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Comparative In Vivo Topical Hemostatic Effect Of Some Medicinal Plants Of Ganye Chiefdom In Adamawa State, Nigeria

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Abstract: ***This study investigated and compared the in vivo topical hemostatic effects of four plant materials (latices of Jatropha curcas and Calotropis procera, stem juice of Musa sapientum and crude leaves juice of Pennisetum pedicellatum) that are topically used against bleeding by traditional healers in Ganye chiefdom in Adamawa State, North-eastern Nigeria. Forty apparently healthy rabbits were used in the experiment. They were randomly divided into 5 groups labeled A, B, C, D and E of 8 rabbits each i.e. for J. curcas, C. procera, M. sapientum, P. pedicellatum and control respectively. The hemostatic effect of the plant materials on experimentally inflicted wounds on rabbits in their respective groups were determined using standard techniques. The result of the study revealed that all the plant materials significantly (P<0.001) reduced bleeding time. The crude leaves extract of P. pedicellatum exhibited the highest efficacy in reducing bleeding time, followed by the stem juice of M. sapientum, then the latices of J. curcas and lastly C. procera in decreasing order. The study validated the basis for the traditional use of these plant materials as hemostatics. Further studies on the hemostatic effect of these plants and on their toxicities were suggested.***

Keywords: Medicinal plants; in vivo hemostasis; topical hemostatic effect; Ganye chiefdom, Nigeria

I. INTRODUCTION

Hemorrhage, the escape of blood from the cardiovascular system to the outside or into body cavity or tissue may lead to shock and death. Three mechanisms operate to reduce blood loss from broken vessels: vascular spasm, platelet plug formation and blood clotting or coagulation [14]. Hemostasis, the cessation of blood flow from damaged blood vessels is a complex process that encompasses blood clotting and involves many clotting factors and Ca^{2+}, enzymes, platelets, and damaged tissues activating each other [10]. Hemostatics are used to arrest or attenuate bleeding and are broadly classified into topical and systemic types [13]. Topical hemostatics are usually applied locally to areas of injury to control local bleeding [16].

Plants and phytochemical products continue to play an important role in medicine [9]. Plants used in traditional medicine provide interesting and still largely unexplored sources for the development of new drugs [5]. Plant medicines are considered to have the advantage still being the most effective, safe and cheaper alternative sources of drugs [7, 2, 15].

In Ganye chiefdom in Adamawa State, North-eastern Nigeria, the latices of *Jatropha curcas* and *Calotropis procera* and the stem juice of *Musa sapientum* and juice obtained from squeezing the leaves of *Pennisetum pedicellatum* are used as traditional remedies for the immediate arrest of bleeding from minor wounds but, the claim for these therapeutic activities have not been scientifically validated. The aim of this study was to investigate the *in vivo* effect of topically applied latices of *J. curcas* and *C. procera* and the stem juice of *M. sapientum* and crude leaves extract of *P. pedicellatum* on hemostasis (bleeding time) in rabbits.

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II. MATERIALS AND METHODS

A. COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Samples of leaves and stems of *C. procera*, *J. curcas*, *M. sapientum* and *P. pedicellatum* were freshly collected from Gangwoki and Sabon Layi in Ganye Local Government Area of Adamawa State, Nigeria and were identified based on standard criteria [1, 8]and confirmed by a taxonomist. Voucher specimens were kept in the Department of Animal Health and Production Technology of Adamawa State College of Agriculture, Ganye.

B. PREPARATION OF PLANT MATERIALS

The latices of the leaves and stems of *C. procera* and *J. curcas* were collected using procedures described earlier [11]. The stem juice of *M. sapientum* was collected based on the methods described by [18] while the fresh leaves of *P. pedicellatum* were cut into small pieces, crushed and ground using a mortar and pestle. 5 mL of distilled water was added to 300g of the ground leaves and macerated. The mixture was then tightly squeezed to yield the juice which was collected into sterile universal bottle. All collected plant materials were used immediately for the experiment.

C. THE EXPERIMENTAL ANIMALS

Forty rabbits of both sexes weighing between 1.2 Kg and 1.8 Kg were used in the experiment. They were randomly divided into 5 groups labeled A, B, C, D and E of 8 rabbits each i.e. for *J. curcas*, *C. procera*, *M. sapientum*, *P. pedicellatum* and control respectively. All animals were handled according to the international guiding principles for biomedical research involving animals [4].

D. DETERMINATION OF BLEEDING TIME

Experimental puncture wounds were inflicted on the ear vein of rabbits using surgical lancet and 2 drops of plant material (stem latices of *J. curcas* and *C. procera* for groups A and B respectively, stem juice of *M. sapientum* for group C and crude leaves juice of *P. pedicellatum* for group D) is placed on the site. 2 drops of distilled water was used for group E (control). The bleeding time were then determined using standard method [3] with slight modification [18].

E. DATA ANALYSIS

Statistical analysis of the data obtained from the study was done using computer software (GraphPad InStat Version 3.10, 32 bit for Windows, by GraphPad Software Inc., USA). The results were expressed as mean and standard deviation (Mean ± SD) and the differences between groups were tested using ANOVA with Tukey-Krammer multiple comparison post test. Difference between means was considered significant at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

The bleeding time of experimental rabbit wounds that were treated with latices of *J. curcas* and *C. procera*, the stem juice of *M. sapientum* and crude leaves extract of *P. pedicellatum* and those of control animals are shown in Table 1.

Rabbit No.	Bleeding Time in Seconds (n=8)				
	Group A	Group B	Group C	Group D	Group E
1	89	84	74	45	130
2	72	71	56	22	98
3	68	82	88	41	103
4	84	98	51	56	91
5	52	78	80	49	141
6	80	62	52	58	100
7	81	86	54	30	132
8	66	84	53	36	114
Mean±SD	$74\pm11.94^{a,b}$	80.63±10.70	63.5 ± 14.77^{c}	$42.13\pm12.48^{b,d}$	$113.63\pm18.55^{a,c,d}$

Table 1: Bleeding Time of Experimental Wounds Treated with Different Plant Materials and that of Control Rabbits

^{a,b,c,d} = Difference between means with same superscripts is statistically significant (P<0.05).

The result indicated that all the plant materials tested for hemostatic activity significantly (P<0.001) decreased mean bleeding time of treated wounds when compared with control. This *in vivo* study revealed that *J. curcas* and *C. procera* latices caused significant decrease in the mean bleeding time of experimental wounds and so did the stem juice of *M. sapientum* and crude leaves extract of *P. pedicellatum.* The mean bleeding time of experimental wounds treated with *P. pedicellatum* crude leaves extract is the lowest and significantly (P<0.05) lower than all the other treatment groups. The result of the study therefore indicated that the crude leaves extract of *P. pedicellatum* exhibit the highest efficacy as a local hemostatic followed by the stem juice of *M. sapientum*, then the latices of *J. curcas* and *C. procera* in decreasing order of efficacy.

In a similar study, *Jatropha gossypifolia* stem latex significantly reduced bleeding time in human subjects [11]. Similar *in vitro* studies on *Jatropha multifida* stem latex resulted in significant reduction in clotting time [6]. The result of bleeding time with regards to *J. curcas* in our study is in agreement with these reports.

Topical hemostatic agents exhibit their effect either through enhancement of coagulant (clotting) factors, or occlusive effect, or vasoconstrictor effect, or by their stuptic and astringent activities and also by enhancement of platelets aggregation [13]. One or a combination of these mechanisms could possibly account for the reduction of bleeding time by these plants. Furthermore, plant latices are substantially applied in traditional systems of medicine to stop bleeding from minor injuries. Proteolytic enzymes present in these latices are known to interfere with the hemostatic and fibrinolytic systems [17]. The latex of *C. procera* contain cysteine protease with both thrombin- and plasmin-like activity acting on fibrinogen and fibrin and consequently on hemostasis [12]. This could also account for the result found in this study.

It is evident from the result of this study that the latices of *J. curcas* and *C. procera*, the stem juice of *M. sapientum* and crude leaves extract of *P. pedicellatum* significantly decreased

bleeding time in experimental wounds in rabbits with *P. pedicellatum* exhibiting the highest efficacy.

IV. CONCLUSION

It is plausible to infer that this study validated the basis for the traditional use of these plants as hemostatics. We recommend further in-depth studies on the hemostatic effects of the plants especially those of *P. pedicellatum* and *M. sapientum* with the view to identifying the active principles and the mechanisms of action involved and the possibility of developing therapeutically viable substances. Toxicity of *J. curcas* and *C. procera* should also be taken into cognizance alongside their therapeutic benefit as both plants are highly toxic.

REFERENCES

[1] Arbonnier, M. (2004). Trees, shrubs and lianas of West African dry zones. Germany: CIRAD, MAGRGRAF Publishers, GMBH, MNHN, Germany
[2] Arifuzzaman, MD, Abdullah, Mannan, A., Sal Binta Imtha, Abedin, J. Sheikh Anwar SM, MD., Zahid Hosen and Barua S. (2011). Evaluation of thrombolytic properties of Nigella sativa, Capsicum frutescens and Brassica oleracea. International Journal of Research Pharmaceutical Sciences, 2(3): 483-487
[3] Brar RS, Sanhu HS, Singh A. (2002). Veterinary Clinical Diagnosis by Laboratory Methods. Kalyani Publishers, India
[4] CIOMS and ICLAS (2012). Council for International Organization of Medical Science and International Council for Laboratory Animal Science. International guiding principles for biomedical research involving animals. c/o World Health Organization 1211, Geneva 27, Switzerland
[5] Cos, P, Vlietinck, AJ, Berghe, DV and Maes, L. (2006) Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J. Ethnopharmacol., 106:290-302.
[6] Dougnon TV, Klotoe JR, Edorh PA, Segbo J, Ategbo JM, Sodipo OA, Dougnon TJ, Dandjesso C, Loko F, Dramane K. (2012). In vitro hemostatic activity screening of sap of Jatropha multifida L. (Euohorbiaceae) used in traditional medicine at Cotonou (Benin). J Phys Pharm Adv 2(6): 227-234
[7] Eisenberg, DM, Davis, RB, Ettner, SL et al. (1998). Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. JAMA, 280: 15691575
[8] FAO (2010). Food and Agriculture Organization. Grassland Index. A searchable catalogue of grass and forage legumes. Retrieved at http://www.fao.org/ag/AGP/AGPC/doc/GBASE/Default.htm on Friday 6th November. 2016
[9] James SA, Bilbiss L and Muhammad BY (2007). Effect of Catharanthus roseus (L) G. Don 1838 aqueous leaf extract on some liver enzymes, serum proteins and vital organs. Science World Journal, 2(1): 5-9
[10] Murray RK, Granner DK, Rodwell VW (2006). Harper's Illustrated Biochemistry. 27th Edition. McGraw Hill Companies, Inc., USA
[11] Oduola T, Adeosun GO, Oduola TA, Avwioro GO, Oyeniyi MA (2005). Mechanism of action of Jatropha gossypifolia stem latex as a haemostic agent. Europian J Gen Med 2(4): 140-143
[12] Ramos MV, Viana CA, Silva AF, Freitas CD, Figueiredo IS, Oliveira RS, et al. (2012). Proteins derived from latex of Calotropis procera maintain coagulation hemostasis in septic mice and exhibit thrombin- and plasmin-like activities. Naunyn Schmiedebergs Arch Pharmacol, 385: 455-463
[13] Sandhu HS (2013). Essentials of Veterinary Pharmacology and Therapeutics. 2nd Edition. Kalyani Publishers, India
[14] Sastry GA and Rao PR (2001). Veterinary Pathology. 27th Edition. CBS Publishers and Distributors Pvt. Ltd., India
[15] Sheik N, Younas N and Akhtar T (2014). Effect of Carica papaya leaf formulation on hematology and serology of normal rat, Biologia (Pakistan) 60(1): 139-142
[16] Turhan N, Bilgili H, Captug O, Kurt M, Shorbagi A, Beyazit Y, Kurt OK, Kosar A, I and Haznedaroglu IC, (2011). Evaluation of hemostatic agent in rabbits, Afr J Tradit Complement Altern Med. 8(1): 51-65
[17] Venkatesha SH, Rajaiah R, Vishwanath BS, (2016). Hemostatic interference of plant latex proteases. SM J Clin Pathol, 1(1): 1002
[18] Weremfo A, Adinortey MB, Pappoe ANM, (2011). Haemostatic effect of stem juice of Musa parasidisiaca L. (Musaceae) in guinea pigs. Adv Biol Res, 5(4): 190-192