

Research Article

ANTIMUTAGENIC PROPERTIES OF MENTHA PIPERITA EXTRACT AGAINST ETHYL METHANE SULPHONATE INDUCED MUTAGENICITY IN MUS MUSCULUS

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ABSTRACT

The antimutagenic properties of an aqueous extract of *Mentha piperita* were evaluated against a known alkylating agent, ethyl methanesulphonate (EMS) in Swiss albino mice (*Mus musculus*). The oral administration of *Mentha* extract (ME) showed a significant reduction in the number of micronuclei as well as other chromosomal abnormalities in newborn Swiss albino mice with respect to the reference group (EMS-alone). The micronuclei reduction was significant from 1.27% to 0.40%. The mutagenicity was induced in Swiss albino mice (*Mus musculus*) before giving *Mentha piperita* extract by injecting once intraperitoneally ethyl methanesulphonate (<300mg/kg bodyweight). The results in both cases were compared with the control group, the animals of which were given double distilled water for six weeks (after weaning) by oral gavages. *Mentha piperita* extract showed a significant chemopreventive action against EMS induced mutagenicity.

Keywords: Antimutagenesis, Mutagenesis, *Mentha piperita*, Ethyl methanesulphonate, Micronucleus.

INTRODUCTION

The use of plant based natural products as chemopreventive agents is drawing a lot of attention and considered to be practically beneficial in certain cell/tissue based systems and animal model systems. It is necessary to provide scientific proof to justify the use of a plant or its active principles for medicinal purposes¹. Modern drugs, plants and plant extracts must be characterized after their pharmacological screening for their pharmacokinetic and pharmacodynamic properties, including toxicity². Cancer chemoprevention is defined as the use of chemicals or dietary components to block, inhibit, or reverse the development of cancer in normal or preneoplastic tissue. A large number of potential chemopreventive agents have been identified and they function by mechanisms directed at all major stages of carcinogenesis^{3, 4, 5}.

Mentha piperita Linn (Family, Labiatae) is aromatic and has stimulant and carminative properties. It currently is being used for alleviating nausea, flatulence and vomiting⁶. *Mentha* extract has antioxidant and antiperoxidant properties^{7, 8, 9}. *Mentha* extract and its oil also showed antibacterial and antifungal activities against *Pseudomonas solanacerum*, *Aspergillus niger*, *Alternaria alternata* and *Fusarium chlamydosporum*, respectively.¹⁰ Vokovic-Gacic and Simic¹² showed that *Mentha* extract could enhance error-free repair of DNA damage. Amman *et al.*¹³ reported that *Mentha piperita* has a chemopreventive effect against the tumorigenicity of Shamma and that this activity could be due to antimutagenic properties. It has been reported that *M.piperita* leaf extract provides protection against radiation-induced alterations in intestinal mucosa¹⁴,

chromosomal damage in bone marrow¹⁵ and blood irradiated mice¹⁶.

Recent focus of cancer chemoprevention is on intermediate biomarkers capable of detecting early changes that can be correlated with inhibition of carcinogenic progression. Short term tests such as the mouse bone marrow micronucleus assay, chromosomal aberration analysis, assessment of antioxidant status and reactive oxygen species (ROS) - induced lipid peroxidation, are widely used in the detection and evaluation of antimutagens and anticarcinogens^{17, 18}. Therefore the present investigation was undertaken to evaluate the antimutagenic activity of *Mentha piperita* leaf extract on ethyl methanesulphonate-induced mutagenicity in Swiss albino mice.

MATERIALS AND METHODS

Swiss albino mice (*Mus musculus*) 6-8 weeks old were brought from Regional Unani Research Centre, University of Kashmir, Hazratbal Srinagar and maintained as an inbred colony. New born mice (<24 hr old) of both the sexes were used for the experiments. The animals were maintained at a temperature of 24°C-27°C and housed in polypropylene cages. After weaning at 3 weeks of age, the animals were fed standard mouse feed (Hindustan Lever, Delhi, India) and provided tap water.

Plant material *M.piperita* Linn. Was collected locally and identified. Freshly collected leaves were air dried, powdered and extracted with double-distilled (DDW) water by refluxing for 36 hrs (12h×3) at 80°C. The extract was vacuum evaporated to a powder. The extract was dissolved in DDW just before oral administration.



Ethyl methanesulphonate (EMS) was purchased from B.M. Scientific Medicates, Karan Nagar, Srinagar and was kept at a temperature below 25°C. Before injecting intraperitoneally, EMS was dissolved in Hank's solution.

Experimental Design

Mice selected from inbred colony were divided into four groups.

Group-I: The animals in this group were administered DDW for three consecutive days to serve as normal.

Group-II: The animals in this group received leaf extract of *M piperita* orally (1g/kg body weight once daily) for six weeks by oral gavage.

Group-III (EMS) This group of animals was injected intraperitoneally with 60mg EMS/kg body weight dissolved in 0.5 ml of Hank's solution.

Group-IV (EMS+ME) the animals in this group were injected intraperitoneally with the same dosage of EMS as in group-iii. After weaning, ME was administered for six weeks by oral gavage as in group-ii

Cytogenetic Studies

(1) Chromosomal Aberration Analysis

Chromosomal aberration analysis in bone marrow cells was performed at the end of experiment. The animals were injected intraperitoneally with 0.1 ml of 0.025% colchicine and sacrificed 2 hours later by cervical dislocation. Both the femurs were dissected out, and the bone marrow was aspirated from both of them, washed, treated hypotonically with 0.6% sodium citrate, fixed in 3:1 methanol: acetic acid and dried. The slides were

prepared and stained with 4% Giemsa stain for 10 minutes. Metaphase plates were prepared by air drying method¹⁹. Chromosomal aberrations were scored under a light microscope. A total of 400 metaphase plates were scored per animal for chromosomal breaks, chromatid breaks, fragments, rings, exchanges and dicentric.

(2) Micronucleus Assay

The method of Schmid²⁰ was employed the bone marrow was employed for the micronucleus assay. After the 9 week treatment protocol, the femurs were dissected out and the bone marrow was flushed out, mixed with a vortex mixer and the cells pelleted by centrifugation at 1100 r.p.m for 7 minutes. The pellet was treated hypotonically with 0.6% sodium citrate, centrifuged twice, fixed in 3:1 methanol: acetic acid and dried. The slides were prepared and stained with 4% Giemsa stain for 10 minutes and air dried. The proportion of polychromatic erythrocytes and normochromatic erythrocytes was estimated from a minimum of 2000 erythrocytes. The micronuclei in these cells were scored and reported as micronuclei per 100 cells.

RESULTS

New born Swiss albino (<24h old) which were injected intraperitoneally once with 60mg of EMS/kg body weight dissolved in 0.5ml of Hank's solution showed significant number of micronuclei and other chromosomal aberrations in comparison to the control group. However when *Mentha* extract was administered after EMS treatment in another group of albino mice, the micronuclei incidence was significantly reduced from 1.27% to 0.4% (Table 1).

Table 1: Comparison of micronuclei in bone marrow cells of Swiss albino mice due to EMS alone and EMS+ME.

Compound	Lethal dose (mgkg ⁻¹ bw)	Dose-treated (mgkg ⁻¹ bw)	No. of Animals	No. of cells analyzed	Erythrocytes with micronuclei	Micronuclei percentage
		Control	03	3013	04	0.13
EMS	300	60	04	4001	51	1.27
EMS + ME		60	04	4005	16	0.40

Table 2: Protective effect of *Mentha* extract on EMS-induced micronucleus frequency in Swiss albino mice, evaluated using Student's t-test (p<0.001)

Treatment Group	No. of micronuclei/1000 cells
Group I: Control	0.28 ± 0.02
Group II: ME	0.24 ± 0.02
Group III: EMS	20.82 ± 1.81 ^b
Group IV: EMS + ME	2.92 ± 0.72 ^b

Group I, DDW alone; Group II, *Mentha* extract alone; Group III, DDW + EM; Group IV, *Mentha* extract + EMS.

^bP < 0.001.

The above statistical comparisons were made between Group I verses Group II; Group III verses Group I; Group III verses Group IV at level of significance <0.001



Table 3: Protective effect of ME on EMS – induced chromosomal aberrations in new born Swiss albino mice (using Student's t-test)

Treatment group	Chromatic breaks (%)	Chromosome breaks (%)	Centric rings (%)	Dicentrics (%)	Exchanges (%)	Fragments (%)
Group I:Control	0.16±0.06	0.00± 0.00	0.00± 0.00	0.00 ±0.00	0.00± 0.00	1.10± 0.37
Group II:ME	0.14±0.05(n.s)	0.00±0.00(n.s)	0.00±0.00(n.s)	0.00±0.00(n.s)	0.00±0.00(n.s)	0.98±0.32(n.s)
Group III:EMS	11.20± 1.3 ^b	6.72± 0.70 ^b	1.42± 0.28 ^b	2.98± 0.62 ^c	1.48± 0.28 ^b	128.42± 7.60 ^b
GroupIV:EMS+ME	1.84± 0.42 ^b	1.20± 0.0 ^b	1.02± 0.02(n.s)	0.94± 0.01 ^a	0.22±0.01 ^c	4.80±1.32 ^b

Each value represents SE/Cell. 400 metaphases were scored per animal.

Statistical comparisons: Group IV versus Group II, Group III versus Group I; Group III versus Group IV.

Level of significance; ^ap<0.05, ^bp<0.001 and ^cp<0.005; n.s(non significant).

A single intraperitoneal injection of EMS (60mg/kg body weight dissolved in 0.5ml of Hank's solution) to Swiss albino mice resulted in significantly increased chromosomal anomalies in bone marrow cells. In group III, significant increases were observed for chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments. The frequency of micronuclei/1000 cells in the group III animals were 20.82±1.81 (compared with a frequency of 0.82±0.02 in the group I control (Table 2).

A treatment with EMS followed by ME (Group IV) resulted in a significant decrease in chromosomal aberrations and micronucleus frequencies compared with those found in EMS-alone group (Table 3).

DISCUSSION

Alkylating agents comprise an important class of genotoxins and the most extensively studied member of this class of chemicals is EMS. EMS is mutagenic in both prokaryotic and eukaryotic test systems, and is an animal carcinogen^{21,22}. It is responsible for converting purine, guanine into O⁶-ethylguanine and results in mis-match base pairing in DNA and hence causes mutation and resulting formation of micro-nuclei and other chromosomal aberrations. The present study demonstrated that oral administration of ME has chemo preventive and anti mutagenic effects against EMS in Swiss albino mice. The ME produced significant reductions in the frequency of chromosomal abnormalities induced by EMS.

Micro-nuclei arise as a consequence of clastogenic or aneugenic action and this end point is widely used to evaluate the genotoxic potential of test agents^{23,24}. Reactive oxygen species (ROS) are usually formed during EMS metabolism. The ROS are highly damaging and their potential for oxidative stress coupled with a deficiency in host anti-oxidant defense mechanisms that was observed in the present study might be important factors contributing to the increase in bone marrow micro-nuclei and chromosome aberrations. Treatment with *Mentha* extract effectively reduced the frequency of EMS-induced bone marrow micro-nuclei as well as the extent of hepatic per oxidation and hence enhanced the anti oxidant status.

The result of the present study showed that pre-treatment of leaf extract of *M. piperita* protects mice from EMS induced mutagenicity by protecting hematopoietic damage to bone marrow. This was observed due to significant decrease in micro-nucleus frequencies compared to those found in Group- III (EMS alone). Damage to the chromosomes is manifested as breaks and fragments which appear as micro-nuclei in the rapidly proliferating cells²⁵. Enhancement in the frequency of micro-nuclei and chromosomal aberrations has also been reported in the bone marrow of mutated mice^{26,27}. It has been reported that *M. piperita* contains anti-oxidants like caffeic acid, rosmarinic acid, eugenol and α -tocopherol. The possible mechanism of chemoprevention by leaf extract of *M.piperita* may be by stimulating /protecting the hematopoietic stem cells against EMS induced free radical damage. The result of the present study suggest that the protective effects of leaf extract of *M. piperita* against EMS induced chromosomal damage in bone marrow may be attributed to the strong anti-oxidants present in the leaf extract of said plant .

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