

Haemostatic Properties of *Chrysophyllum Cainito* L. (Sapotaceae) in Wistar Rat

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Abstract

The measure of certain blood parameters makes it possible to detect certain pathologies such as the hemophilia and leukemia among patients. Indeed, the aggregating and antiaggregating activity of certain substances are known. It is a question in this work of checking the effect of the aqueous extract of *Chrysophyllum cainito* (AECc) on blood. It is a comparative study between AECc and two products of synthesis known for purpose which are the dicynone and the aspirin in vivo in the rats wistar specie *Rattus norvegicus*. The time of bleeding (TB), the number of blood plates, the red blood corpuscle rate, of hemoglobin, the hematocrit and the sedimentation test (TS) were evaluated. During the first week, the evolution of the time of bleeding (TB) is not significant for all the animals. The second week and until the end of the treatment, the TB drops for the animals treated with EACc in an amount-dependant way. Thus the fall is of 4% for the amounts of 300 mg/kg b.w, 17.4% for the amounts of 500 mg/kg b.w and 30.43% for the amounts of 1000 mg/kg b.w at the end of the treatment. In the animals treated with the dicynone, the fall reaches 41.55% at the end of the thirty days of treatment. On the other hand, in the rats treated with the aspirin, the TB increases as from the second week and reached the rise of 17.40% at the end of the thirty days. On the blood plates, AECc varies their number of manner proportions dependant as of the first week on treatment. The number of blood plates increases by 07.69% and 15.38% for the amount of AECc of 300 mg/kg b.w, 15th and 30th day. The amount of 500 mg/kg b.w of AECc makes increase the number of plates of 09.89%, 21.97%, and 25.16%, respectively 5th, 15th and 30th day.

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The concentration of AECc of 1000 mg/kg b.w involves an increase in the plates of 22.31%, 27.36% and 33.07%, respectively with 5th, 15th and 30th day of treatment. The dicynone is managed with the rats with the amount of 500 mg/kg b.w the number of blood plates passes thus from 910 ± 85.83 to 915 ± 88.0 , 918 ± 87.0 then 920 ± 92.0 ; 5th, 15th and 30th days of treatment is 0.55%, 0.88% and 1.09%. This variation is nonsignificant. On the other hand the aspirin managed with the rats with the amount of 500 mg/kg b.w cause a drop in the number of blood plates as of the 5th days with 900 ± 88.2 then with 889 ± 82.2 15th and 750 ± 80.6 the 30th. These drops successive represent rates by -1%, -2.30% and -17.58%. The other factors such as the number of red blood corpuscles the rate of hemoglobin, the hematocrit and also the sedimentation test do not vary significantly before and during the treatment.

Keywords: time of bleeding; rate of blood plates; hemoglobin; hematocrit.

1. Introduction

Chrysophyllum caïnitio or caïnitier is an exotic fruit tree (used in ornamentation of the gardens and avenues) to cultivate for its fruit called "apple of milk" or kainite which picking and does not fall to maturity.

All the parts of the plant are very much used in traditional medicine:

The ripe fruit is eaten to alleviate the ignitions of the laryngitis, pneumonia, the sweetened diabetes, the intestinal disorders [1].

The decoction of the bark is drunk like tonic and gastric stimulant, to stop the acute diarrhea, the dysentery, and gonorrhoea [2].

The decoction of the sheets is used in the treatment of the sweetened diabetes [3].

Our investigations revealed that the decoction of the sheets is used to stop the haemorrhage postpartum in sub-Saharan Africa.

However, according to WHO [4] and the UNICEF [5], the hemorrhages postpartum represent a risk factor of death nursery and infantile (newborn) in Africa and Asia.

This work aims to check the hemostatic activity of the plant and to evaluate its effects of on the hematologic parameters in the rat wistar *Rattus norvegicus* species.

2. Materials and methods

2.1. Materials

2.1.1. Vegetable material

It is primarily composed of fresh sheets of *Chrysophyllum caïnitio* collected in the national floristic center (CNF) of the University of Abidjan.

The fresh sheets are carried to boiling during one hour (1h), in one liter and half of water (1.5l).

Decoction is filtered initially on cotton then on filter paper Wattman n°1. The filtrate obtained is dried with the drying oven with 50°C during sixty two hours according to the method improved by Abo [6].

The dry aqueous extract obtained in the form of powder is weighed and diluted to constitute the extracts tests or the aqueous extract of *Chrysophyllum cainito* (AECc).

2.1.2. Animal material

Rats of Wistar stock, *Rattus norvegicus* species (Muridae), high in the animalery of the Laboratory of Animal Physiology (Training and Research Unit of Biosciences) under conditions standard at the temperature of 24°C were used for the realization of the tests.

2.2. Methods

2.2.1. Experimental methods

2.2.1.1. Time of bleeding

Thirty (30) rats of the two sexes weighing between 100g and 130g were left again in six (6) groups of five (5) rats each one.

The every day by oral way, the rats of the batch n°1 (pilot batch) are nourished with granulated and receive 2 ml of water distilled in addition to the quantity necessary.

The rats of the batches tests 2; 3 and 4 are nourished with same the pellets and respectively receive 2 ml of 300 mg/kg body weight (b.w), 500 mg/kg b.w and 1000 mg /Kg b.w of aqueous extract of *Chrysophyllum cainito* in addition to the quantity of water necessary.

The batch n°5 receives 2 ml of 500 mg/kg b.w of aspirin and the batch n°6 receives 2 ml of 500 mg/kg b.w of dicyclicone in addition to water necessary. The animals are thus treated during thirty days (1 month).

The time of bleeding is given for each rat of each batch before the beginning of experimentation by the method of Duke.

Technique of Duke: After local disinfection with alcohol, an incision of a few millimeters, nonpainful, is carried out using a small point ("microlance") at the end of the tail. As soon as the first blood drops appear, a stop watch is started. The blood drops are collected every 30 seconds on a blotter until the stop of the bleeding. The time of bleeding is thus determined.

2.2.1.2. Numeration formulates blood (NFB)

It is carried out using a hematologic analyzer automated KX-21N^{mc} of Symex on blood taken in a tube containing of the EDTA.

The automat used makes it possible to read directly:

The number of white globules (/μl)

The number of red globules (Red blood corpuscles) (/μl)

The rate of hemoglobin (g/dl)

The rate of hematocrit (%)

The number of plates (/μl)

Average globular volume in hemoglobin

Average globular concentration in hemoglobin

Corpuscular content means of hemoglobin

2.2.1.3. Sedimentation test (TS)

The animals are anaesthetized with the ethylcarbamate (1g/kg b.w). A dissection on the level it neck makes it possible to expose the carotids. The blood is collected in a syringe containing of the anticoagulant.

The blood of each animal of each batch is versed in graduated test-tubes of 10 ml containing 1ml anticoagulant (NaCl 9‰ solution + heparin).

Every hour, the level of the supernatant in each test-tube is noted. This level is equivalent to the drop height of the blood cells evaluated in millimeter per hour (mmh). It is the speed of decantation or blood sedimentation (VS).

2.2.1.4. Phytochemical screening

The description of made up the bioactifs contained in the aqueous extract of *Chrysophyllum cainito* was done by tests of characterization of the chemical compounds. It is based on the principle of induction of chemical reactions in the presence of suitable reagents (see table 1).

2.3. Treatment of the results

***Statistical analyzes**

The computer program GraphPadInstat6 (San Diégo CA, the USA) was used for the statistical analysis of the

results and to draw the graphs.

Values are given as mean followed by standard error of the mean (mean ± sem). The difference between two values is determined by the test in Student-Newmann-Kreul comparison test. It was significant for a probability superior to 5% (p< 0.05) (*).

Table 1: reactions of description of composed in the aqueous extract of *Chrysophyllum cainito*

Required compound	Reaction/Reactive	Results
Polyphenols	Ferricchloride	+
Sterols and polyterpenes	Liebermann-Bouchard	+
Flavonoïdes	The cyanidine	+
Saponosides	Vigorous agitation	-
Quinoidcompounds	Borntraeger	-
Alkaloids	Dragendorff	+
	Bouchardât	+
Tannins catechic	Stiasny	-
Gallic tannins	Hydrochloricacid	-

(+): composed detected (present)

(-): composed not detected (absent)

3. Results

3.1. The time of bleeding (TB) in seconds (S)

Table 2 shows the mean values and the standards errors of the time of bleeding of the rats of each batch for one month of treatment. The time of average bleeding in the rats before treatment is about 240±10.6 seconds.

In the pilot rats, the variation of the TS is not significant during the experimentation.

During the first week, the evolution of the TS is not significant for all the animals (figure1).

The TB drops the second week and until the end of the treatment, for the animals treated with AECc in an amount-dependant way.

Thus the fall is of 4% for the amounts of 300 mg/kg b.w, 17.4% for the amounts of 500 mg/kg b.w and 30.43% for the amounts of 1000 mg/kg b.w at the end of the treatment.

For the animals treated with the dicynone the fall reaches 41.55% at the end of the thirty days of treatment.

On the other hand, in the rats treated with the aspirin, the TB increases as from the second week and reached the rise of 17.40% at the end of the thirty days.

Table 2: evolution of the time of bleeding (TB) according to time

Time (days)	Pilot batch		Batch treated AECc (300 mg/kg b.w)		Batch treated AECc (500 mg/kg b.w)		Batch treated AECc (1000 mg/kg b.w)		Batch treated Aspirin 500 mg/kg b.w)	
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem
0	230	11.4	229	10.2	230	09.7	230	09.9	23.0	10.6
5	233	12.0	228	09.5	222	13.0	200	11.7	240	10.0
15	235	10.6	225	11.1	200	13.0	180	09.5	260	09.4
30	240	10.6	220	10.0	190	11.0	160	10.3	270	09.5
0	230	11.4	229	10.2	230	09.7	230	09.9	23.0	10.6

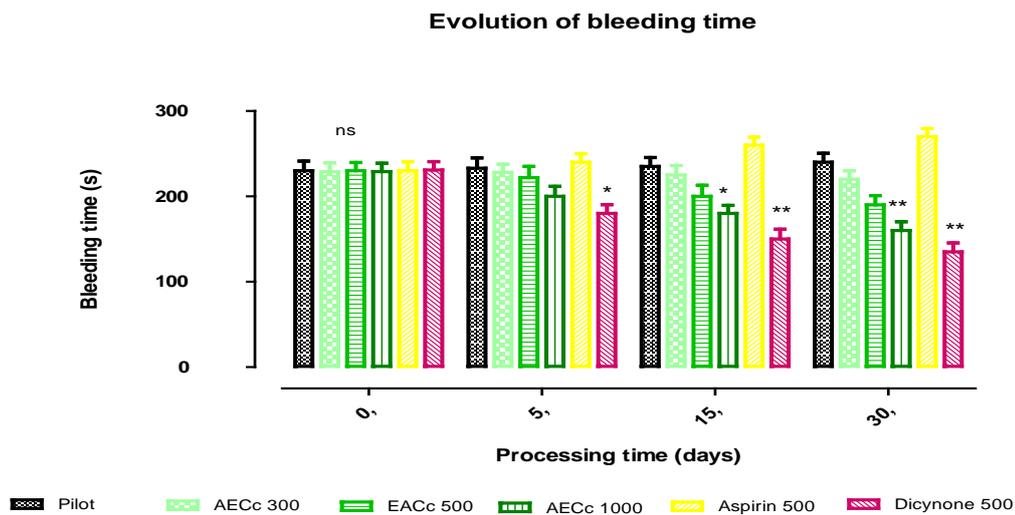


Figure 1: evolution of the time of bleeding according to the processing time

3.2. AECc effect on the blood plates ($10^3/\mu l$)

The rate of blood plates is evaluated before the tests for each rat of each batch. It is about 910 ± 85.83 . Table 3 shows the evolution of the average quantity of the blood plates during the experimentation. In the pilot rats, the variation of the rate of plates is not significant during the experiment. As of the first week of treatment, the rate of blood plates varies in manner proportions dependant compared to the pilot rats. As from the 15th day, the rate of blood plates passes to 980 ± 89.00 , then with 1050 ± 87.67 the 30th day; that is to say a rise of 07.69% and 15.38% for the amount of AECc of 300 mg/kg b.w. The amount of 500 mg/kg b.w of AECc increases the rate of plate as of the 5th day. The rate passes from 910 ± 85.83 to 1000 ± 80.68 , 1110 ± 90.0 and 1139 ± 89.50 . What is equivalent to rates of variation of 09.89%, 21.97%, and 25.16%. This rate is significant as of the second week.

The concentration of AECc of 1000 mg/kg b.w involves a significant growth of the blood plates as of the 5th day. The quantity of the plates passes from 910±85.83 to 1113±85.3, 1159±87.7 and 1211±90.2. That is to say a successive variation of 22.31%, 27.36% and 33.07%. The dicynone managed with the rats with the amount of 500 mg/kg b.w does not involve significant variation of the rate of blood plates. The rate thus passes from 910±85.83 to 915±88.0, 918±87.0 and 920±92.0 5th, 15th and 30th days of treatment. On the other hand the aspirin managed with the rats with the amount of 500 mg/kg b.w cause a drop in the rate of blood plates as of the 5th days with 900±88.2 then with 889±82.2 15th and 750±80.6 the 30th. These drops successive represent rates by -1%,-2.30% and -17.58%. The fall becomes significant only at the end of thirty days of treatment (figure 2).

Table 3: variation of the rate of blood plates according to time (x10³/µl)

Time (days)	Pilot batch		Batch treated EACc (300 mg/kg b.w)		Batch treated EACc (500 mg/kg b.w)		Batch treated EACc (1000 mg/kg b.w)		Batch treated Aspirin 500 mg/kg b.w)		Batch treated Dicynone 500 mg/kg b.w)	
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem
0	910	92.3	909	95.0	910	70.0	910	80.1	910	87.6	910	90.0
5	910	88.9	910	93.5	1000	80.7	1113	85.3	900	88.2	914	88.0
15	913	89.9	980	89.0	1110	90.0	1159	87.7	889	82.2	918	87.0
30	930	83.2	1050	87.7	1139	89.5	1211	90.2	750	80.6	920	92.0
0	910	92.3	909	95.0	910	70.0	910	80.1	910	87.6	910	90.0

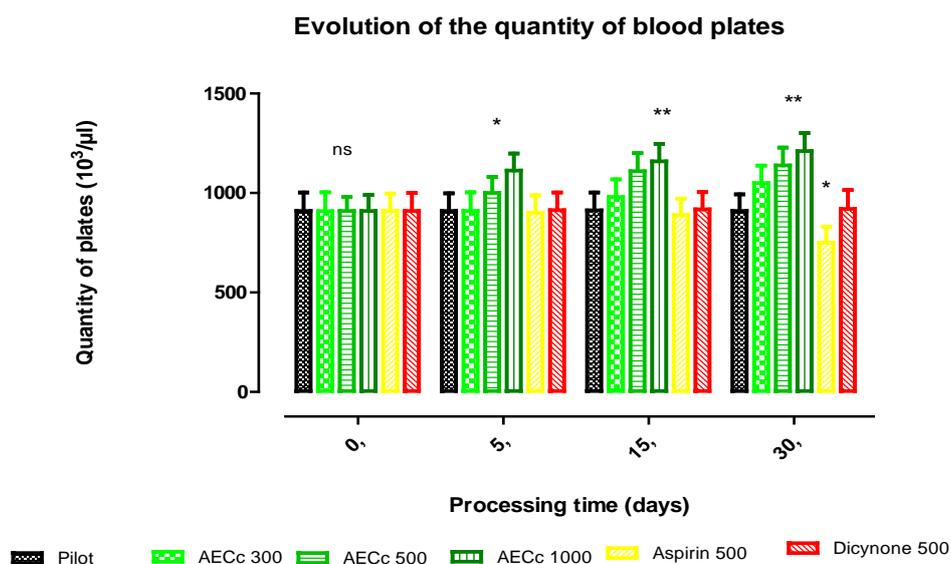


Figure 2: evolution of the volume of blood plates according to the processing time

3.3. AECc effect on the quantity of red blood corpuscles ($10^6/\mu\text{l}$)

The normal quantity of red blood corpuscles for all the rats is estimated at $7.22 \pm 1.80 \times 10^6 \mu\text{l}$.

Table 4 shows the variation of the quantity of red blood corpuscles per batch of the animals subjected to the experimentation.

In the pilot rats one can say that this rate practically does not vary it or the variation is not significant.

The animals treated with EACc with the amounts of 300 mg/kg b.w and 500 mg/kg b.w, the quantity of red blood corpuscles do not vary significantly.

The amount of 1000 mg/kg b.w does not make not varied significantly the red blood corpuscle rate the first 5 days. From the second week of treatment it lowers with $5.90 \pm 1.67 \times 10^6 \mu\text{l}$ and at the end of thirty days with $5.88 \pm 1.69 \times 10^6 \mu\text{l}$ is a reduction of -18%.

At the rats treated with aspirin 500 mg/kg b.w, the red blood corpuscle rate significantly does not vary the first week passing of $7.20 \times 10^6 \mu\text{l}$ with $7.00 \pm 1.68 \times 10^6 \mu\text{l}$ and dice the second week it drops with 5.57 ± 1.75 and $5.30 \pm 1.70 \times 10^6 \mu\text{l}$ is -26.39%.

In the animals treated with the dicynone the variation of the red blood corpuscle rate is nonsignificant. The quantity successively passes from 7.22 ± 1.80 to 7.29 ± 1.67 , 7.35 ± 1.59 and $7.46 \pm 1.70 \times 10^6 \mu\text{l}$ respectively 5th, 15th and 30th days.

Table 4: evolution of the red blood corpuscle rate according to time ($\times 10^6/\mu\text{l}$)

Time (days)	Pilot batch		Batch treated AECc (300 mg/kg b.w)		Batch treated EACc (500 mg/kg b.w)		Batch treated EACc (1000 mg/kg b.w)		Batch treated Aspirin 500 mg/kg b.w)		Batch treated Dicynone 500 mg/kg b.w)	
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem
0	7.22	1.59	7.23	1.98	7.23	1.79	7.22	1.79	7.23	1.80	7.22	1.66
5	7.21	1.69	7.21	1.72	7.20	1.68	7.14	1.46	7.00	1.68	7.29	1.67
15	7.20	1.66	7.19	1.67	7.20	1.58	5.90	1.67	5.57	1.70	7.35	1.59
30	7.19	1.70	7.19	1.90	7.19	1.69	5.88	1.69	5.30	1.75	7.46	1.70
0	7.22	1.59	7.23	1.98	7.23	1.79	7.22	1.79	7.23	1.80	7.22	1.66

3.4. AECc effect on the rate of hemoglobin (g/dl)

Before the treatment, the average rate of hemoglobin in the rats is estimated at $12.35 \pm 2.90 \text{ g/dl}$.

In the pilot rats, the rate of hemoglobin does not vary significantly.

The rats treated with 300 mg/kg b.w, 500 mg/kg b.w and 1000 mg/kg b.w of AECc have a rate of hemoglobin which does not vary significantly compared to the normal.

In the rats treated with 500 mg/kg b.w of dicynone, the variation is not significant during all the experimentation (table 4).

The aspirin with 500 mg/kg b.w, does not have a significant effect on the rate of hemoglobin during the first week of treatment.

During the second week and until the end of the month, the rate of hemoglobin drops slightly (17.07%)

Table 5: evolution of the rate of hemoglobin according to time (g/dl)

Time (days)	Pilot batch		Batch treated EACc (300 mg/kg b.w)		Batch treated EACc (500 mg/kg b.w)		Batch treated EACc (1000 mg/kg b.w)		Batch treated Aspirin 500 mg/kg b.w)		Batch treated Dicynone 500 mg/kg b.w)	
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem
0	12.3	2.82	12.4	2.70	12.4	2.87	12.4	2.76	12.4	2.70	12.3	2.80
5	12.3	2.85	12.4	2.87	12.3	2.78	12.2	2.80	11.6	2.86	12.3	2.89
15	12.3	2.73	12.3	3.05	12.3	2.50	11.4	2.88	11.0	2.99	12.4	2.67
30	12.3	2.78	12.3	3.10	12.3	2.92	11.1	2.96	10.2	3.04	12.4	2.90
0	12.3	2.82	12.4	2.70	12.4	2.87	12.4	2.76	12.4	2.70	12.3	2.80

3.5. AECc effect on the hematocrit (%)

The hematocrit in the rats before the treatment is of 40.81 ± 80 .

In the pilot rats, it does not vary significantly throughout the experimentation (table 6).

For the rats treated with AECc the variation are not significant during the experimentation.

In the same way, the rats treated with the dicynone, the hematocrit does not vary significantly.

The rats which are treated with aspirin 500 mg/kg b.w, the hematocrit drops with $31.25 \pm 7.39\%$ at the end of thirty days. The equivalent of a fall of 23.43% compared to the normal.

Table 6: evolution of the hematocrit according to time (%)

Time (days)	Pilot batch		Batch treated EACc (300 Mg/kg PC)		Batch treated EACc (500 Mg/kg PC)		Batch treated EACc (1000 Mg/kg PC)		Batch treated Aspirin 500 Mg/kg PC)		Batch treated Dicynone 500 Mg/kg PC)	
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem
0	40.8	7.50	40.8	7.40	40.8	7.05	40.8	7.50	40.8	7.28	40.8	7.09
5	40.1	8.24	40.0	8.05	39.8	7.99	37.0	7.80	40.6	7.39	40.6	7.70
15	39.6	8.25	39.8	8.08	39.4	8.02	36.8	8.02	32.5	7.44	39.1	7.96
30	39.0	8.85	39.1	8.96	38.0	8.80	36.3	10.1	31.2	8.03	38.5	8.05
0	40.8	7.50	40.8	7.40	40.8	7.05	40.8	7.50	40.8	7.28	40.8	7.09

3.6. AECc effect on the sedimentation test (VS) in millimeter per hour (mm/h)

The sedimentation test is high in all the hanging tubes the first two hours. Indeed, the blood elements heaviest settle quickly.

In all the tubes the level of clear supernatant, the equivalent drop height of the elements, is around 10 mm (1cm) at the end of the experimentation.

The noted variations are not significant enough between the various tubes at the same time by the multiple test of comparison. Thus, the blood of the pilot animals and that of those having undergone the various treatments have virtually identical VS under our experimental conditions (table 7).

Table 7: evolution of sedimentation test (mm/h)

Time (h)	Pilot batch		Batch treated AECc (300 mg/kg b.w)		Batch treated AECc (500 mg/kg b.w)		Batch treated AECc (1000 mg/kg b.w)		Batch treated Aspirin 500 mg/kg b.w)		Batch treated Dicynone 500 mg/kg b.w)	
	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem	mean	sem
1	3.00	0.15	3.00	0.17	3.5	0.18	3.5	0.15	3.50	0.16	3.00	0.15
2	2.5	0.18	2.5	0.14	2.5	0.17	3.00	0.19	3.00	0.18	2.50	0.16
3	2.00	0.16	2.00	0.18	1.5	0.15	1.5	0.16	1.5	0.20	2.00	0.17
4	1.00	0.19	1.5	0.20	1.5	0.18	1.5	0.10	1.00	0.14	1.50	0.18
5	0.5	0.19	1.00	0.19	1.00	0.17	0.5	0.18	0.50	0.19	1.00	0.20

4. Discussion

The administration of AECc involves a reduction in the time of bleeding. That is similar to the effect of the natural substance extract ethanolic such as *Chromolaena odorata* over the time of bleeding of the albino rats [7].

The time of bleeding is a total test of exploration of the primary education haemostasis appreciated by the stop of a bleeding caused by a cutaneous incision. It is influenced by:

-Plate factors (qualitative + quantitative)

-Factor of von Willebrand [8].

The AECc effect goes as that of the dicynone which has known haemostatic effects. And of the contrary results to that of the aspirin, known anti-haemostatic substance.

Indeed, the dicynone or Ethamsylate increases resistance capillary endothelium and the promotion of the adhesion of the plates [9].

It also inhibits the biosynthesis and the action of the prostaglandins which cause plate disintegration, the vasodilatation and the increase in the capillary permeability [10].

Etamsylate is a haemostatic agent; also support the action aggregating neuroprotector. Its haemostatic action is due to the activation of the formation of thromboplastine of the damaged sites of small blood-vessels and the reduction in the PGI₂ (I₂ prostacyclin) synthesis [11]. It also facilitates the plate aggregation and of adherence, which finally causes the reduction and the stop of the hemorrhage [12].

One observes also an increase in the rate of blood plates in the animals treated with AECc. Whereas this rate does not vary significantly in the animals treated with the dicynone and is in fall at those treated with the aspirin.

AECc would thus have a stimulative action of the thrombopoiesis and the plate release starting from osseous marrow. The increase in the rate of blood plates supports coagulation. The plate cells are responsible for the formation of the plate nail in the event of vascular lesion. Their role is important as well in the primary haemostasis as secondary [13].

The effect AECc is contrary with that of the aspirin. The aspirin (acetylsalicylic acid) is a plate drug antiagregant. It does not inhibit the plate function among patients [14, 15, 16].

On the other factors of the NFS which are the quantity of red blood corpuscles, the rate of hemoglobin and the hematocrit, AECc just as the dicynone do not modify these parameters significantly. Any long-term time and at strong amount of AECc the variations observed can be put in connection with toxicity. For example, in the rat one records a fall of the plate rate after administration at long life of the aspirin.

The proliferation of blood plates in an animal organization in situation normal is source of obstruction to blood

circulation. The cardiovascular accidents and certain cancers occur following a thrombosis [17, 18, 19, 20]

The variations of red blood corpuscles, hemoglobin and the hematocrit are dependant. The significant variations are observed among feeble patients [21, 22, 23].

At the normal subjects, these parameters vary very little. Thus in the animals treated or not, one observes a nonsignificant variation.

The phytochemical screening carried out one shows the presence of compounds polyphenolic and flavonoïdes. Work of N'guessan [24] indicated that the content of flavonoïdes seeds of *Aframomum melegueta* K. Schum. (Zingiberaceae) would be responsible for its effect antihemorrhagic. The sheets of *Ageratum conyzoides* L. (Asteraceae) were used a long time to calm the uterine bleedings, because of polyphenols of the coumarins types which act against the visceral hemorrhages [25]. In the same way, work of Nacoulma [26] and Jairj [27] showed that the strong contents of certain medicinal plants of flavonoïdes are responsible for their effect antihemorrhagic.

5. Conclusion

One can thus conclude that the presence of polyphenols and flavonoïdes in AECc makes substance antihemorrhagic some. Any time, the use in pharmacopeia must be controlled because the strong amounts with long term could involve circulatory troubles. This substance can be used in the event of urgency (hemorrhagic situation) in partnership with the dicynone to accelerate coagulation.

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