



Original Article

Protective effect of *Chuquiraga spinosa* extract on *N*-methyl-nitrosourea (NMU) induced prostate cancer in rats



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ABSTRACT

Background: The main objective was to evaluate the possible protective effect of *Chuquiraga spinosa* extract on *N*-methyl nitrosourea (NMU)-induced prostate cancer in rats and DU-145 cell line.

Materials and methods: Prostate carcinogenesis was induced in 30 male Holtzman rats by providing cyproterone acetate, testosterone, and NMU. The tumors were monitored and hematological and biochemical parameters and frequency of micronucleated polychromatic erythrocytes were recorded. The cell line was assessed by a cytotoxicity assay.

Results: Oral administration of *C. spinosa* extract significantly lowered superoxide dismutase malondialdehyde, NO, C-reactive protein, and prostate-specific antigen levels (all $P < 0.01$ compared with Inductor Group). There was a significant decrease in the frequency of micronucleated polychromatic erythrocytes ($P < 0.05$). *C. spinosa* presented a selectivity index of 17.24 in the cytotoxicity assay.

Conclusions: Considering its anti-inflammatory, antioxidant, and antigenotoxic effects, and important variations on biochemical and hematological parameters, including prostate-specific antigen of *C. spinosa* extract, we conclude that it has a protective effect on NMU-induced prostate cancer in rats and cytotoxicity in the DU-145 cell line.

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1. Introduction

Prostate cancer is the most frequently diagnosed malignancy and the second most common cause of cancer-related death in elderly men in the United States.¹ Chronic inflammation is a potential mediator in the development of many cancers, including prostate cancer.² Cancer patients use medicinal plants because they think that plants have fewer side effects and are less likely to cause dependence.³ It is necessary to evaluate the efficacy and safety of the medicinal plants used by cancer patients with pharmacovigilance studies.⁴ *Chuquiraga spinosa* is traditionally used to treat

prostate diseases in Northern Peru.⁵ Bussman states that despite other plants, *C. spinosa* has proven effects on health. Its antibiotic effect has also been proved.⁶ Casado et al⁷ showed that aqueous and methanol extract of the aerial parts of *C. spinosa* exhibit high antioxidant activity. Also, the methanolic extract administered orally significantly reduced subplantar and ear edema induced in rats. The aqueous and methanol extracts had activity against *Candida albicans* and the aqueous extract showed antifungal activity against *Candida cucumerinum*.⁸ The cytotoxic potential of heliantriol B2, a pentacyclic triterpene isolated from *Chuquiraga erinacea*, has shown antitumor effect in human leukemia cell lines.⁹ Considering the traditional use of *C. spinosa*, its antioxidant properties and relation to *C. erinacea*, we aimed to demonstrate the antitumor effect of ethanol extract of the aerial parts of *C. spinosa* (called Huamanpinta) in prostate cancer induced by *N*-methyl-*N*-nitrosourea (NMU) in rats and DU-145 cell line.

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2. Materials and methods

2.1. Chemicals

NMU was purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade and highest purity.

2.2. Preparation of *C. spinosa* extract

Leaves of *C. spinosa* were collected in El Tambo, Huancayo, Peru. Taxonomic identification was made at the National Herbarium of the National University of San Marcos, Lima, Peru. The voucher specimen of the leaves was deposited (303-USM-2013). The powder material was exhaustively soaked with 96% ethanol with intermittent shaking every day for 7 days. Next, the extract was filtered and concentrated to obtain the solid residue, its final weight was noted, and it was kept refrigerated until further use.

2.3. Qualitative phytochemical screening

The extracts obtained were screened in order to determine the presence of phytochemical constituents, such as alkaloids, terpenoids, quinone, flavonoids, tannins, saponins, steroids, and phenolic compounds, with the standard qualitative phytochemical methods described.¹⁰

2.4. Evaluation of antioxidant activity

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay was carried out according to the procedure described by Mensor et al.¹¹ Ethanol extract (1,900 µL) at different concentrations (1.0 µg/mL, 10.0 µg/mL, and 50.0 µg/mL) and controls (trolox and vitamin C) were allowed to react with 100 µL 0.4mM DPPH in ethanol and reacted at room temperature in the dark for 30 minutes. All tests were performed in triplicate. Absorbance of each sample was measured at 517 nm using a UV/Visible Spectrophotometer (UNICO, UV-2100 United Products and Instruments Inc, Dayton, NJ, USA).

2.5. Animals

Thirty male Holtzman rats weighing 250 ± 20 g were procured from the National Institute of Health, Lima, Peru. The animals were housed in well-ventilated, large, spacious cages in the bioterium of the Faculty of Medicine, National University of San Marcos. The animals received a balanced diet of commercially available pellet rat feed and water *ad libitum*. The rats were kept under a 12-hour light/dark cycle and a temperature of $21 \pm 2^\circ\text{C}$. The experiment began with a 7-day preconditioning period.

2.6. Tumor induction

In all groups, except P80, tumor induction was carried out by following the method of Bosland and Prinsen.¹² After release from quarantine, 36 rats received daily cyproterone acetate (50 mg/kg body weight in sesame oil) by intraperitoneal injection for 18 consecutive days. One day after the final dose of cyproterone acetate, rats received daily subcutaneous injections of testosterone propionate (100 mg/kg body weight in sesame oil) for 3 days. On the day after of testosterone propionate administration, each rat received a single intravenous injection of NMU (50 mg/kg body weight in sterile saline, pH 5.0). Groups were named according to the treatment and dosed in mg/kg. P80 refers to polysorbate 80 and ChS refers to *C. spinosa* ethanolic extract. The negative control group, P80, received 1% polysorbate 80 (50 mg/kg body weight)

orally for 23 weeks. The Inductor Group received only the treatments of cyproterone acetate, testosterone propionate, and NMU. The groups ChS50, ChS250, and ChS500 received oral ethanolic extract of 50 mg/kg, 250 mg/kg, and 500 mg/kg body weight, respectively, for 23 weeks after tumor induction. At the end of the experimental period, the rats were weighed. Blood samples were obtained to assess the biochemical and hematological indicators. The animals were killed by pentobarbital anesthesia (100 mg/kg).

2.7. Hematological parameters

Hemoglobin content was determined spectrophotometrically (B-Hemoglobin, Hemocue, Stockholm, Sweden). The total leukocyte count was performed in a Neubauer chamber. Blood glucose was quantitated using a commercial enzymatic kit (Wiener Lab, Santa Fe, Argentina) obtained from fasted rats. Total cholesterol was estimated by modified Roeschlau et al's¹³ method. High-density lipoprotein-cholesterol level was determined based on the method of Trinder.¹⁴ Triglycerides were estimated by enzymatic GPO-PAP method, as described by Annoni et al.¹⁵ Alanine aminotransferase was determined using the Reitman and Frankel¹⁶ method. Alkaline phosphatase activity was assessed according to King and Armstrong.¹⁷ Urea determination was based upon the cleavage of urea with urease (Berthelot's reaction) according to Fawcett and Scott.¹⁸

2.8. Biochemical parameters

Superoxide dismutase (SOD) was assayed as described by Beauchamp and Fridovich¹⁹ based on the reduction of nitroblue tetrazolium to water-insoluble blue formazan. Lipid peroxidation was detected by the determination of malondialdehyde (MDA) production determined by the method of Begue and Aust.²⁰ NO scavenging assay was performed using the Griess reagent method.²¹ The levels of C-reactive protein (CRP) were determined using Biochemistry VITROS and Integrated system VITROS 5600 (Ortho Clinical Diagnostics Inc, 100 Indigo Creek Drive, Rochester, New York, USA). The amount of prostate-specific antigen (PSA) in mouse serum was quantified using a commercially available ELISA kit (Diagnostics Biochem, Dorchester, ON, Canada) against a standard curve (0.2–50 ng/mL PSA).

2.9. Micronucleus test

The micronucleus test was carried out following Schmid's method.²² Peripheral blood was obtained by cardiac puncture to prepare a blood film. The slides were fixed with absolute methanol and stained with 3% Giemsa. The frequency of micronucleated polychromatic erythrocytes (MNPCEs) based on the observation of 1,000 PCEs per animal was recorded.

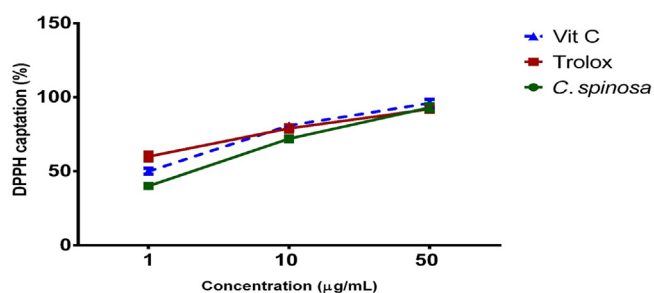
2.10. Cytotoxicity assay in DU-145 cell line

The cell lines DU-145 (prostate carcinoma) and 3T3 (mouse embryonic fibroblasts) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). They were grown at 37°C in a 5% CO_2 atmosphere. DU-145 cell line was grown in minimal essential medium in the presence of 10% fetal bovine serum and 50 µg/mL gentamicin. The 3T3 cell line was grown in Dulbecco's modified Eagle's medium. Cultured cell lines were washed in 3×4 mL Hank's balanced salt solution. Then, 1 mL trypsin/EDTA was added and 10 minutes later, it was eliminated. The cultures were incubated for 8 minutes at 37°C and each culture was resuspended in 2 mL medium. Then, cells were counted using a hemocytometer. Each well of a 96-well plate received 160 µL medium.

Table 1
Phytochemical constituents of *Chuquiraga spinosa* extract.

| Constituent | Test | Result |
|----------------|--------------------|--------|
| Phenols | Ferric chloride | (+++) |
| Protein | Ninhydrin | (+) |
| Steroids | Liebermann–Buchard | (+) |
| Flavonoids | Shinoda | (+++) |
| Saponins | Frothing | (++) |
| Alkaloids | Dragendorff | (++) |
| | Mayer | (+++) |
| | Hager | (-) |
| Anthraquinones | Bornträger | (-) |
| Tannins | Gelatin | (+) |

+++ , large amount; ++ , medium amount; + , small amount; - , absent.

**Fig. 1.** Antioxidant effect of *Chuquiraga spinosa* extract. DPPH, 1,1-diphenyl-2-picrylhydrazyl; Vit C, vitamin C.

For the cytotoxicity assay, there were two plates, 0 (control) and 1 (experimental). Each line was assigned four wells and each well received 160 mL culture medium. They were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 24 hours. Trichloroacetic acid (TCA) was added to Plate 0, to fix the cells and then quantify at time zero. Different dilutions of the extract and 5-fluorouracil (5-FU) were added to Plate 1. To dilute the extract, 5 mg extract and 1 mL dimethyl sulfoxide were mixed and centrifuged at 13,500 g for 10 minutes. The supernatant was the stock of 5 mg/mL, and 8.5 mL of this was diluted in 340 mL medium; with successive dilutions of 1:4. The initial concentration used was 2.5 mg/mL for 5-FU and 250 mg/mL for extracts. Plate 1 was incubated for 48 additional hours. To evaluate antitumor activity, cytotoxicity bioassay method with sulforhodamine B (SRB) was performed as described by Skehan et al.²³ The trial was stopped by adding TCA to the plates. Cells were fixed with TCA and stained for 20 minutes with a solution of 0.4% SRB in 1% acetic acid. The SRB unbound protein was removed by washing with 1% acetic acid. After drying the plates, the protein-bound SRB was solubilized with 10mM Tris base solution [Tris (hydroxymethyl) aminomethane; (pH 10.5)] and the absorbance at 450 nm was read in a microplate reader. The 50%

inhibitory concentration (IC₅₀) was found by linear regression analysis. The extract selectivity index was defined as the ratio of cytotoxicity between normal cells and cancer cells: IC₅₀ (3T3 cell line)/IC₅₀ (tumor cell line), which was > 1 when the cytotoxicity for tumor cells was greater than in normal cells.

2.11. Histological analysis

The prostates were collected from euthanized rats. They were weighed and their volume was determined. The prostates were fixed in Bouin's solution, processed, and embedded in paraffin blocks. Sections were cut at 5-µm thickness and stained with hematoxylin and eosin. The slides were examined under a light microscope (BX51; Olympus, Tokyo, Japan) to observe structural changes and inflammatory and malignant cells, and confirmed by an experienced histopathologist.

2.12. Statistical analysis

Numerical variables were described with measures of central tendency and dispersion, and mean and standard deviation. The normality and homogeneity of variance were evaluated using the Shapiro–Wilk's and Barlett's tests, respectively. Subsequently, analysis of variance with *post hoc* Tukey's test was used with variables that showed significant differences ($P < 0.05$) between the groups. All statistical analysis was performed using SPSS (Chicago, IL, USA) version 18.0 software.

2.13. Ethical considerations

During the entire experimental process, international ethical principles for research using laboratory animals were respected. The rats were killed by intravenous injection of pentobarbital (100 mg/kg). This method led to a rapid and peaceful death, which was acceptable for rats according to recommendations for euthanasia of experimental animals. The protocol was approved by the Institute for Ethics in Health of the National University of San Marcos (01414-R-12-UNMSM).

3. Results

The ethanol extract of *C. spinosa* was predominantly composed of phenolics and flavonoids. Saponins and alkaloids were also in high quantity (Table 1). The percentage of DPPH radical uptake was dose dependent, yielding a dose of 1 µg/mL (40.5%) and 50 µg/mL (90.3%; Fig. 1). Hematological parameters of rats showed no difference between the groups (Table 2). Biochemical parameters showed significantly decreased levels of SOD, MDA, NO, CRP, and PSA in all groups receiving ChS compared with the Inductor Group (all $P < 0.01$; Table 3).

Table 2
Effect of *Chuquiraga spinosa* extract on hematological parameters.

| Parameter treatment | Hb | Hct | Leukocytes | HDL (mg/dL) | Cholesterol (mg/dL) | Triglycerides (mg/dL) | Glucose (mg/dL) | Urea (mg/dL) | ALT (UI/dL) | FA (UI/dL) |
|---------------------|------------|------------|-------------|-------------|---------------------|----------------------------|-----------------|--------------|-------------|--------------|
| P80 | 11.8 ± 0.9 | 37.0 ± 1.9 | 7816 ± 1680 | 30.6 ± 7.0 | 115.5 ± 6.4 | 152.5 ± 14.0 | 100.0 ± 8.5 | 18.5 ± 5.2 | 18.3 ± 8.0 | 135.6 ± 32.8 |
| Inductor | 13.1 ± 1.8 | 38.8 ± 7.1 | 7455 ± 1110 | 41.6 ± 7.2 | 105.3 ± 17.5 | 127.1 ± 13.8 | 90.0 ± 8.8 | 17.5 ± 5.4 | 27.0 ± 13.6 | 128.1 ± 25.0 |
| ChS50 | 11.7 ± 1.5 | 34.8 ± 6.1 | 6578 ± 2547 | 37.4 ± 5.5 | 125.8 ± 23.4 | 145.2 ± 36.2 | 91.0 ± 8.0 | 19.0 ± 6.4 | 23.4 ± 13.0 | 135.2 ± 40.1 |
| ChS250 | 11.5 ± 0.8 | 36.6 ± 1.5 | 7640 ± 1816 | 33.2 ± 8.2 | 124.8 ± 12.4 | 166.6 ± 31.6 ^{a)} | 98.2 ± 8.1 | 18.4 ± 5.8 | 15.8 ± 5.8 | 140.8 ± 33.9 |
| ChS500 | 13.0 ± 1.2 | 39.0 ± 4.6 | 8120 ± 1371 | 41.4 ± 7.5 | 114.4 ± 10.4 | 121.2 ± 18.0 | 95.4 ± 10.0 | 24.0 ± 7.5 | 31.4 ± 14.1 | 130.4 ± 32.9 |

Values are expressed as mean ± standard deviation ($n = 6$). Analysis of variance ($P = 0.034$).

ALT, alanine aminotransferase; ChS50, ChS250, and ChS500, 50 mg/kg, 250 mg/kg, and 500 mg/kg *C. spinosa* ethanolic extract, respectively; FA, fatty acid; Hb, hemoglobin; Hct, hematocrit; HDL, high-density lipoprotein; P80, polysorbate 80.

^{a)} Tukey test ($P = 0.04$) versus Inductor Group.

Table 3
Effect of *Chuquiraga spinosa* extract on biochemical parameters.

| Parameter treatment | SOD (U/mL) | MDA (10^{-6} mol/L) | NO (μ mol/L) | CRP (U/mL) | PSA total (ng/mL) |
|---------------------|--------------------------------|-----------------------------|-------------------------------|-----------------------------|-----------------------------|
| P80 | 116.0 \pm 16.2 ^{a)} | 1.9 \pm 1.3 ^{a)} | 1.2 \pm 0.9 ^{a)} | 0.5 \pm 0.2 ^{a)} | 0.1 \pm 0.0 ^{a)} |
| Inductor | 464.8 \pm 73.9 | 15.9 \pm 3.7 | 137.0 \pm 43.7 | 3.5 \pm 0.4 | 1.2 \pm 0.2 |
| ChS50 | 261.2 \pm 26.5 ^{a)} | 3.0 \pm 0.4 ^{a)} | 74.8 \pm 33.6 ^{b)} | 1.6 \pm 0.7 ^{a)} | 0.3 \pm 0.1 ^{a)} |
| ChS250 | 173.8 \pm 40.7 ^{a)} | 8.0 \pm 3.9 ^{a)} | 64.9 \pm 31.1 ^{a)} | 0.9 \pm 0.1 ^{a)} | 0.4 \pm 0.3 ^{a)} |
| ChS500 | 128.2 \pm 34.6 ^{a)} | 5.0 \pm 2.3 ^{a)} | 104.7 \pm 25.2 | 0.8 \pm 0.4 ^{a)} | 0.6 \pm 0.3 ^{b)} |

Values are expressed as mean \pm standard deviation ($n = 6$). Analysis of variance ($P < 0.01$).

CRP, C-reactive protein; ChS50, ChS250, and ChS500, 50 mg/kg, 250 mg/kg, and 500 mg/kg *C. spinosa* ethanolic extract, respectively; MDA, malondialdehyde; P80, polysorbate 80; PSA, prostate-specific antigen; SOD, superoxide dismutase.

^{a)} Tukey test ($P < 0.01$) versus Inductor Group.

^{b)} Tukey test ($P < 0.05$) versus Inductor Group.

The effects of *C. spinosa* extract on the frequency of MNPCs in the peripheral blood of rats after the experiment are shown in Fig. 3. Examination of the antimutagenicity profile revealed a significant decrease in the frequency of MNPCs in all groups as compared with the NMU group. All groups had a $P < 0.01$.

The weight and volume of the prostate gland were lower in the groups that received *C. spinosa* compared with the Inductor Group. Volume was significantly lowered by the administration of *C. spinosa* extract at a dose of 250 mg/kg ($P < 0.05$; Fig. 2).

Hematoxylin-and-eosin-stained sections of prostate gland from the P80 Group showed normal structure. Tumors from the Inductor Group had altered cellular architecture, with cell hypertrophy, glandular hyperplasia, and dysplasia. It was observed that the ChS50 Group had a reduction of glands, with surrounding fibrosis. In the ChS250 Group, there were colloids and little hypertrophy. The ChS500 Group showed glandular hyperplasia, with cells of different sizes and thickened capsules (Fig. 4). In the study of cytotoxicity, the ethanolic extract of *C. spinosa* presented a selectivity index of 17.24 compared to 0.0037 with 5-FU (Table 4).

4. Discussion

NMU, testosterone, and cyproterone are inducing agents of prostate cancer. In our study, the animals received intraperitoneal cyproterone, which is an antagonist of the release of luteinizing hormone. It inhibits testicular androgens and causes atrophy of prostatic epithelial cells. The subcutaneous administration of testosterone propionate stimulates maximally the proliferation of prostatic epithelial cells, and NMU helps in the increase of epithelial volume.²⁴ Dysplasia is more biologically related to malignant transformation of the prostate, and in this study, all animals that received the tumor-inducing agents showed a lot of dysplasia, especially in groups that had not received treatment. Age possibly influenced the appearance of dysplasia as the predominant pattern.²⁵ In our study, the rats were aged 4 months (young adults), which allowed their tissues to respond less aggressively. The histological pattern found in the rats given ethanol extract of *C. spinosa* showed discrete hyperplasia with fibrotic areas and capsular thickening. The underlying cause of tissue fibrosis is typically a chronic inflammatory response. Recently, it was generally accepted that fibrosis occurs in a confined space, only affecting the area immediately surrounding the site of injury.²⁶ The extract could have generated fibrosis according to histological studies, and this condition also is seen after radiotherapy or chemotherapy, which has been successful in the treatment of tumors.²⁷ Administration of NMU with testosterone generated fibrosis and morphological modifications in the prostate. However, the extract could have prevented prostate cancer progression, with regard to its antioxidant and anti-inflammatory effect determined in this study.

Hematological parameters showed a trend of increased high-density lipoprotein level and decreased total cholesterol level in the ChS500 Group compared to the groups receiving the extract at lower doses. The lipid-lowering effect could directly influence the development of cancer because, in preclinical studies, statins showed promising results in pancreatic, liver, colorectal, and gastric cancer. Statins affect cell division in cancer and are expressed in the inhibition of proliferation, induction of apoptosis, autophagy, and anti-invasion and antimigratory effects.²⁸ The groups that received the extract showed significantly lower values of CRP and NO. The

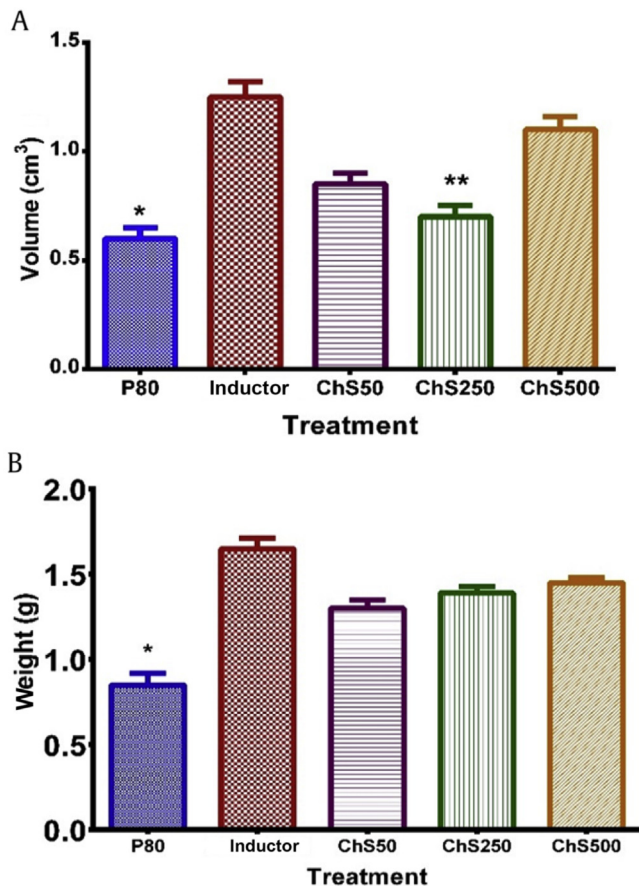


Fig. 2. Effect of *Chuquiraga spinosa* extract on volume and weight of prostate. Each value represents mean \pm standard deviation ($n = 6$). (A) Volume: analysis of variance ($P = 0.012$). (B) Weight: analysis of variance ($P = 0.002$). * Tukey test ($P < 0.01$) versus Inductor Group. ** Tukey test ($P < 0.05$) versus Inductor Group. ChS50, ChS250, and ChS500, 50 mg/kg, 250 mg/kg, and 500 mg/kg *C. spinosa* ethanolic extract, respectively; P80, polysorbate 80.

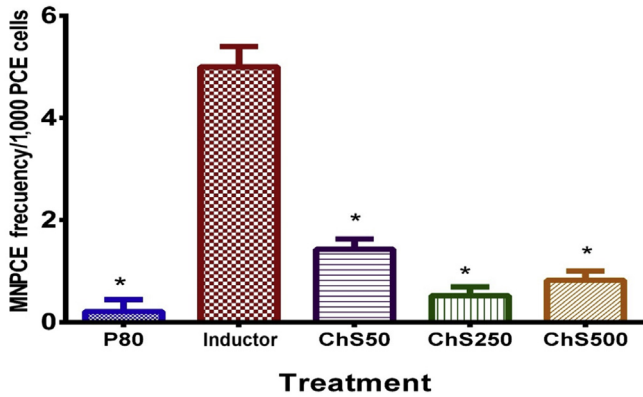


Fig. 3. Effect of *Chuquiraga spinosa* extract on the frequency of MNPCEs. Each value represents mean \pm standard deviation ($n = 6$). Analysis of variance ($P = 0.01$). * Tukey test ($P < 0.01$) versus Inductor Group. ChS50, ChS250, and ChS500, 50 mg/kg, 250 mg/kg, and 500 mg/kg *C. spinosa* ethanolic extract, respectively; MNPCE, micronucleated polychromatic erythrocyte; P80, polysorbate 80.

relationship between chronic inflammation and the development of tumors was studied by Casado et al,⁷ and showed a close relationship between the inflammatory response and increased prevalence of cancer.

The effect of ethanol extract of *C. spinosa* in reducing CRP and NO levels could be due to the presence of flavonoids, which have the property of reducing the inflammatory mediators, which significantly reduces the odds of developing cancer.²⁹ The levels of MDA and SOD were significantly lower in treated groups compared to the Inductor Group. MDA is related to oxidative stress and oxidative degeneration, which is closely linked to the genesis of neoplasms and dysplasia. Joshy et al³⁰ found that reactive oxygen species are important in the pathogenesis of cancer, and cancer cells have many reactive oxygen species that induce malignant phenotypes, elevated levels of vascular endothelial growth factor, angiotensin-2, interleukin-6, tumor necrosis factor- α , and decreased activity of SOD and glutathione peroxidase.³⁰

PSA is a determinant of prostate cancer and is used to detect disease progression and monitor treatment response.³¹ *C. spinosa*

Table 4

Cytotoxicity and IC50 of *Chuquiraga spinosa* extract and 5-FU in DU-145 and 3T3 cell lines.

| Cytotoxic substances | IC50 (ug/mL) | | Selectivity index |
|----------------------|----------------|------------------|-------------------|
| | DU-145 | 3T3 | |
| <i>C. spinosa</i> | 2.98 \pm 0.2 | 51.4 \pm 0.4 | 17.24 |
| Control | — | — | — |
| 5-FU | 9.3 \pm 0.9 | 0.035 \pm 0.01 | 0.0037 |

Values are expressed as mean \pm standard deviation.

5-FU, 5-fluorouracil; IC50, 50% inhibitory concentration.

significantly lowered the levels of PSA in the treated groups with respect to the Induction Group. This could affect the progression of prostate cancer. The level of peripheral blood MNPCEs was significantly lower in the groups treated with *C. spinosa*. The presence of phytochemicals such as terpenes, steroids, and flavonoids may explain the results according to the findings of Casado et al.⁷

C. spinosa showed cytotoxic activity against the DU-145 cell line, exceeding 5-FU activity. However, the cytotoxicity for the 3T3 cell line was minor. It also showed a good security profile by having selectivity rates above unity. Similarly, the degree of dose–effect relationship was significant in all the cell lines. The ethanolic extract of *C. spinosa* presented a selectivity index of 17.24 compared to 0.0037 with 5-FU. Studies with silymarin showed that the alterations in the progression of the cell cycle could be responsible for its anticarcinogenic effect on DU145 prostate carcinoma cells.³² Resveratrol, a polyphenol, inhibits prostate cancer growth and alters mitogenesis. It achieved significant inhibition of the proliferation of DU-145 and PC-3 cells.³³

The groups treated with *C. spinosa* have lower volumes and weights because flavonoids in the plant produce greater activity of caspases 3 and 7 and these have apoptotic activity.

The preventive effect of ethanol extract of *C. spinosa* could be related to secondary metabolites such as tannins, phenolics, flavonoids, and alkaloids (Table 1). These metabolites have a cytoprotective effect.³² The test of antioxidant capacity *in vitro* was compared with trolox and vitamin C, the ethanol extract inhibited 90.3% DPPH radical for 50 μ g/mL. It demonstrates that the *in vitro* antioxidant activity (Fig. 1) goes together with the *in vivo* model.

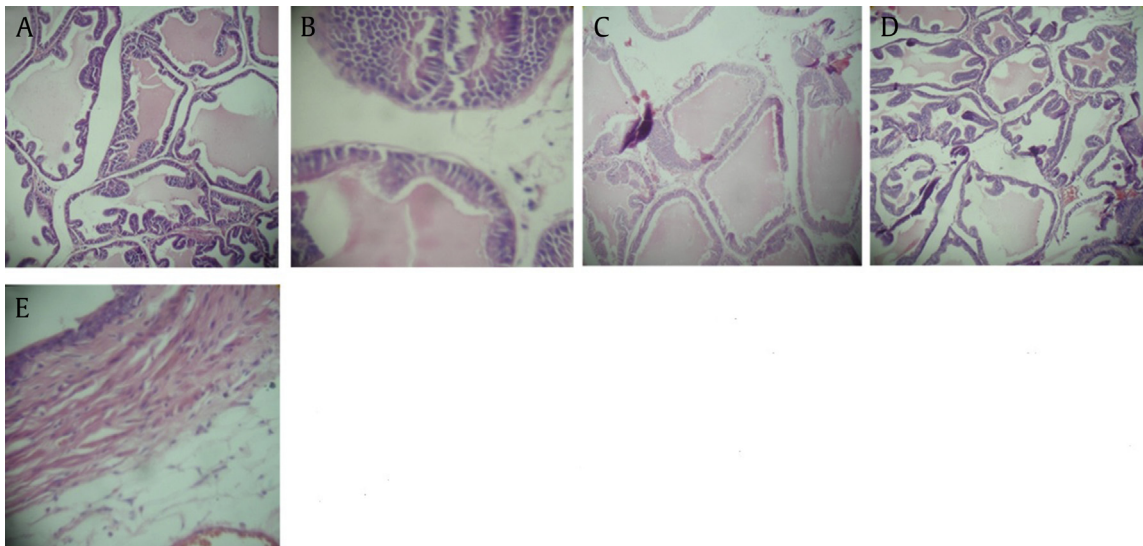


Fig. 4. Hematoxylin and eosin staining of prostates from control and experimental animals (400 \times). (A) P80: normal prostate. (B) Inductor Group: hypertrophy of cells, hyperplasia of glands, and large amount of dysplasia. (C) ChS50: mild reduction in glands with surrounding fibrosis. (D) ChS250: colloids and hypertrophy. (E) ChS500: glandular hyperplasia, cells of different sizes and thickened capsule. ChS50, ChS250, and ChS500, 50 mg/kg, 250 mg/kg, and 500 mg/kg *C. spinosa* ethanolic extract, respectively; P80, polysorbate 80.

This may be because the extract contains phenolic compounds of a flavonoid nature and have demonstrated their property free radical scavengers by donating electrons, it reduces the formation of lipid peroxidation by neutralizing the chain reaction in the formation of reactive oxygen species.³⁴

It is important to mention that a dose-dependent effect was not found. ChS250 had better results in prostate morphology compared to ChS50 and ChS500. The latter has a higher concentration of tannins, which could have an antinutritional effect. They form complexes with dietary components and alter digestive enzymes, preventing the absorption of other beneficial components.³⁵

The limitations of this study included the lack of determination of the metabolites present in the *C. spinosa* extract. Also, its exact mechanism of the extract is unclear. This animal model for prostate cancer was developed in a short time compared with other experimental models *in vivo*, which needed up to 1 year to show dysplasia.

The data indicate the chemopreventive potential in prostate cancer with a high therapeutic index of *Chuquiraga spinosa* extract. However, the complete mechanisms on which these effects are based are unknown. Further studies are needed to identify more precisely the active molecules involved in the protective effect described. Considering the anti-inflammatory, antioxidant, cytotoxic, and antigenotoxic properties of *C. spinosa* extract, we conclude that it has a protective effect on NMU-induced prostate cancer in rats.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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