



GENOTOXIC EFFECTS OF ETHYL METHANESULPHONATE (EMS) IN RATTUS NORVEGICUS

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ABSTRACT

The genotoxic effect of ethyl methanesulphonate, a potent alkylating agent, under various concentrations on bone marrow erythrocytes of *Rattus norvegicus* was studied by means of micronucleus test. 300 mg of EMS per kilogram body weight proved to be lethal. Micronuclei were easily seen and in different controls the micronuclei were found a little 0.1%. The dose effect relationship was obtained. In treated group, incidence of micronuclei was 0.4 to 2.1%. The mutagenic property of EMS at different doses was discussed with respect to micronuclei production from chromosomal aberrations. 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. 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.

Keywords: Ethyl methanesulphonate; Genotoxicity; Micronuclei; *Rattus norvegicus*.

INTRODUCTION

Many alkylating agents have been found to exert a mutagenic effect on a great variety of organisms. A variety of techniques have been developed for the detection of chemical mutagens using different animal systems including bacterial and mammalian system (reviewed by Hollander, 1971-1976)¹. Since a human being can't be used in a direct test, a mammalian in vivo test system is very desirable. Almost all mammalian chemical mutation work has been carried out in the mouse. A rapid screening method for chromosomal aberration by known chemical mutagens or other drugs involves micronucleus test. Erythrocyte micronuclei represent the consequences of chromosomal aberrations induced during preceding mitotic divisions of erythroblasts. Chromosomal fragments lag in anaphase and form micronuclei in the cytoplasm of daughter cells which are clearly visible after these cells have expelled their nuclei. Micronucleus test was reported by Boller and Schmid (1970)², Matter & Schmid (1971)³, Schmid *et al.*(1971)⁴, Tatsuro *et al.*, (1972)⁵, Heddle(1973)⁶, Von Ledebure and Schmid (1973)⁷, Matter and Grauwiler⁸ (1974), Schmid (1973,1975)⁹. Micronucleus testing procedure has a number of important advantages over the analysis of bone marrow metaphase chromosomes. Micronuclei are quite distinct, easily scored structures, and persist for some time and so may be scored at any stage of the cell cycle. Since most of the cells of a tissue are in interphase, there is virtually no time spent finding cells with micronuclei to score. Their recognition is technically much easier than the direct scoring of mitotic chromosomes and is much more rapid. Finally the preparation can be made very simply and rapidly. Boller and Schmid (1970)¹⁰ compared the incidence of nuclear anomalies with the incidence of chromosomal aberrations with strong alkylating mutagens in Chinese

hamster bone marrow cells. They confirmed a high degree of correlation between the frequency of chromosome aberration and the frequency of young polychromatic erythrocytes containing micronuclei. The present study was undertaken in order to evaluate the mutagenic potential of EMS in *Rattus norvegicus* by measuring the frequencies of micronuclei in the bone marrow cells for a known alkylating agent, Ethylmethanesulphonate which proved to induce structural chromosomal aberrations in vivo and in vitro.

MATERIALS AND METHODS

Newly born *Rattus norvegicus*, 4-8 weeks old, weighing approximately 50 gms brought from Regional Unani Research Institute, University of Kashmir, Hazratbal Srinagar, were used in this experiment. They were housed in Polypropylene cages and were maintained at a temperature of 24±3°C. They were fed standard mouse feed (Hindustan Lever, Delhi, India), and provided tap water. Ethylmethane sulphonate was obtained from B.M Medicates, Karanagar Srinagar; Kashmir. EMS was dissolved in Hank's solution and injected intraperitoneally in various doses on two consecutive days. Six hours after second treatment, the rats were sacrificed by cervical dislocation. Another group of *Rattus norvegicus* was given no treatment and were only fed standard mouse feed and tap water and was kept as control group. Two hours before sacrificing, the animals were intraperitoneally injected with 0.5ml of 0.03% colchicine. From the freshly killed rats, both femurs were removed and freed from muscles by the use of gauze and fingers. The proximal end of the femur was carefully cut with scissors until a small opening to the marrow canal became visible. Bone marrow was flushed into a test tube, washed in saline and treated hypotonically with 0.6% sodium citrate. The bone marrow containing test tubes were centrifuged at 1000



rpm for 7 minutes. From the pellet smears were made on slides, air dried and fixed with 3:1(methanol: glacial acetic acid) and air dried again. The slides were stained with 4% Giemsa stain for 10 minutes, washed with distilled water, dehydrated and mounted. Chromosomal aberrations in the form of micronuclei were scored from different groups of rats treated with different doses of EMS under microscope. The results so obtained were compared with the control group. About 1,000 erythrocytes were examined in each experimental animal and control. Micronuclei were recorded in polychromatic as well as monochromatic erythrocytes.

RESULTS

A toxicity test was performed for EMS, to select dose useful in mutagenesis. The concentration of EMS greater than 250 mg/kg body weight killed practically all the animals. The treated dose range, lethal dose and the

result of micronuclei test was indicated in table1. The scored elements are the micronucleated cells and not the number of micronuclei. Young immature erythrocytes (polychromatic) stain differently from older forms. They don't stain red but bluish and they are round with a diameter of about 1/20 to 1/5 of an erythrocytes (fig.1). Micronuclei in immature white cells are not easily distinguished from nuclear lobes or projection. Therefore, they were neglected in scoring in this study. Micronuclei in polychromatic erythrocytes constituted the major proportion in this experiment. An increase in the frequency of polychromatic erythrocytic micronuclei was found with higher doses (Figure 2). Micronuclei percentage in the erythrocytes of control group was just 0.13% and it increased to 2.10% in the group treated with 250mg EMS/kgbw (Table 1). The dosage above 250mg EMS/kgbw proved to be lethal and almost killed all animals.

Table1: Result of the micronuclei test in various groups of *Rattus norvegicus* treated with different doses of EMS (mg/kg/d)

Compound	Lethal dose (mg/kg)	Dose treated (mg/kg/bw)	No. of animals used	No. of cells analysed	Erythrocytes with micronuclei	Micronuclei % age
Ethyl methane sulphonate	300	Control	3	3,013	4	0.13
		100	4	4,005	16	0.40
		150	4	4,046	29	0.72
		200	4	4,001	51	1.27
		250	4	4,003	84	2.10

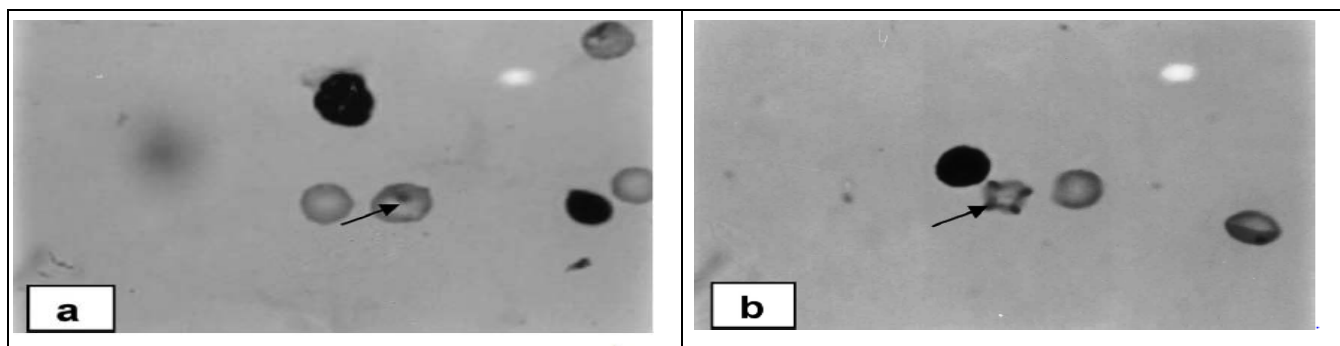


Figure 1: EMS induced micronuclei formation in bone marrow cells of *Rattus norvegicus*

- (a) micronucleated polychromatic erythrocyte (arrow)
- (b) multiple micronucleated polychromatic erythrocyte (arrow).

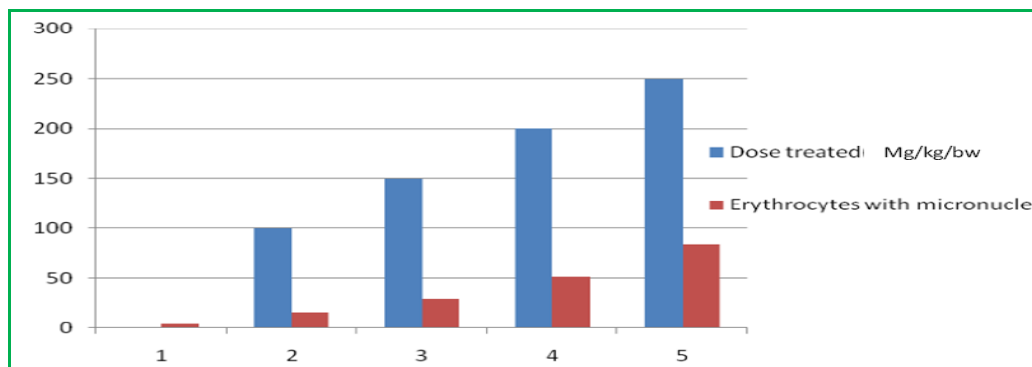


Figure 2: Graph showing increase in the frequency of micronuclei with increase in the concentration of EMS in *Rattus norvegicus*.

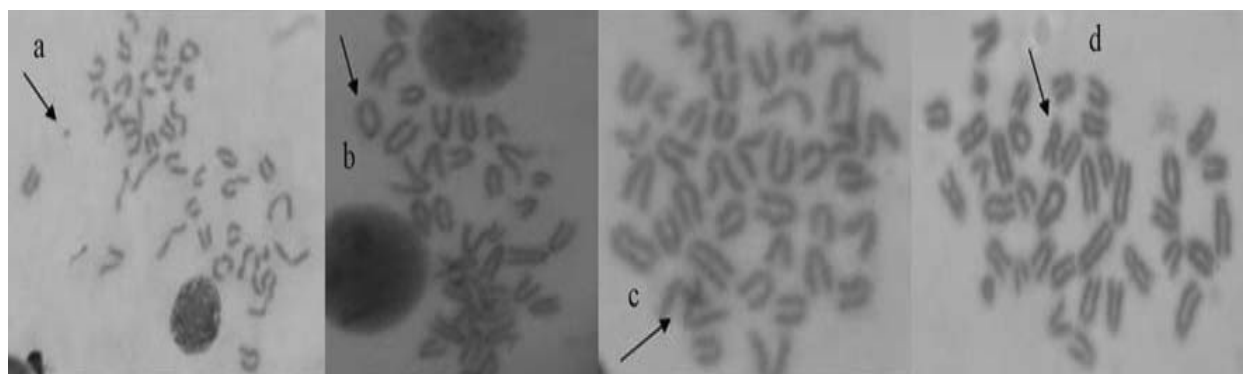


Figure 3: Chromosomal abnormalities induced by EMS in bone marrow cells of *Rattus norvegicus*. (a) Chromatid break, (b) Ring chromosome, (c) chromatid exchange, (d) Dicentric chromosomes.

DISCUSSION AND CONCLUSION

Many drugs or other chemicals, including alkylating agents, have mutagenic activity and they were reported to induce chromosomal aberrations (Cattanach *et al.*, 1968¹¹; Fishbein *et al.*, 1970¹²; Vogel and Rohrborn, 1970¹³; Arakaki and Schmid, 1973¹⁴; Goetz *et al.*, 1975¹⁵; Kihlman, 1975¹⁶; Kirkland and Venitt, 1976¹⁷; Sanger and Eisen, 1976¹⁸; Ishidate and Odashima, 1977¹⁹). The monofunctional alkylating agent EMS has been shown to be highly effective mutagen in a wide variety of organisms. It induces point mutation in barley (Favret, 1960)²⁰ and maize (Neuffer and Fiscor, 1963²¹; Amano and Smith, 1965²²). Also, dominant lethal mutation and translocations have been detected in the offspring of male mice treated with EMS (Partington and Jackson, 1963²³). Moutschen (1965)²⁴ described the chromosome breaking ability of EMS in *Vicia* and she later reported that EMS is efficient in inducing structural changes in chromosomes of mouse (Moutschen, 1969²⁵). Even though EMS can cause chromosome breakage, this is far less impressive in *Drosophila* so that it can produce only a very few translocations (Lim and Synder, 1968²⁶). Cattanach *et al.* (1968²⁷) reported that the same dose of the chemical induced high frequencies of translocations among the F₁ offspring in mice.

Chromosomal changes comprise a major part of the genetic danger to man. However in the past scoring chromosome aberrations have been a time consuming step. Boller and Schmid (1970²⁸) compared the incidence of micronuclei with the incidence of chromosomal aberrations and showed a high degree of correlation. The micronucleus assay system is highly reproducible, very economical; procedure is very fast and requires much less skill on scoring than conventional metaphase chromosome spreads. Furthermore, the reliability of metaphase chromosome analysis in bone marrow proves that there are too small a number of metaphase and those few chromosome spreads don't permit a satisfactory evaluation of the karyotype. Another problem of evaluation of chromosomal abnormalities may be the gap counting. Although some authors show that gaps represent a type of abnormality very characteristic for chemical mutagens (Gibhart, 1971²⁹), only a true break is

likely to give rise to an acentric or fragment and this will appear at the next division as a micronucleus. A gap will either be cured or persist with chromosomal continuity partially intact when no micronucleus will be formed. One way to distinguish between gaps and breaks, then, would be to establish an elevated level of micronuclei formation after treatment with various chemicals. However, the success of micronucleus assay in experiments does not mean that it can replace metaphase analysis in all situations. In many experiments changes in the types of aberrations, changes in the mitotic rate or the proportion of cell death cannot be detected by micronuclei test.

Scoring chromosome lesions by micronuclei test is difficult to quantitate, if their incidence differs from the control values. Negative results or a low incidence with a micronucleus test will not always prove lack of mutagenicity. Some compounds are strong mutagens in lower organisms, mammalian cells *in vitro*, and the host mediated assay and are carcinogenic in mammals (Fishbein, 1970³⁰) yet cause no production of micronuclei in bone marrow erythrocytes. Also it is possible that disparity reflects a dose phenomenon, e.g., the administration low dose of chemicals in which micronuclei can be seen in the absence of chromosome breakages. According to von Ledebur and Schmid (1973³¹) micronucleated polychromatic erythrocytes to a given total number of erythrocytes are very efficient in the range of low mutagenic effects. However, dose effect studies, comprising very high mutagenic effects, require application of both the polychromatic and monochromatic erythrocytes scoring methods. Cattanach *et al.* (1968³²) reported that 240mg/kg of EMS produces a substantial number of translocations. However, Heddle (1973³³) found only an insignificant increase in the frequency of micronuclei with 150mg/kg of EM. In our study, there were only 0.72% of micronuclei with 150mg/kg of EMS but with 250mg/kg they were increased to 2.10%. Chromosomal aberrations also increased with increase in EMS dosage (Fig.3).

The micronuclei test will provide the basis for evaluating unknown drugs and pollutants with regard to their mutagenic effects. However, in order to examine further cytogenetic effects of those compounds, the metaphase

analysis of mouse bone marrow cells *in vivo* and of human peripheral lymphocytes *in vitro* test for evaluation of chromosome aberrations are needed.

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