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Effects of Combined Ethanol Extract of *Anthocleista Vogelii* and *Alstonia Boonei* Stem Barks on Liver Function Indices in Benign Prostatic Hyperplasia Induced Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Objective: This study evaluated the effects of combined ethanol extract of *Anthocleista vogelii* and *Alstonia boonei* (CEAA) stem barks on the liver function indices of benign prostatic hyperplasia (BPH)-induced rats.

Materials and Methods: Thirty (30) male Wistar rats were randomly grouped into five groups (n = 6). BPH was induced subcutaneously with 5 mg/kg/day of testosterone propionate in olive oil. Groups 1-3 served as normal, BPH control (untreated) and standard drug control respectively, while Groups 4 and 5 were BPH-induced rats and treated with 200 and 400 mg/kg/day of the CEAA respectively after one hour of the BPH induction. After twenty-eight days of treatments, biochemical and histopathological analyses were conducted using standard analytical procedures.

Results: The acute toxicity result of CEAA indicated no mortality or adverse reactions. Significant body weight change was only observed in the first week from the group-administered 200 mg/kg/day of CEAA. Liver damage was evident in the BPH control characterized by significant ($P<0.05$) increase in relative liver weight and liver enzymes' (AST, ALT, ALP) activities but a reduction in total protein and globulin levels. Treatment with CEAA attenuated the liver damage by significantly ($P<0.05$) reducing the elevated relative liver weight and liver enzyme function activities and elevated total protein and globulin levels.

Conclusion: The revealed that the combined ethanol extract of *Anthocleista vogelii* and *Alstonia boonei* (CEAA) stem barks possess hepatoprotective effects that could improve liver integrity and functions in BPH induced rats.

Keywords: Benign prostatic hyperplasia, liver marker indices, *Anthocleista vogelii*, *Alstonia boonei*, liver histomorphology

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a histologic diagnosis that refers to the proliferation of smooth muscle and epithelial cells within the prostatic transition zone (Kim *et al*., 2016). The enlarged gland has been proposed to contribute to the overall lower urinary tract symptoms (LUTS) complex through at least two routes: (i) Direct bladder outlet obstruction (BOO) from enlarged tissue (static component) and (ii) Increased smooth muscle tone and resistance within the enlarged gland (dynamic component). BPH affects more than 42% of men from age 51 to 60, 70% of men from 61-70 and as many as 90% of men in their 80s and above (Nickel, 2006). Prostate enlargement often results in lower urinary tract symptoms (LUTS) and this adversely affects the quality of life of such patients. There is as yet no medicinal cure for BPH and the related cancers. Management of BPH has been mainly to provide relief-treatment for the symptoms of the condition. The prostate-specific antigen (PSA) is a

prostate-specific marker, not a prostate cancer (PC) marker.

Current conventional treatment regimens for both BPH and its related cancer have produced very adverse effects (Steineck *et al*., 2002). Apart from being expensive, causing urinary incontinence and erectile dysfunction, some of the adverse effects of conventional therapy include toxicity and growth inhibition to normal cells (Singh *et al*., 2003). Indigenous African medicines are reported to be in very wide use despite the apparent lack of scientific evidence to back their quality, efficacy and safety. This lack of scientific data to back the anecdotal evidence poses a limitation to the use of phytochemicals even though they may be beneficial. Extracts from many plants have been used traditionally over the years in the treatment of many forms of illness. Most elderly men with BPH have limited access to standard medical care or simply cannot afford to foot the cost due to the poverty rate especially in Africa.

Combined herbs are used for the treatment of many diseases (Aslam *et al*., 2015). In the traditional system of Indian medicine, plant formulations and combined extracts of plants are chosen rather than individual ones. Due to synergism, combined extracts confers some benefits which are not available in the single herbal formulation.

In Nigeria, *Anthocleista vogelii* (Fig. 1) is ordinarily called 'cabbage tree' in English, 'Sapo' or 'Apaoro' in Yoruba, 'Kwari' In Hausa, 'Orimi' in Benin and 'Odogwu in Igbo.' The leaves and stem bark are found useful in the management of leprosy, jaundice, bronchitis, and venereal diseases. The stem bark and seeds are likewise utilized as an antipyretic, tonic, and as purgative (Okon *et al*., 2014). Important phytochemicals such as saponins, flavonoids, terpenoids, alkaloids, and steroids are present in the leaf, stem bark and root bark of *Anthocleista vogelii*. Reducing sugar, tannin, phlobatannins, glycosides were found to be absent in both the leaf and the stem bark of the *Anthocleista* species, however, tannins are present in the root bark and anthraquinones in the leaves (Oladimeji *et al*., 2014).

Alstonia boonei De Wild (family = Apocynaceae) is indigenous to Africa (Adotey *et al.,* 2012) (Fig. 2). The bark of *Alstonia boonei* tree is one of the effective analgesic herbs available in nature (Abbiw, 1990). The thick bark cut from the matured tree is commonly used for the following therapeutic purposes: antirheumatic (Abbiw, 1990), anti-inflammatory (Abbiw, 1990), analgesic/pain-killing, antimalaria/antipyretic, antihelminthic, antimicrobial, antidiabetic (mild hypoglycaemic) and antibiotic properties (Hadi *et al*., 2001; Fakae *et al.,* 2001; Toh-Seok *et al*., 1997). *Alstonia boonei* decoction also exerts mild antibacterial and anti-malaria effects (Adotey *et al.,* 2012). This study was aimed at determining the effect of a combined extract of *Anthocleista vogelii* and *Alstonia boonei* stem barks on liver function indices of benign prostate hyperplasia (BPH)-induced rats.

Figure 1: *Anthocleista vogelii* plant

Figure 2: *Alstonia boonei* plant

METHODOLOGY

Collection and identification of plant materials

Anthocleista vogelii and *Alstonia boonei* stem barks were collected from the Rubber Forest at the Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. They were identified and authenticated by Dr Ibe K. Ndukwe, a Taxonomist in the Herbarium section of the Department of Forestry and Environmental Management, MOUAU and assigned voucher numbers FHI 40448 and SAPOBA KENNDY 2084, respectively.

Preparation of plant materials

The plant materials were dried at room temperature for some weeks till they were properly dried. Thereafter, they were dried in the oven at 45℃. The dried plant materials were pulverized into a fine powder using a grinding mill. The powdered samples were stored in air-tight plastic containers.

Extraction of Plant

Powdered plant material was collected in the ratio of 1:1 (i.e 300 mg of coarsely ground sample each, equivalent to 600 mg of both plant samples). The combined plant extract was extracted by dissolving it in 1.5 litres of absolute ethanol for 72 hours. Thereafter, it was filtered and concentrated till the ethanol solvent was completely evaporated in a water bath at 45℃ and the percentage yield was calculated.

Experimental Animals

Male Wistar rats (n = 30) weighing 90-150 g were purchased from the Animal House, Department of Zoology and Environmental Sciences, Faculty of Biological Sciences, University of Nigeria Nsukka, Nigeria. The rats were kept on standard laboratory diet (Vital Feed®) including drinking water to them adapt to the new laboratory condition under a 12-hour light/dark cycle for 2 weeks before the commencement of the full experimental study. The animals were handled in line with the National Institute of Health's guidelines for the care and use of laboratory animals (NIH, 1978, publ. no. 8023; NRC, 1985).

Chemical reagents and drugs

The chemicals (Ethanol, Picric acid, Corn oil, Tween 80) used for this study were of analytical grades. Standard drugs used were Finasteride ® and Testosterone propionate ®

Experimental design

The experimental rats were randomly grouped into five groups (n = 6). Benign prostatic hyperplasia (BPH) was induced in the rats (groups 2-5) by subcutaneous injection of testosterone propionate in olive oil (5 mg/kg body weight) for 28 days consecutively which were further confirmed by histological examination of prostate tissue. The grouping is shown below:

Group 1: Normal control (NC) – animals fed only normal feed, water and normal saline.

Group 2:Negative control (NGC) (BPH control) – animals induced with BPH and untreated.

Group 3:Positive control (PC) (standard control) – animals induced with BPH and treated with 5 mg/kg/day of a standard drug (Finasteride)

Group 4:Treatment group 1 (TG1) – induced with BPH and treated with 200 mg/kg/day of a combined extract of *Anthocleista vogelii* and *Alstonia boonei* (CEAA).

Group 5:Treatment group 2 (TG2) – induced with BPH and treated with 400 mg/kg/day of a combined extract of *Anthocleista vogelii* and *Alstonia boonei* (CEAA).

Sacrifice, Sample Collection and Preparation

After 28 days of the administration, the rats were sacrificed the next day after an overnight fast by cervical dislocation technique and blood samples of the respective rats were collected into sample bottles containing Ethylene Di-amine Tetra Acetic Acid (EDTA) for determination of liver function parameters. The sample bottles were gently shaken to mix up the blood with EDTA to prevent clotting. Liver tissues were harvested, weighed accordingly and stored in 4% formaldehyde for histological examination.

Assays of liver marker enzymes activities and liver function parameters

The alanine transaminase (ALT) and aspartate transaminase (AST) activities were assayed using the methods of Reitman and Frankel (1975) while the serum alkaline phosphatase (ALP) activities were assayed according to the colourimetric method described by Englehardt, 1970. The serum total protein, albumin and globulin concentrations were determined according to the methods of Reinhold (1953). The total bilirubin and direct bilirubin concentrations were determined according to the methods of Jendrassik and Grof (1938).

Examination of the liver histomorphology

Sections of the livers were collected for histopathological examination at the end of the study period. The samples were fixed in 10% phosphate-buffered formalin for a minimum of 48 hours before tissue preparation. The tissues were subsequently trimmed, dehydrated in 4 ascending grades of alcohol (70 %, 80 %, 90 % and 100 %), cleared in 3 grades of xylene and embedded in molten wax. After embedding, the tissue-containing wax blocks were cut into 5 µm thick sections with a rotary microtome, floated in water bathe at 60˚C, placed on clean grease-free glass slides and placed on slide warmer set at 60˚C overnight. The 5µm thick sectioned tissues on glass slides were subsequently cleared in 3 grades of xylene and rehydrated in 3 descending grades of alcohol (90 %, 80 % and 70 %). The sections were then stained with Mayer's hematoxylin for 5 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1 % acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX.

Slide Examination

The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x100, 160 and x400 magnifications.

STATISTICAL ANALYSIS

The data were analysed using Statistical Package and Service Solutions (SPSS). One-way analysis of variance (ANOVA) was applied to observe mean differences between groups and Post hoc Duncan's test was applied to observe which group mean differs. Each of the bars in Figures 3 - 5 represent mean ± standard deviation (n = 6) and those bars with different superscripts indicate levels of significant ($P < 0.05$) difference between any paired means.

RESULTS

Percentage Yield

After extraction and concentration of 600 g of finely ground *A. vogelii* and *A. boonei* stem bark (CEAA), the percentage yield was found to be 1.96 % which is equivalent to 11.76 g.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks on the body weight of benign prostatic hyperplasia (BPH)-induced rats

The result in Figure 3 shows the body weight changes of benign prostatic hyperplasia (BPH)-induced rats treated with CEAA. The result shows that BPH-induced rats treated with 200 mg/kg/day combined extract (TG1) caused a significant ($P<0.05$) decrease in body weight for week 1 when compared with the normal control (NC). Negative control (NGC), positive control (PC) and BPH-induced rats treated with 400 mg/kg/day of CEAA (TG2) all showed no significant ($P>0.05$) change in their body weights. For weeks 2 and 4, the result showed that there was no significant ($P>0.05$) change in the body weight of PC, NGC, TG1 and TG2 when compared with normal control and among themselves.

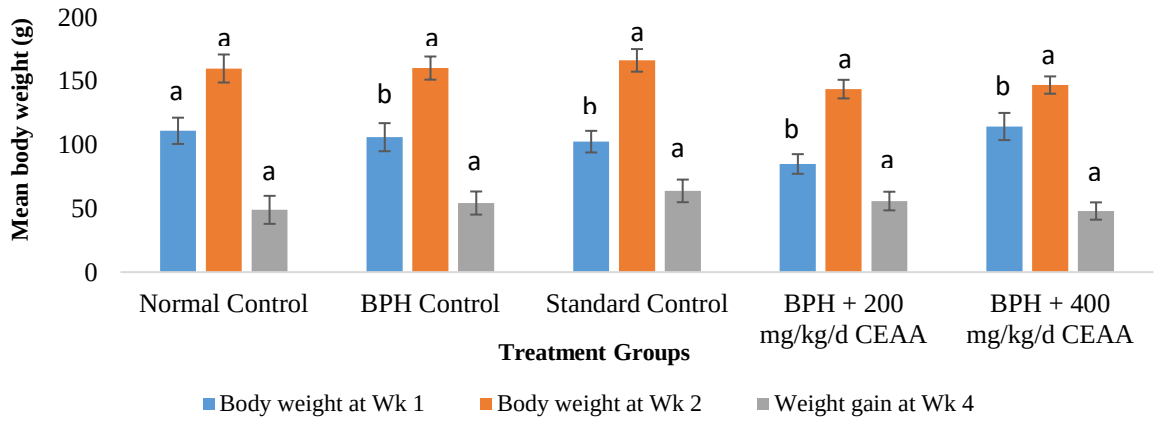

Figure 3: Effects of a combined extract of *Anthocleista vogelii* and *Alstonia boonei* on the body weight of BPH-induced Wistar rats.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the liver weight of benign prostatic hyperplasia (BPH)-induced rats

There was a significant (P<0.05) decrease in liver weight of BPH-induced rats treated with 200 (TG1) and 400 mg/kg/day (TG2) of CEAA when compared with the normal and standard control groups, while there was no significant difference in the liver weight among normal, BPH and standard control groups respectively (Figure 4).

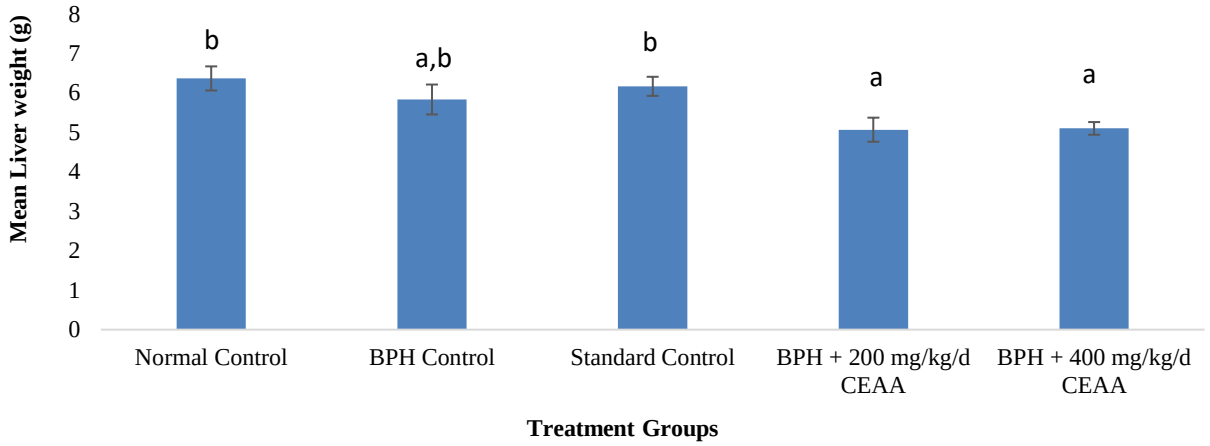

Figure 4: Effect of a combined extract of *Anthocleista vogelii* and *Alstonia boonei* on the liver weight of BPH-induced Wistar rats.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the relative body weight of benign prostatic hyperplasia (BPH)-induced rats

The relative liver weights of the normal, standard and combined extract (200 and 400 mg/kg/day) treatment groups were significantly (P<0.05) lower when compared to BPH-induced control (Fig. 5). However, the relative liver weights of these groups differed insignificantly (P>0.05) among themselves.

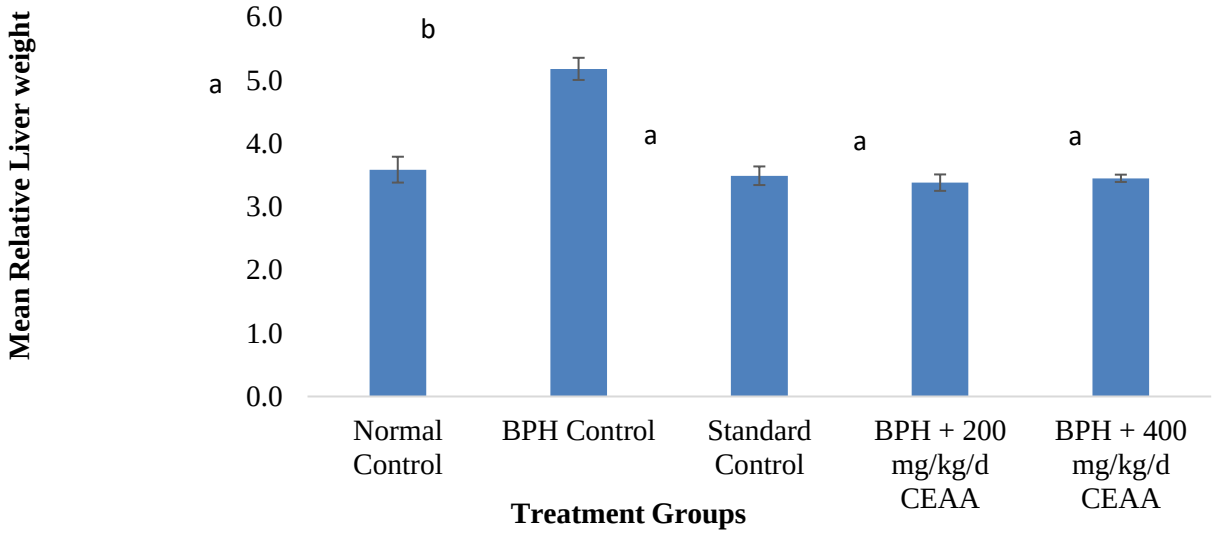

Figure 5: Effect of a combined extract of *Anthocleista vogelii* and *Alstonia boonei* stem bark on the relative liver weight of BPH-induced rats

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the aspartate transaminase activities (AST) of benign prostatic hyperplasia (BPH)-induced rats

There was a significant ($P<0.05$) increase in AST activities observed in BPH-control, Groups 4 and 5 treated with 200 and 400 mg/kg/day of CEAA respectively when compared with the normal control rats (Table 1). The AST activity of BPH-induced group was significantly ($P<0.05$) elevated when compared to all the other groups. No significant ($P>0.05$) difference was observed in the AST activities of normal and standard control groups. On the other hand, there was a significant ($P<0.05$) decrease in AST activity observed in Groups 4 and 5 treated with 200 and 400 mg/kg/day of CEAA when compared with the negative (BPH) control group.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the alanine transaminase activities (ALT) of benign prostatic hyperplasia (BPH)-induced rats

The result of alanine transaminase (ALT) activities of benign prostatic hyperplasia (BPH)-induced rats treated with CEAA which indicated a significant ($P<0.05$) increases in the ALT activities of all the BPH induced rats in comparison with the normal control (Table 1). On the other hand, there was a significant ($P<0.05$) reductions in the ALT activities of all the BPH induced rats treated with CEAA and Finasteride relative to the BPH control. Furthermore, there was no significant ($P>0.05$) difference in ALT activities of BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA when compared with the standard control treated with Finasteride.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the alkaline phosphatase activities (ALP) of benign prostatic hyperplasia (BPH)-induced rats

The result indicated that the ALP activity of BPH control was significantly ($P<0.05$) higher than those treated with CEAA and Finasteride respectively. However, there was a significant ($P<0.05$) increase in ALP activity of standard control and BPH-induced rats treated with 200 mg/kg/day of CEAA when compared with the normal control. Furthermore, all the BPH induced rats treated showed significant ($P<0.05$) decrease in ALP activities when compared with the BPH control. There was no significant difference ($P>0.05$) in ALP activity of BPH induced rats treated with 200 and 400 mg/kg/day of CEAA when compared with the standard control (Table 1).

Table 1: Effect of a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the liver marker enzymes activities of BPH-induced Wistar rats

Groups	AST (iu/L)	ALT (iu/L)	ALP (iu/L)
Normal Control	$20.22+0.77^{a}$	$14.38+0.63^{a}$	$19.77+1.01^{a}$
BPH Control	$33.44+1.19^{d}$	$24.83+0.83^{d}$	$32.63+1.30^{c}$
Standard Control	$20.11+0.51^{a}$	$18.77+0.51^{b,c}$	$24.25+0.38^{b}$
BPH + 200 mg/kg/d CEAA	$27.55+1.39^{c}$	$20.16+0.60^{c}$	$25.80+0.93^{b}$
BPH + 400 mg/kg/d CEAA	$22.85+1.01^{b}$	$17.55+0.75^{b}$	$23.06+1.36^{a,b}$

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the total protein concentrations of benign prostatic hyperplasia (BPH)-induced rats

The result showed that the total protein concentrations of the normal control, standard control and BPH induced rats treated with 200 and 400 mg/kg/day of CEAA was significantly ($P<0.05$) high when compared with the BPH control (Table 2). The BPH control group showed a significant ($P<0.05$) decrease in total protein concentration relative to the normal control group. Additionally, the BPH-induced rats treated with 400 mg/kg/day of CEAA showed a significantly ($P<0.05$) higher total protein concentration when compared with the standard control and PH-induced rats treated with 200 mg/kg/day of CEAA.

Table 2: Effect of a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the liver function indices of BPH-induced Wistar rats

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulins (g/dL)	T. Bil (mg/dL)	D. Bil (mg/dL)
Normal Control	4.72±0.21^{b}	4.24±0.20^{c}	0.87±0.05^{b}	2.14±0.07^{b}	1.20±0.07^{a}
BPH Control	4.07±0.18^{a}	3.22±0.12^{a}	0.33±0.04^{a}	2.40±0.10^{c}	1.74±0.09^{c}
Standard Control	5.04±0.13^{b,c}	3.67±0.19^{b}	0.68±0.07^{b}	1.68±0.06^{a}	1.28±0.05^{a,b}
BPH + 200 mg/kg/d CEAA	5.28±0.14^{c}	4.20±0.16^{c}	0.91±0.02^{b}	2.04±0.10^{b}	1.39±0.06^{a,b}
BPH + 400 mg/kg/d CEAA	6.14±0.24^{d}	4.43±0.15^{c}	1.23±0.05^{c}	2.07±0.08^{b}	1.55±0.05^{b,c}

Values are presented as mean ± standard deviation (n = 6)

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the albumin concentrations of benign prostatic hyperplasia (BPH)-induced rats

There was a significant (P<0.05) decrease in albumin concentration of BPH control when compared with the normal control, standard control and BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA (Table 2). The BPH-induced rats treated with 200 and 400 mg/kg/day of the CEAA showed no significant (P>0.05) difference in albumin concentrations relative to the normal control. It was observed that all the groups showed a significant (P<0.05) difference in albumin concentration when compared with the standard control.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the globulin concentrations of benign prostatic hyperplasia (BPH)-induced rats

The result showed that there was a significant (P<0.05) increase in globulin concentration of all the groups when compared with the BPH control (Table 2). There was a significant (P<0.05) increase in globulin concentration in BPH-induced rats treated with 400 mg/kg/day of CEAA when compared with the normal and standard controls. However, the BPH-induced rats treated with 200 mg/kg/day of CEAA showed no significant (P>0.05) increase in globulin concentration when compared with the normal and standard control groups.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the total bilirubin concentrations of benign prostatic hyperplasia (BPH)-induced rats

There was a significant (P<0.05) elevation of total bilirubin concentration in BPH control group when compared with the other groups (Table 2). The total bilirubin concentration of normal control differs significantly (P<0.05) when compared with BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA. The total bilirubin concentration of standard control was significantly (P<0.05) lower when compared with all the other groups.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the direct bilirubin concentrations of benign prostatic hyperplasia (BPH)-induced rats

There was a significant (P<0.05) increase in direct reacting bilirubin concentration of BPH control and BPH-induced rats treated with 400 mg/kg/day of the CEAA when compared with the normal control (Table 2). The standard control group and BPH-induced rats treated with 200 mg/kg/day of CEAA showed no significant (P>0.05) increase in direct reacting bilirubin concentration relative to the normal control groups. There was no significant (P>0.05) difference in the direct reacting bilirubin concentration of BPH-induced group rats treated with 200 and 400 mg/kg/day of CEAA when compared with the standard control.

Effects of treatment with a combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on the liver histomorphology of benign prostatic hyperplasia (BPH)-induced rats

The sections of the liver presented in Plate 1 showed the normal hepatic histomorphology for laboratory rodents. Normal hepatic lobules consisting of normal hepatocytes arranged in interconnecting cords around the central veins were observed. The hepatic cords are separated by the hepatic sinusoids and are radially arranged around the central veins, terminating at the portal areas in each hepatic lobule which contains the branches of the hepatic artery, hepatic vein and the bile ducts. Similarly, the sections of livers from BPH-induced untreated rats (Plate 2), BPH induced rats treated with Finasteride® (i.e. standard control) in Plate 3, BPH-induced rats treated with 200 and 400 mg/kg/day of the CEAA as shown in the Plates 4 and 5 respectively showed normal liver histomorphologic of normal rodents. The (V); (HV); (HA); and (BD) in Plates 1 – 5 represent central vein, hepatic vein, hepatic artery and bile duct. H&E x160.

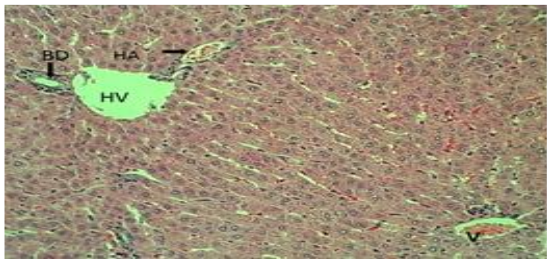

Plate 1: Histomorphology of liver section from normal control rats that were not induced with benign prostatic hyperplasia.

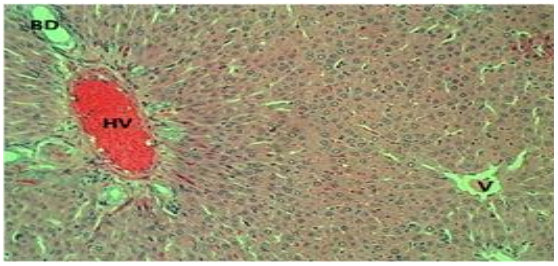

Plate 2: Histomorphology of liver section from benign prostatic hyperplasia-induced rats that were untreated.

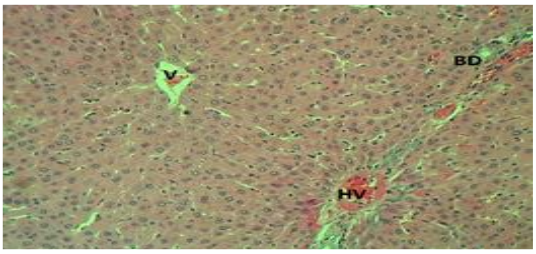

Plate 3: Histomorphology of liver section from benign prostatic hyperplasia-induced rats treated with 5 mg/kg/day of Finastride®(standard drug).

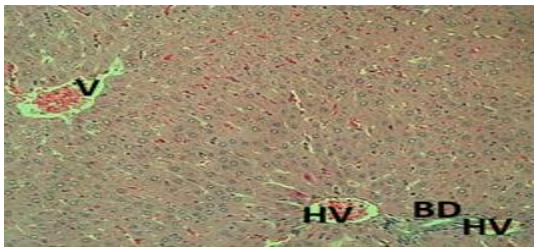

Plate 4: Histomorphology of liver section from benign prostatic hyperplasia-induced rats treated with 200 mg/kg/day of combined extract.

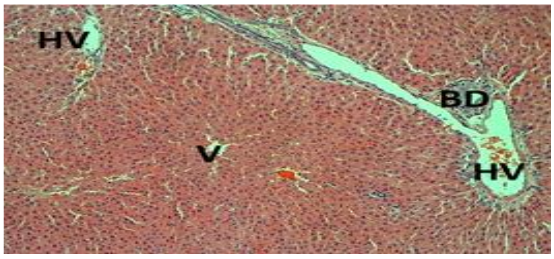

Plate 5: Histomorphology of liver section from benign prostatic hyperplasia-induced rats treated with 400 mg/kg/day of combined extract.

DISCUSSION

The liver function indices and histomorphological changes of benign prostatic hyperplasia (BPH) induced rats treated with combined ethanol extract of *Anthocleista vogelii* and *Alstonia boonei* stem barks (CEAA) were evaluated. It has been established that changes in body weight, organ weight and morphological alterations are associated with pharmacological effects of drugs including their toxicity (Alabi and Akomolafe, 2020). Analyses of toxicology studies are important ends for identification of potentially harmful effects of the combined extract.

From this study, the non-significance variation in weight from week 1 and week 4 in BPH-induced rats treated with combined extract and the normal control suggests that BPH has no adverse effect on the body weight of rats and agrees with findings of Hongcai *et al*. (2018).

BPH-induced rats treated with 200 and 400 mg/kg/day of the combined extract showed significant ($P<0.05$) decrease in liver weight when compared with standard drug – suggesting that the standard drug had more effect on the liver weight than the combined extract. When compared with BPH control, there was also a decrease in the liver weight of the groups treated with combined extract while the standard drug caused an increase. This decrease observed in the liver weights of the CEAA treated rats when coupled their relative liver weights indicate ameliorative effects of the CEAA as their liver weights were appropriate of their reduced body weights.

The liver transaminases aspartate transaminase (AST or SGOT) and alanine transaminase (ALT or SGPT) are useful biomarkers of liver injury in a patient with some degree of intact liver function (Mengel *et al*., 2005). A high liver cell content of these enzymes is

desirable as it is an index of good liver tissue integrity (Oyefuga *et al.,* 2010), and a slightly pronounced and repetitive high level of these enzymes in the serum is an index of liver tissue insults and a state of hepatocellular injury. The current research findings reflect an elevated level of AST in BPH-induced rats, which implies that BPH caused a liver injury. BPH-induced rats treated with 400 mg/kg/day of CEAA showed a decrease in AST activities unlike the increased AST activities observed in the BPH induced rats treated low dose of CEAA. However, CEAA treated groups showed elevated AST activities suggesting that it may not have the same hepatoprotective effects on the BPH induced rats as Finasteride in preventing liver injury but could still help in repairing injured liver and maintaining the liver integrity to some extent.

The alanine transaminase (ALT) activities were in all the BPH induced rats relative to the normal control which indicated that the rats suffered liver injury possibly from the BPH induction. The ALT activities of BPH induced rats treated with CEAA and standard drug respectively decreased unlike the BPH control and could be attributed to the CEAA treated rats possessing more intact liver probably with minor injuries than the BPH control rats. The findings of this study indicate that CEAA could be rich in phytoconstituents with hepatoprotective properties and capable of repairing and ameliorating liver injury most especially at a higher dose.

In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase levels generally reflect hepato-biliary disease (Millán *et al*., 2006). Induction of BPH caused liver damage to the rats. The CEAA treated BPH induced rats and Finasteride treated standard control had marked reductions in the ALP activities contrary to the increased ALP activities observed in the BPH control rats which indicated that CEAA prevents liver injuries in BPH induced rats. The hepatoprotective effects of CEAA linked to the observed reductions in the ALP activities of BPH induced rats treated with it appeared to be dose-dependent with better protective effects at higher doses. The findings from the AST, ALT and ALP activities of the BPH induced rats suggest that patients with BPH should undergo routine liver function tests to evaluate their liver status and maintain a healthy liver. The decreased total protein concentrations in the BPH control rats could be attributed to the inability of the liver to carry out normal biosynthetic function efficiently including protein synthesis due to liver injuries, compromised and functions (Asuk and Ugwu, 2018). However, the increased total protein concentrations in the BPH induced rats treated with CEAA relative to the BPH control indicated recovery of the rats from the negative impact of BPH of the liver and improved protein synthesis by the liver. Likewise, the elevated of globulin concentrations in the CEAA treated BPH induced rats unlike the BPH control, suggests the effectiveness of CEAA in restoring liver integrity which resulted to improved liver functions and increased protein biosynthesis by the hepatocytes including globulin synthesis in line with findings of (Asuk and Ugwu, 2018).

The CEAA showed to be more effective than the standard drug in controlling albumin concentration. There was hypoalbuminemia in the BPH control, which was because of leakage of albumin due to liver damage or inflammation in BPH control group. This is proven by the works of Briscoe *et al*. (2010) who posited that albumin is considered a negative acute-phase protein, and may decrease during acute inflammatory conditions. The CEAA was observed to elevate the level of albumin activities in BPH-induced rats.

Bilirubin is the ultimate breakdown product of haemoglobin and serves as a diagnostic marker of liver and blood disorders. However, it has been recognized that mild hyperbilirubinaemia might have positive health effects (Huang *et al.,* 2003). BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA respectively showed increased total bilirubin concentrations suggesting that CEAA might have a positive health effect on the liver. The BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA did not alter the direct reacting bilirubin concentration, suggesting that the CEAA did not cause any harmful effect.

To elucidate the mechanism by which CEAA exhibit the anti-hepatotoxicity effect which was demonstrated in this study, the sections of the liver presented in Plate 1 showed that the normal hepatic histomorphology for laboratory rodents was similar with the sections of livers from BPH-induced untreated rats. BPH-induced rats treated with Finasteride® (standard control) in Plate 3 and BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA as shown in Plate 4 and 5. This result implies that CEAA has no toxic effect on liver histo-architecture.

CONCLUSION

These results suggest that administration of combined extract of *Anthocleista vogelii* and *Alstonia boonei* stem bark on BPH-induced rats (for 28 days) improved the liver protein, liver integrity, and in overall, conferred protection to the liver cells. Therefore, the combined extract of *Anthocleista vogelii* and *Alstonia boonei* has the potential to be used as a hepatoprotective agent in the management of BPH in terms of liver protection and anti-inflammatory effects.

DECLARATIONS

Ethical Approval

This research work considered and adhered to the standard ethical use of experimental animals. Throughout out the experimentation (acclimatization and exposure periods), all rats were housed at 25° C in stainless steel cages under normal daylight/dark cycle and humid tropical conditions. The rats were allowed free access to rat feed (Vital Feed®, Jos Nigeria) and tap water, and generally received humane care following the guidelines of the National Institute of Health, USA for the ethical treatment of laboratory animals. The ethical clearance was duly obtained from the Ethical Committee of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike with the Ethical Number: MOUAU/VPP/EC/18/003.

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