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Hemostatic Effect of *Jatropha multifida* L. (*Euphorbiaceae*) in Rats Having Coagulation Disorders

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ABSTRACT

Jatropha multifida is a plant whose sap is used in traditional Beninese medicine as haemostatic. Previous work has shown are efficiencies in rapidly causing blood clotting and stopping bleeding in normal rats. The aim of this study was to investigate the haemostatic effects of *J. multifida* sap (SJM) on rats with coagulation disorders. In vivo haemostatic tests were performed on 14 Wistar albino rats including 7 normal rats and 7 warfarin-treated rats (2mg / kg orally for 4 days). After ketamine anesthesia (80 mg / kg) intramuscularly and local application of 2% xylocaine (10 mg / kg); Superficial cuts, saphenous vein and femoral vein were performed simultaneously on the two hind legs of each rat. SJM was applied locally on one of the legs and placebo (0.9% NaCl) on the other. Bleeding Times (TS) were recorded in each case. In normal rats, the results obtained indicated that the SJM topical application significantly reduced TS regardless of the type of cut. Reduction percentages were 39.06%, 46.68% and 47.89%, respectively, with superficial cuts, saphenous vein and femoral vein. In rats pretreated with warfarin, the TS reduction percentages were 71.52%, 61.54% and 66.44%, respectively, with superficial cuts, saphenous vein and femoral vein. The application of SJM significantly decreased TS compared to placebo. SJM has been shown to be effective in stopping hemorrhage in normal rats and those with coagulation disorders.

1. INTRODUCTION

Jatropha multifida, commonly known as the "coral plant", is native to tropical America (from Mexico to Brazil) and grown as an ornamental species [1]. The plant also has insect repellent properties [2], purgatives and antioxidants [3]. The root extract of *J. multifida* has been recognized to prevent infections caused by Staphylococcus aureus at concentrations of 200 g / L [4]. Bark and leaves are used as phytomedicides of neurodermatitis, skin itching and eczema [5]. The stems were used as chewing sticks for dental care in the state of Ekiti in Nigeria [6]. In Benin, sap is used as hemostatic and healing in particular in certain voodoo rituals [7-9]. Traditional use of SJM as hemostatic has been scientifically justified by the recent in vivo work of Dougnon *et al*. [10] which showed a significant decrease in bleeding time after treatment of superficial and deep cuts in normal rats By SJM. Other studies have confirmed in vitro the coagulating power of the sap of *J. multifida* (SJM). Indeed, Dougnon *et al*. [11] demonstrated that the SJM significantly reduces the coagulation time and also has astringent properties. Klotoé *et al*. [12] found a high concentration of calcium and the presence of gallic tannins in SJM. They also showed that the addition of SJM to plasma or SJM serum results in very rapid (<1 second) formation of a protein network. The latter serves as a basis for cell aggregation and stopping bleeding [10][12]. SJM had no significant change in Prothrombin Time (PT) and Activated Cephalin Time (TCA) [12], so its mechanism of action appears to be independent of conventional coagulation factors (II, V, VII, VIII, IX, X, XI, XII and XIII). Thus, the hemostatic action of SJM may be effective in normal subjects and those with coagulation disorders. In order to verify this hypothesis, we carried out the present study, which aims to evaluate in vivo the hemostatic effect of SJM on Wistar rats pretreated with warfarin.

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2. MATERIAL AND METHODS

The work was carried out at the Research Laboratory in Applied Biology of the Polytechnic School of Abomey-Calavi located at the University of Abomey-Calavi (UAC) and the Laboratory of Pharmacology of the Institute of Applied Biomedical Sciences located at the Cotonou fair camp.

2.1 Plant material

It consists of the sap of *J. multifida*. It was directly collected in sterile plastic collection tubes after foliar cleavage of the plant according to the technique indicated by Dougnon *et al*. [10]. The samples were then stored in a refrigerator at 4 ° C.

2.2 Animal equipment

It consists of male Wistar albino rats aged 16-20 weeks and weighs between 240g and 270g. They were kept in the LARBA animal facility at a constant temperature of 22 ± 1 ° C with a cycle of 12 hours in the light and 12 hours in the dark. They were fed with granulated feed and drinking water at will.

2.3 Constitution of batches of rats

A total of 14 male rats were included in this study. They were divided into two batches (of 7 rats each) by randomization:
- lot 1: normal rats without any treatment
- lot 2: Rats pretreated with warfarin (2mg / kg) dissolved in 0.9% NaCl. Oral treatment (gavage) for 4 days (ipip *et al*, 2008). Warfarin is an anti vitamin K, which prevents the synthesis of vitamin k - dependent coagulation factors (II, VII, IX and X). These rats show coagulation disorders.This treatment time was chosen following Guidance for the practical management of warfarin therapy in the treatment of venous thromboembolism [13]. After 4 days of treatment the rate of coagulation factors vitamin k - dependent decreases and so the rats show coagulation disorders.

2.4 Methodology

In each Wistar rat, it was carried out at the level of the two legs, of the superficial and deep cuts of type 1 and 2 according to the protocol described by Dougnon *et al*. [11]. Wistar rats were anesthetized with ketamine (80 mg / kg) intramuscularly. This anesthesia was supplemented by a local administration of Xylocaine 2% (10 mg / kg) at the place of election for total animal insensitisation.

The superficial cuts consisted of incisions one millimeter in length and three millimeters deep at the plantar surface of the rats, one centimeter below the knee using a sterile blade. The cuts were made simultaneously at the level of the two legs.

Deep cuts of Type 1 consisted of severing the saphenous vein two centimeters above the knee. Both veins of the animal were sectioned simultaneously after partial dressing of the rat.

Deep cuts of type 2 consisted of severing the femoral vein, which is the continuation of the saphenous vein, at the level of the groin. Both veins of the animal were sectioned simultaneously after partial dressing of the rat. After each cut, the sap was applied topically to the wound on one of the legs and the physiological water (0.9% NaCl) to the other leg. The applied volumes were 50 µl, 3x50 µl and 4x50 µl for deep, deep type 1, deep type 2 cuttings. The TS was determined in all three cases by a stopwatch. It was defined as bleeding time, the time elapsed between the beginning of the bleeding (as soon as the cut) and the stoppage of the blood flow. At the end of the experiment, the rat is sacrificed by a lethal injection of ketamine

2.5 Statistical analysis

The comparison of the TS mean between the different batches of rats was carried out using the Student t test, with a significance threshold of 5%. The software used is Microsoft Excel 2010 and Stat XL 2011.

3. RESULTS

3.1 Bleeding time in normal rats

Mean TSs obtained in normal rats are summarized in Figure 1. With the superficial cuts, the recorded TS is 91 ± 34 s at the level of the feet treated with physiological water and 56 ± 23 s at the level of the feet treated with the SJM. SJM induced a statistically significant reduction in TS (39.06%) compared to control (p = 0.03). With cuttings of the saphenous veins, the TS recorded were 50 ± 29s at the level of the legs treated with SJM against 95 ± 37s at the feet treated with physiological water. SJM resulted in a statistically significant reduction in TS (47.89%) compared to the control (p = 0.037).
With cuttings of the femoral veins, the TS recorded were 103 ± 26s at the level of the feet treated with physiological water, compared with 55 ± 38s at the level of the legs treated with the SJM. SJM induced a significant (46.68%) reduction in TS versus control (p = 0.037).

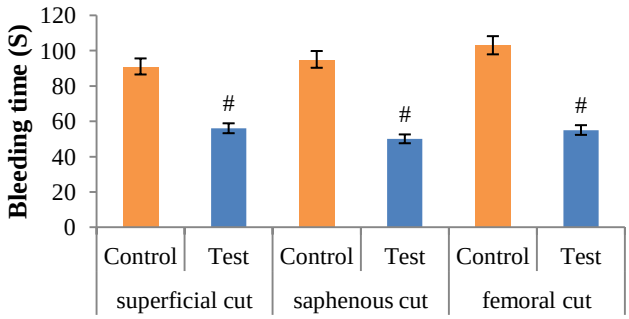

Fig. 1: Bleeding time in normal rats.

3.2. Bleeding time in rats pretreated with warfarin

In the control (non-pretreated rats), the mean TS obtained is summarized in FIG. With the superficial cuts, the TS recorded were 43 ± 7s at the level of the legs treated with the SJM against 151 ± 46s at the feet treated with physiological water. The application of SJM resulted in a significant reduction in TS by

71.52% compared to control (p = 0.002). With cuttings of the saphenous veins, the TS recorded were 40 ± 9s at the level of the legs treated with the SJM against 104 ± 27s at the feet treated with physiological water. SJM induced a TS reduction of 61.54%, statistically significant compared to the control (p = 0.002).
With cuttings of the femoral veins, the recorded TS was 49 ± 11s at the level of the legs treated with the SJM against 146 ± 44s at the feet treated with physiological water. SJM resulted in a TS reduction of 66.44%, statistically significant relative to the control (p = 0.002) (fig 2).

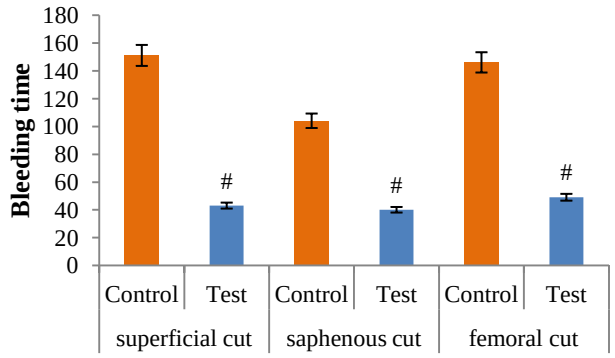

Fig. 2: Bleeding time in rats pretreated with warfarin.

3.3 Comparison of TS of different batches of rats

The comparison between the mean of the TS obtained in the control groups (physiological water) showed that the difference between the batch of rats pretreated with warfarin and the batch of normal rats (warfarin effect) is statistically significant (p = 0.009) . TS was longer in rats pretreated with warfarin (Figure 3). On the other hand, the comparison between the means of TS obtained after the LSU application showed that the difference between the batch of rats pretreated with warfarin and the batch of normal rats (warfarin effect) was not statistically significant (P = 0.935). In other words, the hemostatic efficacy of similar SJM in normal rats and rats pretreated with warfarin (FIG. 3).

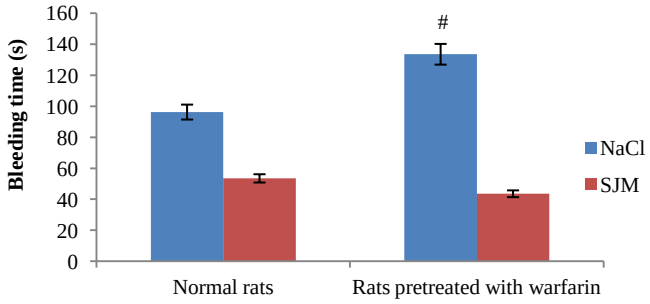

Fig. 3: Comparison of TS of different batches of rats.

4. DISCUSSION

The results obtained on normal rats showed that the topical application of SJM significantly reduced TS compared to placebo. Our results are identical to those of Dougnon *et al*. [11] and confirm the traditional use of SJM as hemostatic [7]; [9]. According to these authors, fresh leaves of *J. multifida* are used as poultices for the treatment of bleeding during certain voodoo rituals. It should be understood, in the light of the results obtained in this study, that it is the sap of the plant that is responsible for this hemostatic effect.

The prolongation of TS shows that the use of warfarin in this study allowed us to create a rat model with coagulation disorders. Warfarin is an anticoagulant of the coumarin family. Its mechanism of action is the inhibition of the synthesis of coagulation factors dependent on vitamin K. According to Gemmati *et al* [14], Warfarin acts by inhibiting the synthesis of vitamin K-dependent clotting factors, which include Factors II, VII, IX, and X, and the anticoagulant proteins C and S. Vitamin K is an essential cofactor for the post ribosomal synthesis of the vitamin K-dependent clotting factors. Vitamin K promotes the biosynthesis of γ-carboxyglutamic acid residues in the proteins that are essential for biological activity. Warfarin is thought to interfere with clotting factor synthesis by inhibition of the C1 subunit of vitamin K epoxide reductase (VKORC1) enzyme complex, thereby reducing the regeneration of vitamin K1 epoxide. 4 days of treatment were essential in this study to have a deficit of all vitamin K dependent factors. Indeed, The mean life of these factors being distinct, after the administration of warfarin a sequential decrease in the plasma concentration of these In Factor VII, Factor IX, Factor X and Factor II [15]. Also Patel, [16] has shown that an anticoagulation effect generally occurs within 24 hours after warfarin administration. However, peak anticoagulant effect may be delayed 72 to 96 hours. This is consistent with the half-lives of the affected vitamin K-dependent clotting factors and anticoagulation proteins: Factor II - 60 hours, VII - 4 to 6 hours, IX - 24 hours, X - 48 to 72 hours, and proteins C and S are approximately 8 hours and 30 hours, respectively.

Factor II called thrombin is the element that allows blood coagulation through the transformation of fibrinogen into fibrin [17].

The deficit in these factors increases TS [18]. From the above, the effectiveness of SJM in rapidly stopping bleeding in rats pretreated with warfarin clearly reflects a mechanism of action independent of these coagulation factors. These results confirm the recent in vitro studies that showed that SJM had no effect on TCA and TQ[10] and its addition to the blood rapidly leads to coagulation through the formation of a protein network Which serves as a basis for cell aggregation [12]. Gallic tannins contained in SJM[11] would play an important role in the formation of the protein network. Their involvement in coagulation was previously reported by Klotoé *et al*. [19] and Dandjesso *et al*., [20] during work on extracts of haemostatic plants. Since SJM causes protein-based coagulation without affecting coagulation factors, it has an advantage over other hemostatics that activates the classical pathways of hemostasis [15], [21].

The effect of SJM on TS is similar to that of Ankaferd Blood Stopper, a mixture of traditional plants (Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica

dioica) which acts on haemostasis by reducing bleeding time[22], [23]. This mix of five plants therefore has the same effect as the only LSU; Which constitutes an additional advantage offered by this plant.

5. CONCLUSION

This study showed the efficacy of SJM to stop haemorrhages in both normal and coagulation disorders. This offers interesting prospects for its use in the treatment of haemorrhages in the presence or absence of haemostasis disorder. The absence of skin toxicity allows for clinical studies.

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Conflict of Interests: There are no conflicts of interest.

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