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Impact of the Aqueous Extract of *Sarcocephalus latifolius* Root Powder on Hemostasis Parameters and Lipid Profile in Wistar Rats

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Abstract

The aim of this study was to evaluate *in vivo* the effect of the aqueous extract of *Sarcocephalus latifolius* root powder on haemostasis parameters and lipid profile in male and female Wistar rats. The plant material consisted exclusively of *Sarcocephalus latifolius* roots harvested in Porto-Novo, southern Benin and certified at the Benin National Herbarium under number YH 790/HNB. The animals were divided into four groups of three rats and gavaged for 14 days. The control rats were given distilled water orally, while the test batches were given 100, 200 and 300 mg/Kg of BW extract respectively. Blood samples were taken on days 7 and 14 of treatment and used to explore the intrinsic and extrinsic pathways of haemostasis. These included parameters such as bleeding time, prothrombin time, activated partial thromboplastin time, thrombin time and platelet count. Lipid parameters were also explored. Parameters such as total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were studied. Data on days D0, D7 and D14 for each haemostasis parameter and lipid profile were expressed as the mean plus or minus the standard error for each batch of animals. The data on day D0 were compared with those on days D7 and D14 to check whether there was a significant difference between the data obtained. The difference between D0 and D14 was tested using the

parametric paired two-sample test using R Studio software and Graphpad Prism 9.5.1 software (733). A significant dose-dependent decrease in bleeding time, prothrombin time, activated partial thromboplastin and thrombin was recorded, with the exception of the platelet count. As regards lipid parameters, there was a significant dose-dependent reduction in LDL cholesterol and triglycerides, and an increase in HDL cholesterol. The results confirm that the roots of *Sarcocephalus latifolius* accelerate the arrest of bleeding, thanks to its ability to stimulate the formation of platelet clots and improve the lipid profile of the animals by eliminating bad cholesterol.

Keywords

Sarcocephalus latifolius, Roots, *In Vivo*, Hemostatic, Lipid Profile

1. Introduction

Coagulation disorders constitute a serious problem in clinical practice because they often cause worrying hemorrhages. Also, they can take on various aspects affecting practically all systems of the human organism [1]. In obstetrics, it is currently established that abnormalities of coagulation factors are responsible for varied clinical pictures ranging from abortions to delivery hemorrhages, including arterial hypertension and its complications, to thromboembolic diseases. A group of processes known as hemostasis work together to halt bleeding [2]. Thus, it seeks to halt bleeding and avoid thrombosis. This standard evaluation is necessary before any procedure, particularly for expectant mothers who are scheduled for a cesarean section. To determine hemostasis, a number of factors are highlighted, including blood platelets, prothrombin level, bleeding time, prothrombin time, and activated partial thromboplastin time. In fact, one test for assessing clotting time is activated partial thromboplastin time. While the Prothrombin time (PT) is a test that can reveal specific deficiencies in coagulation components, a longer coagulation time implies an anomaly of the intrinsic pathway and the final common coagulation pathway [3]. We tested for hemostatic activity to find natural bioactive compounds that could clot blood after analyzing blood coagulation to stop bleeding. Cardiovascular disease-related mortality is rising in developing nations while falling in Western nations [4]. The pathophysiology of cardiovascular disorders is significantly influenced by the circulating lipids and the component lipids of arteries and tissues [5] [6]. Inflammation of the artery walls is caused by an excess of circulating LDL cholesterol [7]. In order to avoid cardiovascular illnesses, it is imperative to find natural bioactive compounds that can act on lipids. Herbal medicine research has grown to be one of the most important scientific topics during the past 20 years [8]. According to ethno-pharmacological research, over 1200 plants are utilized in traditional medicine worldwide for their biological properties, and they have long been employed to prevent or treat a wide range of illnesses [9]. The use of medicinal and food plants is a major part of the medical

treatment of so-called chronic diseases in some traditional, non-industrialized civilizations (China, several African, and Latin nations) [10]. *Sarcocephalus latifolius*, a plant that is primarily rich in phenolic compounds, flavonoids, and tannins, has long been used in Benin to cure hemorrhoids [11] and other common illnesses [12] [13]. Studies on the plant's anti-inflammatory and antioxidant qualities indicate that it might have an impact on significant physiological functions. Few research, meanwhile, have examined its precise effects on lipid profile and hemostasis, two crucial areas for the prevention of cardiovascular disease. Therefore, we set out to evaluate the *Sarcocephalus latifolius* plant's *in vivo* lipid and hemostatic properties.

2. Materials and Methods

2.1. Plant Material

The plant material consists exclusively of the roots of *Sarcocephalus latifolius* collected in Porto-Novo, in southern Benin and certified in the national herbarium of Benin under number YH 790/HNB. After harvest, drying was carried out in a room protected from light and humidity, in order to preserve the integrity of the molecules as much as possible and accelerate drying. The finely cut dried roots were crushed in a hammer mill (type Retshsm 2000) to obtain a powder in order to increase the exchange surface between the solid and the solvent and facilitate extraction. This powder is yellow in color and bitter in flavor.

2.2. Animal Equipment

The animal material consists of Wistar rats (male and female) weighing between 100 and 120 g. These rats are produced by the LPMTA animal facility of the animal physiology department at the University of Abomey-Calavi. All animals have an EOPS health status (free from specific pathogenic organisms). Upon receipt, the rats were weighed and divided into four (4) groups of three (3) rats for an acclimation period before being used in the different experiments. During this period, the animals have free access to food and water and are maintained in an animal room at a constant temperature (22 ± 2)˚C subject to a 12/12 h light/dark cycle. In our laboratory, rats are given rabbit kibble. In fact, the rats are fed a complete food called "Rabbit Fattening". The composition of this food is as follows: corn, cereals, premixed soya-palm kernel cake, BHT, amino acids, limestone, tanica, anti-mold, and rice.

2.3. Method for Preparing the Aqueous Extract of the Powder of the Roots of *Sarcocephalus latifolius*

The extraction was carried out according to the protocol described by Ondele *et al.* 2015 [14] with some modifications:

The protocol for this extraction is as follows:

- Weigh 50 grams of *Sarcocephalus latifolius* root powder, add 500 ml of distilled water, all in a 1L glass bottle then shake on a shaker for 10 min;

- Boil the mixture for 15 minutes;
- After cooling, filter the decoct using a funnel;
- Filter 3 times through hydrophilic cotton;
- Put in the oven at 40˚C for 3 days.

The extraction yield was determined by the ratio between the mass of the dry extract obtained after evaporation and the mass of starting plant material. This yield is given by the following formula:

$$\text{Yield } (\%) = (M_1/M_0) \times 100$$

M_1 = mass of the extract after evaporation, M_0 = mass of the starting plant material

2.4. Treatment

The treatment of the animals was carried out for fourteen (14) days. The extract is administered by oral gavage with a syringe equipped with an esophageal probe. This ensures that the rat has swallowed the expected dose.

Group 1: control rats received only distilled water.

Group 2: These rats were treated with 100 mg/Kg. PC, from the aqueous extract of the powder of the roots of *Sarcocephalus latifolius*.

Group 3: These rats were treated with 200 mg/Kg. PC, from the aqueous extract of the powder of the roots of *Sarcocephalus latifolius*.

Group 4: Rats in this group were treated with 300 mg/Kg from the same extract.

Samples were taken on the first day (D0) before the experiment then on the seventh (D7) and fourteenth (D14) days in order to follow the evolution of the different parameters measured.

2.5. Statistical Calculations

The data on days D0, D7 and D14 for each parameter of hemostasis and lipid profile were expressed as average plus or minus the standard error in each batch of animals. The data on day D0 were compared to those of days D7 and D14 in order to check if there is a significant difference between the data obtained. The normality of the data was first checked using the Shapiro.test script; the difference between D0 and D14 was checked using the paired two-sample parametric test through R Studio software and Graphpad Prism 9.5.1 software (733). For this test, the script t.test (Rate~Period, paired = TRUE, var.equal = TRUE) was used; the histograms showing the average value on D0, D7 and D14 of the parameters in the different batches of animals were produced with the Graphpad Prism 9.5.1 software (733).

2.6. Parameters Evaluated

Bleeding time, Activated partial thromboplastin time, Prothrombin time, Thrombin time Platelets, Triglycerides, Total cholesterol, HDL cholesterol, LDL cholesterol.

3. Results

3.1. Extraction Yield

$$R = (24.75/229.01) \times 100 = 10.80\%$$

After three days, 24.75 g of aqueous extract was obtained, representing a total yield of 10.80%.

3.2. Influence of the Administration of the Aqueous Extract of the Powder of the Roots of *Sarcocephalus latifolius* on the Parameters of Hemostasis

3.2.1. Bleeding Time

Figure 1 shows the variation in bleeding time in rats treated with different doses of the aqueous extract of *Sarcocephalus latifolius.*

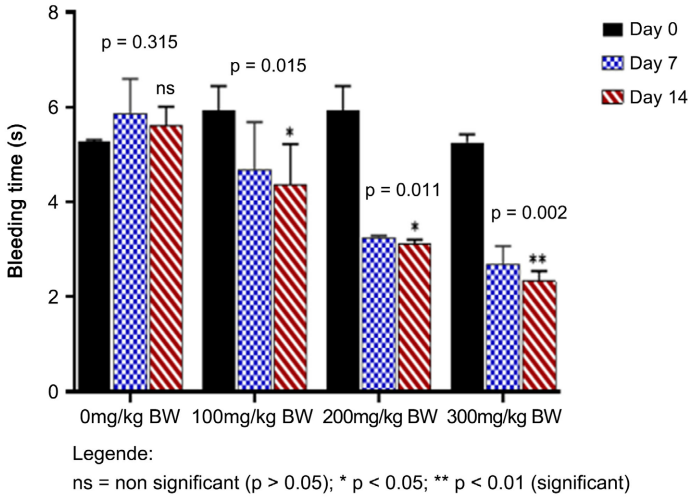

Figure 1. Variation of bleeding time in Wistar rats under the influence of different doses of aqueous extract of Sarcocephalus latifolius root powder.

3.2.2. Activated Partial Thromboplastin Time (APTT)

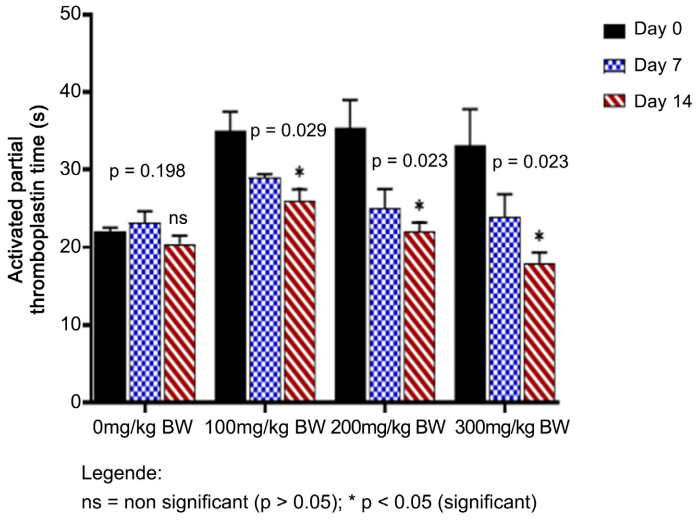

Figure 2. Variation of activated partial thromboplastin time in Wistar rats under the influence of different doses of aqueous extract of *Sarcocephalus latifolius* root powder.

Figure 2 shows the variation in activated partial thromboplastin time in Wistar

rats treated with different doses of the aqueous extract of *Sarcocephalus latifolius*. From D0 to D14, a reduction in the TCA is observed regardless of the dose administered. The analysis of variance showed that the observed reductions are significant ($p < 0.05$) for all batches except the control batch (0mg/kg BW).

3.2.3. Quick Time

The present Figure 3 highlights the evolution of the Quick time in animals treated with different doses of the aqueous extract of *Sarcocephalus latifolius*. It appears from the analysis of this figure that the aqueous extract of *Sarcocephalus latifolius* significantly reduces the quick time for doses of 100 mg/kg. BW ($p < 0.05$) and 200 mg/kg. PC ($p < 0.01$) compared to the control rat.

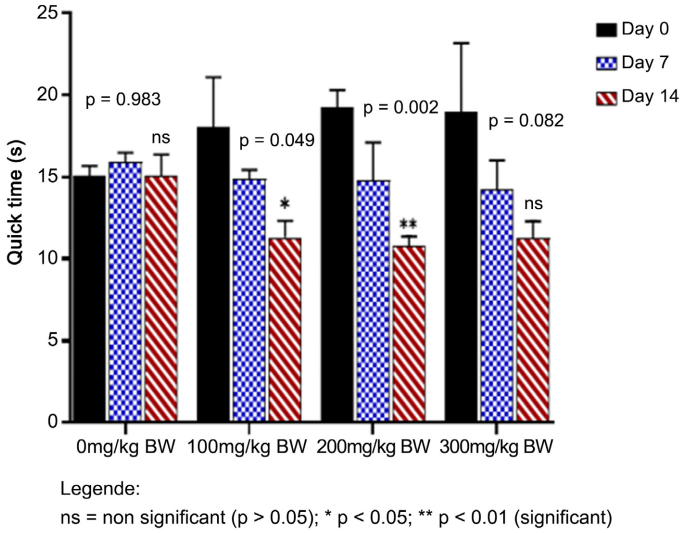

Figure 3. Variation of quick time in Wistar rats under the influence of different doses of aqueous extract of *Sarcocephalus latifolius* root powder.

3.2.4. Thrombin Time

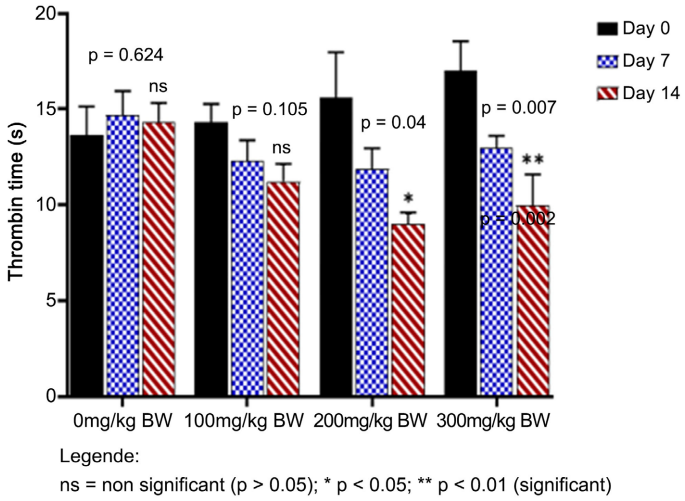

Figure 4. Variation in activated thrombin time in Wistar rats under the influence of different doses of aqueous extract of *Sarcocephalus latifolius* root powder.

Figure 4 above shows the evolution of thrombin time in treated rats.

A tendency to decrease in thrombin time is observed in rats treated at 100 mg/Kg BW. On the other hand, in rats treated at 200 and 300 mg/Kg.BW, the reduction is significant.

3.2.5. Platelets

Figure 5 shows the evolution of the blood platelet level from day 0 to day 14. The statistical analysis reveals a significant increase in the blood platelet level 14 days after treatment at doses of 100 ($p = 0.037$) and 200 ($p = 0.017$) mg/kg BW. The blood platelets of rats having been treated at a dose of 300 mg/kg BW ($p = 0.002$) of the aqueous extract of *Sarcocephalus latifolius* had a very significant increase.

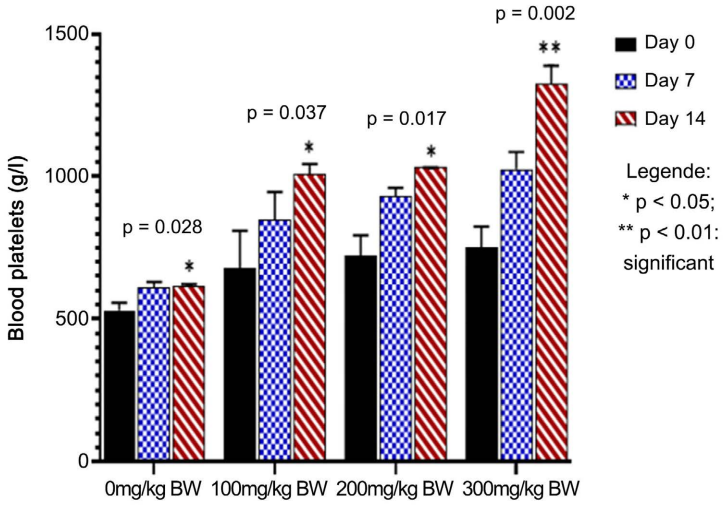

Figure 5. Variation in blood platelet levels in Wistar rats under the influence of different doses of aqueous extract of *Sarcocephalus latifolius* root powder.

3.3. Influence of the Administration of the Aqueous Extract of the Roots of *Sarcocephalus latifolius* on the Lipid Profile of Wistar Rats

3.3.1. Triglycerides

Figure 6 shows the evolution of the concentration of triglycerides under the influence of different doses of the aqueous extract of the powder of the roots of *Sarcocephalus latifolius*. Observation of said figure shows us that the administration of different doses of *Sarcocephalus latifolius* extract compared to the control batch leads to a reduction in triglyceridemia. The analysis of variance shows us that the doses 100 and 300 mg/kg BW of this extract had a significant effect on the variations in the concentration of triglycerides in Wistar rats ($p < 0.05$): a decrease is observed. On the other hand, dose 200 did not have a significant effect on the variation of triglycerides.

3.3.2. Total Cholesterol

Figure 7 shows the evolution of the different concentrations of Total Cholesterol in the different batches of rats as a function of the doses of aqueous extracts of the

roots of *Sar cocephalus latifolius*. This figure shows that the administration of different doses of this extract compared to the control batch leads to a reduction in cholesterol levels ($p < 0.005$).

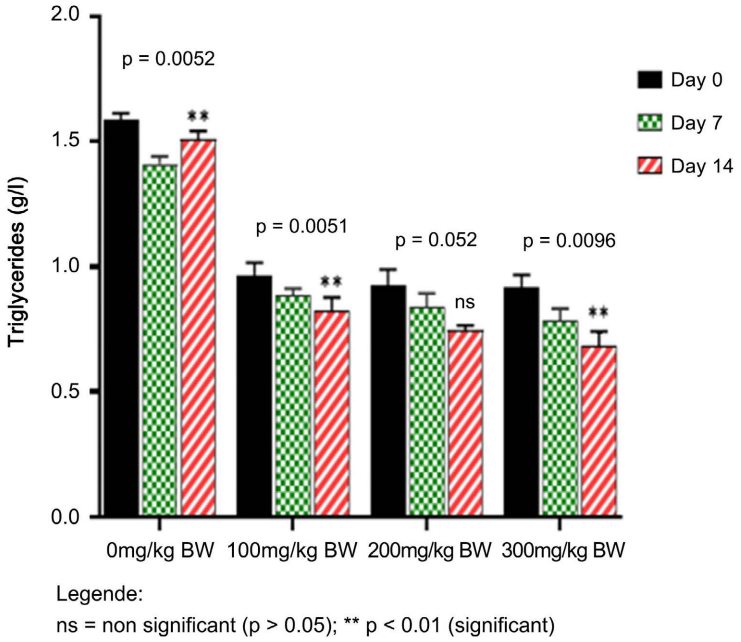

Figure 6. Variation in triglyceridemia under the influence of different doses of aqueous extract of *Sarcocephalus latifolius* root powder.

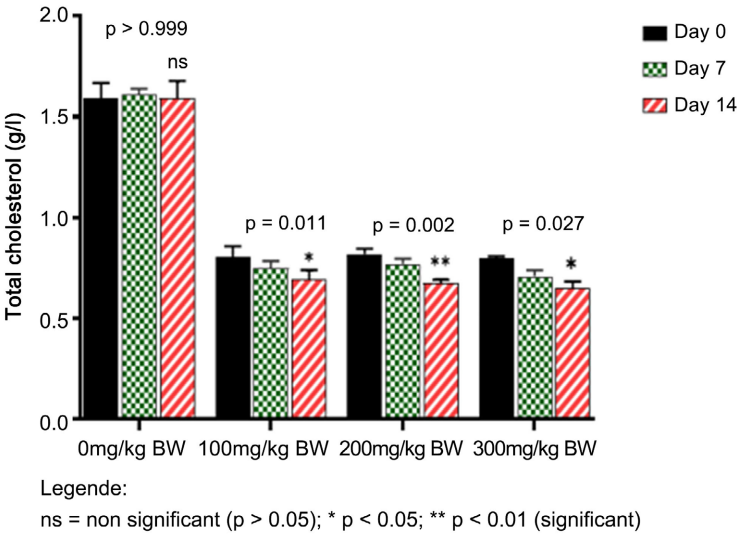

Figure 7. Variation in total cholesterol levels under the influence of different doses of aqueous extract of *Sarcocephalus latifolius* root powder.

3.3.3. HDL Cholesterol

Figure 8 shows the evolution of the different concentrations of HDL cholesterol under the influence of the different doses of the aqueous extract of the roots of *Sarcocephalus latifolius*. The statistics reveal a non-significant increase in rats

treated at 100 mg/kg BW but a significant increase in rats treated at 200 and 300 mg/kg BW.

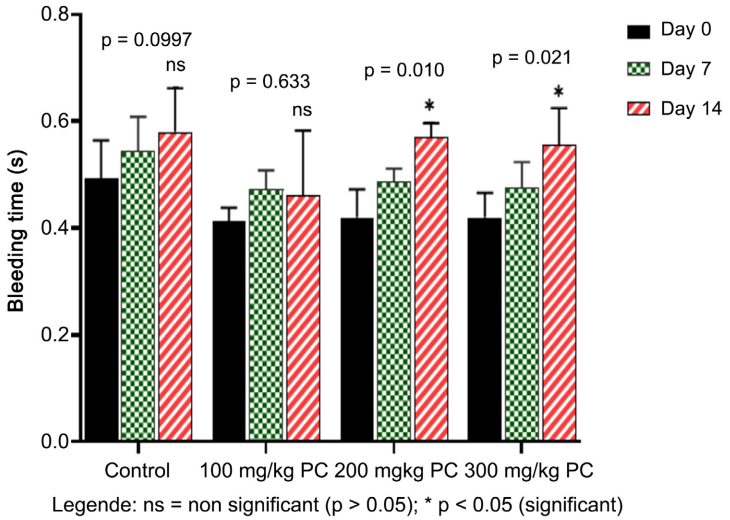

Figure 8. Variation in HDL cholesterol concentrations under the influence of different doses of aqueous extract of *Sarcocephalus latifolius* root powder.

3.3.4. LDL Cholesterol

Figure 9 shows the evolution of the different concentrations of LDL cholesterol under the influence of the different doses of the aqueous extract of the roots of *Sarcocephalus latifolius*. The statistics reveal a non-significant decrease in rats treated at 200 mg/kg BW but a significant decrease in rats treated at 100 and 300 mg/kg BW.

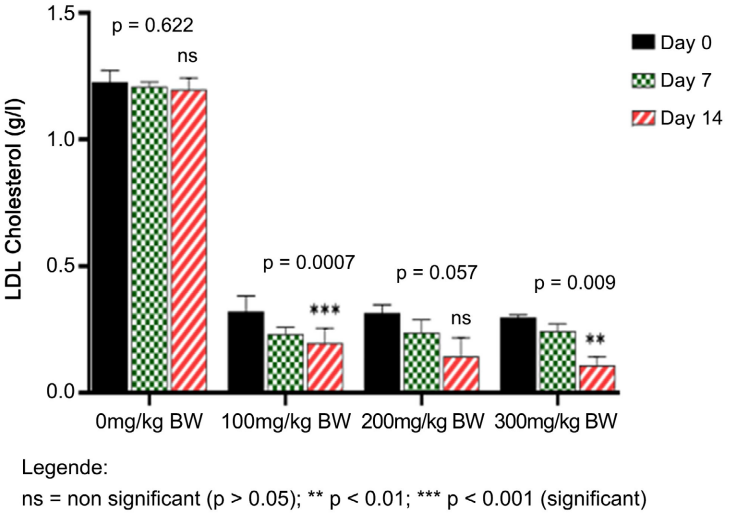

Figure 9. Variation in LDL cholesterol concentrations under the influence of different doses of aqueous extract of *Sarcocephalus latifolius* root powder.

4. Discussion

This study examined the biological activities of an aqueous extract of powdered

Sarcocephalus latifolius roots, evaluating the extract's lipidic and hemostatic properties [13].

The selection of roots and activities mentioned is related to the fact that this plant's organ has long been used as a diuretic [15] [16] and to treat common, especially hemorrhoidal, ailments.

The yield of the resulting aqueous extraction was 10.80%. This extract reduces bleeding time for all dosages (100, 200, and 300 mg/kg BW) when administered in varying doses over a 14-day period. This could be because the powdered extract of the plant's roots contains flavonoids [16]. With Alphonse *et al.*, this outcome was noted [17]. These scientists demonstrated that extract from *Tridax procumbens* decreases the rate of bleeding in Wistar rats. This finding suggests that the aqueous extract of *Sarcocephalus latifolius* roots has both hemostatic and astringent properties. It is important to emphasize that this hemostatic activity promotes vasoconstriction, which is an important parameter in hemostasis. This vasoconstriction is due to the presence of coumarins in this plant. This result was confirmed by Klotoé *et al.* [18]. This plant's coumarins are the cause of this vasoconstriction. By examining extracts from the leaves of *Musa sapientum* L. (Musaceae), medicinal plants frequently offered for sale by herbalists in southern Benin to alleviate bleeding, Klotoé *et al.* [18] verified this finding. Tannins may possibly be the cause of this vasoconstriction. These findings are comparable to those of Aouissa [19]. by studying extracts from the leaves of *Musa sapientum L.* (Musaceae), medicinal plants commonly sold by herbalists in southern Benin for the treatment of bleeding. This vasoconstriction could also be due to the presence of tannins. These results are similar to those found by Aouissa [19]. The latter discovered a connection between the tannin concentration of Mangifera indica leaves and their astringent qualities. Dandjesso *et al.* [20] verified this finding by examining leaf extracts from four medicinal plants that are frequently offered for sale by herbalists in southern Benin to treat bleeding: *Annona senegalensis*, *Newbouldia laevis*, *Cassytha filiformis*, and *Cissampelos mucronata*. In fact, tannins are used to treat hemorrhoids and varicose veins, and they also have a hemostatic and vasoconstrictor action on tiny vessels [21]. According to Bruneton in 2009, tannins used orally are vasoprotective; they limit the loss of fluids and promote tissue regeneration in the event of a superficial injury or burn.

Since platelet aggregation is the initial stage of blood coagulation, the decrease in bleeding time shown in Wistar rats can be explained by the notable rise in platelet numbers [22]. Because of its antioxidant and anti-inflammatory qualities, *Sarcocephalus latifolius* may lessen chronic inflammation, which may otherwise result in secondary thrombocytosis. The extract may stabilize or raise platelet counts by decreasing inflammation [23] [24]. Antioxidant-rich extracts may improve general hematological health and shield platelets from oxidative damage [25], which could raise the platelet count. Certain herbal extracts have the ability to alter coagulation pathways, which in turn can alter platelet counts and function. For instance, a rise in platelet count may result from extracts that affect the ratio

of pro- and anti-coagulant factors [26].

Megakaryocytes, the bone marrow cells that produce platelets, may be directly stimulated by certain plant extracts. As a result, platelet production may rise.

Prothrombin time, activated cephalin time, and thrombin time were among the hemostasis metrics that were seen to decrease in the treated rats during this investigation. In general, these metrics serve as markers of hemostasis equilibrium [27].

Hemostatic activity is linked to the activation of coagulation factors (XII and X). Activation leads to the formation of thrombin which will transform circulating fibrinogen into fibrin, the main constituent of the clot [28].

In the event of vascular injury, the triggering of hemostasis is manifested by vasoconstriction, initiated by the formation of platelet aggregates, depending on the diameter of the damaged vessel.

A significant reduction in the level of LDL cholesterol and triglycerides in treated rats reveals that this extract has an effect on cardiovascular health. Doses of 100, 200 and 300 mg/kg of BW. of the aqueous extract induced a significant increase in HDL cholesterol levels in treated rats. This plant would therefore have a cardio-protective effect [29]. It could be effective against hyperlipidemia since it contains flavonoids which, through their antioxidant effects, could reduce cholesterolemia [30]. Previous studies have demonstrated that flavonoids lower blood cholesterol in rats fed a cholesterol-enriched diet [30].

Likewise, some authors have shown that flavonoids reduce LDL-Cholesterol and increase plasma HDL-Cholesterol and thus correct dyslipidemia [31].

The hypotriglyceridemic effect noted could be due to the repression of the sterol regulatory element binding protein-1C (SREBP-1C) pathway in adipose tissues and the liver and consequently to the reduction in triglyceride synthesis [32]. The aqueous extract of *Sarcocephalus latifolius* protected animals against hypercholesterolemia. Our extract would contain substances capable of protecting animals against hypercholesterolemia. The sterols and polyterpenes present in the extract give it hypocholesterolemic properties, the ability to lower LDL cholesterol, anti-atherogenicity, anti-inflammatory and antioxidant activities [33]. A beneficial effect of *Sarcocephalus latifolius* on the lipid profile was nevertheless clearly demonstrated in this study by an increase in the serum level of HDL-cholesterol and a reduction in the concentration of LDL-cholesterol in the treated rats.

5. Conclusion

The results obtained in this work confirm the importance and therapeutic effect of the *Sarcocephalus latifolius* species. Indeed, the aqueous extract of the roots of *Sarcocephalus latifolius* shortens the bleeding time as well as hemostatic parameters such as prothrombin time, activated partial thromboplastin time and thrombin time and stimulates the formation of platelet nails. On the lipid profile, the beneficial effect of our extract has been proven by facilitating the elimination of bad cholesterol unlike good cholesterol, the content of which increases, highlighting its positive impact on cardiovascular health.

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The phytochemical study and toxicity tests were published in the Arabian Journal of Medicinal and Aromatic Plants [34].

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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