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Haemostatic Effects of Ethanolic Extracts of *Amaranthus hybridus* on Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author BCC designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Author FSAT managed the experimental design and all ethical considerations of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Haemostasis refers to the arrest of bleeding due to vascular damage and involves the intrinsic and extrinsic coagulation pathways which converge at the point of fibrin activation to stop or minimize blood loss. *Amaranthus hybridus* contains a while range of nutritional, chemical and phytochemical constituents which gives it wide range of applications in folk medicine.
Aim: To evaluate the effects of ethanolic extracts of *Amaranthus hybridus* on blood platelet count, prothrombin time (PT) and activated partial thromboplastin time (APTT) using Wistar rat models.
Methodology: Twenty Four (24) adult male Wistar rats were used for the study. The animals were randomly divided into three (3) groups of eight (8) animals each. Oral administration of distilled water for the control group and ethanolic extracts of *Amaranthus hybridus* at 30 and 60 mg/kg lasted for twenty eight (28) days. Platelet count, prothrombin time (PT) and activated partial thromboplastin time (APTT) were determined using standard laboratory methods.
Results: Ethanolic extracts of *Amaranthus hybridus* significantly increased platelet count at 30 mg and 60 mg/kg compared to the control animals ($p<0.05$). Also, it significantly reduced prothrombin time and activated partial thromboplastin time at 30 and 60 mg/kg in a dose dependent manner compared to control animals ($P<0.05$).

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Conclusion: The study shows that ethanolic extract of *Amaranthus hybridus* may have enhanced haemostasis as demonstrated by increased platelet count and reduced prothrombin (PT) and activated partial thromboplastin time (APTT) time.

Keywords: Amaranthus hybridus; haemostasis; platelets; prothrombin time; activated partial thromboplastin time.

1. INTRODUCTION

Haemostasis is the stoppage or arrest of bleeding in response to a damaged blood vessel [1]. It involves a cascade of physiological events which culminates in in the production of a localized clot at the site of vessel injury over a very short period of time, usually seconds to minutes [2,3]. A damaged blood vessel produces a reflex vasoconstriction which slows the loss of blood and thus allows more time for the formation of platelet plug and the initiation of blood coagulation [4]. The process of haemostasis include the formation of a platelet plug followed by an activation of coagulation to form a fibrin mesh, fibrinolysis and the eventual repair of blood vessel [2]. Hence, haemostasis is largely dependent on the platelet count and function as well as on other coagulation parameters and function to achieve a steady balance between the fluid and solid states of blood to prevent pathological thrombosis [3,5]. A hemostatic cascade involves the intrinsic and extrinsic pathways which originate differently but converge at the point of fibrin activation and serves to stabilize the platelet plug [6]. While the intrinsic pathway is activated by trauma within the vascular system, the extrinsic pathway is activated by trauma outside the vascular system [1-6]. Prothrombin time (PT) and activated partial thromboplastin time (APTT) are some of the screening test to assess the process of haemostasis *in vivo*. Prothrombin time is a measure of the time taken for clot formation when thromboplastin (TF) and calcium are added to plasma that is short of platelets [1,7]. It is measure of the extrinsic pathway and hence inhibitors or deficiencies of clotting factors within the extrinsic and final common pathways result in prolongation of the PT [7]. On the other hand, activated partial thromboplastin time (APTT) represents a measure of the time taken for cloth to be formed when phospholipid (partial thromboplastin) and calcium are added to plasma that is short of platelets. It is a measure of the intrinsic system and hence deficiencies of clothing factors and final common pathways will result in a prolongation of APTT [7].

Amaranthus hybridus, commonly called amaranth or pigweed is one of the most harvested and consumed leafy vegetable in Africa [8,9]. It is mostly found in rural areas where they grow as weed in cultivated fields and barnyards and hence the name, “pigweed”. It is an annual monoecious herbaceous plant from the family of *Amaranthaceae* and is characterized by dark long, dull green leaves with hairy and wavy margins. It has abundant small red/green flowers with lenticellular seeds [8,10]. It is generally consumed as a vegetable or grain in many parts of the world. As a grain crop, it is part of the so-called pseudocereals and has been known to possess more nutritional benefits than some cereals [11]. Their leaves are used in making infusions, soups and salads in conjunction with other vegetables. In Nigeria, they are consumed alone or with other leafy vegetables as soup or other delicacies [12]. They are rich nutrients such as protein, carbohydrate potassium, dietary fiber and essential minerals like magnesium, calcium, potassium and iron. They also contain amino acids and vitamins A, B_1, B_2, B_6, C and E [8,13-15] as well as significant quantities of anti-oxidants like phenols, β-carotene, lycopene and anthocyanin, flavonoids and saponins [16-18]. As most indigenous leafy vegetables are known possess medicinal attributes, such is the case of *Amaranthus hybridus* which are noted to be of great value in the folk treatment for boils, burns and indigestion, intestinal bleeding, diarrhea and excessive menstruation as well the treatment of gonorrhea, colic, and eczema [10,15,19]. Research reports have demonstrated and documented its hematopoietic potential [20,21], immuno-modulatory activity [22], antioxidant and hepatoprotective potential [17,23], anti-nociceptive activity [24], antimicrobial activities [25-27] and anti-carcinogenic potentials [23,28]. A recent study [21] suggested that *Amaranthus hybridus* could have some effect on blood coagulation, no study has demonstrated its possible effect on hemostasis. The aim of the present study therefore, is to evaluate the effect ethanolic extracts of *Amaranthus hybridus* on platelet count, prothrombin time and activated

partial thromboplastin time using Wistar rat models.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extract

Fresh leaves of *Amaranthus hybridus* were procured from the popular Choba market, Port Harcourt, Rivers State and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt. The leaves were thoroughly sorted to remove any contaminants and air dried under normal ambient temperature for a period of two (2) weeks. A coarse dry powder was obtained from the dried leaves and used for ethanolic extraction and phytochemical screening according to methods described by Odebiyi and Sofowora [29].

2.2 Research Animals and Experimental Design

Twenty four (24) male Wistar rats, bred at the Animal House of the Department of Human Physiology, University of Port Harcourt were used for the study. The animals were kept under standard environmental conditions at temperature of 27-29°C and 12hour light/dark cycle. The animals were allowed a period of two (2) weeks of acclimatization, during which they had free access to feed and water. The animals were randomly divided into three (3) groups of eight (8) animals each. The control group (group 1) received distilled water while the test groups (groups 2 and 3) received 30 and 60mg/kg of ethanolic extract of *Amaranthus hybridus* respectively. The oral administration of the extracts of *A. hybridus* and distilled water lasted for twenty eight (28) days. All experiments were examined and approved by the appropriate ethics committee.

2.3 Blood Sample Collection and Laboratory Analysis

After the period of the experiment, the animals were anaesthetized using cervical dislocation and blood was collected via cardiac puncture into an EDTA sample bottle for platelet count and 0.25ml tri-sodium citrate anticoagulant sample bottle for the determination of prothrombin time (PT) and activated partial thromboplastin time (APTT). The blood samples were thoroughly mixed together by gentle inversion. Platelet count was determined using an automated Haematological analyzer (Jinan Kinghawk Technology Co. Ltd., China) while PT and APTT were determined using standard laboratory test kits (Agappe Diagnostics Ltd., India) within one (1) hour of sample collection.

2.4 Determination of Prothrombin Time

The PT reagent was pre-warmed at 37°C for ten (10) minutes and 100µL of plasma pipetted into a test cuvette at 37°C and incubated for three (3) minutes. After that, 200µL pre-warmed PT reagent was forcibly added into the test cuvette and the timer was simultaneously started. The time taken for the blood sample to cloth was noted and recorded in seconds.

2.5 Determination of Activated Partial Thromboplastin Time

Reagent 1 (CaCl2) and Reagent 2 (APTT Reagent) were pre-warmed for ten (10) minute at 37°C after which 100µL of test plasma was pipetted in to a test cuvette and incubated for three (3) minutes at 37°C. Also, 100µL of APTT reagent was pipetted into the test cuvette, gently mixed and incubated at 37°C for another three minutes. Finally, 100µL of pre-warmed Reagent1 (CaCl2) was pipetted into the test cuvette and the timer started simultaneously. The time taken by the sample to cloth is noted and recorded in seconds.

2.6 Statistical Analysis

Statistical package for the social sciences (IBM SPSS V.20) was used to analyze the data. The mean and standard error of mean were calculated. Also, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post-hoc analysis were used to determine the difference in means among all animal groups. The mean of the groups were considered significant at $P<0.05$.

3. RESULTS AND DISCUSION

3.1 Results

Fig. 1. shows the mean values for platelet count of Wistar rats after a twenty eight (28) days oral administration of ethanolic extracts of *A. Hybridus* at 30 and 60 mg/kg. The results indicate that there was a significant increase in the mean values of platelets in the test groups

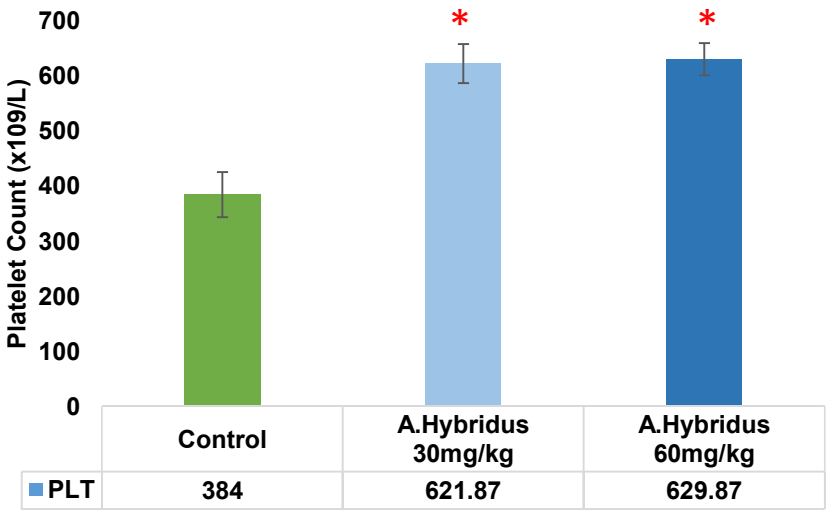

Fig. 1. The effects of ethanolic extracts of *Amaranthus hybridus* on the platelet count of Wistar rats

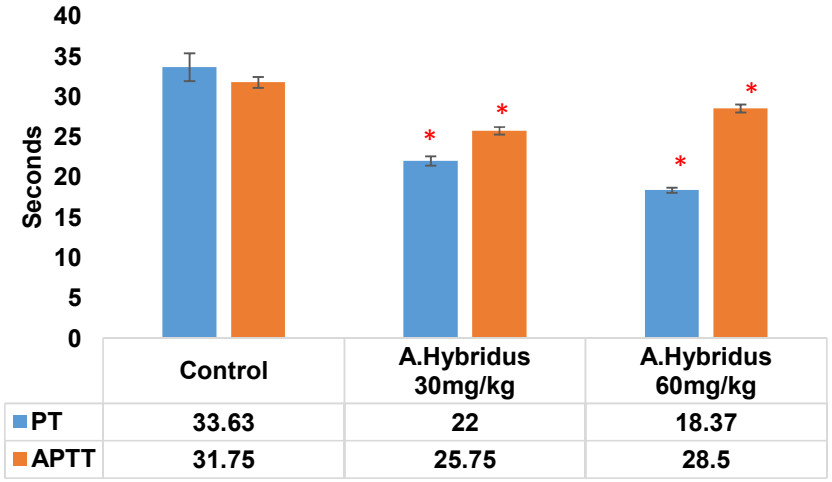

Fig. 2. Effects of ethanolic extracts of *Amaranthus hybridus* prothrombin time and activated partial thromboplastin time of Wistar rats

(groups 1&2) compared to control ($p<0.05$). However, there was no significant difference in the mean values of the test groups ($p>0.05$).

Fig. 2 also show the mean values for prothrombin time and activated partial thromboplastin time of Wistar rats. There was a significant decrease in the mean values of prothrombin time and activated partial thromboplastin time among the test groups compared to the control animals ($p<0.05$). The difference in the mean values of prothrombin time and activated partial thromboplastin was dose dependent as such that there was a significant difference between the test groups ($p<0.05$).

4. DISCUSSION

The process of haemostasis depends to a large extent on platelet count as well as on coagulation parameters via intrinsic and extrinsic pathways. The present study evaluated the effects of ethanolic leaf extracts of *Amaranthus hybridus* on platelet count, prothrombin time and activated partial prothrombin time of Wistar rats.

The study shows a significant increase in the platelet count among the rats that received

ethanolic extract of *A. Hybridus* compared to the control animals ($p<0.05$) with the two doses of the extract (30 and 60mg/kg) causing a 35.5 and 29.2% increase in platelet counts respectively. Platelets (thrombocytes) are non-nucleated cell fragments that are found circulating in the blood where they primarily function to arrest bleeding by adhering to an injured vessel, forming aggregates which eventually stop blood loss [1, 3]. Besides their role in haemostasis, platelets also play pivotal roles in pathophysiological processes like inflammation, wound healing, immunity and tumor and growth metastasis [3, 30,31]. The higher platelet count observed could be attributed the chemical and nutrient composition of *A. Hybridus* which has been shown to play a part in immuno-modulation [22] and the ability to enhance haematopoiesis [20, 21] leading to the formation of red blood cells, white blood cells and platelets [32]. Also, vitamins A and E as contained in *A. Hybridus* have been shown to specifically enhance thrombopoiesis [33-35]. *A. hybridus* contains antioxidants such as Vitamins C and β-carotene which scavenge free radicals, increase protease activity which in turn enhance proplatelets formation [35,36]. Similarly, studies [17,18] have demonstrated that *A. hybridus* is rich in alkaloids and tannins which have the ability to enhance platelet production, reduced platelet destruction and subsequently enhanced their microcirculation [35-37]. Other leaf extracts that have been shown to enhance platelet formation include that of *Carica papaya* [38,39], *Psidium guajava* [40,41], *Ipomoea batatas* [42,43], *Ocimum gratissimum* [44].

This study also showed that prothrombin time was reduced in the test groups compared to control ($p<0.05$) in a dose dependent manner with 34 and 45% decrease observed for 30 and 60 mg/kg test groups respectively. The PT is used to monitor the function of the extrinsic system and common pathway and the level of factor VII which is a vitamin K dependent factor. Since factor VII is synthesized in the liver, the PT can assess the synthetic capacity of the liver as well as the level of vitamin K and other extrinsic factors (FII [prothrombin], FVII, FV, FX and fibrinogen) [1,45]. This may be connected to the antioxidant and hepatoprotective activity [17,28] of *A. hybridus* by possibly protecting the liver and enhancing its production of factor VII and other extrinsic factors that promotes blood coagulation. Extracts of *Ocimum gratissimum* [46], *Ageratum conyzoides* [47], *Typha latifolia* [48] and *Lamiophlomis rotate* [49] have also be shown to reduce PT.

Similarly, the activated partial prothrombin time was reduced in the test groups compared to the control ($p<0.05$) in a dose dependent manner with 18.9 and 10.2% decrease observed for 30 and 60 mg/kg test groups respectively. Unlike PT, the lower dosage of *A. Hybridus* showed greater reduction in APTT. The APTT specifically measures the activity of the intrinsic system and common pathway of the coagulation system and as such will be affected by abnormalities factors XII, XI, VIII, IX as well as the common pathway factors I, II, V and X [1,45]. The APTT is frequently used as a screening test for underlying hemostatic abnormalities like factor XII deficiency or presence of coagulation specific anti-coagulants [1,50]. Reduced APTT have been associated with increased platelet counts, procoagulant tendency [51] and hypercoagulability [52]. The extracts of *A. hybridus* may have via unknown mechanism enhanced the intrinsic pathway leading to reduction in APTT. This may be related to its action on the liver to enhance the production of intrinsic clothing factors. Just like PT, extract of *Ocimum gratissimum* [46], *Ageratum conyzoides* [47] and *Typha latifolia* [48] have been shown to reduce APTT.

5. CONCLUSION

The present study has demonstrated that oral administration of ethanolic leaf extract of *A. hybridus* increased the number of platelets and enhanced both intrinsic and extrinsic coagulation pathways as observed by the reduction in PT and APTT. However, this study is deemed preliminary as more studies are needed to isolate and identify biologically active components of *A. hybridus* which may be responsible for these observed effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments were examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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