Antimutagenic Activity of *Equisetum arvense* Against the Genotoxic Damage Induced by Cyclophosphamide in Mice.

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Abstract

The present study has evaluated the potential of the plant *E. arvense* against the mutagenic effects induced by cyclophosphamide (chemotherapeutic agent) in the bone marrow cells of mice using the CA *in vivo* as the biomarker. The study was performed following 3 protocols: pre-treatment, simultaneous treatment and post-treatment with the aqueous extract of the plant. The results demonstrated that the plant extract was not mutagenic and has a protective effect against the mutagenicity induced by cyclophosphamide. 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 chemo preventive agent.

Keywords: *Equisetum arvense,* anti mutagenicity, chromosomal aberration assay

Introduction

For centuries plants have been prized for their medicinal properties and used empirically as drugs, initially as traditional preparations and then as pure active principles, with this knowledge and practice being passed from generation to generation (Taylor *et al.,* 2001). It has been suggested that the use of antimutagens and anticarcinogens in everyday life can be the most effective procedure for preventing human cancer and genetic diseases (Ferguson, 1994). The antimutagens have been reported almost four decades ago. There have been many reports showing the rising trends of antimutagenic studies with the plant extracts. (Khader *et al*., 2010; Chen *et al*., 2011; El-Sayed and Hussin, 2013).

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. 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 (Monte *et al.,* 2004), antioxidant and antiproliferative (Dragana *et al.,* 2010), antimicrobial (Fathi *et al.,* 2004), hepatoprotective (Oh *et al.,* 2004), antidiabetic (Safiyeh *et al.,* 2007), coagulant and astringent properties (Clute, 1928). Horsetail is mainly used as a diuretic (Jung *et a..*, 1999).

In this study we evaluated the antimutagenic effects of the plant against the damages induced by antineoplastic agent CPA.

Materials and Method

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 identified and authenticated by the Centre of Plant Biodiversity and Taxonomy, Department of Botany, University of Kashmir. A specimen under voucher number KASH-2348 was preserved in the respective department for future reference.

Preparation of the extract

The aerial part of the sterile stem was air dried in the dark at 20±5ºC for 12 days and fine powdered using a domestic blender.1 kg powdered *Equisetum arvense* was soaked overnight in 500 ml of distilled water at room temperature with constant stirring. Next morning the extract was filtered over muslin cloth and the filtrate was centrifuged at 5000 rpm for 10 mins at room temperature. The extract was dried using slow heating method to obtain completely dried powder.

Selection of doses

Aqueous extract was dissolved in distilled water and the concentration given was 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.

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 of Integrative Medicine (IIM). The mice were housed in polypropylene cages under the controlled light and temperature conditions and were given food (commercial mouse pellets) and water *ad libitum* throughout the experiment. The experiment consisted of 6 groups: the treatment groups (pre, simultaneous and post and aqueous extract alone treatment group), the positive control (cyclophosphamide), the negative control/vehicle (distilled water). Each group had 5 animals selected randomly irrespective of the sex.

Chromosome aberration assay in mouse bone marrow

Chromosome preparations were made from mitotically active bone marrow cells following the techniques of Preston *et al.*, (1987). Two hours prior to the tissue sampling, animals were injected intraperitonealy with 0.4 ml of 0.05 % colchicine, to arrest the metaphase stage. The mice were then euthanized using the cervical dislocation method. The euthanasia was held 24 h after the particular treatment ended. Bone marrow was extracted from the femurs and the slides were prepared. The slides

having brightly stained well spread metaphase chromosomes were independently coded and observed under the microscope for CA analysis

Statistical analysis

Statistical analysis was performed with the SPSS (version 20) computer program. To analyze the relationship between the variables, Mann-Whitney U test was performed ($p \leq 0.05$).

Results

Table 1 summarizes the results of the % chromosomal aberrations as well as the percentage of damage reduction by the plant extract following the protocols of pre-treatment, simultaneous treatment and post-treatment and recorded alone

Table 1: Chromosomal aberrations observed during aqueous extract treatment in the bone marrow cells of CPA treated mice.

Treatment group	DOSE mg/kg	TMS	Chromosomal aberrations %							Total abb. Mean(%) ± SD	% Reduction
			Frg	Cr	Csb	Ctb	Cg	St	Ex		
Distilled water (NC)	--	500	2.4	3	0.6	3	1.6	0.2	-	9.2±0.84	-
Cyclophosphamide (PC)	50	500	6	16.2	20.6	21.2	7.2	4.2	0.6	8.8±1.48	-
Aq-EA alone (gavage)	500	500	2	2.2	2	2	1.4	1.2	0.4	9.8±0.89[1]	-
Aq-EA pre-treatment (gavage)	500	500	15	1.2	0.6	2.2	1.2	0.2	0.4	9.6±1.14[a]	65.77
Aq-EA sim-treatment (gavage)	500	500	10.8	5.6	0.4	3	1.8	1.6	0.6	22±1.87[a]	78.52
Aq-EA post-treatment (gavage)	500	500	11.4	7.2	0.6	3	1.2	1.8	0.8	24.8±1.64[a]	73.82

Aq: aqueous extract, EA: E.arvense, TMS: total metaphasic plates studied, Frg: fragment, Cr: chromosomal ring, Csb: chromosome break, Ctb: chromatid break, Gp: gap, St: sticky chromosomes, Ex: exchange. Values with different letter superscript differ significantly (p <0.01: highly significant) from the positive control whereas values with numeric superscript do not differ significantly (p<0.01: highly significant) from the negative control (Mann Whiteny U Test).Gaps have been mentioned but not included in the total aberrations.

During treatment using aqueous extract alone no significant difference from the negative control was shown by the extract given alone in chromosomal aberrations. In Pre-treatment all the animals treated with the aqueous extract of the plant reduced the frequency of the chromosomal aberrations compared to the positive control group indicating its antimutagenic activity. The percentage of reduction of chromosomal damage of the group treated with aqueous extract of the plant was observed to be 65.77%. Animals treated with the extract and CPA simultaneously showed the highest reduction in chromosomal aberration frequency (78.52%) induced by the cyclophosphamide compared to the other two treatment groups. Animals in the post treatment group also reduced the chromosomal damage. The percentage reduction in the chromosomal damage was observed to be 73.82 %.

Discussion

After several decades of research, antimutagenic effect of many naturally occurring compounds extracted from plants has been well established in bacteria and mammalian cells (Ferguson, 2011). Plants like *Acacia salicina* (Boubaker *et al.,* 2011), *Mangifera indica L.* (Morffi *et al.,* 2012), *Phellinus rimosus* (Ajith and Janardhanan, 2011), *Terminalia arjuna* (Kaur *et al.,* 2010), *Uncaria tomentosa* (Caon *et al.,* 2014), *Heterotheca inuloides* (Ruiz-Perez *et al.,* 2014), *Ajuga bracteosa* (Ganaie H.A. *et al.,* 2016) and many more have shown effective antimutagenic properties. Although an antimutagen is not necessarily an anticarcinogen, yet it is an indication of a possible anticarcinogen (DeFlora and Ferguson, 2005; Reid *et a..,* 2006). It was reported previously that several antitumor compounds act through the antimutagenic mechanism (Tsai *et al.,* 1996; Dion *et al.,* 1997; Ikken *et al.,* 1999). Hence, searching for antimutagenic compounds represents a rapidly expanding field of cancer research (Heo *et et al.,* 2001; Ferguson and Philpot, 2008; El-Sayed and Hussin, 2013; El-Sayed *et al.,* 2013).

In the present study mice treated with CPA showed significant ($p<0.001$) increase in aberrant metaphases, CAs (including and excluding gaps). The aqueous extract when given alone did not influence the parameters measured. It was not mutagenic at the time of treatment to the mice.

The use of a simultaneous treatment appeared to identify mechanisms with direct action on the mutagen by inactivating it, which may be classified as desmutagenicity effect (Kada, 1983). Previously, it has been reported that some drugs, dietary components and endogenous biochemicals can function as antimutagens by altering the rates of mutagen absorption and uptake (Waters *et al*., 1998). However, the present experimental design does not rule out the possibility of indirect effects of the *E. arvense* extract during CP exposure. The post treatment could show its antimutagenic potential by playing a role in optimization of DNA repair. The conclusion obtained in the pre-treatment could reflect the effects on the prevention of DNA damage by affecting metabolic pathways, being antioxidant or acting on DNA replication. These action mechanisms, occurring in both pre- and post- treatments, could be called bio-antimutagenicity (Morita *et al.,* 1978) or fidelogenesis. A great variety of antimutagenic agents act through multiple mechanisms to provide protection against diverse mutagens. Noteworthy, the ability of compounds to affect mutagens simultaneously in several different ways significantly increases antimutagenic effectiveness. Hence, searching for such multifunctionally acting antimutagens is of great importance.

Conclusion

A clear negative effect on the induction of chromosomal aberrations by the aqueous extract of *E. arvense* was found. The results of the present study clearly showed that the aqueous extract of *E. arvense* had an antimutagenic and anticlastogenic potential against the mutagenic activity of cyclophosphamide in mice. However, further studies are needed in other test systems so that in the future *E. arvense* can be used in reducing the occurrence of cancers or even as a coadjuvant to chemotherapy to reduce its side effects.

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