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Research Article

The Effect of Malian Herbal Plants Extracts on Hemophilia a Blood *In Vitro*

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Abstract

Introduction: We evaluated the effect of twelve Malian herbal medicine extracts on hemophilia A patients blood *in vitro.*

Methods: Citrated blood from hemophilia A patients (four severe and one moderate) was incubated with an aliquot of each of the twelve plant extracts and the activated prothrombin time (aPTT) and prothrombin time (PT) measured 5 minutes after mixture (T0) and at 30 minutes (T30) post mixing. The tests were done in duplicate, and the control was phosphate buffered saline. Mean values for each extract at T0 and T30 were calculated and compared with statistically methods.

Results: In 3 of the 12 herbal plant extracts seem reduced the aPTT values, also 6 of 12 extracts increased the PT values. The aPPT values (sec, n:10) at T0 and T30 were as follows: *Entada africana* (89.2 ± 17.4 vs 85.7 ± 17.7, OR: 0.98), *Carica papaya* (99.2 ± 24.1 vs 94.0 ± 17.5, OR: 0.74), *Detarium microcarpum* (96.6 ± 11.6 vs 94.6 ± 17.8, p:0.003) and (89.3 ± 15.6 vs 105.7 ± 34, p:0.04) for the pure condition. The PT means values (%, n:13) reached T0 and T30 were respectively (78.7 ± 17.1 vs 78.6 ± 13.8, OR: 0.67) for the pure condition, (92.3 ± 32.4 vs 94.0 ± 31.4, OR: 0.94) *Cassia sieberiana*, (91.3 ± 31.4 vs 99.1 ± 31.6, OR: 0.95) *Pteleopsis suberosa*, (102.5 ± 26.2 vs 111.1 ± 24.9, OR: 0.92) *Pteleopsis suberosa*, (86.0 ± 19.4 vs 93.5 ± 20.2, OR: 0.78) *Erythrina senengalensis*, (84.9 ± 20.3 vs 102.5 ± 27.8, p:0.001, OR: 0.89) *Erythrina senengalensis* and (81.7 ± 17.9 vs 94.6 ± 21.1, OR: 0.88) *Carica papaya*.

Conclusion: Some Malian herbal plants extracts seemed modify the PT and aPTT of blood from severe and moderate hemophilia patients *in vitro.*

Keywords: *Hemophilia A; Plants; Annona; Carica papaya; Mali*

Introduction

Hemophilia A is an X-linked inherited bleeding disorder due to mutations in the Factor *F8* gene with consequent dysfunction or deficiency of FVIII clotting factor which is associated with a life-long spontaneous or traumatic bleeding phenotype [1-3]. The prevalence of

hemophilia A is 1 in 5 000 live births and is the same across all racial and ethnic groups as well as geographic locations. Of the more than 1,125 million global hemophilia population estimated by the World Federation of Hemophilia (WFH) [4], more than 70% have no access to diagnosis or treatment [5,6]. Most of these patients live in developing countries many of which are in Africa [6].

Mali is an African country with a population of 20 million inhabitants and an expected hemophilia population of 2000. Only 200 of the expected 2000 people with hemophilia (PWH) have been identified in Mali, and their access to treatment remains a challenge with all PWH currently reliant on WFH humanitarian Aid Program for the management of bleeds [7]. In Mali and other developing countries with poor access to healthcare facilities and treatment, herbal plants are frequently used in the prehospital setting for the prevention and treatment of bleeding [8,9]. Only a handful of herbal plant extracts are used specifically for the management of prehospital bleeding diathesis in Mali. The extracts are prepared from roots, trunks, barks, and leaves of diverse herbal medicinal plants many of which are widely accepted by the complementary and alternative medicine practitioners. These extracts are prepared to be taken orally or topically in the management of bleeding diatheses.

There is, however, the paucity of data showing that Malian herbal plant preparations do indeed have hemostatic activities. We postulated that extracts from these herbal plants could have a pro-hemostatic effect on the blood of hemophilia A patients *in vitro*. The objective of this study was, therefore, to evaluate the in-vitro hemostatic effect of herbal plants on blood of severe or moderate people with hemophilia.

Materials and Methods

The study was approved by the Human Research Ethics Committee of the National Public Health Research Institute (INRSP) in Mali, and all study participants signed informed consent.

Plants selection, extracts preparation and chemical characterization

We identify the top 10 herbal plants used in this study in Mali through an ethnobotanical survey of actively practicing alternative medicine practitioners [10]. The ten herbal plants identified by practitioners were the *Pteleopsis suberosa* (leaves and stem bark), *Erythrina senegalensis* (trunk and roots barks), stem bark *of Baissea multiflora, Anona senegalensis,* and *Entada africana*, leaves of *Guiera senegalensis, Cassia sieberiana* and *Detarium microcarpum,* seeds and the roots of *Gossypium barbadense* and *Carica papaya*. We have studied twelve parts of the ten selected plants. It was leaves and stem bark of *Pteleopsis suberosa*, trunk and roots barks of *Erythrina senegalensis,* seeds of *Gossypium barbadense,* the roots of *Carica papaya,* stem bark *of* (*Baissea multiflora, Anona senegalensis,* and *Entada Africana*), leaves of (*Guiera senegalensis, Cassia sieberiana,* and *Detarium microcarpum*).

We prepared all twelve extracts of the ten herbal plants using water, dichloromethane, and methanol as solvents depending on the established plant properties [10]. Extracts underwent 24-hour maceration as a 10% weight/volume proportion. The chemical composition of dried and powdered plant material was characterized using chemical group's specific detection reagents and thin layer chromatography (TLC). Briefly, the presence of alkaloids was shown by precipitation of salts and reaction with Mayer's reagent (potassium tetra-iodo mercurate solution). The presence of gallic and catechin tannins was demonstrated by using ferric perchloride. Cyanidin reaction revealed the presence of free flavonoids (flavones and dihydroflavonols). Leucoanthocyans were detected by the cyanidin reaction carried out without the addition of magnesium shavings. The saponosides, which occur widely in plants, were characterized by their foaming power in aqueous solution. The presence of sterols and triterpenes were demonstrated using concentrated sulphuric acid. Several reducing compounds could be detected, oses and holosides and mucilages were detected by adding sulphuric acid and a saturated solution of thymol in ethanol to an aqueous decoction of the samples. Results as expressed as: (-): absence (negative reactions), (+) : traces (slight amount of compounds), (++): present (moderated amount of compounds), (+++): abundant (high amount of compounds) and (++++): very abundant (very high amount of compounds).

Hemophilia blood collection and platelet poor plasma (PPP) preparation

We drew five milliliters of whole blood from hemophilia A volunteers into evacuated tubes (Vacutainer®, Becton-Dickinson) containing 0.129 M trisodium citrate (1vol/9vol blood). The blood sample was incubated with extract solution at the final concentration of 0.25 g/L at the 20°C for 5 and 30 minutes. To establish the best extract/blood concentration, we tested a number of concentrations and found that 0.25 g/L was the optimum concentration not associated with hemolysis

Platelet-poor plasma was prepared by centrifugation of the extract-blood mix at 4000 g for 15 minutes at 20°C. In the controls, phosphate buffered saline (PBS) replaced blood and was handled the same way as the blood samples. All patient were tested at least twice.

Measurement of hemostatic properties of the extracts

An aliquot of each of the extracts suspended in water was prepared. Activated Prothrombin Time (aPTT) and Prothrombin Time (PT) were measured 5 minutes after mixing blood and extract (time 0, T0) and following 30 minutes incubation(T30) in room temperature. The analysis was performed on the STA satellite® coagulation instrument (Diagnostic Stago, New Jersey, USA) using the Stago reagents. The aPTT results of the experiments and controls were measured in seconds and expressed as a ratio. The aPTT results at T0 and T30 were compared. The PT results were expressed as seconds and percentage, and T0 and T30 were compared.

Hemolysis test

Various concentrations of extracts were tested in order to find the concentration with no hemolytic effect. Extracts chromatogram (crude extracts and fractions) were migrated in the solvent system: BAW (60: 15: 25). There were incubated in cool with whole blood for 30 minutes and were washed with distilled water. The appearance of a white spot on a red background indicates the presence of a hemolytic constituent.

Ethical and statistical analysis

Patients were included after obtaining their free consent. The recommendations of Helsinki were respected during the blood drawing process and all others steps of this study. The study was approved by the ethic committee of the National Health Research Institute of Mali.

Each experimental point was performed in duplicate. Results from at least ten experiments were expressed as mean ± SEM and range between minimum and maximum values.

Comparison between variables was performed using the linearly regression to calculate t-test and odds ratio (OR) values. A P-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS 25.0. (IBM, USA).

Results and Discussion

The baseline demographic characteristics of the hemophilia A patients are shown in the table 1.

Patient number	Age (Years)	aPTT (ratio)	PT (%)	Factor VIII level (UI)	Factor IX level (UI)	Fibrinogen (g/dL)
1.	28	2.9	100.0	0.8	74	-
2.	25	3.0	78.6	0.7	78	2.6
3.	17	1.9	100.0	1.7	94	3.7
4.	16	2.5	83.5	0.2	63	2.3
5.	17	3.8	78.0	0.5	142	3.1

Table 1: *Baseline demographic characteristics of hemophilia study participants.*
Four patients with severe hemophilia A conditions and one with moderate hemophilia A, were selected for this study.
All patients were male with a mean age of 20.6 ± 5.5 years.

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The TLC identified chemical groups in each plant material are listed in table 2.

Chemical groups	CSl	PMtb	EStb	EAtb	PMl	GBs	ESrb	AStb	CPr	GSl	BMtb	DMl
Carotenoïds	-	++	+	++	+++	-	+++	-	-	-	-	-
Coumarins	-	+++	+++	+++	++	-	+	-	++	-	-	-
Anthracens	++	-	-	-	-	-	-	+++	-	+++	++	-
C-heterosides	+	-	-	-	-	-	-	+	-	-	+	-
O-heterosides	++	-	-	-	-	-	-	+	-	++	++	-
Flavonoids	++	-	-	-	-	-	-	+	-	-	+	-
Alcaloids	++	+	+	++	+	+++	+	++	-	+	+	-
Saponins	+	++	++	+	+	-	-	++	+	+++	-	+++
Foaming indice of saponins	200	250	200	100	-	-	-	250	166	666	-	500
Tanins	+++	+++	++	+++	+	+++	+	+++	++	+++	+++	+++
Catechins type tanins	+++	+++	+	+++	+	++	+	++	++	+++	+++	+++
Galliques type tanins	-	+++	-	-	-	+	-	+	-	-	-	-
Oses/Holosides	++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
Mucilages	-	-	-	-	+	-	+	+++	++	-	-	+++
Stéroids and triterpens	-	++	+++	+++	++	+++	+	++	++	+++	+++	+++
Leucoanthocyans	+++	+++	-	+++	+	-	+	++	+	+++	+++	+

Table 2: *Results of the characterization of chemical compounds in plants materials.*

CSl: Cassia sieberiana (leaves); PMtb: Pteleopsis suberosa (trunk bark); EStb: Erythrina senegalensis (trunk bark); EAtb: Entada africana (trunk bark); PMl: Pteleopsis suberosa (leaves); GBs: Gossypium barbadense (seeds); ESrb: Erythrina senegalensis (root bark); AStb: Annona senegalensis (trunk bark); CPr: Carica papaya (roots); GSl: Guiera senegalensis (leaves); BMtb: Baissea multiflora (trunk bark); DMl: Detarium microcarpum (leaves).

-: Substance was absent (negative reactions)

+: Substance was present but in traces (slight amount of compounds)

++: Substance was present but in moderate amount.

+++: Compound was present in high amount.

++++: Compound is very abundant.

All plants materials were rich in tannins, carbohydrates, and terpenes. Alkaloids coumarins and carotenoids were found in moderate amounts while flavonoids were found only in *Cassia sieberiana* leaves, *Annona senegalensis* trunk bark and *Baissea multiflora* trunk bark.

After testing the 12 extracts, we have found that three extracts (*Entada africana, Carica papaya, Detarium microcarpum*) strongly modified the aPTT (Figure 1).

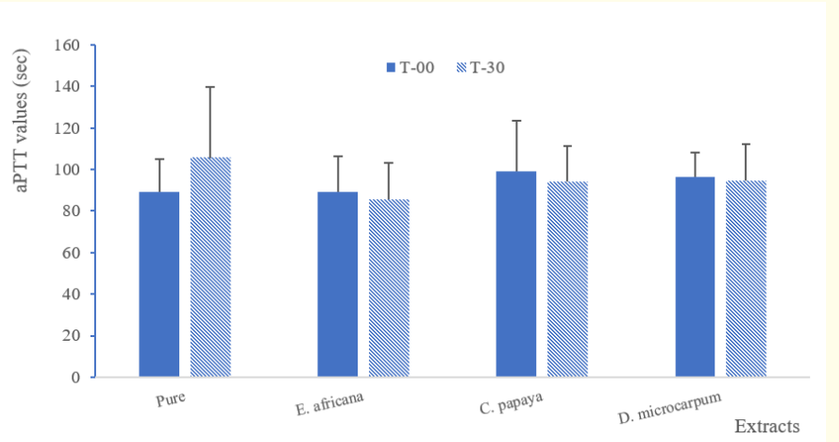

Figure 1: *Effect of extracts on aPTT using blood from hemophilia A patients at T0 and T30 incubation (n:10). aPTT values changes between T0 and T30 for three extracts: E. africana: Entada africana (trunk bark) (p:0.00, OR: 0.98 [0.81-1.12]), C. papaya: Carica papaya (roots) (p:0.02, OR: 0.74 [0.25-1.78]), D. microcarpum: Detarium microcarpum (leaves) (p:0.003, OR: 0.83 [0.24-0.84]) and (89.3 ± 15.6 vs 105.7 ± 34, p:0.04, OR: 0.66 [0.21-0.58]) for the pure condition.*

The aPPT values at T0 and T30 were as follows (results expressed as in sec, n:10) at T0 and T30 were as follows: *Entada africana* (barks) (89.2 ± 17.4 vs 85.7 ± 17.7, p:0.00, OR: 0.98 [0.81-1.12]), *Carica papaya* (roots) (99.2 ± 24.1 vs 94.0 ± 17.5, p:0.02, OR: 0.74 [0.25-1.78]), *Detarium microcarpum* (leaves) (96.6 ± 11.6 vs 94.6 ± 17.8, p:0.003, OR: 0.83 [0.24-0.84]) and (89.3 ± 15.6 vs 105.7 ± 34, p:0.04, OR: 0.66 [0.21-0.58]) for the pure condition.

We have shown that 6 of 12 extracts modified PT values. It was *Carica papaya (*Root), *Erythrina senegalensis* (Barks of the trunk, roots), *Cassia sieberiana* (Leaves) and *Pteleopsis suberosa* (roots and barks), has a probably effect on PT (Figure 2).The PT means values (results expressed as %, n:13) at T0 and T30 were respectively reached T0 and T30 followed by the correlation between this variation between the two stages, were respectively (78.7 ± 17.1 vs 78.6 ± 13.8, p:0.012, OR: 0.67 [0.22 -1.43]) for the pure condition, (92.3 ± 32.4 vs 94.0 ± 31.4, p:0.000, OR: 0.94 [0.72-1.23]) for *Cassia sieberiana* (leaves), (91.3 ± 31.4 vs 99.1 ± 31.6, p: 0.000, OR: 0.95 [0.72 -1.17]) for *Pteleopsis suberosa* (roots), (102.5 ± 26.2 vs 111.1 ± 24.9, p:0.000, OR: 0.93 [0.69-1.26]) for *Pteleopsis suberosa* (barks), (86.0 ± 19.4 vs 93.5 ± 20.2, p: 0.004, OR: 0.78 [0.30 -1.20]) for *Erythrina senengalensis* (roots), (84.9 ± 20.3 vs 102.5 ± 27.8, p:0.001, OR: 0.89 [0.39 - 0.92]), for *Erythrina senengalensis* (barks) and (81.7 ± 17.9 vs 94.6 ± 21.1, p: 0.000, OR: 0.88 [0.47-1.02]) for *Carica papaya* (roots).

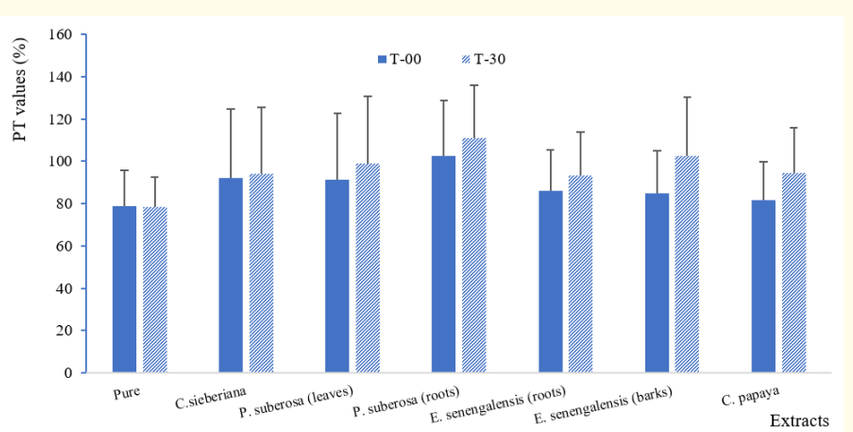

Figure 2: *Effect of extracts on PT using blood from hemophilia A patients at T0 and T30 incubation (n:13). Statistical analysis of different variations for PT values variation between T0 and T30, pure condition (78.7 ± 17.1 vs 78.6 ± 13.8, p:0.012, OR: 0.67 [0.22 -1.43]), Cassia sieberiana (leaves) (92.3 ± 32.4 vs 94.0 ± 31.4, p:0.000, OR: 0.94 [0.72-1.23]), Pteleopsis suberosa (roots) (91.3 ± 31.4 vs 99.1 ± 31.6, p: 0.000, OR: 0.95 [0.72 -1.17]), Pteleopsis suberosa (barks) (102.5 ± 26.2 vs 111.1 ± 24.9, p:0.000, OR: 0.93 [0.69-1.26]), Erythrina senengalensis (roots) (86.0 ± 19.4 vs 93.5 ± 20.2, p: 0.004, OR: 0.78 [0.30 -1.20]) for, Erythrina senengalensis (barks) (84.9 ± 20.3 vs 102.5 ± 27.8, p:0.001, OR: 0.89 [0.39 - 0.92]), and Carica papaya (roots) (81.7 ± 17.9 vs 94.6 ± 21.1, p: 0.000, OR: 0.88 [0.47-1.02]).*

Herbal plants are widely used in many developing countries throughout the world. In Mali, around 80% of the general population use herbal plants as the first line modality to treat many diseases. Our ethnobotanics survey conducted in Dioila district in Mali revealed that more than a hundred plants were used by herbal medicine practitioners to treat bleeding events in their practices. In this study, we evaluated the effects of twelve herbal plant extracts cited by traditional practitioners [10]. Using blood from healthy donors, we have found that some extracts from the twelve plants could modify *in vitro* PT and aPTT [10].

Hemophilia A is known as an X linking inhered bleeding disorders, due to a quantitative defect of factor VIII level. We have postulated that these extracts could modify hemostasis parameters (PT and aPTT) using blood from hemophilia A patients, not on prophylaxis. In this study, we chose 4 severe and 1 moderate hemophilia A patients not on prophylaxis. In this address, we investigated *in vitro* different modifications of PT and aPTT of hemophilia patients when it was incubated with the plant. After testing many other concentrations, finally, all extracts were incubated in whole blood at the final concentration of 0.25 g/dL which was shown to be the highest concentration which was nonhemolytic.

We have found that four of the twelve extracts decreased the aPTT when compared to controls.

The mean value of the aPTT increased at T30 as 89.3 ± 15.6 to 105.7 ± 34 (p:0.04, OR: 0.66 [0.21-0.58]) for the pure condition. This value was highest than the values three extracts namely *Entada africana* (barks) (OR: 0.98 [0.81-1.12]), *Carica papaya* (roots) (OR: 0.74 [0.25-1.78]) and *Detarium microcarpum* (leaves) (p:0.003, OR: 0.83 [0.24-0.84]). The modest change seemed an reduction of the values of aPTT. Besides the modification was statistically signification with *Detarium microcaprum*, but will be strongly difficult to be interpreted. If this results confirmed it could be corresponding to an procoagulant effect of the extract. The hypothesis that the extracts have an effect on coagulation pathway which involve factor VIII could be investigate.

Another important finding in this study is the effect of extracts on PT value. Surely these PT modifications are modest, but need more investigations. The PT changes, if confirmed, suggest an effect of extracts in the extrinsic pathway which include FVII. The role of a high dose of activated factor VIIa in the management of bleeds of hemophilia A patients with the inhibitor is clearly established [11-14]. In developing countries such as Mali, the management of hemophilia A patients with inhibitors remind a serious challenge [15]. Only *Carica papaya* extract modified both PT and APTT which suggest a possible effect on the common coagulation pathway which involves factors X and II.

To our knowledge, this is the first study which investigated the effect of a number of plant extracts on blood from hemophilia A patients *in vitro*. Whilst other studies have studies the hemostatic effects of herbal medicines, none have done these on the blood of PWH [10,16]. In a number of these studies, tannins were shown to have hemostatic effects [10,16,17]. In our study, all extracts were shown to have tannins, but not all extracts with tannins had an impact on the measured aPTT or PT. This suggests that other factors may play a role in the presence of tannins in modifying their hemostatic effects.

This study has a number of limitations which include the small number of hemophilia patients included. The major limitation is the fact that this was a vitro study which may not reflect the *in vivo* effect of the herbal plant extracts.

We believe these results may represent an important initial step in the understanding of the hemostatic effect of herbal medicines which are widely used in many developing countries. The exact mechanism by which the extracts are able to modify the coagulation parameters remains unknown. If confirmed, we believe that our results are encouraging and may open a new era in the management of hemophilia in developing countries.

Conclusion

Some extracts from Malian herbal plants could modify the PT and aPTT of severe and moderate hemophilia patients *in vitro*. If confirmed these study results could open new therapeutic options to improve hemophilia care in developing countries with limited access to clotting factor concentrates.

Conflict of Interest

No conflict interests.

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