

Full Length Research Paper

Effect of ethanol leaf extract of *Gnetum africanum* on testosterone and oestradiol induced benign prostatic hyperplasia

EBENYI Lilian N.^{1*}, YONGABI Kenneth A.^{1,2}, ALI Fredrick U.¹, OMINYI Mathias C¹, ANYANWU Blessing¹ and OGBANSHI Moses E.³

¹Department of Biotechnology, Faculty of Science, Ebonyi State University, P. M. B. 043, Abakaliki, Ebonyi State, Nigeria.

²Department of Public Health, Faculty of Health Sciences, Imo State University, Owerri, Imo State, Nigeria.

³Department of Biochemistry, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Received 28 December, 2019; Accepted 14 April, 2020

The effect of ethanol leaf extract of *Gnetum africanum* was studied in albino rats induced with benign prostatic hyperplasia. The animals (36) were randomly grouped into six with six rats in each group. Testosterone and oestradiol every other day for 28 days was used to induce hyperplasia. The test groups (2 - 4) were treated with 200, 400 and 800 mg/kg body weight of extract for another 28 days. Group 5 was given the standard drug while group 6 served as negative control. At the end of the treatment period, the rats were sacrificed under anesthesia and blood samples collected for biochemical analysis. The results showed that in the animals exposed to the inducing agents increased significantly ($p < 0.05$) in activity of MDA, ACP and PSA levels whereas there was a significant ($p > 0.05$) decrease in the activities of GR, CAT and SOD as compared with the normal control. However, treatment with ethanol leaf extract of *G. africanum* showed significant ($p > 0.05$) increase in the activities of the antioxidant enzymes and significant ($p > 0.05$) decrease in the level of MDA, ACP and PSA in a dose dependent manner as comparable to the normal control. These findings are indication that the extract of *G. africanum* has potential remedial effects on benign prostatic hyperplasia.

Key words: *Gnetum africanum*, oxidative stress, antioxidants, chemopotential, prostate hyperplasia.

INTRODUCTION

Plants which have medicinal values and are widely used for medical purposes are called medicinal plants (Andren et al., 2006). Such plants include various herbs, shrubs and trees. Various parts of the plants including root, leaf, flower, fruit and bark are used to cure diseases. Besides

using them to cure diseases, fatal diseases like cancer can be controlled by daily consumption of such medicinal plants (Bartsch et al., 2002). The use of medicinal plants as remedy for various diseases is the oldest form of healthcare known to mankind. However, since a single

*Corresponding author. E-mail: Lilianebenyi@yahoo.com.

plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain hence the continuous research to discover more potentials (Anand et al., 2008). Due to the increased side effect of synthetic drugs and the fact that human body easily adjusts and become resistant to drugs, there is need to continuously search for medicinal plants for the treatment of common diseases with reduced side effects (Akpanabiatu, 2006).

Cancer is a disease characterized by abnormal cell growth with the potential to invade or spread to other parts of the body. When genetic changes interfere with normal cell processes, cells begin to grow uncontrollably leading to formation of a mass known as tumor. Some tumors are cancerous while others are benign depending on its ability to grow and spread to other parts of the body (National Cancer Institute, 2018).

Benign prostatic hyperplasia (BPH), commonly called prostate enlargement, is a condition characterized by proliferation of the cellular elements of the prostate (Enam et al., 2014). The stromal and glandular epithelial cells are predominantly affected and it is associated mainly with the posterior urethral glands (PUG) as well as the transitional zone (TZ), and to a lesser extent the peripheral zone (PZ). Chronic bladder outlet obstruction (BOO) secondary to BPH may lead to renal insufficiency, urinary retention, gross hematuria, recurrent urinary tract infections and bladder calculi (Esraa et al., 2014). Benign prostatic hyperplasia (BPH) can be a progressive disease if left untreated. They are increased risk of urinary tract infection due to residual urine or urinary stasis caused by incomplete voiding. The prevalence of BPH shows that between the ages of 50-60 years, 50% of men are affected while 90% of men above 80 years old have this condition (Wasserman, 2006). Lifestyle factors such as diet, smoking, exercise, having multiple sex partners and oxidative stress are believed to influence prostate growth (Suzuki et al., 2002). According to Wein et al. (2007), age, androgen and functional androgen receptors, genetics, dyslipidaemia, obesity and diabetes are risk factors associated with BPH while body mass index, diet, smoking, hypertension and sexual malfunction are categorized as possible risk factors (Wein et al., 2007).

Gnetum africanum commonly called wild spinach or afang is a vine Gymnosperm species found natively throughout tropical Africa and belongs to Gnetaceae family (Chindong, 2011). Though bearing leaves, the genus *Gnetum* are gymnosperms, related to pine and other conifers (Soltis et al., 2002). It is a perennial that grows approximately 10 metres long, with thick papery-like leaves growing in groups of three. The leaves may grow approximately 8 cm long, and at maturity the vine will produce small cone-like reproductive structures. The seeds of the vine resemble a fleshy fruit, sized 10-15 mm x 4-8 mm, and are red-orange in color when fully ripe (Bowe et al., 2000). Primarily, *G. africanum* leaves sold typically cut for recipes are used as a vegetable for soups

and stews, commonly called Eru soup or afang soup (Tekwe et al., 2003). *G. africanum* is a great source of protein, essential and non-essential amino acids. It contains high glutamic acid, leucine, and aspartic acid, with low levels of histidine, cysteine and tryptophan. The content of amino acids found in it is similar to recommended levels by the FAO. It has also been found that the levels of iodine are also high in the vine. Fiber levels average approximately 33.4 g/100g of dried Eru leaves, while recommended daily intake of fiber is 30 g (Ali et al., 2011). Medicinally, *G. africanum* has been noted to have anti-inflammatory, anti-carcinogenic and antioxidant potentials (Soltis et al., 2002). In Nigeria, the leaves of *G. africanum* are used by traditional medicine practitioners to treat enlarged spleens, sore throats and as a cathartic (Edet et al., 2005). According to Udoh (2007), the plant is used as a remedy for nausea and regarded as an antidote for certain types of poisons. Also in the Republic of the Congo (Brazzaville), *Gnetum* leaves are used as a dressing for warts, boils and hemorrhoids. The cuttings are used to brew herbal teas to soothe labor pains (Besong et al., 2001). The plant is also used as a medicinal plant in Mozambique. Because of its high fiber content, it is often recommended to ease constipation and to control blood sugar levels in diabetics.

Studies have shown that majority of the rural populace patronize traditional medicine practitioners more due to their level of education, the high cost of conventional treatment and risk of a negative outcome of the surgery. Thus, the aim of this study was to investigate the effect of ethanol leaf extract of *G. africanum* as a natural therapy in the treatment of benign prostatic hyperplasia using animal model.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *G. africanum* were bought from meat market Abakaliki, Ebonyi State, Southeast, Nigeria. Abakaliki city is the capital of Ebonyi State. It is situated at latitude 6.32°N and longitude 8.12°E and 117 m elevation above sea level.

Test animals

Thirty - six healthy and sexually mature male wistar albino rats of 12 weeks old, weighing approximately 100 - 160 kg body weight were purchased from Nsukka Enugu State, Nigeria. The rats were housed in conventional wire mesh cages under standard laboratory conditions. The animals were acclimatized for two weeks under good ventilation with 12 h house light and dark cycle in an animal house at Presco campus, before the commencement of the treatment. Generally, the study was conducted in accordance to the criteria outlined in principles of Laboratory Animal care, 1985.

Extraction

The powdered *G. africanum* leaves were subjected to ethanol

extraction. The ethanol extract was prepared by soaking 200 g of powdered plant leaves in 750 ml of ethanol at room temperature for 48 h. The mixture was shaken at intervals. The mixture was filtered with Muslim cloth and the filtrate was concentrated by evaporation to dryness. The extract obtained was stored in airtight container until when needed for the investigation.

Experimental design

The albino rats were weighed and randomly placed into six different groups of six albino rats in each cage at the end of the two weeks of acclimatization period. Testosterone and oestradiol were administered for the induction of benign prostatic hyperplasia. The hormones, oestradiol at 0.04 mg and testosterone at 0.08 mg per kg body weight, which were all diluted with 0.01 ml of Goya oil, were administered parenteral at the inguinal region using insulin syringe for 21 days.

Group 1 was not induced and served as normal control, while the test groups (2 – 6) were induced with benign prostatic hyperplasia using 0.08 mg/kg of testosterone and 0.04 mg/kg of oestradiol every other day for 28 days. The hormones diluted with 0.01 ml of olive oil were administered parenterally at the inguinal region of the animals using insulin syringe. Hyperplasia was confirmed with PSA analysis. The test groups (2 - 4) were treated with 200, 400 and 800 mg/kg body weight of ethanol leaf extract of *G. africanum* for another 28 days. Group 5 was given the standard drug (finasteride 200 mg/kg) while group 6 which served as negative control was treated with normal saline only. At the end of the treatment period, the rats were sacrificed under anesthesia and blood samples collected for required biochemical analysis (malondialdehyde, (MDA), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), acid phosphatase (ACP) and prostate specific antigen (PSA)).

Biochemical analysis

Determination of oxidative stress parameters; malondialdehyde (MDA), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) were done according to the manufacturer's protocols.

Acid phosphatase determination in serum

Acid phosphatase was analyzed based on enzyme kinetic method (Hillman, 1971). Reagent was reconstituted according to the instruction given. Tubes were labeled; control, test and blank. Spectrophotometer was zeroed with water at 495 nm. And cuvette was settled at temperature of 37°C. About 0.10 ul of sample was incubated at 37°C for 5 min. Then absorbance was read and recorded every minute for 5 min to determine change in acid phosphatase 1 min. The procedure was repeated for each sample. Values were obtained by multiplying the acid phosphatase/minute by the factor.

Non-prostatic acid phosphatase

1.0 ml of reagent was added to appropriately labeled tubes. 10 µl of L. Tartrate reagent was added and mixed. Spectrophotometer was zeroed with 450 nm cuvette and settled at temperature of 37°C. 100 ul of sample was added, mixed and incubated at 37°C for 5 min. Absorbance was read, recorded every minute after incubation for 5 min to determine change in total acid phosphatase 1 min. Values (u/l) were obtained by multiplying change in total acid phosphatase 1 min by the factor. The values were obtained by subtracting the

result of non-prostatic acid phosphatase assay from total acid phosphatase assay.

Prostate specific antigen (PSA) analysis

The PSA Test Device is a semi-quantitative, membrane based immunoassay for the detection of PSA in whole blood, serum or plasma. The membrane is pre-coated with PSA antibodies on the test line region. During testing, the specimen reacts with the particle coated with anti-PSA antibody.

Procedure

About 25 µl of the serum and standard were pipetted into the microplate wells. 100 µl of PSA enzyme reagent was added into each well. Then the mixture in microplate wells was swirled for 30 s to mix and incubated at room temperature for 30 min. The contents of the microplate were discarded. The bottom of the microplate was tapped and blotted with absorbent paper. 350 ul of wash buffer was added to each well, washed and discarded three times. Then, 100 µl of working substrate solution was added into each well and incubated at room temperature for 15 min. 50 ul of stop solution was added and mixed gently for 20 s.

Statistical analysis

Data generated were expressed as mean and standard deviations. Statistical significance of difference was determined by performing one-way Analysis of variance (ANOVA) with post-hoc comparisons between the control group and each of the treated groups by Duncan's multiple comparison tests. $P < 0.05$ was considered statistically significant

RESULTS

The administration of testosterone and oestradiol had a significant increase ($P > 0.05$) in the level of malondialdehyde. And treatment with *G. africanum* methanol leaf extracts had reductions effect in the level of malondialdehyde. The result of malondialdehyde is shown in Figure 1. The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

In Figure 2, the result showed a significant decrease in the level of catalase on the induced rats. Treatment with *G. africanum* leaf extract increased the catalase level in a dose – dependent manner. The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$). From the result in Figure 3, it was observed that testosterone and oestradiol induced hyperplasia and decreased the level of glutathione reductase. Administration of ethanol leaf extract of *G. africanum* increased serum glutathione reductase level significantly compared to the negative control group. The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

In Figure 4, induction of hyperplasia reduced the serum level of super oxide dismutase as seen in negative control group. On administration of *G. africanum* extract, the level of serum SOD significantly increased in a dose

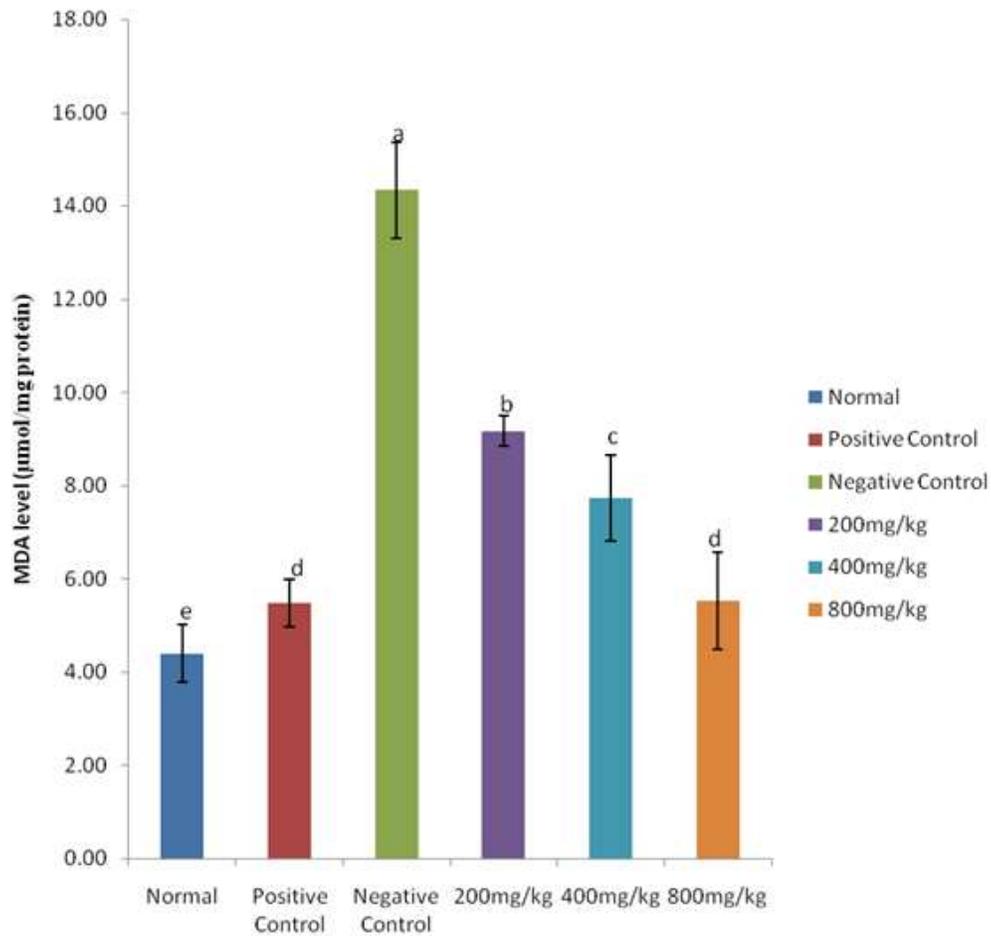


Figure 1. Effect of ethanol leaf extract of *G. africanum* on malondialdehyde level of rats induced with testosterone and oestradiol. The results are mean ± SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

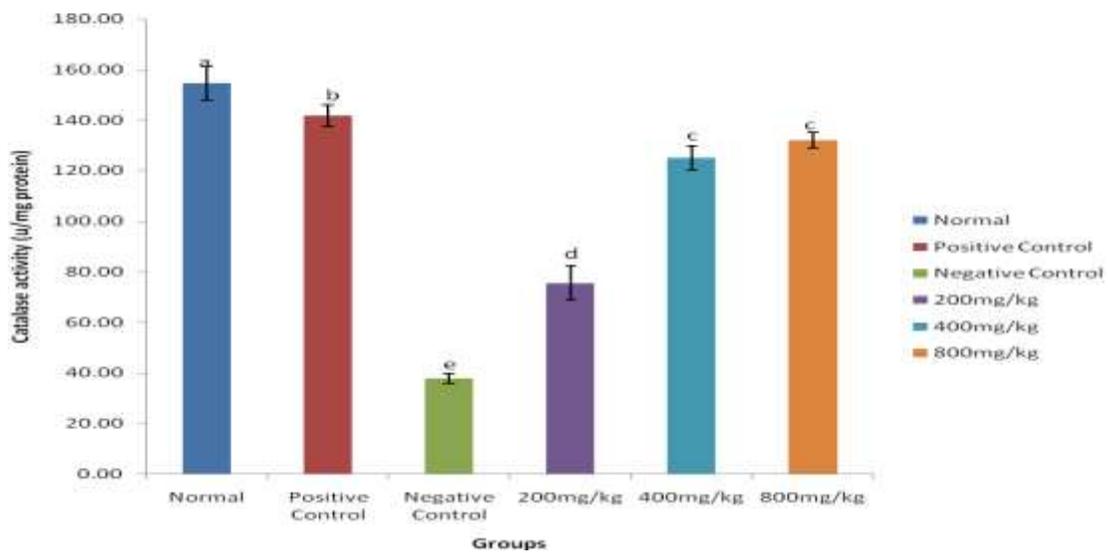


Figure 2. Effect of ethanol leaf extract of *G. africanum* on catalase level of rats induced with testosterone and oestradiol. The results are mean ± SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

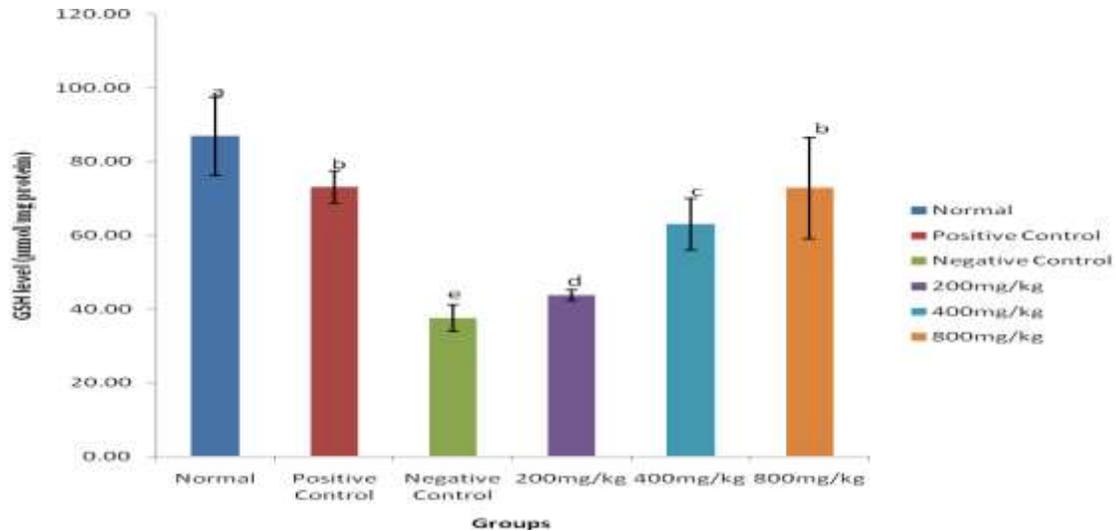


Figure 3. Effect of ethanol leaf extract of *G. africanum* on glutathione reductase level of rats induced with testosterone and oestradiol. The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

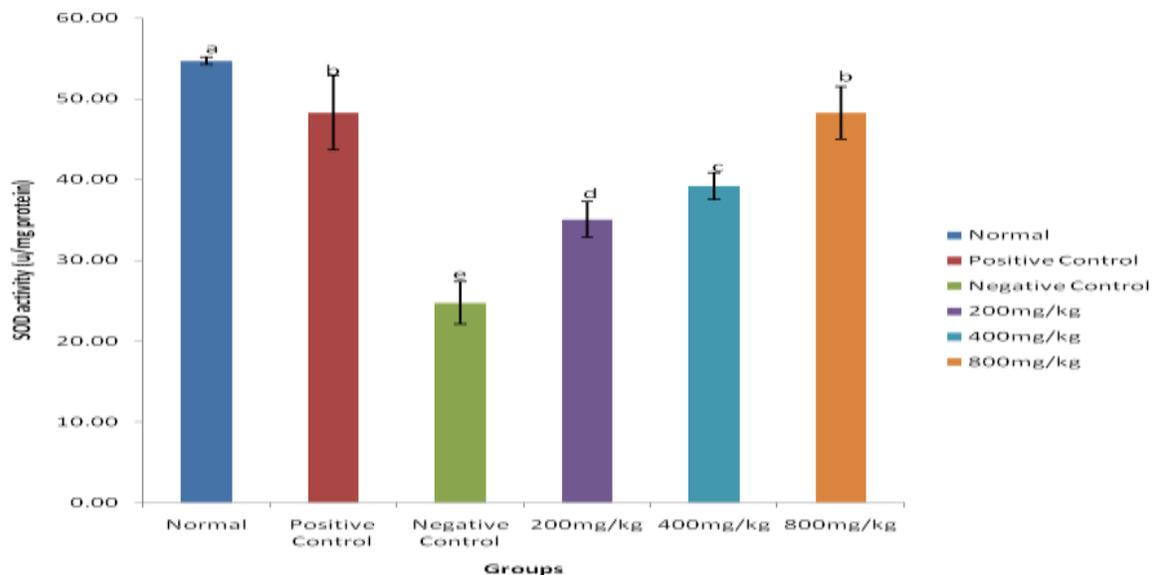


Figure 4. Effect of Ethanol Leaf Extract of *Gnetum africanum* on SOD Level of Rats Induced with Testosterone and Oestradiol. The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

– dependent manner. The result also showed that the highest dosage of the extract, 800 mg/kg was as effective as those treated with the standard drug (positive control group). The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

The administration of testosterone and oestradiol caused a significant reduction in the serum level of acid phosphatase. The highest level was observed in group 6 (negative group) which was induced but not treated.

From the result represented in Figure 5, it can be deduced that ethanol leaf extract of *G. africanum* at 800 mg/kg (Group 1) indicated the most effective dose in the decrease of acid phosphatase level, when compared to other doses (400 and 200 mg/kg). The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

Administration of testosterone and oestradiol caused a significant increase in the level of prostate specific

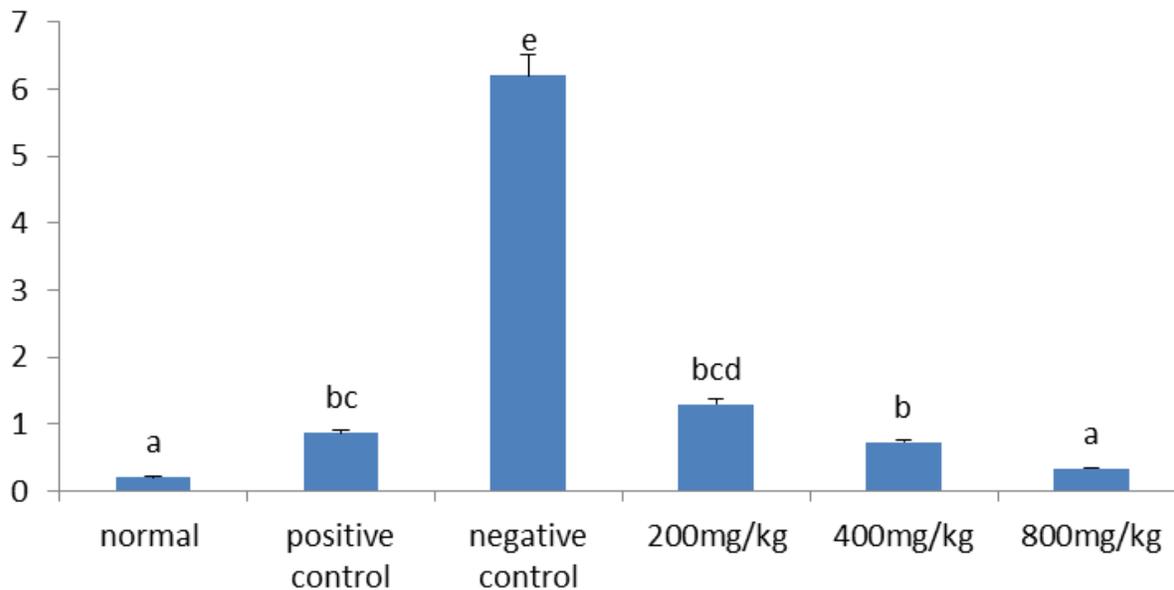


Figure 5. Effect of ethanol leaf extract of *Gnetum africanum* on acid phosphatase level of rats induced with testosterone and oestradiol. The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

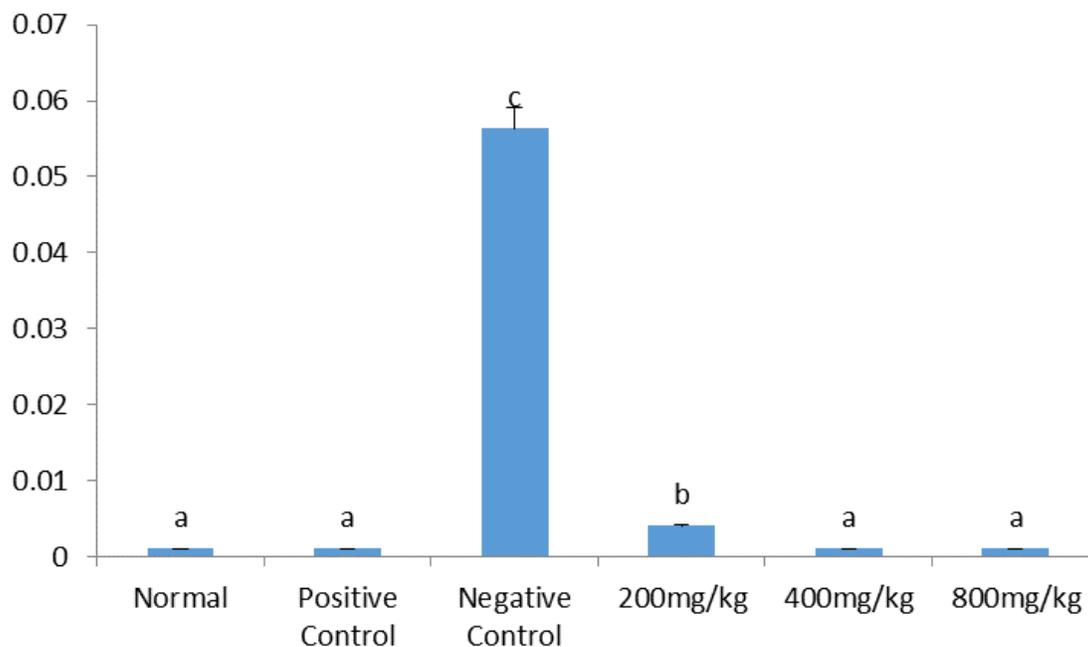


Figure 6. Effect of ethanol leaf extract of *G. africanum* on prostate specific antigen of rats induced with testosterone and oestradiol. The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

antigen. The results revealed that ethanol leaf extracts at 200, 400, and 800 mg/kg respectively showed a relative decrease of prostate specific antigen after the treatment with the extracts. The result also showed that 800 mg/kg

has the most effective reduction when compared to other doses. This is represented in Figure 6. The results are mean \pm SD of 6 rats. Bars with different letters differed significantly ($P < 0.05$).

DISCUSSION

This research investigated the antitumor potential of ethanol leaf extract of *G. africanum* in albino rats induced with benign prostate hyperplasia. Malondialdehyde (MDA) occurs naturally as a marker for checking oxidative stress. When reactive oxygen species degrade polyunsaturated lipids, a reactive aldehyde compound which is one of the many reactive electrophile species that cause toxic stress in cells is formed (De Nunzio et al., 2012). This forms covalent protein adducts called advanced lipoxidation end-products (ALE), in analogy to advanced glycation end-products (AGE). The aldehyde produced which is malondialdehyde acts as a biomarker to measure the level of oxidative stress in an organism. The result of this work showed a significant ($p < 0.05$) reduction of malondialdehyde (MDA) on the group administered with 800 mg/kg of extract when compared to the positive control group. The reduction effect on the malondialdehyde (MDA) after treated with the extracts was observed in a dose dependent manner. The ability of the extract to significantly decrease the level of MDA might be attributed to its high antioxidant activity, mopping up free radicals in the body. This finding is in agreement with the work of Robert and Breyer-Brandwijk (2011). Hence, it is deduced from the result that *G. africanum* has high oxidative property which can be used to reduce oxidative stress.

Cells in the body have a number of mechanisms to protect themselves from the toxic effect of reactive oxygen species (ROS). Intracellular antioxidants like, glutathione, catalase and superoxide dismutase are intracellular reductants, widely distributed in cells and plays major role in catalysis, metabolism and transport (Bowe et al., 2000). These antioxidants protect cells from free radicals, peroxides and other toxic compounds (Ebenyi et al., 2012). In the present study, the effectiveness of *G. africanum* extracts were demonstrated using benign prostatic hyperplasia induced rats as a model for inflammation. The results showed that in the animals exposed to the inducing agents decreased significantly ($P > 0.05$) the activities of GSH, CAT and SOD as compared with the normal control. However, treatment with ethanol leaf extract of *G. africanum*, showed significant ($P < 0.05$) increase in the activities of these antioxidant enzymes in a dose dependent manner as comparable to the normal control. This finding is indicative that the extract of *G. africanum* has high potential antitumor and anti-inflammatory activities.

Acid phosphatase is an enzyme secreted by prostate gland into seminal fluid and is found in concentrations up to 400 times greater in semen than in other body fluids. Different forms of it are found in different organs and their serum levels are used to evaluate the success of the surgical treatment of prostate cancer (Alcaraz et al., 2009). It acts to liberate phosphate under acidic condition and is made in the liver, bone marrow, spleen etc. When

there is increase acid phosphatase in the body, it means that there is a disease condition (Andren et al., 2006). The results showed that ethanol leaf extract of *G. africanum* at 800 mg/kg dose were most effective in the decrease of acid phosphatase level, when compared to other doses (400 and 200 mg/kg). This study indicated that ethanol leaf extracts of *G. africanum* can be used to control acid phosphatase in the body especially those diseases that are associated with benign prostatic hyperplasia. This finding is in agreement with the finding of Bushman (2009).

The results showed that the inducing agents, significantly ($P < 0.05$) increased the activity of PSA levels as compared with the normal control. Prostate specific antigen (PSA) is produced by prostate glandular and endothelial cells. PSA has been detected in various tissues of the male urogenital system but only prostate glandular and endothelial cells secrete it (Bartsch et al., 2002). It can be elevated in malignant conditions such as prostate cancer, and in benign condition such as benign prostatic hyperplasia and prostatitis. Many studies have confirmed that the presence of PSA is the most useful and meaningful tumor marker known for prostate cancer and prostate infection of Benign Prostatic Hyperplasia (BPH).

However, treatment with ethanol leaf extract of *G. africanum*, showed significant ($P > 0.05$) decrease in the level of PSA in a dose dependent manner as comparable to the normal control. Treatment with ethanol leaf extracts at 800 mg/kg showed a relative decrease of prostate specific antigen as compared to other doses. This finding is in agreement with the finding of Andren et al. (2006) who made similar finding using albino rats. These findings are indication that the extract of *G. africanum* has potential remedial effects on benign prostatic hyperplasia

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Akpanbiatu MI, Umoh IB, Uoh FE, Udosen EO (2006). Effect of leaf extracts on some rats serum enzymes of clinical significance: Recent progress in medicinal plant. *Biopharmaceutical* 14:297-304.
- Alcaraz A, Hammerer P, Tubaro A, Schröder FH, Castro R (2009). Is there evidence of a relationship between benign prostatic hyperplasia and prostate cancer? Findings of a literature review. *European Urology* 55(4):864-873.
- Ali F, Assanta MA, Robert C (2011). *Gnetum africanum*: A Wild Food Plant from the African Forest with Many Nutritional and Medicinal Purposes. *Journal of Medicinal Food* 14:1289-1297.
- Anand P, Kunnumakkara AB, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research* 25(9):2097-2116.
- Andren O, Fall K, Franzen L, Andersson SO, Johansson JE, Rubin MA (2006). How well does the Gleason score predict prostate cancer

- death? A 20-year follow up of a population based cohort in Sweden. *Journal of Urology* 175:1337-1340.
- Bartsch G, Rittmaster RS, Klocker H (2002). Dihydrotestosterone and the concept of 5 α -reductase inhibition in human benign prostatic hyperplasia. *World Journal Urology* 19:413-425.
- Besong M, Samalang P, Abia C (2001). Commercialization as an incentive and threat for *Gnetum* spp (eru) in Cameroon. Proceedings of A Workshop on Incentive Measures for Sustainable Use and Conservation of Agro-Biodiversity 14:69-72.
- Bowe L, Michelle GC, Claude W (2000) Phylogeny of Seed plants based on all three Genomic Compartments: Extant gymnosperms are monophyletic and Gnetales, closest relatives are Conifers. *Proceedings of the National Academy of Sciences* 97:4092-4097.
- Bushman W (2009). Etiology, epidemiology and natural history of benign prostatic hyperplasia. *Urology of Clinical North American* 36:403-415.
- Chindong M (2011). Reporting Science in Africa. Agfax.
- De Nunzio C, Aronson W, Freedland SJ, Giovannucci E, Parsons JK (2012). The correlation between metabolic syndrome and prostatic diseases. *European Urology* 61(3):560-570.
- Ebenyi LN, Ibiama UA, Aja PM (2012). Effects of *Allium sativum* extract on paracetamol – induced hepatotoxicity in albino rats. *International Research Journal of Biochemistry and Bioinformatics* 2(5):93-97.
- Edet UU, Ehiabhi OS, Ogunwande IA, Walker TM, Schmidt JM, Setzer WN, Ogunbinu AO, Ekundayo O (2005). Analyses of the volatile constituents and antimicrobial activities of *Gongronemalatifolium* (Benth and *Gnetum africanum* L). *Journal of Essential Oil Bearing Plants* 8:324-329.
- Enam RMN, Joseph MA, Harlow SD, Wei JT (2014). Risk factors for lower urinary tract symptoms in a population-based sample of African-American men. *American Journal of Epidemiology* 117:206-212.
- Esraa A, Nakaya K, Murata E (2014). Benign prostatic hyperplasia (BPH) can be a progressive disease if left untreated. *American Journal of Epidemiology* 121:20-12.
- Hillman U (1971). Acid phosphatase based on enzyme kinetic method. *Journal of Essential Plants* 4:211-719.
- National Cancer Institute (2018). Analyses of the volatile constituents and antimicrobial activities of *Gongronemalatifolium* (Benth and *Gnetum africanum* L). *Journal of Essential Oil Bearing Plants* 4:331-319.
- Robert JM, Breyer-Brandwijk MG (2011). *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. 2nd ed. Livingstone, London, p. 1241.
- Soltis N, Ali S, Nickel JC, Taylor JM, James SA, Cooney KA, Doerr KM (2002). Light microscopic and stereological analysis of the normal human prostate and of benign prostatic hyperplasia. *Journal of Urology* 122:487-491.
- Suzuki K, Koike H, Matsui H, Ono Y, Hasumi M, Nakazato H, Okugi H, Sekine Y, Oki K, Ito K, Amamoto T, Fakubori Y, Korukawa K, Yamanaka H (2002). A soylsoflavone, induces glutathione peroxidase in the human prostate cancer cell lines. *International Journal of Cancer* 99(6):846-852.
- Tekwe C, Ndam N, Nkefor JP (2003). *Gnetum* domestication for Livelihood Improvement and Conservation. *World Agroforestry Congress*.
- Udoh FV (2007). The Effects of *Gnetum africanum* leaf extracts on some biochemical indices and endocrine functions in albino rats. Postgraduate School, University of Calabar, Nigeria, p. 6210.
- Wasserman NF (2006). Benign Prostatic Hyperplasia: A review and ultrasound classification. *Radiology Clinical North American* 44:689-710.
- Wein T, Linuna M, Ali Z, Nakaya K (2007). Regulation of vascular differentiation in leaf primordial during the rhythmic growth of *Gnetum africanum*. *Journal of Botany* 14:202-203.