



RESEARCH ARTICLE

In Vitro Cytotoxic Activity and In Vivo Antiproliferative Activity of Ethyl Acetate Fraction of *Bunium bulbocastanum* Seed Against Prostatic Neoplasia

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Abstract Several in vitro investigations of the therapeutic characteristics of *B. bulbocastanum* have shown that it has cytotoxic, antifungal, and antibacterial effects. It also exhibits antioxidant and anticancer effects. When the ethyl acetate fraction of *B. bulbocastanum* was examined for its phytochemical composition, it was found to be rich in phenolic compounds and had significant cytotoxic effects on PC-3 cell lines. The prostate length-to-weight ratio was significantly higher in vivo in the ethyl acetate fraction-treated group. Compared to the disease control group, histopathological examination of the ethyl acetate-treated group revealed a reduction in inflammation and malignant lesions, confirming its antiproliferative efficacy. According to serum

biochemistry, acid phosphatase and PSA levels in the ethyl acetate fraction treatment group were significantly lower than those in the disease control group. When compared to the disease control group, malondialdehyde levels in the ethyl acetate fraction treatment group were likewise reduced dramatically. However, in the group treated with the ethyl acetate fraction, glutathione levels increased considerably. The ethyl acetate fraction of *B. bulbocastanum* may have cytotoxic and antiproliferative potential, both in vitro and in vivo.

Keywords *Bunium bulbocastanum* · Medicinal herb · Antioxidant · Anticancer · Prostate cancer · Androgen

Significance Statement: *Bunium bulbocastanum* is a popular medicinal plant that is used to treat a number of conditions such as throat infections, colds, fevers, and hyperglycaemia. The cytotoxic properties of ethyl acetate extract is well-known and can be used to prolong proliferative dormancy of the tumour leading to the development of new therapies.

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Introduction

Prostatic neoplasia leading to cancer is one of the most common malignancies in males, claiming the lives of 200,000 men worldwide each year [1]. It is the most prevalent cancer in men, accounting for approximately 33% of all cancers. Males have a one-in-six lifetime probability of developing prostate cancer compared to one-in-two for other areas [1, 2]. Understanding the role of hormones in prostate cancer is a significant step forward in this field. Dr. Huggins and his colleagues at the University of Chicago demonstrated in 1941 that castration, oestrogen therapy, or both usually postpone the development of metastatic prostate cancer for a short period of time, leading to the conclusion that androgens are to blame. This awareness improves the treatment and survival of patients with prostate cancer [2]. Plants have long been employed for therapeutic purposes and India is home to such diverse flora, it stands to reason that it has been included in the ancient Indian medicinal system [3]. Despite the massive

influence and dependence on western medicine as well as remarkable developments in synthetic pharmaceuticals, a substantial portion of the world's population still prefers plant-based products because of their accessibility, efficacy, low cost, and absence of adverse effects [4]. Plant-based compounds have been the most effective sources of medication since the beginning of human civilisation [5]. Herbal medicine is a vital component of medical science. Plant phytochemicals, such as alkaloids, terpenoids, flavonoids, glycosides, and phenolics, have numerous positive and beneficial effects [6]. Most of these outcomes are good and advantageous. These therapeutic plants are thought to be exceptionally helpful because they have few or no adverse effects [7]. The most important thing to remember is that herbal medicine does not rely on the person, age, or gender and is thus safer, but long-term use of synthetic drugs can result in a range of consequences or addiction [6]. *B. bulbocastanum* (BB), also known as Kala jeera, belongs to the Apiaceae family. Its fruit and seeds have long been used as a spice in cooking [8, 9]. BB essential oil is abundant in oxygenated monoterpenes, specifically—terpinene, cumin aldehyde, cymene, and limonene, all of which have antibacterial and antioxidant qualities [10, 11]. Studies have suggested that BB has potent anticancer activity in vitro, but no studies have been conducted on animal models [12, 13]. Hence, this study was performed to elucidate the anticancer potential of BB in Wistar rats with prostate neoplasia.

Methodology

Ethical Approval and Study Centres

After receiving approval from the Institutional Animal Ethics Committee (IAEC/KMC/111/2020), the study was conducted in Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal (MAHE). This study was conducted in collaboration with the Department of Pathology, Kasturba Medical College, Manipal and Department of Pharmacology, Manipal College of Pharmaceutical Sciences, MAHE, Manipal.

Identification and Collection of Raw Material

The seeds of the plant used in this study were collected from Jammu city from the Union territory of Jammu and Kashmir, India, in October 2020 and were validated by Mrs. Usharani S Suvarna, Professor (Associate) & HOD Department of Botany, MGM College, Udupi, Karnataka, India.

Preparation of Seed Extract of *B. bulbocastanum*

The material (seeds) was thoroughly washed, dried in the shade, and finely ground with a blender (grinder). The powder was then placed in two conical flasks and cold macerated with 100% alcohol for 48 h. The extraction was continued until the solvent was colourless. After the extraction was completed, a rotary evaporator was used to extract and evaporate the solvent. The product was stored in a lyophiliser after evaporation to remove moisture.

Qualitative Phytochemical Assay of the Seed of *B. bulbocastanum*

The test was carried out utilising established techniques to determine the preliminary active phytochemicals in alcoholic leaf extract of BB (Gul et al. 2017; Chari et al. 2018).

Total Phenolic and Flavonoid Content

The Folin–Ciocalteu technique was used to determine the total phenolic content of the extract. The gallic acid standard curve was used to calculate the total phenolic content, and the values were expressed as milligramme of gallic acid equivalent per gramme of dry plant material. The total flavonoid content of the crude extract was determined using the aluminium chloride colorimetric method. The total flavonoid content was calculated using a calibration curve and expressed as milligramme quercetin equivalent per gramme of dried plant material. All the tests were performed in duplicates.

Quantitative Phytochemical Analysis by GC–MS of Ethyl Acetate Extract

To evaluate the phytochemical constituents of the BB ethyl acetate seed extract, Shimadzu gas chromatography–mass spectrometry (GCMS-QP2010S) equipment was employed. The acquired MS spectra were matched with the NIST library for chemical identification and conformation, Analytical Research and Metallurgical Laboratories, Pvt., Ltd. (ARML), in Bengaluru, India.

Preliminary Screening of Cytotoxicity by Sulphorhodamine B (SRB) Assay

Based on the assessment of cellular protein content, the SRB assay is used to determine cell density. The cytotoxicity of the ethyl acetate fraction of alcoholic crude extract was assayed on PC-3 cell line, and, for comparison, doxorubicin was used as the standard anticancer medication. In a 96-well format, the approach has been optimised for toxicity screening of drugs against adherent cells. Cell monolayers are fixed with 10% (wt/

vol) trichloroacetic acid and dyed for 30 min after an incubation time, after which the excess dye is washed away with 1% (vol/vol) acetic acid. The protein-bound dye is dissolved in a 10-mM Tris base solution and measured using a microplate reader at 510 nm [14].

In Vivo Anticancer Screening

In vivo anticancer screening was performed using a testosterone-induced prostate cancer model in male Wistar rats inbred at the Central Animal Research Facility (CARF), MAHE, and animals were adapted to the experimental room at 23 ± 3 °C, 12:12 h light, dark cycle, and controlled humidity conditions. The animals were housed in sterile polypropylene cages containing clean/sterile paddy husks as bedding material. Animal care and handling were carried out according to the guidelines of the Institutional Animal Ethics Committee, Manipal.

Testosterone-Induced Prostate Neoplasia Model

Preparation of Testosterone

DMSO was used as a carrier to deliver testosterone to rats. Before each dose, a testosterone solution was freshly prepared. The induction period was 4 weeks long. For 4 weeks, the carcinogen was administered subcutaneously at a dose of 40 mg/kg. After 1 month (30th day) of testosterone administration, blood was collected, and one rat from each group was sacrificed by administering a high dose of thiopentone sodium to check if disease was induced. Neoplasia development was assessed by elevated serum prostate specific antigen (PSA) levels and confirmed histopathologically.

Fraction Preparation

The crude ethanolic extract (130 g) was suspended in 350 ml of distilled water. The suspension was partitioned using ethyl acetate for further separation. The ethyl acetate fraction contained compounds based on their solubility in the crude extract. After further fractionation, it was used for dosing in rats at 50 mg/kg and 100 mg/kg as the test treatment after cancer was induced and confirmed. DMSO was used as a carrier for the ethyl acetate fraction and administered orally. Lupride was administered to rats subcutaneously once disease was induced as a standard treatment for a better comparison of the two treatments (the group and number distribution of the rats are given in Table 1).

Table 1 Distribution of rats and group-wise dosing schedule

Sr. no.	Group	No. of animals	Dose	Dosing schedule
1	Control	6	—	—
2	Disease control	8	40 mg/kg	Daily for 30 days
3	Standard (Lupride) group	8	3 mg/kg	Once in 30 days
4	Test group I	8	50 mg/kg	Daily for 30 days
5	Test group II	8	100 mg/kg	Daily for 30 days

Parameters Monitored in Testosterone-Induced Prostate Cancer Model

Prostate Length and Weight Ratio

The rats were euthanised once the treatment was completed (60th day), and prostate segments were removed and rinsed in phosphate-buffered saline before being preserved in sterile containers with 10% buffered formalin. Per body length of the organ with the organ weight was used to calculate the length and weight ratio.

Collection of Tissue and Blood Sample

Blood samples were collected from the retro-orbital plexus after 1 month of treatment (60th day), and the serum was separated. Blood samples were collected in plain vacutainers and centrifuged for 5 min at 5000 RPM.

Biochemical Estimations

Acid Phosphatase The principle behind the measurement of acid phosphatase activity involves hydrolysis of alpha-naphthyl phosphate by acid phosphatase enzymes in an acidic environment. This hydrolysis reaction generates α -naphthol and phosphate as the products. In this process, alpha-naphthol reacts with a diazo compound, 2-chloro-5-toluene (Fast Red TR Salt), to form an azo dye molecule. This azo dye had a characteristic absorption maximum at 405 nm. The amount of azo dye formed was directly proportional to the acid phosphatase activity present in the sample, and by measuring the absorbance at 405 nm, the total acid phosphatase activity was quantified. Prostatic activity is inhibited when assessed in the presence of tartrate; hence, the prostatic fraction is the difference between total (ACPT) and non-prostatic acid phosphatase (ACPNP).

Prostate-Specific Antigen (PSA) ELISA PSA ELISA is a solid-phase assay that employs the streptavidin–biotin prin-

ciple. In the test blood, PSA binds to two highly specific anti-PSA antibodies tagged with biotin and horseradish peroxidase.

Reduced Glutathione Assay Glutathione reductase is a non-protein compound that contains a sulphhydryl group. It reduces DTNB [5-5'-dithiobis (2-nitrobenzoic acid)] to an intense yellow compound whose absorbance was measured at 412 nm. The higher the absorbance, the greater is the amount of GSH in the sample.

Lipid Peroxidase Assay The extent of peroxidation is determined by the breakdown product of polyunsaturated fatty acids (PUFA). The interaction between thiobarbituric acid and malondialdehyde produces a pink colour that can be observed at 532 nm².

Histopathological Examination of the Prostate

Dissected prostate tissues were stored in 10% formalin. Paraffin-embedded specimens were used for histopathological evaluation of prostate tissues. The specimens were sliced to a thickness of 6 mm and stained with haematoxylin and eosin.

Photography and Presentation of Section

The slides were placed under the microscope when they were thoroughly dried and focused with a 10X lens to capture photos. To distinguish the cellular structure, the acquired image must be compared with the anatomy of a conventional slide.

Statistical Analysis

The data were analysed using Tukey's post hoc test, with $p < 0.05$ being regarded as statistically significant. The analysis above was completed using the GraphPad Prism 5.03 edition (GraphPad Software Inc., La Jolla, CA, USA).

Results and Discussion

- (1) **Phytochemical analysis of ethyl acetate fraction of *B. bulbocastanum*:** Phytochemical screening of the plant extract revealed the presence of terpenoids, saponins, tannins, quinine, carbohydrates, and steroids (Table 2) in the seed extract of BB.
- (2) **Total flavonoid and phenol content:** The total phenolic content in the ethyl acetate fraction was approximately 144-mg gallic acid equivalent (GAE)/100 g, and the

Table 2 Result of phytochemical analysis of *B. bulbocastanum* ethyl acetate fraction

Sr. no.	Phytochemicals	Ethyl acetate fraction
1	Alkaloids	—
2	Flavonoids	—
3	Phenol	—
4	Tannins	+
5	Steroids	++
6	Saponins	—
7	Glycosides	—
8	Terpenoids	++

Table 3 Total phenolic and flavonoid content with the values expressed in Mean $\pm$ SD

Extract	Ethyl acetate fraction (Mean $\pm$ SD)
Total phenolic content	144 $\pm$ 0.21 (RE)/100 g
Total flavonoid content	31 $\pm$ 0.26 (RE)/100 g

total flavonoid content was approximately 31-mg rutin equivalent (RE)/100 g (Table 3).

- (3) **Quantitative analysis by GC–MS:** Approximately 53 compounds were identified by GC–MS analysis (Fig. 1), of which many compounds were plant steroids. Significant quantities of squalene were present in the ethyl acetate fraction. Other major compounds found in ethyl acetate were stigmasterol, tricyclo-triacontane, fucosterol, pregnenolone, alpha-amylin, and many more.
- (4) **Preliminary screening of cytotoxicity by sulphorhodamine B (SRB) assay:** A preliminary cytotoxicity study of the ethyl acetate fraction of the absolute alcoholic extract was performed in PC-3 cell lines. The ethyl acetate seed fraction was found to have potent cytotoxic activity against the standard drug doxorubicin, and the IC₅₀ value was calculated for both compounds. IC₅₀ values were obtained from the SRB assay (Table 4).

In Vivo Anticancer Screening

Prostate Length/Weight Ratio

The prostate length/weight ratio (Fig. 2A) was significantly reduced in the disease control group compared to that in the healthy group ($p < 0.05$). In the treatment group, 50 and 100 mg/kg, the ratio was significantly increased compared than in the disease control group. In the standard treatment

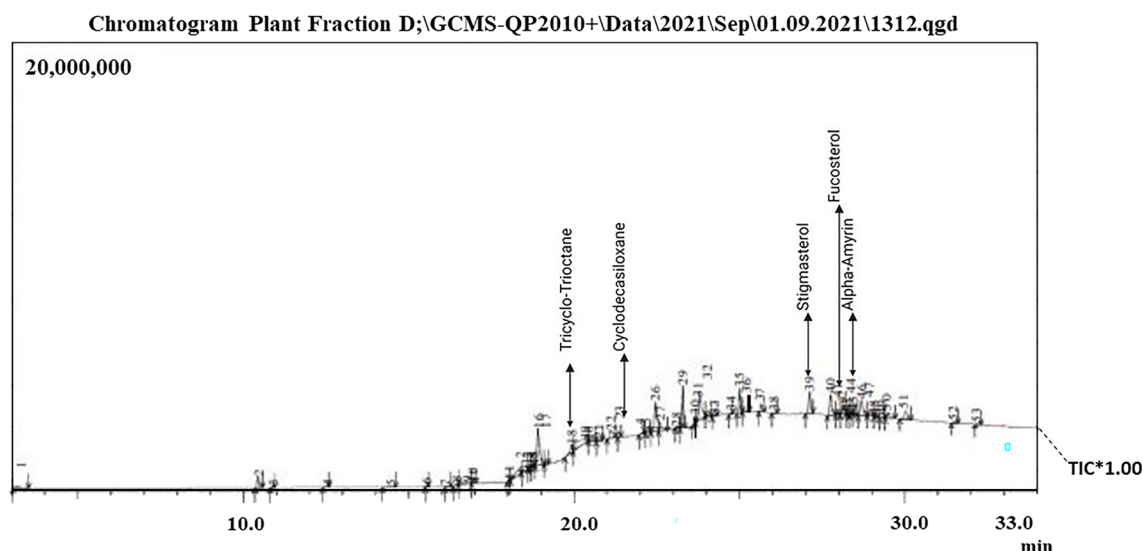


Fig. 1 Chromatogram of GC–MS analysis of ethyl acetate extract of *B. bulbocastanum*

Table 4 Cytotoxicity of ethyl acetate fraction with the standard doxorubicin on PC-3 cell lines

IC ₅₀ of ethyl acetate fraction	4.985 µg/mL
IC ₅₀ of doxorubicin	10.4 µg/mL

group, the ratio was significantly higher than that in the disease control group ($p < 0.05$). A significant difference was observed between the standard treatment group and the 50 and 100 mg/kg treatment groups ($p < 0.05$).

Biochemical Estimations

Acid Phosphatase Acid phosphatase levels (Fig. 2B) were significantly higher in the disease control group than in the healthy controls ($p < 0.05$). Acid phosphatase levels in the 50 and 100 mg/kg treatment groups were significantly lower than in the disease control group ($p < 0.05$). There was no significant difference between the treatment 100 mg/kg group and standard treatment groups ($p > 0.05$), whereas there was a significant difference between the treatment 50 mg/kg group when compared with the standard treatment groups ($p < 0.05$).

Prostate Specific Antigen (PSA) PSA levels were significantly higher in the disease control group than in the healthy controls ($p < 0.05$) (Fig. 2C). There was a significant decrease in the 50 and 100 mg/kg groups compared to that in the disease control group ($p < 0.05$). There was no significant difference between the test and standard treatment groups ($p > 0.05$).

Glutathione Reductase (GSH) The increased GSH levels indicate low oxidative stress. GSH levels in the disease control group were significantly lower than those in the control group ($p < 0.05$) (Fig. 2D). There were no significant differences between the standard treatment and disease control groups ($p > 0.05$). There was a significant increase in GSH levels in the 50 and 100 mg/kg groups compared to the disease control and standard treatment groups ($p < 0.05$).

Lipid Peroxidation (MDA) Lipid peroxidase levels were significantly increased in disease control group compared to healthy control ($p < 0.05$) (Fig. 2E). There was a significant decrease in malondialdehyde in the 50 and 100 mg/kg treatment groups compared with in that the disease control group ($p < 0.05$). The BB ethyl acetate fraction group showed significantly lower levels of malondialdehyde as compared than the standard treatment group ($p < 0.05$).

Histopathological Analysis of Prostate In the control group, normal histological characteristics (Fig. 3A) were observed, including tubules of varying thickness. The lumen was filled with prostatic secretions. The blood arteries were healthy. No loss of contact inhibition is observed. In the disease control group, the tubules were wider than those in the control group (Fig. 3B). The cells were irregular in shape. The walls of the tubules were also thickened. The thickness was reduced by the lumen-lining cells that breached the space. There was a clear loss of contact inhibition. Areas with dilated blood arteries and compressed connective tissues were also observed. The sarcoplasmic texture and distant nuclei were not evident. In the standard treatment group, a normal distribution of the stroma was

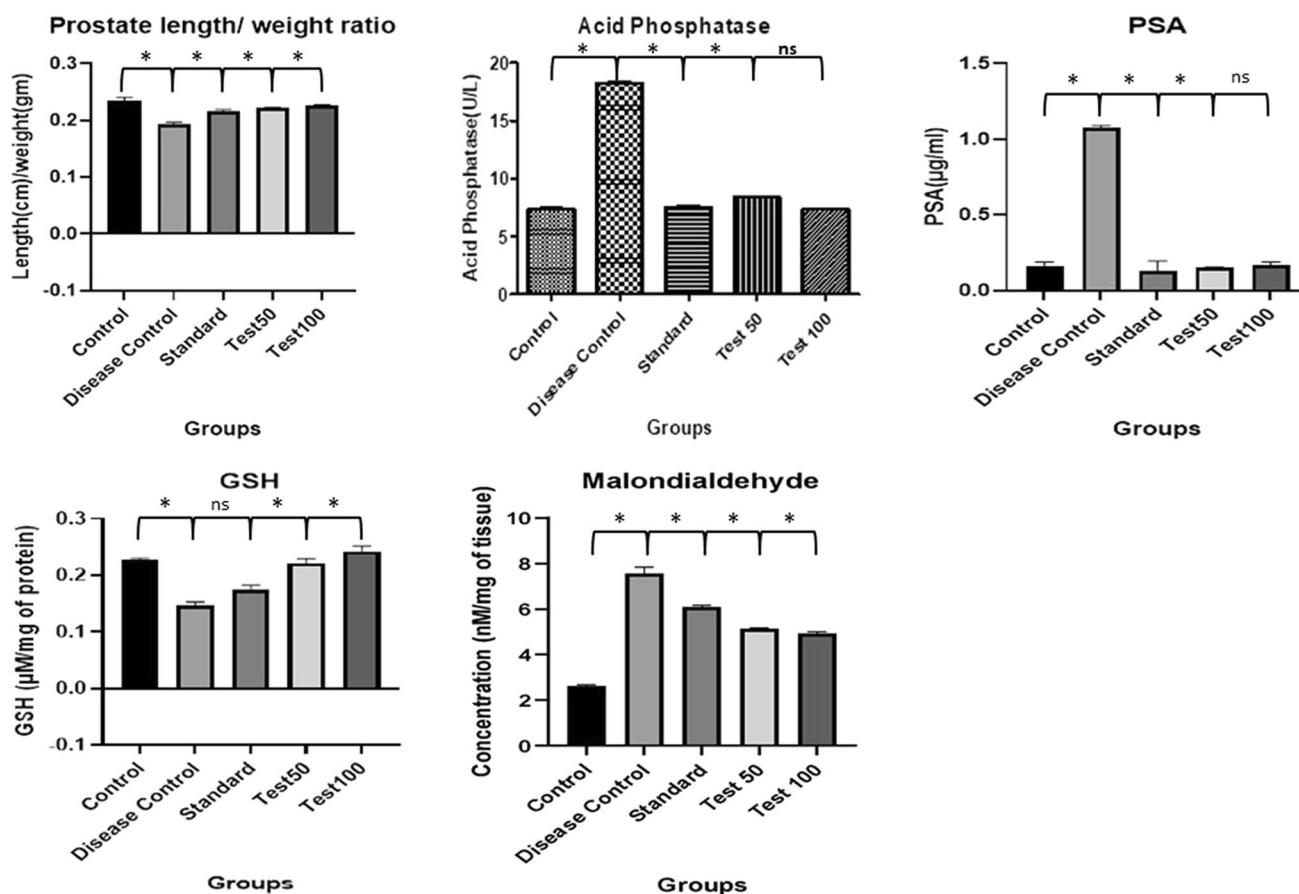


Fig. 2 Effect of BB seed fraction on **A** prostate length/weight ratio, **B** acid phosphatase, **C** PSA, **D** GSH, and **E** malondialdehyde. All values are expressed in $\pm$ SEM (* $p < 0.05$ and $^{ns}p > 0.05$)

observed. Projections into the lumen were not prominent (Fig. 3C). Compared with the testosterone-treated group, several cells with irregular shapes and increased volumes were observed. The thickness of the cell wall was reduced to a greater extent. Cellular vascularisation and the lumen and tubules were normal in the ethyl acetate-treated groups. The epithelium was thicker than that in the control. In the 50 mg/kg group of the test fraction (Fig. 3D), the outer layer of the lumen was thicker than in the 100 mg/kg group (Fig. 3E). Eosinophilic secretion was observed around the lumen. Compared to the disease control group, there was a considerable reduction in the projections of cells in the lumen.

For many years, BB has been used as a medicinal herb for a diverse range of treatments. The medicinal uses of this plant extract have been the subject of much research. As a few studies have shown that various parts of the plant possess antioxidant activity, the seeds were extracted with ethanol and subjected to further fractionation. According to a previous study, ethyl acetate and an aqueous fraction of the plant exhibit antiproliferative activity against Hep2, K-562, MCF7, and NIH3T3 cancer cell lines [8]. Preliminary phytochemical studies showed that the ethyl acetate seed fraction

contains terpenoids, tannins, and steroids (Table 2). The total phenolic and total flavonoid contents were approximately 144-mg gallic acid equivalent (GAE)/100 g and 31-mg rutin equivalent/100 g, respectively (Table 3).

As per the previous data available, the more compounds with the phenolic group present in the plant extract, the more antiproliferative activity is observed [15]. Phenolic compounds like flavonoids add to the medicinal property of the plants [16]. GC-MS analysis of 53 different phytochemicals was found in varying concentrations, including squalene and pregnenolone which are intermediates of steroid metabolism. Other biologically active phytochemicals detected were stigmasterol, tricyclo-triacontane, fucosterol, pregnenolone, cyclodecasiloxane, and alpha-amyrin. Stigmasterol is well-known for its anti-inflammatory action [17], fucosterol is known for its antioxidant activity which is correlated to its anticancer potential [18]. Alpha-amyrin and tricyclo-triacontane are very well-known compounds for their anticancer activity [19, 20]. Preliminary cytotoxicity screening by the SRB assay was performed on PC-3 AR⁺ cell lines using the ethyl acetate fraction of the ethanolic seed extract. PC-3 is an adenocarcinoma of the prostate gland that is widely used

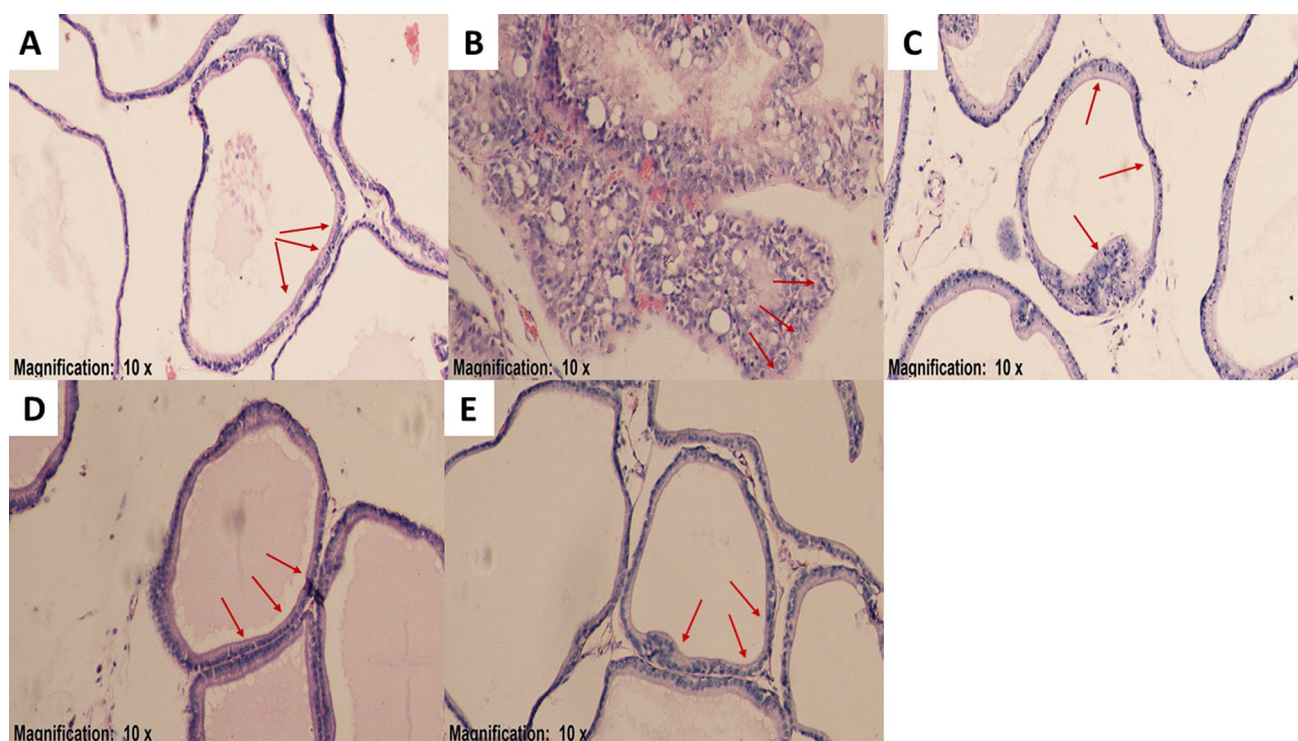


Fig. 3 Histology section of the prostate gland of **A** normal group, **B** disease control group, **C** standard treatment, **D**: test fraction 50 mg/kg treated group, and **E** test fraction 100 mg/kg treated group

for the screening of potent anticancer components [21]. SRB assay is a very sensitive assay when it comes to the screening of cytotoxic agents in plant extracts [14].

Hence, the study revealed that the ethyl acetate fraction possesses significant cytotoxic activity on PC-3 (Table 4), so the further study was conducted with ethyl acetate fraction. In in vivo, based on a prior study, where testosterone was utilised to cause benign prostatic hyperplasia, this study used a high dose of testosterone (40 mg/kg) to promote the growth of prostatic cells in rats [22]. Testosterone is responsible for the growth and division of prostate cancer cells. The cells of the prostate divide and the old cells are replaced by a new set of cells. Cell division is very important for the control of the prostate [23]. When a large amount of testosterone is injected into the animal, testosterone is then utilised by the cells of the prostate, and cell division is initiated, which, in some cases overcomes the cell cycle checkpoints [15, 23]. Rapid cell division increases the demand for energy, which increases mitochondrial metabolism to produce more ATP, resulting in the generation of many reactive oxygen species (ROS) and increasing oxidative stress [24]. Increased oxidative stress can affect genetic material and cause mutations. Mutations can convert many proto-oncogenes to oncogenes and lead to tumours or cancer. Once a tumour is induced, uncontrolled cell division and loss of contact inhibition occur in the cells. For normal respiration (aerobic) to occur

in the cells, the distribution of the cells in the tissue is very important to ensure the supply of adequate nutrients, such as oxygen and glucose [21]. As a result of loss of contact inhibition and uncontrolled cell division, the supply of blood is reduced to the cells, and the cells then start anaerobic respiration which produces more ROS and increases the oxidative stress that can cause further damage to the tissue. After the oral administration of the plant extract in the rats for 4 weeks, it was observed that there was a significant increase ($p < 0.05$) in prostate length/weight ratio in the ethyl acetate treatment groups compared to the standard treatment group (Fig. 2A). Prostate length and weight ratio is precise markers that detect any abnormal growth in the prostate tissue, such as the formation of microadenomas, adenomas, and adenocarcinomas. The length/weight ratio decreases when cancer is induced in animal models [25].

Acid phosphatase levels were significantly elevated in the disease control group compared with those in the control group (Fig. 2B). There was a significant decrease in the level of acid phosphate in the 50 and 100 mg/kg treatment groups ($p < 0.05$), where the 100 mg/kg group was more effective in reducing acid phosphatase levels. Acid phosphatase elevation with high PSA levels is an indicator of prostate cancer [26]. Many studies have shown that high acid phosphatase levels indicate various pathological conditions of the prostate gland [27]. PSA levels were elevated

in the disease control group and significantly reduced in the treatment group ($p < 0.05$). No significant differences were observed between the standard and test treatment groups ($p > 0.05$) (Fig. 2C).

PSA expression is dependent on the Androgen receptor (AR) signalling pathway and can serve as a potent marker to assess the downstream action of the AR pathway. Low levels of PSA can be an indicator of an unfunctional AR pathway due to AR inhibition. PSA levels are very low in circulation but can be increased in cases of any disorder that causes uncontrolled division of prostate cells, and over-expression of PSA genes can exponentially elevate PSA levels [28]. PSA levels are important for the diagnosis of prostate cancer, and both PSA levels and histopathological examination are important for the grading and staging of cancer [29]. Histopathological sections demonstrated a reduction in the lesions and cell density in the ethyl acetate fraction treatment group compared to the disease control group. The cells were healthier and had normal histology in the test fraction treatment group than in the standard treatment group (Fig. 3). The cells in the ethyl acetate treatment group showed normal morphology, and contact inhibition by the cells was observed. In contrast, the disease control group had irregularly shaped cells, and loss of contact inhibition was observed. The overgrowth of cells in the disease control group resulted in the thickening of tubules. GSH is an endogenous enzyme with antioxidant activity that regulates normal free radical levels in the body. In cancer cells, this enzyme is decreased because of an increase in the free radical level. GSH concentrations in the test treatment group were substantially higher and statistically significant ($p < 0.05$) than those in the standard treatment group (Fig. 2D). GSH levels are important markers for indicating damage caused by free radicals in tissues [30]. Malonaldehyde (MDA), a marker of lipid peroxidation, increases during lipid peroxidation due to high oxidative stress which may lead to damage to the cell membrane and various other cell organelles. MDA levels have decreased significantly in the treatment group as compared to the disease control group ($p < 0.05$) which might be due to the potent antioxidant activity of the seed fraction. When compared to standard treatment, the test treatment showed a significant decrease in MDA levels ($p < 0.05$) (Fig. 2E).

Overall, the oxidative stress was significantly reduced in the test fraction-treated group, which indicates that the seed fraction of BB has potent antioxidant activity and can reduce oxidative stress significantly. A similar study showed that lowering MDA levels in the treatment group is a very positive indicator that the plant extract has a protective effect against lipid peroxidation [22]. Antioxidants can prevent the prognosis of cancer by scavenging all the free radicals that may pose threat to normal cells [11]. Having enough levels of antioxidants can prevent the damage caused by the free

radicals to the DNA of the cells which is a widely studied cause of carcinogenesis [11, 24]. The study provides enough evidence that the ethyl acetate fraction of BB of alcoholic seed extract is very efficient to reduce the cancer prognosis in rats and is oncoprotective by action.

Conclusion

According to the findings of this study, the ethyl acetate fraction of alcoholic seed extract of *B. bulbocastanum* showed significant anticancer activity against PC-3 cells. This activity can be attributed to the presence of various bioactive phytochemical constituents in the ethyl acetate seed extract. Based on the in vivo results, the ethyl acetate seed fraction of BB was able to reduce the cancerous lesions of the prostate glands, and the above findings were supported by the results obtained by biochemical estimations, supporting the fact that BB ethyl acetate seed extract showed significant anticancer activity in the testosterone-induced prostate cancer rat model. Based on the results of this study, it can be inferred that the ethyl acetate fraction of the 100% alcoholic seed extract of BB has antioxidant properties and can help prevent the development of prostate cancer. As a result, there is potential in the pharmaceutical sector for even stronger anticancer commercial medications to be created sooner, utilising plant compounds, to successfully treat cancer. Further research should be conducted to identify the elements responsible for the anticancer action.

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Declarations

Ethical Approval The study was carried out after obtaining approval from Institutional Animal Ethics Committee (IAEC/KMC/111/2020), Kasturba Medical College, Manipal, MAHE.

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