalus latifolius* leaves on male reproductive functions in albino rats.

2. Material and methods

2.1 Plant

Samples of fresh leaves of *Sarcocephalus latifolius* were purchased from herbs sellers at Cocody market in Abidjan (Côte d'Ivoire) and authenticated at the National Floristic Center of Félix Houphouet-Boigny University, Abidjan, Côte d'Ivoire, where a specimen remains under the voucher n°-JBUA-321.

2.2 Preparation of plant extract

The washed *Sarcocephalus latifolius* leaves were dried in the shade for one week. The dried plant material was sprayed and 150 g was macerated in 800 ml of distilled water for 24 hours with Micro Vortex stirring. The collected filtrate was evaporated to dryness at 60 °C in an oven to give about 9.6 g of extract powder. This extract was then stored in a refrigerator at 4 °C for the preparation of fresh solutions from the distilled water at the required doses.

2.3 Animals

Forty adult and healthy albino rats (Rattus norvegicus) of about two months, weighing between 140 and 180 g and from the vivarium of ENS (Ecole Normale Supérieure) in Abidjan (Côte d'Ivoire) were used for experimentation. The animals were housed in cages and maintained under standard laboratory conditions (22 ± 3 °C, 12 h of light and 12 h of darkness and 40-60% of moisture), fed with commercial granules of FACI (Côte d'Ivoire) and tap water *ad libitum*. The rats were acclimatized for one week before the start of the experiment.

2.4 Experimental protocol

The rats were weighed and randomly divided into four groups of ten animals. Animals in group I (control) received distilled water. Those in Groups II, III and IV were dosed with 250, 500 and 1000 mg/kg bw of aqueous extract of *Sarcocephalus latifolius*, respectively. The extract and distilled water were administered orally in gastric intubation, daily for 60 consecutive days. After 30 days of treatment, five animals from each group were weighed and sacrificed. The remaining rats were sacrificed, on day sixty-one of the experiment under anesthetic ether, after reading their body weight. Blood samples were taken for hormonal assays. The testis, epididymis, adrenal gland, seminal vesicles, prostate, LABC, Cowper's glands pair and glan were dissected and weighed.

2.5Number, motility and morphology of spermatozoa

The analysis of the sperm was carried out according to the method described by Ngoula *et al*., (2007) [14]. The caudal epididymis was removed, incised and lacerated in 10 ml of a 0.9% saline solution previously heated to 36 °C.

2.6 Sperm count

A sample saline solution of sperm was collected and introduced into the chamber of the Malassez blade and the sperm counting was performed at microscope according Ngoula's method [14, 15].

2.7 Sperm motility

Sperm motility was immediately estimated after sperm collection. A drop of saline sperm solution was placed between the blade and slide and examined under a microscope (Olympus) at X400 magnification. The moving and immobile spermatozoa were rapidly counted on five random fields and the percentage of mobile forms was determined.

2.8 Sperm morphology

To evaluate sperm abnormalities, a smear was performed by spreading the sperm suspension on slides, stained with eosin and dried in ambient air. The colored slides were examined under a microscope using x400 magnification. From one end to the other of the blade, in a population of two hundred spermatozoa, normal and abnormal spermatozoa were estimated. The number of normal spermatozoa was expressed as a percentage of the total number.

2.9 Dosage of hormones

The blood taken from the dry tubes was centrifuged with a Rotofix 32 A centrifuge at 3000 rpm for 4 min. The sera collected are stored at -20 °C in the freezer for the determination of the hormones. The Enzyme Linked Fluorescent Assay (ELFA) technique was used to measure LH, FSH and testosterone levels in serum or plasma using VIDAS (BIOMERIEUX, France).

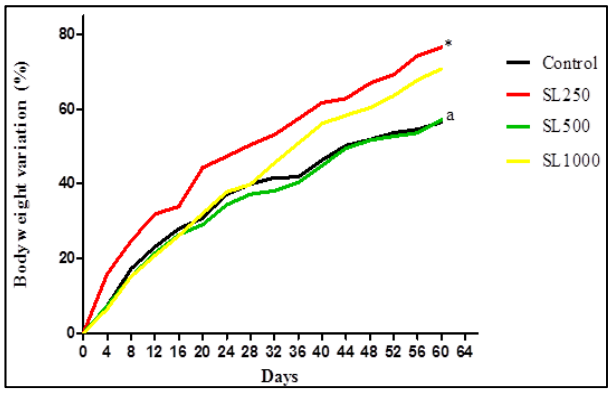
2.10 Statistical analysis

EXCEL and GraphPad are the software used. All data are expressed on average ± SEM. Analysis of variance analysis data (ANOVA) is used to compare the different experimental groups to the control. $P<0.05$ was considered significant.

3. Results

3.1 Effects of the aqueous extract of *Sarcocephalus latifolius* on body weight

When analyzing the results, only the dose of 250 mg/kg bw of aqueous extract of *Sarcocephalus latifolius* leaves induced a significant increase ($p<0.05$) in the body weight of the treated animals compared to controls, rates of 76.54% and 56.48% respectively. In addition, this dose of 250 mg/kg bw of extract had a significant effect ($p<0.05$) compared to that produced by the 500 mg/kg bw dose, a weight gain of 57.17% (Figure 1).



*: $p<0.05$; significant difference in relation to control (distilled water)

a: $p<0.05$; significant difference in relation to the dose of 500 mg/kg bw of *Sarcocephalus latifolius* extract

Fig 1: Effect of *Sarcocephalus latifolius* extract on body weight of adults rats during treatment of 60 days.

3.2 Effects on the weight of the organs (testicles, prostate, seminal vesicle, glans, LABC, adrenal gland)

There was no significant change in the fresh and dry testes ($p>0.05$), either as a function of the dose of the extract administered or as a function of the duration of the treatment, compared with those of the controls. However, after 60 days of gavage, animals receiving 250 mg/kg bw aqueous leaves extract showed significantly reduced testicular weight ($p<0.05$) of 456.6 mg/100g bw, compared to that of rats treated with the 500 mg/kg bw dose of extract, which is 524 mg/100 g bw. This was demonstrated by reference to the control test weight (492.20 mg/100 g bw), a weight loss of 7.23% for the dose of 250 mg/kg bw and a weight gain of 6.46% for that of 500 mg/kg bw (Table 1).

Table 1: Effects of the extract of *Sarcocephalus latifolius* on the weight of androgen-dependent organs of the male reproductive system after 30 and 60 days of treatment.

Organs (mg/100g bw)	State	Duration of treatment							
		30 days				60 days			
		Control	SL250	SL500	SL1000	Control	SL250	SL500	SL1000
Testis	Fresh	538.30 ±40.86	542.40 ±32.64	500.90 ±57.90	484.80 ±39.51	492.20 ±21.68	456.60 ±15.30$^{a'}$	524.00 ±8.99	491.70 ±12.24
	Dry	69.79 ±6.70	70.32 ±4.61	67.22 ±7.18	69.92 ±5.61	63.71 ±5.94	62.95 ±1.11	71.88 ±2.06	65.92 ±1.28
Epididymis	Fresh	159.90 ±9.30	139.90 ±9.53	140.50 ±10.77	164.80 ±14.85	174.30 ±9.00	146.40 ±10.70$^{ca'}$	188.70 ±8.28^{a}	219.00 ±9.43*
	Dry	45.46 ±3.39	39.77 ±4.29	34.34 ±3.21^{a}	50.12 ±4.34	42.09 ±2.40	39.18 ±3.63$^{a'b}$	57.76 ±5.02*	68.37 ±6.30**
Prostate	Fresh	192.80 ±10.39	180.20 ±26.44	202.10 ±24.53	191.20 ±21.54	196.50 ±13.20	215.70 ±9.96	215.20 ±8.26	237.90 ±8.37*
	Dry	_	_	_	_	_	_	_	_
Seminal vesicles	Fresh	375.80 ±27.84	368.00 ±41.79	439.60 ±37.26	397.90 ±25.96	585.7 ±55.11	463.3 ±23.64*	576.5 ±22.78	412.1 ±8.13**
	Dry	_	_	_	_	_	_	_	_
Glan	Fresh	48.44 ±1.25	54.22 ±3.49	57.11 ±3.55	63.72 ±2.86**	62.91 ±5.71	45.70 ±2.33*$^{b'}$	66.62 ±4.15	50.02 ±0.93*$^{a'}$
	Dry	14.24 ±0.53	15.37 ±0.70^{a}	16.80 ±0.85	20.41 ±2.27*	18.27 ±1.42	14.06 ±0.50*$^{a'}$	18.75 ±1.41	13.75 ±1.15*$^{a'}$
Cowper glands	Fresh	19.78 ±1.59	20.77 ±0.62 ^{c}	20.89 ±1.12^{c}	29.77 ±0.82***	27.85 ±2.37	27.51 ±1.66	31.00 ±1.61	33.84 ±1.32
	Dry	6.46 ±0.25	6.82 ±0.13^{b}	6.84 ±0.83^{b}	10.32 ±0.86**	9.22 ±0.79	7.86 ±0.15	9.57 ±0.34	9.61 ±0.77
LABC	Fresh	336.30 ±16.66	296.70 ±12.44^{b}	319.10 ±20.37^{a}	403.00 ±24.90*	377.50 ±18.09	324.50 ±6.46*^{a}	348.80 ±12.37	383.00 ±11.32
	Dry	86.10 ±4.03	79.91 ±4.66^{a}	86.89 ±7.79^{a}	109.30 ±10.12*	95.00 ±6.52	81.48 ±5.58	87.73 ±3.74	96.56 ±5.58
Adrenal glands	Fresh	7.76 ±0.53	7.52 ±0.78	8.96 ±0.49	7.42 ±0.99	7.01 ±0.76	6.38 ±0.33	7.70 ±0.73	8.84 ±0.77
	Dry	3.45 ±0.45	3.23 ±0.12	3.32 ±0.20	2.37 ±0.17*	2.52 ±0.51	2.47 ±0.35	2.71 ±0.15	3.27 ±0.49

*: $p<0.05$; **: $p<0.01$ et ***: $p<0.001$; significant difference with regard of control (distilled water) a: $p<0.05$; b: $p<0.01$ et c: $p<0.001$; significant difference with regard of dose of
1000 mg/kg extract of *Sarcocephalus latifolius*. a': $p<0.05$ et b': $p<0.01$; significant difference with regard of dose of 500 mg/kg bw of extract of *Sarcocephalus latifolius*.

In the epididymis, at the end of the 30 days of treatment, the results showed no significant change ($p>0.05$) in weight (fresh and dry) compared with the control. However, the dry weight of the epididymis is reduced ($p<0.05$) by 24.46% with the dose of 500 mg/kg bw of extract relative to the dose of 1000 mg/kg bw, which makes it grow of 10.25%. After the 60 days of treatment, while the dose of 1000 mg/kg bw of extract significantly increased the fresh and dry weight by 25.64% ($p<0.05$) and 62.44% ($p<0.01$), the 500 mg/kg bw dose of extract increased the dry weight by 37.23% ($p<0.05$) relative to the controls. At this stage, fresh or dry weight intake of the epididymis are dependent doses, but with very significant reductions ($p<0.05$ and $p<0.001$) with the dose of 250 mg/kg bw of extract compared respectively at doses of 500 and 1000 mg/kg bw.

The prostate showed no significant ($p>0.05$) weight change after 30 days of treatment. Only with the dose of 1000 mg/kg bw of aqueous extract, a significant increase ($p<0.05$) of 21.07% of the fresh weight was observed after 60 days of treatment.

At the level of the seminal vesicle, as before, no significant change is observed. However, after the 60 days of administration, a significant decrease of 20.90% ($p<0.05$) and 29.64% ($p<0.01$) was observed at the doses of 250 and 1000 mg/kg bw versus (vs) control.

As for the glans, it is growing at the end of the 30 days of gavage. However, this significant increase of 31.54% ($p<0.01$) at fresh weight and 43.33% ($p<0.05$) at dry weight was observed with the extract dose of 1000 mg/kg bw. The effect of Sarcocephalus latifolius extract on the dry weight of this organ at this dose is significant ($p<0.05$) and greater than that induced by the 500 mg/kg bw dose, which is only 7.93%. On the other hand, the 60 day treatment resulted in a significant ($p<0.05$) decrease of 27.36% and 20.49% at fresh weights, respectively, with doses of 250 and 1000 mg/kg bw, followed by 23.04% and 24.74% at the dry weight level.

As before, the fresh and dry weight of the Cowper glands increased after the 30 days of treatment. However, only the dose of 1000 mg/kg bw had a very significant effect ($p<0.001$ and $p<0.01$), is 50.50% at the fresh weight level and 59.75% at the dry weight, relative to the control. Compared to the dose of 1000 mg/kg bw, doses of 250 and 500 mg/kg bw of extract resulted in very small ($p<0.001$) significant increases in the fresh weight of the Cowper glands to 5% and 5.61% for each dose and 5.57% and 5.88% for dry weight at the respective doses. No significant ($p>0.05$) change in this organ weight was observed after 60 days of treatment.

At the level of the LABC, after 30 days of treatment, only the dose of 1000 mg/kg bw of aqueous extract of Sarcocephalus latifolius

allowed a significant growth (p<0.05) of 19.83% to the fresh and of 26.94% to the dry weight. The doses of 250 and 500 mg/kg bw of extract induced respectively significant decreases of 11.77% (p<0.01) and 5.11% (p<0.05) at the fresh weight. As to dry weights, a significant decrease of 7.19% (p<0.05) for the 250 mg/kg bw dose and a low growth of 0.91% (p<0.05) for the 500 mg/kg bw dose are recorded. After 60 days of treatment, only the reducing effect of the dose of 250 mg/kg bw of the Sarcocephalus latifolius extract on the fresh weight of the LABC is observed. The results show a significant decrease (p<0.05) of 14.04% relative to the control and 15.27% relative to the dose of 1000 mg/kg bw of extract.

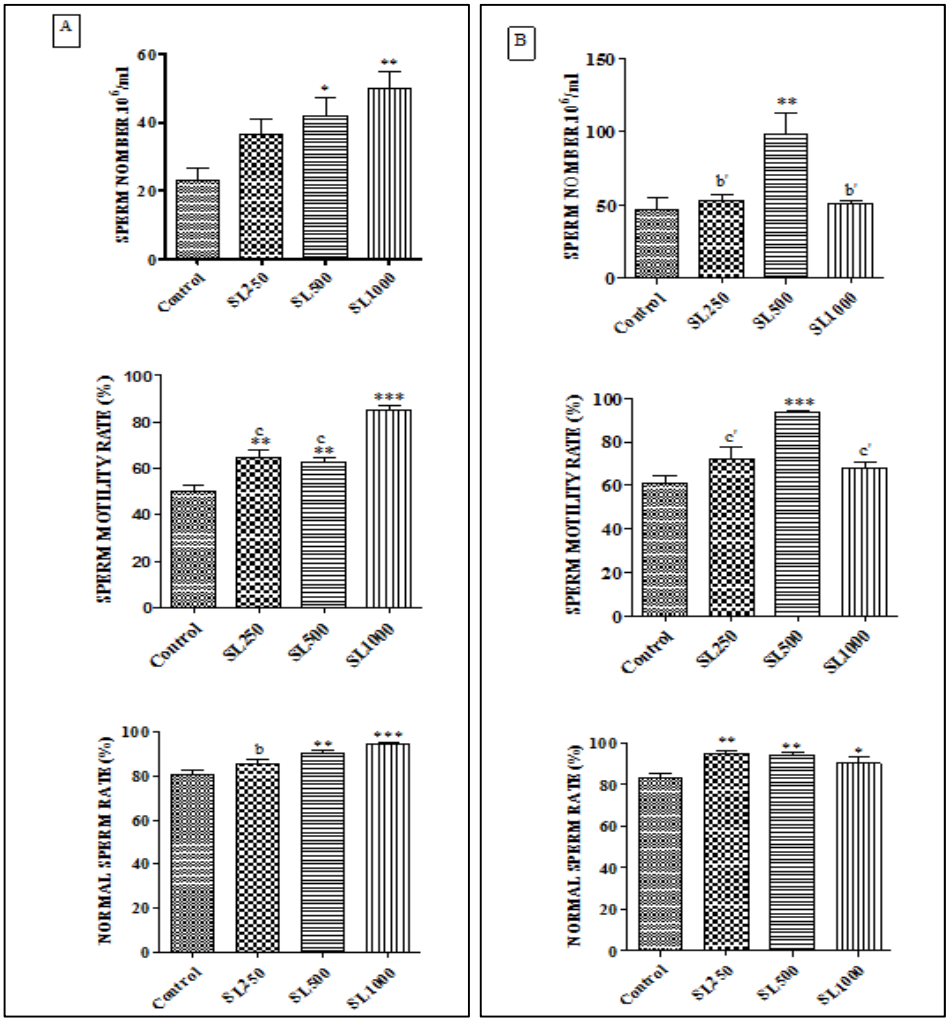
However, in the adrenal glands, only a significant (p<0.05) decrease of 31.30% of the fresh weight at 1000 mg/kg bw of extract compared to the control was noted after 30 days of treatment, whereas after 60 days of treatment no change is observed.

1.3 Effects on sperm concentration, motility and morphology

The concentration of spermatozoa is dose dependent in the case of a "short-term" treatment (30 days), with significant values of 42.04 million/ml (p<0.05) and 50.12 million/ml (p<0.01) for the respective doses of 500 and 1000 mg/kg bw of extract compared to the control (23.08 million/ml). In the case of "long-term" treatment, only the dose of 500 mg/kg bw of extract resulted in a high concentration of spermatozoa. This concentration of spermatozoa of 97.88 million/ml is significant (p<0.01) compared to the control concentration (46.20 million/ml) and also compared to those induced by doses of 250 and 1000 mg/kg bw of extract, estimated at 53.16 (p<0.01) and 51.08 million/ml (p<0.01), respectively.

In the short-term, all doses of extract increased significantly (p<0.01; p<0.01 and p<0.001) sperm motility, respectively, by 64.80; 62.80 and 85.20%, compared to control (50.40%); with a significantly lower (p<0.001 and p<0.001) effect at the doses of 250 and 500 mg/kg bw of extract compared to the dose of 1000 mg/kg bw of extract. However, in the case of long-term treatment, only the dose of 500 mg/kg bw of extract significantly increased (p<0.001) the motility of the spermatozoa by 93.60% compared to control and doses of 250 and 1000 mg/kg bw of extract, respectively of 61.20%, 72.00% and 68.00%.

For normal spermatozoa, 30 days treatment with extract doses of 500 and 1000 mg/kg bw yielded significant levels (p<0.01 and p<0.001) of 90.60 and 94.40% compared to the estimated control of 80.20%. The normality rate of 85.40% produced by the dose of 250 mg/kg bw of extract was significantly (p<0.01) lower than that of the (94.40%) dose of 1000 mg/kg bw of extract. Furthermore, after 60 days of treatment, the results had a significant (p<0.01; p<0.01 and p<0.05) effect of the different doses of extract on sperm normality. The effect of the extract decreases with the increase of the dose compared to the control. Rates of 94.80; 94.10 and 90.10% are then observed according to the following doses of 250; 500 and 1000 mg/kg bw of extract, against a control value of 83.30% (Figure 2).



*: p<0.05; **: p<0.01; ***: p<0.001; significant difference with regard of control (distilled water)
b: p<0.01; c: p<0.001; significant difference with regard of dose of 1000 mg/kg bw extract of *Sarcocephalus latifolius*.
b': p<0.01; c': p<0.001; significant difference with regard of dose of 500 mg/kg bw extract of *Sarcocephalus latifolius*.
A: 30 days of treatment B: 60 days de treatment

Fig 2: Effect of *Sarcocephalus latifolius* extract on the concentration, motility and morphology of adults rats sperm after 30 et 60 days of treatment

3.3 Effects on serum FSH, LH and testosterone levels

FSH levels in animals fed with 250 and 1000 mg/kg bw doses of extract for 30 days are less than 0.10 mIU/ml as in controls, while the rate of 0.61 mIU/ml is observed with the dose of 500 mg/kg bw of extract. After 60 days of treatment, the FSH level remains below 0.10 mIU/ml with the dose of 1000 mg/kg bw of extract, decreases to 0.12 mIU/ml with the dose of 500 mg/kg bw and extracted to 0.15 mIU/ml with the dose of 250 mg/kg bw of extract. These values are lower than the control value (0.17 mIU/ml).

As for LH, in animals treated for 30 days, the levels ranged from less than 0.10 mIU/ml with the dose of 250 mg/kg of bw extract to 0.17 mIU/ml with doses of 500 and 1000 mg/kg bw of extract, compared with 0.18 mIU/ml in the controls. After 60 days of treatment, only animals receiving the 500 mg/kg bw of extract dose had a high LH of 0.21 mIU/ml compared to levels below 0.10 mIU/ml for those treated with doses of 250 and 1000 mg/kg bw of extract, as in the controls.

At the testosterone level, significantly elevated ($p<0.05$, $p<0.05$ and $p<0.05$) levels of 2.91; 2.55 and 3.29 ng/ml were observed in the treated animals for 30 days at the respective doses of 250; 500 and 1000 mg/kg bw of extract relative to the control, estimated at 0.83 ng/ml. However, at the end of the 60 days of treatment, only the dose of 1000 mg/kg bw extract allowed a significantly high testosterone level ($p<0.01$) of 6.35 ng/ml, level of 1.62 ng/ml in the controls. The testosterone levels of 3.10 and 3.31 ng/ml, resulting from the treatments at the respective doses of 250 and 500 mg/k bw of extract are high compared to the control. However, differences between testosterone levels induced by 1000 mg/kg bw of extract and those induced by the two other doses are significantly low ($p<0.05$ and $p<0.05$) (Table 2).

Table 2: Effects of Sarcocephalus latifolius extract on hormone levels steroids in rats after 30 and 60 days of treatment.

	30 days of treatment			60 days of treatment		
	FSH (mUI/ml)	**LH (mUI/ml)**	**Testosterone (ng/ml)**	**FSH (mUI/ml)**	**LH (mUI/ml)**	**Testosterone (ng/ml)**
Control	<0.10	0.18	0.83±0.26	0.17	<0.10	1.62±0.56
SL250	<0.10	<0.10	2.91±0.34*	0.15	<0.10	3.10±0.46[a]
SL500	0.61	0.17	2.55±0.45*	0.12	0.21	3.31±1.04[a]
SL1000	<0.10	0.17	3.29±0.50*	<0.10	<0.10	6.35±0.89**

*: $p<0.05$; significant difference with regard of control (distilled water)

a: $p<0.05$; significant difference with regard of dose of 1000 mg/kg bw of extract of *Sarcocephalus latifolia.*

4. Discussion

Daily oral administration of 1 ml of aqueous extract of *Sarcocephalus latifolius* leaves to male rats during 60 days of treatment did not adversely affect the overall growth of these animals, this would be due to the non-toxic nature of this extract. The dose of 250 mg/kg bw of extract even induced a significant ($p<0.05$) increase in body weight compared to the control, which would be due to the androgenic properties of this plant. Indeed, androgenic substances have anabolic activity [16]. This result is similar to that of Mbongue (2005) [17] on the aqueous extract of dry fruits of *Piper guineense*.

The testes and other accessory reproductive glands are dependent on testicular androgens [18]. Indeed, testosterone plays an important role in the maintenance, growth and function of accessory sexual organs, being itself under the control of pituitary gonadotrophins, such as LH and FSH. In this work, the daily administration of 250; 500 or 1000 mg/kg of bw of *Sarcocephalus latifolius* extract during 30 or 60 days did not significantly reduce or significantly increase ($p>0.05$) the weight of the testes of the treated animals compared to controls. This suggests that this plant extract would not have anti-androgenic effects and would not also have affected testicular spermatogenesis and steroidogenesis, by the dose of extract or the duration of treatment. Indeed, oral administration of the aqueous extract of *Cardiospermum halicacabum* leaves to male rats for 30 days [19] or *Caesalpinia ferrea* for 52 or 104 days [20] did not result in a significant change in the weight of the sex organs, and yet the number, motility, viability of the spermatozoa, serum hormone levels (LH, FSH and testosterone) increased significantly. In addition, the epididymis, although not significantly changed ($p>0.05$) after 30 days of treatment, showed a significant increase in fresh ($p<0.05$) and dry weight ($p<0.01$) at the end of the 60 days of gavage, as well as the prostate, glans, Cowper glands and the LABC which have experienced an increase in fresh and/or dry weight, short and/or long term. The increase in fresh weight would be synonymous with the smooth functioning of the various functions of these organs, whereas the increase in dry weight would reveal the anabolic effects of the extract. All this confirms the androgenic activity of the extract. However, after 60 days of treatment, significant decreases in the fresh and/or dry weight of the seminal vesicles, glans, and LABC observed at 250 and/or 1000 mg/kg bw vs controls suggest that these doses over a long period of treatment can negatively influence fertility. This result is consistent with that of Ikpeme *et al*. (2013) [21], for whom the administration of high doses of *Cylicodiscus gabunensis*, *Nauclea latifolia* or *Araliopsis soyauxii* stem bark extracts over a long period (65 days) would be detrimental to hormones, as some organs of reproduction and thus lead to infertility.

The reduction in dry weight of the adrenal glands after 30 days of treatment would be due to a low activity of these organs in the face of a high availability of testosterone.

The number, motility and morphology (normality) of the spermatozoa are indicative of the quality of the sperm or spermatozoa and therefore of the fertility of the male. In the present work, the aqueous extract of *Sarcocephalus latifolius* induced a significant dose-dependent increase in caudal epididymis spermatozoa in treated rats, with a predominant effect at the 1000 mg/kg bw dose after 30 days of treatment and with the dose of 500 mg/kg bw after 60 days of treatment. The number of spermatozoa is considered an important parameter for evaluating the effect of chemicals on spermatogenesis [22]. It will be said that the extract of *Sarcocephalus latifolius* improves the spermatogenesis. This result corroborates those of Suleiman *et al.* (2014) [23] on the root extract of *Fadogia andersonii* and Woode *et al.* (2011) [24] on the ethanolic fruit extract of *Xylopia aethiopica*. For these authors, the increase in the number of spermatozoa, as well as the increase in the weight of the organs of the reproduction are indicative of an improvement of the fertility. This result differs from that of Sharangouda *et al.* (2010) [25] on the bark extract of *Terminalia bellirica*, for which the reduction in the number of spermatozoa observed is due to an inhibition of the spermatogenesis by the extract.

Similarly, sperm motility is significant and dose-dependent, with a predominance at the 1000 mg/kg bw dose compared to the other doses, after the 30 days of treatment and significant with the 500 mg/kg bw dose after 60 days of treatment. This suggests that the aqueous extract would not have penetrated the blood-testicular barrier and would therefore not have altered the microenvironment of the seminiferous tubules since it has been reported that the decrease in sperm motility caused by chemical agents is due to their ability to impregnate testicular blood [26]. Suleiman *et al.* (2014) [23] reported that the increase in the number and motility of spermatozoa would be due to the presence of alkaloids in the *Fadogia andersonii* extract. This hypothesis seems to be confirmed by the fact that the extract of *Sarcocephalus latifolius* contains alkaloids [27]. Indeed, according to Unnithan (1982) [28] the alkaloids would act on the hypothalamic-pituitary gland to induce the secretion of GnRH and thus stimulate spermatogenesis. This result is similar to that of Memudu *et al.* (2012) [29] with the extract of *Zingibier officinal* in the rat and different from those of Yakubu (2012) [30] on the alkaloidal extract of leaves of *Chromolaena odorata*. But according to Murugan *et al.* (2002) [31] subject to further investigation, some alkaloids such as

those found in *Alangium salviifolium* would induce androgenic effects, while others such as *Hibiscus rosa sinenses*, *Solanum xanthocarpum* and *Striga orabanchioides* would have anti-androgenic effects.

In addition, normal spermatozoa significantly increased at high doses of extract after 30 days of treatment, whereas after 60 days, this significant increase in the different doses of extract was inversely dose-dependent. This result shows that the extract of *Sarcocephalus latifolius* improves the morphology of the spermatozoa or allows the production of spermatozoa of good quality in short and long term. However, knowing that sperm morphology is closely related to fertility, Mc Leod showed in 1951[32] that the percentage of morphological abnormalities was higher in populations of infertile men than in fertile men. In view of these results, the extract of *Sarcocephalus latifolius* improves the germ function of the testis.

According to Etuk and Muhammad (2009) [33] the effect of the extract in increasing the number of spermatozoa would be due to increased blood testosterone levels. This finding is verified since the results show an increase in the different serum testosterone levels. These rates are significant compared to controls at $p<0.05$ after 30 days of treatment for all doses and at $p<0.01$ after 60 days alone dose of 1000 mg/kg bw of extract. This explains the stimulatory effect of the extract on the dependent organs and the quantity and quality of the spermatozoa. Since any change in the circulating androgen would affect the structural and functional integrity of the reproductive organs and thus impair the motility and metabolism of the spermatozoa [34].

Given that the LH (Luteinizing Hormone) and FSH (Follicle Stimulating Hormone) gonadotrophins act on Leydig cells for testosterone synthesis and on Sertoli cells for spermatogenesis control respectively, their study is therefore necessary. Thus, at the LH level, there were no significant differences between controls and doses of 500 and 1000 mg/kg bw of extract, while a low level was observed with the dose of 250 mg/kg bw of extract, after 30 days of treatment. Moreover, after 60 days, only the dose of 500 mg/kg bw of extract shows a high LH level compared to the low control and treated levels obtained. Thus, the aqueous extract of *Sarcocephalus latifolius* administered to male rats in the short and long term with only a small increase in the LH level showed no significant difference with the control. This result is due to the direct action of the extract on the gonads and not at the hypothalamic-pituitary axis. For Rukundo (2007) [8], the active principle in the aqueous extract of roots of *Nauclea latifolia* (*Sarcocephalus latifolius*) is said to be the main target of the testis. According to this author, the androgenomimetic action of this plant is due to the alkaloids it contains, which led to the synthesis of androsten-dione, precursor steroid hormone in the biosynthesis of testosterone. This argument would justify the significant testosterone levels obtained in the face of low LH levels in this study. In addition, the extract could interfere with the functioning of the LHRH receptor or its interaction with LHRH would lead to a decrease in the release of LH [35].

As for FSH, it is similar to the control level for doses of 250 and 1000 mg/kg bw at the end of the 30 days of treatment and relatively high at the dose of 500 mg/kg bw of extract. However, a dose-dependent reduction in FSH level is observed after 60 days of treatment. Since FSH is the hormone stimulating spermatogenesis, its reduction caused a disturbance of spermatogenesis, whereas in this study the number, motility and morphology of the spermatozoa increased significantly in the treated compared to the controls. The leaves extract of *Sarcocephalus latifolius* would also have also disrupted the functioning of the FSHRH receptor or its interaction with FSHRH would have caused a decrease in the release of FSH. This result further reinforces the idea that this extract would act on the testicle. This result corroborates that of Etuk Muhammad (2009) [33] on the bark extract of *Lophira lanceolata* which induced a decrease in FSH, whereas the number of spermatozoa significantly increased in a dose-dependent manner.

5. Conclusion

It was found that the aqueous leaves extract of *Sarcocephalus latifolius* stimulated testicular activity by inhibiting the secretion of pituitary gonadotrophins (LH and FSH). In addition, in the long term (60 days), low-dose (250 mg/kg bw) or very high dose (1000 mg/kg bw) extract therapy could lead to infertility; this being manifested here by a decrease in the relative weight of some androgen-dependent organs, an involution of the number and motility of the spermatozoa and even serum levels of LH and FSH.

6. References

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