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Benign prostatic hyperplasia preventive potential of ethanol leaf extract and fractions of *Cassia alata* L. (Fabaceae) in testosterone-treated rats

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Abstract
Benign prostatic hyperplasia (BPH) is the non-cancerous enlargement of the prostate gland that affects ageing men. The leaf of *Cassia alata* is a known local remedy for the condition. This study examined the anti-BPH activity of the plant's ethanol leaf extract, and its aqueous and chloroform fractions. Plant material was extracted and fractionated to obtain aqueous and chloroform fractions. BPH was induced in rats with subcutaneous testosterone injections except for the normal control group. Extracts and fractions were administered orally, while finasteride was used as the positive control. Treatment lasted for 28 days after which serum testosterone, oestradiol, dihydrotestosterone, prostate-specific antigen, superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde levels were measured. Prostate index and volume were also determined. The extract was safe at administered doses, and dose-dependently reduced serum testosterone, dihydrotestosterone, oestradiol, and prostate-specific antigen levels. It increased superoxide dismutase, catalase, and glutathione activities, while significantly decreasing malondialdehyde levels. Aqueous and chloroform fractions effectively reversed the effects of the extract on serum biochemical and antioxidant levels. Conclusively, the extract is relatively safe, decreased serum markers of BPH with increased serum antioxidant activity observed. Fractions of the extract did not lead to enhanced activity as seen with the whole extract.

Keywords: Anti-BPH; Aqueous fraction; *Cassia alata*; Chloroform fraction; Testosterone

INTRODUCTION

Benign prostatic hyperplasia (BPH), an ageing-related disease in men is characterized by an increase in the size and number of the epithelial and stromal cells of the prostate gland [1]. A clinically significant manifestation of the condition is evidenced in 40 -50% of men aged 50 – 80 years [2]. A prevalence rate of 23.7% and 35.30% have been reported in the South-West and South-East of Nigeria respectively [3,4], while 16.67% was indicated for the South-South, of the country [5]. Globally, the prevalence rate of the disease is estimated at 26.6% [6].

The enlarged prostate is characterised by symptoms such as urinary urgency, frequency, painful urination, excessive passage of urine at night, weak urinary stream,

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hesitancy, incomplete bladder voiding, terminal dribbling, overflow and incontinence [7]. These impact negatively on the quality of life of patients with an associated economic burden linked to the duty of care and prescribed treatment pattern [8]. Importantly, a close link between BPH and the development of bladder and prostate cancers has been established with a high mortality rate closely associated with the latter [9].

Watchful waiting, pharmacotherapeutic agents within the 5-alpha reductase inhibitors (5ARIs), Phosphodiesterase-5 inhibitors (PDE5s) and Alpha-blockers classes of drugs and surgery are management options for the disease [10]. However, the use of orthodox drugs is challenged with high cost and side effects such as lowered libido, impotence, backwards ejaculation [11], while Pain, trauma and high financial burden are linked to the surgical option of treatment. It therefore becomes expedient to search for alternatives in locally available herbal medicines with a history of application in the treatment of BPH and its associated symptoms.

In ethnomedicine, the leaves of *Cassia alata* (*Senna alata*), belonging to the family Leguminoseae are reportedly useful in the management of BPH [12]. The plant is known commonly as "Ringworm bush" "or "Christmas candle" [13]. Local names of the plant include "Asunwon oyinbo" (Yoruba); "Ogalu" (Igbo), and "Ebe-okoria" or "Ahoghamiasun" (Bini). Decoction of various parts of the plant is used in the treatment of wounds, skin and respiratory tract infections, burns, diarrhoea, and constipation. [14]. Antioxidants, antifungal, antibacterial, cytotoxic and thrombolytic activities have been reported for the plant [15-18]. Phytochemical compounds present in the plant include tannins, alkaloids, flavonoids, terpenes, anthraquinone, saponins and phenols. Alatinon, alanonal and β-sitosterol-β-D-glucoside are compounds isolated from the plant [16]. The paucity of information to support the claimed anti-BPH activity gave rise to this study which is aimed at evaluating the acute toxicity and anti-BPH activity of the leaf extract and fractions of the plant, using mice and rats as appropriate. The phytochemical evaluation of the leaves of the plant was also carried out.

EXPERIMENTAL METHODS

Plant collection and authentication. The leaves of *C. alata* were collected within the University of Benin, Ugbowo environs in Ovia North-East Local Government Area of Edo State in July 2023. Identification was done at the Herbarium Unit, Department of Plant Biology and Biotechnology Faculty of Life Sciences, University of Benin, and voucher number UBH-S491 was issued. A voucher specimen was deposited for future reference.

Plant preparation, extraction and fractionation. The stalk of the plant was de-leafed, and the collected leaves were dried under shade for 2 weeks, and, in the oven at 40°C for 30 minutes before grinding with an electric attrition mill (Christy Norris LB7 Mill; Christy Turner Ltd, Suffolk, England). A weighed amount (1.2 kg) of the powdered plant material was extracted in batches with absolute ethanol (2.5 mL) in the Soxhlet apparatus to obtain the ethanol extract (ELECA). The extract was filtered, concentrated *in vacuo,* weighed and preserved in a glass jar at 4ºC in a refrigerator until needed. A weighed amount of the concentrated extract (0.35 kg) was fractioned with chloroform (2.5L) by dissolving it in a mixture of ethanol-water (1: 5), transferring it into separating funnel, and treating with aliquot amount of chloroform (250 mL) to yield the chloroform and aqueous fractions. The aqueous fraction was freeze dried (Hellog AIK-HFD-D; Hello River Industrial Manufacturers, Zhuhai, China), while the chloroform fraction was dried *in vacuo*. Both fractions were weighed (123.52 g and 57.75 g

respectively) and preserved at 4°C in a refrigerator until needed.

Animal handling and care. Seventy-two (72) adult (160-240 g) male albino rats aged 3-4 months and 12 mice (25 - 40 g) aged same as the rats, were obtained from the Animal House of the Anatomy Department, Faculty of Basic Medical Sciences, University of Benin were housed in the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy. The housing conditions include ventilated polypropylene cages, atmospheric humidity of 63±5%, room temperature of 23-28°C), natural day/night cycles. The animals were fed rodent pellets (Chikun® finisher pellet by Olam Agricultural Enterprise, Ibadan) with free access to clean tap water. The housing environment was hygienically maintained by daily cleaning and good ventilation. Ethical approval with reference PEC – 23/022, was obtained from the Faculty of Pharmacy Ethics Committee before proceeding with the study. Animals were handled humanely as much as possible by the standards of the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Animal Research Reporting of In Vivo Experiment (ARRIVE) guidelines for reporting animal research [19].

Acute toxicity test. The acute toxicity test was carried out by Lorke's method [20]. This was carried out in two phases. In phase I, nine mice were randomly assigned into 3 groups of 3 mice each. Animals in groups 1, 2 and 3 received oral administration of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of ELECA respectively and they were monitored frequently for 24 hours for any observable signs of distress, toxicity or mortality. In phase II, three mice were grouped into 3 groups of one mouse each and each group was administered 1600, 2900 and 5000 mg/kg respectively. The mice were also monitored frequently for 24 hours for any observable signs of acute toxicity such as reduction or increase in food and water intake, physical activity, vomiting and/or diarrhoea, and mortality. All administrations were via the oral route.

Test for anti-benign prostatic hyperplasia activity. The method by Akbari et al., [21] with a slight modification was adopted for this study. Seventy-two albino rats were divided into twelve groups of six animals each. Group one, the normal control, received only water and feed and did not receive testosterone injections. The other eleven groups were induced with benign prostatic hyperplasia by administering 4 mg/kg body weight of testosterone in olive oil each day.

Group 2 animals received only water and feed, serving as the negative control. Animals in groups 3, 4, and 5 were administered 100, 200, and 400 mg/kg of ELECA respectively. Groups 6, 7 and 8 animals received 50, 100 and 200 mg/kg of the aqueous fraction and, groups 9, 10 and 11received 50, 100 and 200 mg/kg of the chloroform fraction. Finasteride at a dose of 10 mg/kg/ body weight was administered to group 12 animals and served as positive control. The extract, fractions and finasteride were administered orally, while testosterone in olive oil was administered subcutaneously to induce BPH. Treatments lasted for 28 days.

Animals were weighed, fasted overnight, and sacrificed by cervical dislocation and blood collected via the superior vena cava on day 29. The blood collected was centrifuged at 3000 rpm to obtain the serum, which was used to evaluate levels of testosterone, dihydrotestosterone, **o**estrogen, prostate specific antigen (PSA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA). The prostate gland was equally collected

Assessment of serum biochemical profile. Commercial Enzyme-linked immune-sorbent assay (ELISA) test kits were used to assay for the concentration of serum testosterone, dihydrotestosterone, and **o**estrogen and

prostate-specific antigen as specified by the manufacturer [22].

Assessment of serum antioxidants activity. Serum levels of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GPx) were evaluated using commercial ELISA test kits for specific antioxidant enzymes [22].

Determination of mean prostate index and volume. Harvested prostate gland from each animal was carefully blotted, dry to remove all wetness, weighed and the weight noted. The volume of the prostate was determined using the volume displacement method. The final body weights (BW) of rats in all the groups were obtained, recorded and the Prostatic Index (PI) was calculated as shown in the following formula:

$$PI = PW/BW$$

Where: P1= Prostatic Index; PW = Prostate weight; BW = Body weight

Determination of secondary metabolites. The plant extract was evaluated for different classes of secondary metabolites using standard procedures [23].

Data analysis. The analysis of the data was done using GraphPad InStat software (Version 8.1). The results are presented as means ± standard error of mean (SEM). Statistical analysis was carried out using a one-way analysis of variance (ANOVA), followed by the Dunnett post hoc test to assess significant differences among the means of the various groups. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The oral acute toxicity test revealed nil mortality after 24 hours of treatment with ELECA. However, there was observed increased food intake in the rats treated with 2900 and 5000 mg/kg respectively.

Effect of ethanol leaf extract of *C. alata* on serum biochemical profile. The extract lowered serum testosterone at 100 and 200 mg/kg but increased it significantly at 400 mg/kg ($P<0.05$). It also reduced the serum DHT at lower doses, with an increase at 400 mg/kg. Serum **o**estradiol levels were increased at 100 mg/kg but decreased with higher doses. Serum PSA levels decreased dose-dependently but not significantly, while finasteride significantly lowered PSA levels in animals compared to the negative control group (Table 1).

Effect of ethanol leaf extract of *C. alata* on serum antioxidant activity. The extract significantly increased serum level of SOD activity at 400 mg/kg ($p<0.05$). The serum level of Catalase also increased linearly but not significantly. Glutathione peroxidase showed significant increases at 200 and 400 mg/kg. Both the extract and finasteride significantly reduced serum malondialdehyde levels compared to the negative control (Table 2).

Effect of ethanol leaf extract of *C. alata* on prostate parameters. The extract caused a dose-dependent reduction in mean prostate index (MPI) and mean prostate volume (MPV) compared to the negative control group. Similarly, the finasteride-treated group showed a significant decrease in MPI ($p<0.05$) and MPV ($p<0.001$) compared to the negative control group. Table 3 shows the body weight and prostate parameters for the study groups.

Effect of aqueous fraction of ethanol leaf Extract of *C. alata* on serum biochemical profile. The aqueous extract had varying effects on the serum levels of profiled biochemicals. Both the fraction and Finasteride at tested doses influenced testosterone and DHT similarly. 50, 100, and 200 mg/kg doses of the fraction increased **o**estradiol levels. Conversely, finasteride reduced PSA levels, whereas the fraction at 100 and 200 mg/kg increased serum PSA levels compared to the observed concentrations in normal and negative control animals (Table 4).

Table 1: Effect of ethanol leaf extract of *C. alata* on some serum biochemical profile

Group/Dose	Test. (ng/dL)	DHT (ng/dL)	Oest. (pg/mL)	PSA (ng/mL)
Normal control	2.83±0.31	0.72±0.02	84.02±3.61	2.34±0.41
Negative control	3.96±0.33	0.90±0.01	76.73±1.51	3.99±0.20
100 mg/kg	2.62±0.12	0.86±0.04	125.73±5.06	3.63±0.35
200 mg/kg	2.96±0.20	0.80±0.03	91.59±4.53	3.41±0.11
400 mg/kg	2.90±0.15	0.90±0.02	86.06±6.42	2.71±0.29*
Finas. 10 mg/kg	2.91±0.23	0.91±0.05	95.68±5.91	2.11±0.32**

Finas =Finasteride; Oest. = Oestrogen; Test. Testosterone; PAS = Prostate specific antigen. *p< 0.05; **p<0.001

Table 2: Effect of ethanol leaf extract of *C. alata* on serum antioxidant activity

Group/Dose	SOD (U/mL)	CAT (u/mL)	GPx (mM)	MDA (µmol/L)
Normal control	53.73±1.01	8.75±0.13	295.00±11.20	111.83±5.62
Negative control	49.24±2.19	7.94±0.11	239.32±6.50	192.55±3.99
100 mg/kg	53.64±3.32	8.13±0.12	250.54±12.22	171.67±11.30
200 mg/kg	59.22±3.41	9.53±0.15	259.22±7.59	140.19±6.62
400 mg/kg	65.23±5.29	9.14±0.18	272.81±9019*	134.03±5.18*
Finas. 10 mg/kg	58.11±2.25	8.02±0.12	277.37±7.21	109.67±5.55*

CAT= Catalase; Finas = Finasteride; GPx = Glutathione peroxidase; MDA = Malondialdehyde; SOD = Superoxide dismutase. p< 0.05.

Table 3: Effect of ethanol leave extract of *C. alata* on prostate parameters

Treatment	Mean body weight (g)	Mean prostate weight (mg)	Prostate Index (mg/g)	Mean prostate volume (mL)
Normal control	163.00 ± 2.43	0.08±0.00	0.50± 0.00	0.21± 0.02
Negative control	170.83 ± 2.19	0.38±0.01	0.22±0.01	0.40± 0.01
100 mg/kg	166.94 ± 1.78	0.30±0.05	0.18±0.03	0.24± 0.04*
200 mg/kg	170.20 ± 5.28	0.28±0.02	0.16±0.01	0.26± 0.04*
400 mg/kg	162.64 ± 4.27	0.23±0.03	0.14±0.02	0.23± 0.03*
Finas. (10 mg/kg)	169.30 ± 6.03	0.11±0.01	0.07±0.01*	0.15± 0.08**

Finas = Finasteride. Data is presented as mean of n =6±S.E.M. *p<0.05; **p<0.001

Table 4: Effect of Aqueous fraction of ethanol leaf extract of *C. alata* on serum biochemical profile

Group/Dose	Test. ((ng/dL)	DHT (ng/dL)	Oest. (pg/mL)	PSA (ng/mL)
Normal control	3.66±0.14	0.57±0.02	55.44±8.73	2.87±0.24
Negative control	4.27±0.33	0.66±0.05	39.23±2.51	2.90±0.06
50 mg/kg	3.63±0.17	0.57±0.02	50.33±8.03	3.97±0.56
100 mg/kg	3.80±0.23	0.59±0.04	50.80±3.85	5.20±0.10**
200 mg/kg	2.87±0.17	0.530±0.03	50.17±2.68	4.20±0.45*
Finas. 10 mg/kg	3.80±0.31	0.59±0.04	42.63±1.37	2.11±0.32*

Finas =Finasteride; Oest. = Oestrogen; Test. Testosterone; PAS = Prostate specific antigen. *p< 0.05;**p<0.001.

Table 5: Effect of Aqueous fraction of ethanol leaf extract of *C. alata* on serum antioxidant activity

Group/dose	SOD (U/mL)	CAT (n/mL)	GPx (uM)	MDA (µmol/L
Normal control	53.73±1.01	8.75±0.13	295.00±11.20	111.83±5.62
Negative control	49.24±2.19	7.94±0.11	239.32±6.50	192.55±3.99
50 mg/kg	48.27±2.32	6.34±0.15	168.22±8.13	215.67±2.17
100 mg/kg	45.41±1.34	5.11±0.12	210.51±9.53	231.11±5.69
200 mg/kg	41.01±3.29	5.45±0.19	185.40±9.14	243.43±4.30
Finas. 10 mg/kg	58.11±2.25	8.02±0.12	277.37±7.21	109.67±5.55

CAT= Catalase; Finas = Finasteride; GPx = Glutathione peroxidase; MDA = Malondialdehyde; SOD = Superoxide dismutase.

Table 6: Effect of aqueous fraction of ethanol leaf extract of *C. alata* on prostate parameters

Treatment	Mean body weight (g)	Mean prostate weight (mg)	Prostate Index (mg/g)	Mean prostate volume (mL)
Normal control	209.97 ± 27.67	31.05 ± 0.19	0.15 ± 0.09	0.40 ± 0.18
Negative control	219.78 ± 32.08	48.90 ± 0.11	0.22 ± 0.05	0.72 ± 0.11
50 mg/kg	243.52 ± 28.29	64.35 ± 0.11	0.26 ± 0.08	0.68 ± 0.11
100 mg/kg	230.93 ± 20.98	75.14 ± 0.12	0.32 ± 0.08	0.77 ± 0.15
200 mg/kg	204.95 ± 25.42	80.23 ± 0.13	0.39 ± 0.03	0.87 ± 0.16
Finas. (10 mg/kg)	236.68 ± 33.33	50.33 ± 0.08	0.21 ± 0.06	0.45 ± 0.10

Finas = Finasteride. Data is presented as mean ± S.E.M; n=6

Table 7: Effect of Chloroform fraction of *C. alata* ethanol leaf extract on serum biochemical profile

Groups/Dose	Test. (ng/dL)	DHT (ng/dL)	Oest. (pg/mL)	PSA (ng/mL)
Normal control	3.67±0.14	0.57±0.02	55.40±8.73	2.87±0.24
Negative control	4.27±0.34	0.67±0.05	39.23±2.50	2.98±0.05
50 mg/kg	3.83±0.15	0.60±0.02	46.37±4.29	2.77±0.52
100 mg/kg	4.37±0.73	0.70±0.01	51.50±6.50*	3.53±0.20
200 mg/kg	4.07±0.50	0.63±0.08	47.07±1.57	2.90±0.17
Finas. 10 mg/kg	3.80±0.30	0.60±0.04	42.65±1.37	3.03±0.21

Finas =Finasteride; Oest. = Oestrogen; Test. Testosterone; PAS = Prostate specific antigen.*p< 0.05.

Table 8: Effect of Chloroform fraction of *C. alata* ethanol leaf extract on serum antioxidant activity

Groups/dose	SOD (U/mL)	CAT (µ/mL)	GPx (mM)	MDA (µ/moL)
Normal control	53.67±2.60	9.37±0.00	257.33±13.34	107.21±33
Negative control	50.33±0.67	7.00±1.52	239.67±16.70	98.59±5.90
50 mg/kg	51.43±3.38	8.00±0.58	250.00±15.95	101.33±6.89
100 mg/kg	44.67±1.66	7.67±0.61	209.47±3.76	85.67±1.20
200 mg/kg	50.00±2.08	8.00±0.58	236.67±11.05	97.37±7.40
Finas. 10 mg/kg	55.33±0.67	9.00±0.00	260.00±4.50	108.00±1.15

CAT= Catalase; Finas = Finasteride; Gpx = Glutathione peroxidase; MDA = Malondialdehyde; SOD = Superoxide dismutase.

Table 9: Effects of chloroform fraction of ethanol leaf extract of *C. alata* on prostate parameters

Treatment	Mean prostate weight (mg)	Mean body weight (g)	Prostate Index (mg/g)	Mean prostate volume (mL)
Normal control	0.31 ± 0.19	209.97 ± 27.67	0.14 ± 0.09	0.40 ± 0.18
Negative control	0.48 ± 0.11	219.78 ± 32.08	0.22 ± 0.05	0.72 ± 0.11
50 mg/kg	0.52 ± 0.13	224.80 ± 8.38	0.23 ± 0.05	0.72 ± 0.11
100 mg/kg	0.72 ± 0.16	220.71 ± 39.92	0.33 ± 0.09	0.83 ± 0.08
200 mg/kg	0.67 ± 0.08	233.58 ± 18.10	0.29 ± 0.04	0.77 ± 0.08
Finas. (10 mg/kg)	0.50 ± 0.08	236.68 ± 33.33	0.22 ± 0.06	0.45 ± 0.10

Finas. = Finasteride

Table 10: Secondary metabolites detected in *C. alata*

Secondary Metabolite	Present/Absent
Anthracene derivative	Present
Alkaloid	Present
Cyanogenetic glycoside	Present
Cardiac glycoside	Present
Flavonoids	Present
Saponin	Present
Tannins	Present

Effect of aqueous fraction of ethanol leaf extract of *C. alata* on serum antioxidant activity. At doses of 50 and 100 mg/kg, SOD levels and activity in the treated animals were reduced compared to the negative control group. However, at 200 mg/kg, serum level of

SOD was significantly increased. At 50 mg/kg, serum CAT levels and activity were lower than in the negative control group, with increases recorded at 100 and 200 mg/kg. A dose-dependent increase in glutathione peroxidase levels was observed with the 50, 100, and 200 mg/kg doses. Finasteride-treated animals had higher GPx levels than the negative control animals. A linear [or dose dependent] increase in serum level and activity of MDA was observed in animals that were treated with different doses of the fraction, with the Finasteride-treated animals having higher serum MDA concentrations, compared to negative controls (Table 5).

Effect of aqueous fraction of ethanol leaf extract of *C. alata* on prostate parameters. The fraction caused a dose-dependent increase in the mean prostate index and volume of treated animals, while Finasteride caused mean prostate index and volume changes in the animals comparable to that observed in the normal control animals (Table 6)**.**

Effect of chloroform fraction of ethanol leaf extract of *C. alata* on serum biochemical profile. At 50 and 200 mg/kg, the chloroform fraction decreased serum testosterone levels. However, it was increased by the100 mg/kg dose. These changes were not statistically significant ($P<0.05$). Similarly, dihydrotestosterone levels were decreased by 50 and 200 mg/kg doses, but increased by 100 mg/kg, with no statistical significance ($P<0.05$) recorded. Oestradiol levels increased slightly at all doses compared to the negative control, while PSA levels were decreased by 50 and 200 mg/kg but increased at 100 mg/kg. (Table 7).

Effect of chloroform fraction of ethanol leaf extract of *C. alata* on serum antioxidant activity. Decreases in serum concentrations of SOD, CAT, GPx, and MDA activities in the treatment groups compared to negative control group was observed. At100 mg/kg dose, the activity of these enzymes was lower than in the negative control group. The 200 mg/kg dose resulted in higher activity than the 100 mg/kg group but still lower than the negative control group. The highest activity for each enzyme was observed at the 50 mg/kg dose, and it was slightly lower than in the normal control and finasteride groups. However, these effects were not statistically significant at $P < 0.05$. Details are presented in Table 8.

Effects of chloroform fraction of the ethanol extract of the leaves of *C. alata* on prostate parameters. The chloroform fraction of the ethanol leaf extract of *C. alata* was observed to cause a dose-dependent increase in the mean prostate volume and prostate index in the treated groups compared to the negative group, while finasteride caused a decrease in both parameters. The details of the result parameters are presented in Table 9.

Phytochemical screening of the ethanol leaf extract of *C. alata* revealed the presence of alkaloids, saponin, anthraquinones, tannin, flavonoid and cardiac glycosides (Table 10).

DISCUSSION

This study investigated the effects of ethanol leaf extract of *C. alata* and its aqueous and chloroform fractions on benign prostatic hyperplasia (BPH). It assessed how *C. alata* extract and its fractions influence some parameters closely associated with the disease. Acute toxicity refers to the harmful effects produced by a substance due to one or more exposures to it within 24 hours or less. The lethal dose (LD_{50}), which kills 50% of the test animals, is a key measure of acute toxicity used for screening chemicals and drugs [24]. In test with Swiss albino male mice, Fatmawati et al [18], reported no obvious toxicity in animals treated with 3,000 mg/kg of the ethanolic leaf extract of *C. alata*. In the present study, the ethanolic leaf extract of *C. alata* at an oral dose of 5000 mg/kg showed no mortality or toxic effects in mice. This suggests an estimated LD_{50} greater than 5000 mg/kg.

Androgen levels have long been studied as major influencers of prostatic growth, with **o**estrogen/**o**estradiol possibly playing a role as well. Androgen/androgen receptor signa**l**ling plays a crucial role in the development of BPH, and the blockade of this signa**l**ling decreases BPH volume and can relieve urinary symptoms [25]. The hormones considered in this study were testosterone, dihydrotestosterone, and **o**estradiol.

Testosterone, an androgen produced by the Leydig cells of the testes, is the main circulating androgen in men and is critical for the virilization of Wolffian-duct structures, spermatogenesis, and hypothalamic-pituitary-testes feedback inhibition of sex steroid production [26]. Etim et al, [27] reported that exogenous testosterone administration resulted in an elevation in serum testosterone levels and the development of BPH. The balance between proliferation and programmed cell destruction (apoptosis) in the prostate is controlled by androgens. In the prostate, testosterone is irreversibly converted to dihydrotestosterone (DTH) by 5-alpha reductase (5-AR) [28]. A decrease in serum testosterone levels in the 100 and 200 mg/kg and finasteride-treated animals compared to the negative suggests that the extract at low and medium dose altered the serum concentration of circulating testosterone by either inhibiting its release from the testes or promoting its metabolism and conversion to DHT. The rats treated with varying doses of the aqueous fraction of the *C. alata* ethanol leaf extract showed a dose-dependent increase in serum testosterone levels, while varying results obtained with chloroform fraction of the extract indicate that the aqueous and chloroform fractions have less ability to reduce serum testosterone levels compared to the ethanol leaf extract of the plant.

Dihydrotestosterone (DHT) is synthesized from testosterone by the action of 5α-reductase. It binds to androgen receptors in the prostate tissues, influencing the growth of the prostate and elevation of PSA levels [29]. The ethanol extract of *C. alata* induced a decrease in serum DHT level, while the aqueous and chloroform fractions caused an increase. This suggests that the extract likely blocked the conversion of testosterone to DHT and or increase its conversion to other end products such as oestradiol. The aqueous and chloroform fractions likely enhanced the conversion of testosterone to DHT.

Oestradiol (E2) is a steroid hormone and the major female sex hormone. Its presence in males has long been established. Hess and Cooke [30] have reported the relationship between endogenous oestradiol signalling and male reproduction. Xu et al [31] suggested a direct connection between prostate enlargement and an increase in intraprostatic oestradiol. Similarly, Wynder et al [32] provided evidence for the role of oestradiol in the aetiology of BPH. Aromatase, an enzyme expressed in various male organs and tissues, including Leydig cells and seminiferous epithelium catalyse the conversion of testosterone, into oestradiol [33]. Oestradiol interacts with oestradiol receptors (ER) distributed across various male lower urinary tract and reproductive tissues, such as the bladder, prostate, urethra, and testis. The presence of both ERα and ERβ is reported in the prostatic stroma and epithelium of normal and hyperplastic prostate tissues. High oestradiol induces prostatic cell continued growth, even with diminished testosterone as seen in older men. This is due to signal amplification of androgens by oestradiol [24,27]. The activity exhibited by the extract at the low and medium doses suggests that it has the potential to attenuate the production and activity of E2 at the different levels that amplify prostate hyperplasia. This activity was reversed by the chloroform and aqueous fractions.

Prostate-specific antigen (PSA) is a serine protease produced almost exclusively by the epithelial cells of the prostate gland [34]. It acts as a sensitive, non-specific marker for

prostate-related conditions, including both benign and malignant processes. Elevated serum PSA levels indicate various prostate-related issues such as prostatitis, prostate cancer, and benign prostatic hyperplasia (BPH) [35]. Observations from this study showed a decrease in the serum PSA levels comparable to that seen with finasteride in extract treated rats. These findings are consistent with those reported by Onyeabo et al [37], who observed a reduction in PSA levels in benign prostatic hyperplastic rats treated with the methanolic extract of *Curcuma longa.* Previous research demonstrated that treatment with finasteride resulted in a 2.2-fold decrease in elevated serum PSA levels in male rats injected with testosterone [38]. The aqueous and chloroform fractions caused a dose-dependent increase in serum PSA levels. Overall, these observations suggest that the ethanol leaf extract of *C. alata* can mitigate the effect of exogenously administered testosterone-induced increases in PSA levels, whereas the aqueous and chloroform fractions exhibited opposite effects.

The prostate is susceptible to oxidative DNA damage due to rapid cell turnover and fewer DNA repair enzymes. Elevated testosterone levels are linked to increased reactive oxygen species (ROS) production, leading to oxidative stress in the prostate via androgen receptor signalling [34,39]. This imbalance can cause an enlarged prostate and lower urinary tract symptoms (LUTs) [40]. Antioxidant enzymes act to protect prostate tissue from oxidative stress, which is implicated in BPH aetiology [41]. This study evaluated the serum antioxidants superoxide dismutase (SOD), catalase enzyme, glutathione peroxidase, and malondialdehyde.

SOD catalyses the conversion of superoxide radicals into molecular oxygen and hydrogen peroxide, which protects cells from ROS damage [42]. The study reported a significant increase in SOD activity in the extract-treated groups. The results are consistent with a study on the ethanolic leaf extract of *Cassia sieberiana* [43]. The aqueous and chloroform fractions exhibited varied activities, mainly, a decrease in SOD activity.

Catalase enzyme breaks down hydrogen peroxide and regulates its cellular levels to protect cells from oxidative damage [44]. Hydrogen peroxide in prostate tissue is linked to ROS damage and BPH. Reduced catalase activity has been reported in testosterone-induced BPH animals [44]. Catalase activity was increased by the extract and, decreased by the aqueous and chloroform fractions.

Glutathione peroxidase (GPx) catalyses the reduction of free radicals to alcohol and oxygen. It primarily facilitates the reduction of lipid hydroperoxides to their corresponding alcohols and decreases free hydrogen peroxide to water. A notable decrease in GPx activity has been observed in patients with BPH compared to healthy individuals, thereby establishing a relationship between BPH and GPx [45]. The extract demonstrated a significant dose-dependent increase in glutathione activity, compared to the negative control group. The aqueous and chloroform fractions reversed the increased GPx activity observed with the extract. These findings indicate that the ethanol leaf extract exhibits greater GPx activity than the aqueous and chloroform fractions.

Studies on antioxidant activity in induced BPH have shown that high ROS levels are usually neutralized by natural mechanisms like SOD, CAT, and GPx enzymes. The degree of ROS-induced damage repair can be gauged by the increased levels of these enzymes, indicating enhanced antioxidant protection [42]. Therefore, a stronger antioxidant defence system aids in managing benign prostatic hyperplasia.

Malondialdehyde (MDA) is a final product of polyunsaturated fatty acids peroxidation, and its levels rise significantly under oxidative stress and BPH [46]. The

extract groups showed a dose-dependent and significant decrease in MDA compared to the negative group. The aqueous and chloroform fractions did not match the extract's efficacy.

The prostate index (PI) reflects the relationship between the prostate and body weight. An increase in body weight leads to an increase in prostate weight resulting in a high PI Prostate weight and volume indicate gland size as prostatic cell proliferation increases both. Although the clinical value in PI in benign prostatic hyperplasia is not well established, men with high adiposity and BMI are believed to be more prone to BPH [25]. Prostate weight is an imperfect measure of volume [47], however, a significant correlation between PV and PSA have been established and aids in the diagnosis and monitoring of BPH [35, 48]. Extract treatments significantly reduced PI and PV dose-dependently, while the aqueous and chloroform fractions increased both PI and PV.

Phytochemical screening is employed to analyse plant extracts for potential categories of secondary metabolites. The ethanol leaf extract of *C. alata* revealed the presence of alkaloids, cardiac glycosides, anthraquinones, cyanogenetic glycosides, tannins, flavonoids, and saponins. As stated by Oladeji et al [15], bioactive compounds such as anthraquinone and flavonoids significantly contribute to the free radical scavenging and antioxidant activity observed in many plants, including the leaf extract of *C. alata*. For instance, diacerein, an anthraquinone isolated from *Rumex crispus* (Polygonaceae), has been reported to notably reduce prostate weight and index, while enhancing the activities of prostatic SOD, glutathione (GSH), and CAT. It also diminished prostatic lipid peroxidation in testosterone-induced BPH rats [48]. Additionally, flavonoids such as apigenin-4′-*O*-α-l-rhamnopyranoside, luteolin, and apigenin 7-*O*-β-d-glucopyranosyl-4′-O-α-l-rhamnopyranoside, isolated from *Pteris multifida* (Pteridaceae), exhibited significant anti-BPH activity in treated rats [49]. The potential anti-benign prostatic hyperplasia ameliorating effect of the ethanol leaf extract of *C. alata* identified in this study can be attributed to the presence of these phytochemical groups, individually or in combination.

The significance of fractioning a crude extract lies in its ability to facilitate the isolation and identification of specific compounds or groups of compounds with activity in bioassay-guided processes for further study [50]. However, in this study, fractionation did not enhance the activity of any of the fractions. Instead, there was a decrease in activity compared to the crude extract, suggesting that the active components work synergistically or additively in the crude extract, which was lost during fractionation. Thongphichai et al. [51] reported that fractionation of the ethanolic crude extract of *Crinum latifolium* to isolate two alkaloids, lycorine and 6α-hydroxybuphanidrine, led to a loss of the potential anti-BPH activity observed in the crude extract.

Conclusion. Conclusively, this study provide evidence that shows that the ethanol extract of *C. alata* is relatively safe when administered to mice up to 5000 mg/kg. Also, the extract has potential to ameliorate benign prostatic hyperplasia in rats, supporting its claimed use in ethnomedicine to manage the disease. Separating the constituents of the extract into polar and non-polar fractions led to loss of observed activity.

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