# GENOTOXIC EFFECT OF METHOTREXATE ON BONE MARROW CHROMOSOMES AND DNA OF MALE ALBINO MICE *Mus musculus*

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TO


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# APPROVAL SHEET

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# ABSTRACT

Methotrexate (MTX), a chemical analogue of folic acid, is an antineoplastic, antirheumatic and antipsoriatic drug which is used in a variety of clinical schedules and combination therapy regimens in man. The present work is mainly concerned with the study of the genotoxicity of methotrexate on bone marrow chromosomes and DNA content of male albino mice *Mus musculus.*

Sixty male albino mice, 16-18 weeks old weighing approximately mean 24 ± 2 g were used in the present study and randomly divided into four groups; one control and three methotrexate treated groups. Each group consisted of fifteen mice. The control group was injected intraperitoneally with 1ml/kg b.wt. distilled water, the solvent of methotrexate, while treated group (1) was intraperitoneally injected with methotrexate 2.5 mg/kg b.wt., single dose at the first day of the experiment and sacrificed by cervical dislocation after 24, 48 and 72 hour of treatment, treated group (2) was intraperitoneally injected with methotrexate 5 mg/kg b.wt., single dose at the first day of the experiment and sacrificed by cervical dislocation after 24, 48 and 72 hour of treatment and treated group (3) was intraperitoneally injected with methotrexate 10 mg/kg b.wt., single dose at the first day of experiment and sacrificed by cervical dislocation after 24, 48 and 72 hour of treatment.

Results of the present study indicated that the three tested doses of methotrexate induced structural and numerical chromosomal aberrations in bone marrow cells of the treated mice which were highly significant increased ($P < 0.001$) by dose and time of treatment. Structural aberrations were chromosomal and chromatid gap, fragment, centromeric attenuation, deletion, centric fusion, ring formation, end to end association and beaded chromosomes. While, numerical aberration was in the form of polyploidy.

Also, methotrexate treatment decreased the mitotic index in bone marrow cells of treated mice with 2.5, 5, 10 mg MTX/kg b.wt. and after 24, 48 and 72 hour of treatment in comparison with the control by increasing dose and time of treatment. A little different average of percentages of mitotic indices (18 % & 14 %) was observed between control & group 1 after 24 hr of treatment, respectively and a highly elevated average of percentages of mitotic indices (18 % & 1.2 %) was observed between the control and group 3 after 72 hr of treatment, respectively.

Micronucleus assay results showed that methotrexate treatment induced genotoxicity in bone marrow cells and the number of micronucleated polychromatic erythrocytes (MNPCEs) was gradually increased significantly ($P < 0.001$) by the increase of dose and time in treated groups with methotrexate when compared to the control one. Also, cytotoxicity test showed that the ratio of polychromatic erythrocytes/normochromatic erythrocytes was gradually increased

significantly ($P < 0.001$) by the increase of dose and time in treated groups with methotrexate when compared to the control one.

The current results of comet assay indicated that treatment with methotrexate significantly ($P < 0.001$) increased DNA damage in the blood leukocytes in dose and time dependent manner.

Results of randomly amplified polymorphism DNA-polymerase chain reaction **(**RAPD-PCR) analysis showed different range of DNA modifications in the methotrexate treated groups after 24, 48 and 72 hour of treatmet in comparison with untreated control.

Results of the present study indicated that methotrexate treatment induced genotoxic effect on bone marrow chromosomes and DNA content of male albino mice even after a low dose and single treatment. Therefore, the therapeutic uses of methotrexate should be restricted to a very narrow range border.

**Key words:** Methotrexate, Mice, Chromosomes, DNA, RAPD-PCR, Comet assay, Micronucleus.

# Contents

# List of Tables