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Effect of Hydroalcoholic Extract of Stem Bark of *Bridelia ferruginea* on Blood Coagulation

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Abstract: *Bridelia ferruginea* is a plant commonly used in several regions of the world for its various properties. It is offered to certain patients with cerebrovascular accidents. It is with this in mind that this study is undertaken in order to verify whether *B. ferruginea* has an activity on blood coagulation. *Materiel and method:* Stem bark of *B. ferruginea* was collected at Noepe (24 km northwest of Lome). Hydroalcoholic extract (50/50) was obtained by evaporation. Male Wistar rats were used for the tests. Qualitative screening was done according to conventional methods and total phenols were quantified. Some parameters of coagulation (blood platelets, aPTT, PR and fibrinogen) were determined. Oxidative stress was induced by Fe-NTA. Results were analysed by GraphPad®Prism 8.4.2. Analysis of variance (ANOVA) was used to compare several groups. The difference between groups was determined by Tukey's test and considered significant at $p<0.05$. *Results:* Hydroalcoholic extract of *B. ferruginea* increased aPTT and decreased PR. Compared to the negative control (distilled water), oxidative stress was responsible in vivo for an increase in platelets blood number and PR and a decrease in aPTT and fibrinogen. The hydroalcoholic extract of *B. ferruginea* caused a decrease in platelets blood number (at low concentration), PR and fibrinogen level and an increase in the platelets blood number (at high concentration) and aPTT. Hydroalcoholic extract of *B. ferruginea* decreased MDA levels and increased GSH levels in rats subjected to oxidative stress. *Conclusion:* The present study proved existence of antioxidant and anticoagulant activities of *B. ferruginea* stem bark. There is a link between oxidative stress and coagulation. Future studies may better elucidate interaction between oxidative stress and coagulation mechanisms involved.

Keywords: *Bridelia ferruginea*, Oxidative Stress, Blood Coagulation

1. Introduction

Before the advent of modern medicine, plants were used worldwide for healing and curing various illnesses. According to the World Health Organization (WHO), about 80% of the populations of developing countries use traditional medicine and in particular herbal medicine for their health care needs for cultural tradition or lack of other alternatives reasons [1]. On the continents of Africa and Asia, plants are used as the first line of treatment for pathologies such as malaria, diabetes, hypertension, sickle cell disease, skin diseases and, quite recently, opportunistic infections due to HIV/AIDS [1]. According to the West African Health Organization (WAHO), more than 120 pharmaceuticals in common use today are derived from plants, most of which come from tropical regions of the world [2]. Nowadays,

biological activities of plants are better elucidated and their use in several pathologies are more refined. Thrombotic pathologies are more frequent [3] and often herbal treatments are offered empirically, or in a proven way. During a survey on traditional treatments, *Bridelia ferruginea* was often offered to patients with cerebral vasculary diseases.

To verify whether *B. ferruginea*, apart from its many properties, could also have anticoagulant properties, the present study was then carried out.

2. Materiel and Method

2.1. Plant and Extraction

The stem bark of *B. ferruginea* was collected at Noepe, 24 km in Northwest of Lome. Botanical identification (number: Togo 15867) was carried out in Department of Botany, Faculty of Sciences, University of Lome.

The stem bark was washed, shade dried, ground and macerated in a hydroalcoholic solution (50/50) for 72 hours with stirring at laboratory temperature. The macerate underwent a double filtration (hydrophilic cotton then filter paper). The filtrate was evaporated at 45°C using Rotavapor IKA RV 10 Digital until a dry powder was obtained. The extract was stored in the refrigerator (4±2°C).

2.2. Animal

Male Wistar rats weighing an average of 152±20 g, reared in the Department of Animal Physiology of the University of Lomé were used for the study. These rats were acclimatized to laboratory conditions (alternating 12 hours of light and 12 hours of darkness, temperature of 24-25°C), with free access to water and food.

2.3. Phytochemical Screening

2.3.1. Qualitative Determination

The qualitative determination of phytochemicals was carried out according to conventional methods [4].

2.3.2. Total phenols Determination

The method of Shetty *et al.* [5] modified by Kagnou H *et al.* was used. To 0.05 mL of the extract, 0.25 mL of Folin-Ciocalteu diluted to the 10^{th} were added. After 5 minutes of reaction, 0.2 mL of carbonate sodium (Na_2CO_3) at 75 g/L were added to the mixed. The mixture was well homogenized and the whole was incubated for 30 minutes at room temperature away from light and the samples were read at 765 nm against a blank using a UV Visible spectrophotometer (5100B Spectrophotometer). The standard range of 10 to 100 µg/mL was prepared under the same conditions as the extract. The calibration curve was plotted in using the different concentrations of gallic acid and the results were expressed in milligram equivalent of gallic acid per gram of dry extract (mg EqAG/g ED) [6].

2.4. Induction of Coagulation by Stress

Rats were randomly divided into five groups of 8 rats each: negative control (distilled water), stressed control (distilled water), positive control (acenocoumarol 0.1 mg/kg), test-groups (extract respectively at 50 and 100 mg/kg). Distilled water, acenocoumarol and extract were administered orally each day for seven consecutive days to each rat depending on the group. Each rat received 10 ml/kg of preparation solution.

On the 5^{th} day, 9 ml/kg of Fe-NTA freshly prepared was administered by intraperitoneal injection to all rats except those of group 1 (negative control). Ferric nitrate solutions [$Fe(NO_3)_3$] (0.16 mmol/kg body weight) was mixed with a molar excess (four times) of disodium nitrilotriacetic salt [$C_6H_9NO_6$] (0.64 mmol/kg body weight) and the pH was adjusted to 7.4 with sodium bicarbonate solution [7].

On the 8th day, after a 12 hour fast, each rat underwent ether anesthesia in a closed container and blood samples were taken from the retro-orbital sinus. The platelet count was performed on a Humacount 5Diff® hematology counter of Human®. The determination of activated partial thromboplastin time (aPTT), the level of prothrombin ratio (PR) and fibrinogen were carried out on blood coagulation instrument StarMax® of Stago®.

Rats were then sacrified and liver was harvested.

2.5. Malondialdehyde (MDA) Determination in Liver

The method of Patlolla *et al.* was used. The removed liver was rinsed in 9 g/L NaCl, homogenize on potter homogenizer in a 10 mM Tris-HCl buffer pH 7.5. The reaction solution contained 650 µL of 1-methyl-2-phenylindole activated by acetonitrile, 250 µL of MDA or liver homogenate, 150 µL of the HCl solution with the concentration of 12 N and 10 µL of BHT (Butylated Hydroxy Toluene) solution at a concentration of 0.1 M. The tubes were incubated at 45°C for 60 minutes. The tubes were then centrifuged at 1881.59 g per minute for 10 minutes. The standard concentrations of MDA used are 0.625; 1.25; 2.5; 5 and 10 nM. The absorbance was read at the wavelength of 586 nm [8].

2.6. Reduced Glutathione (GSH) Assay

Glutathione level was evaluated according to the method of Sedlak and Lindsay [9]. To 50 µL of the homogenate or of GSH are added 150 µL of 0.2 M Tris, pH = 8.2; 10 µL of the solution of 0.01 M 5,5-dithiobis-2-nitrobenzoic acid (DNTB) and 790 µl of methanol. The tubes are homogenized and then incubated for 30 min at room temperature. The concentrations of GSH standards used were 800, 600, 400, 200, 100 and 50 µg/mL. The absorbance was read with a spectrophotometer at 412 nm.

2.7. Statistical Analysis

GraphPad ® Prism 8.4.2 software was used for the analysis of the results These results were expressed as mean value with standard error of mean (m ± ESM). Analysis of variance (ANOVA) was used to compare several groups. The difference between groups was determined by Tukey's test. The results were considered significant at $p < 0.05$.

3. Results

3.1. Extraction Yield and Phytochemicals Screening

The yield of hydroalcoholic extraction of *B. ferruginea* stem bark was 24.7%. Phytochemical analysis revealed presence of phenols, tannins, flavonoids, reducing sugars and coumarins and absence of alkaloids, terpenes, saponins, anthracenes and cardiotonic glycosides.

The calibration curve for gallic acid was established by linear regression ($y = 0.007952x + 0.0007857$ with $R^2 = 0.9998$). The total polyphenol content was 34.924% Eq AG/gES ± 2.821.

3.2. Actions of B. ferruginea on Blood Coagulation

The results of the appreciation of the induction of oxidative stress, platelet count, determination of aPTT, PR and fibrinogen in rats or not and the rats not subjected to the stress are shown in figures 1 to 4.

Stress caused an increase of 23.67% in the number of platelets compared to the negative control. A reduction of number of platelets was noted in the rats treated with acenocoumarol (18.13%), extract at 50 mg/kg (10.80%) and extract at 100 mg/kg (12.49%) compared to the negative control. Stress-induced increase in platelet count was inhibited by the administration of acenocoumarol or *B ferruginea* extract.

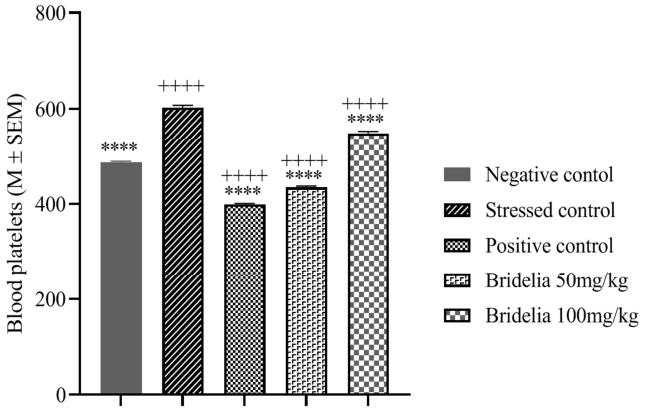

Figure 1. *Effect of hydroalcoholic extract of B. ferruginea on the number of blood platelets in stressed rats.*

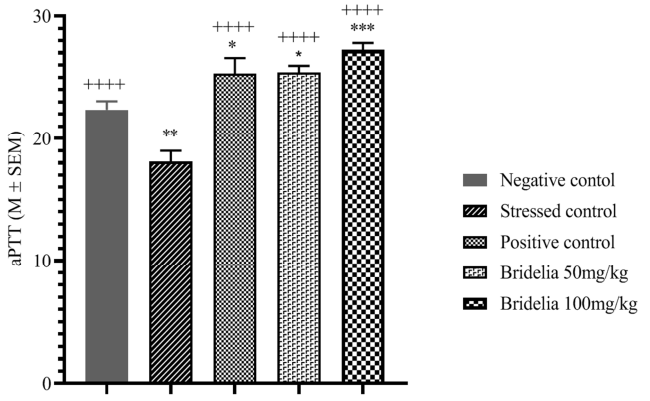

Figure 2. *Effect of the hydroalcoholic extract of B. ferruginea on the aPTT in stressed rats.*

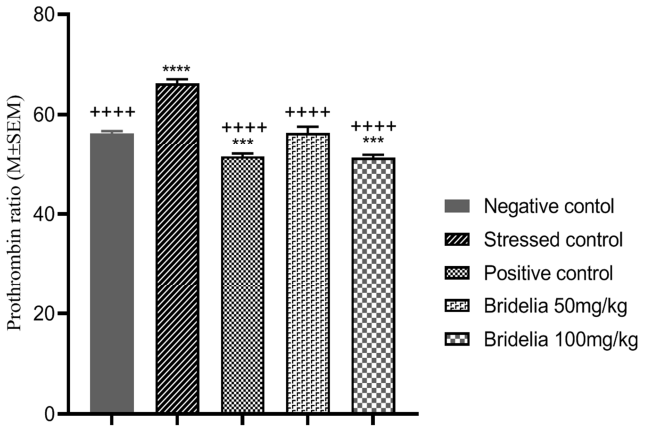

Figure 3. *Effect of hydroalcoholic extract of B. ferruginea on the PR in stressed rats.*

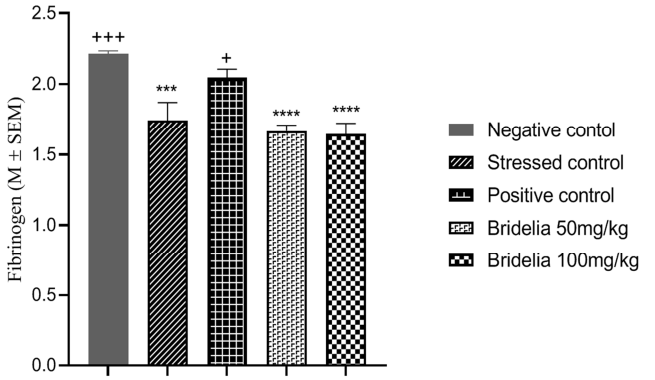

Figure 4. *Effect of hydroalcoholic extract of B. ferruginea on the fibrinogen in stressed rats.*

p> 0.05 = non-significant difference; *: p <0.05; **: p <0.01; ***: p <0.001; ****: p <0.0001
(*): Comparison with the negative control (distilled water)
(+): Comparison with the stressed control (no treatment)

Decrease in aPTT was reduced by 54.87% in the untreated stressed rats compared to the negative control group. An increase in aPTT was noted in stressed rats treated with acenocoumarol (13.15%) or the extract at 50 mg/kg (13.60%) or 100 mg/kg (21.82%) compared to the negative control group. Stress-induced decrease in aPTT was inhibited by administration of acenocoumarol or *B ferruginea* extract. This inhibition was more pronounced with the high dose *B. ferruginea* extract.

In stressed rats, PR levels was increased (17.78%) compared to the negative control. A decrease in PR was noted in stressed rats treated with acenocoumarol (8.44%) or the extract at 100 mg/kg (8.89%) compared to the negative control group. There was no difference between stressed rats treated with *B. ferruginea* extract at 50 mg/kg and the negative control group. *B. ferruginea* extract strongly inhibited the effect of oxidative stress.

Fibrinogen level decreased with oxidative stress by 21.27% compared to the negative control. Only acenocoumarol inhibited the effect of oxidative stress on fibrinigen level. *B. ferruginea* extract has no effect on fibrinogen level.

3.3. Antioxidant Activities of B. ferruginea

Effects of hydroalcoholic extract of *B. ferruginea* stem bark on the levels of MDA and GSH in the liver of stressed rats are shown in figures 5 and 6.

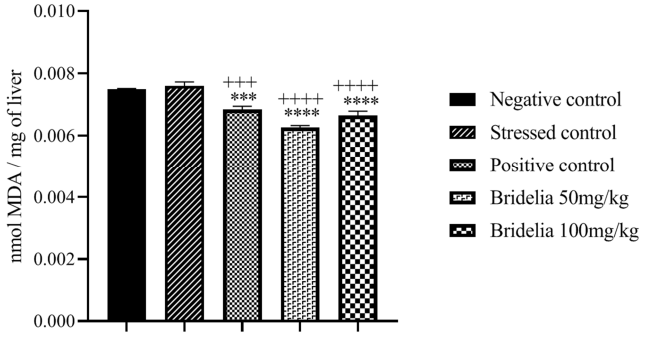

Figure 5. *Effet of hydroalcoholic extract of B. ferruginea on the level of MDA in the liver of stress rats.*

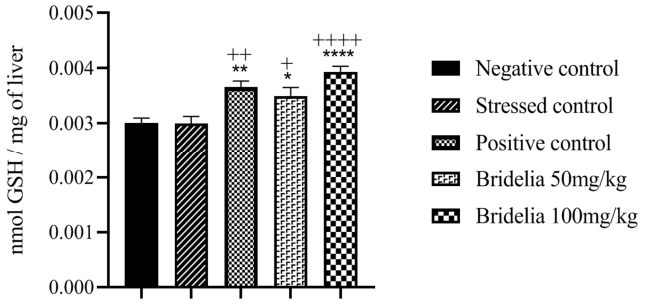

Figure 6. *Effet of hydroalcoholic extract of B. ferruginea on the level of GSH in the liver of stressed rats.*

p> 0.05 = non-significant difference; *: p <0.05; **: p <0.01; ***: p <0.001; ***: p <0.0001
(+): Comparison with the negative control (distilled water)
(*): Comparison with the stressed control (no treatment)

MDA level is reduced in stressed rats and treated with acenocoumarol or different doses of extracts of *B. ferruginea*. The effect is more pronounced with the lower dose of *B. ferruginea* extract. The level of GSH lever is significantly increased in rats which were stressed and treated with acenocoumarol or the different doses of extracts of *B. ferruginea*. The effect is greater with a higher dose of *B. ferruginea* extract. *B. ferruginea* extract has an antioxidant effect.

4. Discussion

Phenols are present in the hydroalcoholic stem bark extract of *B. ferruginosa*. Amios *et al*. established that polyphenols have the ability to regulate a cellular processes and molecular diversity by interaction with protein targets, conferring on them anti-atherogenic, anti-inflammatory, anti-thrombotic, anti-carcinogenic and neuroprotective properties. Polyphenols are also able to decrease other risk factors for cardiovascular diseases involved in the metabolic syndrome (hyperglycemia, high lipid level, insulin resistance, abdominal obesity and arterial hypertension) [10].

Low levels of MDA and high levels of GSH in the liver of treated animals compared to controls shown that *B. ferruginea* had antioxidant activities [11].

Oxidative stress in untreated rats resulted in a significant increase in the number of platelets, as well as an increase in aPTT and decrease in PR and fibrinogen (statistically significant difference between negative control rats and stressed control rats). Stress therefore induced haemostasis disorder. Our results are similar to the studies of Salvemini *et al*. [12] and Pawlak *et al.* [13].

B. ferruginea extract impacted significantly on the effect of oxidative stress on hemostasis.

Acenocoumarol, an antivitamin K, was sed as a reference drug. Acenocoumarol inhibits reduction of vitamin K by vitamin K reductase. This prevents the carboxylation of certain residues of glutamic acid near the N-terminals of vitamin K dependent coagulation factors: II, VII, IX and X. Carboxylation of glutamic acid is important for the interaction between these coagulation factors and calcium. Without this interaction, coagulation cannot occur. Both extrinsic (via factors VII, X and II) and intrinsic (via factors IX, X and II) are affected by acenocoumarol [14]. Acenocoumarol will thus modify the PR (extrinsic route) and the aPTT (extrinsic route).

The evaluation of results obtained in stressed rats treated by hydroalcoholic stem bark extract of *B. ferruginea* was similar to that obtained in those treated by acénocoumarol for platelet count, aPTT and PR. High concentration of extract use gave opposite effect of that induced by acenocoumarol.

Oxydative stress caused modifications in parameters affecting hemostasis. The hydroalcoholic extract of stem bark of *B. ferruginea* neutralized hemostasis disorders induce by oxidative stress. The extract of *B. ferruginea* can therefore be considered to have anticoagulant proprieties.

5. Conclusion

Oxidative stress disrupts coagulation. The hydroalcoholic extract of *B. ferruginea* stem bark showed antioxidant properties and consequently corrected coagulation disorders caused by oxidative stress. With *B. ferruginea* action on aPTT and PR, we conclude that the plant has anticoagulant activity. Further studies may help to determine the different mechanisms.

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