

Full Length Research Paper

Haemostatic effect of methanolic leaf extract of *Ageratum conyzoides* in albino rats

O. Bamidele^{1*}, A. M. Akinnuga³, M. M. C. Anyakudo¹, O. A. Ojo¹, G. B. Ojo², J. O. Olorunfemi⁴
and O. P. Johnson⁴

¹Department of Physiology, Bowen University, Iwo, Osun State, Nigeria.

²Department of Anatomy, Bowen University, Iwo, Osun State, Nigeria.

³Department of Nursing Science, Lead City University, Ibadan, Oyo State, Nigeria.

⁴Department of Physiology, Madonna University, Okija, Anambra State, Nigeria.

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Scientific reports on the haemostatic effects of *Ageratum conyzoides*, Asteraceae in experimental animals are relatively scanty. But their uses in wound care in many indigenous tribes around the world have been widely reported. This experiment was therefore designed to scientifically test the possible haemostatic effect of methanolic leaf extract of *A. conyzoides* using albino rat as a model. Twenty albino rats weighing 100 to 170 g were divided into four groups of five rats each. Group I served as control and received 10 ml/kg of vehicle (normal saline) while Group II, III, and IV served as the experimental groups and were given 250, 500 and 750 mg/kg of the methanolic leaf extract of *A. conyzoides* once a day for two weeks. All the doses were administered intraperitoneally. In all groups, the blood samples were obtained by cardiac puncture under chloroform anaesthesia to determine prothrombin time, clotting time and plasma fibrinogen concentration. A skin puncture was made quickly using disposable lancet to determine bleeding time. The results obtained indicate that methanolic leaf extract of *A. conyzoides* significantly decreased ($p < 0.05$) the bleeding time, prothrombin time and clotting time respectively in a dose dependent manner. In contrast, plasma fibrinogen concentration significantly increased ($p < 0.05$). The study suggests that methanolic leaf extract of *A. conyzoides* possesses haemostatic activities and possibly interacts with both intrinsic and extrinsic pathways.

Key words: *Ageratum conyzoides*, bleeding time, prothrombin time, clotting time, plasma fibrinogen concentration, haemostasis, albino rats.

INTRODUCTION

Ageratum conyzoides is an erect herbaceous, annual, 30 to 80 cm tall with stems covered with fine white hairs. Its leaves are opposite, pubescent with long petioles and include glandular trichomes. The inflorescences contain 30 to 50 pink flowers arranged as acorymb and are self-incompatible (Jhansi and Ramamijam, 1987; Kaul and Nee Longini, 1989; Ramamijam and Kalpana, 1992; Kleinschmidt, 1993). Among Yoruba of the South western Nigeria, it is known as Imi esu (Oladejo et al.,

2003), Efik as ikongifoien, and Igbo as akwukwonwosinaka (Iwu, 1993). The plant is commonly found in South eastern North America and Central America, but the centre of origin is in Central America and the Caribbean (Baker, 1965; Lorenzi, 1982; Correa, 1984; Cruz, 1985).

Moreover, *A. conyzoides* is reported to have a long history of medicinal uses in several countries of the world. Traditional communities in India use this specie as a bactericide, anti-dysentery and anti lithic (Borthakur and Baruah, 1987). In Brazil, its aqueous extract has been extensively used to treat colic, cold, fevers, diarrhoea, rheumatism and spasms (Penna, 1992; Jaccoud, 1961; Correa, 1984; Cruz, 1985; Marques et al., 1988; Negrelle

*Corresponding author. E-mail: femi_bayo_is@yahoo.co.uk. Tel: +2348067463528.

et al., 1988; Oliveira et al., 1993). Also, in Cameroon and Congo, its traditional use is to treat fever, rheumatism, headache and colic (Menut et al., 1993; Bioka et al., 1993). In Central Africa it is used to treat pneumonia, but the common use is to cure wounds and burns (Durodola, 1977). Likewise, the plant is used by the Fipa in South Africa as application to fresh wounds (Watts et al., 1962). Oladejo et al. (2003) reported the extract of the plant to have a wound healing enhancing action. Although, there are numerous reports about the use of *A. conyzoides* in treatment of wounds and burns, there is presently scarcity of information on its effects on haemostasis in humans. Therefore, the current work was undertaken to investigate the possible haemostatic effects of the methanolic leaf extract of *A. conyzoides* using albino rats as a model.

MATERIALS AND METHODS

Animal model

Twenty wistar strain albino rats of both sexes weighing between 100 – 170 g were used. The rats were divided into four (4) groups of five (5) animals each. The rats were purchased from the College of Medical Sciences' animal house of the University of Nigeria, Nsukka. The rats were housed in wire mesh cages under standard conditions (temperature, 25 - 29°C, 12 h light and 12 h dark cycle) and allowed to acclimatize for 3 weeks. The rats were fed with standard pellets diet and water given *ad libitum*.

Plant materials

The fresh leaves of *A. conyzoides* were collected in some parts of Port-Harcourt, Nigeria. The botanical identification and authentication was done by the Chief Herbarium Officer of the Department of Biological Sciences, University of Port Harcourt, River State, Nigeria. The leaves were washed in tap water and shade-dried after which they were reduced into fine powder by grinding and soaked for 72 h in methanol (70% v/v, BDH) at room temperature. It was then filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The filtrate was then concentrated using a rotary vacuum evaporator to obtain the solid mass. The solid extract was then re-dissolved in normal saline and stored in capped bottles in a refrigerator at 4°C until required.

Experimental procedure

This study was carried out on 4 groups of rat; each group contained 5 rats and was placed in a different cage for proper identification. The control group was given (10 mg/kg) normal saline while test groups were given different concentrations (250, 500 and 750 mg/kg) of methanolic leaf extract of *A. conyzoides* intraperitoneally (Akah et al., 2010). The treatment regimens lasted for 2 weeks. All procedures involving the use of animals in this study complied to the guiding principles for research involving animals as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals (World Medical Association, 2002).

Group I (Control)

In this group, 5 rats were used. Each rat received 10 mg/kg of

normal saline intraperitoneally for a period of 2 weeks.

Group II (low dose)

Also, 5 rats were used in this group. Each rat received a low dose of methanolic leaf extract of *A. conyzoides* intraperitoneally (250 mg/kg) for a period of 2 weeks.

Group III (medium dose)

There were 5 rats used in this group. Each rat received a medium dose of methanolic leaf extract of *A. conyzoides* intraperitoneally (500 mg/kg) for a period of 2 weeks.

Group IV (high dose)

There were 5 rats used in this group. Each rat received a high dose of methanolic leaf extract of *A. conyzoides* intraperitoneally (750 mg/kg) for a period of 2 weeks. At the end of treatment, the rats were anaesthetised with chloroform and blood samples were collected by cardiac puncture into sample vials containing sodium citrate in ratio 1:9 with the blood with aid of a 5 ml syringe. Only blood samples used for determination of clotting time were collected in anticoagulant-free vials.

Sample analysis

Determination of bleeding time

This was determined using a modified Duke method (Ochei and Kolhatkar, 2000). A skin puncture was made quickly using disposable lancet and the stopwatch was started as soon as bleeding started. The puncture was dabbed with filter paper every 15 s until the paper no longer stained red with blood. Bleeding time was then taken as the time when the blood stopped flowing from the puncture.

Determination of prothrombin time

Blood was collected into sample vials containing 3.2% sodium citrate (as specified in the prothrombin time (PT) test kit used) in the ratio 1:9 with the blood sample. The blood was then centrifuged at 1000 g for 15 min to obtain platelet poor plasma. Thromboplastin PT-S was placed in a water bath at 37°C; and 0.1 ml of test plasma was also put into a test tube and placed in the water bath to pre-warm to 37°C. A 0.2 ml of warmed thromboplastin PT-S was then forcibly added to the test plasma and the stopwatch was started. The tube was tilted repeatedly until a clot was formed and the time taken for clot to form was noted. This was repeated for all the blood samples (five in each group). Precaution was taken to perform test within 3 h of blood collection since the labile factor deteriorates quickly at room temperature.

Determination of clotting time

Blood was taken directly from the heart to avoid contamination with tissue thromboplastin (0.8 ml from each rat). A 0.2 ml of blood was then delivered into four glass test tubes that had previously been warmed and maintained at 37°C and the tubes immediately placed in a 37°C water bath to mimic the temperature of the internal environment. The stopwatch was started immediately the blood was delivered into the glass test tubes and the tubes were continually

Table 1. Haemostatic effects of methanolic leaf extract of *Ageratum conyzoides* in albino rats.

Haemostatic indices	Group I (Control)	Group II (250 mg/kg)	Group III (500 mg/kg)	Group IV (750 mg/kg)
Bleeding time (s)	121.0 ± 13.0	88.6 ± 21.3	81.8 ± 13.2*	60.6 ± 8.9*
Prothrombin time (s)	39.8 ± 6.9	22.0 ± 3.5*	21.2 ± 2.2*	18.0 ± 2.8*
Clotting time (s)	328.5 ± 10.2	88.3 ± 18.9*	72.5 ± 10.2*	33.4 ± 6.7*
Plasma fibrinogen concentration (mg/dl)	247.0 ± 10.4	266.2 ± 10.4*	273.2 ± 12.9*	291.8 ± 7.0*

Values are expressed as mean ± S.E.M. *p < 0.05, (n=6).

tilted at 40 s intervals (until blood in them stopped flowing when tilted at an angle of 90°), starting with the first, to see and note the time when the blood clotted. The clotting time was taken as the average of the times blood clotted in the four tubes.

Determination of fibrinogen concentration

Plasma fibrinogen concentration was determined as defined by the clot weight method of Ingram (1961) though with modifications to accommodate the procedure for use of thrombin time (TT) test kit as given by the manufacturers of the kit. Blood was collected with the aid of plastic disposable syringes into sample vials containing 3.2% sodium citrate in the ratio 1:9 with blood. Blood plasma was obtained by centrifuging blood in a stopped vial at 1000 g for 10 min. 0.2 ml of the test plasma was put into a test tube and incubated in a water bath for 3 min at 37°C. 0.2 ml of thrombin time-reagent was added to test plasma, mixed and the clot formed harvested with a wooden applicator stick. The resulting clot was transferred into a tube containing acetone to dry and harden for about 10 min; the acetone was decanted and the clot placed on a filter paper for the remaining acetone to evaporate. The clot was then recovered and weighed. The process of fibrinogen concentration determination was completed within 3 h of blood collection. Thus, fibrinogen concentration of citrated plasma in mg/dl equals clot weight (mg) divided by plasma volume (dl).

Statistical analysis

All data were presented as mean ± SEM. The one way ANOVA was used to analyze the data, followed by a post-hoc test (LSD). The results were considered significant at p values of less than 0.05.

RESULT

Bleeding time

The extract decreased bleeding time in rats of the experimental group in comparison with the control group. The mean bleeding time in control group was 121.0 ± 13.0 s while those of group II, III and IV were 88.6 ± 9.5, 81.8 ± 13.2 and 60.0 ± 8.9 s respectively. The analysis showed that the decrease was not significant in group II at p > 0.05 but significant in group III and IV when compared independently with the control. The effect was most significant in the group administered the highest dose of the extract, group IV.

Prothrombin time

The mean prothrombin time in the control group was 39.8 ± 6.9 s while those of group II, III and IV were 22.0 ± 3.5, 21.2 ± 2.2 and 18.0 ± 2.8 s respectively as shown in Table 1. There was significant decrease (p < 0.05) of prothrombin time in the experimental groups compared with the control group. The decrease was dose dependent with most significant decrease in group IV.

Clotting time

The mean clotting time in the control group was 328.5 ± 18.9 s while those of group II, III and IV were 88.3 ± 18.9, 72.5 ± 10.2 and 33.4 ± 6.7 s respectively as shown in Table 1. There was also significant decrease (p < 0.05) of clotting time in the experimental groups compared with the control group. The decrease was also dose dependent.

Plasma fibrinogen concentration

The mean plasma fibrinogen concentration in the control group was 247.0 ± 10.4mg/dl while those of group II, III and IV were 266.2 ± 10.4, 273.2 ± 12.9 and 291.8 ± 7.0 mg/dl respectively as shown in Table 1. There was significant increase (p < 0.05) of plasma fibrinogen concentration in the experimental groups compared with the control group. The increase was dose dependent.

DISCUSSION

This study was carried out to evaluate the potentials of *A. conyzoides* on the haemostatic mechanism, with primary interest on how it affects bleeding time, prothrombin time, clotting time and plasma fibrinogen concentration. The methanolic leaf extract of *A. conyzoides* exhibited haemostatic activities by decreasing bleeding, prothrombin and clotting times and also by increasing plasma fibrinogen concentration. These indices are measure of blood coagulation. While clotting time

measure the intrinsic pathway, the prothrombin time measures the extrinsic pathway of blood coagulation. Fibrinogen concentration is critical to the formation of stable fibrin clot (Ochei and Kolhatkar, 2000). The reported decrease in bleeding time in this study is consistent with the findings of Akah et al. (1988) when aqueous leaf extract of the plant was used.

The results showed significant decrease in prothrombin time. Since prothrombin is a screening test for the extrinsic clotting system, that is, factor VII and can also detect deficiencies of factor V, X, prothrombin and fibrinogen (Monica, 2000), it follows then that the decrease in prothrombin time by the extract may be as a result of increase in the concentration of prothrombin or one of the other extrinsic clotting factors. These results agree with the reported findings of Essien et al. (1985). They reported decrease in (PT) and partial thromboplastin time (PTT) by *Fagara xanthoxyloides*, but contrasted with the observed effect of *Wobenzyme darages*, which increased PT in albino rats as reported by Korpin et al. (1997).

Clotting time test is a qualitative measurement of factors involved in the intrinsic pathway (Ochei and Kolhatkar, 2000; Dapper et al., 2007). Therefore, deficiency in the factors of the intrinsic pathway (I, II, V, VIII, IX, X, XI, XII) will affect the result. From the results obtained, there was significant decrease in clotting time, reflecting that there was an increase in one or more of the clotting factors involved in the intrinsic pathway. These results correlate with the report by Okoli et al. (2007) on the haemostatic activities of the leaf extract of *Aspilia Africana* which arrested bleeding from fresh wounds by reducing both bleeding and clotting times.

Plasma fibrinogen concentration was increased significantly by methanolic leaf extract of *A. conyzoides*. Fibrinogen is synthesized in the liver and its synthesis is not dependent on the presence of vitamin K. Thrombin acts upon fibrinogen to remove four low-molecular weight peptides from each molecule, forming a molecule of fibrin monomer which can polymerize with other fibrin monomer molecules, thus forming fibrin (Guyton and Hall, 2004). Therefore, increase in fibrinogen concentration thus facilitates the rate of fibrin polymer formation which ultimately leads to more effective clot formation.

Composition of *A. conyzoides* includes flavonoids, alkaloids, essential oils and tannins, many of which are biologically active (Oladejo et al., 2003). Tannins have been implicated in the haemostatic activity of plants where they arrest bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs (Okoli et al., 2007). Also, *A. conyzoides* plays an essential role in the synthesis of vitamin K by healing gastrointestinal disorders (Penna, 1921; Jaccoud, 1961; Correa, 1984; Cruz, 1985; Marques et al., 1988; Negrelle et al., 1988; Oliveira et al., 1993; Menut et al., 1993; Bioka et al., 1993). Vitamin K formed contributes to normal formation of prothrombin as well as a few other clotting factors which are responsible for the positive haemostatic effect.

In conclusion, methanolic leaf extract of *A. conyzoides* effectively decreased bleeding, prothrombin, and clotting times and increased plasma fibrinogen concentration. Thus, indicating a positive haemostatic effect. This further provides a rationale for the use of the leaves of *A. conyzoides* in the wound management in traditional medical practice.

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