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Anti-Toxic and Anti-Carcinogenic Activities of the Trunk Bark of *Sarcocephalus Pobeguinii*

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Abstract :

Background

Sarcocephalus pobeguinii, because of these virtues, is used in food as well as in therapy. The works noted in the literature concern both total extracts or different organs demonstrating antimalarial, antibacterial, antibiotic and antioxidant properties. The evaluation of toxicity is based on adequate qualitative or quantitative studies. Toxicity can be assessed on epidemiological, experimental in vivo, in vitro and theoretical modelling studies.

Objective

The purpose of this study is to evaluate anti-toxic and anti-carcinogenic of trunk bark of Sarcocephalus pobeguinii.

Method

10 g of powder from the trunk bark of Sarcocephalus pobeguinii was introduced in 100 mL. After lyophilization of the extract, the single dose of 2000 mg/kg of Sarcocephalus pobeguinii was administered to 5 male and female mice and their behaviour was observed for 1 day. The 0 mg/kg, 10 mg/kg, 50 mg/kg and 100 mg/kg doses of the extract were prepared and administered daily to 4 male and female rats per batch for 45 and 90 days. At the end of each experiment, the rats were sacrificed and ALT and AST levels were determined. Doses of 1 mg/kg and 0.5 mg/kg of the extract were tested in vitro on MCF-7 cancer cells.

Results

The mortality rate of 2000 mg/kg was 0%. The weight of female rats was decreased by 42.64% at 100 mg/kg. The increase in ALT activity at 50 mg/kg was 44.95% and 100 mg/kg (43.92%) while the increase in ALT activity was 59.68%. Percent survival of MCF-7 cells was 120% at 1 mg/kg.

Conclusion

Sarcocephalus pobeguinii is no toxic, no carcinogenic and requires further studies.

Keywords : *Sarcocepahalus pobeguinii, toxicity, cancer*

1. INTRODUCTION

Toxicity is the measure of a substance's ability to cause adverse and unhealthy effects on all life forms. It can be conducted on an animal, a bacterium or plant, or a substructure of an organism such as the liver [1, 2].

The toxicity assessment is based on adequate non-measurable or measurable studies. Depending on the duration of exposure of the organism to the administered substance, a distinction is made between acute toxicity, subacute or (short-term) toxicity, sub chronic toxicity and chronic toxicity [3].

In sub-Saharan Africa, cancer now accounts for 10% to 20% of the chronic pathologies observed. It primarily affects women between 45 and 55 years of age. The "classic" cancers of the breast, cervix and uterus for women and prostate for men are the most common. But Africa also produces its own specificities. Published estimates predict a 45% increase in mortality by 2025 [4].

Because of the high cost of treatment and the inaccessibility of treatment centers, populations resort to medicinal plants while being more or less unaware of their toxicity. *Sarcocephalus pobeguinii (syn. Nauclea pobeguinii)* is commonly used in traditional medicine. In Benue State, Nigeria, *Sarcocephalus pobeguinii* stem bark is boiled and administered orally to treat abdominal pain and stomach ache [5]. In Guinea Conakry, leaves and bark of *Sarcocephalus pobeguinii* are recommended for the treatment of diarrhoea, dysentery, cholera and hypertension [6]. The root decoction is anti anthelmintic [7]. The decoction of *Sarcocephalus pobeguinii s*tem bark is antiseptic, anti-infectious [8].

In Cameroon, in the upper valley of the Nyong forest, *Sarcocephalus pobeguinii* bark is used to prevent some problems of abortion [9].

2. Materials and Methods

2.1. Plant Material and Preparation of the Extract

The trunk bark of *Sarcocephalus pobeguinii*, harvested on the outskirts of Lambaréné in Gabon in January 2010 during the short dry season. The bark was dried in the shade and powdered. 100 g of powder from the bark of the trunk of *Sarcocephalus pobeguinii* were boiled for 15 minutes in 1000 mL of distilled water.

2.2. Biological Material

Male and female albino wistar mice and rats aged about two months and weighing 18-28 g and 160-200 g, respectively, from the animal house of the Department of Traditional Medicine in Bamako, Mali, were used to study the acute and subchronic toxicity of the extract. These animals were acclimatised for one week before the start of the experiment and were placed 5 per cage with free access to water and food. A live human MCF-7 cancer cell line was also used.

2.3. *In Vivo* Evaluation of Toxicity

2.3.1. Evaluation of the Acute Toxicity of the Extract of Sarcocephalus Pobeguinii

10 mice were acclimatized for 2 weeks and fasted with free access to water for 24 hours. After the fasting period, the animals were weighed and given the extract (2000 mg/kg) by gavage and again deprived of food for 3-4 hours. The animals were observed individually at least once during the first 30 minutes after treatment. Particular attention was paid during the first 2 hours after administration. Locomotion, exploration, aggressiveness, sensitivity to touch and noise, appearance and colour of faeces and mortality or morbidity of treated animals were observed in comparison with control animals.

2.3.2. Subchronic Toxicity of Sarcocephalus Pobeguinii Extract

32 rats were divided into 4 batches of 4 males and 4 females each. The first batch, considered a control batch, received distilled water (10 mL/kg); the second batch received plant extract at 10 mg/kg; the third batch was treated with extract at 50 mg/kg and the fourth batch with plant extract at 100 mg/kg. The animals received a single daily dose of the extract by gavage for 13 weeks. Half of the animals were sacrificed on day 45 of treatment and the rest on day 90. The aorta, liver, heart and kidneys were removed, weighed and fixed in 10% formalin.

The arteriovenous blood was collected and left to rest for 30 minutes and then centrifuged at 3000 rpm for 5 minutes. Serum was stored at -20°C for subsequent determination of alanine transaminase (ALAT) and aspartate transaminase (ASAT).

2.4. Determination of Serum Transaminases

100 µl of the sample and 500 µl of ALAT and ASAT buffer were homogenized and incubated for 30 minutes in a water bath to which 500 µl of the coloured reagent was added. After homogenisation and

incubation for 20 minutes at room temperature, 5 ml of diluted NaOH were added and the absorbance was read 505 nm against the blank. The activity of the ALAT and ASAT corresponding to the optical densities read was determined via the calibration curves of ALAT and ASAT.

2.5. *In Vitro* Evaluation of Toxicity on MCF-7 Cancer Cells

The antiproliferative potential of the extract was evaluated by microculture on the proliferation of a human MCF-7 cancer cell line. The cells were inoculated in 96-well plates at 2,104 cells per well in 200 µL of culture medium for 24 hours. The cells were then treated for 48 hours with or without 0.5 and 1 mg/mL extracts in phosphate buffer solution. After 30 min incubation, the cells are washed with phosphate buffered saline (PBS) solution and then incubated with 0.1 mL MTT (2mg/mL) for 4 hours at 37°C. The absorbance corresponding to the solubilized Formazan crystals (reflecting the relative viability of the cell number (%V) was determined at 570 nm with a Lasystems Multiskan MS microplate reader.

2.6. Statistical Analyses

The results were expressed as mean ± ESM (standard error on the mean). Statistical analysis was performed using the analysis of variance (ANOVA) followed by Dunnett's test for comparison of the batches to each other. The probability values $P < 0.05$ were considered significant.

3. Results and Discussion

3.1. Effects of *Sarcocephalus Pobeguinii* Extract 2 Hours after Administration to Mice

It was evaluated on the behaviour and mortality rate of mice. The main behavioural responses of the mice 2 hours after gavage of a single dose of the extract are noted in Table I.

Table1. *Behaviour of mice 2 hours after 2000 mg/kg of Sarcocephalus pobeguinii extract*

Extract dose (mg/kg)	Locomotion	Exploration	Agression	Sensitivity		Faeces	
				Touch	Noise	Appearance	Color
0 mg/kg	N	N	N	N	N	G	No
2000 mg/kg	D	D	D-	D-	D	P	M

Normal (N); Slightly diminished (D); Diminished (D-); Granular (G); Pasty (P); Black (No); Brown (M).

At 2000 mg/kg, exploration, aggressiveness and sensitivity to touch and noise decreased compared to controls. Stools were pasty brown in colour. These behaviours disappeared after 2 hours of observation. There were no deaths in the mice after treatment at 2000 mg/kg.

3.2. Effects of *Sarcocephalus Pobeguinii* Extract on Body Weight Changes in Rats

Figures 1 and 2 show the evolution of the body mass of male and female rats treated with different doses of the extract for 13 weeks.

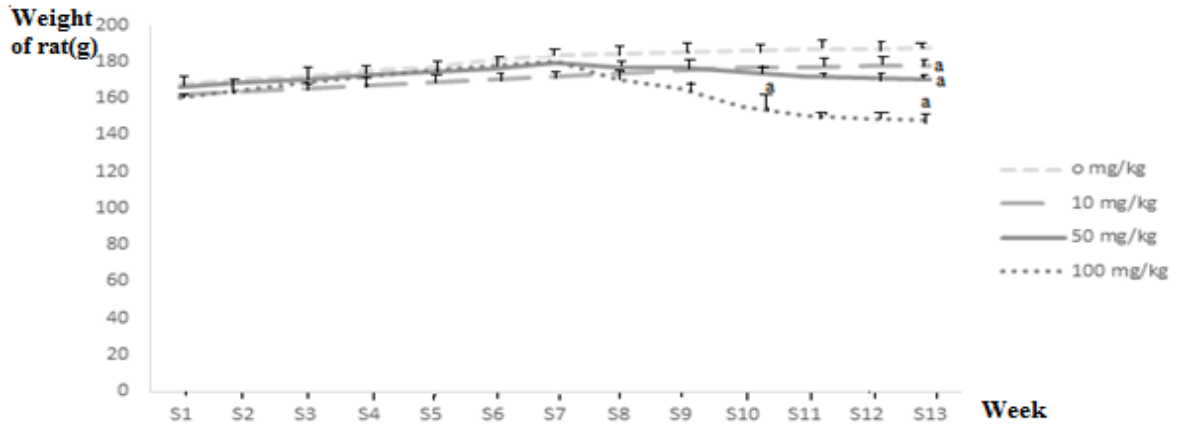

Figure1. *Evolution of body weight of male rats for 13 weeks*

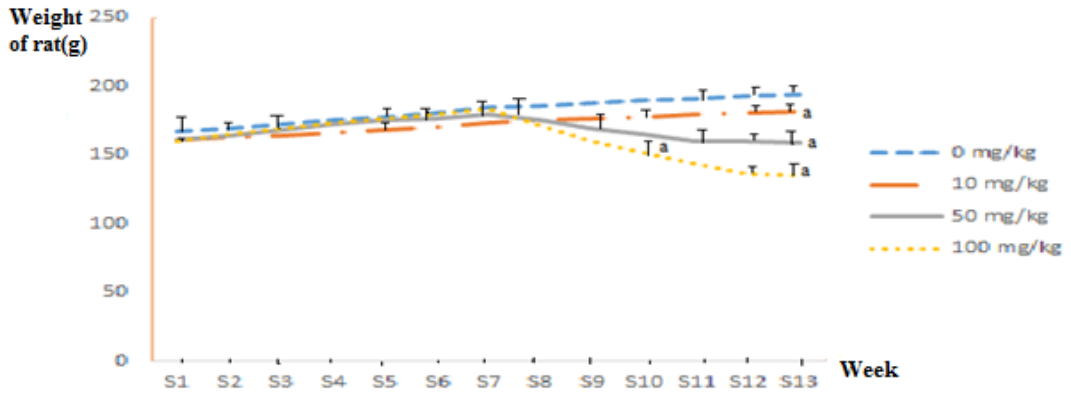

Figure2. *Evolution of body weight of female rats for 13 weeks*

From week eight onwards, body weight was significantly ($p<0,05$) reduced in the 100 mg/kg dose group compared to the control group. At the end of the thirteenth week, the decrease in body weight is more pronounced in the rats receiving the extract at the 100 mg/kg dose. Male rat weights were decreased by 10.40% and 26.92% for rats receiving 50 mg/kg and 100 mg/kg respectively.

3.3. Effect of the Extract on Transaminase Activity

Tables II and III show the effects of the extract on the activity of alanine transaminase and aspartate transaminase in animals after 45 and 90 days of treatment respectively.

Table2. *Effects of the extract on the activity of ALAT and ASAT after 45 days of treatment*

	0 mg/kg	**10 mg/kg**	**50 mg/kg**	**100 mg/kg**
ALAT (U/I)	60 ± 3,51	64 ± 1,45	109 ± 2,65	107 ± 1,33
ASAT (U/I)	200 ± 2,04	235 ± 2,85	433 ± 1,76	496 ± 1,09

The increase in ALT activity was 44.95% at the 50 mg/kg dose and 43.92% at the 100 mg/kg dose compared to control, while the increase in AST was 53.81% and 59.68% at the respective doses.

Table3. *ALT and AST levels after 90 days of treatment*

	0 mg/kg	**10 mg/kg**	**50 mg/kg**	**100 mg/kg**
ALAT (U/I)	51 ± 0,23	51 ± 2,14	67 ± 1,07	52 ± 0,78
ASAT (U/I)	216 ± 2,36	219 ± 1,98	209 ± 0,47	200 ± 1,53

ALAT activity at 50 mg/kg and 100 mg/kg increased by 23.88% and 1.92%, respectively, compared to control. In contrast, the activity of AST decreased 3.24% and 8% at the respective doses.

3.4. Effects of the Extract on Live Human Cancer Cells MCF-7

Figure 3 shows the effects of the extract on the viability and growth of MCF-7 cells.

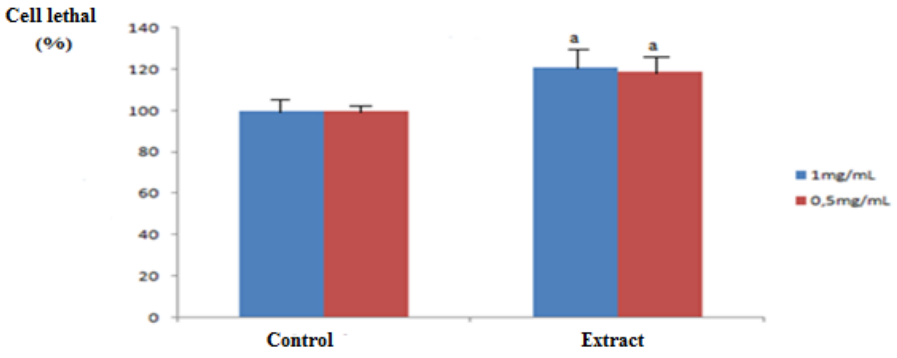

Figure3. *Effects of extract on MCF-7 cells*

MCF-7 cell lethal was 120% and 118% at 1 mg/kg and 0.5 mg/kg, respectively, indicating no toxicity.

The subchronic toxicity results show that serum transaminase activity was significantly increased, suggesting hepatic and renal impairment. In the assessment of liver damage caused by hepatotoxic substances, the determination of levels of enzymes such as ALT and AST is widely used [10, 11]. Necrosis or membrane damage in hepatocytes results in the release of these enzymes into the

circulation, and these enzymes can therefore be assayed in serum. Elevated levels of AST are indicative of liver, heart, and muscle disease. ALT, which is more specific to the liver, is therefore a better indicator of liver function [12]. The extract of Sarcocephalus pobeguinii caused an increase in transaminase activity. The decrease in weight more or less equally affected certain organs. A change in weight was noted in the heart and kidney and the hypertrophy observed in the liver was accentuated. This could indicate hyperactivity in the liver due to the extract. Organ hypertrophy may also be related to organ infection or inflammation, since the relative increase or decrease in weight is often a sign of underlying disease or organ damage [13]. The change in organ weight is a good indicator of toxicity after exposure to a toxic substance [14,15].

The proliferation of MCF-7 cancer cells thus reflects an absence of toxicity, confirming the results of acute toxicity, from which no animal losses have been recorded. Over the last two decades, numerous studies have described the great importance of natural products in the development of new pharmaceutical products [16]. However, much work has been done on the toxic effects of natural products [17,18].

4. CONCLUSION

The aqueous extract of the trunk bark of *Sarcocephalus pobeguinii* is no toxic and no carcinogenic but it requires further pharmacological studies.

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