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Effect Of Aqueous And Ethanol Leaves Extracts Of Carica Papaya (Caricaceae) On Hemostasis In Rabbits

Aji, S.B.

Tarimbuka, L.I.

Lamurde, K.J.

Solomsi A.A.

Department of Animal Health and Production Technology

Tsunda, B.M.

Department of Science Laboratory Technology, Adamawa State College of Agriculture, Ganye, Adamawa State, Nigeria

Abstract: ***Most clinically available systemic hemostatics are not without their shortcomings. This necessitates the search for new products especially from plants as they provide wider pharmacognostic opportunities. Carica papaya is a nutritionally and medicinally beneficial plant. The leaves are used in traditional medicine as hemostatic in Ganye chiefdom of Adamawa State in Nigeria. This study sought to examine the effect of aqueous and ethanol leaves extracts of C. papaya on hemostasis in rabbits. Twenty four rabbits of both sexes, weighing between 1 Kg and 1.7 Kg were divided into 3 groups labeled A, B and C and used in the experiment. Group A and B were treated with daily oral dosage of 1000 mg per Kg body weight of the aqueous and ethanol extracts respectively for 5 days. Group C received only distilled water for the same period. At the end of the fifth day, all animals were used for the determination of whole blood clotting and bleeding time using standard techniques. The result of the study revealed that both extracts significantly reduced clotting time but did not likewise affect the bleeding time in the rabbits. This result validated the claim for the traditional use of C. papaya leaves as a hemostatic. Further studies to reveal the mechanism of action by which these extracts reduced clotting time and the active principles involved and also to determine the hemostatic effect of the extracts using heparin, vitamin K and aspirin pretreated and other subjects and on the extracts' toxicity were recommended.***

Keywords: ***Carica papaya leaves, aqueous extract, ethanol extract, hemostasis, rabbits***

I. INTRODUCTION

Hemorrhage may result to anemia, shock and death [14] and it is the leading cause of maternal mortality worldwide [9] with about 84% of cases unpredictable [4]. The ability of the body to control hemorrhage (hemostasis) following vascular injury is paramount to continued survival and contributes to homeostasis in part. Hemostasis involves the clotting of blood while maintaining blood in a fluid state within the vascular system and subsequent fibrinolysis following repair of the injured tissue. A collection of complex interrelated systemic mechanisms operates to maintain this balance between coagulation and anticoagulation [8].

Hemostatics or hemostatic agents are used primarily to arrest bleeding or to control oozing from minute blood vessels. They are also important in naturally occurring pathological conditions like hemophilia and fibrinolytic states which may arise after surgery. These agents are broadly classified into topical and systemic hemostatics. Presently, most of the clinically available systemic hemostatics are not without their shortcomings e.g. vitamin K is restricted to use in vitamin K related anticoagulation only and has slow therapeutic response of about 24 hours, aminocaproic and tranexamic acids have bad potential side effects, aprotinin preparations are now completely withdrawn from the market due to increased risk of complications and deaths, protamine sulphate has restricted use only as heparin antagonist and may in fact exacerbate bleeding due to its inherent anticoagulant activity. The same is potentially dangerous to people allergic to fish. Adrenochrome monosemicarbazone has questionable efficacy as a hemostatic,

while ethamsylate, desmopressin, rutin and caboprost have limited uses [13]. These demerits spur the need to explore materials with potential hemostatic effect with the hope of making novel discoveries.

Plants used in traditional medicine provide an interesting and still largely unexplored source for the development of new drugs [6]. Although the pharmacological properties of several plants have been demonstrated, and not fewer than 170,000 bioactive molecules have been identified from plants, relatively few plants have been evaluated for their therapeutic properties and some pharmacological activities such as hemostatic effect is among the least explored [12].

Carica papaya L. (paw paw) belongs to the family Caricaceae. Every part of the plant is used medicinally with the latex been widely used as hemostatic and coagulant and as vermifuge [2]. In Ganye chiefdom of Adamawa State in Nigeria, orally administered infusion of leaves of *C. papaya* is used in traditional medicine as a hemostatic especially postpartum and against other hemorrhagic conditions. The claim for this hemostatic activity has not been scientifically validated. Therefore, this study sought to examine the effect of orally administered aqueous and ethanol leaves extracts of *C. papaya* on hemostasis (whole blood clotting time and bleeding time) in rabbits.

II. MATERIALS AND METHODS

A. COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

Fresh leaves of *Carica papaya* were collected from Gangwoki, in Ganye Local Government Area in Adamawa State, Nigeria. The collected plant materials were identified based on standard criteria [2]. Voucher specimen was kept in the Department of Animal Health Laboratory, College of Agriculture Ganye. The collected *C. papaya* leaves were air-dried, crushed, pulverized into fine powder and stored in polythene bag before extraction.

B. PREPARATION OF ETHANOL EXTRACT

One hundred and fifty grammes (150g) of the powdered leaves of *C. papaya* was mixed with 750 mL of 95% v/v ethanol (BDH Chemical Ltd, Poole, England) and allowed to dissolve over a period of 24 hours. It was then thoroughly mixed, passed through a sieve to remove coarse plant material and then filtered through Whatman No. 1 filter paper and concentrated at 75 ^{o}C in a hot water bath until dryness. The ethanol extract was stored at 4^{o}C pending use. A stock solution of 250mg of extract per mL of distilled water was prepared for oral administration when required.

C. PREPARATION OF AQUEOUS EXTRACT

One hundred and fifty grammes (150g) of the finely pulverized leaves powder of *C. papaya* was mixed with 750 mL of distilled water and allowed to dissolve over a period of 24 hours and processed using the same procedure as described for the ethanol extract above except that the evaporation in hot water bath was done at 100 ^{o}C.

D. THE EXPERIMENTAL ANIMALS

Twenty four rabbits of both sexes weighing between 1Kg (1000g) and 1.7Kg (1700g) where purchased from a farmer and kept in deep litter housing with good ventilation and lightening. They were given commercial feeds (*Vital Feed*, Plateau State, Nigeria) and clean tap water *ad libitum*. They were allowed to adapt to the environment for two weeks before the commencement of the experiment. All animals were handled according to the guidelines for research and evaluation of traditional medicine using animal model [17] and the international guiding principles for biomedical research involving animals [5].

E. TREATMENT OF THE ANIMALS

The rabbits were randomly separated into 3 groups of 8 rabbits per group. The groups were labeled group A, B and C. Group A were treated with 1000mg per Kg of body weight of aqueous extract of *C. papaya* orally for 5 days. Group B received 1000mg per Kg of ethanol extract of *C. papaya* orally for the same period while group C (control) were given only distilled water for the same period. At the end of the 5th day, all rabbits were used for the determination of whole blood clotting and bleeding time using standard techniques.

F. DETERMINATION OF WHOLE BLOOD CLOTTING TIME

This was done using the capillary tube method [3]. A capillary tube 15 cm long and 1 mm in diameter is filled with blood from ear vein puncture after discarding the first few drops. The stopwatch is started as soon as blood appears in the tube. The tube is kept horizontal between the thumb and index finger of both hands and a small piece is broken after every 30 seconds. The time interval between the appearance of the blood in the tube and the appearance of fibrin strand is the coagulation time (whole blood clotting time).

G. DETERMINATION OF BLEEDING TIME

A small area around the ear vein is shaved with a razor blade and cleaned with methylated spirit followed by cotton wool partially soaked in sterile distilled water. The bleeding time was then determined using standard method [3]. A deep puncture wound was made with a surgical lancet on the ear vein. The stopwatch was started when the first blood appear. The accumulated blood is removed with filter paper after every 30 seconds without touching the skin. The stopwatch is stopped when no longer blood appears at puncture site. The time taken is recorded as the bleeding time.

H. DATA ANALYSIS

The data obtained were carefully subjected to statistical analysis using computer statistical software, GraphPad InStat Version 3.10, 32 bit for Windows by GraphPad® Software

Inc., San Diego, CA, USA. Results were expressed as mean and standard deviation (Mean ± SD). Differences between groups were analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparison post test to compare treatment and control groups. Level of significance of differences between means was considered at $P < 0.05$.

III. RESULTS AND DISCUSSIONS

A. OUTCOME OF EXTRACTION OF THE PLANT MATERIALS

The extraction of 150 g of air-dried leaves powder of *C. papaya* in 750 mL of 95% ethanol yielded 20.3 g of extract giving an extract yield of 13.53% w/w. The extraction of the same quantity of the plant material in 750 mL of distilled water yielded 21.6 g of extract giving an extract yield of 14.4% w/w. The ethanol extract is black in color and has a bitter taste while its aqueous counterpart is dark brown in color but also has a bitter taste.

B. WHOLE BLOOD CLOTTING AND BLEEDING TIME OF C. PAPAYA EXTRACTS-TREATED AND CONTROL RABBITS

The result of the clotting time of blood of rabbits treated with *C. papaya* aqueous and ethanol extracts and control subjects (Table1) indicated that the mean clotting time of both extract treated groups are significantly lower ($P<0.05$) than those of the control animals however, there is no significant differences ($P>0.05$) between the mean values of clotting times of *C. papaya* aqueous extract and ethanol extract treated groups of rabbits. The bleeding times of wounds of *C. papaya* aqueous and ethanol extracts-treated and control rabbits (Table 2) indicated that although the mean bleeding time values of the extracts-treated rabbits were lower than that of control group, the differences between them is not statistically ($P>0.05$) significant.

Rabbit No	Clotting Time in Seconds (n=8)		
	Group A	Group B	Group C
1	90	60	210
2	60	60	150
3	90	60	120
4	60	60	120
5	60	60	150
6	60	60	120
7	90	60	210
8	90	60	120
Mean±SD	75±16.04[b]	60±0[a]	150±39.28[a,b]

[a, b] = Difference between means with same superscripts is statistically significant (P<0.05)

Table 1: Whole Blood Clotting Time of C. papaya Leaves Aqueous and Ethanol Extracts-treated and Control Rabbits

Rabbit No	Bleeding Time in Seconds (n=8)		
	Group A	Group B	Group C
1	72	118	130
2	75	112	70
3	120	102	103
4	74	78	71
5	74	78	141
6	72	80	80
7	72	96	132
8	75	106	114
Mean±SD	79.25±16.52[a]	96.25±15.94[a]	105.13±28.64[a]

[a] = Difference between means with same superscripts is not statistically significant (P>0.05).

Table 2: Bleeding Time of C. papaya Leaves Aqueous and Ethanol Extracts-treated and Control Rabbits

The study revealed that treatment of rabbits with oral dosage of 1000mg/Kg body weight of both aqueous and ethanol leaves extracts of *C. papaya* for five days significantly decreased clotting time in the treatment groups when compared to the control subjects. The same treatment however, did not produce significant difference between bleeding times of the treated and control groups although the mean values of bleeding time of treated groups appear lower than that of control group.

The study did not attempt to explore the exact mechanism by which these extracts decreased blood clotting time however, the role of plant latex proteases (including that of *C. papaya*) in hemostasis and their mechanism of action on blood coagulation and fibrinolytic pathways are well elaborated [16]. Earlier report indicated that the latex of *C. papaya* contain a cysteine protease known as papain which was demonstrated to have thrombin-like activity and directly induce fibrin clotting by cleaving the Aα and Bβ chains of fibrinogen molecule at specific sites, releasing fibrinopeptides similar to thrombin and inducing fibrin formation by the polymerization of activated fibrinogen. Papain was also reported to have factor XIIIa-like activity catalyzing cross-linkages between adjacent fibrin monomers similar to factor XIIIa. This intermolecular cross-linking of fibrinogen monomers by papain leads to γ-chain dimmers, trimers and tetramers similar thrombin-factor XIIIa-stabilized fibrin [7]. The aqueous and ethanol extracts of *C. papaya* used in this study may contain papain and the above established mechanism may account for the decrease in blood clotting time observed in this study. It is also likely that the extracts decreased the clotting time by promoting the hepatic synthesis of clotting factors and proteins concerned with hemostasis. The role of factors I (fibrinogen), VIII (hemophilia A factor) and IX (hemophilia B factor) in this regard and the consequence of their deficiency in prolonging clotting time are well spelt out [3]. Another hypothesis is that the extracts either contain vitamin K or promote the hepatic synthesis of vitamin K or its dependent clotting factors (factors II,VII, IX and X) as the role of vitamin K and these factors in promoting hemostasis are also well established [13]. *C. papaya* leaves extract have been reported to increase platelets count [1, 10, 11, 15]. It could also be possible that the extracts enhanced the production of platelets which in turn enhanced blood clotting.

IV. CONCLUSION

This study revealed that both the aqueous and ethanol extracts of the leaves of *C. papaya* significantly reduced whole blood clotting time. This result validated the claim for

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the traditional use of the leaves of *C. papaya* as a hemostatic agent and confirmed the basis for its use as such in Ganye chiefdom especially postpartum and in other hemorrhagic conditions. These findings trigger the need to examine the mechanism of action by which *C. papaya* aqueous and ethanol leaves extracts reduce clotting time and the active principle involved and also to determine their hemostatic effects using heparin, vitamin K and aspirin pretreated and other subjects. Toxicity study is also another area that should be explored.

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