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ANTICANCER ACTIVITY OF CASSIA SENNA (L) AGAINST PROSTATE CARCINOGENESIS

[*]Mr. Ayush B. Chavhan[1], Dr. Ranajit D. Tijare[2], Mr. Omkar S. Daware[3]

[1]B. Pharma Student Ishwar Deshmukh Institute of Pharmacy, Digras Maharashtra, India

[2]Principal, Department of Pharmacogonosy, Ishwar Deshmukh Institute of Pharmacy, Digras.

[3]B. Pharma Student Ishwar Deshmukh Institute of Pharmacy, Digras Maharashtra, India

Sant Gadge Baba Amravti University, Amravati, Maharashtra, India

ABSTRACT

In Western nations, prostate cancer (PC) is the most prevalent male non-cutaneous tumour. Since testosterone's metabolic conversion to 5 dihydrotestosterone (DHT) through 5 reductase, as well as genetic predisposition and promotion pathways, play a role in the development of prostate cancer. In the current investigation, testosterone and N-Methyl N-Nitroso Urea (MNU) were used as test subjects for the Cassia senna ethanolic extract's (4-month, 150 mg/kg body weight) anticancer efficacy. After treatment with an ethanolic extract of C. senna, biochemical measurements such as those of prostate acid phosphatase, lipid peroxidation, enzymic antioxidants, and non-enzymatic antioxidants activity were recovered. Histopathological analysis shown important alterations, such as hyperplastic prostatic acini and malignant proliferation of ductal epithelial cells in the prostate and seminal vesicle of carcinogen-induced rats. After receiving the C. senna extract, the prostate showed typical, flow-dilated ducts, acini with regular epithelial lining, and seminal vesicles showed partially hyperplastic and partially flattened epithelium. Based on these data, it is concluded that the ethanolic extract of C. senna effectively inhibits the growth of prostate tumours in an in vivo model that induces prostate carcinogenesis using testosterone and N-methyl N-nitrosourea.

Key words: MNU, Testosterone, Cassia senna, prostate carcinogenesis, antioxidants.

1.INTRODUCTION

One of the most common forms of human cancer in the West is prostate cancer. among the USA and several west European nations, prostate cancer is the most often diagnosed cancer and the second most common cause of cancer-related mortality among men[1]. Prostate cancer has the fifth highest incidence and fourth highest fatality rate in India[2]. Age-related changes in testosterone levels have been hypothesised to have a significant role in the development of benign prostatic hyperplasia and prostate cancer[3]. The aetiology of prostate cancer is unclear despite substantial investigation. The condition is thought to have multiple factors contributing to it, including genetic, hormonal, nutritional, age, race, and environmental factors. Prostate cancer's initiation and progression are mostly governed by unknown processes. Lack of acceptable animal models is one of the factors contributing to the delayed progress. Animal carcinogenesis models do exist, but they are all based on either the single sex hormone testosterone or a combination of testosterone and oestrogen[5]

The benefit of increasing the incidence of prostate carcinogenesis in Sprague-Dawley rats is the combination of testosterone with the carcinogen N-methyl-N-nitrosourea.[5].The development of prostatic intra epithelial neoplasia and hyperplasia to dysplasia in only five months of treatment is one benefit of this particular paradigm.

There is no known method that can effectively prevent prostate cancer in people, and both therapeutic drugs and surgical techniques only provide momentary advantages [6]. Prostate cancer prevention has received relatively minimal attention, despite major advancements in cancer chemoprevention in other organ locations. Despite the fact that the prostate is an organ where efficient chemopreventive regimens could significantly reduce morbidity and mortality, this is the case.[7]

Alternative treatments for cancer or tumours that are safe and effective include natural therapies made from medicinal herbs. The use of medicinal plants in contemporary medicine for cancer therapy or prevention is a crucial component. According to a WHO assessment, more than 30% of pharmaceutical preparations are based on plants, and roughly 80% of the world's population relies on medicinal plants for their healthcare requirements[8]. In Fragaria vesca (strawberries) and Rubus idaeus (raspberries), elagicacid and a wide variety of flavonoids, carotenoids, and terpenoids have been identified to be responsible for antioxidant action. These substances obstruct a number of hormone functions and metabolic pathways linked to the growth of cancer.[9]

However, a dichloromethane extract of Athrixia elata's seeds and leaves showed some growth-inhibitory, cytostatic, and cytotoxic activities against the melanoma, breast cancer, and renal cancer cell lines TK10, MCF7, and UACC62 [10]. Significant levels of carnosol and ursolic acid, two powerful antioxidants with anticancer properties, are found in the herb rosmarinus officinalis (rosemary).[11]

Senna, also known as Cassia senna (L.) (Caesalpinaceae), is a significant medicinal plant[12]. One of the most popular herbal laxatives is it [13]. The anthraquinone glycosides, particularly the sennosides A and B, are primarily responsible for C. senna's therapeutic effects[12]. Up to 7% of sennosides can be found in the

dried leaves and pods of C. senna [15]. Consequently, the search for potent anti-cancer medications is an important research area. Therefore, efforts are being conducted to find naturally occurring anticarcinogens that would stop, slow down, or reverse the development of cancer. As a result, a chemoprevention study was carried out to determine how Cassia senna affected Wistar rats' prostate cancer.

2.MATERIALS AND METHODS

2.1 Plant Collection and Extract preparation

The Cassia senna leaves were gathered at Pollachi, Tamil Nadu, India. Dr. G.V.S. Murthy from the Tamil Nadu Agricultural University Campus in Coimbatore, Tamil Nadu, India, verified its authenticity. For future use, a voucher specimen was stored in the lab (BSI/SC/5/23/09- 10/Tech-238). Using 500 cc of 95% ethanol, the powdered leaves of C. senna (100 g) were extracted.

2.2 Chemicals

Testosterone was purchased from SD Fine Chemicals, Mumbai, MNU (N-Me-thyl N-Nitroso Urea) was purchased from Sigma U.S.A. and Propylene glycolwas purchased from Qualigens Fine Chemicals, Mumbai.

2.3 Preliminary Phytochemical Screening

The phytochemical screening of C.Senna was performed as per procedure.[16],[17]

2.4 Animals Used

In the current investigation, male albino Wistar rats weighing between 160 and 180 g were employed. Throughout the trial, the animals were kept in roomy, spacious cages and were allowed unlimited access to food and water. The Institutional Animal Ethical Committee, established by the Indian government to serve CPCSEA, gave its approval to the study.

2.5 Induction of prostatic carcinogenesis using carcinogen and hormone

Step 1: For three days, rats were given daily intraperitoneal injections of 100 mg/kg of testosterone dissolved in 0.3 ml of propylene glycol.

Step 2: All rats were given a single intravenous dosage of N-methyl N-nitrosourea (MNU) diluted in saline at a concentration of 10 mg/ml through the tail vein one day following the last testosterone treatment.

Step 3: Rats were given daily intraperitoneal injections of 2 mg/kg of testosterone for 60 days starting one week following MNU treatment.

.6 Experimental design

Four groups of experimental animals were created. Six rats each comprise each group. Group I served as a control group (propylene glycol); Group II rats were given a carcinogen (MNU) and a hormone (T) to induce prostate cancer; Group III rats were given a crude ethanolic extract of C. senna at a dose of twice as

much (150 mg/kg body weight) every week for 16 weeks; and Group IV rats were given a dose of twice as much (150 mg/kg body weight) every week for 16 weeks.

2.7 Biochemical estimations done in the rat prostate gland and seminal vesicle

Following the treatment period, the rats were slaughtered under chloroform anaesthesia, and the prostate was taken from the adhering connective tissue. It was then carefully weighed and utilised for biochemical estimates after being repeatedly rinsed with physiological saline. Prostatic acid phosphatase[18], enzyme-based antioxidants like SOD [19], Catalase [20], and GPx [21], as well as non-enzyme-based antioxidants like Vitamin C [22] and reduced glutathione [23] levels.

2.8 Histological Observations

Prostate gland and seminal vesicle separated lobes, in part, were fixed in 10% neutral buffered formalin. The lobes were embedded in paraffin and stained for histological analysis after fixing. It was done in Chennai's Vaishnavi Histopathology Lab.

2.9 Statistical Analysis

The values were expressed as mean ± SD. The statistical analysis was carried out

by one-way analysis of variance using SPSS (version 10) statistical analysis

program. Statistical significance was considered at $p<0.05$.

3.RESULTS

3.1 Phytochemical Screening

According to the results of the phytochemical screening, the extract's main chemical components were flavonoids, tannins, steroids, alkaloids, saponins, oils and fats, carbohydrates, amino acids, and proteins.

3.2 Body weight of prostate and seminal vesicle

The impact of the crude ethanolic extract of C. senna during MNU and testosterone-induced prostate carcinogenesis on the body weight of Wistar rats is shown in Figure 1. Rats treated with MNU+T had considerably lower body weights than the control group. When compared to MNU+T treated rats, simultaneous treatment with C.senna treated rats did not result in any weight reduction.

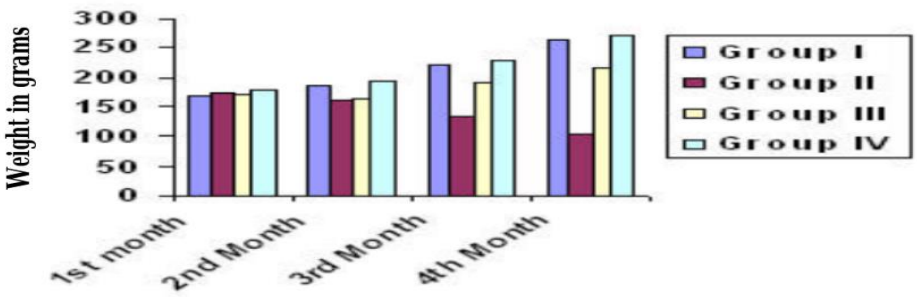

Figure 1: Average body weight of Experimental rats.

3.3 Organ weight of prostate and seminal vesicle

The impact of the crude ethanolic extract of C. senna during MNU and testosterone-induced prostate carcinogenesis on the body weight of Wistar rats is shown in Figure 1. Rats treated with MNU+T had considerably lower body weights than the control group. When compared to MNU+T treated rats, simultaneous treatment with C.senna treated rats did not result in any weight reduction.

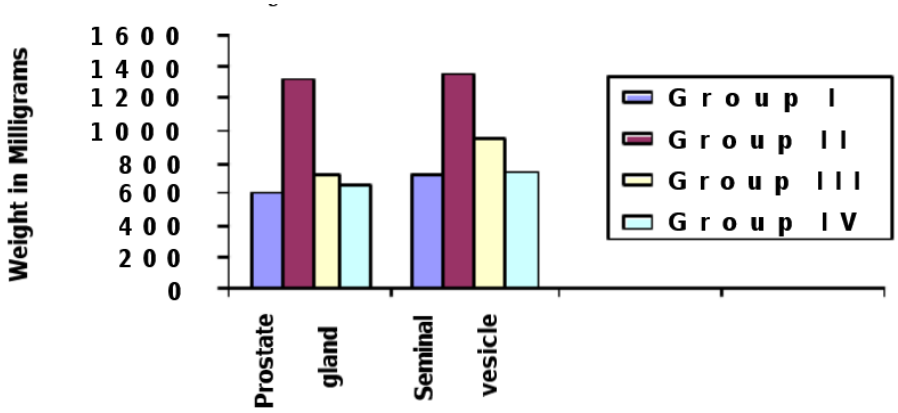

Figure 2: Average organ weight of prostate and seminal vesicle of Experimental rats.

3.4 Prostatic acid phosphatase

The impact of the crude ethanolic extract of C. senna on acid phosphatase in the prostate and seminal vesicle is shown in Figure 3. When the ethanol extract of C. senna was provided, a substantial drop in the level of acid phosphatase was seen in group III, and there was a significant increase in acid phosphatase in group II. When just the plant extract was administered to group IV, the levels matched those of group I's norm.

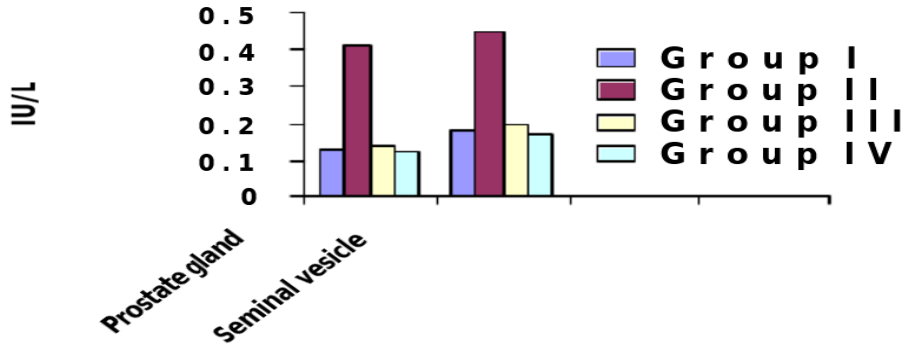

Figure 3: Level of Acid Phosphatase in prostate gland and seminal vesicle of Experimental rats.

3.5 Antioxidants and Lipid peroxidation

The enzymes SOD, CAT, and GPx are crucial free radical scavengers. According to the current data, as shown in Figures 4 and 5, Group III antioxidant enzymes were found to be significantly more abundant in prostate and seminal vesicles than Group II antioxidant enzymes.

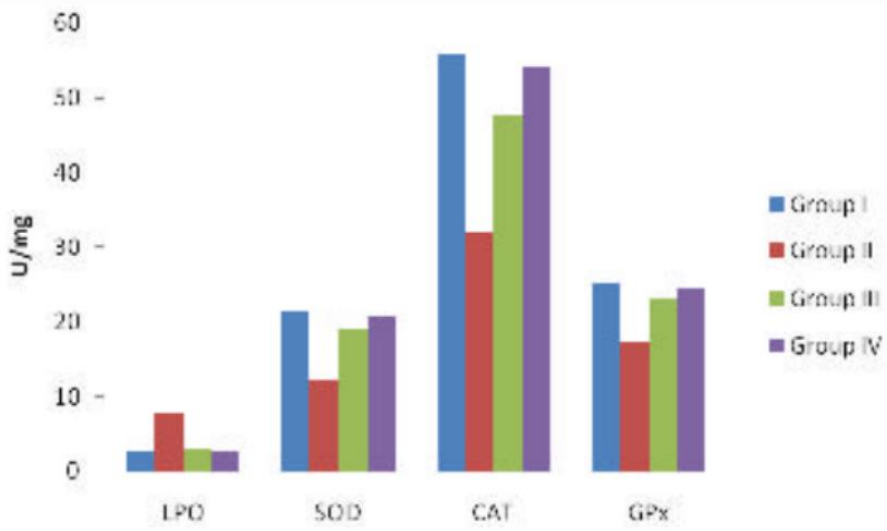

Figure 4: Levels of LPO, SOD, CAT and GPx in prostate tissue.

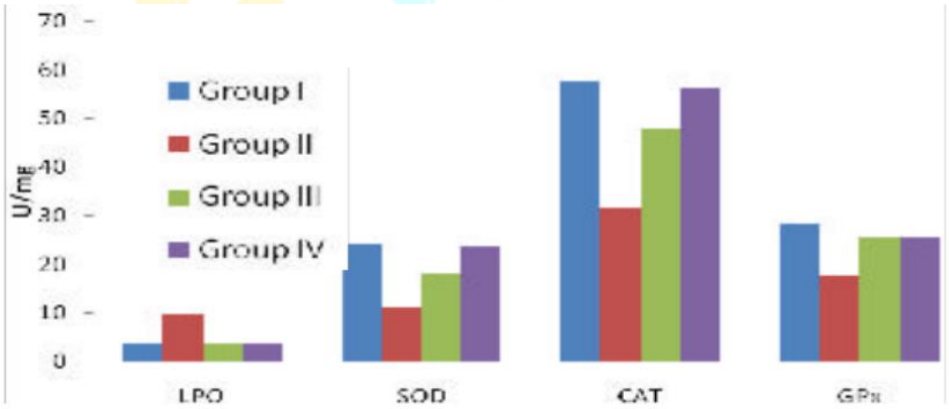

Figure 5: Levels of LPO, SOD, CAT and GPx in seminal vesicle.

In the prostate gland and seminal vesicle tissue homogenate of several groups of rats, the activity of non-enzymic antioxidants such GSH and Vitamin C was examined. The results are depicted in Figure 6. The non-enzymic antioxidants significantly decreased in group II. However, group III showed a considerable increase in non-enzymic antioxidants following administration of C. senna ethanolic extract. Following treatment with the ethanolic extract of C. senna, the increased degree of lipid peroxidation in group II dramatically decreased in group III.

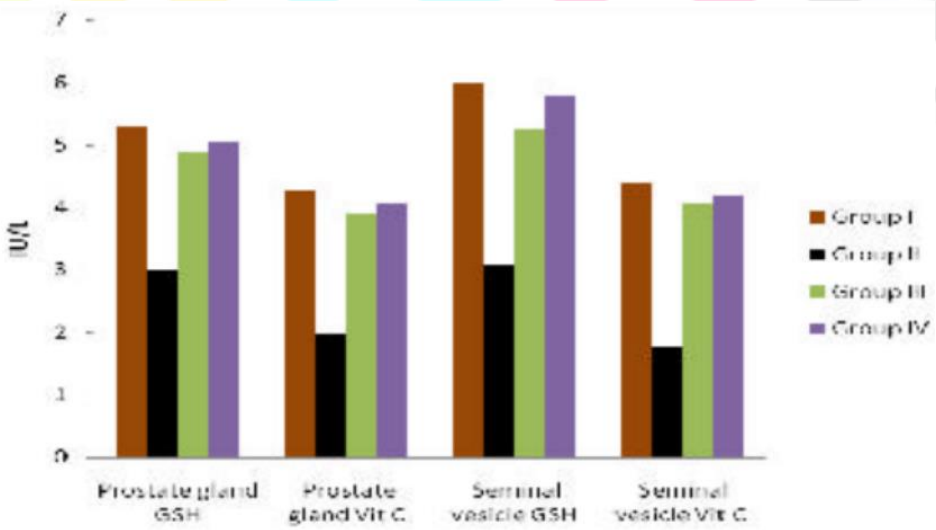

Figure 6: Levels of GSH and Vitamin C in prostate and seminal vesicle.

3.6 Histopathological Studies

The Histopathological examination of prostate gland and seminal vesicle (Figure 7 and Figure 8) revealed that the normal architecture was disturbed bycarcinogen induction. In the prostate gland of rats treated with C.senna the

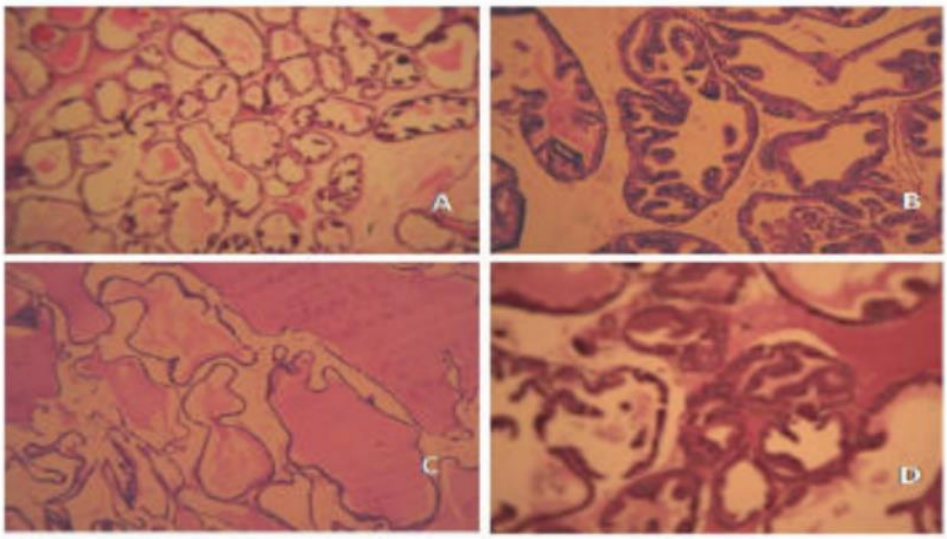

Figure 7: Histopathology of Prostate Gland.

A. Control: A single layer of columnar epithelium lines the acini in the prostate.

B. Carcinogen + Hormone- Demonstrates hyperplastic prostatic acini and glands with epithelial lining that penetrates through Papillary foldings and budding into the lumen. **C. Carcinogen + Hormone + Drug** – Displays dilated and normal acini and glands with columnar lining and minor papillary folds. **D Drug Control**: Dilated ducts with flattened epithelial lining and retained secretion are visible. H&E-stained sections (100x)

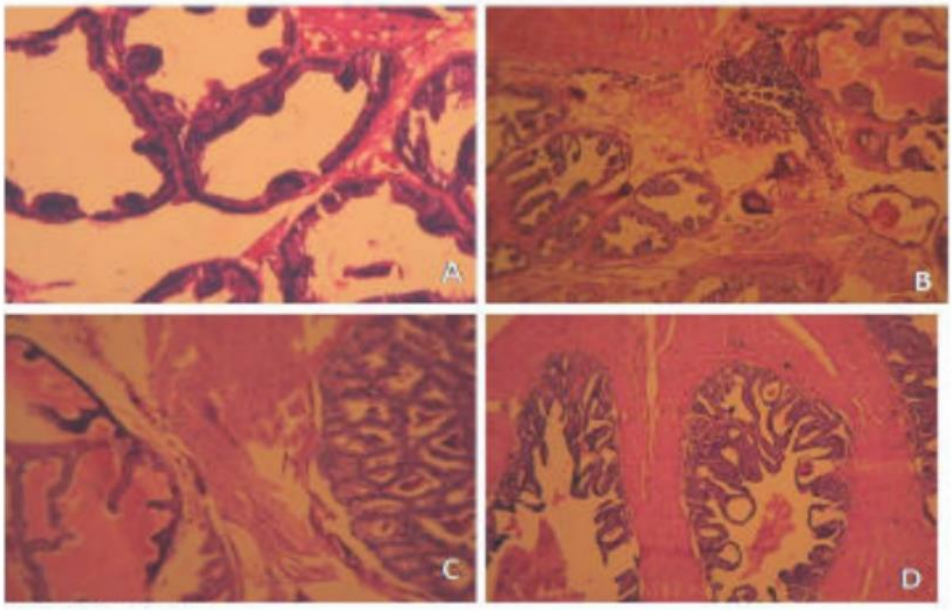

Figure 8: Histopathology of Seminal Vesicle.

A. Control: A seminal vesicle with a normal mucosal lining and modest papillary folds. **B. Carcinogen + Hormone** – Seminal Vesicle – Demonstrates Malignant Proliferation of Ductal Epithelial Cells with Tightly

Packed Tall Papil- lary Infoldings, Lined by Hyperchromatic Nuclei. **C. Drug + Hormone + Carcinogen** + Acini Lobules divided by fibrous septa. Mild cystic dilatation can be seen on the ducts' inner projection. **D-Drug Control**: Dilated ducts with flattened epithelial lining and retained secretion are visible.

H&E-stained sections (100x)

Flow dilated ducts and acini with regular columnar epithelial lining preserved the natural architecture. Rats treated with C. senna had partially hyperplastic and partially flattened epithelium in their seminal vesicles.

4.DISCUSSION

Tribal healers typically prescribe herbal preparations made from a single plant component or a combination of many plant parts. They think that combining various plant parts quickly heals illnesses. Many clinicians and researchers now view cancer chemoprevention using antioxidant approaches as a key strategy for inhibiting, delaying, or even reversing the process of carcinogenesis . It has been suggested that this approach has a good potential for offering significant fundamental benefits to public health.[24]

Alkaloids, tannins, steroids, saponins, flavonoids, oils and lipids, carbohydrate, amino acids, and proteins can all be found in the crude extract of C. senna, according to a phytochemical screening. It is known that some of their active ingredients, such as flavonoids, are utilised to treat carcinogenic activity.A medication that contains alkaloids also shown antitumor action [25]. Many substances, including alkaloids, glycosides, tannins, and flavonoids, have been shown to have strong antioxidant activity [26]. As a result, any of these ingredients may be the cause of this plant's anti-tumour effects.

The activity of acid phosphatase was analysed in prostate and seminal vessicle tissue homogenate of different groups of rats and the increased level of prostaticacid phosphatase was decreased after treatment with ethanolic extract of C.senna.Thus the decreased activity of prostatic acid phosphatase inhibits the prostate cancer initiation. The prostate is a major organ, which secretes acid phosphatase and the level of serum acid phosphatase of prostatic origin increase markedly in human with extensive or metabolic carcinoma of the prostate [27]. This enzyme is an excellent marker of androgen dependent function of rat prostatic tissue [18].[28]

One of the key mechanisms by which ROS initiate oxidative damage is lipid peroxidation, which has been associated with altered membrane structure and enzyme activation. It starts when a hydrogen atom is removed from a PUFA side chain in the membrane [29]. The current study's increase in tissue MDA levels shows that lipid peroxidation has progressed. On the other hand, of the other parameters examined for the demonstration of oxidative stress, the changes discovered either in the form of the inhibition or stimulation of the activity of antioxidant enzymes points out to the generation of a high level of free radicals in tissues and also establishes that antioxidant enzymes play an active role in the conversion of free radicals to reactive oxygen species. A mutually beneficial team of defence against reactive oxygen species (ROS) is made up of SOD, CAT, and GPx. By reducing the steady state of O2, SOD is the first enzyme implicated in

the antioxidant defence. A hemoprotein called CAT, which is found in the peroxisomes, catalyses the conversion of H2O2 into water and oxygen. A selenoenzyme called GPx, which is mostly found in the liver, catalyses the reaction between hydroperoxides and reduced glutathione to produce glutathione disulphide (GSSG) and the hydroperoxide's reduction product [30]. Figure VI displays the activity of non-antioxidant enzymes like GSH and Vitamin C that were examined in the prostate gland homogenate of various rat groups. Antioxidant systems including glutathione (GSH), ceruloplasmin, superoxide dismutase (SOD), antioxidant vitamins (A, C, and E), lipid peroxidation, catalase, glutathione (GSH), and glutathione peroxidase (GSH-Px), which is the basis of many pathological processes, guard the cells from this damage. [31],[32]

Using Testosterone and N-methyl N-nitrosourea to induce prostate cancer in an in vivo model, it is reported that the ethanolic extract of C. senna has good anticancer efficacy.

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