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Full Length Research Paper

Evaluation of the pharmacological activity of ethanol leaf extract of *Acalypha torta* (Muell)

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ABSTRACT
The effects of ethanol extract of the leaves of *Acalypha torta* on spontaneous contraction of isolated rabbit gut, rat blood platelet aggregation and other haematological indices were investigated. The height of contraction of the isolated rabbit gut was 0.80 ± 0.03 cm on stabilization. Following the introduction of *A.torta* extract (2.5 mg), the height increased to 1.7 ± 0.4(i.e 112.5% increase). Higher concentrations of the extract (5.0, 7.5 and 10.0 mg) did not alter the size of response . Histamine and acetylcholine standards produced opposite effects on the isolated rabbit gut. While histamine (0.002µg) abolished contraction, acetylcholine(0.002µg) enhanced contraction. Administration of the extract (10.0 mg) after incubation with acetylcholine reversed the effect of acetylcholine suggesting that the extract antagonizes the actions of acetylcholine and may possess antidiarrhoeal potential. Extract (5.0 mg/ml) also inhibited $CaCl_2$ – induced platelet aggregation by 81.72% (i.e from 87.95 ± 0.16 to 16.08 ± 0.08 % aggregation). Administration of 15.0 mg/ml the extract completely inhibited the aggregatory effect of $CaCl_2$. This indicates antithrombotic or thrombolytic activity of the extract. A significant ($p < 0.05$) increase in haemoglobin concentration (from 12.85 ± 0.1 to 14.43 ± 0.33 g/dl) was observed at 50.0 mg/kg body wt. of extract. Decreased white blood cell numbers were recorded.

Keywords: *Acalypha torta*, rabbit smooth muscle, antidiarrheal, antiaggregatory, thrombolytic, calcium antagonist.

1.0 INTRODUCTION
The use of medicinal plant products in the treatment and management of acute and chronic disorders is now prevalent worldwide, most especially in developing and underdeveloped countries of the world (Venukumar and Latha, 2002; Malaya *et al*., 2004). WHO estimates depicted that in Africa, up to 80% of the population rely on traditional medicine and in India, about 65% of the people employ these medicinal plants (Ashwanden, 2001). Scientific evidence of the efficacy of these herbal pharmaceuticals is beginning to emerge through series of preclinical and clinical researches.

Acalypha torta (Muell) belongs to the family Euphorbiaceae and is widely distributed all over the world, particularly in the tropics and sub-tropical Africa, Asia and America. This ornamental plant is popularly employed in Nigerian tradomedicine for the treatment of malaria, stomach upset, dermatitis, bacterial and fungal infections (Irobi and Banso, 1994), and hypertension (Ezekwesili *et al*., 2008). The evidence of the antimicrobial (Irobi and Banso, 1994), hypolipidaemic (Ezekwesili *et al*., 2008), anti-inflammatory (Ogbunugafor *et al*., 2011), and antihypertensive (Ezekwesili *et al*., 2012) activities of ethanol extract of the leaves of *A. torta* have been scientifically established. The response of the vital organs such as liver, kidney, heart, lungs and spleen to ethanol leaf extract of *A. torta* have also been documented (Ezekwesili *et al*., 2011). The presence of pharmacologically active phytochemicals such as alkaloids, flavonoids, saponins, tannins and glycosides in the extracts of the leaves of *A. torta* (Ezekwesili *et al*.,2012) and *A. wilkesiana* (Nwinuka *et al*., 2008) have also been reported.

This study was therefore designed to assess scientifically the effects of the ethanol extract of *A. torta* on spontaneous contraction of intestinal smooth muscle, human blood platelet aggregation and other haematological indices using conventional assay techniques.

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2.0 MATERIALS AND METHODS

2.1 Plant materials

Mature leaves of *A. torta* Muell were collected from Abagana in Njikoka Local Government Area of Anambra State, Nigeria, in March, 2011. They were identified and authenticated at the International Centre for Ethnomedicine and Drug Development (INTERCEDD) and Voucher specimen No.8256, was prepared and kept in the herbarium.

2.2 Animals

Adult male Wistar albino rats weighing ~140g and albino rabbits ~2.0kg were purchased from the Department of Pharmacology, College of Medicine, University of Nigeria Teaching Hospital, Enugu. All the experimental animals were kept at the animal house of The Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, for one week before use to allow for acclimatization.

2.3 Chemicals

All the chemicals used were of analytical grade and were products of BDH Ltd, Poole, England. The biochemicals (acetylcholine and histamine standards) used were manufactured by Sigma Chemical Company, U.S.A.

2.4 Extraction

Four hundred grams(400g) of dried and pulverized leaves of *Acalypha torta* were defatted by soaking in 2.0 litres of chloroform-methanol (2:1) at room temperature for 72h. Three changes of solvents were made at 24h intervals. The extract was filtered through cheese cloth and Whatman no.1 filter paper and the filtrate was discarded. The residue from chloroform-methanol (2:1) extraction was then dried and re-extracted thrice in 2.0 litres of ethanol at room temperature and at 24h intervals. After filtration, the filtrate was evaporated to obtain the thick brown slurry which was refrigerated and used as the crude ethanol extract.

2.5 Assay of isolated rabbit gut contraction

The effect of the extract on intestinal motility was investigated using the method of Finkleman (1930). Male rabbits weighing approximately 2.0kg were fasted overnight and then sacrificed by stunning on the head.In each experiment, the animal was dissected and the abdomen exposed. Lengths (2.0 cm) of the ileum were cut and mounted in an organ bath containing Tyrode's solution of the following composition (in g/l): NaCl, 8.0; KCL, 0.2; MgCl, 0.2; $NaHPO_4$, 0.5; $NaHCO_3$, 1.0; and glucose, 1.0. The solution was quickly and carefully aerated and was allowed time to stabilize at 37^{o}C. One end of the tissue was tied to a hook on the aerator and the other end to the Chymograph. After 30minutes of equilibration, responses following contact of the tissue with varying doses (2.5 to 10.0mg) of the extract, histamine (0.002µg) and acetylcholine (0.002µg) were recorded. Drug-tissue contact time of 2 minutes was maintained. The tissue was washed thrice between additions of drugs.

2.6 Blood platelet aggregatory activity study

The method of Born and Cross (1963) was adopted. Human blood samples were obtained from healthy adult male subjects who had not taken any drug for at least one week. Blood samples (10.0ml) were collected by venepuncture into plastic anticoagulant (3.8% trisodium citrate) tubes and centrifuged at 300 rpm for 15 minutes. The supernatants were drawn out and used as platelet-rich plasma (PRP). Reaction medium (2.5ml) containing normal saline (2.0ml) and PRP (0.5ml) served as the control, whereas varying concentrations of the extract (5.0 to 15.0 mg/ml final concentrations) were included in the test media and allowed 15 seconds incubation with platelet-rich plasma before the induction of aggregation. Aggregation of platelets was induced by the addition of 4.0 mM $CaCl_2$ (0.1ml) and the absorbance at 600nm monitored for 5 minutes using Spectrophotometer. Appropriate blanks containing the extract but without PRP were used.

2.7 Study of extract's effects on haematological parameters

Fifteen male Wistar albino rats weighing ~155.5g were divided into three groups of five animals per group according to their weights. Animals in test groups 1 and 2 received the extract orally at a daily dose of 50 and 100mg/kg body wt. respectively, whereas animals in group 3 served as the control rats and were given a daily dose of normal saline (1.0ml/kg body wt.) orally. The duration of

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treatment was 28days. At the end of 28days, the rats were anaesthetized with chloroform, sacrificed and blood samples carefully collected through cardiac puncture into anticoagulant sample bottles containing ethylene diamine tetra-acetic acid (EDTA). Haemoglobin content of samples was determined by the Cyanmeth method as recommended by International Committee for Standardization of Haematology (ICSH, 1978) whereas total red blood cells and white blood cells counts were estimated using the visual method of Dacie and Lewis (1975). The percentage packed cell volume was determined according to the haematocrit method of Alexander and Grifiths (1993). The time taken for $CaCl_2$ –induced clotting of rat blood samples were also estimated by the method of Mayer (1955).

2.8 Statistical Analysis

Data were analysed statistically using ANOVA and Bonferroni's Multiple Comparison Test.

3.0 RESULTS AND DISCUSSION

In our study, results presented in Fig. 1 show spontaneous contraction of the rabbit gut in the absence of drugs. The average height of contraction on stabilization was 0.80 ± 0.03cm. Incubation of the gut with *A. torta* extract (2.5mg) increased the height of contraction to 1.70 ± 0.4cm (i.e 112.5% increase), but the observed tone was not remarkably different from the normal tone of the isolated gut. Higher concentrations of the extract (5.0, 7.5 and 10.0mg) did not alter the size of the response.

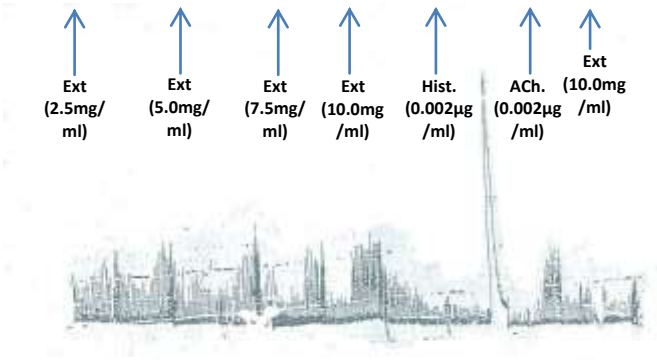

Figure 1: Chymograph tracings showing the effects of varying concentrations of *A. torta* extract, histamine and acetylcholine on spontaneous contraction of isolated rabbit gut. *A. torta* extract(10.0mg/ml) reversed the effect of acetylcholine(0.002µg/ml). Histamine (0.002µg) abolished this spontaneous contraction of isolated rabbit gut, whereas acetylcholine (0.002µg) induced prolonged contraction of the tissue. Acetylcholine is a biochemical that is well-known for it's peristaltic activity on the intestine (intestinal motility) thereby causing diarrhoea (Vianna-Jorge *et al*., 2003).

Histamine, on the other hand, produces opposite effects to acetylcholine (Ariyoshi *et al*., 1985). Therefore, they are usually regarded as physiological antagonists. Since the extract (10.0mg) completely reversed the intestinal contraction caused by contact of the tissue with acetylcholine, it may be deduced that the extract possesses antidiarrhoeal property.

Cardiovascular diseases are among the major causes of death worldwide and are believed to be exacerbated by blood platelet dysfunctions (Golino *et al*., 2005). The effect of *A. torta* extract on $CaCl_2$-induced platelet aggregation is shown in Table 1. *A. torta per se* did not have any significant ($p>0.05$) effect on the aggregatory property of blood platelets in the absence of $CaCl_2$. Addition of $CaCl_2$ (4mM) into the medium caused 87.95% aggregatory effect. Incubation of the platelet-rich plasma with 5.0 mg/ml of *A. torta* extract decreased the aggregatory response by 81.72% (i.e from 87.95 ± 0.16 to 16.08 ± 0.08). This activity was statistically significant ($p<0.001$) and increased with the dose of the extract. At the largest dose of 15.0mg/ml, the extract completely abolished the aggregatory effect of $CaCl_2$. Since blood platelets participate is pathological thrombosis leading to such conditions as myocardial infarction, stroke, embolism and peripheral vascular thrombosis (Berkow *et al*., 1999; Imram *et al*., 2012) inhibition of platelet aggregation by the extract is indicative of it's possible role as an antithrombotic or thrombolytic agent and could be useful in the management of the above named disorders.

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Table 1: Effect of ethanol extract of *A. torta* on percentage platelet aggregation induced by $CaCl_2$.

Group	1min	2min	3min	4min	5min
A. torta (10.mg/ml)	1.00 ± 0.07	3.46 ± 0.06	0.81 ± 0.05	1.38 ± 0.09	1.49 ± 0.04
Normal saline + $CaCl_2$ (4.0mM,0.1ml),Control.	15.60 ± 0.14	41.68± 0.10	61.98±0.08	84.15±0.85	87.95±16
A. torta (5mg/ml) + $CaCl_2$	8.25±0.05*	11.60±0.05*	13.76±0.10*	17.21±0.03*	16.08±0.08 *
A. torta (10mg/ml) + $CaCl_2$	2.66±0.06**	8.13±0.95**	10.46±0.09**	9.20±0.19**	7.08±0.08**
A. torta (15mg/ml) + $CaCl_2$	1.28±0.06**	1.08±0.08**	0.40±0.05**	0.49±0.03**	0.72±0.01**

Values are means±standard errors of means. n=5.Comparison was made after ANOVA using Bonferroni's Multiple Comparison Test. * is significantly different from control at p<0.001. ** p<0.0001 when compared with control.

The extract also delayed $CaCl_2$-induced blood clotting (Table 2) by 31.2%. Blood clotting is a normal physiological process that prevents an individual from bleeding to death when a blood vessel is ruptured. However, this can pose health problems when the clotting occurs within intact healthy blood vessels, and is not degraded after due time. The end result may also be any of the diseases associated with blood clotting such as pulmonary embolism, stroke and heart attack.

These findings indicate antagonism of calcium-utilizing processes. Calcium ion is involved in several physiological and biochemical processes in the body such as excitation-contraction of vascular and skeletal smooth muscles, excitation-secretion processes of the secretory glands, blood coagulation and blood platelet aggregation. Over activation of any of these haemostatic mechanisms as a result of abnormal calcium ion metabolism could trigger the development of any of the cardiovascular diseases. Thus calcium ion antagonists are therapeutically useful in the management of hypertension, platelet adhesion and aggregation dysfunctions (Robert *et al*., 1999). In addition, the anticoagulant and antiplatelet aggregatory activity of phenolic compounds and flavonoids have been reported (Imran *et al*., 2012). Ezekwesili *et al*., 2012, reported the presence of flavonoids in *A. torta* (Muell) ethanolic leaf extract. These phytochemicals may, therefore, be responsible for the observed antiplatelet activity of the extract.

Results presented in table 2 also showed that ethanol extract of *A. torta* leaves at both doses of 50 and 100 mg/kg body wt. triggered slight but statistically significant (p<0.05) increases in rat blood haemoglobin concentration, PCV and RBC counts. No significant difference was observed between the two groups treated with varying doses of the extract. However, both groups showed significant decreases at p<0.01 in white blood cell numbers. These findings may suggest the stimulatory effect of *A. torta* extract on the physiological systems responsible for the proliferation of the red blood cells such as the bone marrow, as well as the erythropoietin system. On the other hand, the biogenesis of WBC was suppressed and this may imply immunosuppressive potential of some chemical components of the extract.

Table 2: Effects of treatment with ethanol extract of *A.torta* on haematological indices in rats.

Haematological index	Normal saline (control)	*A. torta* extract, 50mg/kg wt.	*A. torta* extract, 100mg/kg wt.
Hb (g/dl)	12.85 ± 0.10	14.43 ± 0.33*	14.50±1.80*
PCV (%)	39.10 ± 0.46	45.30 ± 0.81	46.05 ± 0.38
RBC ($x10^6/mm^3$)	6.20 ± 0.17	8.05 ± 0.94	8.85 ± 0.01
WBC ($x10^3/mm^3$)	12.87 ± 1.25	5.15 ± 0.72**	4.05 ± 0.15**
Clotting time (mins)	7.37 ± 0.01	7.57 ± 0.06	9.67 ± 0.03

Values are means ± standard errors of means. n = 5. * represents significant difference at p<0.05, ** represents significance at p <0.01 when compared with the control group.

These observations are in agreement with earlier report (Sule *et al*., 2012) that supplementation of animal diet with *Acalypha wilkesiana* herb improved the PCV, Hb, RBC and MCV, but WBC and neutrophils were decreased. This could be a property that is common to the *Acalypha* species of the Euphorbiaceae family.

CONCLUSION

Findings from our investigations suggest that ethanol extract of *A. torta* Muell leaves inhibited spontaneous contraction of rabbit

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intestinal smooth muscle, human blood platelet aggregation and blood clotting. It enhanced RBC proliferation, but suppressed WBC formation. These revealed plausible antidiarrhoeal, antithrombotic and immunosuppressive activities of *A. torta* Muell herb. However, it is equally necessary that the actual modes of actions be studied and the phytochemical constituents mediating these actions identified, isolated and characterized.

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