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Effect Of Methanolic Seed Extract Of *Persea Americana* (Avocado Pear) On Prothrombin Time And Activated Partial Thromboplastin Time In Mice.

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ABSTRACT

Twenty (20) adult albino mice were used in the study to determine the effect of methanolic seed extract of *Persea americana* on prothrombin time (PT) and activated partial thromboplastin time (APTT) test. The mice were obtained and kept for 2 weeks to acclimatize. They were weighed and divided into 5 groups. Group A served as control without the extract. Groups B to E were orally administered with graded doses of 200mg, 400 mg, 800 mg and 1600mg/kg body weight per mice daily for 28 days. Blood samples were collected through the median canthus into ti-sodium citrate anticoagulant containers for the analysis of PT and APTT, using standard operative procedure. The analysis was carried out at the Haematology Laboratory of University of Nigeria Teaching Hospital (UNTH) Enugu. The results showed a prolonged APTT time at all the doses of the extract when compared with the control ($P<0.05$). The prothrombin time at the dosage of 200mg/kg did not differ when compared with the control ($P>0.05$). The increase in PT and APTT was dose dependent. This result pattern suggests that the extract causes prolonged prothrombin time and APTT at various concentrations possibly due to its high potassium content. The extract can be recommended in anticoagulant therapy since it prolongs PT and APTT.

Key words: *Persea americana*, effect, PT, APTT, coagulation, mice.

INTRODUCTION

Herbal medicine has become an important component of health care delivery system in many countries around the world (Kolawole et al. 2014) and there has been tremendous improvement in healthcare with the combination of phyto-medicine and orthodox medicine in most part of the world, especially in Africa, Asia, etc. (Baum, 2007). About 80% of world population presently depends on herbal remedies for some aspects of primary health care (Yasir et al. 2010). This is because medicinal plants are mostly rich sources of compounds that possess therapeutic effects, in addition to the affordability and easy accessibility of Herbal remedies, especially by people in developing countries (Kolawole et al. 2014).

Persea americana (avocado pear) is widely found in America, Africa and the tropics. The leaf is simple, finely toothed, glossy and green in colour. The fruit has a bell shape with colour mostly green or brown (Oyeyemi and Oyeyemi, 2015). The flesh of the fruit is white and juicy with a hard seed inside. The plant (widely known as Alligator pear) is native to Mexico but is also found in many tropical countries (Oyeyemi and Oyeyemi, 2015). The plant has several species and its tree can grow up to 20m high. Apart from the nutritional value of *Persea americana*, extracts from the leaf and seed of the plant have been found to be of good medicinal value (Ojewole et al. 2007, Owolabi et al. 2005).

The seed and leaf extracts are traditionally used to treat hypertension (Ozoula et al. 2009). The therapeutic value of *P. americana* in some disease conditions has been scientifically validated. The seed extract has been reported to lower blood pressure in normotensive and hypertensive rat models (Imafidon and Amaechina, 2010; Arukwe et al.

2012). Other morphorlogical parts of the plant have been demonstrated to possess medicinal properties such as anti-inflammatory, analgesic and antioxidant properties (Ojewole and Amabeoku, 2006). Phytochemical analysis of *P. americana* leaves revealed the presence of flavonoids, saponins, alkaloids, tannins, reducing sugars (Adeyemi et al. 2002; Owolabi et al. 2010) and mineral compounds (Arukwe et al. 2012). It has been discovered that extracts of the leaf and seed of *Persea americana* reduce fever, heart diseases, obesity, cancer risks and helps in wound healing (Anderson, 1990).

Coagulation is a complex process by which blood forms clots. Coagulation is highly conserved throughout biology and in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component (Astrup and Kjeldsen, 1973). The coagulation cascade of secondary heamostasis has two pathways' which lead to fibrin formation (Ambrus and Mink, 1964). These are the contact activation pathway (formerly known as the intrinsic pathway), and the tissue factor pathway (formerly known as the extrinsic pathway) (Ambrus and Mink, 1964) (Astrup and Kjeldsen, 1973).

In spite of its numerous therapeutic potentials, coagulation studies on the plant parts of *Persea americana* is scanty. Such studies are essential to rule-out possibility of prolonged clotting and excessive bleeding in the event of injury, especially with increased consumption of the plant parts. In this study, the effect of increasing doses of *P. americana* methanolic fruit extracts on the extrinsic and intrinsic pathways of blood coagulation (Hemostasis) in mice was assessed, by estimation of the PT and APTT respectively.

MATERIAL AND METHOD

Experimental Animals

Twenty (20) mice, procured from the Department of Veterinary Medicine, University of Nigeria Nsukka were used for the study. The mice were kept in clean steel cages ate the temperature of 25± 2 °C and fed with super starter feed and water. They were acclimatized for 2 weeks before the commencement of the study.

Preparation of the Plant Extract.

The fruits of *Persea americana* were obtained from the local market (Ogbete Main Market, Enugu) and the seeds were extracted and allowed to shade dry and grounded into fine powder and weighed. About 500mg were dissolved in 98% methanol into a 5 liter container and was well corked. The mixture was agitated at intervals for 48 hours to ensure that it became a complete homogenous mixture.

The extract was filtered and the filtrate was allowed to evaporate to dryness at room temperature and the residue was weighed. Exactly 10 grams of the residue was mixed with 3% tween 80 solution to get the crude extract that was used for the experiment. Graded doses (200mg, 400mg, 800mg and 1600mg/kg body weight) of the extract were administered to experimental groups B-E respectively, whereas group A served as control receiving only 3% tween 80 solution. The extracts were administered orally for 28days, after which blood samples were collected.

Sample Collection and Processing.

Two (2) mls of venous blood was collected from the animals via the medial canthus of the eye with the aid of heparinized capillary tube, and delivered into a sample container containing 0.1ml of trisodium citrate anticoagulant. The contents were well-mixed and centrifuged at 1500rpm for 10 mins and the plasma was immediately removed and transferred into another glass tube and kept in plastic racks at room temperature for PT and APTT tests. Plasma from apparently-healthy individual was used as control.

Analytical Methods:

Determination of Activated Partial Thrombin Time (APTT) was done using the Hemoscann® Test Kit, manufactured by Quimica Clinica Aplicada S.A (QCA). Prothrombin time was determined using tissue thromboplastin and calcium chloride (Ochei and Kolhatkar, 2004).

The data from the study was analyzed with SPSS computer soft ware, version 20.0 and the difference between individual means were analyzed using students t-test and $P<0.05$ considered significant.

RESULT

Table 1 shows the mean ± SD of prothrombin time (PT) and activated partial thromboplastin time (APTT) values in the treated and control mice. The table shows a non-significant increase (P>0.05) in PT in the mice treated with 200mg/kg compared with the control, whereas significant increase (P<0.05)was observed in the remaining groups, ranging from 22.0 ± 0.4 seconds in mice treated with 400mg/kg to 23.1± 0.6 seconds in mice treated with 1600mg/kg, compared to the control. APTT was also significantly increased in a dose-dependent fashion, from 62.4 ± 2.1 seconds in mice treated with (200mg/kg) to 82.3± 1.7 seconds in mice treated with 1600mg/kg (P<0.05).

DISCUSSION

Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel). Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor (Furie and Furie, 2005). Platelets immediately form a plug at the site of injury; this is called primary heamostasis. Secondary heamostasis occurs simultaneously: proteins in the blood plasma, called coagulation factors or clotting factors, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Furie and Furie, 2005). Haemostatic dysfunction, however, arises from any alteration of this complex system, leading to pathologic thrombosis or vascular occlusion by thrombus fragments (Soronnadi and Neboh, 2014).

The World Health Organization (WHO) Alma Ata (1978) declaration in primary Health care indeed paved way for the official recognition of traditional medicine as a source of primary healthcare (Arukwe *et al*., 2012).

The present study examined the effect of methanolic seed extract of *Persea americana* on PT (extrinsic pathway) and APTT (intrinsic pathway) in mice. The result showed that at the dosage of 200mg/kg of the extract, the APTT was prolonged significantly when compared to the control (P<0.05) whereas PT did not show significant difference (P>0.05). Both PT and APTT were significantly prolonged (P<0.05) at the dosage of 400mg/kg, 800mg/kg and 1600mg/kg compared with the controls. The changes in PT and APTT resulting from the administration of graded doses of the extract may be due to the physicochemical constituents of the extract which includes potassium. The seed of *P. americana* has been reported to have high potassium content (100.83 ± 5.64 mg/100g) (Arukwe et al. 2012).

The prolonged PT and APTT in the present study may also be attributed to the calcium content of the plant seed compared to the other plant parts. The seeds of the plant have been shown to have the least calcium content compared to the other parts (Arukwe et al. 2012). Arukwe et al. (2012) reported the calcium compositions of *P. americana* leaf, fruit and seed as 56.13 ± 3.31 mg/100g, 210.08 ± 0.17 mg/100g, and 14.15± 3.01 mg/100g respectively.

Calcium has been shown to be crucial for effective blood coagulation and is required by both the intrinsic and extrinsic pathways. Zinc which plays a part in wound healing was

TABLE 1:
The Coagulation Parameters in the Control Mice and those Treated with graded Doses of the Extract.

Parameters	Control (A)	Test (B) (200mg/kg)	Test (C) (400mg/kg)	Test (D) (800mg/kg)	Test (E) (1600mg/kg)	P -value
PT (secs)	16.8 ± 0.5	19.4 ± 0.5	22.0 ± 0.4 *	22.5± 0.2*	23.1 ± 0.6*	P<0.05
APTT (secs)	62.4 ± 2.1	71.6 ± 1.3*	77.0 ± 4.0*	79.8 ± 1.7*	82.3 ± 1.7*	P<0.05

* = Statistically significant compared to control.

also shown to be lowest in the seeds of *P. americana* (0.09 ± 0.01 mg/100g) compared to the leaf and fruit (7.21 ± 2.62 mg/100g and 0.64 ± 0.03mg/100g) respectively (Arukwe *et al*., 2012).

The study by Oyeyemi and Oyeyemi (2015) reported that prolong administration of aqueous extracts of the seed and leaf of *Persea americana* might cause inflammation or damage of the liver cells. This may also affect the availability of these essential elements required for effective coagulation since the liver has been known to be a central organ involved in intermediary metabolism. The seed of *P. americana* contains 13.6% tannin, 13.25% starch, and the dried seed contains 1.33% of yellow wax containing sterol and organic acid. The seed oil contains capric acid 0.6%, myristic 1.7%, palmitic acid 23.4%, stearic acid 8.7%, oleic acid 15.1%, linoleic acid 24.1%, and linolenic acid 2.5% (Imafidon and Okunrobo, 2009). In addition, the seed extract may also possibly contain sufficient vitamin C, which may have led to the observed effect. A previous study has reported that vitamin C administration effectively reverses coagulation disorders caused by long term smoking (Sorronnadi et al, 2013).

Haemostatic dysfunction can result in increased risk of haemorrhage or thrombosis (Ariens et al. 1999; Soronnadi and Neboh, 2014). Whereas the result of the study has shown that the extract can be applied as coagulation therapy, adequate care should be taken in administration of the extracts in very high doses in treatment of other conditions, to avoid haemorrhage which may result from excessive prolonged PT and APTT.

CONCLUSION

The study shows that administration of methanolic seed extract of *P. americana* significantly affected the intrinsic pathway (APTT) more than the extrinsic pathway (PT) in treated mice. Although both pathways were affected, the extrinsic pathway (PT) was however significantly affected at higher doses of the extract whereas all the doses administered caused a significant change in the intrinsic pathway (APTT) compared to the control mice.

P. americana methanolic seed extract can be useful in anticoagulant therapy in treatment of coagulation disorders. However, consumption in treatment of other conditions should be properly monitored to avoid doses capable of causing prolonged coagulation.

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