Toxicology Reports 4 (2017) 226–233

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Amelioration of the cyclophosphamide induced genotoxic damage in mice by the ethanolic extract of *Equisetum arvense*

Jasbir Kour[a], Md Niamat Ali[a,*], Hilal Ahmad Ganaie[a,*], Nahida Tabassum[b]

[a] Cytogenetics & Molecular Biology Research Laboratory, Centre of Research for Development, University of Kashmir, Srinagar 190 006, JK, India
[b] Department of Pharmaceutical Sciences, University of Kashmir, Srinagar 190 006, JK, India

ARTICLE INFO

Keywords:
Equisetum arvense
Antimutagenicity
Chromosomal aberration assay
Mitotic index
GC–MS analysis

ABSTRACT

In the present study, we evaluated the potential of the plant *E. arvense* against the cytotoxic and mutagenic effects induced by cyclophosphamide (chemotherapeutic agent) in the bone marrow cells of mice using the Chromosome assay (CA) and Mitotic index (MI) *in vivo* as the biomarkers. The study was performed following 3 protocols: pre-treatment, simultaneous treatment and post-treatment with the ethanolic extract of the plant. The results demonstrated that the plant extract was not cytotoxic and mutagenic and has a protective effect against the mutagenicity induced by cyclophosphamide in pre, simultaneous and post treatments and against its cytotoxicity as well. Because of its ability to prevent chromosomal damage, *E. arvense* is likely to open an interesting field concerning its possible use in clinical applications, most importantly in cancer as a chemopreventive agent or even as a coadjuvant to chemotherapy to reduce the side effects associated with it.

1. Introduction

Medicinal plants have always been on the vanguard whether regarding the treatment of a number of ailments or even cancer. Over decades plants have been prized for their medicinal properties and used pragmatically as drugs, initially as traditional preparations and then as pure active principles, with this knowledge and practice being passed from generation to generation [1]. It has been suggested that the use of antimutagens/anticarcinogens in everyday life can be the most effective way to avert human cancer and genetic diseases [2]. The bioactive compounds in medicinal plants act as a blueprint to block or reverse carcinogenesis at early stages [3]. Moreover, they are considered to be an inexpensive, effective and easily applicable approach to control cancer [4]. Herbal medicines remain an important component of the health care system. Medicinal plants are the food supplements which have not only nutritional value but therapeutic value as well. The medicinal value of plants is due to the presence of secondary metabolites which includes alkaloids, saponins, terpenoids, flavonoids, tannins, sterols and phenolic compounds. Hence the importance of any plant lies in its biologically active principles. Almost four decades ago, the antimutagens were reported. Many reports have shown the rising trends of antimutagenic studies with the plant extracts. [5–7].

Medicinal plants and their extracts have been used by man from prehistoric times to cure various diseases and this has resulted in the discovery of some very important drugs. It is now been well established that the traditional herbal therapies contain a diverse array of chemopreventive agents as well [8].

Equisetum arvense, commonly known as the field horsetail or common horsetail (Sehetband or Brahmgund locally in Kashmir), is a very common, bushy perennial herb native to the northern hemisphere. It is a member of a very primitive family of plants. It is distributed throughout Canada and the USA except the southeast (Florida, Georgia, Alabama, Louisiana, Mississippi, and Tennessee), throughout Europe and Asia south to Turkey, Iran, the Himalayas and across China (except the southeastern part), Korea and Japan [9]. *Equisetum* is the only living genus of the order Equisetales and the class Sphenopsida. The plant mostly occurs in marshes, swamps, ditches, river banks, open fields, open woods, and fill areas, such as road sides, and railroad embankments.

Horsetail is a strange looking plant with creeping, string like rootstock and roots at the nodes that produce numerous hollow stems. Phytochemically, the plant is found to have a wide array of secondary metabolites which contribute to the medicinal properties of the plant.

The plant *Equisetum arvense* is a folk medicine and its extract is used locally to treat tuberculosis, edema, kidney and bladder stones, urinary tract infections, incontinence, acidity and dyspepsia, ulcers and wounds, bleeding etc. Reports are available regarding its anti-inflammatory, antinociceptive [10], antioxidant and antiproliferative [11], antimicrobial [12], hepatoprotective [13], antidiabetic [14], coagulant and astringent properties [15]. Horsetail is mainly used as a diuretic

* Corresponding authors.
E-mail addresses: mdniamat@hotmail.com (M.N. Ali), hilal.scholar@kashmiruniversity.net (H.A. Ganaie).

http://dx.doi.org/10.1016/j.toxrep.2017.05.001
Received 3 February 2017; Received in revised form 8 May 2017; Accepted 9 May 2017
Available online 11 May 2017

[16]. The ancient Greeks used horsetail in the treatment of wounds.

Mutagenicity/antimutagenicity assays have been used to identify potential antimutagens and anticarcinogens providing opportunities for the development of new drugs that can be used in the prevention and treatment of neoplasies and other genetic diseases [17]. Among the bioassays to assess DNA damage in mammalian cells in vivo, the chromosomal aberration test is an effective short term assay because the occurance of the chromosomal aberrations is one of the most important biological consequence of exposure of an organism to the genotoxic agent. *In vivo* mammalian chromosomal aberration test has been successfully used for the detection of structural aberrations induced by the test substance in the bone marrow cells of animals, usually rodents [18–20]. The advantages of the CA assay are related to the cell by cell approach, an accurate identification of most of the chromosome mutation types, a possible co-detection of mitotic indices and the precise scoring by image analysis.

There have been improvements in the cancer treatment which have increased the survival rates dramatically among patients in the recent time [21]. But the treatments like radiotherapy and chemotherapy have proven genotoxic and mutagenic and have led to the development of secondary, treatment related conditions. This includes further cancers, as well as predisposes survivors to various non-malignant diseases that can often evince in the offspring [22]. The efficacy of present chemotherapeutics has been limited by its toxicity and for the cells developing resistance against the therapy [23]. Studies on mice have also shown that anticancer drug exposure can result in the genomic instability acting across multiple generations [24]. Chemotherapy cancer treatment cases involve invariable usage of antineoplastic agents which kill neoplastic tissue as they are toxic to rapidly proliferating cells. However in the process, they can damage normal proliferating cells as well. This lowers their therapeutic index. Thus long term use of these antineoplastic agents has become a subject of increasing concern [25].

More than 100 chemotherapy drugs are used in treating cancer, either alone or in combination with other drugs or treatments. One such drug is Cyclophosphamide. Cyclophosphamide (CPA) which belongs to the class of oxazaphosphorines is an alkylating agent. It had been listed as one of the most successful chemotherapy drugs on the World Health Organizations List of Essential Medicines. Cyclophosphamide possesses marked immunosuppressant properties against both humoral and cellular immunity because of which it has been extensively used to treat a variety of childhood and adult malignancies [26–28] including breast cancer, lymphomas and leukemias, retinoblastoma, small cell lung cancer, ovarian cancer, sarcomas and multiple myeloma [29], since its initial synthesis in 1958. But over the time its use has been declining because of its adverse genetic effects. The chemical is activated by the hepatic P-450 cytochrome and its metabolites phosphoramide mustard and acrolein are linked to its antineoplastic and other toxic side effects [30,31].

CPA has been recommended to be used as a positive control chemical in genetic toxicity tests [32,33]. CPA has also been extensively tested by various mutagenicity/antimutagenicity assays to induce dominant lethal mutations, micronuclei, DNA damage and generation of free radicals or Reactive Oxygen Species (ROS) *in vivo* [2].

Thus based on all the pharmacological properties of *Equisetum arvense*, especially the antioxidant effect which is desirable for the antimutagenic property, in this study we evaluated the anticytotoxic and antimutagenic effects and its corresponding benefits to humans with the possible mechanisms of actionof this plant against the damages induced by antineoplastic agent CPA.

Certain plants, however, can also directly induce mutations and/or chromosome aberrations under certain conditions. It is reasonable that while some medicinal plants may suppress the effects of mutagens, others may have toxic or mutagenic effects [34]. However, it is very important to investigate any negative effect of the plants to the animals, if any. So the mutagenicity of the plant *E. arvense* was also studied before evaluating its antimutagenic potential.

2. Materials and method

2.1. Plant material

Equisetum arvense was collected at the sterile stage from Hajibal area of Baramulla district, 1577 mt above sea level, J & K. The plant was collected randomly from the area. The plant was identified and authenticated by the Centre of Biodiversity and Plant Taxonomy, Department of Botany, University of Kashmir. A specimen under voucher number KASH-2348 was preserved in the respective department for future reference.

2.2. Preparation of the extract

The aerial part of the sterile stem was air dried in the dark at 20 ± 5 °C for 12 days. The shade dried plant material was fine powdered using a domestic blender. After being macerated to fine powder, 1 kg powdered *Equisetum arvense* was subjected to hot extraction using the soxhlet apparatus. The powdered material was packed in the soxhlet apparatus and exhaustively extracted with ethanol at a desired temperature for 16–20 h continuously. The extract was filtered using Whatman's filter paper no. 1. The residue was discarded and the filtrate collected and concentrated to dryness under reduced pressure using Heidolph rotary evaporator (R-215). The final yield of the extract was also calculated and found to be 98.6 g (on dry weight basis of the crude material).

2.3. GC–MS analysis

GC–MS analysis of the extract was carried out with GCMS-QP2010 Plus, Shimadzu, Japan fitted with programmable head space auto sampler and auto injector. The capillary column used was DB-1/RTX-MS (30 m) with helium as a carrier gas, at a flow rate of 3 ml/min with 1 µl injection volume. Samples were analyzed with the column held initially at 100 °C for 2 min after injection, then increased to 170 °C with 10 °C/min heating ramp without hold and increased to 215 °C with 5 °C/min heating ramp for 8 min. Then the final temperature was increased to 240 °C with 10 °C/min heating ramp for 15 min. The injections were performed in split mode (30:1) at 250 °C. Detector and injector temperatures were 260 °C and 250 °C, respectively. Pressure was established as 76.2 kPa and the sample was run for 70 min. Temperature and nominal initial flow for flameionization detector (FID) were set as 230 °C and 3.1 ml/min, correspondingly. MS parameters were as follows: scan range (*m/z*): 40–650 atomic mass units (AMU) under the electron impact (EI) ionization (70 eV). The constituent compounds were determined by comparing their retention times and mass weights with those of authentic samples obtained by GC and as well as the mass spectra from the Wiley libraries and National Institute of Standards and Technology (NIST) database.

2.4. Selection of doses

Ethanol extract was dissolved in distilled water and the choice of the concentration was based on the maximum dose used in other experimental works on this plant i.e. 500 mg/kg of body weight (bw). The treatments were performed orally (gavage). The alkylating agent cyclophosphamide (CAS No. 6055-19-2, Himedia) was used as the positive control. It was diluted with water and administered intraperitonealy at a dose of 50 mg/kg of the body weight.

2.5. Animals and treatment

Swiss albino mice (*Mus musculus*), 5–6 weeks old and weighing 30 ± 5 g were randomly selected and supplied by the Indian Institute