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Hemostatic Effects Of Extracts Of Leaves Of *Annona Senegalensis* In Rabbits

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Abstract: ***The leaves of Annona senegalensis is used in traditional medicine as a hemostatic agent in Ganye Local Government Area of Adamawa State in Nigeria, but the claim for its hemostatic activity has not been scientifically validated. The need to validate this claim necessitated this study. The effect of orally administered aqueous and ethanolic leaves extract of A. senegalensis on whole blood clotting time and bleeding time in rabbits were determined using standard methods. Twenty four rabbits divided into 3 groups of 8 rabbits per groups and labeled group A, B and C, were used in the experiment. Each rabbit in Groups A and B (the experimental groups) were given 1000 mg/Kg of aqueous and ethanolic leaves extracts of A. senegalensis respectively for 5 days (the approximate dose and treatment period were adopted from its traditional usage) while those in Group C (the control group) was given 5mL of distilled water for the same period. The result of the study revealed that the aqueous extract did not affect bleeding time but significantly (P<0.05) reduced clotting time while the ethanolic extract significantly (P<0.05) increased bleeding time but had no effect on the clotting time. It was concluded that orally administered aqueous leaves extract of A. senegalensis reduced clotting time while its ethanol counterpart increased bleeding time but did not affect the clotting time in rabbits and that these findings supported the basis for the traditional use of the aqueous leaves extract of A. senegalensis as a hemostatic agent in the study area, but not its ethanolic extract, which indeed increased bleeding time. Further detailed studies of these effects were recommended with the view to throw more light on the mechanisms of action, the active principle(s) involved and its possible effects on coagulants and anticoagulants pretreated subjects.***

Keywords: ***Annona senegalensis, Leaves extracts, Hemostasis, Rabbits***

I. INTRODUCTION

Hematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health. Hemostasis is the process which causes bleeding to stop, meaning to keep blood within a damaged blood vessel (Saliu *et al*., 2012). Bleeding is a major cause of maternal mortality worldwide (USAID, 2006). Hemostasis is the prevention or control of blood loss from damaged blood vessels. It is a complex phenomenon that includes physical and biochemical changes. It starts with the injury to tissue and blood vessels and terminates in the formation of solid clot that seal the vascular endothelium and involves 3 main processes namely vascular constriction, platelets activation and adhesion and blood coagulation/fibrin formation (Sandhu, 2013). Hemostatics (hemostatic drugs) are agents which prevents or attenuate bleeding. These agents are primarily used to arrest bleeding or to control oozing of blood from minute blood vessels. Hemostatics are useful also in some naturally occurring pathological condition like haemophilia and in fibrinolytic state (Sandhu, 2013).

Many leafy vegetables and weeds abound in tropical Africa that could be used in the management of hematological abnormalities (Saliu *et al*., 2012) and these plants are used alone or in combination. Several studies have been conducted on the hemostatic effect of different plants including *Zingiber officinale, Thymus vulgaris* and *Acacia arabica* (Raaof *et al*., 2013), *Chromoleana odorata* (Akomas and Ijioma, 2014), *Moringa oleifera* (Otitoju *et al*., 2014) and have yielded various results. Previous reports have shown that plant

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materials are also employed as hemostatics to control bleeding. For example, Vetigel is a new plant-based gel that stops traumatic bleeding in just 15 seconds. It works by using plant-based haemophilic polymer made from polysaccharides that grab unto the blood and form a mesh that seals over the wound without any need to apply pressure. Also, Ankaferd Blood Stopper (ABS), which has been used as a traditional medicine represents an alternative treatment modality for many kinds of bleeding that are resistant to conventional methods (Beyazit *et al*., 2010). This plant product which contains material obtained from 5 different plants is currently been officially approved for use in Turkey and Bosnia-Herzegovina as a topical hemostatic agent for external post-surgical and post-dental surgery bleeding (Turhan *et al*., 2011). *Cassytha filiformis* ans *Annona senegalensis* leaves extract has astringent property that could act on primary hemostasis through vasoconstriction (Dandjesso *et al*., 2012) also, ethanolic extract of *Chromolaena odorata* leaves has been found to reduce bleeding and clotting times and may be useful in managing bleeding problems and accelerating wound healing (Akomas and Ijioma, 2014).

The leaves of *Annona senegalensis* is used in traditional medicine in Ganye Local Government Area and its environs in Adamawa State of Nigeria for immediate arrest of bleeding (especially following circumcision) and in wound healing management, but the claim for this pharmacological activity has not been tested in a controlled experiment. It is in view of this that this research is been undertaken. Therefore, the aim of this study is to determine the effect of aqueous and ethanolic leaves extract of *Annona senegalensis* on hemostasis in rabbits. The specific objectives are to determine the effects of the extracts on whole blood clotting time and bleeding time in the experimental animals.

II. MATERIALS AND METHODS

A. COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

Fresh leaves of *Annona senegalensis* were collected from Gangwoki, in Ganye Local Government Area in Adamawa State, Nigeria. The collected plant materials were identified based on standard criteria described by Arbonnier (2004). Vourcher specimen was kept in the Department of Animal Health Laboratory, College of Agriculture Ganye.

B. PREPARATION OF THE AQUEOUS EXTRACT

The collected leaves were air-dried, crushed, pulverized into fine powder and stored in polythene bag before extraction. One hundred and fifty grammes (150g) of the dried leaves powder of *A. senegalensis* was mixed with 750 mL of hot distilled water after boiling. It was then allowed to cool down, 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 resulting aqueous extract was then stored at 4^{o}C pending use. A stock solution of 250mg per mL of the aqueous extract in distilled water was prepared for oral administration when required.

C. PREPARATION OF ETHANOLIC EXTRACT

One hundred and fifty grammes (150g) of the dried leaves powder of *A. senegalensis* 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, sieve, filtered, concentrated until dryness and stored using the same procedure described above for the aqueous extract. A stock solution of 250mg of the ethanolic extract per mL of distilled water was prepared for oral administration when required.

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 (WHO, 2000) and the international guiding principles for biomedical research involving animals (CIOMS, 1985). Ethical clearance was granted by the Headship of the Department of Animal Health Technology, College of Agriculture Ganye.

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. Each rabbit in Groups A and B were treated with 1000mg per Kg of body weight of aqueous and ethanolic leaves extract of *A. senegalensis* respectively for 5 days while those in group C (control) received 5mL of distilled water for the same period. At the end of the fifth day, each 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 based on the capillary method described by Brar *et al.* (2002). 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 recorded as the coagulation time (whole blood coagulation time).

ISSN: 2394-4404

G. DETERMINATION OF BLEEDING TIME

A small area around the ear vein is shaved with a razor blade and cleaned with methylated spirit and followed by cotton wool partially soaked in sterile distilled water. The bleeding time was then determined as described by Brar *et al* (2002). A deep puncture wound was made with a No. 2 Bard Parker blade 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 from the study were carefully subjected to statistical analysis using ANOVA with Tukey multiple comparison post-test by computer statistical software (GraphPad InStat Version 3.10, 32 bit for Windows, by GraphPad Software Inc., USA). Results were expressed as mean and standard deviation (Mean ± SD). Level of significance of difference between means was considered at $P < 0.05$.

III. RESULTS AND DISCUSSIONS

A. RESULT OF EXTRACTION OF PLANT MATERIAL

The extraction of 150 g of air-dried leaves powder of *A. senegalensis* in 750 mL of distilled water yielded 22.13g of extract giving an extract yield of 14.75% w/w. On the other, the same amount of air-dried leaves powder of *A. senegalensis* in 750 mL of 95% ethanol yielded 31.25 g of extract giving an extract yield of 20.83% w/w. The aqueous extract produced is brown while the ethanol extract is black in color. Both extracts are tasteless and bland.

B. EFFECT OF LEAVES EXTRACTS OF *A. SENEGALENSIS* ON WHOLE BLOOD CLOTTING TIME AND BLEEDING TIME OF RABBITS

The result of treatment of rabbits with aqueous and ethanolic leaves extracts of *A. senegalensis* on their clotting time of blood and those of control animals are shown in Table 1. The result indicated that the aqueous leaves extract significantly ($P<0.05$) reduced clotting time of blood of treated rabbits. However, the ethanolic extract had no significant ($P>0.05$) effect on the clotting time of blood of treated subjects when compared to the control rabbits. The bleeding time of wounds of rabbits treated with aqueous and ethanolic leaves extracts of *A. senegalensis* and those of the control group are shown in Table 2. The result revealed that the differences between the mean bleeding time of the aqueous extract treated group and that of the control group is not statistically significant ($P>0.05$). Conversely, the ethanolic extract significantly ($P<0.05$) increased the bleeding time in treated rabbits when compared to that of the control subjects.

It is observed from the findings in this study that treatment of rabbits with oral dosage of aqueous leaves extract of *A senegalensis* at 1000 mg/Kg for 5 days significantly decreased the clotting time of their blood. Although this research did not attempt to explore the exact mechanism by which the aqueous extract decreased blood clotting time in rabbits, a hypothesis could hold that the extract possibly increased the synthesis of blood platelets and consequently decreased clotting time. Another possibility is that as the deficiency of clotting factors (factors I - fibrinogen, VIII - hemophilia A factor and IX - hemophilia B factor) and proteins concerned with hemostasis are known to prolong clotting time (Brar *et al*., 2002), the aqueous extract could have possibly decreased the clotting time by enhancing the hepatic synthesis of these hemostatic factors and / or proteins. Another possibility is that the extract could either contain vitamin K or promote the hepatic synthesis of vitamin K or its dependent clotting factors (factors II, VII, IX and X) all of which are well known to promote hemostasis (Sandhu, 2013).

Rabbit No.	Clotting Time in Seconds (n=8)		
	Group A (Aqueous extract)	Group B (Ethanol extract)	Group C (Control)
1	90	150	210
2	120	180	150
3	90	150	120
4	90	90	120
5	120	150	150
6	120	150	120
7	90	150	210
8	90	180	120
Mean±SD	101.25 ± 15.53^{ab}	150 ± 27.78^{b}	150 ± 39.28^{a}

^{ab} = Difference between means with same superscripts is statistically significant (P<0.05)

Table 1: Whole blood clotting time of rabbits treated with leaves extracts of A. senegalensis

Rabbit No.	Bleeding Time in Seconds (n=8)		
	Group A (Aqueous extract)	Group B (Ethanol extract)	Group C (Control)
1	122	250	130
2	103	147	70
3	87	148	103
4	90	173	71
5	79	178	141
6	132	146	80
7	118	151	132
8	84	142	94
Mean±SD	101.88 ± 19.92^{b}	166.88 ± 36.11^{ab}	102.63 ± 28.63^{a}

^{ab} = Difference between means with same superscripts is statistically significant (P<0.05)

Table 2: Bleeding time of rabbits treated with leaves extracts of A. senegalensis

This study also revealed that the ethanolic extract of *A. senegalensis* significantly increased the bleeding time of blood of the experimental animals. The exact mechanism by which the ethanolic extract increased the bleeding time is not understood as far as this study is concerned, but it could be suggested that the extract probably produced this effect by suppressing the hepatic synthesis of clotting factors and proteins concerned with hemostasis. It could also be that the extract either contain anti-vitamin K substance, or suppress the

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hepatic synthesis of vitamin K or its dependent clotting factors (factors II,VII, IX and X) and anticoagulant proteins C and S because the role of these factors in promoting hemostasis are well established (Sandhu, 2013). Another suggestion is that the extract could have possibly acted by promoting the activities of, or accelerating the synthesis of the coagulation inhibitory factors and consequently suppressing the coagulatory activities of factors II and X and enhancing the neutralization of factors IX, XI, XII, XIII and kallikrein by coagulation inhibitory factors. The role of coagulation inhibitory factors in preventing coagulation is also well established (Sandhu, 2013) and forms the basis for heparin activity as an anticoagulant.

IV. CONCLUSION

It is concluded from the result of this study that oral administration of aqueous leaves extract of *A. senegalensis* to rabbits at a dosage rate of 1000mg/Kg for 5 day significantly decreased blood clotting time but did not affect their whole blood bleeding time, while similar regime of ethanol extract only increased bleeding time. The above finding supported the claim for the traditional use of aqueous extract of the leaves of *A. senegalensis* as a hemostatic agent in Ganye Local Government Area and its environs but not its ethanol counterpart. Therefore, further studies on *A. senegalensis* leaves extracts' mechanism of action, active principle(s) involved and effects on coagulant and anticoagulants pretreated subjects are recommended.

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