MECHANISM OF ACTION OF JATROPHA GOSSYPIFOLIA STEM LATEX AS A HAEMOSTATIC AGENT

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Aim: Jatropha gossypifolia belongs to the family "Euphorbiacea". The coagulant activity and the mechanism of action of Jatropha gossypifolia stem latex as a haemostatic agent were investigated.

Methods: The mechanism of action was investigated using doubling dilution technique by mixing neat (undiluted) and serial dilutions of the latex with 30% bovine albumin.

Results: The results of whole blood clotting time using Lee and White method and bleeding time using Ivy's method were significantly reduced ($P < 0.05$) when stem latex was introduced than when the tests were performed without stem latex. This means that the stem latex possesses procoagulant activity.

Conclusion: The result of this test showed that precipitation of coagulant factors is the mechanism of action of its haemostatic activity.

Keywords: Jatropha gossypifolia, haemostatic agent, mechanism.

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INTRODUCTION

Jatropha gossypifolia belongs to the family "Euphorbiacea". Other species are Jatropha curcas, Jatropha glandulifera, Jatropha tanjorensis, Jatropha multifida, Jatropha podagrica and Jatropha intergerrima. It is a bushy, gregarious shrub of about 1.8meters in height. The leaves are 3-5 lobed, palmately, 20cm glandular hairs. The flowers are red-crimson or purple in corymbs, with greenish seeds in capsule (1,2).

Different parts of J. gossypifolia are used in different countries in many ways. The leaves of J. gossypifolia are used for intermittent fevers, carbuncles, eczema, itches, sores on the tongues of babies, swollen mammae, stomachache, and venereal disease (3). The leaf decoction is used for bathing wounds (4). The bark contains the alkaloid jatrophine and a lignan (jatroiden) is found in its stem (5,6). Seeds are emetic, purgative, and used for body pain.

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Plant is antibiotic, insecticidal and used for toothache and as blood purifier (3).

The normal haemostatic mechanism involves normal functions of blood vessels, platelets and the blood coagulation. Vessels with muscular coats contract following injury thus helping to arrest blood loss; the contraction is aided by the release of vasoconstrictors such as angiotensin II (7).

When the vessel wall is damaged, the subendothelial structures, including basement membrane, collagen and microfibrils, are exposed. The platelets adhere to the exposed damaged endothelium, the outer membrane becomes stickier so that other platelets can adhere to it form platelet aggregates (platelet plug). The platelets release certain substances (agonists) such as adenine diphosphate (ADP), serotonin, adrenaline, prostaglandin derivatives e.g. thromboxane A_2 which promote platelet aggregation (8).

The coagulation cascade leading to the generation of thrombin and the formation of a fibrin clot is classically divided into parts: the slow intrinsic and fast extrinsic pathways. Both intrinsic and extrinsic pathways terminate in the final common pathways.

In the intrinsic pathway, factor XII is activated by collagen and other negatively charged components of the subendothelium. Activation of factor XII leads to the sequential activation of factors XI, IX, VIII (as co-factor) X and prothrombin. In the extrinsic pathway, tissue factor (TF) complexes with factor VII with sequential activation of factors VII, X and prothrombin. Both intrinsic and extrinsic pathways terminate in the final common pathway where activated factor X in association with the co-factor V in the presence of phospholipids and calcium converts prothrombin into thrombin. Thrombin in turn converts fibrinogen to fibrin monomers. These monomers combine spontaneously into dimers which assemble to form the fibrin polymer to consolidate the thrombus. The final fibrin thrombus forms a meshwork, which reinforces the platelet plug (9).

In the modern concept of coagulation, factor VII binds to TF where it is rapidly activated to factor VIIa. The resulting TF–VIIa complex activate some factor X to Xa and factor IX to IXa. The generation of Xa results in the intervention of tissue factor pathway inhibitor (TFPI) which binds factor Xa and then form a complex with factor VIIa and TF, which bind and inhibits factor Xa directly. Based on the present understanding, the contact factors XI, XII, High molecular weight Kininogen, Prekallikrein play no role in coagulation. Subjects deficient in these factors do not have expected severe bleeding disorders (10,11).

The stem latex of Jatropha gossypifolia is routinely used in southern Nigeria by herbalists, rural dwellers and some people in urban centers to stop bleeding from nose, gum and skin, hence the aim of present study is to investigate the coagulant property and the mechanism of action of the stem latex of Jatropha gossypifolia as a haemostatic agent.

MATERIALS AND METHODS

Method of collection of stem latex

The stem of growing Jatropha gossypifolia plants were cut and the fluid coming out collected into a clean sterile universal bottle.

Investigation of coagulant activity of Jatropha gossypifolia stem latex

Coagulation screening tests (whole blood clotting and bleeding time tests) were performed with the stem latex within 6 hours of collection on thirty healthy subjects.

Whole Blood Clotting Time (12)

Eight Khan tubes were arranged in a water bath at 37^0C, into four of these tubes (test), 0.1ml each of the latex was added and nothing was added to the remaining four tubes (control). 8ml of blood was collected from each subject by clean venepuncture and 1ml was added into each of the tubes, immediately the blood started flowing into the syringe, a stop watch was started, the tubes were observed for clot and the clotting time taken. The average of the clotting time of the four tubes with stem latex (test) and the four tubes without stem (control) were taken as the clotting time respectively.

Bleeding Time (13)

A sphygmomanometer cuff was tied to the fore arm and pressure increased to 40mmHg. After sterilizing the skin with 70% methyl alcohol, three punctures were made on the fore arm with a lancet, care was taken to avoid puncturing blood vessels and the stop watch started. Filter paper was used to absorb blood coming out and time taken for bleeding to stop was recorded, the average was taken as bleeding time (control). The procedure was repeated on the second arm but after puncturing the skin and the blood started coming out, a drop of stem latex was dropped on each of the puncture sites and the time taken for bleeding to stop was recorded (test), the average taken as the bleeding time.

Investigation of mechanism of action of Jatropha gossypifolia stem latex

This was investigated by adding 0.1ml of the neat latex (undiluted) to 1ml of 30% bovine albumin. Serial dilutions of latex were then made with distilled water: 1/2, 1/4, 1/8, 1/16 1/32, 1/64, 1/128, and 1/512, 0.1ml of each dilution was added to 1ml of 30% bovine albumin.

Statistic analysis

The mean and standard deviation and the level of significance for the difference between means were computed by SPSS 6 .

Table 1. Clotting time with and without Stem Latex

	Group 1	Group 2
Parameter	Without stem latex	With stem latex
Clotting time	6 minutes 25 seconds	5 seconds
Bleeding time	2 minutes 20 seconds	2 seconds

RESULTS

The clotting time without adding stem latex was 6 minute 25 seconds while it was 5 seconds when stem latex was added. The difference was statistically significant ($p<0.05$). The reference range for whole blood clotting time is 6 to 9 minutes at 37^0C (12).

The bleeding time without adding stem latex was 2 minutes 20 seconds while it was 2 seconds on addition of stem latex. The difference was statistically significant ($p<0.05$). The reference range for bleeding time is 2-5 minutes (13). The results of the reaction between the neat and serial dilutions of the latex with 30% bovine were shown in Table 1.

DISCUSSION

Coagulant activity of the stem latex of Jatropha gossypifolia was demonstrated based on the findings of this study. The reduced clotting and bleeding times (Table 1) recorded in the experiment with stem latex as compared to those without stem latex was an evidence that the stem latex possess coagulating agent thereby providing scientific basis for its use as a haemostatic agent.

It was established from the reaction of the neat latex, its serial dilution, with the 30% bovine albumin that the latex is a protein precipitant (Table 2). All coagulation factors are proteins except factor IV (calcium), hence the mechanism of action of the stem latex is by precipitation of coagulation factors. The explanation for the shortened bleeding and clotting times observed on addition of stem latex might be that the coagulation factors are precipitated thereby bringing the coagulation factors into close contact, then the activation of coagulation cascade leading to the generation of thrombin and formation of a fibrin clot took place in a matter of seconds as compared to the control experiment that took minutes to complete. Since some coagulation disorders are treated with drugs with coagulant properties, the findings of this study is worth being explored for clinical trials.

In conclusion, there is no doubt that the stem latex of Jatropha gossypifolia has coagulant properties but work needs to be done on toxicity studies to eliminate any dangerous side effects before it can be safely recommended for intravascular treatment. This means that the active chemical must be extracted and characterized.

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Table 2. Mechanism of action of Coagulant activity of Jatropha gossypifolia stem latex

Tube Number	1	2	3	4	5	6	7	8	9	10
Dilution	Neat	1/2	1/4	1/8	1/16	1/32	1/64	1/28	1/256	1/512
Distilled water	__	0.1^a	0.1^a	0.1^a	0.1^a	0.1^a	0.1^a	0.1^a	0.1^a	0.1^c
Stem latex	0.1	0.1^b	0.1^b	0.1^b	0.1^b	0.1^b	0.1^b	0.1^b	0.1^b	0.1^d
30% Bovine Albumin	1	1	1	1	1	1	1	1	1	1
Result	C	C	C	PC	GP	GP	CS	SCS	SCS	SCS

a-b: mixed and 0.1ml transferred, c-d: 0.1ml discarded, C = complete clot, PC = partial clot, GP = Granular precipitate, CS= cloudy precipitate, SCS = slightly cloudy precipitate

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