OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article **Biochemistry**

Effects of methanol extract of *Allium sativa* (MEAS) on hepatic and renal function biomarkers and Lipid profile of Testosterone propionate induced Benign Prostatic Hyperplasia male Rats

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DOI: 10.36348/sjmps.2023.v09i05.003 | **Received:** 23.03.2023 | **Accepted:** 05.05.2023 | **Published:** 13.05.2023

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Abstract

The increasing incidence of mortality due to benign prostatic hyperplasia (BPH) in ageing men globally is of serious concern and needed collective efforts to arrest it. The benign prostatic hyperplasia result from excessive growth of the prostate gland due to proliferating cells which impairs urethral functions by compressing it. This present study evaluated the effect of methanol extract of *Allium sativa* (MEAS) on lipid profile, renal and hepatic function biomarkers of testosterone induced benign prostate hyperplasia in male rats. The study was done using 25 adult male albino rats, divided into 5 groups, which comprises of normal control, negative control, positive control, and BPH induced rats treated with 200 and 400 mg/kg /day of MEAS respectively. Hepatic and renal biomarkers (ALT, AST, ALP, Urea, Creatinine) and lipid profile (HDL, LDL, TAG, CHOL were evaluated. The BPH induction caused significant ($p<0.05$) increases in lipid profile with exception HDL; hepatic and renal biomarkers of the negative control when compared with the normal control. The MEAS treated BPH-induced rats had significant ($p>0.05$) reduction in the biochemical biomarkers with significant ($p<0.05$) increase HDL. The findings of this study indicated that BPH impairs cellular functionality and treatment of BPH with methanol extract of Allium sativa showed to be effective. Therefore, extract of Allium sativa is a potential agent for the treatment of benign prostatic hyperplasia.

Keywords: benign prostatic hyperplasia, hepatic biomarkers, renal biomarkers, lipid profile, *Allium sativa*.

INTRODUCTION

The most common non-cutaneous form of cancer in older men, benign prostatic hyperplasia (BPH) is characterized by increasing glandular and stromal tissue hyperplasia, which results in an enlarged prostate. Lower urinary tract symptoms (LUTS), such as obstructive symptoms like hesitancy, poor intermittent stream, and the sensation of incomplete bladder emptying, as well as irritative symptoms like increased frequency, urgency, and nocturia, are caused by the rapid growth of stromal and epithelial elements in the prostate (Karunasagara et al., 2020). The quality of life is eventually affected by the narrowing of the urethra, which can lead to increased frequency, urgency, and hesitancy of urination as well as reduced urine flow (Cai *et al*., 2018). Despite several attempts throughout the years, the pathophysiology of BPH is still not fully understood (Cai *et al*., 2018). According to earlier research, aging-related increases in testosterone to DHT conversion, which is mediated by prostate 5α-reductase, are a critical change in the emergence of BPH. Increased levels of prostatic DHT encourage excessive prostatic stromal and epithelial cell growth, which results in hyperplasia (Jeon et al., 2017). "Studies have shown that BPH patients had greater levels of the male hormone dihydrotestosterone (DHT) than healthy males. Therefore, androgens play a key role in the development of BPH. Due to its high affinity for androgen receptors (AR), DHT is a more potent androgen than testosterone; 5α-reductase (5AR), an enzyme that transforms testosterone to DHT, strongly contributes to BPH development" (Choi *et al*., 2019). Therefore, a number of medications have been created with the goal of lowering DHT levels. In particular, finasteride, a commercially available 5α -reductase inhibitor, has been used to treat BPH. By preventing the

conversion of testosterone to DHT, 5α -reductase inhibitors prevent the onset of BPH (Jeon *et al*., 2017).

However, the use of these medications is restricted due to their negative effects, which include nasal congestion, a decline in libido, and ejaculatory or erectile dysfunction (Cai *et al*., 2018). Different effective treatments for BPH include medications, minimally invasive therapies, and surgery. However, more research is required to determine whether certain medicinal herbs might treat testosterone propionate-induced benign prostatic hyperplasia in male Wistar rats in a way that is both inexpensive and natural. *Allium sativum* is one such typical medicinal plant with a range of health advantages. *"Allium sativum* has a high medicinal value and is used to cure a variety of human diseases. It has anti-inflammatory, rheumatological, ulcer inhibiting, anticholinergic, analgesic, antimicrobial, antistress, antidiabetics, anticancer, liver protection, anthelmintics, antioxidants, antifungal, and wound healing properties, as well as properties that help with asthma, arthritis, chronic fever, tuberculosis, runny nose, malaria, leprosy, skin discoloration, and itching, indigestion, colic, enlarged spleen, hemorrhoids, fistula, bone fracture, gout, urinary tract disease, diabetes, kidney stones, anemia, jaundice, epilepsy, cataract, and night blindness" (Tesfaye, 2021). The present study aims at evaluating the effect of methanol extract of *Allium sativum* on the hepatic, renal, and lipid profile of Testosterone propionate-induced benign prostatic hyperplasia male Rats.

MATERIALS AND METHODS

Plant Collection and Preparation of Plant Sample

The fresh plant sample of *Allium sativum* was collected from the Ekeapkara community in Osisioma L.G.A of Abia State, Nigeria. The plant was identified by a botanist at the Department of Biology and Environmental Microbiology Federal Polytechnic Nekede, Imo State. The fresh sample of *Allium sativum* was washed with distilled water and then allowed to get dried in a dust-free environment for ten days. The dried sample was blended using an electronic blender.

Preparation of Plant extract

One thousand gram (1000g) of powdered leaves was macerated in 2.5L of 95% methanol at room temperature for 72h. It was continuously mixed and then filtered using a filter paper (Whatman size No.1). The filtrate was concentrated using a water bath at 45^0c. The methanol extract of Allium sativa (MEAS) was refrigerated in air tight container.

Phytochemical Screening

Qualitative and quantitative phytochemical analysis of MEAS was carried out on the extract using standard procedure of John De Britto et al. (2013).

Acute Toxicity (LD_{50})

The median lethal dose (LD50) of the extract and fraction were determined by the method of Lorke (1983). Six groups of three adult albino-mice each weighing between 14 and 22g were used for this study.

Experimental animal

A total of 25 matured male albino rats weighing between 150 – 250 kg were used for this study. They were obtained from the Veterinary Medicine Department animal house in the Michael Okpara University of Agriculture Umudike. The rats were housed in conventional wire cages under standard laboratory conditions and allowed to acclimatize for two weeks before use. They were given standard feed and drinking water *ad libitum*. Care of experimental animals was taken as per the guidelines given by NRC (2011) and approval for animal studies was obtained from the Ethical Committee of the Department of Pharmaceutical Technology, Federal Polytechnic Nekede with the no Ethical Clearance Number.

BPH induction

In all groups, except normal control were induced BPH using testosterone administered to the rats for 28days following the method of Bosland and Prinsen (1990).

Experimental design

The animals were weighed and divided into five (5) groups with five (5) animals each. The five groups were: Group 1 (Normal control), Group 2 (negative control: induced benign prostatic hyperplasia for 28 days without treatment), Group 3 (positive control administered with finasteride), Group 4 (administered 200mg/kg of MEAS), and Group 5 (administered 400mg/kg of MEAS). After 28 days of treatment, the rats were sacrificed using cervical dislocation and sera will be collected using cardiac puncture for biochemical and cellular toxicity analysis.

Biochemical and cellular toxicity analysis

Triglycerides, high density lipoprotein cholesterol (HDL), were measured using kits from Randox Laboratory (Randox co. UK) following protocol described by manufacturer. Total cholesterol was calculated using Friedewald equation. Serum liver markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Total Bilirubin. were measured using semiautomated biochemistry analyzer according to the procedure of Ayza et al. (2020) and Onyegeme-Okerenta et al. (2022). Serum kidney markers (Urea, Uric acid and creatinine) were analyzed according to the standard principles and procedures outlined in the kit manufacturer's manual (Kedir *et al.*, 2022; Onyegeme-Okerenta et al., 2022).

Statistical analysis

Statistical analysis was carried out using SPSS version 23 for Windows (IBM Statistics for Social

Sciences). One-way analysis of variance (ANOVA) followed by Duncan's posthoc test for multiple comparisons was performed to determine differences between treatment groups. A p-value less than 0.05 was considered statistically significant. Results were expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Acute toxicity study

Following Lorke's method for acute toxicity evaluation, methanol extract of *Allium sativa* showed no toxicity even at the highest dose of 5000mg/kg thus indicating the plant is safe at the doses used in this study

Table 1: Qualitative phytochemical test results

S/no	Test	Test method	Result
1	Proteins	Millon's test	-
		Ninhydrin test	-
2	Carbohydrates	Iodine test	+
		Benedict's test	+
3	Phenols	Litmus test	++
4	Tannins	Geletin test	++
5	Flavonoids	Shinoda test	++
		Alkaline reagent test	+
6	Saponins	Frothing test	+++
7	Glycosides	Liebermann's test	+
		Salkowski's test	+
8	Steroids		+
9	Terpenoids		+
10	Alkaloids		++

Key: "-": Absent, "+": present in trace amount, "++": Present in moderate amount, "+++": Present in abundant amount.

Table 2: Quantitative phytochemical test results

Sample	Values
Total phenols	43.16 ± 0.74 mg/Ggae
Total flavonoids	47.11 ± 2.05 (mgQE/g)
Tannins	26.45% ± 1.35
Alkaloids	39.18 ± 0.55
Saponins	38.70 ± 3.50
Terpenoids	29.28 ± 0.48

The values are mean ± SD of triplicate determination

Effects of methanol extract of Allium sativa (MEAS) on the Serum aspartate transaminase (AST) activity

The result of aspartate transaminase (AST) activities of BPH-induced rats treated with methanol extract of Allium sativa (MEAS) showed no significant ($p<0.05$) increase in the AST activities when compared with normal control rats (figure 1). Likewise, the BPH induced rats treated with finasteride (positive control) showed no significant ($p>0.05$) increase in AST activities as compared with normal control rats. However, the positive, 200mg/kg and 400mg/kg of MEAS had significantly ($p<0.05$) lower AST activities relative to BPH control (negative control), while there was significant ($p<0.05$) increase in the AST activities of BPH control (negative control) compared to the normal rats.

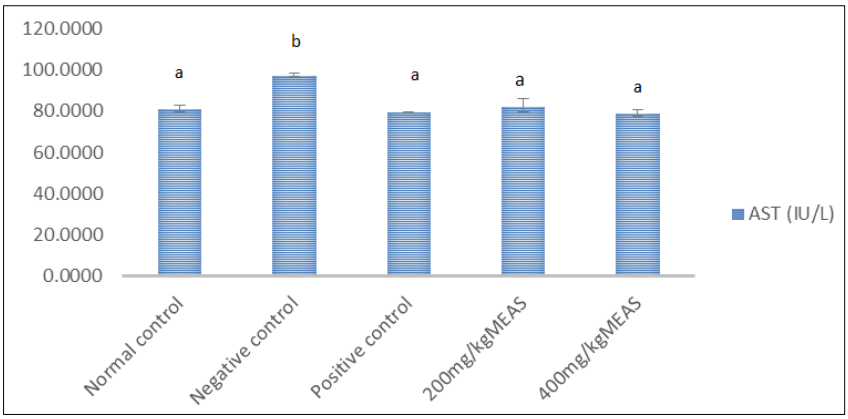

Figure 1: Effects of methanol extract of Allium sativa (MEAS) on the Serum aspartate transaminase (AST) activity of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum alanine transaminase (ALT) activity

The result of alanine transaminase (ALT) activities of BPH-induced rats treated with 400mg/kg methanol extract of Allium sativa (MEAS) showed no significant ($p<0.05$) increase in the ALT activities when compared with normal control and positive control rats. Likewise, the BPH induced rats treated with finasteride (positive control) showed no significant ($p>0.05$) increase in ALT activities as compared with normal control rats. However, the positive, 200mg/kg and 400mg/kg of MEAS had significantly ($p<0.05$) lower ALT activities compared to BPH control (negative control), while there was significant ($p<0.05$) increase in the ALT activities of BPH control (negative control) compared to the normal rats (figure 2).

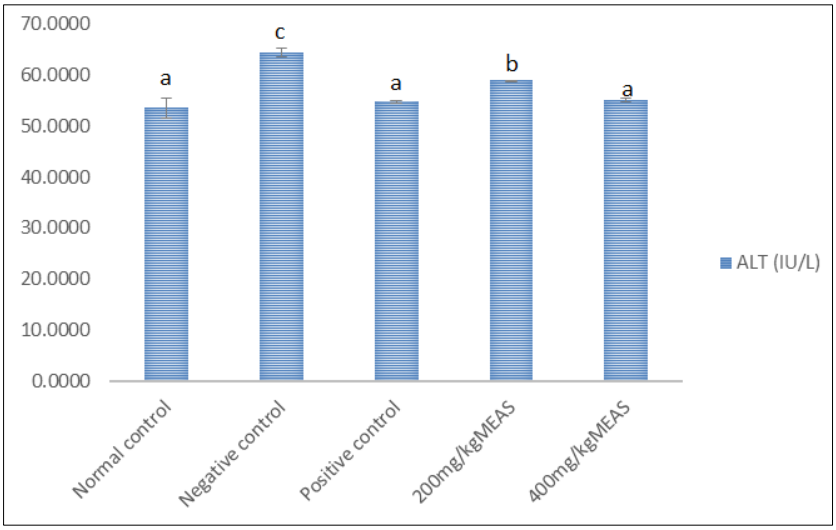

Figure 2: Effects of methanol extract of Allium sativa (MEAS) on the Serum alanine transaminase (ALT) activity of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum alkaline phosphatase (ALP) activity

The alkaline phosphatase (ALP) activities of BPH-induced rats treated with methanol extract of Allium sativa was shown in the figure 3. The result showed that there were significant ($p<0.05$) increase in the ALP activities of BPH control (negative control) when compared with the normal control. However, the standard control treated with 5 mg/kg/day of finasteride and the BPH-induced rats treated with the MEAS at a dose of 400 mg/kg/day showed significant ($p<0.05$) decrease in the ALP activities relative to normal control, while BPH-induced rats treated with the MEAS at a dose of 200 mg/kg/day showed no significant ($p<0.05$) decrease in the ALP activities as compared with positive control rather there was significant ($p<0.05$) decrease when compared with the negative control. Furthermore, the BPH-induced rats treated with 200 mg/kg/day of the MEAS showed no significant ($p>0.05$) increase in the ALP activities as compared with the normal control.

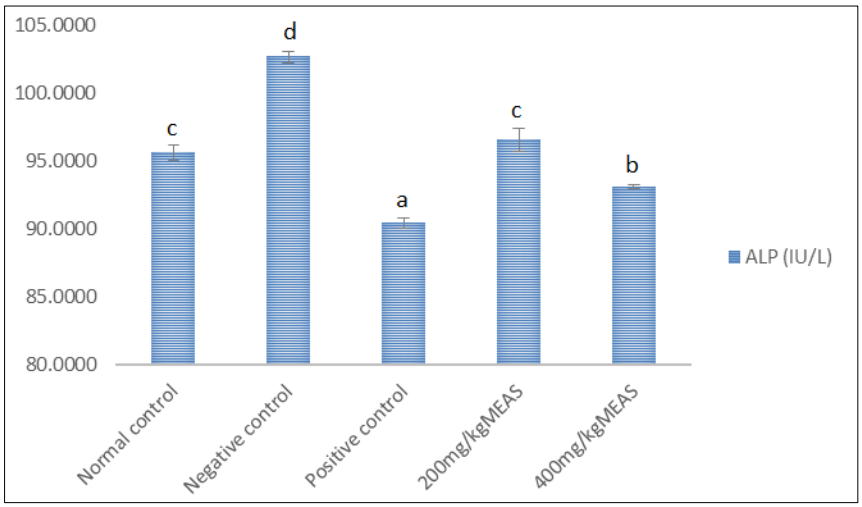

Figure 3: Effects of methanol extract of Allium sativa (MEAS) on the Serum alkaline phosphatase (ALP) activity of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum total bilirubin concentrations

The total bilirubin concentrations in BPH-induced rats treated with methanol extract of Allium sativa was showed in figure 4. The result indicated that the positive control and BPH induced rats treated with 200 and 400 mg/kg/day of the MEAS, respectively showed significant ($p<0.05$) decrease in the total bilirubin concentrations when compared with the BPH control (negative control) while the BPH control showed significant ($p<0.05$) increase in the total bilirubin compared to the normal control. Furthermore, the postive control and the BPH induced rats treated with 200 and 400 mg/kg/day respectively showed no significant ($p>0.05$) difference in the total bilirubin concentrations when compared with the normal control.

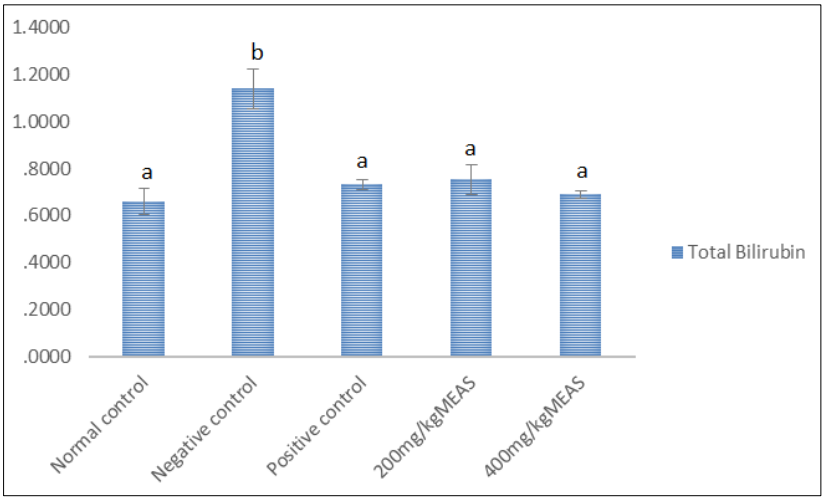

Figure 4: Effects of methanol extract of Allium sativa (MEAS) on the Serum total bilirubin concentrations of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum creatinine concentrations

The creatinine concentrations in BPH-induced rats treated with methanol extract of Allium sativa was showed in figure 5. The result showed that the positive control and BPH induced rats treated with 200 and 400 mg/kg/day of the MEAS, respectively showed significant ($p<0.05$) decrease in the creatinine concentrations when compared with the BPH control (negative control) while the BPH control showed significant ($p<0.05$) increase in the creatinine concentration compared to the normal control. The rats treated with 5mg/kg/d finasteride (positive control) and the BPH rats treated with 400mg/kg MEAS showed no significant ($p<0.05$) difference in the creatinine concentration compared to the normal control. Furthermore, the BPH induced rats treated with 200 mg/kg/day MEAS showed no significant ($p>0.05$) decrease in the creatinine concentrations when compared with the positive control.

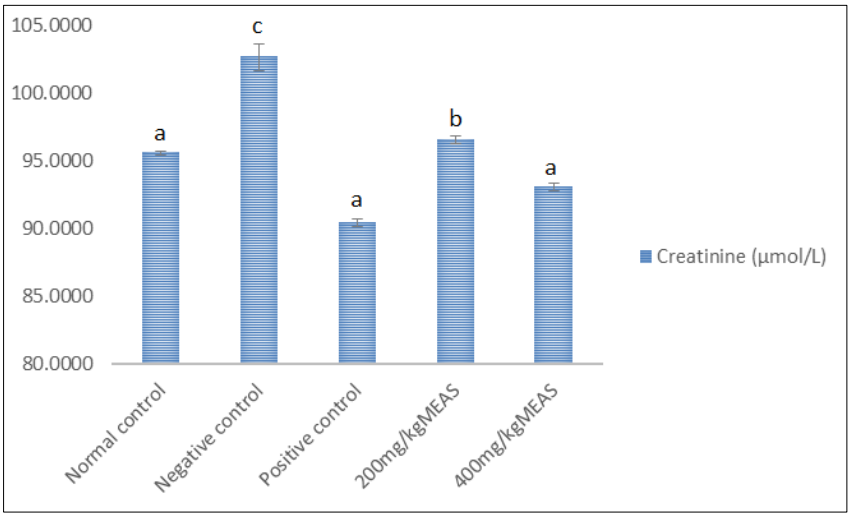

Figure 5: Effects of methanol extract of Allium sativa (MEAS) on the Serum creatinine concentrations of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum Urea concentrations

The urea concentrations in BPH-induced rats treated with methanol extract of Allium sativa was showed in figure 6. The result showed that the positive control and BPH induced rats treated with 200 and 400 mg/kg/day of the MEAS, respectively showed significant ($p<0.05$) decrease in the urea concentrations when compared with the BPH control (negative control) while the BPH control showed significant ($p<0.05$) increase in the urea concentration compared to the normal control. The rats treated with 5mg/kg/d finasteride (positive control) and the BPH rats treated with 400mg/kg MEAS showed no significant ($p<0.05$) difference in the urea concentration compared to the normal control. Furthermore, the BPH induced rats treated with 200 mg/kg/day MEAS showed no significant ($p>0.05$) increase in the urea concentrations when compared with the normal control.

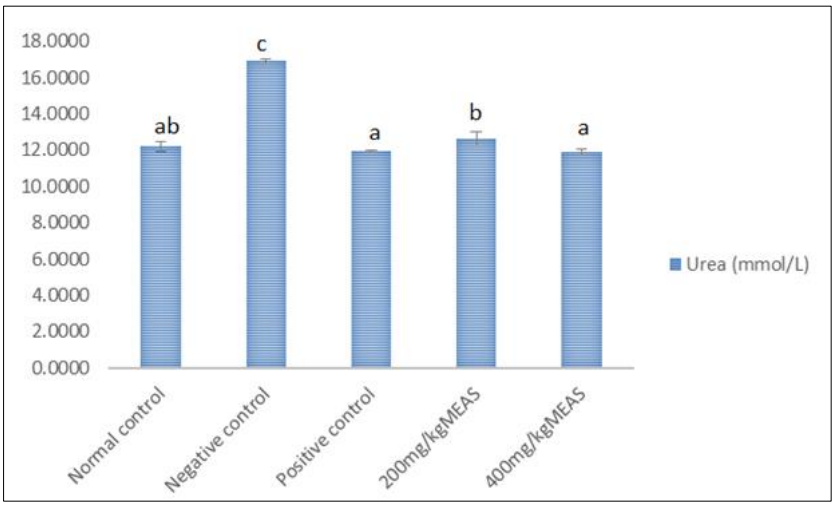

Figure 6: Effects of methanol extract of Allium sativa (MEAS) on the Serum Urea concentrations of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum Uric acid concentrations.

The uric acid concentrations in BPH-induced rats treated with methanol extract of Allium sativa was showed in figure 7. The result showed that the positive control and BPH induced rats treated with 200 and 400 mg/kg/day of the MEAS, respectively showed significant ($p<0.05$) decrease in the uric acid concentrations when compared with the BPH control (negative control) while the BPH control showed significant ($p<0.05$) increase in the uric acid concentration compared to the normal control. The rats treated with 5mg/kg/d finasteride (positive control) and the BPH rats treated with 400mg/kg MEAS showed no significant ($p<0.05$) difference in the uric acid concentration. Furthermore, the BPH induced rats treated with 200 mg/kg/day MEAS showed no significant ($p>0.05$) increase in the uric acid concentrations when compared with the normal control and positive control.

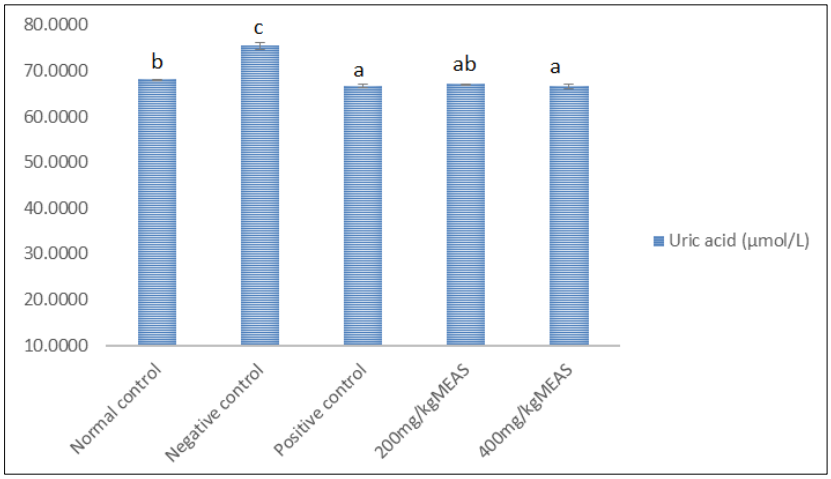

Figure 7: Effects of methanol extract of Allium sativa (MEAS) on the Serum Uric acid concentrations of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum triacylglycerol (TAG) concentrations

The TAG concentrations in BPH-induced rats treated with methanol extract of Allium sativa was showed in figure 8. The result showed that the BPH induced rats treated with 200 and 400 mg/kg/day of the MEAS, respectively and the positive control showed significant ($p<0.05$) decrease in the TAG concentrations when compared with the BPH control (negative control) while the BPH control showed significant ($p<0.05$) increase in the TAG concentration compared to the normal control. The rats treated with 5mg/kg/d finasteride (positive control) and the BPH rats treated with 400mg/kg MEAS showed no significant ($p<0.05$) difference in the TAG concentration compared to normal control. Furthermore, the BPH induced rats treated with 200 mg/kg/day MEAS showed no significant ($p>0.05$) increase in the TAG concentrations when compared with the normal control.

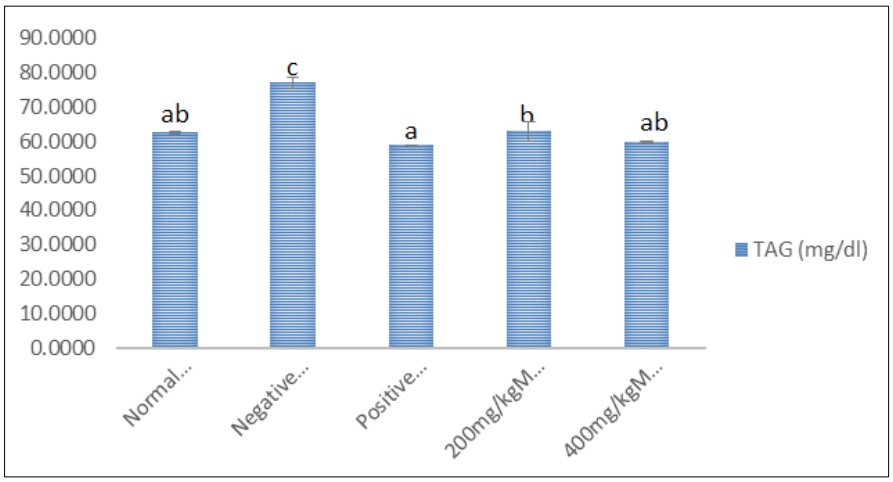

Figure 8: Effects of methanol extract of Allium sativa (MEAS) on the Serum triacylglycerol (TAG) concentrations of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum cholesterol (CHOL) concentrations

The cholesterol concentrations in BPH-induced rats treated with methanol extract of Allium sativa was showed in figure 9. The result showed that the BPH induced rats treated with 200 and 400 mg/kg/day of the MEAS respectively and the positive control showed significant ($p<0.05$) decrease in the choleaterol concentrations when compared with the BPH control (negative control) while the BPH control showed significant ($p<0.05$) increase in the cholesterol concentration compared to the normal control. The rats treated with 5mg/kg/d finasteride (positive control) and the BPH rats treated with 400mg/kg MEAS showed no significant ($p<0.05$) difference in the cholesterol concentration compared to normal control.

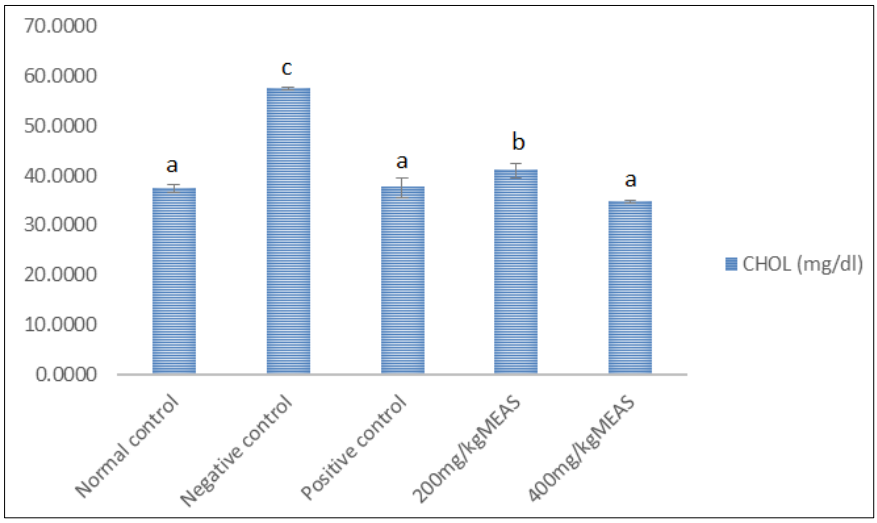

Figure 9: Effects of methanol extract of Allium sativa (MEAS) on the Serum cholesterol (CHOL) concentrations of Benign Prostatic Hyperplasia Induced Rats

Effects of methanol extract of Allium sativa (MEAS) on the Serum High density lipoprotein (HDL) concentrations

The HDL concentrations in BPH-induced rats treated with methanol extract of Allium sativa was showed in figure 10. The result showed that the BPH induced rats treated with 200 and 400 mg/kg/day of the MEAS respectively and the positive control showed significant ($p<0.05$) increase in the HDL concentrations when compared with the BPH control (negative control) while the BPH control showed significant ($p<0.05$) decrease in the HDL concentration compared to the normal control. The rats treated with 5mg/kg/d finasteride (positive control) showed no significant ($p>0.05$) difference in the HDL concentration compared to normal control. Furthermore, the BPH rats treated with 400mg/kg MEAS showed significant ($p<0.05$) increase in the HDL concentration compared to positive control.

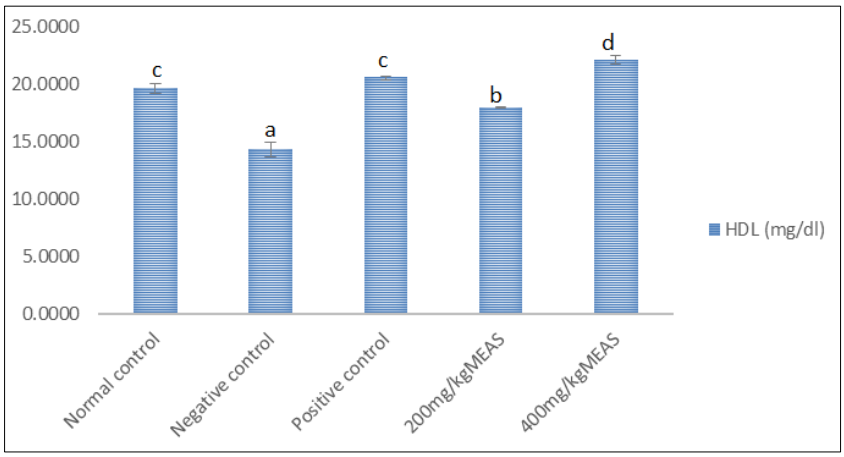

Figure 10: Effects of methanol extract of Allium sativa (MEAS) on the Serum High density lipoprotein (HDL) concentrations of Benign Prostatic Hyperplasia Induced Rat

DISCUSSION

The effects of methanol extract of *Allium sativa* (MEAS) on hepatic and renal function biomarkers and lipid profile of testosterone propionate induced Benign Prostatic Hyperplasia male rats was evaluated in this study. The phytochemicals present in the plant extract was also evaluated.

Phytochemicals from plants when ingested are extremely important in the control of disease (Ajiboye et al., 2013). The protective role of phytochemicals may be associated with their antioxidant activity, since overproduction of oxidants (reactive oxygen species and reactive nitrogen species) in the human body is involved in the pathogenesis of many chronic diseases (Zhang et al., 2015). One of the most typical plant components is a flavonoid. They have been credited to the polyphenolic substances in them that have antioxidant properties (Benito *et al*., 2002). This study indicates that methanol extract of *Allium sativa* possess phenol, alkaloid, tannins and saponins in moderate amount.

The liver and kidneys are two important mammalian organs that play a key role in the body's numerous metabolic processes (Onyegeme-Okerenta et al., 2022). There is a correlation between the kidney and benign prostate hyperplasia (BPH). BPH with urinary retention has the tendency to result in kidney dysfunction and several risk factors associated with BPH might influence deterioration in kidney function (Zamzami et al., 2021).

Liver dysfunction was evident with significant increase in the studied liver function biomarkers of the BPH induced untreated rats. The effect of MEAS on the liver of testosterone propionate-induced benign prostatic hyperplasia in male rats revealed a significant decrease in AST, ALT, ALP activities and Total Bilirubin concentration. Renal functionality was investigated by checking the concentration of urea, creatinine, and uric acid in the serum. The MEAS significantly reduced the urea, creatinine, and uric acid compared the BPH untreated rats (negative control). However, the findings of the ability of Allium sativa to protect the kidney and liver was in agreement to the study of Aprioku and Amah-Tariah (2017), where the aqueous garlic bulb extract reduced elevated hepatic enzymes and renal biochemical indices in alloxan-induced elevations of plasma biochemical factors of renal and hepatic functions.

There is a scientific report on the correlation between hyperlipidia and benign prostatic hyperplasia (Erbay and Ceyhun, 2022). Recent clinical and basic research evidence has demonstrated a possible linkage of cholesterol to benign prostatic hyperplasia. Accumulation of cholesterol within the lipid raft component of the cellular plasma membrane may stimulate signaling pathways that promote prostate tumor growth and progression (Yat-Ching, 2011).

Kayode et al. (2022) reported alteration in the biochemical and lipid profile of testosterone propionate-induced benign prostatic hyperplasia in male Wistar rats treated with ketogenic diet.

The significantly elevated serum TAG concentration in the BPH negative control relative to the normal control, positive control and treated groups indicates the adverse effect of BPH on lipid metabolism, which promoted hyperlipidaemia in the BPH rats. The elevated serum TAG level in the BPH untreated group agrees with Uroko et al. (2023), who reported that high serum TAG level is associated with BPH pathogenesis. The BPH untreated rats could have experienced impaired carbohydrate catabolism and conversion of excess amount of free fatty acids resulting in increased serum TAG concentration due to the decline in the transport of TAG to the liver for catabolism because of the inadequate amount of HDL needed for the vehicle. The lower HDL cholesterol level in the BPH control is consistent with research by Gacci et al. (2017) and Uroko et al. (2023), which found that lower HDL levels are linked to prostate enlargement and that hyperlipidaemia can hasten the progression of BPH by encouraging prostate cell inflammation. The MEAS significantly restored the alteration in the lipid profile, which was evident in the reduced TAG, total cholesterol, LDL and increased HDL of the MEAS treated groups.

CONCLUSIONS

Allium sativum were found to possess great potential in the management of benign prostatic hyperplasia, this may be attributed to the levels of phytochemicals contained in the plant. The findings of this study showed that treatment of BPH rats with MEAS significantly restored the altered lipid profile, Liver and kidney function biomarkers. The findings suggest that MEAS could prevent hyperlipidaemia, liver damage and renal malfunctions associated with BPH.

Conflict of interest

The authors declare that no conflict of interest exists with respect to this work.

Funding

The research was funded by Tertiary Education Trust Fund (TETFund) Nigeria through the Institution Base Research (IBR) Project Grant 2022 (TETF/DR&D/CE/POLY/NEKEDE/IBR/2022/Vol. II).

Acknowledgements

The authors sincerely acknowledge Tertiary Education Trust Fund (TETFund) Nigeria for the financial support. We also acknowledge the Management of Federal Polytechnic Nekede Owerri for creating enabling environment for research.

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