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(RESEARCH ARTICLE)

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Evaluation of the hemostatic properties of Aqueòus extract of *Bambusa vulgaris* Schrad ex. J.C. Wendl (POACEAE) leaves in Wistar rats

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Abstract

This study focused on *Bambusa vulgaris*, a species belonging to the Poaceae family, commonly used traditionally in several regions of Africa and Asia to treat various ailments;

This work aims to evaluate the hemostatic properties of an aqueous extract of *Bambusa vulgaris* leaves (EABv) in Wistar rats.

To do this, 5 groups of 4 rats each were formed. Every day, orally, the rats in group 1 (control group) received 2 ml of distilled water. Those in groups 2 and 3 (test groups) received the doses, 500 mg/kg BW and 1000 mg/kg BW of EABv respectively, while groups 4 and 5 received 500 mg/kg BW of aspirin and 500 mg/kg BW of dicynone respectively. The animals were treated for 28 days and each week, the bleeding time and blood count were performed. After the 28 days of treatment, the sedimentation rate was also determined.

The results showed that a dose of 1000 mg/kg BW of EABv significantly reduced bleeding time and increased platelet count and sedimentation rate. EABv administered at a dose of 1000 mg/kg BW did not significantly decrease red blood cell count, hemoglobin level, or hematocrit, unlike aspirin.

These results indicate that the extract exhibits hemostatic properties, but its use at high doses could cause adverse effects on blood cells.

Thus, the dose of 500 mg/kg BW of EABv seems the most appropriate because it exerts a hemostatic effect without altering hematological parameters. These results justify the use of this plant by traditional therapists to treat coagulation disorders.

Keywords: *Bambusa vulgaris*; Sedimentation Rate; Hemostatic Parameters; Bleeding Time; Blood Count

1. Introduction

Medicinal plants are often the primary therapeutic resource, particularly in rural areas where access to modern healthcare is limited [1].

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In Ivory Coast, traditional medicine has experienced unprecedented expansion over the years and remains the mainstay of primary healthcare for most residents, due to its geographical, economic, and cultural accessibility [2]. According to the [3], the population living below the poverty line in Côte d'Ivoire is estimated at approximately 44%. The high cost of patient care in modern hospitals, whether public or private, and the near absence of social security coverage lead a large portion of the population to turn to traditional medicine.

Bambusa vulgaris, a bamboo species belonging to the Poaceae family, has been traditionally used in medicine in several regions of Africa and Asia. Thus, [4] this plant is used to treat various conditions, such as typhoid fever, malaria, diabetes and high blood pressure. According to traditional pharmacopoeia, a decoction of *Bambusa vulgaris* leaves is used to treat high blood pressure [5]. The hypoglycemic [6], antimicrobial [7] and toxicity [8] effects of this species have been revealed.

Worldwide, an estimated half a million women die each year from causes related to pregnancy, childbirth, or the postpartum period [1]. Given the high number of maternal deaths, this study aims to demonstrate the hemostatic effects of an aqueous extract of *Bambusa vulgaris* (Poaceae) in wistar.

2. Materials and methods

2.1. Plant Material

The plant material consists primarily of fresh *Bambusa vulgaris* leaves harvested from the Agban National Gendarmerie barracks (Abidjan, Ivory Coast) on Tuesday, May 6, 2025. This plant was identified and authenticated at the National Floristic Center (CNF) of the Félix Houphouët-Boigny University, in comparison with the center's herbarium UCJ006804.

2.2. Animal Material

Rats of the species *Rattus norvegicus* (Muridae), Wistar strain, raised under the same conditions as the mice, were used for all other tests, namely bleeding time, complete blood count (CBC), and sedimentation rate.

2.3. Study Methods

2.3.1. Preparation of the aqueous extract of Bambusa vulgaris (Poaceae) leaves

The extract was prepared according to the method described by [9]. To prepare an aqueous extract of *Bambusa vulgaris*, 400 g of fresh leaves of this plant were boiled for 1 hour in 4 liters of distilled water. The resulting decoction was filtered first through white cloth (poplin), then through absorbent cotton, and finally through Whatman No. 2 filter paper. The collected filtrate was dried in an oven (Vacutherm Vacuum Oven, France) at 40°C for 72 hours.

The preparation of this aqueous extract is summarized in the diagram in Figure 1. After drying, the aqueous extract of *Bambusa vulgaris* (EABv) was presented in powder form. This product (EABv) is used for toxicity tests in mice and pharmacological studies in rats.

2.3.2. Method for Studying Pharmacological Effects

Measurement of Bleeding Time

For bleeding time, 20 *Rattus norvegicus* (Muridae), Wistar strain, male and female (100-130 g) were divided into 5 groups of 4 rats each. Every day, orally, rats in group 1 (control group) received 2 ml of distilled water. Those in groups 2, and 3, (test groups) received doses of 500 mg/kg BW, and 1000mg/kg BW of EABv, respectively, while groups 4 and 5 received 500 mg/kg BW of aspirin and 500 mg/kg BW of dicynone, respectively. The animals were treated for 28 days. Bleeding time was determined for each animal before the start of treatment and then weekly during treatment, using the Duke method. In fact, the tip of each rat's tail is cut off, causing bleeding. A stopwatch is started as soon as the animal begins to bleed. White paper is used to wipe away the blood every 15 seconds. As soon as the bleeding stops, the stopwatch is stopped and the time recorded as the bleeding time for each animal is noted.

Complete Blood Count

Complete blood count is performed using a Sysmex KX-21 NMC automated hematology analyzer on whole blood collected in a tube containing EDTA. The automated analyzer used directly provides the following hematological parameters (white blood cell count/µL, red blood cell count/µL, hemoglobin level, hematocrit level, platelet count/µL,

mean corpuscular hemoglobin volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin content).

Blood Sedimentation Rate Measurement

The measuring equipment consists of EDTA tubes, 1 and 5 ml syringes, and a ruler. After all the bleeding time and CBC tests, the rats from each group are sacrificed and their blood is collected for this test.

Blood is collected directly into the EDTA tubes. Using the ruler, the height of the solution poured is measured. This height must be at the same level in the other tubes.

The tubes are finally placed in a vertical position. The height of the blood supernatant or plasma in each tube is measured per hour using a millimeter ruler. The value of this height in relation to time makes it possible to determine the sedimentation rate or ESR.

2.4. Results Processing

2.4.1. Statistical Analysis

The computer program GraphPad Instat (San Diego, CA, USA) was used for statistical analysis of the results. Values are given as the mean, followed by the standard error of the mean (SEM). Statistical differences between results were determined using ANOVA, followed by the Tukey-Kramer multiple comparison test. Significance was determined for $p < 0.05$, with:

- $p > 0.05$, the observed difference was not significant (ns);
- $p < 0.05$, the observed difference was not significant (*);
- $p < 0.01$, the observed difference was significant (**);
- $p < 0.001$, the observed difference was highly significant (***).

Graphs

The computer program GraphPad Prism 8 (San Diego, CA, USA) is used to plot the graphs. Sigmoid curves are plotted after transforming the x-axis values into decimal logarithms.

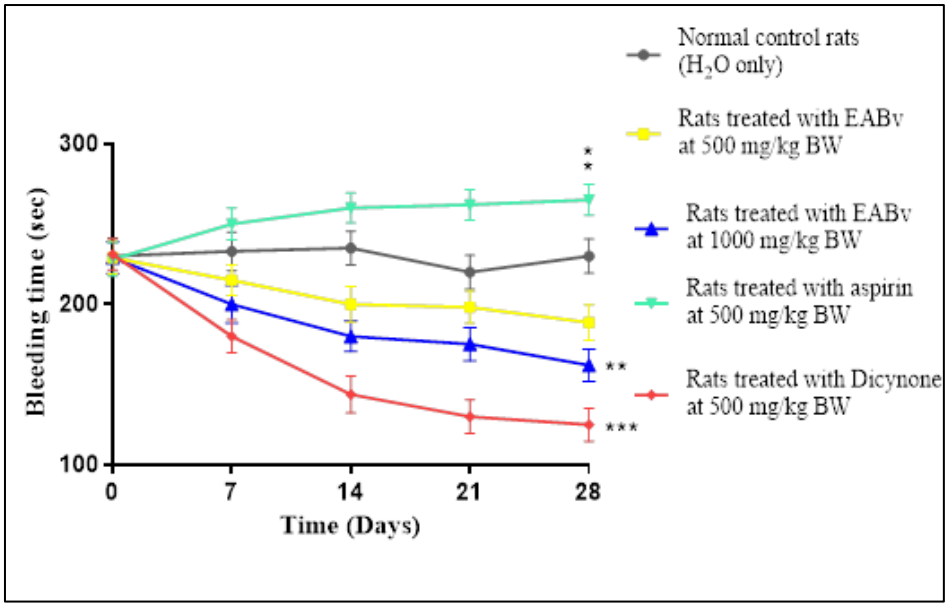
3. Results

3.1. Studies of an aqueous extract of *Bambusa vulgaris* (EABv) on hemostasis

3.1.1. Effect of Bambusa vulgaris (EABv) on bleeding time

The bleeding time of control rats did not vary significantly throughout the experiment (Figure 1). This time was approximately 229.8 s ± 11.6 at the end of the experiment. When rats were treated with the aqueous extract of *Bambusa vulgaris* (EABv) at a dose of 500 mg/kg BW, the bleeding time decreased from 228.9 s ± 10.2 at the beginning of the experiment to 188.70 s ± 11.01 after 28 days of treatment. This represents a 17.56% reduction in bleeding time. Also, EABv at a dose of 1000 mg/kg BW, causes a significant decrease ($P < 0.05$) in the bleeding time of rats treated for 28 days. Indeed, the bleeding time goes from 229.05s ± 9.9 before treatment, to 162.02s ± 10.2 after 28 days of treatment. This represents a decrease in bleeding time of around 28.83%.

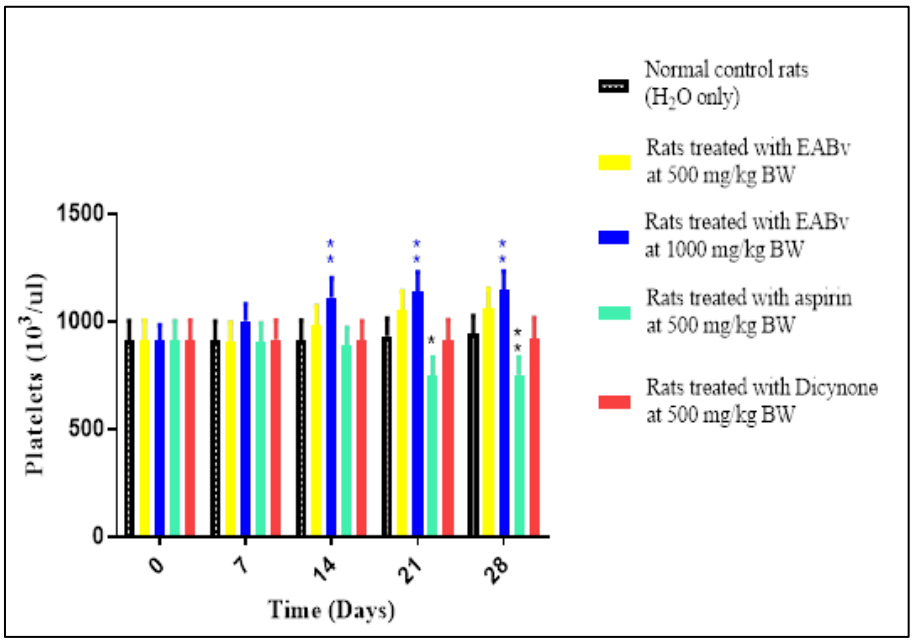
Oral administration of dicynone at a dose of 500 mg/kg BW also caused a significant decrease ($P < 0.05$) in bleeding time, which decreased from 231s ± 9.8 before treatment to 125.07s ± 10.2 after treatment (45.85% reduction). However, when rats were treated with aspirin at a dose of 500 mg/kg BW, there was a significant increase in bleeding time, which increased from 228s ± 10.6 before treatment to 265.01s ± 9.7 at the end of the experiment.



p <0.01 (**), p <0.001 (***) compared to the control

Figure 1 Effect of *Bambusa vulgaris* (EABv) on bleeding time

3.1.2. Effect of Bambusa vulgaris (EABv) on blood platelets as a function of time



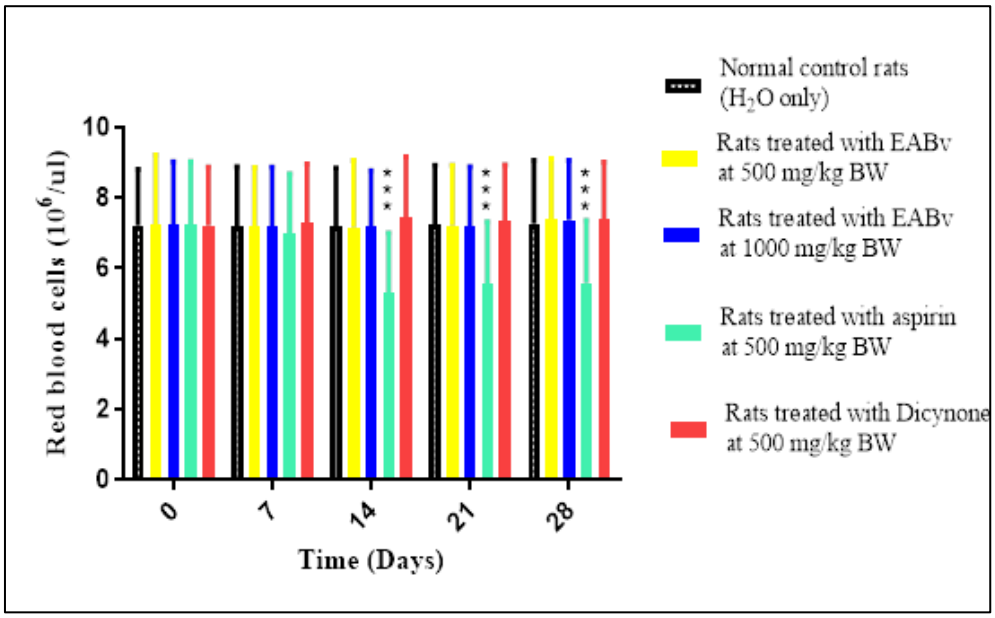
p <0.05 (*), p <0.01 (**) compared to the control group

Figure 2 Effect of *Bambusa vulgaris* (EABv) on blood platelets over time

The blood platelet count of the control rats remained constant throughout the experiment (Figure 2). It was around 929.02 ± 83.20 (10^3/µl) at the end of the experiment. The aqueous extract of *Bambusa vulgaris* administered at a dose of 500 mg/kg BW had no significant effect ($P > 0.05$) on the blood platelet count compared to the platelet count of the control rats. On the other hand, when rats were treated with the dose of 1000 mg/kg BW, the number of blood platelets increased from 913.45 ± 70 (10^3/ul) at the beginning of the experiment, to 1142.5 ± 90.86 (10^3/ul) after 28 days of treatment. This is an increase of 25.08% in the number of blood platelets. Oral administration of dicynone at a dose of 500 mg/kg BW, does not cause any effect on the number of blood platelets after 28 days of treatment. However, treatment of rats with aspirin at a dose of 500 mg/kg BW, causes a significant decrease in the number of blood platelets, which goes from 912.5 ± 87.6 (10^3/ul) before treatment, to 752.04 ± 80.4 (10^3/ul), or 17.58% after 28 days of treatment.

3.2. Effect of *Bambusa vulgaris* (EABv) on red blood cell count

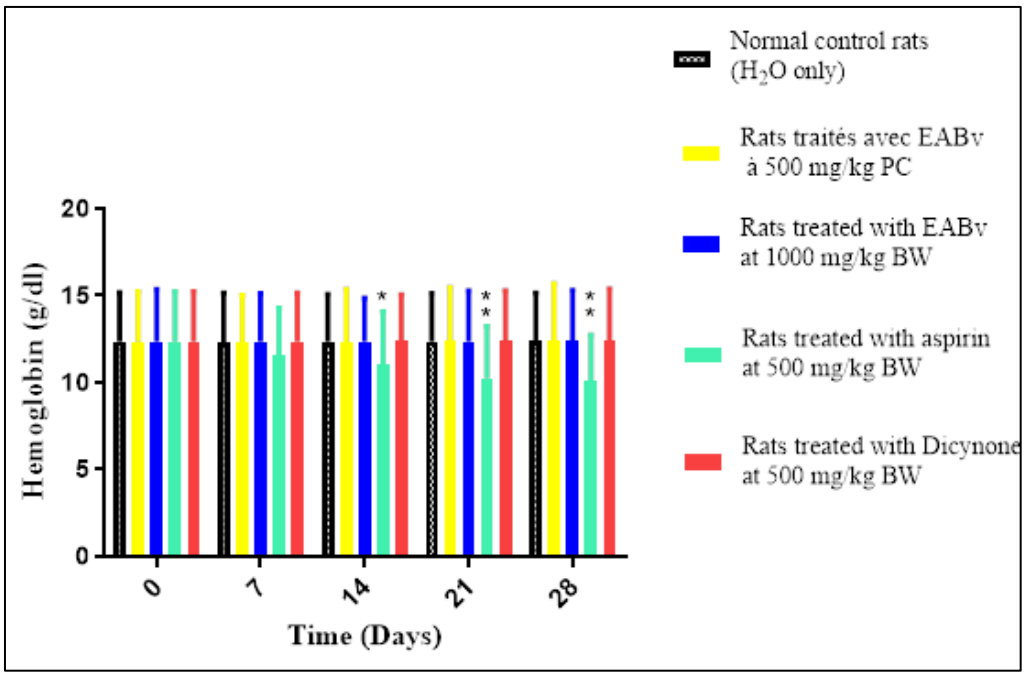
The red blood cell count of the control rats did not vary significantly throughout the experiment (Figure 3). This number was around 7.23 ± 1.70 (10^6 /µl) at the end of the experiment. Administration of EABv at doses of 500 mg/kg BW and 1000 mg/kg BW did not result in a significant change in the red blood cell count compared to the control group. Administration of dicynone at a dose of 500 mg/kg BW did not cause a significant change in the red blood cell count after treatment compared to the control group. This number is of the order of 7.35.10^6 /ul ± 1.59. However, when rats are treated with aspirin at a dose of 500 mg/kg BW, the number of red blood cells decreases (7.23.10^6/ul ± 1.80) to 5.57.10^6 /ul ± 1.75 at the end of the experiment.



p <0.01(**) compared to the control group

Figure 3 Effect of *Bambusa vulgaris* (EABv) on red blood cells over time

3.3. Effect of *Bambusa vulgaris* (EABv) on hemoglobin levels

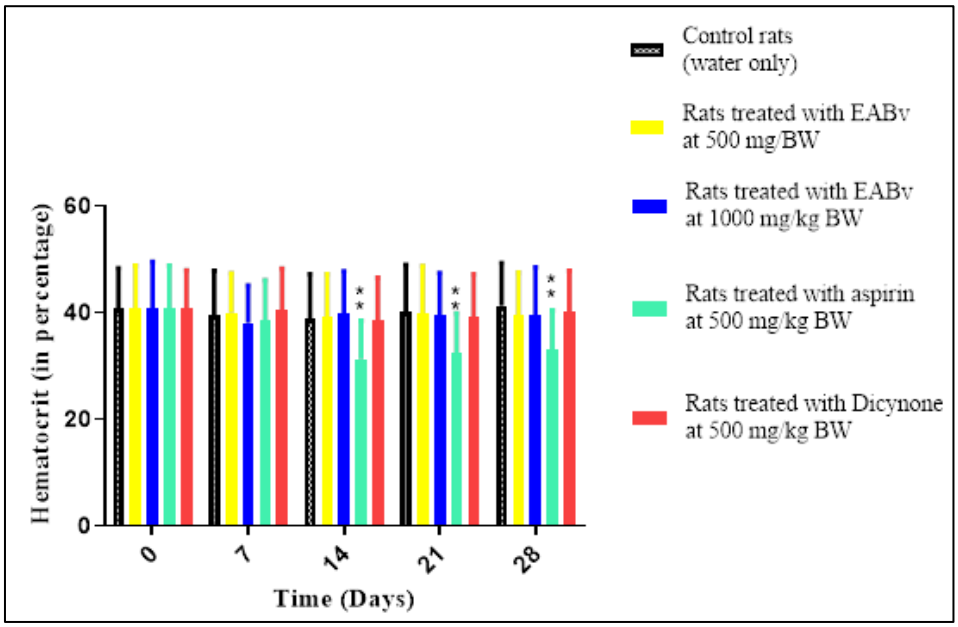


p <0.05 (*), p <0.01 (**) compared to the control group

Figure 4 Effect of *Bambusa vulgaris* (EABv) on blood hemoglobin over time

The hemoglobin level of the control rats did not vary significantly throughout the experiment (Figure 4). This level was around 12.35 ± 2.78 g/dL at the end of the experiment. Administration of EABv at doses of 500 mg/kg BW and 1000 mg/kg BW did not result in any significant change in hemoglobin levels compared to the control group. Administration of dicynone at a dose of 500 mg/kg BW did not cause any significant change in hemoglobin levels after treatment compared to the control group. This rate is of the order of 12.38 g/dl ± 2.90. However, when the rats are treated with aspirin at a dose of 500 mg/kg BW, the hemoglobin level decreases significantly and goes from 12.36 g/dl ± 2.86 to 10.12 g/dl ± 2.99 at the end of the experiment, a decrease of 18.12%.

3.3.1. Effect of Bambusa vulgaris (EABv) on hematocrit



p <0.01(**) compared to the control

Figure 5 Effect of *Bambusa vulgaris* (EABv) on hematocrit as a function of time

The hematocrit of the control rats did not vary significantly throughout the experiment (Figure 5). This rate was around 40.7% ± 4.85 at the end of the experiment. Administration of EABv at doses of 500 mg/kg BW did not result in a significant change in hematocrit compared to the control group.

However, the hematocrit of the group receiving 1000 mg/kg BW decreased insignificantly. Indeed, the hematocrit goes from 40.71% ± 6.5 before treatment to 39.5% ± 5.80 after treatment. The administration of dicynone at a dose of 500 mg/kg BW does not cause a significant variation in the hematocrit after treatment compared to the control group. This rate is around 40.72% ± 5.05. However, when the rats are treated with aspirin at a dose of 500 mg/kg BW, a significant decrease in the hematocrit is noted, which goes from 40.79% ± 6.03 before treatment to 33.02% ± 5.39 at the end of the experiment, i.e. a decrease of 19.05% in the hematocrit.

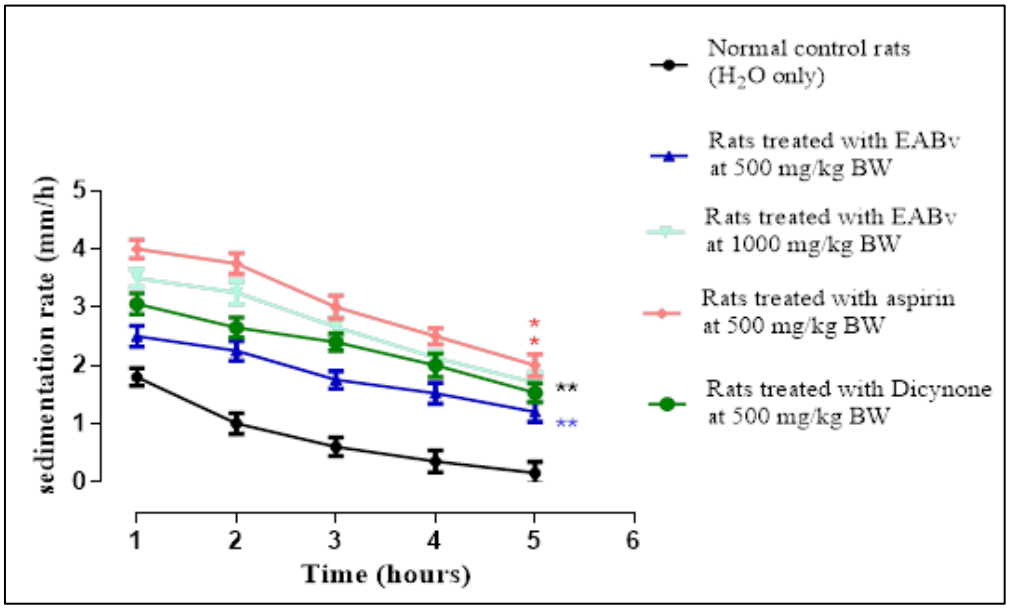
3.4. Effect of *Bambusa vulgaris* (EABv) on erythrocyte sedimentation rate

In this series of experiments, the blood used was that of rats from the first two tests. Indeed, after the bleeding time and CBC tests, the animals were sacrificed and their blood sedimentation rate was measured. The objective of this study was to measure the deposition time of suspended elements in the plasma of a given volume of blood rendered incoagulable with EDTA.

In all the experimental tubes, the sedimentation rate at the first hour is relatively faster because the supernatant column is always longer. This descent of elements decreases from the first to the fifth hour (Figure 6). Moreover, this rate, at each time period, is always higher compared to the control.

From 1.8 mm/h at the first hour, the rate in the control tube drops to 0.5 mm at the fifth hour. Whereas in the blood tubes treated with EABv, the sedimentation rate is always higher at all time periods from the lowest dose received to the highest (from 500 mg/kg BW to 1000 mg/kg BW). The sedimentation rate in the blood tubes from rats treated with

dicynone is high when compared to the control. It is more or less equal to that observed in the blood tubes treated with 500 mg/kg BW of EABv.



p <0.01(**) compared to control

Figure 6 Effect *of Bambusa vulgaris* (EABv) on sedimentation rate as a function of time

4. Discussion

Administration of *Bambusa vulgaris* aqueous extract (BAEv) at doses of 500 and 1000 mg/kg BW significantly reduced bleeding time. The effect of BAEv on bleeding time was similar to that of the ethanolic extract of *Chromolaena odorata* in albino rats [10].

These results suggest that BAEv may contain active substances with hemostatic effects. Platelet count increased significantly at a dose of 1000 mg/kg BW. This suggests that the decrease in bleeding time is due to the synthesis and activation of blood platelets. BAEv therefore stimulates the synthesis and activation of blood platelets. On the other hand, aspirin (reference substance) administered at a dose of 500 mg/kg BW causes a significant increase ($P<0.01$) in bleeding time after 28 days of treatment while decreasing the number of blood platelets. This result confirms that aspirin has anti-platelet properties.

Like dicynone (reference substance), EABv reduces bleeding time. However, EABv and dicynone act through two different mechanisms of action. Indeed, dicynone acts on the first stage of hemostasis (endothelial-platelet interaction), improving platelet adhesion and restoring capillary resistance. The results obtained therefore justify the traditional use of this plant for stopping bleeding and wound healing.

Furthermore, administration of EABv, at a dose of 500 mg/kg BW, had no significant effect ($P > 0.05$) on hematological parameters (red blood cell count, hemoglobin level, hematocrit), as did dicynone. This means that for these doses (500 and 1000 mg/kg BW), EABv has a similar effect to that of dicynone on the CBC. On the other hand, aspirin, which is a substance that increases coagulation time, significantly modifies these parameters.

EABv administered at a dose of 1000 mg/kg BW does not cause a significant decrease in red blood cell count, hemoglobin level, or hematocrit, unlike aspirin. However, a downward trend in these parameters was observed starting at this dose, suggesting a potential risk of hemolytic anemia at higher doses.

Furthermore, the 1000 mg/kg BW dose of EABv significantly reduced bleeding time and increased platelet count. These results indicate that the extract exhibits hemostatic properties, but its use at high doses could lead to adverse effects on blood cells. Thus, the 500 mg/kg BW dose of EABv appears to be the most appropriate because it exerts a hemostatic effect without significantly altering hemostatic parameters.

The effect of EABv on the sedimentation rate reveals that this rate increases with the dose received. Indeed, within the same time period, the higher the dose, the higher the rate. The increase in the blood sedimentation rate of rats treated with doses of 300 mg/kg BW and 500 mg/kg BW could be explained in two ways:

Either the blood would become more viscous, in which case EABv would behave like *Hibiscus sabdariffa* (Malvaceae) studied by [11]. The active ingredient of EABv would therefore form a complex with a blood factor that is of the enzyme-substrate type. It is therefore because this complex is certainly relatively heavy, that the elements settle more quickly by gravity.

Either the blood would become less viscous or more fluid, in this case, the blood in the presence of EABv would behave like a perfect liquid and would explain the use of EABv in the treatment of diabetes [12].

At a dose of 1000 mg/kg BW, we observed that rats were anemic. Therefore, the elevated erythrocyte sedimentation rate at this dose could be largely due to this disorder, as the erythrocyte sedimentation rate increases in cases of anemia, regardless of the cause [13].

The high erythrocyte sedimentation rate observed in rats treated with aspirin is explained by the fact that aspirin makes the blood much thinner than dicynone and EABv.

As for the increased blood sedimentation rate in the dicynone-treated group, it should be noted that this reference product is known to increase platelet aggregation. Indeed, platelet aggregation is responsible for the formation of the platelet plug. This plug is important in the clotting process. Dicynone would therefore make the blood much more viscous in the animals than aspirin and the controls.

5. Conclusion

The 1000 mg/kg BW dose of EABv significantly reduced bleeding time and increased platelet count and sedimentation rate. Unlike aspirin, EABv administered at a dose of 1000 mg/kg BW did not significantly decrease red blood cell count, hemoglobin level, or hematocrit. However, a downward trend in these parameters was observed starting at this dose, suggesting a potential risk of hemolytic anemia at higher doses. These results indicate that the extract exhibits hemostatic properties, but its use at high doses could lead to adverse effects on blood cells.

Thus, the 500 mg/kg BW dose of EABv appears to be the most appropriate because it exerts a hemostatic effect without significantly altering hematological parameters. These results justify the use of this plant by traditional healers to treat coagulation disorders.

Compliance with ethical standards

Acknowledgements

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Disclosure of conflict of interest

The authors declare no conflict of interest

Statement of ethical approval

Animals were treated according to the standard standards of laboratory animals published by the Interdisplinary Principles and Guidelines for the Use of Animals in Research, Testing and Education issued by the New York Academy of Science Adhoc Committee on Animal Research and the National Institute of Animal Health Hygiene and use of laboratory animals (Ivory Coast).

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