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DRUG DISCOVERY

Haemostatic activities of *Jatropha curcas* (Linn) in rats

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ABSTRACTS

Backgroud: Hypocoagulable state and, or haemophilia can cause death due to excessive blood loss from minor trauma, post partum haemorrhage, motor traffic accidents or surgical incision. *Jatropha curcas* is a medicinal plant used by traditional medicine healers in treatment of fresh wound/cuts, post partum heamorrhage, bleeding from umbilical stump and as a purgative.

Aim: To carry out some in-vivo and in-vitro haemostatic studies on *Jatropha curcas* in rats and to scientifically demonstrate its possible coagulant activity.

Methods: The leaves of *Jatropha curcas* were harvested, air dried, ground and macerated in equal volumes of water and ethanol for 24hours. Sieved separately, filtered using Whatman filter paper and evaporated to dryness. The phytochemistry and acute toxicity testing were done. Adult white Wistar rats of average weight of 110grams were divided into cages of 5 animals per cage and housed in a standard animal house for one week acclimatization. Extracts of *Jatropha curcas* were orally administered daily for four weeks. Tail bleeding was used for bleeding time while whole blood clotting time, platelet count, prothrombin and activated partial thromboplastin time were done on whole blood collected through orbital sinus. All samples where necessary, were sent to the Laboratory as soon as collected. Data collected were statistically analysed with SPSS version 16 and P.Values ≤ 0.5 were considered as significant.

Results: Acute toxicity for both extracts was 28.28mg/kg (IP). Phytochemistry revealed the presence of alkaloids, resin, oils, saponinis, flavonoids, tannins and glycosides. The ethanolic extract had a bleeding time of 4 ± 6.5, the aqueous had 3.44 ± 3.14 while non treated animals had 6 ± 8. In the same vein whole blood clotting time was shortened by both extracts. The prothrombin, activated partial thromboplastin time and platelets count were normal and same with control.

Conclusion: The leave extracts of *Jatropha curcas* possess coagulation property justifying its use in treatment of bleeding disorder in folklore medicine. Its mechanism of action may stem from enhancement of carboxylation of trans-carboxylic acid (glutamate) residue, mimicking that of vitamin K or aggregation of platelets.

Key words: Haemostosis, Coagulation, *Jatropha curcas*, Bleeding time, Medicinal plants.

1. INTRODUCTION

Haemostasis is the body's normal physiological response for the prevention of blood loss from an injured vessel as well the maintenance of fluidity in an intact vessel. It functions in the blocking of any vascular breech and helps generally, to ensure blood fluidity and blood vessel integrity thus simultaneously involving coagulation and fibrinolysis (Azikiwe et al., 2008; James et al., 2013). Bleeding disorders may occur in haemorrhagic diseases of the new born resulting in life threatening umbilical stump bleeding and or neonatal jaundice, deficiency in vitamin K, frank case of haemophilia, disseminated intravascular coagulation, life threatening post partum haemorrhage, thrombocythaemia, thrombocytopenia, poor platelets function and platelet storage diseases as well lack of ascorbic acid (Azikiwe et al., 2007; Azikiwe et al., 2008; James et al., 2013). Blood or massive blood loss commonly leads to anaemia of varying degree and in life threatening post partum haemorrhage, a consequential pituitary gonadal shock may occur leading to subsequent secondary infertility (Magon et al., 2013). Most cases of clinical haemorrhagic disorder are treated with blood transfusion, clotting factor replacement, administration of vitamin K or desmopressin (Ozgonenel *et al* 2007; Azikiwe *et al*., 2008, James *et al*., 2013).

Most medicinal plants are well documented in literature to possess varying pharmacological activities that have proven good therapeutic value like their orthodox drugs counterpart. But unlike their orthodox drugs counterpart, medicinal plants exhibit much less or no unwanted side/adverse effects (Azikiwe et al., 2007; Azikiwe et al., 2009, Amazu et al., 2010). A good number of medicinal plants have been shown to inhibit coagulation processes thus could acts as antithrombotic or fibrolytic agents (Yamamoto et al 2005; Prasad et al., 2007; Azikiwe et al., 2007; Ezike et al., 2010; Li et al., 2013). In most cases of bleeding or haemorrhagic disorders which abound in our localities, the medicinal plants like *Ankafared* comes quite handy (Goker et al., 2008). Other medicinal plants like *Ocimum gratissium,* a pepper testing tropical plant; *Moringa oleifera* a native tree of northern India and Nigerian Zobo drink of *Hibiscus sabdariffa* have been shown to possess coagulating activity in experimental animals (Bola et al., 2008; Dahot, 2008).

The plant *Jatropha curcas* from the family *Euphorbiaceae*, is commonly called Physic nut, Barbados nut, Black vomit nut, *Curcas bean, Kukcichaole,* Purge nut*,* Purgeer boontjie, *Purging nut tree* (Juliette et al., 2006). In Igbo dialect of Delta State, Nigeria, it's called *Olokpo* and commonly used to fence garden as both seeds and stems are easily propagated. The leaves are dark green or greenish-yellow, alternates, simple, ovate to slightly lobed with 3.5 indentation up to 1.5cm wild petioles with 10cm [4 inches] long (Abioye, 2007). Flowers are usually yellow-green, Borne in axils of the leaves and being small are mostly hidden by foliage. Fruits are small capsule-like round fruits, about 2.5-4cm when immature becoming dark brown when ripe and splitting to release 2 or 3 black seeds each about 2cm long. The pulp of the seeds is white, soft in texture and oily. A fresh cut on the stem produces latex that has been shown to exhibit both pro and coagulant activities (Osoniyi and Onajobi, 2007; Odusote et al., 2008).

In India, the leaves are used in folklore medicine and possess antidiabetic activity (Patil et al., 2011). The roots have demonstrable antdirrhoae activity while the seeds are purgatives (Mujumdar et al., 2011). Biodiesel was obtained by transesterification of *Jatropha curcus* oil with anhydrous methanol, ethanol, and various mixtures of methanol/ethanol system (Kumar et al., 2012). The methane yield was highest with a pool of all components of the plant (Gunaseelan, 2009; Bhatta et al., 2012) while Mainali (2012) made a catalytic good vegetable oil yield from the seeds.

2. MATERIALS AND METHOD

2.1. Materials

Fleshy stems and leaves of *Jatropha curcas* were harvested from Omoku, Rivers State, Nigeria while taxonomy was done at the Department of Botany of the University of Benin. Mice of average weight of 21grams and rats of average weight of 110grams were bought from the Department of physiology of the University of Port Harcourt, Nigeria.

2.2. Methods

The leaves of were air dried for 5 days at room temperature, ground into a coarse powder. To a litre each of water or ethanol, 120grams of the coarse powder was macerated and allowed to stand at room temperature for 24hours. They

Table 1

A Comparison of Phytochemistry Result of ethanol and aqueous extracts

TESTED SUBSTANCE	RESULT Aqueous	RESULT Ethanol
Carbohydrate	+	+
Reducing sugar	+	+
Alkaloids	+++	+ + ++
Saponins	++	+ +
Flavonoids	+++	+ + ++
Steroids and Terpernoids	+	+
Proteins	+	+
Tannins	++	+ + +
Glycosides	++	+ +
Resins	++	++
Acidic compounds	_	-
Fats/Oils	+++	++++

KEY = *Negative sign (-) = not present, + = present, ++ = moderately present, +++ = significantly present, ++++ = abundantly present. It may be appreciated that ethanol extract had a richer yield than aqueous thus suggestive of the semi-polar nature of the active components of the plant.*

were separately sieved, filtered using Whatman filter paper and evaporated to dryness using Rotary evaporator. The residues were scraped out and weighed to deduce the percentage yield of each solvent.

PHYTOCHEMICAL ANALYSIS was carried out based on the methods described by Trease and Evans (2004). The extracts were appropriately tested for the presence of alkaloids, saponins, flavonoids, steroids, terpenoids, proteins, tannins, carbohydrates, reducing sugars, glycosides, resins and acidic compounds, oil.

Lethal Toxicity test: Acute toxicity testing was done using a total of 13 adult mice of average weight of 21grams at two stages of Lorke's 1983 method to obtain the LD50. Extrapolation of one fifth of LD50 was made as possible effective dose to study the activities.

In-vivo animal study: Adult white Wistar rats of average weight of 110grams were divided into cages of 5 animals per cage and housed in a standard animal house for one week acclimatization. Extracts of *Jatropha curcas* were orally administered daily for four weeks. Tail bleeding was used for bleeding time while whole blood clotting time, platelet count, prothrombin and activated partial thromboplastin time were done on whole blood collected through orbital sinus. All samples where necessary, were sent to the Laboratory as soon as collected.

Statistics: Data collected were statistically analysed using SPSS version 16.0. P.Values ≤ 0.5 were considered as significant. Results where possible were presented as mean ± standard error of mean.

4. RESULTS

The extraction yield was 4% and 7% for aqueous and ethanol respectively. Phytochemistry revealed the presence of alkaloids, resin, oils, saponinis, flavonoids, tannins and glycosides (Table 1). Acute toxicity for both extracts was 28.28mg/kg (IP) which was deduced from a geometry mean of 40mg/kg being the least dose that killed an animal and 20mg/kg as the highest dose that did not kill any animal (Lorke's 1983) (Table 2 a and b). The ethanolic extract had a bleeding time of 4 ± 6.5, the aqueous had 3.44 ± 3.14 while non treated animals had 6 ± 8. In the same vein whole blood clotting time was shortened by both extracts. The prothrombin, activated partial thromboplastin time and platelets count were normal and same with control (Table 3). Table 3 shows the pattern of activity on haemostasis. The ethanolic extract decreased bleeding time significantly lower that of aqueous ($P<0.03$) and more significantly lower than that of non-treated (Control) animals ($P<0.002$). The same pattern played out in those of whole blood clotting time.

5. DISCUSSION

In our present study, the ethanolic extract exhibited higher levels of active components than aqueous extract. It also had a higher lowering effect on bleeding and whole blood clotting time. These effects may stem from the presence of the phytochemical components. Alkaloids, tannins, flavonoids and saponins have been shown to possess coagulation properties (Azikiwe et al., 2007; Azikiwe et al., 2009). Goker et al, (2008) earlier demonstrated that *Ankafared* had inhibitory effect on fibrinogen while Beyazit et al., (2010) postulated that mechanism of coagulation by medicinal plants is via constricting action on the endothelial vessels. Precipitation of clotting factors was demonstrated as mechanism of coagulation by *Jatropha gossypifolia* stem (Oduola et al., 2005).

Considering the numerous uses of *Jatropha curcas* across the globe, its mechanism of coagulation of blood may cut across blood vessels constriction, inhibition on clotting factors activity or activation of endothelin. Our study however could not demonstrate any effect on prothrombin time that relates primarily to extrinsic pathway and activated partial thromboplastin time that relates to the intrinsic pathway with essential involvement of factor Vii. But, we may liken its mechanism of anticoagulation to that of vitamin K, the most popular drug used in coagulation control. Desmopressin, is a vasopressor agent that activates the von-Willebrand factor to cause coagulation especially in haemophilia. Constriction of vessels and muscles is a major mechanism of wound healing and tissue apposition. *Jatropha curcas* may therefore primarily cause blood vessels constriction followed by vitamin-K like action to cause

Table 2

LD_{50} (Lethal Dose) Study based on Lorke's method of 1983

NO. of Animals	DOSE	NUMBER OF DEATH
STAGE 1(Table 2a):		
3	10mg/kg	0
3	100mg/kg	3
3	1000mg/kg	3
STAGE 2(Table 2b):		
NO. of Animals	DOSE	NUMDER OF DEATH
1	10mg/kg	0
1	20mg/kg	0
1	40mg/kg	1

Table 2 shows stages 1 and 2 of the LD50 testing. The LD50 was deduced as the geometry mean of 40, the least dose that caused death and 20, the highest dose that caused no death. Both solvent extractions had equal lethal dose toxicity.

Table 3

Haemostatic study in rats

Solvent Group	Bleeding Time (Sec)	Whole Blood Clotting Time (Mins)	Prothrombin Time (Sec)	Activated Partial Thro Time (Sec)	Platelets Count (µl)
Ethanol	4 ± 6.5	20.46±7.8	11.2±0.8	29.0±2	155,000±151
Aqueous	3.44 ± 3.14	21.37±7.8	11.1±1.1	28±2.4	155,000±102
Water(Cont)	6 ± 8	23.46±7.8	11.2±1.2	28.9±5	154,500±220

Table 3 shows the pattern of activity on haemostasis. The ethanolic extract decreased bleeding lower that of aqueous ($P<0.03$) and significantly lower than that of non-treated (Control) animals ($P<0.002$). The same pattern played out in those of whole blood clotting time but no effect on prothrombin and Activated partial thromboplastin time. Platelet counts were essentially the same.

both wound healing and blood coagulation. This is further buttressed in our finding that there was no effect on platelets number but, on platelets functionality. Most likely through platelets increased aggregation possibly brought about via increased in vasoconstriction and platelets release.

Plant tannins as rumen modifiers are better than chemicals or antibiotic-based modifiers since these compounds are natural products which are environmentally friendly and therefore have a better acceptance with regard to feed safety issues. Tropical plants containing phenols such as tannins were found to suppress or eliminate protozoa from the rumen and reduce methane and ammonia production (Bhatta et al., 2012). Most medicinal plants are well documented in literature to possess varying pharmacological activities that have proven good therapeutic value like their orthodox drugs counterpart. But unlike their orthodox drugs counterpart, medicinal plants exhibit much less or no unwanted side/adverse effects (Azikiwe et al., 2007; Azikiwe et al., 2009, Amazu et al., 2010). For ages plants have been used in treatment of many ailments with resounding success but, less toxicity. They are also readily available and low or virtually no cost.

6. CONCLUSION

The leave extracts of *Jatropha curcas* possess coagulation property justifying its use in treatment of bleeding disorder in folklore medicine. Its mechanism of action may stem from enhancement of carboxylation of trans-carboxylic acid (glutamate) residue, mimicking that of vitamin K or aggregation of platelets.

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