



# **Therapeutic Effect of Ethanol Extract of *Anthocleista vogelii* Stem Bark in the Treatment of Jaundice on Paracetamol-induced Hepatotoxicity in Adult Wistar Rats**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

This study investigated therapeutic effect of ethanol extract of *Anthocleista vogelii* stem bark (EEAV) in the treatment of jaundice on paracetamol-induced hepatotoxicity in adult wistar rats. A total of 30 wistar rats weighing between 160-200 g were distributed into 6 groups comprising group 1 (normal control), group 2 (paracetamol 2 g/Kg bw), group 3 (paracetamol 2 g/Kg bw + Silymarin 100mg/Kg bw) and groups 4-6 (paracetamol 2 g/Kg bw each + 250, 500, & 1000 mg/Kg bw of EEAV, respectively). Experiment lasted for a period of 16 days. Paracetamol induction elevated liver indices and enzyme activities. However, administration of EEAV significantly reduced ( $p \leq 0.05$ ) these effects in groups 3-6 when compared to group 2. A significant elevation ( $p \leq 0.05$ ) was reported in creatinine, urea, sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ) when group 2 was compared with group 1. Haematological study showed that packed cell volume (PCV), haemoglobin (Hb) and red blood cell (RBC) levels significantly increased ( $p \leq 0.05$ ) in treatment groups 3-6 when compared to group 2. The catalase (CAT) activity increased significantly ( $p \leq 0.05$ ) in groups 3, 4 and 6 when compared to group 2. Liver histology showed normal hepatocytes architecture with normal central vein in group 1.

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Group 2 revealed a histologically distorted liver tissue while there was regeneration of hepatic cells in groups 3-6. The study revealed that EEA V could serve as potent herbal therapeutic agent in the treatment of jaundice triggered by paracetamol induction in adult wistar rats.

**Keywords:** Ethanol extract; *Anthocleista vogelii*; stem bark; paracetamol; hepatotoxicity; jaundice; wistar rats.

## 1. INTRODUCTION

The role of herbal medicine in meeting healthcare needs of the populace especially those from developing countries cannot be over emphasized. Sizeable numbers of the people living in developing nations still depend on the use of traditional medicine derived from plants [1]. The history on the use of traditional medicine to manage numerous diseases is as old as human existence [2]. People living in rural areas, depend more on traditional medicine as modern systems are either not affordable or out of reach [1]. The affordability and accessibility of traditional medicine in meeting primary health care needs have endeared the people throughout Africa, Asia and Latin America to it. Traditional medicine is considered as part of everyday life and well-being for some people, which is also often part of a wider belief system for them [3]. According to World Health Organization [4] there has been tremendous increase in the patronage of traditional medicine in the last twenty years. However, there is still a significant lack of research data in this field. Going by the current trend of awareness and acceptability, medicinal plants have a positive prospect and future because a good number of the plants are yet to be studied in medical practice [2]. So, plants which are considered to have chemical components that are potential sources of drug are recommended for further scientific research to discover more of its medicinal potential.

Several researches have been conducted on *Anthocleista vogelii* plant and different parts of the plant are identified in ethno-medicine to be effective in the management and treatment of various diseases [5]. It is commonly found in the tropical rainforest (Fig. 1). They are effective in treating stomach aches, constipation, haemorrhoids, malaria, fever, diabetes, typhoid hypertension, syphilis etc. [6]. Besides their medicinal uses, *A. vogelii*'s leaf is found suitable in the making of cleaners, tobacco, abrasives, snuff etc. Its wood is also utilized in making inks, mordants, dyes, tattoos and stains; the timber is utilized in carving and similar applications; the

branches and trunk are used in forestry, hunting, farming, and in making of fishing tool [7]. Phytochemicals such as steroids, terpenoids, saponins, alkaloids and flavonoids are found in the root, stem bark and leaf of *A. vogeli* [8]. It has been observed that the aqueous stem bark of *A. vogelii* provoked an LD50 greater than 5000 mg/kg bw in an acute toxicity study [9]. Various researches have shown that jaundice rank high among most conditions that present patients with liver and biliary diseases and other extra hepatobiliary causes [10]. The liver is susceptible to toxic effects associated with air pollutants and drugs such as paracetamol According to Bishayi et al. [11]. The cause of jaundice is the imbalance between bilirubin production and excretion and they further described it as the appearance of yellow pigmentation in the skin, sclera and mucous membranes. Although, jaundice happens to people of all ages but neonatal jaundice has been a regular occurrence in more than 60% of normal newborns during their first seven days of life [12]. Thus, there is urgent need to investigate medicinal plants that may confer protective effects on the liver. This study therefore, investigated the therapeutic effect of ethanol extract of *Anthocleista vogelii* stem bark (EEAV) in the treatment of jaundice on paracetamol-induced hepatotoxicity in adult wistar rats.



**Fig. 1. *Anthocleista vogelii* plant**  
Source:[http://www.westafricanplants.senckenberg.de/root/index.php?page\\_id=14&id=3459](http://www.westafricanplants.senckenberg.de/root/index.php?page_id=14&id=3459)(retrieved 12/12/19)

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Plant sample collection

The fresh stem barks of *Anthocleista vogelii* were collected around the vicinity of University of Port Harcourt, Rivers State, Nigeria in the month of May, 2019. Plant species was identified in the Herbarium of Plant Science and Biotechnology Department, University of Port-Harcourt, Nigeria.

#### 2.1.2 Experimental animals

A total of thirty (30) Wistar adult rats weighing between 160 g and 200 g were used in this study. They were bought, housed and acclimatized in the animal house of Biochemistry Department, University of Port Harcourt, Rivers State, Nigeria. Animals were kept under normal temperature and humidity, and half-day light and half-day darkness cycle. During acclimatization, the animals had free access to animal grower feed and clean water *ad libitum*.

### 2.2 Methods

#### 2.2.1 Preparation of plant sample and extraction

The plant stem barks were sorted to remove debris and air-dried under shade at normal room temperature for two weeks. The dried stem barks were powdered using an electric grinder with a 60µm mesh size, neatly packed and subsequently used for extraction. Extraction was carried out using hot Soxhlet extraction methods according to Erhman [13] as modified by Onyeike and Ayalogu [14]. The powdered plant materials were weighed by sensitive digital weighing balance. Six hundred (600) grams of powdered sample (stem bark) was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was then placed in a conical flask and one litre of 80% ethanol added. Extraction was carried out for one hour and this was concentrated in a rotary evaporator at 50°C for 30 minutes. The yield of the extract was 3.8% w/w.

#### 2.3 Experimental Design

The animals were distributed into six (6) groups of five (5) rats each comprising group 1(normal control, fed with rat feed + water), group 2-negative control (paracetamol 2 g/Kg bw), group 3-positive control (paracetamol 2 g/Kg bw. and silymarin suspension 100 mg/Kg bw orally),

group 4 (paracetamol 2 g/Kg bw and 250 mg/Kg bw of EEAV orally), Group 5(paracetamol 2 g/kg b.w. and 500 mg/Kg bw of EEAV orally), and Group 6(paracetamol 2 g/Kg bw and 1000 mg/Kg bw of EEAV orally). Paracetamol (2 g/Kg bw orally) was administered for 5 days. After which, either silymarin or EEAV was administered for 10 days, based on the experimental design. Aqueous stem bark of *A. vogelii* has been reported to provoke an LD<sub>50</sub> greater than 5000 mg/kg in an acute toxicity study [9], which informed the choice of doses for this study.

#### 2.4 Collection of Blood and Tissue Organs for Analysis

The animals were anaesthetized and sacrificed on day 16. The blood samples were collected by cutting the jugular vein of the rats. The blood samples for biochemical analyses were collected in a plain bottle and left to coagulate and the coagulated blood samples were centrifuged at 3,000rpm for 15mins. Serum was separated and used for the kidney indices (creatinine and urea) and liver enzymes (ALP, AST and ALT) analysis [15]. The blood samples for haematological indices were stored in ethylene diaminetetraacetate (EDTA) bottles and automated haematology analyzer (BC-3200) was used to assess the haematological parameters [16]. The liver were homogenized and kept in normal saline in plain bottles for antioxidant (GSH, CAT, SOD and MDA) analysis by the method of Kunwar and Priyadarsini [17], while the livers were kept in 10% formalin in plain bottles for histopathological studies [18]. Biuret method was used for Total Protein (TP) analysis, while albumin (ALB) concentration was analysed using the bromocresol green (BCG) method. Serum Total Bilirubin (TB) was analysed spectrophotometrically at 578 nm, while creatinine and urea were measured using PriestestEasyLab Biochemistry Analyzer. Electrolytes were estimated using automated EUC machine Mindrays BS 800 Chemistry [19].

#### 2.5 Histological Examination

The liver of the experimental animals were subjected to histological analysis using the method of Kumar et al. [18].

#### 2.6 Statistical Analysis

Results were analyzed using Statistical Package for Social Science (IBM-SPSS version 22). Significant difference between the treatment

groups and the control groups was determined using one way analysis of variance (ANOVA). A probability value of less than 5% ( $p \leq 0.05$ ) was considered to be significant, and values were expressed as means  $\pm$  standard deviation.

### 3. RESULTS

#### 3.1 Effect of EEAV on Liver Indices

The effect of EEAV on some liver indices of paracetamol-induced hepatotoxicity in rats is as presented in Table 1. Paracetamol caused significant increase ( $p \leq 0.05$ ) in total bilirubin (TB) and conjugated bilirubin (CB) levels in all animals when negative control group was compared to the normal control group. However, the effect was not significant in both the total protein and albumin levels. There were significant decreases ( $p \leq 0.05$ ) in the levels of TB and CB at all the treatment doses (250 mg, 500 mg, and 1000 mg) of EEAV when compared to the negative control group. Both the standard drug and the plant extract (at different concentrations) reflected a decrease in Total Protein (TP) and albumin (ALB) levels when compared to the negative control group.

#### 3.2 Effect of EEAV on Liver Enzymes

The effect of EEAV on some liver enzymes of paracetamol-induced hepatotoxicity in rats is as presented in Table 2. There were significant increases ( $p \leq 0.05$ ) in liver enzymes (AST, ALT and ALP) in negative control group when compared to normal control group. The positive control group also showed significant changes ( $p \leq 0.05$ ) in all the liver enzymes in comparison to the negative control group. Different dose levels of 250 mg and 500 mg of EEAV also presented a significant difference ( $p \leq 0.05$ ) for both ALT and ALP when compared to the negative control

group. There was no significant change ( $p \geq 0.05$ ) on the AST in all the doses of EEAV when compared to negative control group.

#### 3.3 Effect of EEAV on Kidney Indices

The effect of EEAV on some kidney parameters of paracetamol-induced hepatotoxicity in rats is as presented in Table 3. A significant elevation ( $p \leq 0.05$ ) was reported in Creatinine, Urea, Sodium ( $\text{Na}^+$ ), and Chloride ( $\text{Cl}^-$ ) when group 2 was compared with group 1. Meanwhile, no significant change ( $p \geq 0.05$ ) was observed in Potassium ( $\text{K}^+$ ) and Bicarbonate ( $\text{HCO}_3^-$ ) when group 2 was compared with group 1. The administration of the standard drug (Silymarin) caused significant changes ( $p \leq 0.05$ ) in creatinine, sodium, urea, and chloride levels when compared with group 2. Whereas, no significant difference ( $p \geq 0.05$ ) was observed in the levels of bicarbonate and potassium when group 3 was compared with group 2. Also, no significant change ( $p \geq 0.05$ ) was observed in creatinine, potassium, sodium, and bicarbonate levels when all the doses of the extract were compared with group 2. At 500 mg of EEAV, a significant reduction ( $p \leq 0.05$ ) was observed in chloride when compared with group 2. All the doses of the extract present a significant decrease ( $p \leq 0.05$ ) in urea level when compared with group 2.

#### 3.4 Effect of EEAV on Hematological Parameters

The effect of EEAV on some hematological parameters of paracetamol-induced hepatotoxicity in rats is as shown in Table 4. The paracetamol caused significant reduction ( $p \leq 0.05$ ) in the PCV, Hb and RBC levels, whereas there was significant increase ( $p \leq 0.05$ ) in the WBC and the platelet levels. The

**Table 1. Effect of EEAV on some liver indices of paracetamol-induced hepatotoxicity in wistar rats**

Group	TB ( $\mu\text{mol/l}$ )	CB ( $\mu\text{mol/l}$ )	TP (g/l)	ALB (g/l)
Normal Control	10.80 $\pm$ 0.82 <sup>d</sup>	4.46 $\pm$ 0.98 <sup>d</sup>	70.80 $\pm$ 2.86 <sup>a</sup>	35.60 $\pm$ 3.71 <sup>a</sup>
Negative Control	21.64 $\pm$ 4.82 <sup>a</sup>	8.52 $\pm$ 1.02 <sup>a</sup>	71.00 $\pm$ 6.44 <sup>b</sup>	40.20 $\pm$ 3.74 <sup>b</sup>
Positive Control	2.88 $\pm$ 0.33 <sup>ab</sup>	1.70 $\pm$ 0.12 <sup>ab</sup>	56.60 $\pm$ 1.34 <sup>ab</sup>	36.40 $\pm$ 2.51 <sup>ab</sup>
250 mg EEAV	5.26 $\pm$ 0.78 <sup>ab</sup>	3.14 $\pm$ 0.35 <sup>b</sup>	61.80 $\pm$ 3.83 <sup>ab</sup>	36.00 $\pm$ 0.84 <sup>ab</sup>
500 mg EEAV	4.26 $\pm$ 0.49 <sup>ab</sup>	2.98 $\pm$ 0.29 <sup>b</sup>	52.80 $\pm$ 2.95 <sup>ab</sup>	37.40 $\pm$ 1.67 <sup>ab</sup>
1000 mg EEAV	4.58 $\pm$ 1.04 <sup>ab</sup>	3.16 $\pm$ 0.80 <sup>b</sup>	53.60 $\pm$ 1.34 <sup>ab</sup>	28.60 $\pm$ 1.34 <sup>c</sup>

Values represent Mean  $\pm$  Standard Deviation,  $n=5$ . Superscript (a) represents a significant difference from normal control group at  $p \leq 0.05$  down the column; Superscript (b) represents a significant difference from negative control group at  $p \leq 0.05$  down the column. Superscript (c) represents not significant difference from normal and negative control. TB= Total bilirubin, CB= Conjugate bilirubin, TP= Total protein, ALB= Albumin

**Table 2. Effect of EEAV on liver enzymes of paracetamol-induced hepatotoxicity in wistar rats**

Group	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal Control	91.20±12.60 <sup>b</sup>	20.40±1.34 <sup>b</sup>	53.00±3.67 <sup>b</sup>
Negative Control	115.00±0.00 <sup>a</sup>	35.60±1.34 <sup>a</sup>	85.00±15.92 <sup>a</sup>
Positive Control	99.60±5.37 <sup>b</sup>	17.80±1.64 <sup>b</sup>	30.60±1.34 <sup>ab</sup>
250 mg EEAV	111.20±7.43 <sup>a</sup>	22.80±3.83 <sup>b</sup>	37.80±11.14 <sup>b</sup>
500 mg EEAV	108.40±12.68	13.60±1.34 <sup>b</sup>	35.60±7.23 <sup>b</sup>
1000 mg EEAV	117.60±6.54 <sup>a</sup>	27.60±4.67	41.40±6.66 <sup>b</sup>

Values represent Mean ± Standard Deviation, n=5. Superscript (a) represents a significant difference from normal control group at  $p \leq 0.05$  down the column; Superscript (b) represents a significant difference from negative control group at  $p \leq 0.05$  down the column. AST= Aspartate transaminase, ALT= Alanine transaminase, ALP= Alkaline phosphatase

**Table 3. Effect of EEAV on kidney indices of paracetamol-induced hepatotoxicity in wistar rats**

Group	Creatinine (mmol/l)	Urea ( $\mu\text{mol/l}$ )	Na <sup>+</sup> (mmol/l)	K <sup>+</sup> (mmol/l)	Cl <sup>-</sup> (mmol/l)	HCO <sub>3</sub> <sup>-</sup> (mmol/l)
Normal Control	125.60±9.81	3.54±1.27 <sup>b</sup>	117.67±2.52	3.03±0.21	94.00±2.00 <sup>b</sup>	24.00±2.00 <sup>a</sup>
Negative Control	139.00±8.22 <sup>a</sup>	8.58±0.96 <sup>a</sup>	136.67±10.41 <sup>a</sup>	4.47±1.31	108.00±5.29 <sup>a</sup>	26.67±1.15
Positive Control	122.40±9.40	2.92±0.20 <sup>b</sup>	125.00±11.79	4.20±1.18	95.33±3.51	25.33±1.15
250 mg EEAV	149.80±3.27 <sup>a</sup>	6.82±0.33 <sup>ab</sup>	150.67±4.04 <sup>a</sup>	6.03±0.76	98.00±6.00	24.00±3.45
500 mg EEAV	131.80±2.05	3.40±0.30 <sup>b</sup>	132.33±2.52	5.06±0.59	94.00±1.00 <sup>b</sup>	24.67±1.15
1000 mg EEAV	152.60±7.37 <sup>a</sup>	3.22±0.20 <sup>b</sup>	154.67±9.29 <sup>a</sup>	6.57±1.10 <sup>a</sup>	95.33±3.51	28.00±2.00

Values represent Mean ± Standard Deviation, n=5. Superscript (a) denotes a significant difference from normal control group at  $p \leq 0.05$  down the column; Superscript (b) denotes a significant difference from negative control group at  $p \leq 0.05$  down the column

**Table 4. Effect of EEAV on haematological parameters of paracetamol-induced hepatotoxicity in rats**

Group	PCV (%)	Hb (g/dl)	RBC ( $\times 10^{12}/\text{l}$ )	WBC ( $\times 10^9/\text{l}$ )	Platelet ( $\times 10^9/\text{l}$ )
Normal Control	38.40±1.67 <sup>b</sup>	13.48±1.42 <sup>b</sup>	5.56±0.13 <sup>b</sup>	9.30±1.02 <sup>b</sup>	189.00±24.90 <sup>b</sup>
Negative Control	26.00±2.45 <sup>a</sup>	8.68±0.82 <sup>a</sup>	3.84±0.21 <sup>a</sup>	13.64±1.96 <sup>a</sup>	248.80±15.58 <sup>a</sup>
Positive Control	36.80±2.95 <sup>b</sup>	12.14±1.06 <sup>b</sup>	5.14±0.70 <sup>b</sup>	12.22±1.15	267.20±19.70
250mg EEAV	42.80±2.17 <sup>b</sup>	14.26±0.73 <sup>b</sup>	6.08±0.22 <sup>b</sup>	7.10±0.30 <sup>b</sup>	191.00±22.75 <sup>b</sup>
500mg EEAV	37.60±2.07 <sup>b</sup>	12.54±0.71 <sup>b</sup>	5.44±0.37 <sup>b</sup>	9.82±1.06 <sup>b</sup>	233.20±18.44
1000mg EEAV	38.20±0.84 <sup>b</sup>	12.74±0.29 <sup>b</sup>	5.56±0.25 <sup>b</sup>	9.82±1.06 <sup>b</sup>	232.40±17.29

Values represent Mean ± Standard Deviation, n=5. Superscript (a) represents a significant difference from normal control group at  $p \leq 0.05$  down the column; Superscript (b) represents a significant difference from negative control group at  $p \leq 0.05$  down the column

plant extract had significant changes ( $p \leq 0.05$ ) at 250 mg/Kg bw of EEAV in all the haematological parameters when compared to negative control group. At 500 mg/Kg bw and 1000 mg/Kg bw of EEAV, the plant extract had a significant difference ( $p \leq 0.05$ ) in PCV, Hb, RBC and WBC in comparison to negative control group. There was no significant change ( $p \geq 0.05$ ) in the platelet levels for both 500 mg and 1000 mg of EEAV when compared to negative control group.

### 3.5 Effect of EEAV on Oxidative Stress Markers

The effect of EEAV on some oxidative stress markers of paracetamol-induced hepatotoxicity in rats is as presented in Table 5. There was a

significant decrease ( $p \leq 0.05$ ) in GSH, CAT, and SOD in negative control group when compared to normal control group. There was also a significant increase ( $p \leq 0.05$ ) in MDA in negative control group when compared to normal control group. Positive control (group 3) gave a significant increase ( $p \leq 0.05$ ) in CAT and SOD when compared to the negative control (group 2). There was a significant increase ( $p \leq 0.05$ ) in CAT at 250 mg and 1000 mg of the extract when compared to negative control group.

### 3.6 Histopathological Results on the Liver

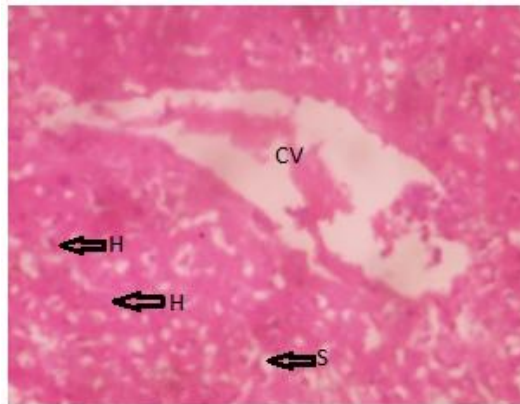
Histopathological study revealed that control group showed normal hepatic cells with normal

central vein patent. Whereas, negative control group showed a histologically distorted liver tissue. The histopathological examination of treatment groups administered with silymarin and various doses of EEAV showed histologically regenerated liver tissues.

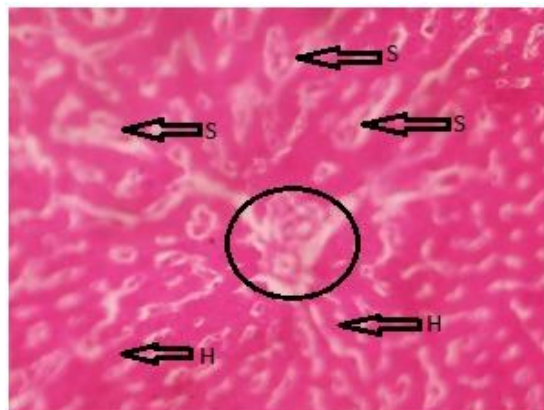
**Table 5. Effect of EEAV on oxidative stress markers of paracetamol-induced hepatotoxicity in wistar rats**

Group	GSH (u/ml)	CAT (u/g)	SOD (u/ml/l)	MDA (µmol/ml)
Normal Control	1.42±0.35 <sup>b</sup>	2.43±0.78 <sup>b</sup>	0.71±0.04 <sup>b</sup>	0.28±0.04 <sup>b</sup>
Negative Control	1.25±0.17 <sup>a</sup>	1.24±0.18 <sup>a</sup>	0.43±0.09 <sup>a</sup>	0.55±0.08 <sup>a</sup>
Positive Control	1.33±0.19	2.49±0.80 <sup>b</sup>	0.68±0.08 <sup>b</sup>	0.46±0.13 <sup>b</sup>
250 mg EEAV	1.46±0.06	1.71±0.75 <sup>b</sup>	0.56±0.10 <sup>b</sup>	0.44±0.08 <sup>b</sup>
500 mg EEAV	1.54±0.21 <sup>b</sup>	1.34±0.38 <sup>a</sup>	0.48±0.11 <sup>a</sup>	0.72±0.12 <sup>c</sup>
1000 mg EEAV	1.31±0.09	1.94±0.71 <sup>b</sup>	0.41±0.17 <sup>a</sup>	0.69±0.10 <sup>c</sup>

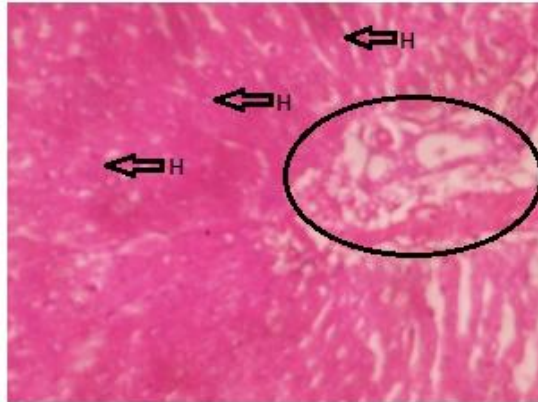
Values represent Mean ± Standard Deviation, n=5. Superscript (a) represents a significant difference from normal control group at p≤0.05 down the column; Superscript (b) represents a significant difference from negative control group at p≤0.05 down the column. Superscript (c) represents not significant difference from normal and negative control



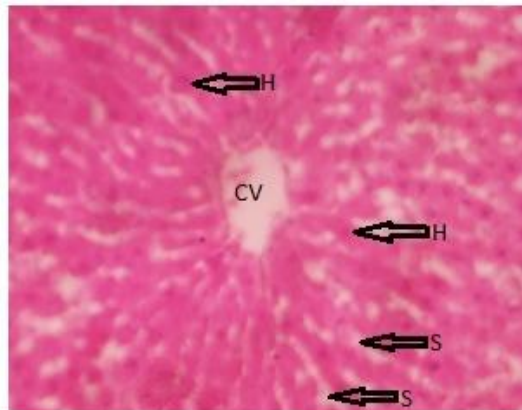
**Plate 1. Photomicrograph of a liver tissue from normal control group (Group 1). H&E staining; magnification X400. This shows a histologically normal liver tissue with central vein patent. The sinusoids and hepatocytes are normal and arrowed**



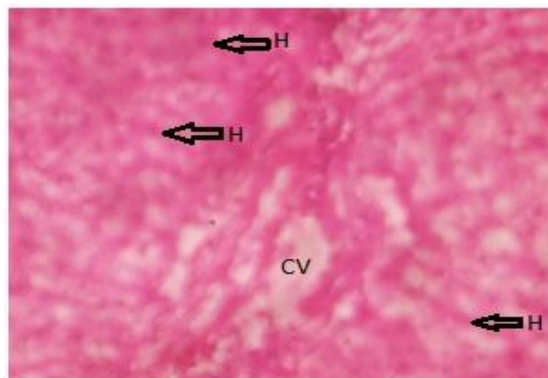
**Plate 2. Photomicrograph of a liver tissue from negative control group (Group 2). H&E staining; magnification X400. The findings showed a histologically distorted liver tissue with normal hepatocytes. Normal portal triad encircled but the sinusoids are filled with inflammatory cells**



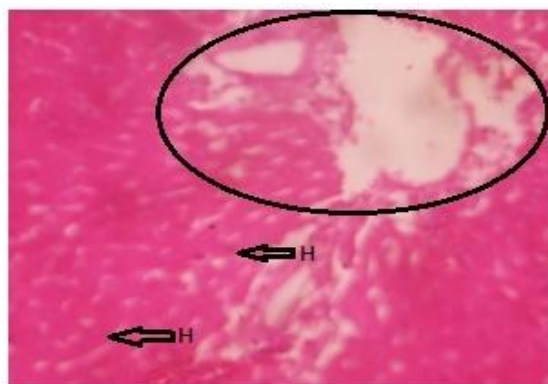
**Plate 3.** Photomicrograph of a liver tissue from positive control group (Group 3). H&E staining; magnification X400. This revealed a histologically regenerated liver tissue with central vein patent. The hepatocytes are normal and arrowed



**Plate 4.** Photomicrograph of a liver tissue from Group 4 (EEAV 250 mg/kg). H&E staining; magnification X400. The findings showed a histologically regenerated liver tissue showing central vein (CV) patent. Cords of hepatocytes (liver cells) radiating away from CV. The sinusoids are well dilated



**Plate 5.** Photomicrograph of a liver tissue from Group 5 (EEAV 500 mg/kg). H&E staining; magnification X400. The findings revealed a histologically regenerated liver tissue showing central vein (CV) patent, normal hepatocytes, and with a well dilated sinusoids



**Plate 6. Photomicrograph of a liver tissue from Group 6 (EEAV 1000 mg/kg). H&E staining; magnification X400. The result showed a histologically regenerated liver tissue. The portal tract is encircled and normal hepatocytes arrowed**

#### 4. DISCUSSION

Paracetamol is a commonly-used antipyretic and analgesic drug, mostly available over-the-counter. It is highly effective but can result to liver damage if taken in overdose [20]. Previous studies have shown that paracetamol-induced hyperbilirubinemic rats showed significant elevation in total bilirubin levels an indication of hepatotoxicity which is associated with a condition called jaundice. Induction with paracetamol leads to excess hemolysis of the red blood cells (RBCs) which led to over production of the bilirubin thereby causing hyperbilirubinemia [21]. The present work shows that paracetamol-treated rats showed significant elevation in conjugated bilirubin, total bilirubin, ALT, AST and ALP activities and this is a pointer to severe hepatocyte necrosis because of generation of N-Acetyl-P-Benzoquinone Imine (NAPQI) and saturation of glucuronide and sulfate routes of metabolism of paracetamol [22]. Meanwhile, most of the elevated liver indices were significantly reduced by all the doses of EEAV. Serum bilirubin examination was to test liver's ability to metabolize drugs and transport organic anions [23]. An underlying disease is suspected when liver function examination results are abnormal and level of total bilirubin is more than 17  $\mu\text{mol/L}$  [24]. According to Jegede et al. [8] the antioxidant, antihepatotoxicity, free radical scavenging, and reno-protective properties of the plant can be explained based on the presence of active constituents like saponins, flavonoids, steroids, terpenoids, and alkaloids in the plant stem bark.

When there is liver damage caused by overdose of paracetamol, it affects hepatocytes transport

function and causes plasma membrane leakage, thereby resulting in an increase serum hepatic enzyme levels. The ALT, AST and ALP are important serum pointers to liver injury that are normally considered to monitor the function of the liver [25]. In this study, the 2g/Kg bw of paracetamol, resulted in significant elevation ( $p \leq 0.05$ ) of levels of liver enzymes like AST, ALT and ALP when compared with group 1, as anticipated. The administration of silymarin also showed significant decrease ( $p \leq 0.05$ ) in all the liver enzymes in comparison to group 2. The extract (EEAV) at 250 mg and 500 mg doses also decreased the levels of both ALT and ALP significantly when compared with group 2. However, no significant changes ( $p \geq 0.05$ ) were recorded in all the doses of EEAV on the AST when compared with group 2. EEAV at 1000mg dose level presented a significant change ( $p \leq 0.05$ ) only in ALP when compared with group 2 (Table 2). Hepatocellular damage leads to high level of ALT and AST in the bloodstream. However, elevations in ALT levels generally are more specific for liver damage than AST because ALT is found mainly in the liver, while AST is not only found in the liver but also in erythrocytes and skeletal muscles [26].

Creatinine estimation is necessary in jaundice investigation because creatinine measurement is negatively interfered by bilirubin [27]. When the kidneys are not working well, creatinine stays in the blood. In this study, significant increase ( $p \leq 0.05$ ) was noticed in creatinine level when group 2 was compared with group 1. Silymarin was able to restore the level of creatinine to normal but the extract (EEAV) at various doses did not make any significant change. According to Chaudhary [27], elevated urea levels denote



reduced kidney function. Urea clearance is the amount of blood, which contains the urea excreted in a minute by kidneys. In this study, urea level also increased significantly ( $p \leq 0.05$ ) when group 2 was compared with the group 1. The standard drug and the extract, at all doses, were able to reduce the urea, at 500 mg/kg and 1000 mg/kg dose levels. High levels of electrolytes [Sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), bicarbonate ( $\text{HCO}_3^-$ ), and Chloride ( $\text{Cl}^-$ )] in the body are pointers to kidney dysfunction. In this work, all the electrolytes except bicarbonate were elevated when group 2 was compared with group 1. The use of the standard drug (Silymarin) caused significant changes ( $p \leq 0.05$ ) in sodium and chloride levels when group 3 was compared to group 2. However, no significant difference ( $p \geq 0.05$ ) was recorded in the levels of potassium and bicarbonate when group 3 was compared with group 2. The extract (EEAV) had no significant effect on the levels of potassium, sodium and bicarbonate when all the doses of the extract were compared with group 2.

Liver disease usually, comes with various hematological abnormalities and research has shown that high dose of paracetamol causes alteration in the hematological parameters in rats [28]. In this present study, paracetamol-induced hepatic damage caused a significant reduction of hematological parameters like PCV, Hb, and RBC as compared to group 1. Though an increase was found in WBC and platelets levels. WBC are cells in the blood that help fight infections and some diseases. When the volume of white cells in the blood is more than normal, it is called leukocytosis, and it usually happens when there is an infection in the body [29]. The results clearly revealed that paracetamol caused destruction of RBC, decrease of Hb concentration because of reduction in the ability of the blood to transport oxygen and depletion of the volume of oxygen delivered to the tissues. This is also responsible for decreased PCV value in paracetamol group [25]. The administration of silymarin restored the PCV, Hb, and RBC to control baseline. After treatment with EEAV for 10 days, there was significant improvement, even better than the standard drug (silymarin), in all the haematological parameters for all the doses of the extract. The 250 mg/Kg bw gave the highest levels for PCV, Hb and RBC.

Antioxidants (flavonoids, polyphenols and carotenoid) are produced by organisms to inhibit the unrestrained generation of oxidants and triggered oxygen species, or prevent their reactions with biological structures [30,31]. They

include superoxide dismutase (SOD) that decontaminates the superoxide ion, catalase (CAT), which handles hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and glutathione peroxidase (GPx) that detoxifies cellular peroxides. These enzymes frustrate oxidative damage, aim to destroy damaged molecules and replace damaged ones [32]. The outcomes of this work reported a significant decrease ( $p \leq 0.05$ ) in reduced glutathione (GSH), superoxide peroxidase (SOD), and catalase (CAT) in paracetamol-hepatic-damage-induced group in comparison with normal control group. The extract (EEAV) increased the GSH and the CAT significantly ( $p \leq 0.05$ ) at 250 mg/Kg bw, whereas 1000 mg/Kg bw dose of the extract increased the CAT significantly when compared to negative control group. The GSH was also increased significantly ( $p \leq 0.05$ ) by the extract at the dose of 500 mg/Kg bw as compared to negative control group. According to Dwivedi et al. [25], malondialdehyde (MDA) level is commonly evaluated to determine oxidant production in terms of lipid peroxidation injury of the liver when treated with paracetamol. The oxidant enzymes are also enhanced along with protein oxidation during liver tissue injury caused by paracetamol. Findings from this study, showed a significant increase ( $p \leq 0.05$ ) in MDA when group 2 was compared with group 1. Also, the extract further increased the level of MDA significantly at 500 mg/Kg bw and 1000 mg/Kg bw when compared to group 1.

Histopathological examination is carried out in order to recognize specific microscopic structural changes in a diseased organ tissue. Histopathological study revealed that normal control group showed normal hepatic cells with normal central vein patent. The hepatocytes and sinusoids are normal and arrowed. Whereas, negative control group showed a histologically distorted liver tissue with normal hepatocytes. According to Thapa and Walia [24], hyperbilirubinemia caused by acute viral hepatitis is directly proportional to the degree of histological injury of hepatocytes. The histopathological examination of treatment groups administered with various doses of EEAV showed a histologically regenerated liver tissues showing central vein (CV) patent. Cords of hepatocytes (liver cells) radiating away from CV and the sinusoids are well dilated.

## 5. CONCLUSIONS

The outcome of this study clearly indicates that the ethanol extract of the stem bark of *Anthocleista vogelii* (EEAV) ameliorated the

jaundiced condition triggered by paracetamol-induced hepatotoxicity via the restoration of the elevated bilirubin levels as well as other biochemical parameters. The plant therefore could serve as potent herbal drug for the correction of jaundiced condition.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

As per international standard or university standard guideline written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Oladele AT, Alade GO, Omobuwajo OR. Medicinal plants conservation and cultivation by traditional medicine practitioners (TMPs) in Aiyedaade Local Government Area of Osun State. *Agriculture and Biology Journal of North America*. 2011; 2(3):476-487.
2. Jamshidi-Kia F, Zahra Lorigooni Z, Hossein Amini-Khoei H. Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*. 2018; 7(1):1-7.
3. Ekeopara CA, Ugoha AM. The contributions of African traditional medicine to Nigeria's health care delivery system. *IOSR Journal of Humanities and Social Science*. 2017;22(5):32-43.
4. World Health Organization. WHO monographs on selected medicinal plants. World Health Organization. 2005;4.
5. Igoli JO, Ogaji OG, Tor-Anyiin TA, Igoli NP. Traditional medicine practice amongst the Igede People of Nigeria. Part II. *Journal of Traditional Complementary and Alternative Medicines*. 2005;2(2):134-152.
6. Olubomehin OO, Abo KA, Ajaiyeoba EO. Alpha-amylase inhibitory activity of two *Anthocleista* species and *in vivo* rat model anti-diabetic activities of *Anthocleista djalonensis* extracts and fractions. *Journal of Ethnopharmacol*. 2013;146:811-814.
7. Anyanwu GO, Nisar-ur-Rehman RT, Onyeneke CE, Rau K. Medicinal plants of the genus *Anthocleista*- A review of their ethnobotany, phytochemistry and pharmacology. *Journal of Ethnopharmacology*. 2015;175:648-667.
8. Jegede IA, Ibrahim JA, Kunle OF. Phytochemical and pharmacognostic studies of the leaf and stem-bark of *Anthocleista vogelii* (Planch). *Journal of Medical Plant Resources*. 2011;5:6136-6139.
9. Sunday RM, Ilesanmi OR, Obuotor EM. Antioxidant property and phenolic content of *Anthocleista vogelii* root ethanolic extract and fractions. *British Biotechnology Journal*. 2016;14(2):1-8.
10. Beckingham IJ, Ryder SD. ABC of diseases of liver, pancreas, and biliary system: Investigation of liver and biliary disease; 2001. [Retrieved August 20, 2019] Available:<https://www.bmj.com/content/322/7281/282.1>
11. Bishayi B, Roychowdhury S, Ghosh S, Sengupta M. Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl<sub>4</sub> intoxicated mature albino rats. *Journal of Toxicol Science*. 2002; 27(3):139-146.
12. Karen T, Bucher HU, Fauchère JC. Comparison of a new transcutaneous bilirubinometer (Bilimed) with serum bilirubin measurements in preterm and full-term infants. *BMC Pediatr*; 2009. [Retrieved August 20, 2019] Available:<https://www.ncbi.nlm.nih.gov/pubmed/19909530>
13. Ehrman T. Standard method for the determination of extractive in biomass. NREL CAT Task, Laboratory Analytical Procedure. 1994;10:1-7.
14. Onyeike EN, Ayalogu EO. Techniques of proximate analysis of foods and food products. In E. N. Onyeike, *Research Techniques in Biological and Chemical Sciences* (1<sup>st</sup> ed.). Owerri, Nigeria.: Springfield Publishers Ltd; 2003.
15. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvic transaminases. *American Journal of Clinical Pathology*. 1957;28:56-63.
16. Tietz NW, Pruden FL, Siggard-Anderson O. Electrolytes blood gasses and acid base balance. In: *Textbook of Clinical Chemistry*, Saunders, Philadelphia. 1986;118-119.

17. Kunwar A, Priyadarsini KI. Free radicals, oxidative stress and importance of antioxidants in human health. *Journal of Medical and Allied Sciences*. 2011;1(2):53-60.
18. Kumar KV, Naidu MUR, Shifow AA, Ratnakar KS. Protocol protects against gentamicin-induced nephrotoxicity in rats. *Indian J Pharmacol*. 2000;32(2):108-113.
19. Varley H, Gowenlok AH, Bel MC. *Practical biochemistry*. Heinmann, Lonely. 1976; 389-391.
20. Mahmood ND, Mamat SS, Kamisan FH, Yahya F, Kamarolzaman FF, Nasir N, Mohtarrudin N, Tohid SF, Zakaria ZA. Amelioration of paracetamol-induced hepatotoxicity in rat by the administration of methanol extract of *Muntingia calabura* L. leaves. *Bio Med Research International*. 2014;1-10.
21. Patil RA, Makwana AB. Anti-hyperbilirubinemic and wound healing activity of aqueous extract of *Calotropis procera* leaves in Wistar rats. *Indian Journal of Pharmacology*. 2015;47(4):398-402.
22. Heard KJ. Acetylcysteine for acetaminophen poisoning. *N Engl J Med*. 2008;359:285-392.
23. Petrie M, McKay G, Fisher M. Silymarin. *practical diabetes*. 2015;32(4):148-150.
24. Thapa BR, Walia A. Liver function tests and their interpretation. *Indian Journal of Pediatrics*. 2007;74:662-671.
25. Dwivedi VK, Mishra J, Shrivastava A. Efficacy study of livartha against paracetamol induced hepatotoxicity in adult sprague dawley rats. *Journal of Drug Metabolism and Toxicology*. 2015;5(6):1-7.
26. Robert C, Husted TR. Causes and evaluation of mildly elevated liver transaminase levels. *American Family Physician*. 2011;84(9):1003-1008.
27. Chaudhary SS, Shah JP, Mahato RV. Interference of bilirubin in creatinine value measurement by jaffe kinetic method. *Nepalese Association for Clinical Chemistry*. 2015;1(1):25-28.
28. Oyediji KO, Bolarinwa AF, Ojeniran SS. Effect of paracetamol (Acetaminophen) on haematological and reproductive parameters in male albino rats. *Journal of Pharm Biol Science*. 2013;4:65-70.
29. Moyer NL. What Is leukocytosis? *Healthline*. 2018;1. [Retrieved October 7, 2019] Available:<https://www.healthline.com/health/leukocytosis>
30. Riley PA. Free radicals in biology: Oxidative stress and the effects of ionizing radiation. *International Journal of Radiate Biology*. 1994;65:27-33.
31. Ogunka-Nnoka CU, Ohwokevwo OA, Onyeike EN. Nutraceutical potentials of *Spilanthes filicualis*. *Global Journal of Science Frontier Research*. 2019;19:41-47.
32. Somogyi A, Rosta K, Pusztai P, Tulassay Z, Nagy GS. Antioxidant measurements. *Physiological Measurement*. 2007;28:41-55.

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