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Phytochemical Study, Acute Toxicity and Fertility Potential Effect of *Sarcocephalus latifolius* (Smith) on the Histology of Wistar Rats Testicles

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Authors' contributions

This work was carried out in collaboration between all authors. Author BMAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AME and ZNE managed the analyses of the study. Author KK managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Sarcocephalus latifolius is a popular medicinal plant used in treatment of many ailments basically in West Africa and particularly in Ivory Coast. Thereby, this study aims to find out the major chemical groups in the aqueous leaf extract of *Sarcocephalus latifolius*, its acute toxicity and its fertility potential. In this perspective, a phytochemical study to determine chemical groups was carried out. Furthermore, the acute oral toxicity study was conducted according to OECD guideline 423, using three female rats sequentially. As for the fertility study, it was performed on the histology of the testes of forty albino rats of 60 days of age weighing between 130 g and 170 g and treated for 30 and 60 days, at doses of 250; 500 and 1000 mg/kg body weight from the aqueous extract of *Sarcocephalus latifolius*. The phytochemical screaming of the aqueous leaf extract of *Sarcocephalus latifolius* revealed the presence of polyterpene sterols, polyphenols, flavonoids,

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quinonics and alkaloids. In addition, the acute oral toxicity assay did not reveal any signs of toxicity, morbidity or mortality at studied doses. Finally, the histology of testes of the albino rats treated with the plant extract showed a more intense spermatogenesis, seminiferous tubules and more developed interstitial tissue compared to control. To sum up *Sarcocephalus latifolius*, although rich in phytochemical compounds, might not be toxic in a single dose and might have androgenic effects.

Keywords: Sarcocephalus latifolius; phytochemical compounds; acute toxicity; OECD 423; androgenic effects.

1. INTRODUCTION

Ivory Coast is located in West Africa and this country has a rich pharmacopoeia. Among these wide range of medicinal plant, we can quote *Sarcocephalus latifolius*, a rubiaceae found in the savannas of Sub-saharan Africa.

Sarcocephalus latifolius is a sarmentose shrub that can reach 12 meters in height [1]. It has a large, elliptical, opposite leaves, a large terminal glomeruli composed of small white flowers. Its fruit is a fleshy berry, red when ripe and with pink flesh.

This rubiaceae is one of the most widely used traditional medicines in Africa [2]. It is involved in the treatment of fever, gastrointestinal disorders, edemas, sleep diseases, hemorrhoids, wounds [3,4], amenorrhea, infertility [2,4], diabetes, hypertension [5,6], cough, measles, dysentery and bilharzia [7,8,9]. In Ivory Coast, this plant is widely used in the treatment of malaria [10,11].

In order to contribute to the valorization of the Ivorian pharmacopoeia, this study was conducted to determine the major chemical groups contained in this rubiaceae, its acute toxicity and its fertility effect in male rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Sarcocephalus latifolius* were purchased in Cocody market in the district of Abidjan, Ivory Coast. They were then authenticated at the National Floristic Center (NFC) of the University of Felix Houphouet-Boigny (Abidjan, Ivory Coast), where a voucher specimen was kept.

2.2 Extract Preparation

After washing the fresh leaves thoroughly, they were dried at room temperature and ground. 150 g of the powder was macerated in 800 ml of distilled water using an electric stirring in a Micro-Vortex for 24 h, protected from light in beakers covered with aluminum foil. The macerated was filtered through poplin cloths, then with Wattman No.1 filter paper and frozen before being dried in an oven.

2.3 Animal Material

Adult young male albino rats, of the Wistar strain, from the Teacher Training School vivarium in Abidjan were used for the experiment. These animals were fed with food pellets from the company FACI (Abidjan, Ivory Coast). They had free access to water. In the vivarium, the animals were subjected to a temperature of 22 ± 3°C with 40 to 60% humidity and a photoperiod of 12 hours of light and 12 hours of darkness.

2.4 Phytochemical Study

The characterization of the major chemical groups was carried out according to the methods used by Bleu [12].

2.5 Acute Oral Toxicity

This study was conducted using the acute toxicity method described in OECD Guideline 423 [13].

The test is a sequential process, using a small group of animals per stage. Sufficient information on acute toxicity was obtained for classification purposes. A specified dose of the substance was orally administered orally to a group of animals. The substance was tested by a sequential process in which three rats were used at each stage. The absence or manifestation of mortality in a group that received a single dose determines the next step, i.e.:

- stopping the test,
- administration of the same dose to three additional animals,

- administration of the immediately higher or lower dose to three additional animals.

Paragraph 22 of the OECD Guideline 423 states two types of test: The limit test and the main test. The limit test is used when the experimenter has information on the toxicity of the extract and that this could be above the regulatory limit dose.

Paragraph 23 suggests that a dose limit of 2000 mg/kg bw can be performed with six animals (three per stage). Exceptionally, a limit test at a dose level of 5000 mg/kg can be carried out with three animals (see Annex). If substance-related mortality occurs, it may be necessary to test the next lower dose.

The rats in the experiment were deprived of food 4 hours before and after force-feeding, while having free access to water. The animals were given a single dose of 1ml of extract. The limit dose of 2000 mg/kg bw was chosen as the initial dose, taking into account the study of Taïwe et al. [14] estimating the LD_{50} (lethal dose 50%) by the oral route at more than 14000 mg/kg bw of aqueous extract of the roots of *Sarcocephalus latifolius*. The experiment with the same dose was repeated on three other rats after 14 days of observations. Then, after another 14 days of observations, a third group of three rats received a single dose of 5000 mg/kg bw. The LD_{50} was then estimated.

During the different treatments, the animals were individually observed after treatment, at least once during the first 30 minutes, periodically during the first 24 h, with particular attention given during the first four hours and every day thereafter, for 14 days. According to OECD protocol 423, all rats were observed at least twice a day for the purpose of registering symptoms of poor health or changes in behavior.

The following parameters were observed: Appearance of body hair, stretching, tremors, convulsions, salivation, diarrhea, appearance of the eyes, lethargy, sleep and coma, breathing, state of nervousness and behavior. The time of death, if it occurred, is also recorded.

2.6 Animal Treatment

Forty rats of 60 days of age weighing between 130 g and 170 g were divided into four groups of ten rats. The animals were treated with distilled water and the aqueous leaf extract of *Sarcocephalus latifolius* as follows:

Group 1: distilled water (control)
Group 2: 250 mg/kg bw of SL extract
Group 3: 500 mg/kg bw of SL extract
Group 4: 1000 mg/kg bw of SL extract

The aqueous extract of *Sarcocephalus latifolius* leaves, as well as distilled water were administered orally by gastric intubation, daily for several days. After 30 days of treatment, 5 animals from each group were weighed and sacrificed under anesthetic ether. The others were after sixty days of experimentation. The left testis of each rat was isolated and placed in 10% formalin for histopathological study.

2.7 Histopathological Study

The collected left testes were first stored in labeled vials containing 10% formalin for tissue fixation. Then, dehydrated in alcohol baths of increasing degrees (80°; 90°; 100° and 100°) for respectively 1 h, 2 h, 2 h and 2 h. Then, they are thinned in three baths of toluene for 1 hour, 2 hours and 2 hours respectively, and finally, impregnated in two baths of liquid paraffin in an oven at 60° C for 2 hours, then 3 hours. After inclusion of these organs in paraffin in ambient air and using cassettes and molds, histological sections 5 µm thick, of these blocks previously hardened in the freezer, were made with a Leica type microtome. RM 2125 RTS, then mounted on slats. The sections were then deparaffinized in an oven at 58° C for 30 min, then in three successive toluene baths of 15 min each, before being rehydrated in alcohol baths of decreasing degrees (100°, 95° and 75°), 5 min each. Rinsed with distilled water, they were then stained with Hematoxylin-Eosin, and then observed under an Olympus CKX41-type microscope (Germany). Observations were made at different magnifications (× 40, × 100 and × 400), in order to detect any pathological lesions in the tissues. The photographs and measurements on the slides were made on the computer using Videomet software.

2.8 Statistic Study

Excel and GraphPad Prism 5 were used for processing the results obtained. The means were expressed in ± SD, then compared on ANOVA-One Way according to the Newman-Keuls test. The variance $p < 0.05$ was considered significant.

3. RESULTS

3.1 Phytochemical Study

The result of the phytochemical analysis of the aqueous extract of *Sarcocephalus latifolius* leaves was confined in Table 1.

3.2 Acute Oral Toxicity

The rats treated at doses of 2000 and 5000 mg/kg bw with the extract showed no change in behavior or apparent signs of toxicity compared to those given distilled water. No morbidity or death was observed in these rats within minutes, hours or days after force-feeding (Table 2).

3.3 Effects of the Aqueous Extract of *Sarcocephalus latifolius* on the Histology of the Testes

Cross-sections of the testes showed the same architecture, both in controls and in rats treated with the aqueous extract of *Sarcocephalus latifolius*. The germ cells of spermatogonia, spermatocytes, spermatids and spermatozoa, as well as Leydig and Sertoli cells are all present there. The extract improved spermatogenesis (Fig. 1) and induced a significant increase ($p < 0.001$) in the diameter of the seminiferous tubules at different doses of the extract compared to controls, after 30 days of treatment (Table 3). After 60 days of treatment, histological sections revealed more intense spermatogenesis with the dose of 500 mg/kg bw of the extract compared to the control and the other doses of the extract. The distance between the seminiferous tubules (interstitial space), as well as the diameter of the seminiferous tubules of the rats treated at a dose of 1000 mg/kg bw experienced a significant increase (respectively $p < 0.01$ and $p < 0.001$) compared to controls and to those treated at doses of 250 and 500 mg/kg bw.

Table 1. Chemical composition of the aqueous leaf extract of *Sarcocephalus latifolius*

Chemical compounds	Presence
Sterols and polyterpenes	+
Polyphenols	+
Flavonoids	+
Tannins	-
Quinonic substances	+
Alkaloids	+
Saponoids	-
Reducing sugars	-

+: present; -: absent

Table 2. Effects of the acute oral toxicity of the aqueous extract of *Sarcocephalus latifolius*

	Dose (mg/kg bw)	Number of death	Mortality rate
Aqueous extract of *Sarcocephalus latifolius*	2000	0/3	0%
	5000	0/3	0%

Table 3. Effects of *Sarcocephalus latifolius* extract on the diameter of seminiferous tubules and width of the interstitial space

Duration of treatment	30 days				60 days			
	Control	SL250	SL500	SL1000	Control	SL250	SL500	SL1000
Diameter of seminiferous tubules (µm)	450,5 ±8,5	595,8 ±24,6***	611,5 ±22,8***	621,7 ±18,1***	537,6 ±17,9	477,9 ±11,1*$^{ca''}$	531,5 ±10,8^{c}	776,2 ±22,0***
Interstitial tissue width (µm)	25,9 ±1,9	27,4 ±3,1	23,4 ±0,8	28,4 ±2,1	27,8 ±2,7	27,4 ±0,6^{b}	27,3 ±1,1^{b}	36,40 ±1,7**

**: p <0,05 ; **: p<0,01; ***: p<0,001; significant difference compared to the control (distilled water)*
b : p<0,01; c: p<0,001; significantly different at a dose of 1000 mg/kg bw of Sarcocephalus latifolius extract
a'': p <0,01 significantly different at a dose of 500 mg/kg bw of Sarcocephalus latifolius extract

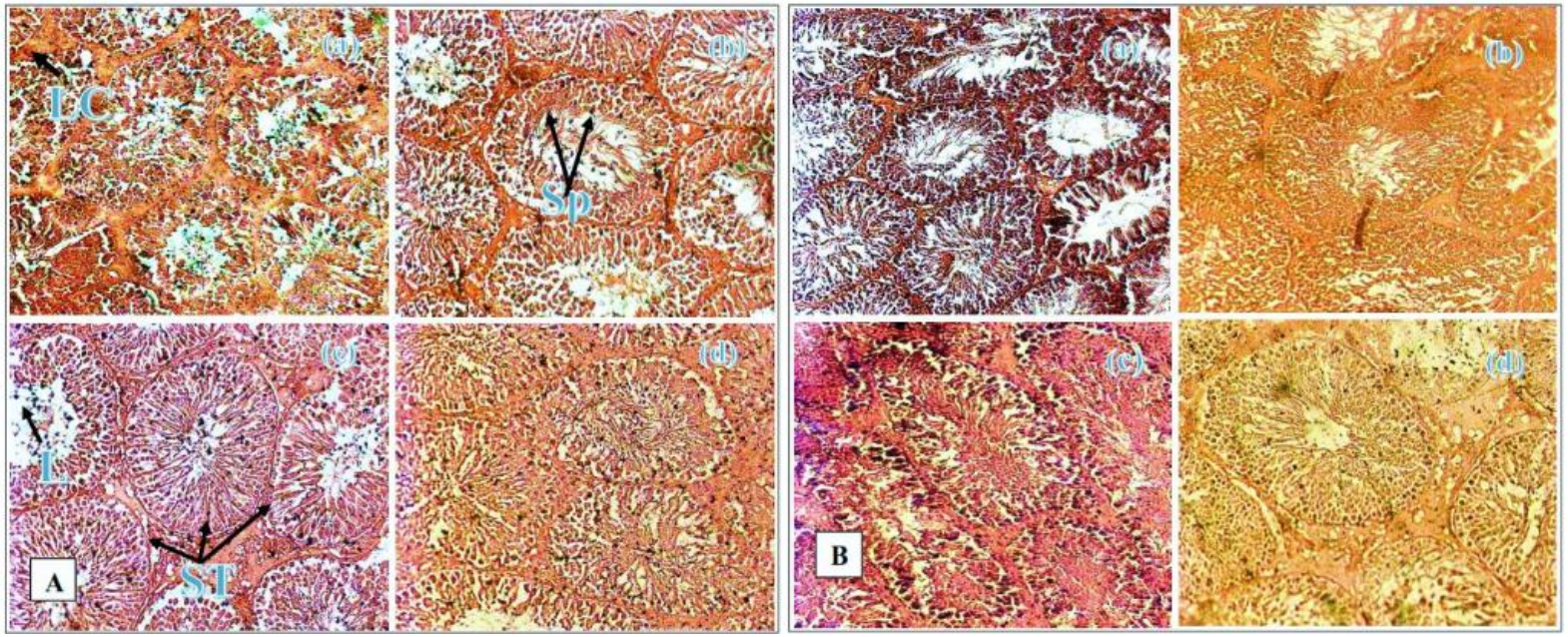

Fig. 1. Photomicrographs of rat testis sections after 30 and 60 days of treatment

(a): Control; (b): 250 mg/kg bw of Sarcocephalus latifolius (SL); (c): 500 mg/kg bw of SL; (d): 1000 mg/kg bw of SL.
LC: Leydig cell; Sp: sperm; F: flagella; L: Lumen of the seminiferous tube; ST: Seminiferous tubules;
A: 30 days of treatment; B: 60 days of treatment. (Hematoxylin-Eosin; X 100)

4. DISCUSSION

The phytochemical study of the aqueous leaf extract of *Sarcocephalus latifolius* revealed the presence of alkaloids, flavonoids, polyterpene sterols, polyphenols and quinonic substances and the absence of tannins, saponoids and reducing sugars. These compounds were demonstrated by Badiaga [15], in the leaves of *Sarcocephalus latifolius* collected in Mali. Alkaloids, flavonoids, saponins, phenols, tannins and vitamins are known for their various biological and pharmacological activities [16,17,18,19]. Thus, the many chemical families possessed by this plant could justify its traditional use in the treatment of many ailments [2].

However, it is wise to determine its level of toxicity, in order to prevent the risks associated with its use.

The acute toxicity study of the aqueous leaf extract of *Sarcocephalus latifolius* was performed according to the acute toxicity class method of OECD Guideline 423. This method is not intended to calculate the LD_{50} value, but rather to determine the toxicity class. Doses of 2000 and 5000 mg/kg bw did not induce any mortality, morbidity or visible signs of toxicity in the treated rats. These results confirm those of Kouadio et al. [20] and Taïwe et al. [14]. Indeed, Kouadio et al. [20] estimated the LD_{50} of the aqueous extract bark of the stems of this rubiaceae at more than 18 g/kg bw in mice and rats. Taïwe et al. [14], for their part, determined an LD_{50} of the aqueous extract of the roots of this plant greater than 14000 mg/kg bw after administration by oral route and 2197.85 mg/kg bw by intraperitoneally. This shows that acute toxicity occurs after oral administration at a very high doses. The LD_{50} of the aqueous leaf extract of *Sarcocephalus latifolius* could be greater than 5000 mg/kg bw. According to the Globally Harmonized Classification System (GHS) of the OECD guideline, *Sarcocephalus latifolius* could belong to class 5 or would not be classified with regard to its acute toxicity.

The histology of the testes of the rats was not affected by treatment with the different doses of the aqueous leaf extract of *Sarcocephalus latifolius*, regardless of the duration. Indeed, spermatogenesis could take place normally and even more intensely in the treated than in the controls, inside the seminiferous tubes. This could be due to the high testosterone level in these treated and the high concentration of sperm [21]. In addition, the size of the seminiferous tubes increased significantly ($p < 0.001$) with the different doses of extracts after 30 days of treatment and with the dose of 1000 mg/kg bw after 60 days ($p < 0.01$), compared to controls, as well as interstitial tissue at a dose of 1000 mg/kg bw after 60 days ($p < 0.01$). The growth of the seminiferous tubes would be due to a more active spermatogenesis in the treated. While the increase in the size of the interstitial tissue may result from the action of mature Leydig cells present in this tissue [22], whose function is to secrete testosterone. These results agree with those of the work of many authors, including those of Khouri et al. [23], in male mice treated with the extract of *Orchis anatolica*, for 35 days and of Bordbar et al. [22] with the extract of ginger in the rat rendered infertile and treated for 48 days. It follows from these facts that the aqueous leaf extract of *Sarcocephalus latifolius* could not have toxic effects on testes, but rather an androgenic effect.

5. CONCLUSION

These studies demonstrated that the aqueous leaf extract of *Sarcocephalus latifolius* contains a wide range of phytochemical compounds, is non-toxic in a single dose and has an androgenic effect on the testes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animals were handled according to the departement of Biosciences (University of Felix Houphouet-Boigny) ethical committee guidelines on the use and care of expérimental animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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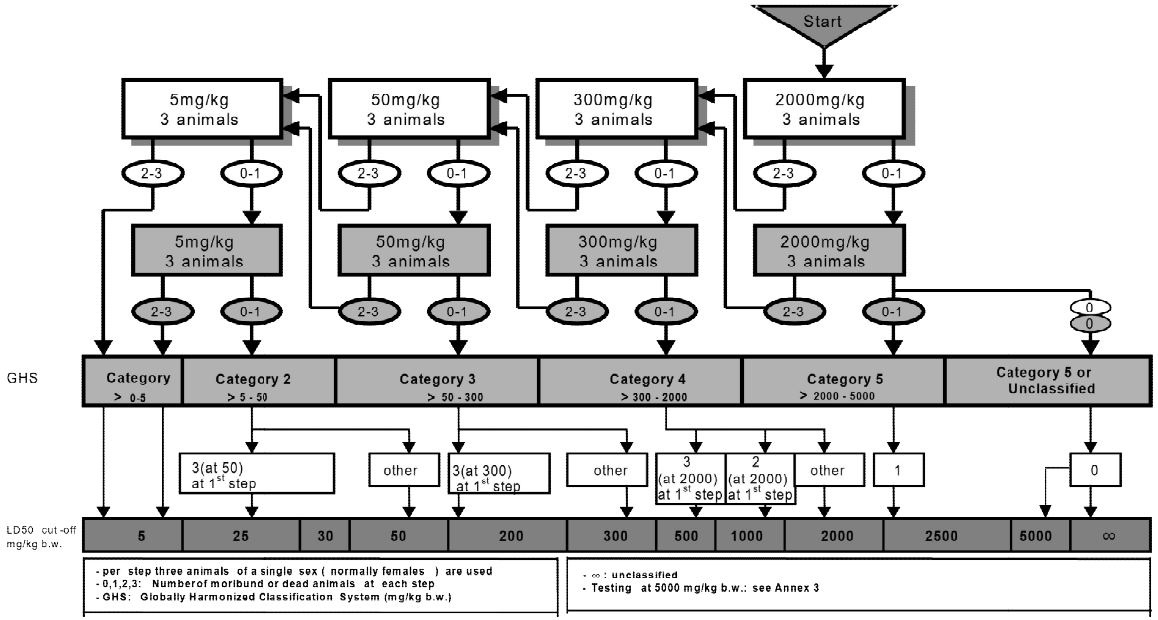
ANNEX



Annex. Test procedure with a starting dose of 2000 mg/kg body weight

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