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Original Article

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

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A B S T R A C T

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.

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 al[7] 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] Ethanolic 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 ± 2°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's[13] 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 Frankel[16] 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 Fridovich[19] 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.

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