

**Effect of Ethanolic Fruit Extract of *Adenopus Breviflorus*
(*Lagenaria Breviflora* Robert) on Hematological Indices in
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Effect of Ethanolic Fruit Extract of *Adenopus Breviflorus* (*Lagenaria Breviflora* Robert) on Hematological Indices in Male Albino Wistar Rats

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Abstract

The present study was carried out to investigate the effect of ethanolic fruit extract of *Adenopus breviflorus* (EEAB) on hematological indices in male albino rats. Phytochemical screening was carried out on the fruit of *A. breviflorus* sample. The effect of the extract on the histology of the liver tissues was also investigated. Thirty (30) male albino rats were randomly divided into 5 groups (n= 6) rats each. Group 1 served as the control and was administered 2mL distilled water. The remaining groups received 100, 200, 300 and 400 mg/kg/b.w.t/day of EEAB respectively orally over a period of 28 days. Twenty-four hours after the last administration, the effect of administration of this extract on hematological parameters were determined in the plasma using standard techniques and the data were analyzed using ANOVA and the level of significance was at $P<0.05$. Phytochemical studies revealed the presence of saponins (triterpenoids). The oral lethal dose (LD₅₀) of the ethanolic extract was determined to be above 5000mg/kg BW. Results also indicate that treatment with all doses of EEAB caused no significant change in the hematological parameters except the platelets (PLT) that was significantly decreased ($P<0.05$) in animals administered 400mg/kg/b.w.t of EEAB when compared with the control. Routine H and E histological study revealed no features of hepatotoxicity. The study suggests that ethanolic fruit extract of *A. breviflorus* did not exhibit any significant effect ($P<0.05$) on hematological indices of albino rats at all the administered doses of the extract while it produced a significant reduction on platelet count at administered dose of 400mg/kg b.w.t and may be considered safe at all the tested doses.

Keywords: *Adenopus breviflora*, fruit extracts, phytochemical screening, hematology, rats.

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Introduction

The indigenous medicinal plants in Nigeria form an important component of the natural wealth of the Country. Most of these plants have been used indiscriminately by large percentage of people for managing various diseased states without actually knowing how relief is brought about or its safety/toxicity risk. One of such plant is *Adenopus breviflorus*.

Adenopus breviflorus Benth is a tree plant, commonly known as "*Lagenaria breviflora* Robert", belongs to the family Cucurbitaceae (Yasuyuki *et al.*, 2005; Hanno *et al.*, 2009). It is a perennial climber ascending to the forest canopy, occurring from Senegal to the West Cameroons, and generally widespread in tropical Africa. The family is a diverse family of plants in the temperate zones but also thrives in hot arid regions of the world (Weihrauch and Teter, 1994). In Nigeria, different tribal groups have their indigenous names as: "Ogbenwa" in Igbo, "Tagiri" in Yoruba) and so on (Burkill, 1995). The leaves are scab rid and sand papery. The stem when crushed has an unpleasant smell and a decoction from it is said to be used in Africa for headache and as a vermifuge (Ajayi *et al.*, 2002). Its seeds and fruits have been used in folk medicine since antiquity.

The fruit of *Lagenaria breviflora* Robert is widely used in folklore medicine in West Africa as herbal remedy for the treatment of measles, digestive disorders, and as wound antiseptics (e.g. umbilical incision wound) in man, while the livestock farmers use it for the treatment of Newcastle disease and coccidiosis in various animal species, especially poultry (Sonaiya, 1999). Laboratory investigations have shown evidences in support of its anti-implantation activity (Elujoba *et al.*, 1990), miracidicidal and cercaricidal activities (Ajayi *et al.*, 2002) and antibacterial activity (Tomori *et al.*, 2007). In addition to its medicinal application, so much has been reported on the taxonomy (Morimoto *et al.*, 2005) and chemical constituents of the plant (Elujoba and El-Alfy, 1986; Elujoba *et al.*, 1991; Esuoso and Bayer, 1998; Esuoso *et al.*, 2000). However, despite the acclaimed and documented uses, there appears to be

a paucity of information from literature on the effects of administration of the extract of the whole fruit of *A. breviflorus* on hematological indices. Therefore, the present study WAS designed to investigate the effect of EEAB on hematological indices in male albino rats.

Materials and Methods

Experimental Animals

Thirty male albino rats weighing between 180 to 200 g were obtained from the Central Animal House, Faculty of Basic Medical Sciences, Ebonyi State University, Abakaliki, Nigeria. They were housed in netted cages under standard laboratory conditions and were fed with standard rat's pellets (Pfizer Feeds LTD, Enugu, Nigeria) and tap water provided *ad-libitum*. Excess feeds and water were removed and replaced daily. The rats were allowed to stabilize for 2 weeks before commencement of the experiment. The experimental procedures and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by National Institute of Health (NIH, 1985); all protocol and procedure were approved by Animal Ethics Committee of the University with reference number (EBSU/REC/BM14/011).

Plant Materials

The fresh fruit of *Adenopus breviflorus* was collected at the back of Food Processing Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The fruit sample was identified and authenticated as *A. breviflorus* by Dr. A.T.J. Ogunkunle in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Authenticated voucher specimen (LHO 227) was deposited in the Herbarium of the same department.

Extraction and Preparation of Extract

The whole fruit of *Adenopus breviflorus* was rinsed with distilled water and reduced into smaller sizes. The smaller sizes were air dried in the shade for 12 to 14 days and then pulverized to coarse powder, and stored in an air tight container until required. 1000g of the powdered fruit was weighed and suspended in 2.5liters of 96% ethanol and

shaken vigorously at intervals, this was allowed to stand for 48 hours in a dark room environment. Thereafter it was filtered by a mesh cloth and the filtered was concentrated into a greenish-brown syrupy mass in a rotary evaporator with a temperature of about 40°C under reduced pressure for 3h. The extract syrup formed was left over a water bath for final concentration into solid paste which gave a percentage yield of 21.8%. The concentrate was later reconstituted in sterile distilled water to give the required doses of 100, 200, 300, 400 mg/kg/ body weight in 2ml of the vehicle respectively using tween-80 as suspending agent. The solutions were prepared fresh on the day of experiment prior to the administration.

Preliminary Phytochemical Screening

The ethanolic fruit extract of the plant was subjected to various qualitative phytochemical tests, to identify the secondary metabolites; saponins, tannins, terpenes, steroids, alkaloids, flavonoids and cardiac glycosides present in the fruit. The methods of analysis employed were those described by Trease and Evans (1983) and Sofowora (1993).

Acute Oral Toxicity Study

The lethal dose (LD₅₀) of the ethanolic fruit extract of *A. breviflorus* was determined by the method of Lorke (1983) and Sandow (1979) using thirteen (13) adult rats of both sexes. In the first phase rats were divided into three groups of three (3) rats each and were treated with the ethanolic fruit extract of *A. breviflorus* at doses of 10, 100 and 1000mg/kg body weight orally. They were observed for 24 hours for signs of toxicity. In the second phase four rats were divided into four (4) groups of one rat each and were also treated with the ethanolic fruit extract *A. breviflorus* at doses of 1000, 1600, 2900 and 5000mg/kg body weight (p.o). The median lethal dose (LD₅₀) was calculated using the second phase.

Experimental Design and Animal Treatment

A total of thirty (30) Wistar rats were used for the study. The rats were randomly divided into five (5) groups consisting of six (6) animals each. The rats in group 1 received 2mL of distilled water daily while the experimental groups 2, 3, 4 and 5 received

2mL graded doses of 100, 200, 300 and 400mg/kg body weight of the ethanolic fruit extract of *A. breviflorus* respectively orally via an orogastric syringe for twenty-eight days. Twenty-four hours after the last administration, the animals were anesthetized with sodium pentobarbital (30mg/kg, intraperitoneally). When the rats became unconscious, the neck area was quickly cleared of fur and skin to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sharp sterile blade. Blood samples were collected from each rat into EDTA sample bottles for hematological analysis.

Gross Necropsy and Observation

During the period of experimentation, all the animals used were subjected to a detailed gross necropsy that included careful examination of the external surface of the body, all orifices and cranial, thoracic and abdominal cavities. Behavioral changes, depression, salivation, diarrhea, muscular weakness and sedation were also observed.

Hematological Analysis

The effects of the extract on red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) count, lymphocytes (LYM) count and neutrophils (NEUT) count were analyzed using an automated hematological analyzer (SYSMEX KX-21: SYSMEX CORPORATION, JAPAN).

Hepatotoxicity Study

After the blood samples were taken, the rats abdomen were opened and the liver excised, rinsed with saline and fixed in 10% buffered formalin solution. The liver was routinely processed; paraffin sections of 10 µm thick were obtained and stained with hematoxylin and eosin for histo-pathological signs of toxicity (Luna, 1968). Thereafter microscopic slides were photographed and interpreted.

Statistical Analysis

The data were statistically evaluated by one way ANOVA. Comparison between treatment and control group were made by Student's t- test then followed with Fisher's exact. Differences between groups were considered significant at ($P < 0.05$). Data are presented as mean \pm standard error of the mean ($M \pm SEM$).

Results

Phytochemical Screening

The results of phytochemical analysis indicate that the extract contains saponins (tripterpenoids).

Acute Oral Toxicity Test

The oral administration of the ethanolic fruit extract of *A. breviflorus* to albino rats up to the dose of 5000mg/kg BW did not record any mortality. However, a mild clinical sign of writhing, increase in motor activity and tremors in rats treated with the dosage of 5000mg/kg BW was observed. Thus, the LD_{50} was determined to be above 5000mg/kg BW.

Hematology Results

Administration of EEAB for 28 days produced no significant change in the hematological parameters at ($P > 0.05$) except the platelets (260.00 ± 76.676) in animals administered 400mg/kg B.W of EEAB that was significantly ($P < 0.05$) reduced when compared with the control group (666.75 ± 55.328) (Table 1).

Table 1: Hematological parameters of male wistar rats after 28 days of oral administration of ethanolic fruit extract *Adenopus breviflorus*.

Parameters	Control (2ml of D.W)	Group B (100mg/kg B.W) of EEAB	Group C (200mg/kg B.W) of EEAB	Group D (300mg/kg B.W) of EEAB	Group E (400mg/kg B.W) of EEAB
WBC ($\times 10^3/\mu l$)	5.23 \pm 0.43	9.78 \pm 2.08	7.93 \pm 2.45	8.28 \pm 2.31	5.65 \pm 1.37
RBC ($\times 10^6/\mu l$)	7.70 \pm 0.15	7.57 \pm 0.15	6.20 \pm 0.77	8.35 \pm 0.89	7.24 \pm 0.85
Hb (g/dl)	12.48 \pm 0.84	13.15 \pm 0.37	9.30 \pm 1.87	12.15 \pm 0.93	9.80 \pm 2.57
PCV (%)	43.50 \pm 0.65	43.18 \pm 0.80	34.38 \pm 4.63	46.00 \pm 5.58	40.55 \pm 4.27
MCV (fl)	56.60 \pm 1.43	57.08 \pm 1.48	55.30 \pm 1.78	54.88 \pm 1.23	56.28 \pm 1.55
MCH (pg)	16.18 \pm 0.98	17.36 \pm 0.61	14.58 \pm 1.31	14.92 \pm 1.51	13.40 \pm 3.61
MCHC (g/dl)	28.75 \pm 2.09	30.45 \pm 0.35	26.33 \pm 2.35	27.30 \pm 2.95	23.55 \pm 6.01
PLT ($\times 10^3/\mu l$)	666.75 \pm 55.33	457.25 \pm 135.16	440.00 \pm 84.55	411.75 \pm 140.38	260.00 \pm 76.68*
LYM (%)	79.43 \pm 1.40	73.95 \pm 7.44	74.68 \pm 5.57	76.85 \pm 5.69	71.35 \pm 4.66
NEUT (%)	20.58 \pm 1.40	25.30 \pm 7.68	25.32 \pm 5.57	23.15 \pm 5.69	28.65 \pm 4.66

(WBC, White blood cell; RBC, Red blood cell; Hb, Hemoglobin; PCV, Packed cell volume; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PLT, Platelet; LYM, Lymphocyte; NEUT, Neutrophils). Values expressed as mean \pm SEM (n = 6), (* = $P < 0.05$) \rightarrow Significant, DW and EEAB means Distilled water and Ethanolic Extract of *A. breviflorus* respectively.

Hepatotoxicity Findings

The liver section from group 1 (control, 2ml distilled water) showed normal architecture of the liver (Figure 1). Similarly, the liver sections from

group of animals administered EEAB orally at all doses of the extract revealed no features of hepatotoxicity (Figure 2, 3, 4, and 5) when compared with the control.

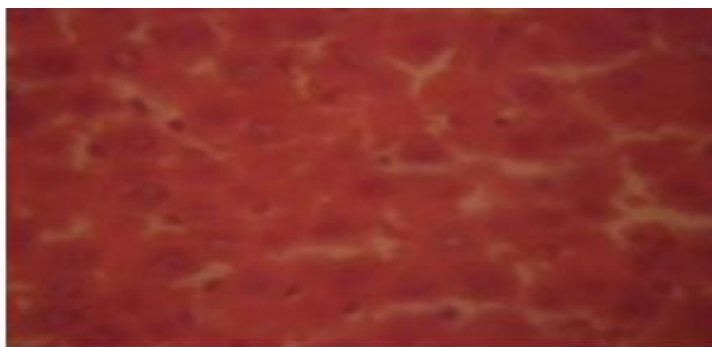


Fig. 1: Photomicrograph of the liver of control rat given 2ml distilled water showing normal arrangement of hepatocytes with nuclei, sinusoid, portal triad and central vein (H&E stain, 400x).

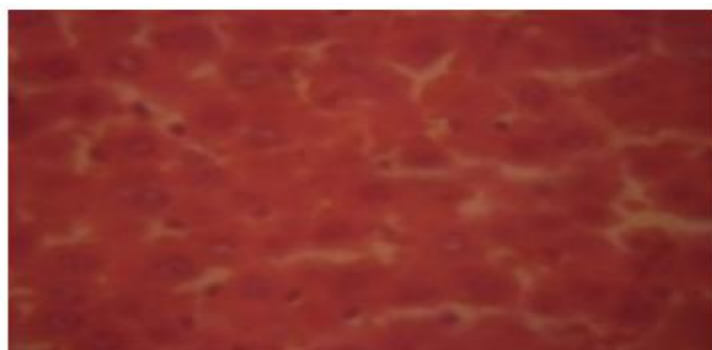


Fig. 2: Photomicrograph of the liver of rats administered 100mg/kg body weight of EEAB, showing normal hepatocytes with nuclei, central vein, sinusoid and portal triad (H&E stain, 400x).

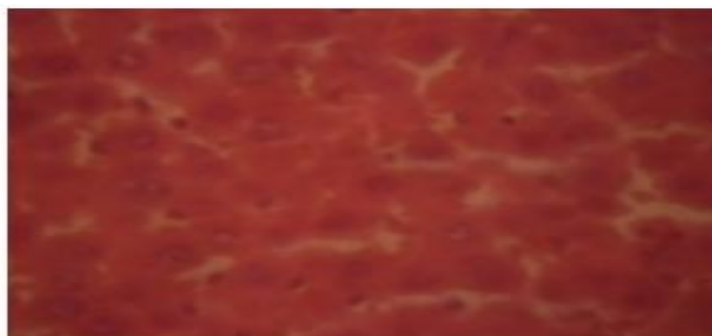


Fig. 3: Photomicrograph of the liver of rats administered 200mg/kg body weight of EEAB, showing normal hepatocytes with nuclei, central vein, sinusoid and portal triad (H&E stain, 400x).

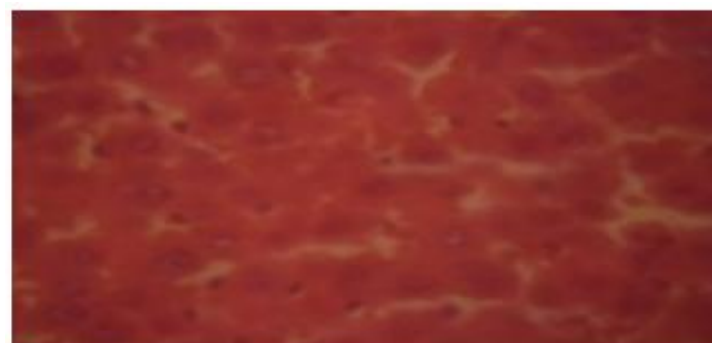


Fig. 4: Photomicrograph of the liver of rats administered 300mg/kg body weight of EEAB, showing normal hepatocytes with nuclei, central vein, sinusoid and portal triad (H&E stain, 400x).

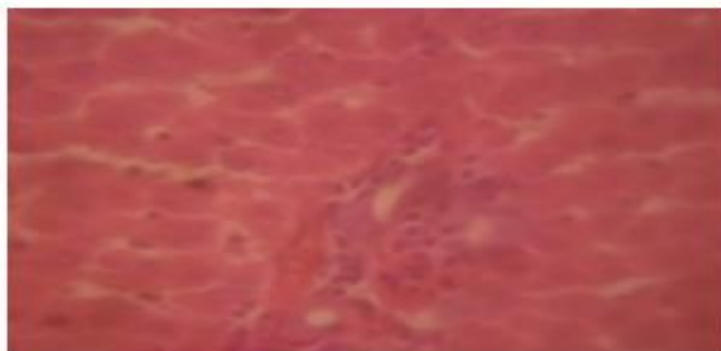


Fig. 5: Photomicrograph of the liver of rats administered 400mg/kg body weight of EEAB, showing relatively normal hepatocytes with nuclei, central vein, sinusoid, portal triad and some inflammatory cells scattered all over (H&E stain, 400x).

Discussion

Phytochemical studies revealed the presence of saponins without tannins, therefore is described as tripterpenoids. The acute toxicity LD₅₀ of ethanolic fruit extract of *A. breviflorus* in albino rats was determined to be above 5000mg/kg body weight according to the method of Lorke (1983) and Sandow (1979). The elimination of toxic substances is just one of the many functions of the liver. The liver converts nutrients into energy, forms proteins and stores carbohydrates. However, this organ can be remarkably resilient in the elimination of toxins and their other functions can be damaged in the process (Sembulingam and Prema Sembulingam, 2005).

Toxicity studies of herbal extract in animals are commonly used to assess potential health risk in humans, caused by intrinsic adverse effects of chemical compounds of plant extracts (Ashafa and Olunu, 2011). The deleterious effects of these extracts may be accompanied or preceded by clinical signs of toxicity such as salivation, loss of hair, changes in animal eye color, decreased respiratory rate and motor activity. Such toxicity testing is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity, when data are translated from animal studies (Olson *et al.*, 2000). At an administered dose of 400 mg/kg body weight of ethanolic fruit extract of *A. breviflorus*, all rats

displayed normal behavioral, neurological and autonomic profiles. No mortality or morbidity was observed in the rats at 400mg/kg body weight of the extract. This is an indication that the extract may have been well tolerated by the rats.

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal (Ashafa *et al.*, 2009). It can also be used to explain blood relating functions of chemical compounds/plant extract (Yakubu *et al.*, 2007). Hematological parameters provide information regarding the status of bone marrow activity and hemolysis. The various hematological indices investigated in this study as shown in Table 1 are useful parameters that can be employed to assess the toxic potentials of plant extracts in living systems (Sunmonu and Oloyede, 2010).

Treatment of male wistar rats with *Adenopus breviflorus* produced no significant changes in total WBC and this suggests that the overall immune function has not been compromised. White blood cells, or leucocytes, are vitally important in the disposal of damaged and ageing tissues and in the immune responses which protect body from infections and cancer cell proliferation. Treatment of male wistar rats with *Adenopus breviflorus* produced no significant changes in RBC counts and some indices relating to it (Hb, PCV, MCV, MCH, and MCHC). MCV, MCH and MCHC relates to

individual red blood cells while Hb, RBC and PCV are associated with the total population of red blood cells (Ashafa *et al.*, 2011). The non-significant effect of the extract on RBCs might be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) was not altered. In the present study, the administration ethanolic fruit extract *A. breviflorus* did not exhibit any significant effects on the hematological indices investigated, except the platelet count (Table 1). There was a significant reduction in platelet count at a dose of 400mg/kg of EEAB when compared with the control. The significant reduction in platelet count (Tables 1) may be due to inhibitory effect on thrombopoietin production (Kaushansky, 1995; Li *et al.*, 1999). Reduction in platelets count in experimental animals has been reported to indicate adverse effect on the oxygen carrying capacity of the blood as well as thrombopoietin (McLellan *et al.*, 2003). Results from this study show that the platelet count was altered signifying that the oxygen carrying capacity of the blood was affected when this extract was administered at a dose of 400mg/kg body weight to the male Wistar rats. This suggests that *A. breviflorus* do not have the potential to stimulate thrombopoietin production (Blood and Radostitis, 1989). Toxicity studies on administered doses of *A. breviflorus* fruit extract in the range of 100mg/kg to 400mg/kg body weight did not show any sign of hepatotoxicity in normal male wistar rats. We had reported similar findings earlier for the plant extract in our previous study (Balogun *et al.*, 2014).

Conclusion

The study suggests that ethanolic fruit extract of *A. breviflorus* did not exhibit any significant effect ($p > 0.05$) on hematological indices of albino rats at all the administered doses of the extract while it produced a significant reduction on platelet count at a dose of 400mg/kg BW. Though the ethanolic fruit extract of *A. breviflorus* did not cause either mortality or obvious morphological toxic effect, it may be considered safe at all the administered doses.

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