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Impact of Ethanolic Extract of *Ficus umbellata* (Vahl.) Leaves On The Blood-Thinning Process In Wistar Rats Made Obese By A Hypercaloric Diet

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Abstract

Obesity is a factor favoring hypercoagulation of the blood and exposes the body to the risk of venous or arterial thrombosis, responsible for cardiovascular disease. The main aim of this study is to contribute to the prevention of thromboembolic diseases by evaluating the effect of ethanolic extract of *Ficus umbellata* (Vahl.) leaves on the blood-thinning process in obese Wistar rats. The in-vitro anticoagulant activity test revealed that in the presence of the plant extract at concentrations of 10, 30 and 50 mg/mL, no coagulation was observed when the coagulate was observed in the control tube. Hemostasis parameters (bleeding time, coagulation time, prothrombin time, activated partial thromboplastin time, fibrinogen, platelets and D-dimer) were determined in obese rats treated with different doses of the ethanolic extract of *Ficus umbelleta* leaves and aspirin® used as the reference molecule. The results revealed a dose-dependent prolongation of parameters such as bleeding time, Quick time, coagulation time, activated partial thromboplastin time and thrombin time for doses of 100, 300 and 500 mg/Kg body weight. The effect of the extract is similar to that of aspirin 100 mg. Ethanolic extract of *Ficus umbelleta* leaves and aspirin® liquefy blood by the same mechanism. Treated animals also showed an increase in fibrinogen levels, correlated with a reduction in platelet and D-dimer levels. The extract has been shown to inhibit platelet aggregation and slow blood coagulation.

Keywords
Hypercoagulation, Fluidification, Platelet, Hemostasis.

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INTRODUCTION :

Pathologies associated with lipid accumulation in the human body, such as obesity and atherosclerosis, are becoming increasingly important health issues. These pathologies are generally the consequence of our modern lifestyles, which allow easy and rapid access to rich and abundant food, caloric intake in excess of energy expenditure, coupled with a permanent sedentary lifestyle and a lack of physical activity. The term obesity is derived from the Latin "obesus" meaning to fatten[1] and is defined as an abnormal or excessive accumulation of body fat that leads to adverse health consequences[2] . It favors the onset of numerous diseases, due to excess body fat or a chronic inflammatory state linked to excess abdominal adipose tissue. The most frequent complications are type 2 diabetes, osteoarthritis and, above all, haemostasis and coagulation disorders.

Hypercoagulation of the blood is an excess of coagulation that exposes it to the risk of venous or arterial thrombosis [3] and is the cause of cardiovascular diseases such as myocardial infarction, ischemic stroke, deep vein thrombosis or pulmonary embolism[4]. Blood clots (thrombus) obstruct a vein and impede blood flow [5]. Curative treatment relies primarily on the prescription of anticoagulants, also known as blood thinners. Oral anticoagulants are used to prevent thrombosis [6].

Previous work has revealed that *Ficus umbelleta* leaves, used in traditional African medicine, possess numerous pharmacological effects such as antioxidant, anesthetic and anti-inflammatory effects[7]. *Ficus umbellata* belongs to the Ficus genus and the Moraceae family. It is a plant that can reach 6 to 10 m in height, whose various parts are used in traditional medicine to treat various diseases[8].

The aim of this study is to contribute to the prevention of thromboembolic diseases by evaluating the effect of ethanolic extract of *Ficus umbellata* (Vahl.) leaves on the blood-thinning process of obeses rats.

MATERIALS AND METHODS

MATERIAL

Plant material

The plant material used is the ethanolic extract of *Ficus umbellata* (Vahl.) leaf powder. These leaves were harvested in May 2022 in the commune of Abomey Calavi, southern Benin.

Animal material

The animal material consists of Wistar rats made obese by a hypercaloric diet for 30 days. The diet consisted of 50g of fattening granulated feed, 50g of a mixture of dry sausage and cookies, cheese, potato chips, chocolate, peanuts and sugar water.

The manipulations took place at the Laboratory of Pharmacology and Improved Traditional Medicines (LPMTA), Faculty of Science and Technology (FAST), University of Abomey-Calavi (UAC) and with the Experimental and Clinical Biology Unit (UBEC), Biotechnology Research Laboratory Medical and Pharmaceutical (LaRBiMeP), National School of Biosciences and Biotechnology of Dassa-Zoumé (ENSBBA), National University of Science, Technology and Engineering of Abomey (UNSTIM) in Benin.

METHOD

Preparation of ethanolic extract

The collected sample was dried for three (3) weeks. It was then reduced to powder and stored in a suitable container for further handling.

Extraction was dictated by bibliographical information on the chemistry of plant constituents. The solvent used was ethanol. Extraction took place in 3 stages : maceration, filtration and evaporation. 50 g of *F. umbellata* leaf powder is placed in 500 ml of ethanol.

After maceration (for 72 hours), the product was filtered using filter paper, then absorbent cotton placed in a funnel connected to a suction pump to speed up filtration. After a few minutes, an exclusively liquid solution was obtained (the operation was repeated three times in succession). The filtrate obtained was placed in an oven at 45°C to evaporate the ethanol. The dried extract was then scraped, weighed and the yield calculated.

Induction of experimental obesity

In order to generate significant weight gain and thus provide a good model of obesity, the animals were fed a hypercaloric diet. This consisted of 50g of fattening granulated feed (pre-mixed cotton and palm kernel cakes, amino acids, limestone, dicalcium phosphate, wheat and rice bran) and 50g of a mixture of sausage-dried cookies, cheese, potato chips, chocolate and peanuts in equal proportions. Their drink is nothing but 100mg/mL sugar-sweetened water. This diet induces obesity as a result of hyperphagia[9].

The indication of obesity in rats is confirmed by monitoring the animals' body weight gain and lipid profile.

Table 1 : Composition of standard and high-calorie diets (g/100g)

Parameters	Standard diet	High-calorie diet
Lipids	5.44	35.56
Carbohydrates	11.26	27.33
Proteins	14.06	19.36

***In vitro* Blood coagulation inhibition test**

The effect of the extracts was tested on whole blood according to the method used by RAMDE-TIENDREBEOGO [10]. Four (4) glass tubes rinsed with physiological fluid, dried and labelled were prepared. 100µl (of different concentrations) of the test extract were placed in 3 labeled tubes (T_1, T_2, T_3). The test extract was not added to the control tube (T_0). 2ml of rat whole blood was drawn from each tube (T_0, T_1, T_2, T_3). Tubes were capped with carded cotton and immediately placed in a water bath at 37°C without shaking. After 3 minutes, the tubes were removed from the water bath, tilted at a 45° angle to determine the presence or absence of coagulation. In the absence of coagulation, the tube was returned to the water bath and examined every 30 seconds until coagulation was complete (no flow when the tube was turned upside down). As soon as the blood in the first tube has coagulated, the second tube is examined and the coagulation time recorded. The test is summarized in Table 2.

Table 2: Summary of the in vitro blood coagulation inhibition test

Tubes	Extract concentration (mg/ml)
Tube T_0	0 mg/ml +2ml whole blood
Tube T_1	10mg/ml (100µl) +2ml whole blood
Tube T_2	30mg/ml (100µl) +2ml whole blood
Tube T_3	50mg/ml (100µl) +2ml whole blood

Evaluation of the in-vivo anticoagulant activity of the ethanolic extract of *Ficus umbellata* leaves.

Obese and non-obese rats were divided into six (6) groups of 3 rats each and kept under the same conditions. They were treated for 28 days with the ethanolic extract of Ficus umbellata leaves. In order to assess the effect of different doses of the extract on blood coagulation, the two important intrinsic and extrinsic pathways of hemostasis were explored. The parameters evaluated were Coagulation time (CT), Prothrombin time (PT), Thrombin time (TT), Activated partial thromboplastin time (APTT), platelet content, fibrinogen level, D-dimer level.

Animals were divided as follows:

Batch 1 : healthy controls force-fed with distilled water (negative control)

Batch 2 : Obese controls gavaged with distilled water (positive control)

Batch 3 : Obese rats gavaged with 100mg of aspirin®

Batch 4 : Obese rats gavaged with 100 mg/kg BW of extract

Batch 5 : Obese rats gavaged with 300 mg/kg BW extract

Batch 6 : Obese rats gavaged with 500 mg/kg BW extract

Blood samples were taken on days (D) 0, 14 and 28 (D 0, D14 and D28).

Determination of activated partial thromboplastin time.

The activated partial thromboplastin time (APTT) is the time taken for deplaquetted plasma to which a contact factor activator and cephalin (platelet factor III substitute) have been added, after which coagulation is triggered using calcium.

BIOCK reagent recalcifies plasma in the presence of a standardized quantity of cephalin (platelet substitute) and a factor XII activator (kaolin). Kaolin offers the double advantage of easy reading and shorter reading time.

▪ **Procedure**

Centrifuge blood samples at 3500 rpm for 10 minutes ;

- Switch on the SUNNYMED SY-BO31 instrument and wait;

-Heat the APTT reagent (Activated Partial Thromboplastin) ;

-Take 80µl of calcium chloride and heat in a water bath ;

-Take 40µl of serum and 40µl of APTT reagent in another foam;

-Add 40µl of calcium chloride to the previous mixture and take the reading;

-Then record the activated partial thromboplastin rate (APTT) values obtained. Leave for 3 minutes in the device.

Determination of Quick Time (QT)

This is the clotting time of deplated citrated plasma after the addition of thromboplastin and calcium. It is expressed in seconds.

The clotting time is determined at 37°C in the presence of tissue thromboplastin and calcium. The Quick Time (TQ) measured in this way can be converted into the prothrombin rate.

▪ **Procedure**

- Switch on instrument ;
- Heat the PT (Prothrombin Rate) reagent;
- Take 40µl of serum and heat for 2 minutes ;
- Add 80µl of PT reagent and take the reading;
- Record results.

Determination of Thrombin Time.

Make a double determination per sample: Proceed in the same way with the control plasma.

Table 3 : Procedure for Thrombin Time Determination

Plasma	100µl
Incubate for exactly 2 min at 37°C	
Thrombicalci-test	100µl
Record the clotting time	

Platelet count measurement.

Hematological parameters were measured using a "SYSMEX XN-330" automated system. This machine counts white blood cells, red blood cells and platelets.

Quantitative determination of fibrinogen by the Clauss method

The reagent can be used with manual, mechanical or photooptical techniques, or with any instrument designed to detect clot formation.

- Prepare a 1/10 dilution of sample and controls in Imidazole Buffer : 50µl sample + 450µl Imidazole Buffer.
- Prepare Calibrator dilutions in Imidazole Buffer
- In 0.2ml of each dilution, add 20µl of Kaolin Solution and set at room temperature at 37ºC for 4-6 minutes. Add 0.1ml bovine thrombin and time clot formation. Do not bring bovine thrombin to room temperature.
- Calculate the mean of the clotting time duplicates immediately after the reaction is complete. Use the five Calibrator points to create a curve of the times obtained (s) against the fibrinogen concentration values of each Calibrator dilution (mg/dl).

Bleeding time experiment

Bleeding time was performed according to the DUKE method. The anterior surface of the shaved rat tail was carefully cleaned with absorbent cotton swabbed in 70% ethanol. Using disposable lancets, a 3 mm deep laceration was made on the shaved area. Bleeding time corresponds to the duration of blood flow.

Statistical analysis of data

Data collected before and after treatment with the extract were tabulated and entered into Excel 2013. Normality and homogeneity of variances were checked with R Studio software using the Shapiro.test and Levene.test respectively; comparison of pre- and post-treatment data was carried out using the parametric paired two-sample test with R Studio software. Histograms showing the comparison of the mean rate of each parameter were produced using Graphpad Prism 9.5.1 (733) software. Significance is declared when the probability value P-value is less than 0.05.

Ethical considerations

The study was approved by the appropriate institutional animal ethics committee, in accordance with the "Principles of Laboratory Animal Care" of the Ecole Polytechnique d'Abomey-Calavi (EPAC) of the University of Abomey-Calavi (UAC) for Health Research in Benin. Participation is voluntary and the information collected is confidential. The survey has been approved and informed consent has been obtained. No information is shared with anyone other than those involved in the study.

RESULTS

Indication of obesity in rats :

The indication of obesity in rats is confirmed by monitoring the animals' body weight gain and lipid profile.

• **Body weight**

Figure 1 shows the variation in rat body weight between D0 and D30 according to the diet to which they were subjected. The body weight of rats on a high-calorie diet increased compared with those on a standard diet. This variation was highly significant at day 30 ($p<0.001$).

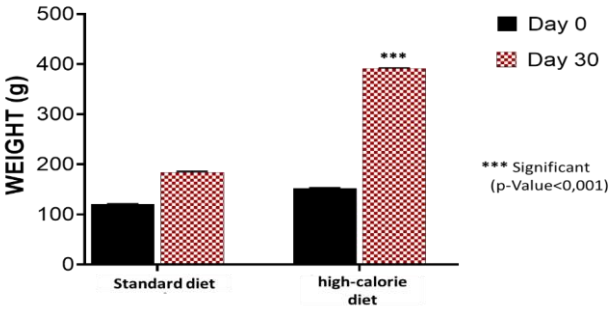


Fig 1 : Variation in body weight of rats on a hypercaloric diet

- **Variation in blood glucose levels (g/l)**

Figure 2 shows the blood glucose levels of wistar rats on a standard diet and those on a high-calorie diet. Blood glucose levels in experimental rats show variations compared with control values. A highly significant increase (p<0.001) was noted on day 30 in rats on the hypercaloric diet.

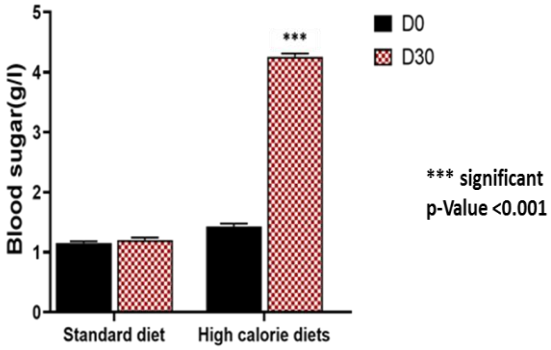


Fig 2 : Variation in blood glucose levels in rats fed a hypercaloric diet during obesity induction.

- **Changes in blood protein (g/l)**

Figure 3 shows the blood protein levels of Wistar rats fed a standard diet and those fed a hypercaloric diet. The blood protein content of experimental rats shows variations compared with control values. A highly significant increase (P<0.001) was noted on day 30 in animals on the hypercaloric diet. No significant variation (P>0.05) was observed in animals on the standard diet.

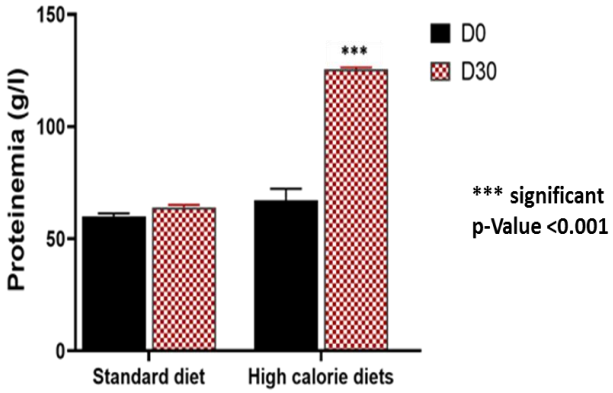


Fig 3 Variation in protein levels in rats fed a hypercaloric diet

- **Variation in triglyceride levels (g/l)**

Figure 4 shows blood triglyceride levels in Wistar rats on standard and high-calorie diets. Blood triglyceride levels in experimental rats show variations compared with control values. A highly significant increase (p<0.001) was noted on day 30 in animals on the hypercaloric diet. No significant variation (P>0.05) was observed in animals on the standard diet.

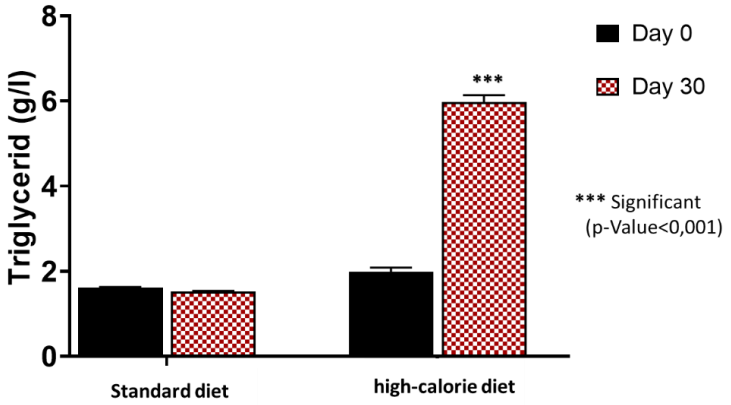


Fig 4 : Variation in triglyceride levels in rats fed a hypercaloric diet during obesity induction.

• **Variation in total cholesterol levels (g/l)**

Figure 5 shows the variation in total cholesterol levels in Wistar rats on standard and high-calorie diets.

There was an increase in total cholesterol levels in animals on the high-calorie diet, but not in those on the standard diet by day 30.

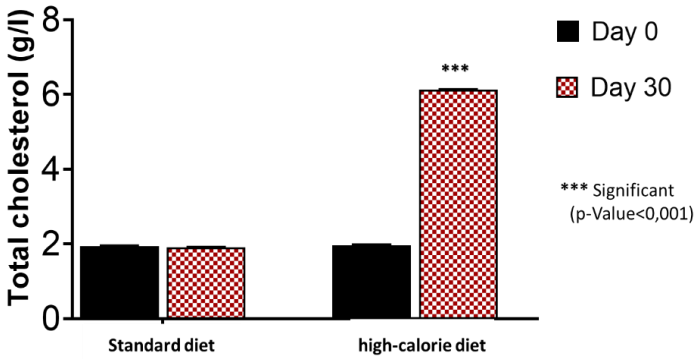


Fig 5: Variation in total cholesterol levels in rats on a hypercaloric diet during obesity induction.

***In vitro* blood coagulation inhibition test :**

• **Variation of coagulation time in obese versus healthy rats**

In the absence of extract, the mean blood clotting time of obese rats was 3.41 ± 0.22 min, while that of healthy subjects was 5.67± 0.47 min. Healthy rats therefore showed a longer coagulation time than obese subjects. As a result, the blood of an obese subject coagulates more rapidly than that of a healthy rat.

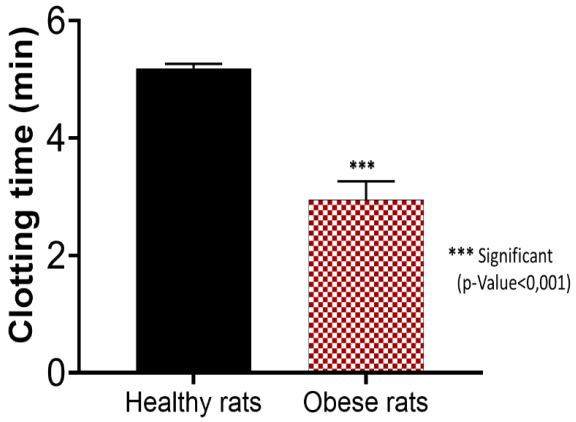


Fig 6: Variation in clotting time (min) between obese and healthy rats.

- **Inhibition of blood coagulation in vitro under the effect of different extract concentrations**

In the presence of plant extracts at different concentrations (10, 30 and 50 mg/ml), no coagulation was observed in the test tubes (T_1, T_2, T_3) at the same time as blood coagulated in the control tube.

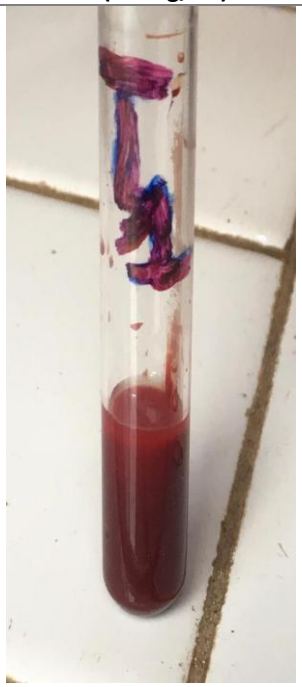
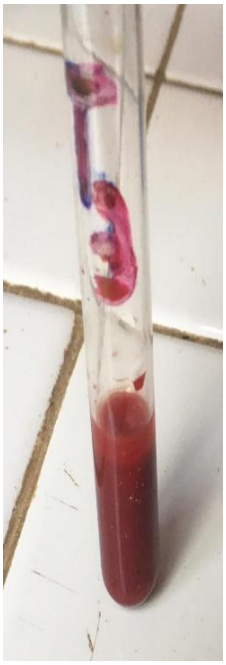
The higher the concentration of ethanolic extract from *F.umbellata* leaves in the tubes, the longer the coagulation time.

Table 4: Results of the in vitro blood coagulation inhibition test

Extract concentration (mg/ml)	Clotting time (min)
Control	3.41 ± 0.22 min
10 mg/ml (100µl)	12.53±2.7 min**
30 mg/ml (100µl)	14.09±0.73 min***
50 mg/ml (100µl)	15.49±0.9 min***

: significant difference at P < 0.01 *: significant difference at P < 0.001

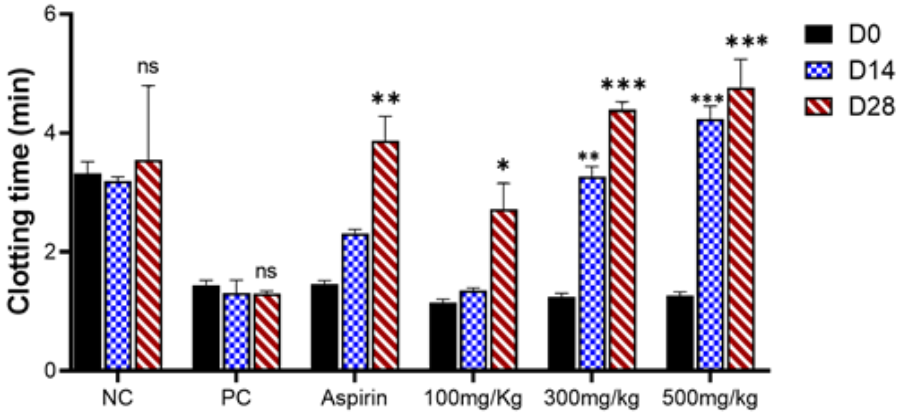
Table 5 : Images of in vitro blood coagulation inhibition test results

Control (T_0)	T_1 (10 mg/ml)	T_2 (30 mg/ml)	T_3 (50 mg/ml)
			
Formation of blood clots	**No coagulation**	**No coagulation**	**No coagulation (very fluid blood)**

Evaluation of the in-vivo anticoagulant activity of ethanolic extract of *Ficus umbellata* leaves :

• Variation in coagulation time (min)

Analysis of figure 7 reveals that, compared with healthy rats (negative controls), the mean blood clotting time is very short ($p<0.01$) in obese control rats (positive controls) given distilled water during the 28-day experiment. Between the first (1st) and fourteenth (14th) days of experimentation, there was a tendency for the mean coagulation time to increase ($p>0.05$) in obese rats treated with 100mg/kg BW extract and those treated with the reference molecule (Aspirin), but the increase was highly significant ($p<0.001$) on day 28. The mean blood clotting time of obese rats treated with 300 mg/Kg and 500 mg/kg BW of the extract increased significantly ($p<0.01$) from day 14 and progressed to day 28. Healthy subjects (negative controls) showed a longer mean clotting time than obese rats (positive controls).



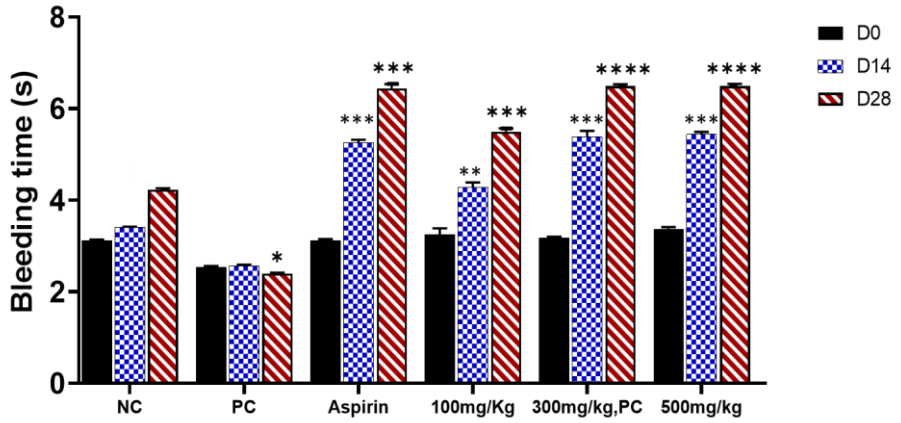
Legende : ns = non-significant ; NC : Negative control ; PC : Positive control
The results expressed are the average clotting time of rats on days 0, 14 and 28.
*** p< 0.05 ; ** p< 0.01 ; *** p< 0.001 : Satatiscally significant compared to Day 0**

Fig 7: Variation in blood clotting time *in vivo* in obese rats treated with ethanolic extract of *F. umbellata* leaves

• Variation in bleeding time (min)

The test results presented in figure 8 shows that healthy subjects (negative controls) showed a higher mean bleeding time ($p<0.05$) than untreated obese rats (positive controls).

Between the first (1st) and fourteenth (14th) days of experimentation, obese rats treated with 100mg/kg BW of the extract showed an increase in mean bleeding time ($p<0.01$). The increase was more pronounced ($p<0.001$) in rats treated with 300 mg/kg and 500 mg/kg BW of the extract, and continued until the 28th day of treatment. The effect of the extract is close to that of Aspirin.



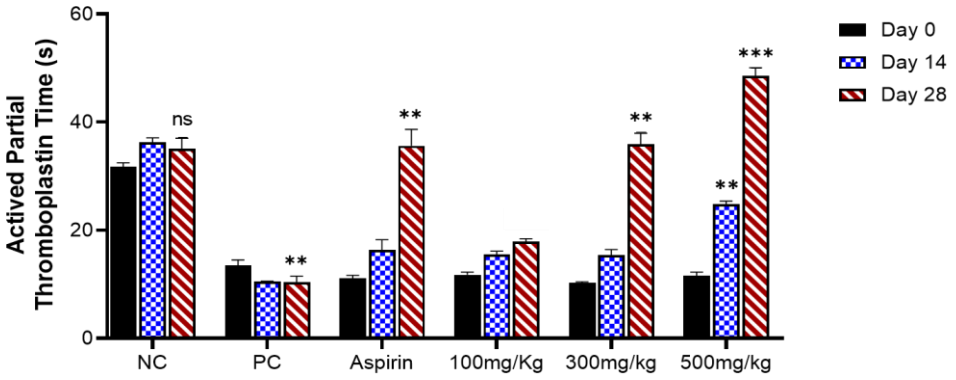
Legende : ns = non-significant ; NC : Negative control ; PC : Positive control
The results expressed are the average Bleeding time of rats on days 0, 14 and 28.
*** p< 0.05 ; *** p< 0.001 ; **** p<0.0001: Satatiscally significant compared to Day 0**

Fig 8: Variation in bleeding time recorded in obese rats treated with ethanolic extract of *F. umbellata* leaves.

- **Variation in Activated Partial Thromboplastin Time (APTT)**

Analysis of figure 9 reveals that the activated partial thromboplastin time (APTT) recorded in untreated obese rats (positive controls) is shorter ($p<0.001$) than in healthy rats (negative controls).

This figure shows that the activated partial thromboplastin time (APTT) recorded in treated obese rats was prolonged following administration of the ethanolic extract of *F. umbellata* leaves. This lengthening was observed from day 14 onwards, and was significant ($p<0.01$) only for the 500mg/kg dose. At day 28, the variation in APTT was highly significant ($p<0.01$) in rats treated with aspirin and at doses of 300 ($p<0.01$) and 500 mg/kg BW ($p<0.001$).



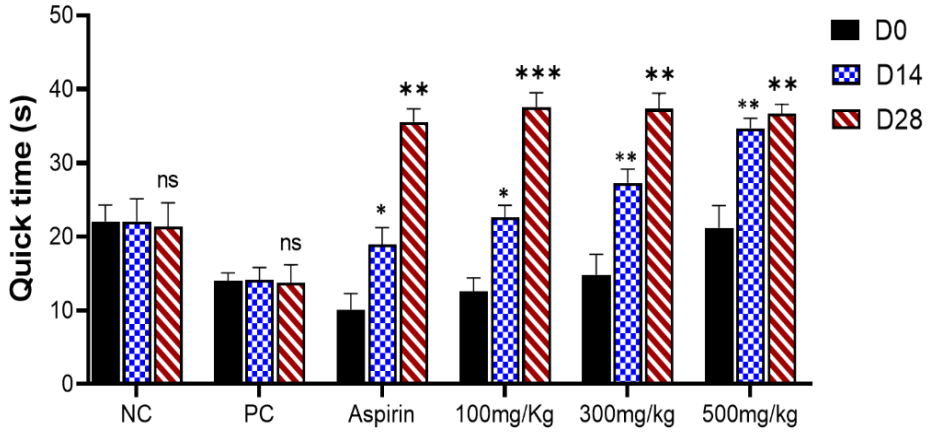
Legende : ns = non significant ; NC : Negative control ; PC : Positive control
The results expressed are the average Actived Partial Thromboplastin Time (s) of rats on days 0, 14 and 28.
**** p< 0.01 ; *** p< 0.001 : Satatiscally significant compared to Day 0**

Fig 9: Variation in activated partial thromboplastin time (APTT) recorded in obese rats treated with ethanolic extract of *F. umbellata* leaves.

• Variation in Quick time (s)

The Quick time (prothrombin time) recorded in untreated obese rats (positive controls) was significantly shorter (p<0.01) than in healthy rats (negative controls).

This figure shows that the prothrombin time recorded in treated obese rats lengthens following administration of the extract from day 14 onwards, and the lengthening is more pronounced with doses of 300 and 500 mg/kg BW, as well as with aspirin on day 28.

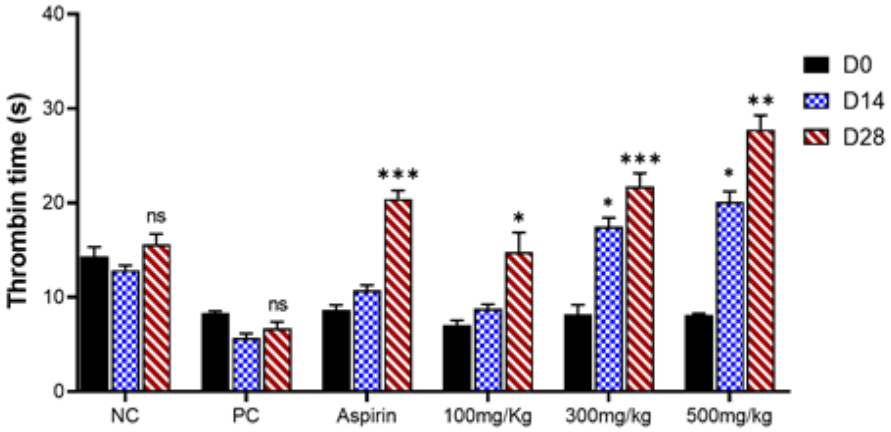


Legende : ns = non-significant ; NC : Negative control ; PC : Positive control
The results expressed are the average Quick time of rats on days 0, 14 and 28. *p<0.05
**** p< 0.01 ; *** p< 0.001 : Satatiscally significant compared to Day 0**

Fig 10: Variation in prothrombin time (Quick time) recorded in obese rats treated with ethanolic extract of *F. umbellata* leaves.

• Variation in thrombin time (s)

Figure 11 shows that, irrespective of the dose of extract administered (100, 300 and 500 mg/kg BW), thrombin time increased in obeses rats. For a dose of 100mg/kg BW, thrombin time tended to lengthen (p>0.05) at day 14 and lengthened significantly (p<0.01) afterwards. For doses of 300 and 500 mg/Kg BW, the lengthening is more pronounced (p<0.001) from day 14 onwards.



Legende : ns = non-significant ; NC : Negative control ; PC : Positive control
The results expressed are the average thrombin time of rats on days 0, 14 and 28.
*** p< 0.05 ; ** p< 0.01 ; *** p< 0.001 : Satatiscally significant compared to Day 0**

Fig 11 : Variation in thrombin time recorded in obese rats treated with ethanolic extract of *F. umbellata* leaves
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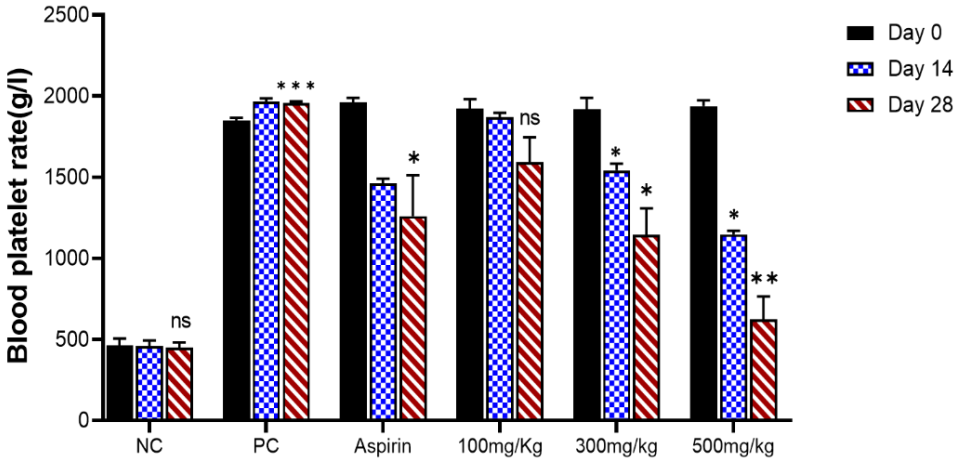
- **Variation in blood platelet rate (g/l)**

The blood platelet content of untreated obese rats is very high (p<0.01) compared with that of healthy rats (Figure 12).

The blood platelet content of experimental rats showed variations compared with that of obese control rats (Positive control). A significant (p<0.05) and very significant (p<0.01) decrease was noted at 14 and 28 days respectively in rats treated with 300 and 500 mg/kg BW. Analysis of figure 12 shows that blood platelet levels also fell in obese rats treated with the reference molecule (aspirin). However, subjects treated with 500 mg/kg showed a lower platelet count than those treated with aspirin.

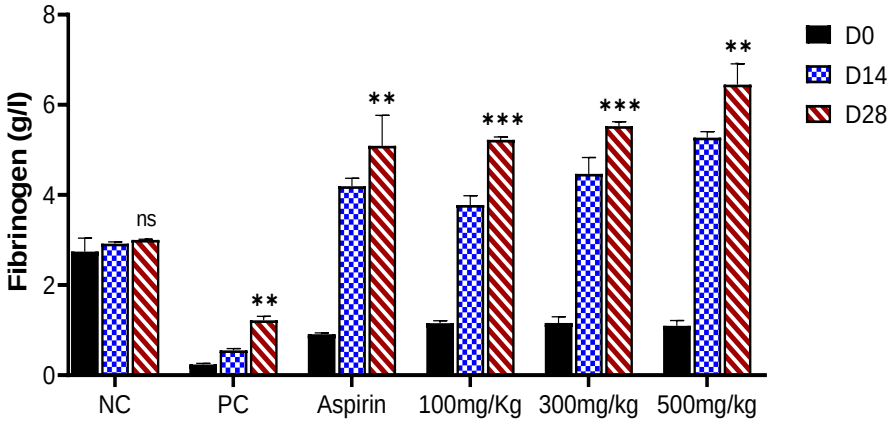


Legende : ns = non-significant ; NC : Negative control ; PC : Positive control
The results expressed are the average blood platelets of rats on days 0, 14 and 28.
*** p< 0.05 ; ** p< 0.01 ; *** p< 0.001 : Satatiscally significant compared to Day 0**

Fig 12: Variation in blood platelet content in obese rats treated with ethanolic extract of *F. umbellata leaves.*

- **Variation in fibrinogen levels (g/l)**

Fibrinogen levels were significantly (p<0.05) higher in healthy rats (negative control) than in the untreated obese group (positive control). Serum studies carried out on obese rats treated with the extract show a highly significant (p<0.01) increase in fibrinogen levels for doses of 100, 300 and 500 mg/kg. BW from day 14 onwards. This increase continued until day 28 (p<0.001), and was also noted with aspirin.



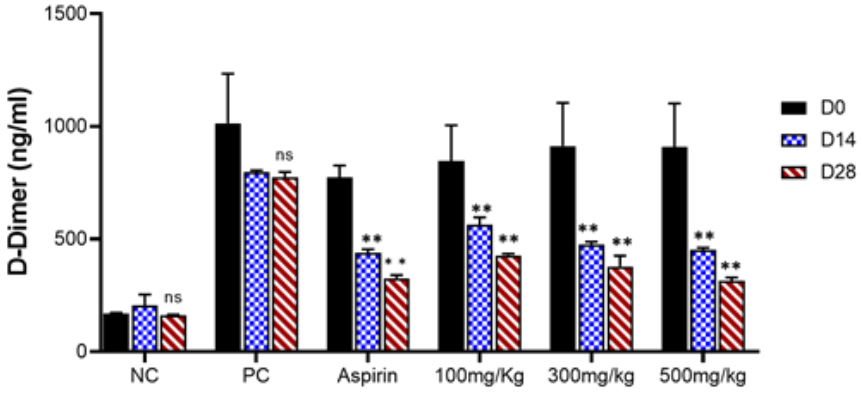
Legende : ns = non-significant ; NC : Negative control ; PC : Positive control
The results expressed are the average Fibrinogen of rats on days 0, 14 and 28.
**** p< 0.01 ; *** p< 0.001 : Satatiscally significant compared to Day 0**

Fig 13 : Changes in fibrinogen levels in obese rats treated with ethanolic extract of *F. umbellata* leaves.

• Variation in D-Dimer levels (ng/ml)

Figure 14 shows that D-Dimer levels are very high (p<0.01) in obese rats (Positive control). Administration of different doses of Ethanolic extract of *F. umbellata* leaves (100, 300 and 500 mg/Kg. BW) and aspirin reduced D-Dimer levels by day 14. Analysis of variance shows that the variation is highly significant (p<0.01) from day 14 onwards. The effect of the extract is close to that of the reference molecule (aspirin).

D-Dimer levels were significantly (p<0.01) higher in the untreated obese group (positive control) than in the healthy group (negative control).



Legende : ns = non-significant ; NC : Negative control ; PC : Positive control
The results expressed are the average D-Dimer of rats on days 0, 14 and 28.
*** p< 0.05 ; ** p< 0.01 : Satatiscally significant compared to Day 0**

Fig 14 : Variation in D-Dimer levels in obese rats treated with ethanolic extract of *F. umbellata* leaves.

DISCUSSION

The aim of the present study was to demonstrate the in vivo and in vitro anticoagulant properties of the ethanolic extract of *Ficus umbelleta* leaves. For this purpose, an experimental obesity model was used. Wistar rats were fed a hypercaloric diet for 30 days. Obesity is an abnormal or excessive accumulation of body fat that leads to adverse health consequences[11]. Our results showed a change in the lipid profile and an increase in the weight of animals subjected to such a diet. Plasma concentrations of cholesterol, triglycerides and glucose also increased. These results are in line with those of Araujo, who showed that lipid levels in obese rats were higher than in control rats on the Standard diet[12]. This is explained by the high level of lipids in the diet. These findings are partly consistent with those of Zambou, who showed that overweight is strongly associated with high blood triglyceride levels, reduced HDL cholesterol and increased LDL cholesterol[13].

According to Dalila Chabane, the combination of these abnormalities puts individuals at high risk of atherosclerosis[14]. Bouchard-Mercier demonstrated that dyslipidemia is characterized by increased triglycerides due to hyperproduction of liver VLDL or increased LDL or decreased HDL cholesterol[15]. It is itself associated with the development of atheromatous plaque and strongly linked to cardiovascular complications [16]. Indeed, obesity is often associated with the presence of atherosclerosis, and from a very early age. Our study revealed permanent hyperglycemia in rats fed a hypercaloric diet. This result is in line with those of Chaumont who showed that insulin resistance is the link between obesity and type 2 diabetes[17]. Antonopoulos observed in obese subjects subjected to caloric restriction or exercise, a considerable improvement in systemic insulin sensitivity in those who had reduced their amount of visceral adipose tissue [18]. In this study, we also observed a disturbance in haemostasis parameters (Bleeding time, Clotting time, Quick time, Activated partial thromboplastin time, Thrombin time, fibrinogen, blood platelets and D-Dimer) in rats on a high-calorie diet. Indeed, a significant decrease in Quick time, Activated partial thromboplastin time, clotting time and thrombin was recorded in obese animals (Positive Controls), reflecting an increase in blood viscosity. This result is in agreement with that of Nolan who showed that an increase in blood viscosity is associated with an increase in body mass and is due to the release of profibrinogen and plasminogen activator inhibitor from adipocytes with a decrease in plasminogen activator[19]. Obesity is a hypercoagulable condition, with increased levels of certain coagulation factors. This increase is moreover responsible for a clear shortening of the Quick time (QT) and the Activated partial thromboplastin time [20]. The blood platelet count detected in animals on a high-calorie diet is excessively high. This situation favors clot formation (thrombosis), leading to vascular, venous and arterial obstruction [21]. This risk is confirmed by the lowering of fibrinogen levels leading to fibrin and D-dimer clot formation detected. Indeed, according to Lim, D-dimers are molecules of variable size resulting from the specific degradation of fibrin and are used in the diagnosis of venous thromboembolic diseases[22]. These results are in line with those obtained by Mamad during a study on the profile of Von Willebrand factor in pregnancy[23]. At doses of 10, 30 and 50 µl, ethanolic extract of dry *Ficus umbelleta* leaves prolongs plasma clotting time in vitro. In fact, no coagulation was observed in the test tubes at the same time as coagulate was observed in the control tube. As a result, this extract slows down plasma coagulation. This result is an indication of the anticoagulant and anti-thrombotic activity of the ethanolic extract of *Ficus umbelleta* leaves. Hemostasis parameters (Bleeding time, Clotting time, Quick time, Activated partial thromboplastin time, Thrombin time, fibrinogen, blood platelets and D-Dimer) were determined in obese rats treated with different doses of this extract and with aspirin used as the reference molecule. Results showed a dose-dependent prolongation of parameters such as bleeding time, prothrombin time, coagulation time, activated partial thromboplastin time and thrombin time at doses of 100, 300 and 500 mg/kg. The effect of this extract is similar to that of aspirin 100mg. Ethanolic extract of Ficus umbelleta leaves and aspirin liquefy blood by the same mechanism. Treated animals also showed an increase in fibrinogen levels, correlated with a reduction in platelet and D-dimer levels. According to the literature, the prolongation of the Quick time and Activated partial thromboplastin time is more marked in patients on oral anticoagulants, which can increase the INR by around 10%. The observed reduction in blood platelet levels is thought to be linked to inhibition of platelet aggregate formation, which is the first step in blood coagulation [24] . Our results are in line with those of Kim, who found that Turmeric prevents platelet aggregation and hence clot formation [25], which helps and stimulates blood circulation and significantly prolongs activated partial thromboplastin time and Quick and inhibits thrombin activities. The anticoagulant and anti-thrombotic effect observed could be linked to the presence of flavonoids in this plant. Indeed, it has been reported that *Ficus umbelleta* leaves are rich in flavonoids. Flavonoids have been shown to help keep arteries relaxed (vasodilation). They have anticoagulant and platelet anti-aggregant potential, preventing platelets from clumping together to form a clot[26]. As a result, they reduce blood clotting and make it more fluid. They limit the oxidation of blood lipids and contribute to the fight against atherosclerotic plaques [27]. Some tannins are also antioxidants. Among other properties, they are considered cardio-protective, anti-inflammatory, anti-carcinogenic and anti-mutagenic[28]. These protective effects are mainly linked to their ability to act as free radical scavengers and activate antioxidant enzymes [29]. As for total polyphenols, they constitute one of the most important groups of compounds present in plants, where they are widely distributed. These compounds are reported to exhibit anticarcinogenic, anti-inflammatory, antiatherogenic, antithrombotic, immune and

analgesic properties and exert these functions as antioxidants[30]. Fibrinogen (Factor I), a protein synthesized in the liver, is a blood component used to form the clot. Its determination helps us to assess alterations in coagulation mechanisms[31]. In the present study, a significant decrease in fibrinogen levels was noted in all treated rats, irrespective of the dose administered. According to Clauss, low values were observed in the case of thrombolytic therapies, validating the thrombolytic and therefore anticoagulant activity of the ethanolic extract of *Ficus umbelleta* leaves[31]. It was demonstrated in this study that this extract lengthens thrombin time (TT) and bleeding time, which according to Michel, indicate the presence of antithrombin.

CONCLUSION

The aim of the present study was to evaluate the effects of ethanolic extract of *Ficus umbellata* (Vahl.) leaves on the blood-thinning process in obese Wistar rats. The results showed that this extract was able to lengthen blood clotting time in vitro and in vivo in obeses rats. The study also demonstrated an increase in activated partial thromboplastin time, prothrombin time and thrombin time following treatment of the animals. The extract also demonstrated its ability to inhibit platelet aggregation, slowing down the formation of the first stage of blood coagulation and thus demonstrating an anticoagulant property.

Conflicts of Interest

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

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