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Combined Spermacoce Radiata and *Hypselodelphys Poggeana Extract* (CESH) Protect Against Oxidative Stress and Enhances Haematological Parameters in Benign Prostatic Hyperplasia-induced Rats

Robert Ikechukwu Uroko[1*], Favour Matthew Awah[1], Chinedu Aguwamba[3], Mercylyn Ezinne Uche[2], Ikenna Obiwuru[2], Chinomso Friday Aaron[2], Ezichi Favour Ofoezie[1]

[1] Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

[2] Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria

[3] Biochemistry Unit, Department of Chemical Sciences, Faculty of Sciences, Clifford University, Owerrinta, Abia State, Nigeria

ABSTRACT

This study investigated the therapeutic effect of a combined extract of *Spermacoce radiata* and *Hypselodelphys poggeana* (CESH) on oxidative markers and haematological parameters in benign prostatic hyperplasia (BPH) induced rats. The study adopted five groups containing equal numbers of rats (n = 6), including normal control, BPH control, Finasteride control, BPH-induced rats treated with 200 mg/kg CESH, and BPH-induced rats treated with 600 mg/kg CESH. The rats were induced BPH by the subcutaneous administration of a 5 mg/kg testosterone propionate injection. At the same time, treatment finasteride and CESH to the respective groups were given orally 60 minutes after the BPH induction for 28 uninterrupted days. The induction of BPH with testosterone propionate injection caused a significant reduction in the serum levels of haematological parameters, including haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), and platelet counts of the BPH control compared with normal control. The glutathione (GSH) concentration, glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione S-transferase, and catalase activities decreased significantly in the BPH control relative to the normal control. The BPH control had elevated white blood cell (WBC), and malondialdehyde (MDA) concentrations contrary to the high WBC and MDA in the normal control and CESH treated BPH induced rats, respectively. Conversely, the Hb, PCV, platelet count, GPx, SOD, catalase, GST, and GSH increased significantly in the finasteride and CESH-treated BPH-induced rats, respectively, compared to the BPH control. These findings show that CESH attenuates adverse effects of BPH on antioxidant parameters and oxidative markers, which may prevent BPH progression.

Keywords: Oxidative stress; Antioxidants parameters; Haematological parameters; Prostate enlargement; Medicinal plants

INTRODUCTION

The prostrate is an ovoid-shaped sex hormone-dependent organ, and it is positioned close to the bladder and performs peripheral reproductive functions via nourishment and transport of sperm cells. Benign prostatic hyperplasia (BPH) is a non-malignant proliferation ailment that affects ageing men. It is one of the most common diseases among senior men, with 50% of men above 60 years suffering from this condition and 80% of ageing men above 80 years showing histologic evidence (Agrawal *et al.,* 2012; Ajayi and Abrahama, 2018). It results from the hyperplasia of the prostatic stromal that develops in the transition zone's periurethral regions, resulting in the obstruction of the bladder outlet by narrowing the urethra (La Vignera *et al*., 2016).

***Corresponding author : Robert Ikechukwu Uroko**
Email : ir.uroko@mouau.edu.ng

Urinary urgency, nocturia, frequent urination, obstructive/voiding (partial voiding of the bladder and reduced urinary flow), and lower urinary tract symptoms (LUTS) are some of the clinical manifestations of BPH. It is important to note that these symptoms and their severity are affected by ethnicity and race (Ajayi and Abrahama, 2018).

Undisrupted distribution and excretion mechanism of smooth prostatic muscles are necessary to remove prostatic fluid from the prostate to the ejaculate. This process is severely disrupted in ageing groups with BPH. Age-related prostate function decline is caused by oxidative stress, local tissue inflammation, hormonal disruption, alteration in smooth muscle function, prostate fibrosis, growth, and enlargement (Taoka and Kakehi, 2017: Liu *et al*., 2019). For example, one experiment has shown that decreasing androgen levels mediates the proliferation of myofibroblasts and fibroblasts in smooth muscle

tissue due to stromal remodelling (Kajiwara *et al*., 2020). Also, inflame-ageing, which is minimal, sterile systemic inflammation resulting from stimulation of proinflammatory cytokines from senescent cells, plays a vital role during the onset of BPH. The proinflammatory cytokines contribute to the pathogenesis of BPH via the proliferation of senescent cells within the BPH epithelium and the expression of senescence-related beta-galactosidase in aged men with BPH (Choi *et al*., 2000; Jiang *et al*., 2019). Inflame-ageing depletes cellular defence against oxidative stress and is connected with the onset of BPH (Zuo *et al*., 2017). Thus, phytonutrients that restore cellular redox homeostasis may be a novel therapeutic approach for BPH management and treatment.

The increasing incidence and prevalence of BPH among ageing men could lead to a high population of men with BPH, which could adversely increase pressure on the already stressed health facilities, the decline in life expectancy of the affected men, and more burden to their relatives and government. The lack of effective drugs and adequate surgical procedures has resulted in a burgeoning interest to research on novel therapy to treat and manage BPH (Mbaka *et al*., 2013).

Spermacoce radiata (DC.) Sieber ex Hiern is a potent medicinal plant from the family of *Rubiaceae*. Plant extracts formulated using different parts of *Spermacoce radiata* are effective therapeutic agents against many diseases, such as microbial infections, venereal diseases, and prostate enlargement. It is erect and partly woody hairy and distributed across the African continent, especially in West African countries. *Spermacoce* species have been therapeutically effective against headache, conjunctivitis, gallstones, inflammation, and haemorrhoids due to various phytochemicals, including alkaloids, terpenoids, flavonoids, and phenolics (Parrotta, 2001; Subramanya *et al*., 2015). Other pharmacological properties attributed to *Spermacoce* species entail antioxidative stress, larvicidal, antitumour, and anti-gastrointestinal tract disorders (Conserva and Ferreira Júnior, 2012). Plant extract formulated with *Spermacoce radiata* and *Hypselodelphys poggeana* possesses hepatoprotective properties against BPH-induced hepatic injury and dysfunction in rats (Uroko et al., 2022). *Hypselodelphys poggeana* (K Schum.) Milne-Redh belongs to the Marantaceae family and is partially woody like *Spermacoce radiata* but has unique violet and white flowers commonly found across tropical rainforests worldwide. *Hypselodelphys poggeana* possesses various therapeutic potentials, including anti-infertility, anti-dyslipidaemia, renal protection, hepatoprotective, antidepressant, and prevention of prostate enlargement (Burkill, 1995; Uroko *et al*. (2022b). The high medicinal potentials and wide applications of *Spermacoce radiata* and *Hypselodelphys poggeana* in traditional medicine have prompted the need for this study on the therapeutic effect of a combined extract of *Spermacoce radiata* and *Hypselodelphys poggeana* (CESH) on oxidative markers and haematological parameters in benign prostatic hyperplasia (BPH) induced rats.

METHODOLOGY

Materials

Plant materials

We collected fresh *Spermacoce radiata,* and *Hypselodelphys poggeana* leaves from Ahia Eke Ndume, Umuahia, Abia State, followed by identification and authentication at the Herbarium at the Michael Okpara University of Agriculture, Umudike (MOUAU) with voucher numbers Jones FHI 13749 and 2694-5 (Preuss 1899) respectively.

Preparation of combined ethanol extract of *S. radiata and H. poggeana* leaves (CESH)

Leaf materials were hand-picked, thoroughly cleaned with running water, and then air-dried at room temperature. Following pulverisation of the dried leaves with an electric blender, 350 g of both powders (i.e., 700 g of combined ground powder at a ratio of 50:50) was then immersed in 2.5 L of absolute ethanol for three days and filtered with Whatman No. 1 filter paper. The solution obtained was dried in a water bath at 50°C till the ethanol solvent evaporated entirely, and the percentage yield was calculated. Combining the plants in the proportion used in this study mimics the pattern of their use in folktale medicine and aligns with an earlier report (Uroko *et al*., 2020).

Collection and acclimatisation of experimental animals

Following ethically approved guidelines for the study, 30 mature male Wistar albino rats weighing 155 – 165 g were obtained from the animal care unit of the Department of Veterinary Medicine, MOUAU. The rats were acclimatised for three weeks at the animal house of the Department of Biochemistry, College of Natural Sciences, MOUAU, with access to standard animal feed (Vital Feeds®) and water *ad libitum.*

Table I. Haematological indices of BPH induced rats treated with CESH

Parameters	Normal control	BPH control	Finasteride control	BPH + 200 mg/kg CESH	BPH + 600 mg/kg CESH
Hb (g/dl)	11.75&±0.21^{b}	9.11±0.67^{a}	13.13±0.23^{b,c}	13.84±0.22^{b,c}	14.72±0.87^{c}
PCV (%)	53.13±2.89^{b}	44.32±2.09^{a}	53.11±1.56^{b}	55.43±0.66^{b}	62.21±2.12^{b}
WBC (x10^{3}/mm^{3})	68.24±5.25^{a}	89.23±4.61^{b}	65.22±2.31^{a}	69.56±3.46^{a}	73.67±5.33^{a}
RBC(x10^{6}/mm3)	151.44±2.69^{b}	126.55±2.33^{a}	181.67±8.46^{b}	150.23±.3.41^{b}	160.02±3.34^{b}
Platelet (x10^{3}/mm^{3})	255.44±6.88^{b}	163.13±5.28^{a}	223.33±6.16^{b}	234.33±7.45^{b}	246.77±7.64^{b}

Values are presented as mean ± standard deviation (n = 6), and values with different superscripts are significantly (P<0.05) different from any paired mean across the row.

Methods

BPH Induction

The rats were induced BPH by the subcutaneous administration of a 5 mg/kg testosterone propionate injection masked in olive oil (2:1 v/v) for 28 consecutive days.

Experimental design

Thirty acclimatised mature male Wistar albino rats were selected into five groups containing an equal number of rats (n = 6). The five groups comprise the normal control, BPH control, Finasteride control, BPH+200 mg/kg CESH, and BPH+600 mg/kg CESH. The normal control received 1 ml/kg distilled water mixed with olive oil (1:1; v/v), and the BPH control received 5 mg/kg testosterone propionate injection subcutaneously for 28 uninterrupted days without any other treatment. The Finasteride control, BPH+200 mg/kg CESH, and BPH+600 mg/kg CESH were administered 5 mg/kg testosterone propionate injection subcutaneously for 28 uninterrupted days and orally treated with 5 mg/kg Finasteride, 200 and 600 mg/kg CESH respectively an hour after the testosterone administration. The rats fasted overnight after the final treatments on the 28th day. They were anaesthetised on the 29th day by intraperitoneal administration of a mild pentobarbital dose (25 mg/kg). After a few minutes, blood samples were collected from the rats for biochemical analyses via cardiac puncture.

Determinations of haematological and antioxidant parameters

Catalase activity (CAT), glutathione peroxidase activity (GPx), and superoxide dismutase activity (SOD) were determined by the methods of Aebi (1983) Ursini *et al*. (1985) and Xin *et al*. (1991), respectively. The malonaldehyde concentration (MDA) was determined according to the procedure outlined by Ohkawa *et al*. (1979). The haematological parameters, including red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), and differential white blood cell count (WBC) were determined through methods described by Dacie and Lewis (1991).

Statistical Analysis

The data collated from the study were subjected to a one-way analysis of variance and the different means compared by a Duncan multiple range comparison test. The statistically significant difference was 95% confidence (P < 0.05).

RESULT AND DISCUSSION

Effects of CESH on haematological indices of BPH-induced rats

Many medicinal plants, including Spermacoce radiata and *Hypselodelphys poggeana*, have been found helpful in managing BPH, and their mechanism of action and effects on some biochemical, physiological, and haematological parameters have not been investigated to ascertain their therapeutic and toxicity potentials. The role of medicinal plants in improving the well-being of humankind cannot be over-emphasised, having been reported that many therapeutic agents are from medicinal plants. Previous research has shown that 50% of prescription drugs are made from herbal isolates Dhami (2013). This study elevated the therapeutic effect of a combined extract of *Spermacoce radiata and Hypselodelphys poggeana* (CESH) haematological parameters and on oxidative markers in benign prostatic hyperplasia (BPH) induced rats.

The haemoglobin (Hb) concentrations in Table I showed significant (P<0.05) reductions in the Hb concentrations of the benign prostatic hyperplasia (BPH) induced untreated rats (BPH control) in comparison with the normal control. The BPH-induced rats treated with 5 mg/kg finasteride (finasteride control) and 600 mg/kg

CESH respectively, indicated significant (P<0.05) increases in the Hb concentration when compared with the normal control. The BPH-induced rats treated with 200 mg/kg CESH had no significant (P>0.05) increase in the Hb concentration relative to the normal control. On the contrary, the BPH-induced rats treated with 5 mg/kg finasteride, 200 and 600 mg/kg CESH respectively, showed significantly (P<0.05) elevated Hb concentrations compared with the BPH control. There was no significant (P>0.05) difference between the Hb concentrations in the finasteride and CESH-treated BPH-induced rats.

Our results reveal that induction of BPH in experimental animals significantly decreased serum Hb concentration; however, treatment with finasteride and CESH extract did not only restore Hb levels but significantly increased the Hb concentration of treated animals compared to the control. Notwithstanding, treatment with low and high doses of CESH produced effects comparable to those of the standard drug regarding Hb concentration. The impact of CESH on the haematological parameters aligns with previous reports of boosting and replenishing medicinal plants' roles in CCl_4-induced oxidative stress (Uroko *et al*., 2018). Significantly restored haemoglobin synthesis may be attributable to the synergistic impact of phytochemicals in the CESH.

The packed cell volume (PCV) counts of BPH-induced rats in Table I showed significant (P<0.05) reductions in the PCV counts of the BPH control rats when compared with the normal control. Also, there was no substantial reduction in the PCV counts of the BPH-induced rats treated with 5 mg/kg finasteride, 200 mg/kg CESH, and no significant (P>0.05) increase in PCV count of the BPH-induced rats treated with 600 mg/kg CESH relative to the normal control. All the BPH-induced rats treated with 5 mg/kg finasteride, 200 and 600 mg/kg CESH had significantly (P<0.05) higher PCV counts than the BPH control, respectively. No significant (P>0.05) difference was observed between the PCV counts of the BPH-induced rats treated with 200 and 600 mg/kg CESH respectively.

Since BPH induction decreased PCV counts remarkably, it can be inferred that it either decreased erythropoiesis, increased red blood cell destruction, or increased plasma volume. However, treatment with finasteride and graded doses of CESH after BPH induction comparably increased PCV counts compared with the untreated group. Thus, restoring PCV counts to basal levels is in line with decreased RBC counts in the BPH control group as against increased RBC counts observed in the treatment groups. Increased PCV counts may be ascribable to the phytochemicals, antioxidant, and pharmacological properties, such as flavonoids, phenolics, sterols, triterpenes, quinones, coumarins, and alkaloids, and lots more present in the CESH, which may promote the synthesis of new blood cells (Sharma *et al*., 2017).

The result in Table I indicated a significant (P<0.05) increase in the white blood cell count (WBC) counts of BPH control in comparison with the WBC counts of the normal control, while there were no significant (P>0.05) decreases in the WBC counts of BPH induced rats treated with graded doses of CESH relative to the normal control. While the finasteride-treated BPH-induced rats showed no significant (P>0.05) reduction in WBC counts relative to the normal control. The BPH-induced rats treated with 200 and 600 mg/kg CESH indicated no significant (P>0.05) increase in WBC count, respectively, compared with WBC counts of the BPH-induced rats treated with mg/kg finasteride.

Significantly increased WBC count in the BPH control aligns with prior studies and is attributable to increased immune and phagocytic response due to high levels of ROS cum free radicals caused by BPH induction (Ugwu *et al*., 2019). Increased free radicals trigger an immune response resulting in increased synthesis and recruitment of WBCs to impede further spread and clear existing radicals (Ijioma *et al*., 2019). While the induction of BPH significantly increased WBC counts, treatment with finasteride and low and high doses of CESH significantly reduced WBC counts to normal levels. The phytonutrients in the CESH might have inhibited 5-alpha reductase, regulating prolactin-induced prostatic growth, preserving detrusor, bladder, and renal function, and exerting anti-inflammatory and free radical effects scavenging activities in line with Sharma *et al*., 2017.

The red blood cell (RBC) counts of BPH control rats in Table I showed a significant (P<0.05) reduction in comparison with the RBC counts of the normal control rats. In contrast, a slight increase in RBC counts was observed in the BPH-induced rats treated with 5 mg/kg CESH and 600 mg/kg CESH, respectively, compared with the RBC counts of the normal control rats. Furthermore, the BPH-induced rats treated with 200 mg/kg CESH showed no significant (P>0.05) decrease in RBC relative to the normal control. Contrarily, the BPH-induced rats treated with 5 mg/kg finasteride, 200 and 600 mg/kg CESH had significantly (P<0.05) elevated RBC counts relative to the RBC counts of the BPH control. However, the RBC counts of BPH-induced rats treated with 600

mg/kg CESH were not significantly (P>0.05) higher than those of the BPH-induced rats treated with 200 mg/kg CESH.

In this study, BPH induction significantly decreased RBC counts attributable to increased erythrocyte osmotic fragility following impaired redox balance leading to anaemic condition. Uncontrolled oxidative stress may shorten the lifespan of RBC and induce hepatic damage and anaemia (Bamishaiye *et al*., 2009; Uroko *et al*., 2018). However, improved RBC count following treatment with CESH indicated the pharmacological effect of its bioactive compositions, which might have stimulated the erythropoietic stem cell activities, thus increasing RBC synthesis in the bone marrow. Also, the high iron levels in most herbal treatments implicate the elevation of RBC counts (Uroko *et al*., 2018).

The data in Table I indicated a significant (P<0.05) reduction in the platelet count of BPH control and a slight decrease in the platelet count of BPH-induced rats treated with 5 mg/kg CESH, 200 and 600 mg/kg CESH when compared with the normal control. The BPH-induced rats treated with finasteride and graded doses of CESH had significantly (P<0.05) increased platelet counts relative to the platelet counts of the BPH control rats.

Mean platelet volume could help to distinguish prostate cancer from Benign Prostatic Hyperplasia (Fu *et al*., 2018). In this study, the BPH control group showed abnormally reduced platelet levels indicating impaired immune response in BPH-induced animals. While treatment with low-dose CESH produced a similar effect as finasteride, high-dose treatment significantly increased and restored platelets to normal levels. Thus, adequate consumption of the CESH within the safe dose range may sufficiently restore the oxidative effect of BPH induction, as exemplified in restored platelets and other haematological parameters. Previous studies suggest that platelets play vital roles in autoimmunity, haemostasis, and inflammation; functional platelet activation could improve host response to therapeutic intervention (Fu *et al*., 2018).

Effects of CESH on differential white blood cell counts of BPH-induced rats

Table II indicated no significant (P>0.05) elevation in the neutrophil counts of BPH control and BPH-induced rats treated with 200 mg/kg CESH compared with the normal control. There were mild reductions in the neutrophil counts of the BPH-induced rats treated with finasteride and 600 mg/kg CESH relative to the normal control and BPH control, respectively.

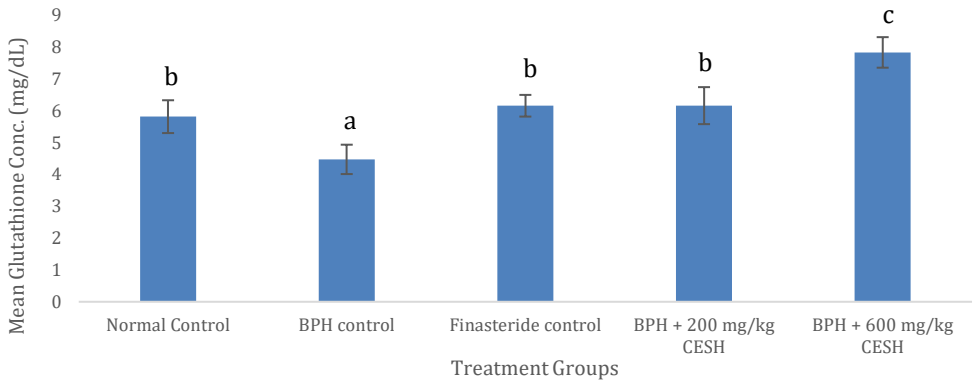
There were no significant (P>0.05) reductions in the percentage leucocyte counts of the BPH-induced rats in Table 2 treated with 5 mg/kg finasteride, 200 mg/kg CESH, and BPH control in comparison with the leucocytes count of the normal control. However, the leucocyte counts of the BPH-induced rats treated with 600 mg/kg CESH increased not significantly (P>0.05) when compared with the leucocyte counts of the normal control rats.

The percentage eosinophil counts of the BPH-induced rats in Table 2 showed significant (P<0.05) decreases in the eosinophil of the BPH control, BPH-induced rats treated with 5 mg/kg finasteride, 200 and 600 mg/kg CESH respectively when compared with the normal control. Also, there was no significant (P>0.05) between the eosinophil counts of BPH induced treated with finasteride and graded doses of CESH. Furthermore, no detectable levels of basophils and monocytes in the normal control rats, BPH control, finasteride, and graded doses of CESH treated BPH induced rats. These findings showed that BPH did not affect the neutrophil, lymphocytes, and eosinophil counts, contrary to Ugwu *et al*. (2019).

Effects of CESH on GSH concentrations, GPx, and GST activities of BPH-induced rats

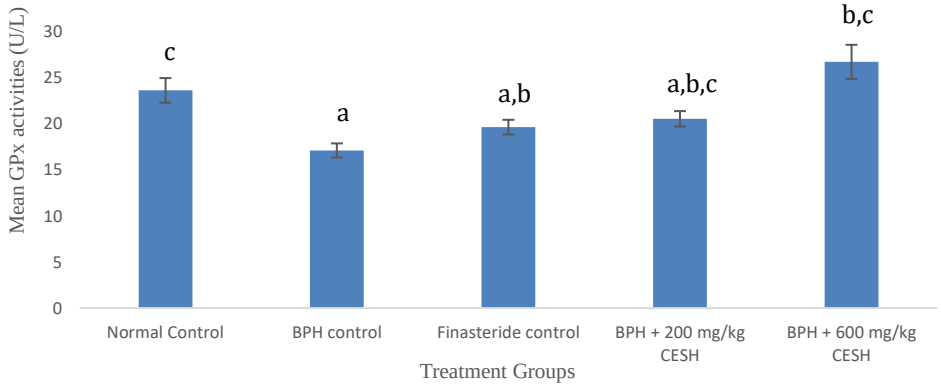
The glutathione peroxidase (GPx) activities of BPH-induced rats in Figure 1 indicated significant (P<0.05) reductions in the GPx activities of the BPH control, finasteride control, and BPH-induced rats treated with 200 mg/kg CESH in comparison with the normal control. Contrarily, the GPx activities of BPH-induced rats treated with 600 mg/kg CESH insignificantly (P>0.05) reduced relative to the normal control. The finasteride control and BPH-induced rats treated with 200 mg/kg CESH showed an insignificant (P>0.05) increase in GPx activities in comparison with the BPH control, unlike the GPx activities of the BPH-induced rats treated with 600 mg/kg CESH that increased significantly (P<0.05) relative to the BPH control. Also, there were insignificant (P>0.05) increases in the GPx activities of BPH-induced rats treated with 200 and 600 mg/kg CESH compared to the finasteride control.

It was evidenced in Figure 2 that the glutathione (GSH) concentrations of the BPH control rats significantly (P<0.05) decreased relative to the GSH concentration of the normal control. Whereas the GSH concentration of the finasteride and BPH-induced rats treated with 200 mg/kg CESH insignificantly (P>0.05) increased, and the GSH concentration of the BPH-induced rats treated with 600 mg/kg CESH increased significantly (P<0.05) when compared with the



Each of the bars is presented as mean ± standard deviation (n = 6); and bars with different superscripts are significantly (P < 0.05) different with any paired mean.

Figure 1. GSH concentrations of BPH-induced rats treated with CESH



Each of the bars is presented as mean ± standard deviation (n = 6); and bars with different superscripts are significantly (P < 0.05) different with any paired mean.

Figure 2. GPx activities of BPH-induced rats treated with CESH

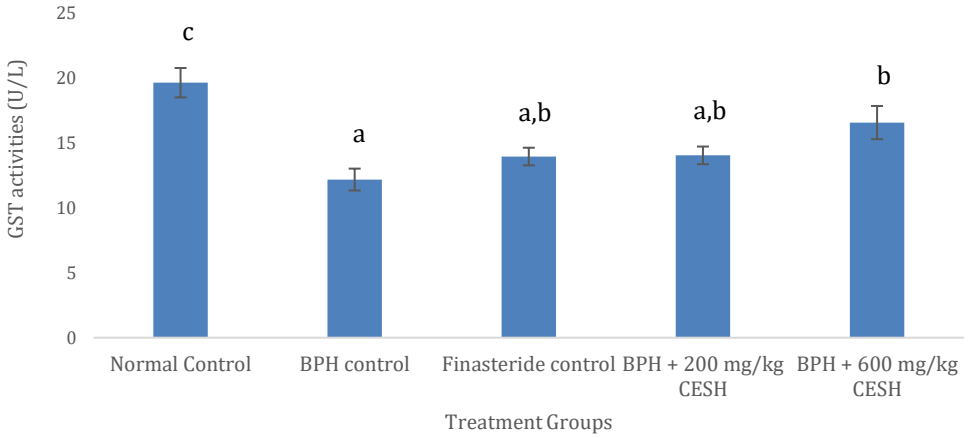
Table II. Differential white blood cell counts of BPH induced rats treated with CESH

Treatments Groups	Normal Control	BPH control	Finasteride Control	BPH + 200 mg/kg CESH	BPH + 600 mg/kg CESH
Neutrophil (%)	62.89±5.33[a]	68.97±6.34[a]	62.25±3.04[a]	64.68±2.20[a]	60.67±3.13[a]
Lymphocytes (%)	35.33±2.15[a]	30.67±3.02[a]	34.67±5.16[a]	33.67±3.22[a]	38.67±4.11[a]
Eosinophil (%)	3.00±0.04b	2.00±0.00[a]	2.00±0.00[a]	2.33±0.05[a]	2.00±0.00[a]

Values are presented as mean ± standard deviation (n = 6), and values with different superscripts are significantly (P<0.05) different from any paired mean across the row.

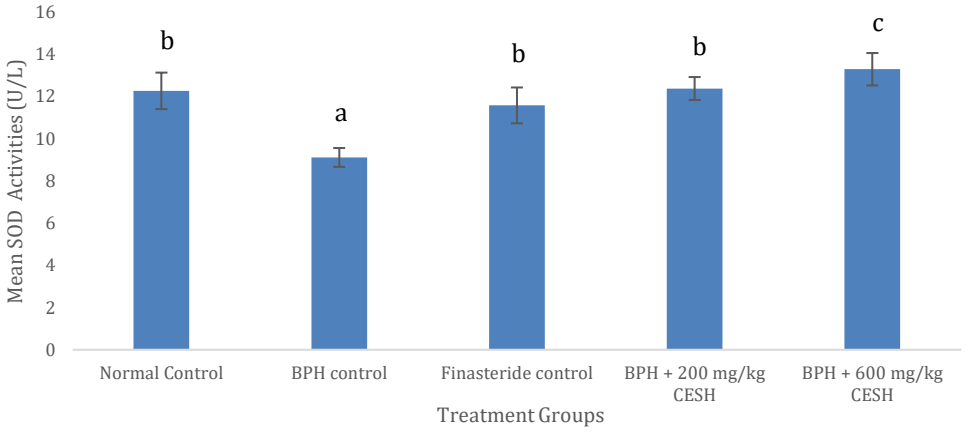
GSH concentration of the normal control rats. The GSH concentrations observed in the finasteride control and BPH-induced rats administered 200 and 600 mg/kg CESH respectively, were significantly (P<0.05) elevated compared with the GSH concentration of the BPH control rats. Similarly, the GSH concentration of BPH-induced rats administered 600 mg/kg CESH was significantly (P<0.05) elevated compared to the GSH concentration of the finasteride control.

The glutathione transferase (GST) activities of BPH-induced rats in figure 3 showed significant (P<0.05) reductions in the GST activities of the BPH control, finasteride control, and BPH-induced rats administered 200 and 600 mg/kg CESH, respectively, compared with the normal control. Contrarily, the finasteride control and BPH-induced rats that received 200 and 600 mg/kg CESH, respectively, showed significant (P<0.05) increases in the GST activities relative to the GST



Each of the bars is presented as mean ± standard deviation (n = 6); and bars with different superscripts are significantly (P < 0.05) different with any paired mean.

Figure 3. GST activities of BPH-induced rats treated with CESH



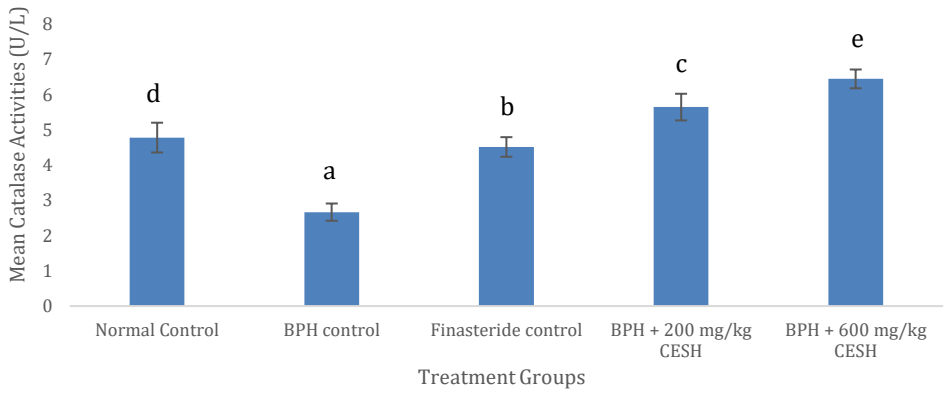
Each of the bars is presented as mean ± standard deviation (n = 6); and bars with different superscripts are significantly (P < 0.05) different with any paired mean.

Figure 4. SOD activities of BPH-induced rats treated with CESH

activities of the BPH control rats. Besides, insignificant (P<0.05) increases in the GST activities of BPH-induced rats administered 200 and 600 mg/kg CESH respectively, compared with the GST activities of the finasteride control.

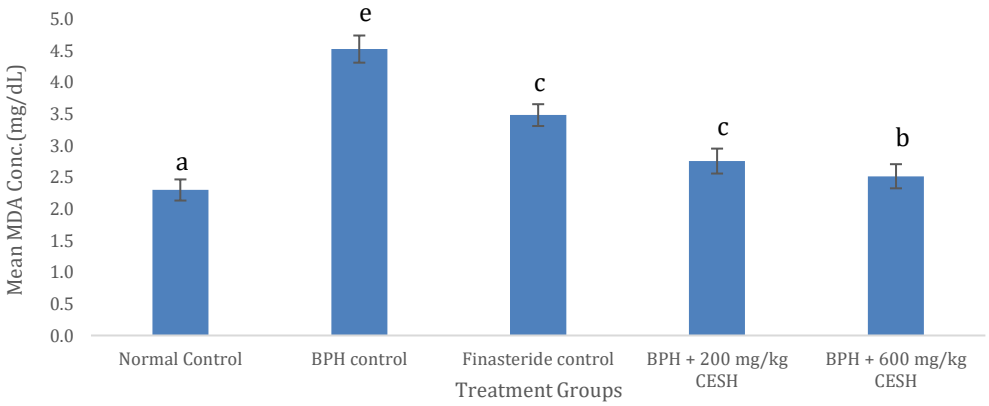
Effects of CESH on SOD activities of BPH-induced rats

The BPH-control and BPH-induced rats treated with 600 mg/kg CESH showed a significant (P<0.05) decrease and increase in SOD activities, respectively, when compared with the SOD activities of the normal control rats (Figure 4). The SOD activities of the finasteride control rats showed a slight decrease insignificantly; however, the SOD activities of the BPH-induced rats treated with 200 mg/kg CESH increased insignificantly (P>0.05) in comparison with the SOD activities of the normal control rats. It was also observed that the SOD activities of the finasteride control and BPH-induced rats treated with 200 and 600 mg/kg CESH respectively, increased significantly (P<0.05) relative to the SOD activities of the BPH control rats. At the same time, there were increases in the SOD activities of the BPH-induced rats treated with graded doses of CESH compared with the finasteride-treated BPH-induced rats. The SOD activities observed in the BPH-induced rats treated with 600 mg/kg CESH were significantly (P<0.05)



Each of the bars is presented as mean ± standard deviation (n = 6); and bars with different superscripts are significantly (P < 0.05) different with any paired mean.

Figure 5. CAT activities of BPH-induced rats treated with CESH



Each of the bars is presented as mean ± standard deviation (n = 6); and bars with different superscripts are significantly (P < 0.05) different with any paired mean.

Figure 6. MDA concentrations of BPH-induced rats treated with CESH

high when compared with the SOD activities of the finasteride control rats.

The significantly reduced SOD activities in the BPH control rats compared to the normal control align with findings from studies by Uroko *et al*. (2018) and Ikeyi *et al*. (2020). In contrast, the substantially increased SOD activities in the BPH-induced rats treated with finasteride and CESH compared to the normal control suggest the therapeutic effects of each treatment agent. Furthermore, the treatment group which received a high dose (600 mg/kg) of CESH had SOD activities that were significantly higher than the normal control. Increased SOD levels imply sufficient dismutase of excess superoxide radicals associated with BPH induction; to hydrogen peroxide and molecular oxygen. In tandem with a report by Ikeyi *et al*. (2020), our result indicates that herbal treatments contain antioxidant photochemical such as terpenoids, phenols, flavonoids, and saponins that can scavenge free radicals. Thus, the CESH may attenuate BPH by curbing oxidative damage.

Effects of CESH on catalase CAT activities in BPH-induced rats

The result in Figure 5 indicated significant (P<0.05) reductions in the catalase activities of the BPH control, finasteride control, and BPH-induced rats treated with 200 mg/kg CESH, respectively when compared with the catalase activities of the normal control rats. However, the catalase activities of the BPH-induced rats treated with 600 mg/kg CESH significantly (P<0.05) increased relative to the catalase activities of the normal control rats. Similarly, in the finasteride control, BPH-induced rats treated with 200 and 600 mg/kg

CESH had significantly (P<0.05) elevated catalase activities compared with the BPH control. Also, the BPH-induced rats treated with 200 and 600 mg/kg CESH had significantly (P<0.05) increased catalase activities compared with the catalase activities of the finasteride control rats.

The BPH control showed very varied CAT activities; treatment with finasteride and low-dose CESH following BPH induction improved CAT activities. However, this improvement remained significantly below the CAT activity of the normal control. Only treatment with high doses of CESH restored CAT levels significantly above the normal levels. The CESH might have stabilised the enzyme protein promoting its role in converting excess hydrogen peroxide to water and molecular oxygen. Decreased CAT activities following BPH induction are in line with previous findings by Uroko *et al*. (2018) and Ikeyi *et al*. (2020), which are attributable to inactivation and decreased synthesis of the enzyme, probably via inhibition of the gene responsible for CAT synthesis.

Effects of CESH on MDA of BPH-induced rats

The MDA concentrations of BPH-induced rats in figure 6 indicated significant (P<0.05) increases in the MDA concentrations of the BPH control, finasteride control, and BPH-induced rats administered 200 and 600 mg/kg CESH respectively, compared with the MDA concentration of the normal control rats. Also, the MDA concentrations of the finasteride control and BPH-induced rats that received 200 and 600 mg/kg CESH, respectively, were significantly (P<0.05) reduced in relationship with the MDA concentration of the BPH control. Besides, the MDA concentration of the BPH-induced rats administered 600 mg/kg CESH was significantly (P<0.05) reduced when compared with the MDA concentration of the finasteride control.

A cellular injury could trigger lipid peroxidation resulting in increased MDA production, which serves as a biomarker for monitoring such damage (Uroko *et al*., 2022b). The findings of this study show that the BPH induction in experimental rats significantly raised MDA concentration above normal levels. MDA and 4-hydroxyalkanals are the decomposition products of polyunsaturated fatty acid peroxides resulting in oxidative stress, mutagenesis, and uncontrolled cellular proliferation (Ikeyi *et al*., 2020). Although there was an improvement in the MDA following treatment with 200 mg/kg CESH was comparable to finasteride, the amount of MDA levels indicated oxidative stress in the rats. A further significant decrease in MDA levels was observed following treatment with 600 mg/kg CESH, but this was still significantly above normal safe levels. Considering the high safety margin of CESH, an increased dose of CESH could sufficiently decrease MDA levels without posing toxicological threats to the animals (Uroko et al., 2022b). Additional dietary antioxidant supplementation and lifestyle changes can synergise in effectively ameliorating lipid peroxidation associated with BPH. The antioxidant effects of CESH against BPH-associated oxidative damage are attributable to its high contents of antioxidant phytochemicals such as flavonoids, polyphenols, terpenoids, tannins, saponins, phenols, polyterpenes, steroids, and alkaloids (Uroko *et al*., 2022a). Although our study provides meaningful preclinical evidence on the antioxidant effects of CESH in ameliorating BPH, it leaves an opportunity for further works on the specific solvent fraction or isolated phytonutrient responsible for the antioxidant effects; molecular docking studies on the binding characteristics of this phytonutrient vis-a-vis the active pharmaceutical ingredient of existing commercial drugs such as finasteride; further clinical studies and mechanism of action.

CONCLUSION

The findings of this study reveal that treating BPH with graded doses of CESH can restore the antioxidant status and normalise haematological indices. Our result indicates that 600 mg/kg CESH is a more viable treatment for BPH than finasteride, as shown by the improved antioxidant status and haematological healing. Also, considering the cost and side effects of synthetic drugs, therapeutic preparations such as CESH, rich in antioxidant phytochemicals, can serve as viable alternatives after thorough clinical studies.

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REFERENCES

Aebi, H.E., 1983, 'Catalase. In: Bergmeyer HU, (ed) Methods of Enzymatic Analysis' 3rd edn. Deefield Beach, pp. 273-285, Weinheim, Florida.

Agrawal, M., Nahata, A. & Dixit, V.K., 2012, 'Protective effects of Echinops echi-natus on testosterone-induced prostatic hyperplasia in rats', *Eur. J. Integr. Med.* 4, 177-185.

Ajayi, A. & Abrahama, K., 2018, 'Understanding the role of oestrogen in the development of benign prostatic hyperplasia', *African J. Urol*. 24, 93–97.

Bamishaiye, E., Muhammad, N. & Bamishaiye, O., 2009, 'Haematological parameters of albino rats fed on tiger nuts (*Cyperus esculentus*) tuber oil meal-based diet. *Internet J. Nutr. Wellness.* 10, 1-5.

Burkill, H.M., 'The Useful Plants of West Tropical Africa', Volume 1, 2ndEdition, Royal Botanic Gardens, Kew. 1995.

Choi, J., Shendrik, I., Peacocke, M., Peehl, D., Buttyan, R., Ikeguchi, E.F., Katz, A.E. & Benson, M.C., 2000, 'Expression of senescence associated beta-galactosidase in enlarged prostates from men with benign prostatic hyperplasia', Urol. 56, 160-166.

Conserva, L.M. & Ferreira Júnior, J.C., 2012, 'Borreria and Spermacoce species (Rubiaceae): A review of their ethnomedicinal properties, chemical constituents, and biological activities', *Pharmacogn. Rev.* 6(11), 46-55.

Dacie, J.V. & Lewis, S.M., 1991, 'Practical Haematology. Edingburgh: Churchhill Livingstone 7: 535-44.

Dhami, N., 2013. Trends in Pharmacognosy: a modern science of natural medicines. *J. Herb. Med*. 3(4), 123-131

Fu, S., Zhang, X., Niu, Y. & Wang, R.T., 2018, 'Prostate specific antigen, mean platelet volume, and platelet distribution width in combination to discriminate prostate cancer from benign prostate hyperplasia', *Asian Pac. J. Cancer Prev.* 19(3) 699-702

Güzel, A., 2021, 'Can Preoperative Haematological parameters predict pathology results in patients undergoing open prostatectomy due to benign prostatic hyperplasia? A Retrospective Study. *Meandros Med. Dent. J*. 22, 47-52.

Ijioma, S.N., Osim, E.E., Nwankwo, A.A., Nwosu, C.O. & Ekeleme, C.M., 2019, 'Antioxidant potentials and effects on the hematology and osmotic fragility scores of a polyherbal formulation used in Southeast Nigeria', J. Basic. Clin. Physiol. Pharmacol. *30*(4), 10.1515/jbcpp-2017-0099.

Ikeyi, A.P., Okagu, I.U., Ezeanyika, L.U.S. & Alumanah, E.O., 2020, 'Zapoteca portoricensis root crude methanol extract and its fractions normalises aberrations associated with benign prostatic hyperplasia in rats', *All Life*. 13(1), 360-372.

Jiang, S., Song, C.S. & Chatterjee, B., 2019, 'Stimulation of prostate cells by the senescence phenotype of epithelial and stromal cells: Implication for benign prostate hyperplasia', *FASEB, BioAdv.* 1, 353-363.

Kajiwara, S., Ishii, K., Sasaki, T., Kato, M., Nishikawa, K., Kanda, H., Arima, K., Watanabe, M. & Sugimura, Y., 2020, 'Castration-induced stromal remodelling disrupts the reconstituted prostate epithelial structure', *Lab. Invest.* 100, 670-681.

La Vignera, S., Condorelli, R.A., Russo, G.I., Morgia, G. & Calogero, A.E. 2016, 'Endocrine control of benign prostatic hyperplasia. *Androl.* 4(3), 404-411.

Liu, T.T., Thomas, S., Mclean, D.T., Roldan-Alzate, A., Hernando, D., Ricke, E.A. & Ricke, W.A., 2019. Prostate enlargement and altered urinary function are part of the aging process. Aging (Albany NY). 11(9), 2653-2669

Mbaka, G.O., Ogbonnia, S.O. & Olawunmi, O. 2013. The effects of ethanol extract of Raphia hookeri seed on exogenous testosterone and estradiol induced benign prostatic hyperplasia in adult male rats. *J. Morphol. Sci.* 30(4), 235–243.

Nwandu, C.J., Nyananyo, B.L. & Ozimede, C.O., 2019, 'Phytochemical screening of leaf extracts of eleven selected tropical plants species from eastern and southern Nigeria', *J. Appl. SCI. Environ. Manag.* 23(10), 1867-1873.

Ohkawa, H., Ohishi, N. & Yagi, K., 1979, 'Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction', *Anal. Biochem*, 95(2), 351-358.

Parrotta, J.A., 2001, 'Healing Plants of Peninsular India. Wallingford', pp.614-615. CAB International Publishing.

Shah, A., Shah, A.A., Nandakumar, K. & Lobo, R., 2021, 'Mechanistic targets for BPH and prostate cancer: A review', *Environ. Health Rev.* 36(2), 261-270.

Sharma, M., Chadha, R. & Dhingra, N., 2017, 'Phytotherapeutic agents for benign prostatic hyperplasia: an overview', *Mini-Rev. Med. Chem.* 17(14), 1346-1363.

Subramanya, M.D., Pai, S.R., Upadhya, V., Ankad, G.M., Bhagwat, S.S. & Hegde, H.V., 2015, 'Total polyphenolic contents and in vitro antioxidant properties of eight Sida species from Western Ghats, India', *J. Ayurveda. Integr*. Med. 6, 24-8.

Taoka, R. & Kakehi, Y., 2017, 'The influence of asymptomatic inflammatory prostatitis on the onset and progression of lower urinary tract symptoms in men with histologic benign prostatic hyperplasia', *Asian J. Urology.* 4, 158-163.

Ugwu, M.N., Asuk, A.A., Eteng, M.U. & Emuru, E.O., 2019, 'Evaluation of impact of some selected plants on haematological parameters of testosterone and estradiol induced benign

prostatic hyperplasia adult male rats', *Int. Blood Res. Rev*. 10(1), 1-15.

Uroko, R.I., Simeon, I.E., Oluomachi, N.U., Chidiogo, A.O., Amarachi, A. & Cosmos, C.A., 2018, 'Therapeutic effects of methanolic extract of *Funtumia africana* leaves on antioxidants and hematological indices of carbon tetrachloride-induced oxidative stress in rats', *Drug Invent. Today.* 12 (1),114-122.

Uroko, R.I., Ijioma, S.N., Ogwo, E.U. & Awah, F.M., 2022b, 'Combined ethanol extract of *Hypselodelphys poggeana* and *Spermacoce radiata* leaves ameliorate benign prostatic hyperplasia in rats via modulation of serum sex hormonal levels', *J. Res. Pharm.*; 26(4), 742-752.

Uroko, R.I., Nweje-Anyalowu, P.C., Aaron, C.F. & Chukwu, C.N. 2022a, 'Combined extract of *Spermacoce radiata* (DC.) Hiern and *Hypselodelphys poggeana* (K.Schum.) Milne-Redh leaves (CEESH) confer hepatoprotection in rat induced benign prostatic hyperplasia', *Plant Biotechnol. Persa.* 4(1), 78-88

Uroko RI, Nwuke CP, Egba SI, Uchenna ON, Nwaekwu, FE, Anyanwu, PO (2020) Effects of combined ethanolicic extracts of Funtumia Africana and Abutilon Mauritianum leaves on sex Hormones and prostate indices of benign prostatic Hyperplasia induced rats, Animal Research International 17(3):3892 – 3902

Ursini, F., Maiorino, M. & Gregolin, C., 1985, 'The selenoenzyme phospholipid hydroperoxide glutathione peroxidase', Biochim. Biophys. Acta. 839, 62-70.

Xin, Z., Waterman, D.F., Hemken, R.W. & Harmon, R.J., 1991, Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers', *J. Dairy Sci.* 74, 3078-3085.

Zuo, L., Prather, E.R., Stetskiv, M., Garrison, D.E., Meade, J.R., Peace, T.I. & Zhou, T., 2019, 'Inflammaging and oxidative stress in human diseases: From molecular mechanisms to novel treatments', *Int. J. Mol. Sci. 20*(18), 4472.