

RESEARCH

Open Access



Phytochemical insights, antioxidant activity and therapeutic potential of *Cassia senna* against prostate cancer in Wistar albino rats

Guru Kumar Dugganaboyana¹, Mohan Krishna Gunjevu Kannankumar¹, Sahana Shivaramakrishna¹, Manikandan Vani Raju², Meenakshi Kaniyur Chandrasekaran², Rathai Muthaiyan Ahalliya^{2,3}, Nirmaladevi Panneerselvam^{2,3}, Chella Perumal Palanisamy⁴ and Gopalakrishnan Velliyur Kanniappan^{5*} 

*Correspondence:

Gopalakrishnan Velliyur Kanniappan
vkgopalakrishnan@gmail.com

¹Present address: Division of Biochemistry, School of Life Sciences, JSS Academy of Higher Education and Research, Mysuru, Karnataka 570 015, India

²Department of Biochemistry, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu 641021, India

³Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu 641021, India

⁴Department of Dermatology, Saveetha Medical College & Hospital Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai, Tamil Nadu 602 105, India

⁵Department of Physiology, Saveetha Medical College & Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai, Tamil Nadu 602 105, India

Abstract

Prostate cancer is the second most common disease and a major worldwide health concern for males. This study investigates the phytochemical antioxidant properties of *Cassia senna* and its therapeutic potential against prostate cancer in Wistar albino rats. The Phytochemical screening was conducted to identify the presence of bioactive compounds. The radical scavenging activities, including DPPH, ABTS, hydroxyl, superoxide and nitric oxide assays were performed to evaluate antioxidant capacity. The prostate cancer was induced in rats using testosterone and N-methyl-N-nitrosourea (MNU). The experimental groups were treated with *Cassia senna* extract and finasteride to compare therapeutic outcomes. The effects were assessed by weight, enzymes, blood, lipids and histology. The phytochemical screening revealed the presence of significant secondary metabolites in the ethanolic extract, including flavonoids, tannins, alkaloids, steroids, saponins, carbohydrates, and amino acids. The ethanolic extract contained high levels of polyphenols (75.29 ± 0.22 mg/g), flavonoids (35.25 ± 1.15 mg/g), and tannins (180.62 ± 1.29 mg/g), along with moderate levels of vitamin C (19.40 ± 0.30 mg/g) and carotenoids (4.21 ± 0.20 mg/g). Vitamin E was present in smaller amounts (3.50 ± 0.25 mg/g). The ethanolic extract demonstrated significant antioxidant activity. Prostate cancer-induced rats treated with *Cassia senna* showed significant improvement in body and organ weights, serum enzyme levels, lipid profiles, and hematological markers compared to untreated groups. The study suggests that *Cassia senna* may be effective against prostate cancer by reducing oxidative stress, and further research is needed to explore its mechanisms and potential use in treatment.

Article highlights

- Ethanolic extract of *Cassia senna* having significant antioxidant activity.
- Prostate cancer-induced rats treated with *Cassia senna* showed significant improvement in body, organ weights, enzyme levels, lipid profiles, and hematological markers compared to untreated groups.
- *Cassia senna* is promising as a complementary or alternative therapeutic candidate for prostate cancer.



Keywords *Cassia senna*, Secondary metabolites, Antioxidants, Prostate cancer, Wistar albino rats

1 Introduction

Cancer has been recognized as a leading cause of death. In 2020, around 19.3 million new cancer cases and 10 million cancer-related deaths were reported globally [1]. Prostate cancer remains a significant global health concern, as the second most frequent cancer and the fifth highest cause of cancer-related deaths among men globally [2]. According to recent data, there were 1,414,259 new cases and 375,304 deaths worldwide in 2020, with high-income nations having the highest incidence rates due to widespread prostate-specific antigen (PSA) screening. However, mortality rates have decreased in many affluent countries, because of breakthroughs in therapy and early detection. Between 2000 and 2019, the annual prostate cancer death rate in the United States decreased by 1.22% [3]. Still, variations persist, African-American men have the greatest incidence and deaths because of aggressive disease subtypes and inadequate healthcare access [4]. Regional trends indicate rising instances in Asia and Eastern Europe, whereas incidence rates are stable in many resource-rich countries. One of the critical factors contributing to the development and progression of prostate cancer is hormonal imbalance, particularly elevated levels of androgens such as testosterone. Testosterone, the primary male sex hormone, plays a vital role in prostate growth and function. However, excessive or prolonged androgen stimulation can promote abnormal cell proliferation, leading to prostatic hyperplasia and eventually carcinogenesis. In experimental models, the administration of exogenous testosterone in combination with carcinogens like MNU has been shown to reliably induce prostate cancer, mimicking hormonal and oxidative environments observed in human disease. This makes testosterone a experimental agent for prostate tumor induction [5].

Anti-inflammatory and antioxidant defense systems play a crucial role in maintaining cellular homeostasis and protecting against a wide range of diseases, including cancer. Inflammation, though a natural immune response to harmful stimuli such as pathogens or damaged cells, can become chronic and contribute to tumor development [6]. Inflammation is a natural immune response to harmful stimuli such as pathogens or damaged cells; however, when it becomes chronic, it can contribute significantly to tumor development. Chronic inflammation leads to the sustained production of reactive oxygen species (ROS), which in excess can disrupt cellular homeostasis. Elevated ROS levels are a hallmark of many cancers, including prostate cancer, and are closely linked to mitochondrial dysfunction [7]. In prostate cancer cells, excessive ROS production damages key cellular components, particularly mitochondrial DNA. Damage to mitochondrial DNA impairs oxidative phosphorylation, resulting in further ROS generation and establishing a vicious cycle of oxidative stress. This accumulation of ROS promotes genetic instability, activates oncogenic signaling pathways, and enhances tumor progression and survival. Thus, oxidative stress plays a central role in the initiation and progression of prostate cancer, highlighting the therapeutic relevance of targeting ROS-mediated damage [8]. The critical involvement of oxidative stress in the development and progression of cancer, particularly prostate cancer, has led to increasing interest in natural strategies aimed at restoring redox balance and counteracting ROS-induced damage.

Medicinal plants have long been recognized for their therapeutic potential, particularly due to their antioxidant and anti-inflammatory properties, which can help modulate oxidative stress and support cellular defense mechanisms. Much of this protective activity is attributed to phytochemicals, a diverse group of naturally occurring bioactive compounds found in plants. These compounds, often working synergistically with dietary fibers and nutrients, contribute to the plant's overall medicinal value. In recent years, interest in herbal medicine has surged as researchers seek safer, more sustainable alternatives to synthetic drugs [9]. Both laboratory and epidemiological studies increasingly suggest that phytochemicals possess strong antimutagenic and anticarcinogenic properties. In vitro and in vivo research has demonstrated that these compounds can suppress angiogenesis, inhibit cell proliferation, induce apoptosis, and delay metastasis a key mechanism relevant to cancer prevention and therapy [10].

Cassia senna L. (Caesalpinaceae) (known as “senna”) is an important medicinal herb. Senna is one of the most popular herbal laxatives [11]. The anthraquinone glycosides, particularly sennosides, are responsible for senna's medicinal properties. It is a varied, branched, tall shrub that can reach 1.8 m in height. Pinnate pubescent leaves, leaflets pale green to bluish green, 3 to 9 pairs, lanceolate or elliptic, varied on the same plant, 1.5 to 5 cm × 0.4 to 2 cm. Flowers brilliant yellow in erect, terminal racemes; pods light green when young to dark brown or black when old; flat, thin, oblong pubescent, 3.5 to 7 cm × 0.2 to 2.5 cm. Seeds of dark brown color appeared. This genus is distributed widely in North East Africa, Arabia, and India. Within India, it has been imported, naturalized in some areas, and grown [12]. We hypothesize that oxidative stress plays a critical role in the initiation and progression of prostate cancer, and that phytochemicals such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds with antioxidant properties such as those present in *Cassia senna* can mitigate carcinogenesis by scavenging ROS, restoring redox balance, and modulating key signaling pathways involved in cell proliferation, apoptosis, and inflammation.

This study explores the phytochemical antioxidant activities of *Cassia senna* and its potential therapeutic effects against prostate cancer in Wistar albino rats. Known for its rich composition of bioactive compounds such as flavonoids, tannins, and phenolics, *Cassia senna* exhibits strong antioxidative properties that help reduce oxidative stress a key factor in prostate cancer progression. By enhancing antioxidant enzyme activity and mitigating lipid peroxidation, *Cassia senna* may play a vital role in combating oxidative damage and inhibiting tumor growth. This investigation aims to uncover its therapeutic potential, providing valuable insights into its application for managing prostate cancer.

2 Materials and methods

2.1 Collection and identification of plant material

The leaves of *Cassia senna* L. were collected from Pollachi, Tamil Nadu, India. It was authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Agricultural University Campus, Coimbatore, Tamil Nadu, India. A voucher specimen has been deposited in a laboratory for future reference (BSI/SC/5/23/09–10/Tech-238).

2.2 Preparation of extracts

The leaves were spread out and dried at room temperature for 8–10 days. After drying, the leaves were blended into a fine powder with an electric blender. The powdered

(100 g) leaf material was stored in an airtight container at room temperature, protected from light and moisture, and used within four weeks to minimize potential degradation of phytochemicals, as this time frame is reported to maintain stability of major secondary metabolites under controlled conditions [13]. The powdered sample was subjected to sequential solvent extraction using the following solvents in order of increasing polarity: petroleum ether, chloroform, ethyl acetate, ethanol, and water. Each extraction was carried out separately, and the resulting extracts were concentrated and stored for further analysis.

2.3 Total yield of extracts

The total yield of several *C. senna* extracts was determined by measuring the actual yield, which is the amount of product produced throughout the experiment. Next, calculate the theoretical yield, which is the greatest amount of product that can be produced using stoichiometric calculations and the limiting reactant in the chemical reaction.

$$\text{Yield Percentage} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100$$

2.4 Phytochemical screening

The phytochemical screening of the *C. senna* extracts were carried out by the method followed by [14] with some modifications.

2.5 Quantification of bioactive compounds

2.5.1 Estimation of total phenol content

The total phenolic content of the extract was determined using the Folin–Ciocalteu method [15], with slight modifications, and gallic acid was used as the standard. A volume of 0.5 mL of the extract was mixed with 2.5 mL of 10% aqueous Folin–Ciocalteu reagent and 1.0 mL of methanol. The mixture was stirred and allowed to stand for 5 min. Subsequently, 2.0 mL of 5% aqueous sodium carbonate (Na_2CO_3) solution was added. The reaction mixture was then incubated at 40 °C for one hour, after which the absorbance was measured at 765 nm using a UV-Visible spectrophotometer.

2.5.2 Estimation of total flavonoid content

The total flavonoid content of the extract was estimated using a previously described method [15], with slight modifications. A 0.5 mL sample solution was mixed with 0.5 mL of 2% aluminum chloride (AlCl_3) in ethanol. The mixture was incubated at room temperature for one hour, during which a yellow coloration developed. The absorbance of the resulting solution was then measured at 420 nm using a UV-visible spectrophotometer. A standard calibration curve was prepared using quercetin, and the total flavonoid content was expressed as quercetin equivalents.

2.5.3 Estimation of tannin content

The tannin content of the extract was estimated using the method described by [16]. A series of standard solutions containing 0.2–1.0 mL of tannic acid was prepared in test tubes. Separately, 0.5 mL of the extract solution was added to another test tube. The volume in each tube was adjusted to 3 mL with distilled water, while 3 mL of distilled water alone served as the blank. To each test tube, 5 mL of 20% sodium carbonate (Na_2CO_3)

solution was added, followed by 2.5 mL of Folin–Dennis reagent. The mixtures were incubated at room temperature for 30 min. After incubation, the absorbance was measured at 700 nm using a UV-visible spectrophotometer, with reference to the reagent blank. The tannin concentration in the extract was determined using a standard calibration curve constructed with tannic acid and expressed as tannic acid equivalents.

2.5.4 Estimation of vitamin C

Vitamin C content was estimated using the method described by [17]. For the assay, 0.5 mL and 1.0 mL aliquots of the extract supernatant were taken and the volume was made up to 2 mL with 4% trichloroacetic acid (TCA). Separately, 0.2–1.0 mL of the working standard solution containing 20–100 µg of ascorbic acid was pipetted into clean, dry test tubes and similarly brought up to 2 mL with 4% TCA. To each tube, 2,4-dinitrophenylhydrazine (DNPH) reagent was added, followed by two drops of 10% thiourea solution. The tubes were incubated at 37 °C for 3 h to allow the formation of osazones. After incubation, the osazones were dissolved in 2.5 mL of 85% sulfuric acid, which was added dropwise in cold water to avoid a rise in temperature. A reagent blank was prepared using DNPH and thiourea, followed by sulfuric acid, without any sample or standard. After allowing the color to develop for 30 min at room temperature, the absorbance was measured at 540 nm using a UV-visible spectrophotometer. The ascorbic acid content was calculated using the standard curve and expressed as mg/g or per 100 g of sample.

2.5.5 Estimation of vitamin E

Vitamin E content was estimated following the method described by [18], using α -tocopherol as the standard. A total of 1.5 mL of plant extract, 1.5 mL of standard solution, and 1.5 mL of distilled water were added to three separate centrifuge tubes labeled as test, standard, and blank, respectively. To the test and blank tubes, 1.5 mL of ethanol was added, while 1.5 mL of distilled water was added to the standard tube. Subsequently, 1.5 mL of xylene was added to each tube. The tubes were stoppered, mixed thoroughly, and centrifuged. From the xylene layer of each tube, 1.0 mL was carefully transferred to new stoppered tubes, ensuring no contamination from the ethanol or protein layers. To each of these tubes, 1.0 mL of 2,2'-dipyridyl reagent was added and mixed well. Then, 1.5 mL of each mixture was transferred into spectrophotometer cuvettes, and the absorbance of the test and standard samples was measured against the blank at 450 nm. Following this, 0.33 mL of ferric chloride solution was added to each cuvette, starting with the blank. The mixtures were thoroughly mixed, and after exactly 1.5 min, a second absorbance reading was taken at 520 nm. The vitamin E (α -tocopherol) content was calculated using a standard curve and expressed as mg/g of sample.

$$\text{Vitamin E } (\mu\text{g}) = \frac{\Delta A_{520 \text{ nm}} - \Delta A_{450 \text{ nm}} (\times \text{conc of standard} \times 0.29) \text{ total volume}}{\Delta A_{520 \text{ nm}} \times \text{Volume for experiment} \times \text{weight of sample}}$$

2.5.6 Estimation of total carotenoids

Carotenoid content was estimated according to the method described by [19]. A 1–2 g portion of the sample was saponified by adding a small amount of 12% alcoholic potassium hydroxide, and the mixture was shaken for 30 min at 37 °C. The saponified mixture was then transferred and extracted with petroleum ether (boiling range 60–80 °C). To

remove non-carotenoid impurities, calcium carbonate was added to the mixture. After phase separation, the lower aqueous layer was discarded, and the upper petroleum ether layer was collected. This extraction process was repeated until the petroleum ether layer became colorless, indicating complete extraction of carotenoids. The pooled petroleum ether extracts were collected in a volumetric flask, filtered through glass wool, and treated with anhydrous sodium sulfate to eliminate any turbidity caused by moisture. The final volume of the petroleum ether extract was recorded, and the optical density of the sample was measured at 450 nm using a UV-visible spectrophotometer. Lycopene was used as the standard for quantification.

$$\text{Total carotenoids } (\mu\text{g}) = \frac{(\text{Optical density of the sample} \times 4 \times \text{Volume of the sample} \times 100)}{\text{Weight of the sample}}$$

2.6 In vitro radical scavenging assay

2.6.1 DPPH radical activity

DPPH radical scavenging assay by the method of [20]. Sample and standard concentrations (20, 40, 60, 80, and 100 $\mu\text{g/mL}$) were measured in separate test tubes. To make the volume 500 μL , methanol was added. These test tubes were vortexed with 5 mL of a 0.1 mM methanolic DPPH solution. The tubes were left to stand at room temperature for 20 min. The control was made as described above, but without extract and methanol was used to correct the baseline. Changes in the sample's absorbance were measured at 517 nm.

Butylated hydroxytoluene (BHT) was taken as a reference standard. The percentage inhibition against concentration curve was plotted and the concentration required for 50% radical inhibition was represented as the IC_{50} value.

2.6.2 Reducing power capacity

Reducing power was estimated by the method of [21]. The extracts were prepared at various concentrations (20, 40, 60, 80, and 100 $\mu\text{g/mL}$). 2.5 mL of sodium phosphate buffer was added to each extract in test tubes, followed by 2.5 mL of 1% potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] solution. The content was vortexed and incubated at 50°C for 20 min. After incubation, 2.5 mL of 10% TCA was added to each tube and centrifuged at 3000 g for 10 min. The supernatant was mixed with 5 mL of deionized water. To each test tube, add 1 mL of 1% ferric chloride and incubate for 10 min at 35°C. The extract's reducing power was related to its concentration. Increasing the absorbance of the reaction mixture indicated a reduction in power. Butylated hydroxy toluene (BHT) was used as a reference standard. The absorbance was measured at 700 nm.

2.6.3 ABTS radical scavenging activity

ABTS radical scavenging assay was determined by the method of [22] 7 mM of ABTS was dissolved in water, and 2.45 mM potassium persulfate was combined in a 1:1 ratio to produce ABTS cation radicals. This mixture was held at room temperature in the dark for 12–16 h before use. Following that, methanol was used to dilute the ABTS solution, yielding an absorbance of 0.700 at 734 nm. The standard and sample were divided into aliquots (20, 40, 60, 80, and 100 $\mu\text{g/mL}$) and diluted with methanol to 300 μL . The solutions were incubated for 30 min at room temperature, and the absorbance was measured

using a spectrophotometer. Trolox served as the standard. The ABTS scavenging % was estimated using the following formula:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.6.4 Hydroxyl radical scavenging assay activity

Hydroxyl radical scavenging assay was determined by the method of [23]. Various concentrations (20, 40, 60, 80, and 100 µg/mL) of extract were added to 1 mL of iron ethylene diamine tetra acetic acid (EDTA) solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). To activate the reaction, add 0.5 mL of ascorbic acid (0.22%) and incubate in a water bath at 80–90 °C for 15 min. After incubation, 1 mL of ice-cold TCA (17.5% w/v) was added to end the reaction. 3 mL of Nash reagent was applied and left at room temperature for 15 min. The reaction mixture without sample served as a control. The specificity of the achieved color was determined spectroscopically at 412 nm in comparison to a reagent blank. BHT was used as a standard. The proportion of hydroxyl radical scavenging activity can be calculated using the following formula:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.6.5 Superoxide radical scavenging activity

Superoxide radical scavenging assay was assayed by the method of [24]. Various concentrations (20, 40, 60, 80, and 100 µg/mL) of standard BHT solutions were taken in separate test tubes. Plant sample solutions of 20, 40, 60, 80, and 100 µg/mL were taken in another set of test tubes. Acetone was used to fill all of the tubes to 3 mL. As a control, 3 mL of alcohol was used. Each tube was filled with 1 mL of Nitro Blue Tetrazolium (NBT), NADH and Phenazine methyl sulphate (PMS). All tubes were incubated for 5 min at room temperature. The absorbance was measured at 560 nanometers. The absorbance of reaction mixture decreased, indicating greater superoxide anion scavenging activity. The following formula calculates the percentage of radical scavenging activity:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.6.6 Nitric oxide radical scavenging activity

Nitric oxide radical scavenging assay was determined by the method of [25]. The reaction mixture (3 mL) contains 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffered saline (PBS) and 0.5 mL of extract or standard solution (20, 40, 60, 80, and 100 µg/mL) and incubated at 25 °C for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted out and mixed with 1 mL of sulfanilic acid reagent and allowed to stand for 5 min for completing diazotization. Then 1 mL of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25 °C. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. BHT was used as a standard. Percentage inhibition was calculated as follows.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.7 Experimental groups and dosage

2.7.1 Selection of animals and diet

Wistar Strain male albino rats weighing between 160 and 180 g were obtained from Karpagam Arts and Science College (Autonomous), Animal House, Coimbatore, India. The study has been approved by the Institutional Animal Ethical Committee, constituted for the purpose of CPCSEA, Government of India. The rats were housed in large, spacious cages and allowed to consume an appropriate diet for 16 weeks, with water available ad libitum. Rats were fed a conventional rat pellet diet (Karnataka Agro Food Corporation Limited, Bangalore, India).

2.7.2 Preparation of the leaves extract for animal study

The freshly picked leaves were washed with distilled water and dried in the shade. CS leaves were employed in the form of a crude 99% ethanolic extract, which was made using the conventional method of medicine. 500 g of the shade-dried and powdered leaves were extracted with 2500 mL of 99% ethanol in the cold for 72 h. The extract was filtered, yielding a crude residue. The residue was dissolved in water and provided orally to the experimental rats.

2.7.3 Experimental design

For the in vivo study, a total of 30 male rats were randomly divided into five groups, with six rats per group. Serum and tissue samples were collected post-treatment to assess various biochemical parameters. The experimental protocol was as follows:

- Group 1: Normal Control (Healthy controls).
- Group 2: Prostate Cancer Induced (Untreated).
- Group 3: Prostate Cancer + 150 mg/kg CS.
- Group 4: *Cassia senna* Extract Control (CS alone).
- Group 5: Prostate Cancer + Standard Drug (25 mg/kg bw finasteride).

The animals in groups 2, 3, and 5 were induced with prostate cancer by administering 100 mg/kg body weight (bw) testosterone in 0.3 mL of propylene glycol via intraperitoneal injection daily for three days. One day after the final testosterone dose, rats received 50 mg/kg bw of MNU intravenously via the tail vein (dissolved in saline at 10 mg/mL) for one week. Beginning one week after MNU administration, rats received 2 mg/kg bw of testosterone intraperitoneally each day for 60 days to promote tumor development.

Treatment lasted for 16 weeks, after which blood and organ samples were collected for biochemical and histopathological examination. The prostate and seminal vesicles were carefully

collected after euthanasia, with the prostate excised for tumor evaluation and histopathological analysis. The seminal vesicles were isolated to evaluate any treatment-induced changes. These tissues were fixed in formalin for further histological examination.

Table 1 The total yield of various extracts of *Cassia Senna*

Solvents	Cassia senna (mg/100 g)
Petroleum ether	75
Chloroform	127
Ethyl acetate	225
Ethanol	500
Water	190

Table 2 Phytochemical screening of *Cassia senna* extracts

Secondary metabolites	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
Alkaloids	–	–	–	+	–
Steroids	+	+	–	+	+
Flavonoids	+	+	+	+	+
Terpenoids	–	–	–	+	–
Amino acids and proteins	+	+	+	+	+
Carbohydrates	–	+	+	+	+
Cardioglycosides	+	+	+	–	+
Saponins	+	+	–	+	+
Oils & fats	–	–	–	–	–
Tannins	+	+	+	+	+

‘+’ Present ‘–’ Absent

2.8 Statistical analysis

All results were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to evaluate differences between the experimental groups. A p-value less than 0.05 was considered statistically significant. Significant results were indicated with an asterisk (*), while non-significant results were denoted as “ns”. All statistical analyses were conducted using GraphPad Prism version 10.0.

3 Results

3.1 Total yield

The total yield of various extracts was given in Table 1. The ethanolic extract of CS the showed the maximum yield of approximately 500 mg. Petroleum ether extract produced showed the lowest yield of 75 mg.

3.2 Phytochemical analysis

The phytochemical analysis obtained qualitatively by all the extracts of *Cassia senna* is presents in the following table.

The preliminary phytochemical analysis of different extracts was given in Table 2. The ethanolic extract of CS revealed the presence of the major chemical constituents of the extract were flavonoids, alkaloids, tannins, steroids, saponins, oils and fats, carbohydrates, amino acids and proteins. The ethanolic extract exhibited a higher number of secondary metabolites compared to extracts obtained using other solvents.

3.3 Quantification of bioactive compounds

The quantification of bioactive compounds was given in Table 3. The CS had a significant phytochemical composition including polyphenols (75.29 ± 0.22 mg/g GAE), flavonoids (35.25 ± 1.15 mg/g quercetin equivalent), and a high tannin content (180.62 ± 1.29 mg/g

Table 3 Total polyphenols, total flavonoids, total tannins, and vitamin C, vitamin E and total carotenoids in the ethanolic extract of *Cassia senna* (CS)

Bioactive compounds	CS
Total polyphenols (mg/g of gallic acid equivalent)	75.29 ± 0.22
Total flavonoids (mg/g of quercetin equivalent)	35.25 ± 1.15
Tannins (mg/g of tannic acid equivalent)	180.62 ± 1.29
Vitamin C (mg/g of ascorbic acid equivalent)	19.40 ± 0.30
Vitamin E (mg/g of α -tocopherol equivalent)	3.50 ± 0.25

Values are taken as a Mean $\pm$ of three individual experiments

Table 4 IC₅₀ values of CS and standards for antioxidant assays

Parameters	Standard/Sample	IC ₅₀ Concentration (μ g/mL)
DPPH Radical Activity	BHT	42.25 ± 2.38
	CS	63.55 ± 3.45
Reducing Power Capacity	BHT	40.25 ± 0.96
	CS	51.82 ± 2.57
ABTS Radical Scavenging Activity	Trolox	48.24 ± 1.96
	CS	63.32 ± 3.55
Hydroxyl radical scavenging activity	BHT	40.08 ± 2.28
	CS	55.30 ± 2.45
Superoxide Radical Scavenging Activity	BHT	47.43 ± 1.75
	CS	58.64 ± 1.48
Nitric Oxide Radical Scavenging Activity	BHT	48.14 ± 1.78
	CS	69.42 ± 2.56

Values are taken as a Mean $\pm$ of three individual experiments

tannic acid equivalent). The extract included vitamin C (19.40 ± 0.30 mg/g ascorbic acid equivalent), vitamin E (3.50 ± 0.25 mg/g α -tocopherol equivalent), and total carotenoids (4.21 ± 0.20 mg/g lycopene equivalent). These findings show that CS contains a high concentration of polyphenols and tannins, as well as moderate quantities of flavonoids, vitamin C, and carotenoids and a low concentration of vitamin E indicating that it has the potential to be a natural antioxidant.

3.4 Antioxidant activity

The IC₅₀ values of CS were shown in Table 4. The antioxidant activity of CS was measured using several assays and compared to standard antioxidants. CS had a greater IC₅₀ value for DPPH radical activity (63.55 ± 3.45 μ g/mL) compared to BHT (42.25 ± 2.38 μ g/mL). Similarly, its reducing power capacity was slightly lower (51.82 ± 2.57 μ g/mL) than that of BHT (40.25 ± 0.96 μ g/mL). CS has a higher IC₅₀ value for ABTS radical scavenging (63.32 ± 3.55 μ g/mL) compared to Trolox (48.24 ± 1.96 μ g/mL). CS exhibited IC₅₀ value (55.30 ± 2.45 μ g/mL) than BHT (40.08 ± 2.28 μ g/mL) for hydroxyl radical activity. Superoxide radical showed (47.43 ± 1.75 μ g/mL) and (58.64 ± 1.48 μ g/mL) for BHT and CS. Finally, the nitric oxide scavenging activity showed (69.42 ± 2.56 μ g/mL) for CS and (48.14 ± 1.78 μ g/mL) for BHT. These findings suggest that CS has significant antioxidant activity, but slightly lower than standard values in various assays.

3.5 Changes in body and organ weight

The body weight of rats in all experimental groups was monitored throughout the study. Group I (Normal control) rats exhibited a steady and consistent increase in body weight, reflecting normal physiological growth without any adverse effects. In contrast, Group II (Untreated– Prostate cancer-induced) showed a significant decline in body weight over time (Fig. 1), indicative of systemic toxicity and cachexia associated with MNU and testosterone-induced prostate cancer. Group III (Prostate cancer + CS) demonstrated a notable improvement in body weight compared to Group II. The gradual increase in weight suggests that treatment with *Cassia senna* (CS) extract helped mitigate cancer-induced weight loss, pointing toward its potential therapeutic and protective effect. Group IV (CS alone) maintained body weight levels comparable to the control group, confirming that CS extract alone did not adversely affect body mass or general health. Group V (Prostate cancer + Finasteride) also showed moderate weight recovery; however, the extent of weight gain was slightly lower than in the CS-treated group, indicating that while Finasteride exhibited therapeutic benefit, CS may offer comparable or slightly superior restorative effects in terms of body weight maintenance. These observations reinforce the potential of *Cassia senna* as a supportive treatment in mitigating prostate cancer-associated systemic effects. While alterations in body weight offer supportive evidence of systemic effects linked to cancer, they are not conclusive indicators of prostate cancer development. Hence, to validate the induction of prostate carcinogenesis, we further conducted histopathological analysis of prostate tissues.

3.6 Hematological parameters

Figure 2 revealed that the testosterone and MNU-induced group (Group II– Untreated) had significantly altered blood parameters compared to the Normal control (Group I). Specifically, cancer-induced rats exhibited decreased RBC and platelet counts, reduced hemoglobin levels, and elevated WBC counts, indicating hematological disruptions due to prostate cancer induction. Treatment with *Cassia senna* extract (Group III– Prostate cancer + CS) improved these parameters, as evidenced by increased RBC and hemoglobin levels and reduced WBC counts. Similar improvements were observed in the Finasteride-treated group (Group V). Rats treated with CS alone (Group IV) showed hematological values comparable to the control group, confirming the non-toxic nature of the extract.

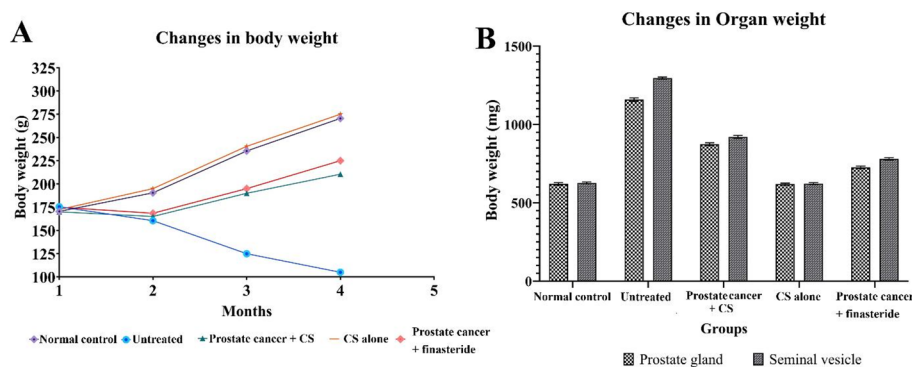


Fig. 1 **A** Alterations in the body weight over time (months) across five groups **B** Changes in the organ weight of prostate gland and seminal vesicles of various groups

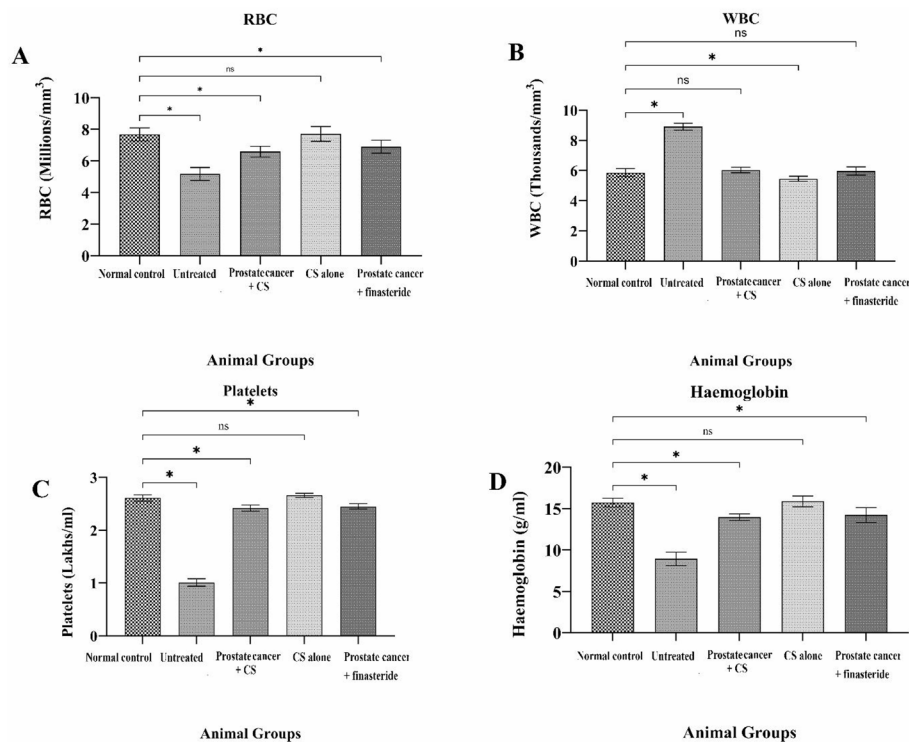


Fig. 2 Hematological parameters across experimental groups. **A** Red blood cell (RBC) count (millions/mm³) **B** White blood cell (WBC) count (thousands/mm³) **C** Platelet count (lakhs/mL) **D** Hemoglobin concentration (g/mL) across groups

3.7 Liver and renal parameters

Figure 3 illustrates the changes in serum marker enzyme levels among control and experimental groups. Rats in Group II (Untreated– Prostate cancer-induced) exhibited a significant increase ($p < 0.05$) in serum levels of AST, ALT, ALP, and LDH compared to Group I (Normal control), indicating hepatic and cellular damage associated with cancer induction using testosterone and MNU. Elevated LDH levels reflect increased tumor activity and metabolic turnover. However, treatment with Cassia senna extract in Group III (Prostate cancer + CS) for 16 weeks significantly normalized these enzyme levels, suggesting a protective and therapeutic effect. Similarly, Group V (Prostate cancer + Finasteride) also showed substantial restoration of enzyme markers toward normal values. Notably, Group IV (CS alone) maintained enzyme levels comparable to the normal control, confirming the safety of CS extract. Additionally, renal function was assessed through measurements of urea, uric acid, and creatinine. In Group II, urea and creatinine levels increased significantly ($p < 0.05$), while uric acid levels decreased, indicating renal stress and altered purine metabolism. Treatment with CS (Group III) and Finasteride (Group V) improved these parameters, demonstrating the nephroprotective potential of the interventions.

Measurement of Antioxidant enzymes, Nucleic acids and protein levels in Prostate Gland and Seminal Vesicles

Figures 4 and 5 illustrate the levels of antioxidant enzymes, nucleic acids, and lipid peroxidation in the prostates and seminal vesicles of control and experimental rats. In Group II (Untreated– Prostate cancer-induced), induction with testosterone and MNU resulted in a significant reduction ($p < 0.05$) in enzymatic antioxidants including

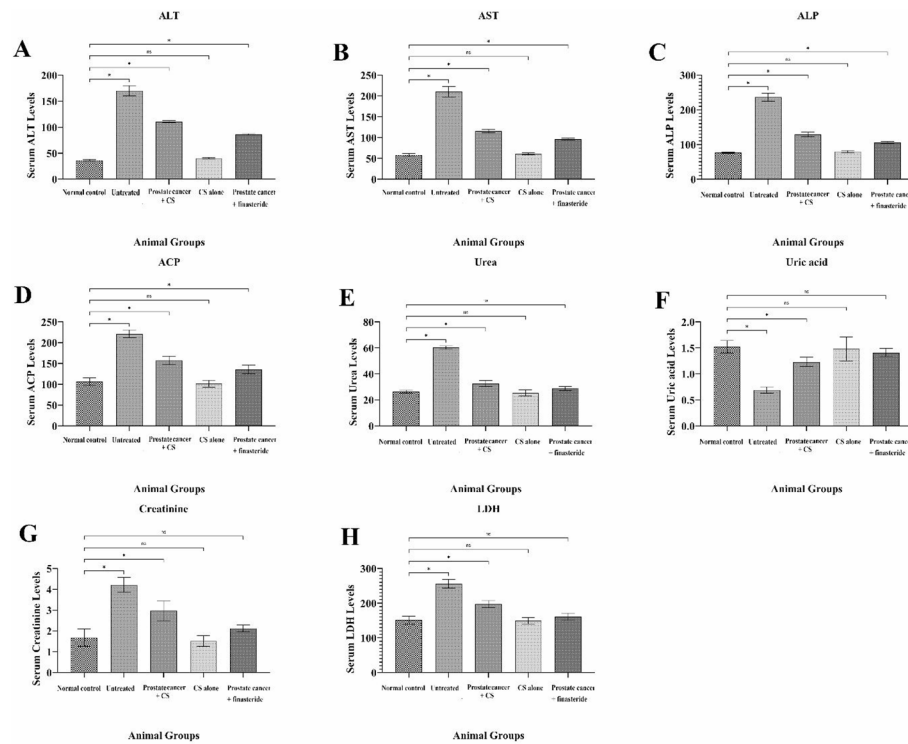


Fig. 3 Biochemical analysis of serum parameters across experimental groups **A** ALT **B** AST **C** ALP **D** ACP **E** Urea **F** Uric acid **G** Creatinine **H** LDH

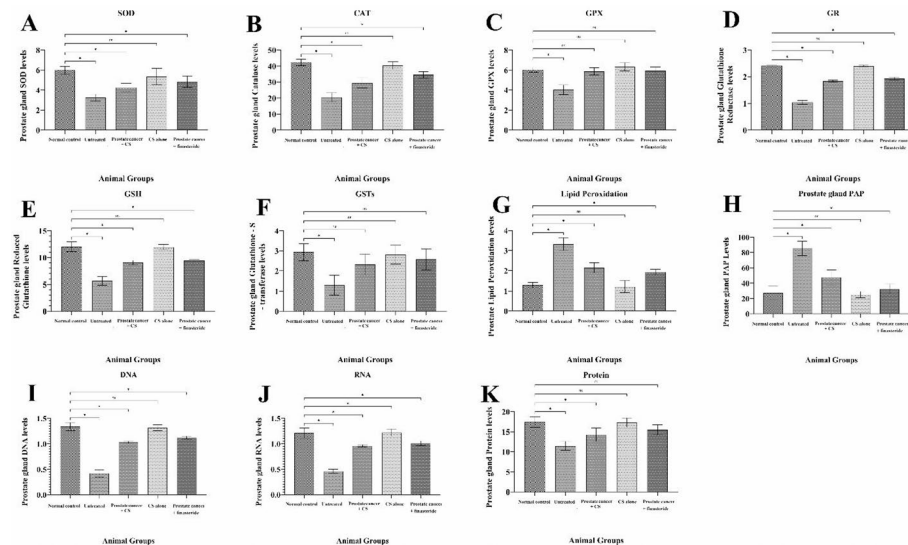


Fig. 4 Assessment of antioxidant enzymes, oxidative stress markers and nucleic acids in prostate tissue across experimental groups. **A** SOD **B** CAT **C** GPx **D** GR **E** GSH **F** GST **G** Lipid peroxidation **H** PAP levels **I** DNA **J** RNA **K** Protein content

superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR), compared to Group I (Normal control). These reductions indicate oxidative stress and impaired cellular defense mechanisms. This reduction reflects elevated oxidative stress in prostate tissues, a hallmark of prostate cancer, and is consistent with the Warburg effect, where cancer cells

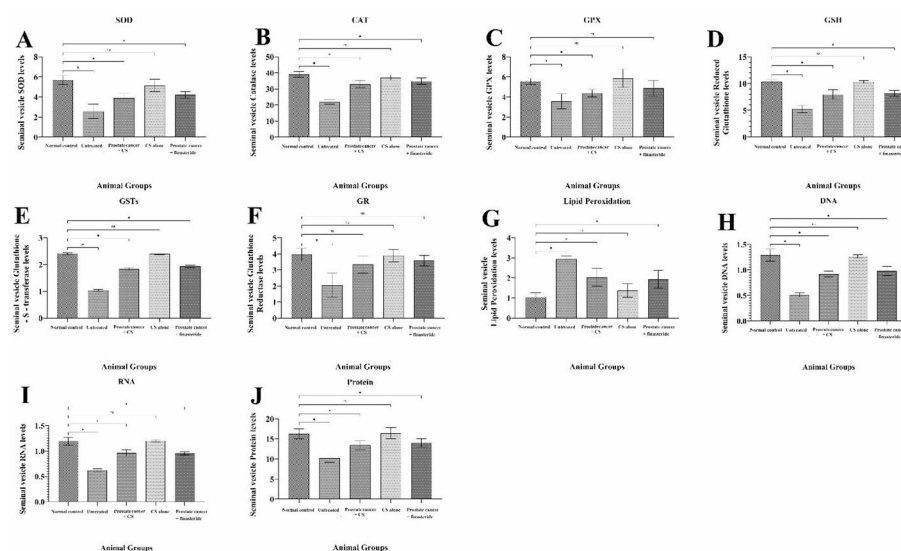


Fig. 5 Assessment of antioxidant enzymes, oxidative stress markers and nucleic acids in seminal vesicles across experimental groups. **A** SOD **B** CAT **C** GPX **D** GSH **E** GSTs **F** GR **G** Lipid peroxidation **H** DNA **I** RNA **J** Protein content

produce high levels of ROS due to a shift toward glycolytic metabolism and mitochondrial dysfunction.

Treatment with *Cassia senna* extract (Group III– Prostate cancer + CS) and finasteride (Group V– Prostate cancer + Finasteride) significantly elevated the levels of these antioxidant enzymes, restoring them toward normal values ($p < 0.05$). Group IV (CS alone) showed antioxidant levels comparable to the control group, suggesting that CS has no adverse effect on oxidative status in healthy tissues.

Furthermore, DNA and RNA content were markedly reduced in the prostate and seminal vesicle tissues of Group II rats, indicating nucleic acid damage associated with cancer induction. Both CS (Group III) and Finasteride (Group V) treatments significantly restored nucleic acid levels, while CS alone (Group IV) maintained values similar to the control group.

Lipid peroxidation (LPO) levels were significantly elevated in Group II, confirming enhanced oxidative damage. Oral administration of CS and Finasteride effectively reduced LPO levels in the prostate and seminal vesicles ($p < 0.05$), bringing them close to those observed in normal controls. Again, no significant differences were noted between Group IV (CS alone) and Group I (Normal control), confirming the antioxidant safety profile of *Cassia senna* extract.

3.8 Serum biochemical parameters and lipid profile

Figure 6 presents the serum biochemical and lipid profile parameters of all experimental rat groups. In Group II (Untreated– Prostate cancer-induced), a significant increase ($p < 0.05$) was observed in total cholesterol, triglycerides, low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), alongside a marked reduction in high-density lipoprotein (HDL), when compared to Group I (Normal control). Oral administration of *Cassia senna* (Group III– Prostate cancer + CS) for 16 weeks effectively restored these values toward normal levels ($p < 0.05$), indicating hypolipidemic activity. A similar pattern was seen in Group V (Prostate cancer + Finasteride). Notably, Group IV (CS alone)

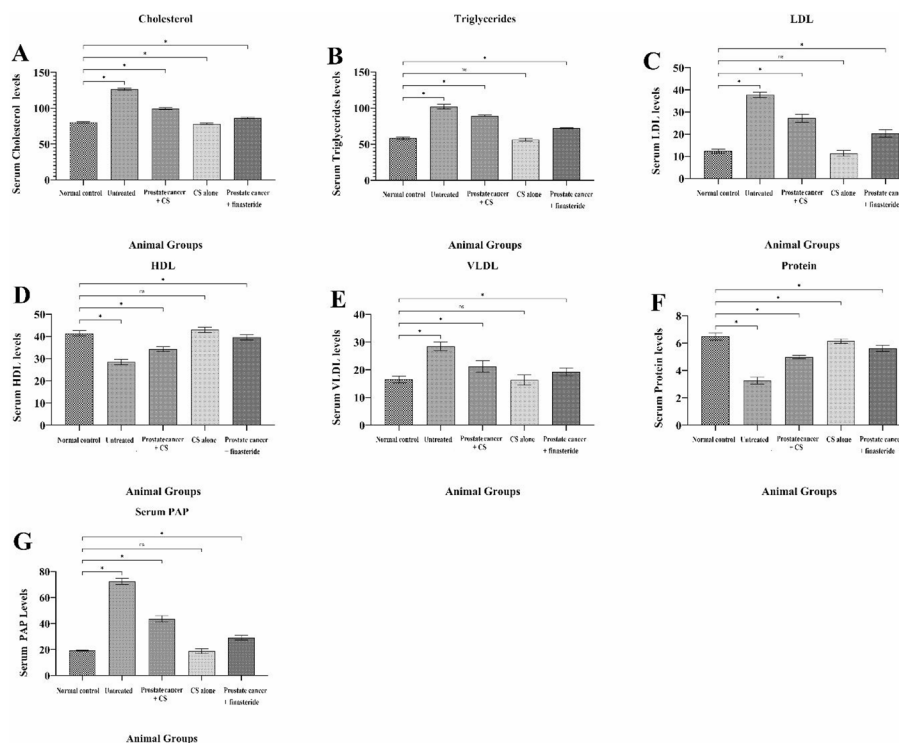


Fig. 6 Comparative analysis of serum biochemical parameters in different experimental rat groups. **A** Cholesterol **B** Triglycerides **C** Low-Density Lipoprotein (LDL) **D** High-Density Lipoprotein (HDL) **E** Very Low-Density Lipoprotein (VLDL) **F** Total Protein and **G** Serum Prostatic Acid Phosphatase (PAP)

exhibited no significant deviation from the control, further supporting the safety of the extract.

Serum total protein levels were significantly reduced in Group II, reflecting impaired metabolic function due to cancer induction. Treatment with CS (Group III) partially restored protein levels, although they remained slightly below normal ($p < 0.05$). Protein levels in Group IV (CS alone) were comparable to the control, indicating a protective effect of CS. In contrast, Group V (Prostate cancer + Finasteride) also showed lower protein levels than controls, suggesting limited efficacy of finasteride in restoring protein balance.

Additionally, serum prostatic acid phosphatase (PAP), a prostate-specific cancer marker, was significantly elevated ($p < 0.05$) in Group II, confirming disease progression. CS treatment (Group III) substantially reduced PAP levels ($p < 0.05$), indicating therapeutic benefit. Group IV had PAP levels similar to the control group, supporting its preventive role. However, Group V (Finasteride-treated) showed no significant improvement in PAP levels compared to the untreated cancer group, suggesting limited effectiveness in reducing disease-specific markers. PAP, in particular, is considered a more specific and sensitive biomarker of prostate malignancy. The marked rise in PAP indicates that the model likely progressed toward a prostate cancer within the study period.

3.9 Histopathological examination of prostate gland and seminal vesicle

Histopathological analysis of the prostate gland and seminal vesicle in control and experimental rats is presented in Figs. 7 and 8. Tissue sections were stained and examined at $\times 100$ magnification.

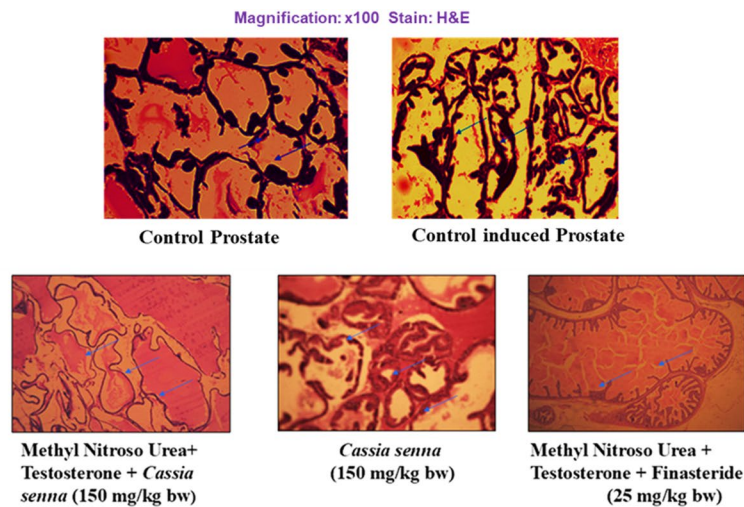


Fig. 7 Histopathological study of Prostate gland in control and experimental rats

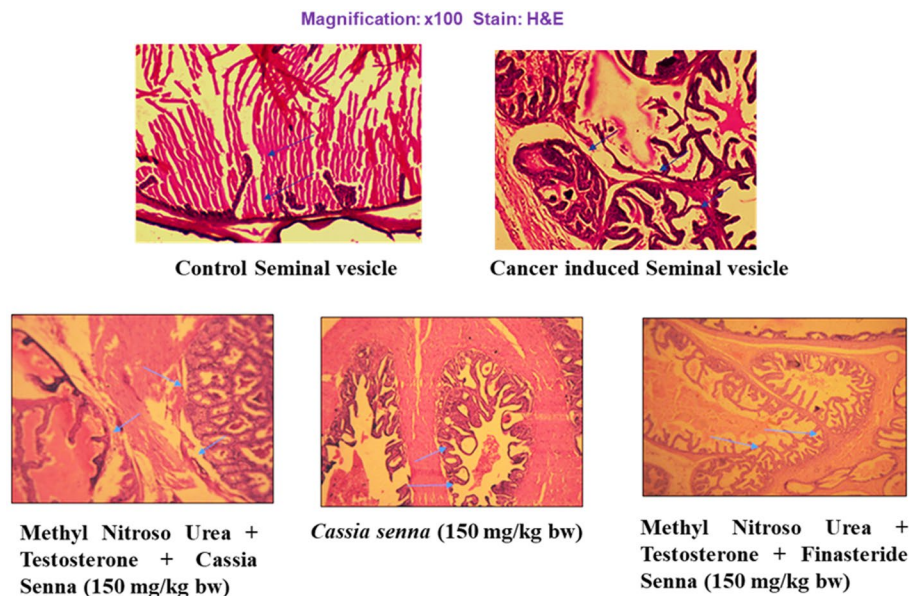


Fig. 8 Histopathological study of Seminal vesicle in control and experimental rats

In Group I (Normal control), the prostate exhibited acini lined by a single layer of columnar epithelium, while the seminal vesicles showed a ductal lumen with typical mucosal architecture forming small papillary folds, consistent with normal histology reported in healthy rodents [26].

In contrast, Group II (Untreated– Prostate cancer-induced) rats displayed marked histological alterations, including hyperplastic prostatic acini and glandular structures lined with proliferating epithelium forming papillary folds and budding into the luminal space, indicative of neoplastic transformation. The seminal vesicles in this group showed malignant proliferation of ductal epithelial cells arranged in closely packed formations, as described in prostate cancer progression models [27].

Group III (Prostate cancer + *Cassia senna*) rats showed significant histological recovery, with relatively normal and dilated acini and glands lined with columnar epithelium,

presence of minor papillary folds, and acini lobules separated by fibrous septa. The seminal vesicle showed inner duct projections with mild cystic dilatation, indicating reduced pathological changes, suggesting the therapeutic potential of *Cassia senna* in ameliorating prostate cancer-induced histological damage [28].

In Group IV (CS alone), the prostate and seminal vesicles retained normal histoarchitecture, suggesting no adverse effect of *Cassia senna* treatment on healthy tissue.

Group V (Prostate cancer + Finasteride) rats exhibited nearly normal histological features of the prostate and seminal vesicles, including dilated ducts with flattened epithelium and retained secretions, along with fibrous septa separating lobules, consistent with the well-established therapeutic action of finasteride in prostate cancer [29].

4 Discussion

The ethanolic extract of *Cassia senna* revealed the presence of key bioactive compounds such as flavonoids, tannins, alkaloids and saponins. Flavonoids and Tannins are known for their antioxidant and anti-inflammatory properties, which help mitigate oxidative stress and reduce inflammation. Omer et al. (2022) reported high flavonoid and phenolic content in *Senna alexandrina*, which supports our findings of *Cassia senna*'s antioxidant potential. Previous studies have shown that *Cassia senna* contains important bioactive compounds like phenolics and flavonoids, which contribute to its antioxidant activity. Flavonoids, alkaloids, and saponins in *Cassia senna* have been shown to exhibit diverse biological activities, such as immune modulation and antimicrobial effects. These compounds likely contribute to the observed therapeutic effects through a multi-targeted approach [30]. The results from our study are similar to these earlier findings, showing good levels of these compounds, especially in the ethanol and aqueous extracts. Some differences were seen, which could be due to changes in plant source, environment, or how the extracts were prepared. The antioxidant activity we observed also supports earlier reports, suggesting that *Cassia senna* may help protect against damage caused by oxidative stress [31, 34]. The ethanolic extract of *Cassia senna* demonstrated antioxidant activity through DPPH, ABTS, and hydroxyl radical scavenging assays. The DPPH Scavenging Activity showed moderate antioxidant potential whereas ABTS Assay demonstrated effective radical scavenging activity attributed to high flavonoid and polyphenol content. Ahmed et al. (2016) evaluated *Cassia angustifolia* and observed strong antioxidant activity, with IC_{50} values as low as 2.41 $\mu\text{g/mL}$ in DPPH assays [11]. Al-Naqqash et al. (2023) reported an IC_{50} of 26.7 $\mu\text{g/mL}$ for DPPH scavenging activity in *Cassia acutifolia*, which aligns closely with the antioxidant potential of *Cassia senna* [32]. The body weight of rats in all experimental groups was recorded throughout the study. Group I (Normal control) rats exhibited a steady increase in body weight, indicating normal growth. Group II (Untreated– Prostate cancer-induced) showed a significant reduction in body weight, reflecting systemic toxicity. Group III (Prostate cancer + CS) showed improved body weight compared to Group II, suggesting the protective effect of *Cassia senna*. Group IV (CS alone) maintained normal weight, while Group V (Prostate cancer + Finasteride) also showed moderate weight gain, though less definite than Group III. These findings suggest a potential restorative effect of *Cassia senna* in cancer-induced weight loss. In terms of haematological parameters, the cancer control group showed a lower RBC count and hemoglobin, elevated WBCs, and reduced platelet levels, indicating haematological disruption due to carcinogen exposure. Treatment with *Cassia*

senna significantly improved these parameters, which supports the plant's role in restoring hematological balance. Serum enzyme markers (AST, ALT, ALP, and LDH) were significantly elevated in MNU and testosterone-induced rats, indicative of tissue damage. Oral administration of *Cassia senna* for 16 weeks reversed these levels to near-normal. A similar tendency was observed in the finasteride-treated group. These results align with those of Fan et al. [33], who reported normalization of liver enzymes with botanical extracts such as *Hibiscus sabdariffa* in experimental cancer models.

Zhang et al. [29] demonstrated that plant-based antioxidants effectively mitigate oxidative stress by enhancing the activity of these enzymes, thereby contributing to reduced tumor progression.

Lipid peroxidation measured by malondialdehyde levels, was evidently increased in cancer induced control rats. Elevated malondialdehyde disrupts cell membranes and is associated with cancer development. Treatment with *Cassia senna* significantly reduced LPO levels, indicating restored membrane stability and reduced oxidative stress. Furthermore, nucleic acid content (DNA and RNA) was significantly reduced in prostate cancer-induced rats, while *Cassia senna* treatment significantly restored these levels. This indicates a protective effect on cellular genetic material, further supporting its anticancer potential. In lipid profile analysis, the cancer induced group showed significantly increased total cholesterol, triglycerides, LDL, and VLDL levels and decreased HDL. Treatment with *Cassia senna* normalized these parameters to near control levels. This finding supports the extract's lipid-regulating effect, comparable to finasteride. Moreover, the *Cassia senna*-treated group alone maintained lipid profiles similar to the control, indicating no adverse metabolic effect. Serum proteins were also evaluated. The cancer induced group exhibited a significant reduction in serum protein levels, while *Cassia senna* treatment partially restored them. PAP (Prostatic Acid Phosphatase) levels, a specific marker for prostate pathology, were markedly elevated in cancer-induced rats and significantly reduced following treatment with *Cassia senna*. These results highlight its therapeutic effect on prostate-specific cancer markers. Histological analysis revealed that cancer induced rats showed prostatic hyperplasia and dysplasia, and malignant features in the seminal vesicle. In contrast, *Cassia senna*-treated rats showed recovery of tissue architecture with normalized epithelial linings and reduced abnormal growth. These histological improvements reinforce the curative role of the extract. When compared with finasteride, a known anti-androgenic drug, *Cassia senna* showed comparable efficacy in most evaluated parameters, including biochemical markers, antioxidant enzymes, lipid profile, and histology. This supports the impression that *Cassia senna* may serve as a complementary or alternative therapy with potentially fewer side effects. In conclusion, this study validates the role of *Cassia senna* as a potent anticancer agent. Its antioxidant, biochemical, and histopathological effects align with findings from recent literature. Its comparable efficacy to finasteride highlights its therapeutic potential and warrants further studies to explore its molecular mechanisms and potential for clinical use.

5 Conclusion

This study highlights the therapeutic potential of *Cassia senna* extract as an anticancer agent, demonstrating significant antioxidant, enzymatic, and histopathological restorative effects in a testosterone- and MNU induced prostate carcinogenesis model. The

ethanolic extract, rich in flavonoids, tannins, and other bioactive compounds, effectively normalized oxidative stress markers, lipid profiles, and enzymatic antioxidant activities while reducing lipid peroxidation and restoring tissue architecture. These results not only align with but also expand upon existing literature by showcasing the extract's comparable efficacy to standard drugs like finasteride, underscoring its promise as a complementary or alternative therapeutic candidate for prostate cancer. Further studies are warranted to elucidate its molecular mechanisms and potential clinical applications.

Acknowledgements

The Authors acknowledge the support and infrastructure offered by the authorities of JSS Academy of Higher Education and Research (JSSAHER), Mysuru, India. The authors thanks to the authorities of Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India for carryout the animal studies.

Author contributions

GD: Conceptualization; Methodology; Investigation; Data curation; Writing—original draft. MGK: Resources; Statistical Analysis. SS: Resources; Statistical Analysis. MVR: Resources; Drafting the article. MKC—Resources; Drafting the article. RMA: Resources; review & editing. NP: Review & editing. CPP: Validation; review & editing. GVK: Conceptualization; Methodology; Supervision; Project Administration; Validation; Writing—review & editing.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Ethics approval and consent to participate

This research work was approved by the Institutional Animal Ethical Committee, (IAEC) with IAEC reference No. 739 / 03 / abc / CPCSEA under the rules set forth by the Government of India's CPCSEA commission. No participants involved in this study.

Consent for publication

No any research involved using personal data and case reports.

Competing interests

The authors declare no competing interests.

Received: 8 February 2025 / Accepted: 23 June 2025

Published online: 14 October 2025

References

1. Kim JH, Dareowolabi BO, Thiruvengadam R, Moon E-Y. Application of nanotechnology and phytochemicals in anticancer therapy. *Pharmaceutics*. 2024;16(9):1169. <https://doi.org/10.3390/PHARMACEUTICS16091169>.
2. Rawla P. Epidemiology of prostate Cancer. *World J Oncol*. 2019;10(2):63–89. <https://doi.org/10.14740/WJON11191>.
3. Wang L, Lu B, He M, Wang Y, Wang Z, Du L. Prostate Cancer incidence and mortality: global status and Temporal trends in 89 countries from 2000 to 2019. *Front Public Health*. 2022;10:811044. <https://doi.org/10.3389/FPUH.2022.811044>.
4. Siegel DA, O'Neil ME, Richards TB, Dowling NF, Weir HK. Prostate cancer incidence and survival, by stage and Race/Ethnicity—United states, 2001–2017. *MMWR Morb Mortal Wkly Rep*. 2020;69(41):1473–80. <https://doi.org/10.15585/MMWR.MM6941A1>.
5. Culp MB, Soerjomataram I, Efstathiou JA, Bray F, Jemal A. Recent global patterns in prostate cancer incidence and mortality rates. *Eur Urol*. 2020;77(1):38–52. <https://doi.org/10.1016/J.EURURO.2019.08.005>.
6. Sankiritha H, Thirumani L, Alex A, Neha B, Vimal S, Madar IH. Systematic evaluation of *Aegle marmelos*-Derived compounds: potential therapeutic agents against inflammation and oxidative stress. *Cureus*. 2024. <https://doi.org/10.7759/CUREUS.57499>.
7. Ammar HO, Shamma RN, Elbatanony RSE, Khater B. Antioxidants in cancer therapy: recent trends in application of nanotechnology for enhanced delivery. *Sci Pharm*. 2020;88(1):5. <https://doi.org/10.3390/SCIPHARM88010005>.
8. Veskokouk AS, Tsatsakis AM, Kouretas D. Dietary oxidative stress and antioxidant defense with an emphasis on plant extract administration. *Cell Stress Chaperones*. 2012;17(1):11–21. <https://doi.org/10.1007/S12192-011-0293-3>.
9. Sivapragasam G, Devi G, Ragavan T, Suryadevara ND, Sridewi N, Obaid NA, Alharbi S, S. A., Arulselvan PA. Comparative phytochemical characterization of *Moringa oleifera* plant parts by different solvent extraction. *Indian J Pharm Educ Res*. 2024;58(2):s648–59. <https://doi.org/10.5530/IJPER.58.2S.69>.
10. Rudzińska A, Juchaniuk P, Oberda J, Wiśniewska J, Wojdan W, Szklener K, Mańdziuk S. Phytochemicals in cancer treatment and cancer prevention—review on epidemiological data and clinical trials. *Nutrients*. 2023;15(8):1896. <https://doi.org/10.3390/NU15081896>.

11. Ahmed SI, Hayat MQ, Tahir M, Mansoor Q, Ismail M, Keck K, Bates RB. Pharmacologically active flavonoids from the anti-cancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. *BMC Complement Altern Med*. 2016;16(1):1–9. <https://doi.org/10.1186/S12906-016-1443-Z>.
12. Ganesh S, Arivarasu L, Rajeshkumar S, Thangavelu L. Anti-Inflammatory activity of *Cassia auriculata* flower extract. *J Pharm Res Int*. 2021;396–403. <https://doi.org/10.9734/JPRI/2021/V33I62A35614>.
13. Kapcum C, Uriyapongson J. Effects of storage conditions on phytochemical and stability of purple corn cob extract powder. *Food Sci Technol*. 2018;38(1):301–5. <https://doi.org/10.1590/1678-457x.23217>.
14. Dhanapal DP, Kaniyur Chandrasekaran M, Vani Raju M, Muthaiyan Ahalliya R, Sundarraj R, Dugganaboyana G, Kanniappan V. Hepatoprotective effects of ethanolic extract of *Corallocarpus Epigaeus* roots on nitrobenzene-induced liver injury in Wistar rats. *J Herbs Spices Med Plants*. 2024;1–17. <https://doi.org/10.1080/10496475.2024.2423279>.
15. Wairata J, Fadlan A, Setyo Purnomo A, Taher M, Ersam T. Total phenolic and flavonoid contents, antioxidant, antidiabetic and antiplasmodial activities of *Garcinia Forbesii* king: A correlation study. *Arab J Chem*. 2022;15(2):103541. <https://doi.org/10.1016/J.ARABJC.2021.103541>.
16. Baek MW, Choi HR, Hwang IG, Tilahun S, Jeong CS. Prediction of tannin content and quality parameters in astringent persimmons from visible and near-infrared spectroscopy. *Front Plant Sci*. 2023;14:1260644. <https://doi.org/10.3389/FPLS.2023.1260644>.
17. Iqbal MA, Yaqub M. Determination of vitamin C from human urine and blood. *J Pak Med Assoc*. 1980;30(3):69–72.
18. Jargar JG, Hattiwale SH, Das S, Dhundasi SA, Das KK. A modified simple method for determination of serum α -tocopherol (Vitamin E). *J Basic Clin Physiol Pharmacol*. 2012;23(1):45–8. <https://doi.org/10.1515/JBCPP-2011-0033>.
19. Zhou X, Huang W, Kong W, Ye H, Dong Y, Casa R. Assessment of leaf carotenoids content with a new carotenoid index: development and validation on experimental and model data. *Int J Appl Earth Obs Geoinf*. 2017;57:24–35. <https://doi.org/10.1016/J.JAG.2016.12.005>.
20. Raju MV, Sekar S, Chandrasekaran MK, Rajendran MS, Ahalliya RM. Evaluation of the antioxidant activity of novel South Indian plant *Commelina forskolii* and its prospective anticancer activity in hep G2 cells. *Indian J Nat Prod Resour*. 2024;15(1):65–72. <https://doi.org/10.56042/IJNPR.V15I1.4468>.
21. Vijayalakshmi M, Ruckmani K. Ferric reducing anti-oxidant power assay in plant extract. *Bangladesh J Pharmacol*. 2016;11(3):570–2. <https://doi.org/10.3329/BJPV11I3.27663>.
22. Kaniyur Chandrasekaran M, Vani Raju M, Duraisamy B, Kanniappan V, G., Muthaiyan Ahalliya R. Investigating the antioxidant properties and GC-MS profile of Indian native medicinal flower *Pandanus odorifer* and assessing its cytotoxic effects on HT-29 cells. *Int J Ayurvedic Med*. 2024;15(3):650–9. <https://doi.org/10.47552/IJAM.V15I3.4971>.
23. Pleh A, Mahmutović L, Hromić-Jahjefendić A. Evaluation of phytochemical antioxidant levels by hydrogen peroxide scavenging assay. *Bioeng Stud*. 2021;2(1):1–10. <https://doi.org/10.37868/BES.V2I1.ID178>.
24. Sampath S, Kalimuthu B, Veeramani V, Janardhanam S, Baran MA, Chellan R. Evaluation of total antioxidant and free radical scavenging activities of *Callistemon citrinus* (Curtis) Skeels extracts by biochemical and electron paramagnetic resonance analyses. *RSC Adv*. 2016;6(15):12382–90. <https://doi.org/10.1039/C5RA24410B>.
25. Karthik DVP, Darling DR, David C. Vitro nitric oxide scavenging activity of ethanol extract of *Canthium Coramandelium*. *Int J Pharma Bio Sci*. 2015;6(4):695–700. <http://ijpbs.net/details.php?article=4642>.
26. Oliveira DSM, Dzinic S, Bonfil AI, Saliganan AD, Sheng S, Bonfil RD. The mouse prostate: a basic anatomical and histological guideline. *Bosnian J Basic Med Sci*. 2016. <https://doi.org/10.17305/bjbms.2016.917>.
27. Bosland MC, Schlicht MJ, Horton L, McCormick DL. The MNU plus testosterone rat model of prostate carcinogenesis. *Toxicol Pathol*. 2022;50(4):478–96. <https://doi.org/10.1177/01926233221096345>.
28. Leje IU, Wali U, Yeldu MH, Alhassan HM, Wasagu IZ, Abubakar U, Abubakar BH, Evuti HA, Bello M. Effects of aqueous fruit extract of *Annona muricata* on testosterone propionate induced benign prostate hyperplasia (BPH) in male Wistar rats. *Asian J Res Biochem*. 2024;14(4):72–83. <https://doi.org/10.9734/AJRB/2024/V14I4295>.
29. Zhang MG, Wu W, Zhang CM, Wang XJ, Gao PJ, Lu YL, Shen ZJ. Effects of oral finasteride on erectile function in a rat model. *J Sex Med*. 2012;9(5):1328–36. <https://doi.org/10.1111/J.1743-6109.2012.02661.X>.
30. Omer HAA, Caprioli G, Abouelenein D, Mustafa AM, Uba AI, Ak G, Ozturk RB, Zengin G, Yagi S. Phenolic profile, antioxidant and enzyme inhibitory activities of leaves from two *Cassia* and two *Senna* species. *Molecules*. 2022;27(17):5590. <https://doi.org/10.3390/MOLECULES27175590>.
31. Omer HAA, Caprioli G, Abouelenein D, Mustafa AM, Uba AI, Ak G, Ozturk RB, Zengin G, Yagi S. Phenolic profile, antioxidant and enzyme inhibitory activities of leaves from two *Cassia* and two *Senna* species. *Molecules*. 2022;27(17):5590. <https://doi.org/10.3390/molecules27175590>.
32. Al-Naqqash ZAE, Tawfeeq TA, Eldalawy R. Phytochemical and Anti-Oxidant Investigation of *Alexandrian senna* L. Leaves Cultivated in Iraq. In: IOP Conference Series: Earth and Environmental Science. 2023. vol. 1158(7). <https://doi.org/10.1088/1755-1315/1158/7/072022>.
33. Fan X, Wang Y, Jiang T, Cai W, Jin Y, Niu Y, Zhu H, Bu Y. B-Myb mediates proliferation and migration of non-small-cell lung cancer via suppressing IGFBP3. *Int J Mol Sci*. 2018;19(5):1479. <https://doi.org/10.3390/IJMS19051479>.
34. Doifode T, Giridharan VV, Generoso JS, Bhatti G, Collodel A, Schulz PE, Forlenza OV, Barichello T. The impact of the microbiota-gut-brain axis on Alzheimer's disease pathophysiology. *Pharmacol Res*. 2021;164:105314. <https://doi.org/10.1016/J.PHRS.2020.105314>.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.