

Research Article



Effect of Ethanol Extract of *Adenopus breviflorus* (Roberty) on Hematological and Plasma Biochemical Parameters in Male Wistar Rats

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ABSTRACT

This study aims at investigating the effect of ethanol extract of *Adenopus breviflorus* (EEAB) on hematological and plasmabiochemical parameters in albino rats. Eight hundred grams of air-dried *Adenopus breviflorus* fruits were cold macerated in 70 % ethanol and concentrated using water-bath. Twenty eight male albino rats (150-200 g) were divided into control (distilled water) and EEAB-treated (62.5, 125, 250 mg/kg) groups (7 per group) for the hematological and biochemical assays. The animals were orally treated on daily basis for 30 days. Red Blood cell (RBC) count and Total White Blood Cell (TWBC) count were determined using the hemocytometer. Activities of plasma Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), as well as levels of total protein, globulin, albumin, creatinine and urea were determined by spectrophotometry. Data were analyzed using descriptive statistics and ANOVA at $p=0.05$. Treatment of rats with 250 mg/kg caused a significant ($p<0.05$) increase in PCV value relative to the control, while, 125 mg/kg and 250 mg/kg caused a significant ($p<0.05$) decrease in TWBC count relative to the control. The EEAB (125 mg/kg, 250 mg/kg) produced a significant ($p<0.05$) decrease in platelet count relative to the control. The EEAB (250 mg/kg) induced a significant ($p<0.05$) increase in monocyte value relative to the control. The EEAB (62.5 mg/kg, 125 mg/kg) caused significant ($p<0.05$) increments in total protein, albumin and globulin levels relative to their respective controls. Also, EEAB (62.5 mg/kg, 250 mg/kg) caused significant ($p<0.05$) increments in AST and ALT activities relative to their respective controls. It can be concluded that *Adenopus breviflorus* probably has a little toxic effect and a lot of beneficial potentialities on the hematological functions and blood chemistry of male Wistar rats.

Keywords: *Adenopus breviflorus*, Total white blood cell counts, Alanine amino transferase, Creatinine, Rats.

INTRODUCTION

Adenopus breviflorus (Roberty) belongs to the family of Cucurbitaceae which are mostly prostrate or climbing herbaceous annuals. It is commonly called Wild colocynth in English language, "Ogbenwa" in Ibo language and "Tagiri" by Yoruba language speaking people of Nigeria¹.

The plant is used medicinally as a purgative in Tanganyika and as a vermifuge in Nigeria¹. A decoction from the plant is said to be used in Nigeria for headache¹. The plant is used for money-making charms by the Yoruba herbalists of South-West Nigeria². It is used in West Africa for a wide range of gastrointestinal disorders and measles in man. It is also used as an anticonvulsant, sedative and pain killer³.

Pharmacologically, it is used as an anti-implantation agent⁴, abortifacient⁵, broad spectrum antibacterial agent⁶ as well as an anti-oxidant and anti-ulcerogenic agent⁷.

Since this plant has been reported to be used locally as a blood tonic, this study therefore aims to authenticate the veracity of this claim.

MATERIALS AND METHODS

Experimental animals

Adult male albino mice weighing between 150-200 g bred in the Pre-Clinical Animal House of the College of

Medicine and Health Sciences, AfeBabalola University were used. They were housed under standard laboratory conditions and had free access to feed and water. They were acclimatized for two weeks to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of AfeBabalola University Ethics Committee on guiding principles on care and use of animals.

Plant material

Fresh samples of *Adenopus breviflorus* fruit were bought in Bodija Market, Ibadan, and were authenticated in the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan where a voucher specimen (No. FHI.108336) was deposited in their Herbarium.

Preparation of crude ethanol extract

Large quantity (7.5 kg) of fresh specimens of the whole fruit of *Adenopus breviflorus* were washed free of debris and pulverized using mortar and pestle and air-dried for eight weeks.

The resultant dried specimens (800.0 g) were macerated and extracted with 70 % ethanol (1:2 wt./vol.) for 72 hours at room temperature (26-28 °C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The 70 % ethanol was later evaporated using water-bath to give a percentage yield of



8.6 % of the starting sample. The dried sample was reconstituted in distilled water to make up test solutions of known concentration.

Experimental design

Twenty eight male albino rats were randomly divided into four groups, with each consisting of seven animals. The four groups were subjected to the following oral treatments once a day for thirty (30) days and the dosages of EEAB used in this study were in accordance with those reported by [2]:

Group I: received 0.5 ml/100 g of distilled water as control group

Group II: received 62.5 mg/kg of EEAB

Group III: received 125 mg/kg of EEAB

Group IV: received 250 mg/kg of EEAB

Collection of blood samples

Twenty four hours (day 31) after the last dosing of all the groups, blood samples were collected from all the animals through the medial cantus with heparinized capillary tubes into EDTA bottles for hematological and plasma biochemical analyses. Before assays, the blood was centrifuged for 5 minutes using a bench top centrifuge (Centromix) and the plasma were used for the determination of the biochemical parameters.

Determination of hematological parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the Improved Neubauerhemocytometer method. The hemoglobin (Hb) concentration was determined according to⁸ using the cyanomethemoglobin method. The packed cell volume (PCV) was determined by the micro-hematocrit method according to⁹. Schilling method of differential leukocyte count was used to determine the distribution of the various white blood cells¹⁰. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were computed according to⁸.

Determination of plasma biochemical parameters

The total protein concentration was determined using the Biuret method¹¹ and the albumin concentration by the method of¹². The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration. Activities of plasma alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to the method of¹³. The levels of creatinine, urea and alkaline phosphatase were determined using the method of¹⁴. All the above biochemical parameters were determined in the plasma using the Randox kits.

Statistical analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparison between the control and experimental groups was done using one-way

analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The effects of varying doses of EEAB on hematological and plasma biochemical parameters after treatment of rats for 30 days are shown in Tables 1 and 2 respectively.

Treatment of rats with 250 mg/kg caused a significant ($p < 0.05$) increase in PCV value relative to the control, while treatment of rats with 62.5 mg/kg and 125 mg/kg produced no significant ($p > 0.05$) changes in PCV values relative to the control. Treatment of rats with all the doses of EEAB (62.5 mg/kg, 125 mg/kg, 250 mg/kg) produced no significant ($p > 0.05$) changes Hb, RBC, MCV, MCHC, MCH values relative to their respective controls. Treatment of rats with 125 mg/kg and 250 mg/kg of EEAB caused significant ($p < 0.05$) decreases in TWBC counts relative to the control, while treatment of rats with 62.5 mg/kg caused no significant ($p > 0.05$) change in TWBC counts relative to the control. Treatment of rats with 125 mg/kg and 250 mg/kg of EEAB produced significant ($p < 0.05$) decreases in platelet counts relative to the control, while treatment of rats with 62.5 mg/kg caused no significant ($p > 0.05$) change in platelet count relative to the control.

Administration of all the treatment doses of EEAB (62.5 mg/kg, 125 mg/kg and 250 mg/kg) to the rats produced no significant ($p > 0.05$) changes in neutrophil, lymphocyte and eosinophil values relative to their respective controls. Treatment of rats with 250 mg/kg of EEAB caused a significant ($p < 0.05$) increase in monocytes values relative to the control, while 62.5 mg/kg and 125 mg/kg produced no significant ($p > 0.05$) changes in monocytes values relative to the control.

Treatment of rats with 62.5 mg/kg and 125 mg/kg of EEAB caused a significant ($p < 0.05$) increase in total protein level relative to the control, but 250 mg/kg of EEAB caused no significant ($p > 0.05$) change in total protein level relative to the control. Treatment of rats with 62.5 mg/kg and 125 mg/kg of EEAB caused significant ($p < 0.05$) increases in albumin levels relative to the control, while 250 mg/kg of EEAB caused no significant ($p > 0.05$) change in albumin level relative to the control. Administration of 62.5 mg/kg and 125 mg/kg of EEAB to rats produced significant ($p < 0.05$) increases in globulin levels relative to the control, while 250 mg/kg of EEAB caused no significant ($p > 0.05$) change in globulin level. Treatment of rats with 62.5 mg/kg caused a significant ($p < 0.05$) increase in creatinine level relative to the control while 125 mg/kg and 250 mg/kg caused no significant ($p > 0.05$) changes in creatinine level relative to the control. Treatment of rats with 125 mg/kg caused a significant ($p < 0.05$) increase in urea level relative to the control, while 62.5 mg/kg and 250 mg/kg caused no significant ($p > 0.05$) changes in urea level relative to the control.



Table 1: Effect of 30 days treatment with varying doses of EEAB on hematological parameters

Parameters	Control	62.5 mg/kg	125 mg/kg	250 mg/kg
PCV (%)	41.71±2.09	44.00±1.44	44.57±0.84	47.25±1.25*
Hb (g/dL)	13.99±0.73	14.77±0.41	15.03±0.55	15.35±0.57
RBC ($\times 10^6/\mu\text{L}$)	7.06±0.31	7.41±0.26	7.52±0.14	7.82±0.32
MCV (fL)	58.91±0.48	59.42±0.90	59.31±0.30	58.07±2.14
MCHC (g/dL)	37.79±4.15	33.60±0.21	33.65±0.63	32.80±0.37
MCH (pg)	19.69±0.25	19.87±0.32	19.96±0.39	19.87±0.39
TWBC ($\times 10^3/\mu\text{L}$)	8.10±0.74	7.75±0.50	6.00±0.54*	5.25±0.56*
Platelets ($10^5/\mu\text{L}$)	1.78±0.12	1.62±0.06	1.39±0.13*	1.24±0.15*
Neutrophils (%)	33.86±2.76	45.00±9.00	44.43±5.25	46.50±4.29
Lymphocytes (%)	58.86±3.70	48.33±8.53	48.14±4.86	45.00±4.42
Eosinophils (%)	3.86±1.10	3.33±0.72	4.14±0.80	3.00±0.41
Monocytes (%)	3.14±0.63	3.50±0.76	3.71±0.47	5.75±1.11*

*Significant different as compared with control group at $p < 0.05$ **Table 2:** Effect of 30 days treatment with varying doses of EEAB on Plasma Biochemical Parameters

Parameters	Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Total Protein (gm%)	6.93±0.34	8.47±0.29*	8.00±0.22*	7.80±0.24
Albumin (gm%)	4.43±0.10	4.85±0.15*	4.83±0.11*	4.53±0.11
Globulin (gm%)	2.86±0.05	3.62±0.16*	3.46±0.16*	3.28±0.25
Creatinine ($\mu\text{mol/L}$)	0.44±0.05	0.73±0.07*	0.66±0.09	0.38±0.08
Urea (mg/dL)	14.86±0.26	15.50±0.43	16.71±0.29*	14.75±0.48
ALP (IU/L)	94.43±7.65	106.83±9.30	94.14±8.32	97.75±4.13
AST (μL)	38.29±0.42	42.17±1.28*	37.29±0.42	42.00±1.41*
ALT (μL)	27.00±0.44	30.00±0.73*	26.14±0.40	30.50±0.65*

*Significant different as compared with control group at $p < 0.05$

Treatment of rats with all the doses of EEAB (62.5 mg/kg, 125 mg/kg and 250 mg/kg) produced no significant ($p > 0.05$) changes in ALP activity relative to the control. Treatment of rats with 62.5 mg/kg and 250 mg/kg caused significant ($p < 0.05$) increases in AST and ALT activities relative to their respective controls, while 125 mg/kg caused no significant ($p > 0.05$) change in AST and ALT activities relative to their respective controls.

DISCUSSION

The result has shown that the extract has no significant effect on the RBC and indices relating to it (Hb, MCV, MCH and MCHC). This could indicate that the extract does not have the potential to stimulate erythropoietin release from the kidneys which is the humoral regulator of RBC production¹⁵. It could also indicate that there were no changes in the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since RBC and hemoglobin are very important in transferring respiratory gases¹⁶. It has been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia¹⁷; thus, the extract (EEAB) may not have the potential to induce anemia or polycythemia. Also, the extract (EEAB) may not have adverse effects on the bone marrow, kidney and

hemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and hemoglobin metabolism¹⁸. Similar results were reported by¹⁹ in *Fadogiaagretis* extract treated rats. There was a significant increase in the PCV value after treatment of rats with the extract which probably indicates polycythemia. Contrary results were reported by²⁰ in the *A. Cordifolia*, *P. amarus*, *P. muellerianus* and *S. virosa* extracts treated rats. The extract caused significant decrease in the TWBC value; this probably indicates a reduction in the ability of the body to defend against invading organisms²¹. Contrary result was report by²² in *Viscum album* extract treated rats. The extract caused significant decrease in the platelet value which probably indicates a reduction in the hemostatic function of the body. Contrary result was reported by²³ in *Portulacaoleracea* ergosterol isolate treated rats. The extract caused no significant change in neutrophil count which probably indicates that it has no effect in the ability of the body to attack and destroy invading bacteria, viruses and other injurious agents (phagocytosis). Contrary result was reported by²⁴ in *Dennettiatripetala* extract treated rats. The extract caused no significant change in lymphocyte value which probably indicates that



it has no effect in the acquired immune response of the body. Contrary result was reported by²⁵ in *Pelargonium reniforme* extract treated rats. The extract caused no significant change in eosinophil value which could indicate that it has no effect in the anti-allergic and anti-parasitic infectious responses of the body. Contrary result was reported by²⁶ in *Arctotisactotoides* extract treated rats and mice. The extract induced a significant increase in monocyte value which probably indicates an enhancement in the phagocytic function of the body²⁷. Contrary result was reported by²⁸ in Neem (*Azadirachtaindica*) treated chickens.

The results of the plasma biochemical study have shown that treatment of rats with the extract caused significant increase in the total protein level. This could indicate an enhancement in the buffering capacity of blood as well as an increase in colloid osmotic pressure which could prevent loss of fluid from the capillaries, since plasma proteins have been reported to be responsible for 15 % of the buffering capacity of blood²¹ and that osmotic pressure caused by the plasma proteins (called colloid osmotic pressure) tends to cause fluid movement by osmosis from the interstitial spaces into the blood²⁷. Similar result was reported by²⁹ in the *Perseaamericana* extract treated rats. The significant increase in the albumin level induced by the extract could indicate an increase in the plasma levels of metals, ions, fatty acids, amino acids, bilirubin and enzymes; since it has been reported that albumin serves as a carrier for metals, ions, fatty acids, amino acids, bilirubin, enzymes and drugs²¹. Similar result was reported by³⁰ in *Enicostemmaaxillare* extract treated rats. The extract produced a significant increase in the globulin level which probably indicates an enhancement in both the natural and acquired immunity of the body against invading organisms, since it has been reported that globulins are principally responsible for the body's both natural and acquired immunity against invading organisms²⁷. Similar result was reported by²⁵ in *Pelargoniumreniforme* extract treated rats. The extract caused significant increase in creatinine level. This probably indicates the induction of renal impairments by the extract since creatinine levels is used to measure the extent of renal impairment. Similar result was reported by³¹ in rats treated with *Mucunapruriens* extract. The extract caused significant increase in urea level. This probably indicates nephrotoxicism, since urea and creatinine have been reported to be markers of kidney functions³². Contrary result was reported by³³ in *Passifloraedulis* extract treated rats. The extract caused no significant change in ALP level. This probably indicates the absence of cholestasis, since ALP has been reported to be a marker of cholestasis³⁴. Similar result was reported by³⁵ in *Mangiferaindica* extract treated rats.

The significant increase in the activity of AST caused by the extract could indicate an induction of tissue necrosis, since it has been reported that elevation in the activity of AST can be associated with cell necrosis of many tissues, pathology involving the skeletal or cardiac muscle and /

or hepatic parenchyma, allows leakage of large amounts of this enzyme into the blood³⁶. Contrary result was reported by³⁷ in *Murrayakoenigii* extracts treated rats. The extract caused a significant increase in the activity of ALT which probably indicates an induction of hepatic damage, since it has been reported that ALT is present in the liver and other cells and is particularly useful in measuring hepatic necrosis, especially in small animals¹³. Similar result was reported by³⁸ in *Phyllantusamarus* extract treated rats.

CONCLUSION

In conclusion, this study has shown that the crude extract of *Adenopus breviflorus* has a little toxic effect and a lot of beneficial potentialities on the hematological functions and blood chemistry of rats. However, the effect of crude extract of this fruit on human hematological functions and blood chemistry are unknown; nevertheless, considering these findings in animal model, it is recommended that caution should be exercised by the locals in the consumption of *Adenopus breviflorus* as a blood tonic in order to prevent nephrotoxicity and hepatotoxicity.

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