Antigenotoxic effects of rutin against methotrexate
genotoxicity in Swiss albino mice

Ashoka Ch ¹ and Mohammed S. Mustak²*

¹Department of Zoology, Government Science College, Nrupathunga Road,
Bangalore-560 001, Karnataka, India.
²Departments of Applied Zoology, Mangalore University, Mangalagangothri - 574 199
Mangalore, Karnataka, India.
*For Correspondence Address : msmustak@gmail.com;
msmustak@mangaloreuniversity.ac.in

Abstract
Cancer chemoprevention with natural phytochemical compounds is an emerging strategy to prevent, impede, delay, or cure cancer. The aim of the study is to evaluate the antineoplasticogenic potency of the rutin, a flavonoid to modulate the side effects induced by the anticancer drug Methotrexate. Methotrexate is an antimetabolite drug broadly used in the treatment of cancer and autoimmune diseases which causes an array of many side effects. Different doses of rutin (50, 100 and 150 mg/kg bw) were given once daily for five days orally and methotrexate (20mg/kgbw) was administered on the fourth day intraperitoneally. To understand the protective effect of rutin against methotrexate side effects, micronucleus test and chromosome aberration tests were performed at 48h post methotrexate administration. The results showed that 20mg/kgbw of MTX significantly induced MN in polychromatic erythrocytes (p<0.05) and resulted in significant increase in total chromosomal aberrations (41.8±3.70 %; p<0.05). Further, pre-treatment with the flavonoid rutin reduced the MNPCE and chromosomal aberrations in bone marrow and MN NCE in peripheral blood in comparison to MTX group. The Rutin supported the recovery from the mitotic suppression compared to MTX treated mice. Thus, the present study implies the major therapeutic use of rutin against genotoxic effects of methotrexate.

Keywords: Methotrexate, Rutin, Micronucleus, Mitotic Index, Polychromatic Erythrocytes, Chromosomal aberrations.

Introduction
In recent years, there have been considerable efforts to find naturally occurring substances that can inhibit, reverse, or retard mutagenesis (1-3). Flavonoids are a large group of plant secondary metabolites (4) that have attracted considerable interest because of their beneficial effects in humans; they have been reported to have antiviral (5), antiallergic (6), anti-inflammatory (7-9), antitumor (10-12), anti-radiation (13) and antioxidant activities (14,15). They are ubiquitous in fruits and vegetables that are regularly consumed by humans (16). More than 6000 different flavonoids (17) have been identified, many of which are responsible for their attractive colours of flowers(18) fruits and leaves. All these aspects justify the intense interest in flavonoids which has been manifested over several decades (19).

Methotrexate (MTX) is an anti-cancer drug developed during 1940s as a specific antagonist of folic acid, showing inhibitory effects on de novo synthesis of purine and pyrimidine nucleotides (20,21). Basic principle of therapeutic efficacy of
MTX is due to the inhibition of dihydrofolate reductase, a key enzyme in the folic acid metabolism (22). Studies have shown that MTX induce short and long term toxic effects including genotoxicity in mouse somatic and germinal cells (23). Previously, our study showed the cytogenetic toxicity of methotrexate in mouse bone marrow using chromosomal aberration, mitotic index and micronucleus assays (24). Studies have shown that Bone marrow (24), gastrointestinal mucosa and hair are particularly vulnerable to MTX (25). The MTX is not selective for the cancer cells, it can affect the normal tissues and so prolonged use of MTX has been associated with various organ toxicity (26). Recently, many secondary metabolites are tried to ameliorate the toxicity associated with MTX. The protective effects of vitamin E and Cornus mas fruit extract were studied on methotrexate-induced cytotoxicity in sperms of adult mice and jejunal mucosal damage in rats (27). Yuncu et al. 2015 (28) studied the protective effects of vitamin E and L-carnitine against MTX-induced injury in rat testis and reported the elevation in malondialdehyde (MDA) levels and the amelioration in superoxide dismutase levels. The compounds such as resveratrol and famotidine were also found significantly prevent the MTX induced elevation of the MDA, 8-OH/Guanosine and myeloperoxidase (MPO) parameters and decreased the levels of GSH in the duodenal and jejunal tissues (29). The beta glucan, an antioxidant also showed protective effect on MTX induced testicular damage in rats (30).

Recently, more emphasis has been given to the discovery of genoprotective agents from the natural products and their isolated compounds against the damaging effects of chemicals. The Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside the flavonoid has the pharmacological properties like antioxidant, anti-inflammatory, anti-apoptotic, and anti-autophagic effects and have been exploited in human medicine and nutrition (31). Conventionally, it is used as an antimicrobial, antifungal, and anti-allergic agent. The recent studies have shown its multispectral pharmacological benefits for the treatment of various chronic diseases, such as cancer, diabetes, hypertension, and hypercholesterolemia (32-36). Further, studies demonstrated that orally administered rutin significantly attenuated memory deficits in Alzheimer’s disease transgenic mice, by decreasing oligomeric Aβ level, increased super oxide dismutase activity and glutathione/glutathione disulphide ratio, reduced glutathione disulphide and MDA levels, and decreased IL-1 and IL-6 levels in the brain suggesting that rutin is a promising agent for Alzheimer’s disease treatment (37,38). However, antigenotoxic effects of rutin against the MTX induced genotoxicity/clastogenic damage in Swiss albino mice were not yet studied. Therefore, the present study was aimed to evaluate protective role of rutin against the MTX induced the genotoxicity/clastogenicity through micronucleus assay and chromosomal aberrations.

Materials and Methods
Chemicals: Methotrexate (MTX, C_{20}H_{22}N_{8}O_{5}, CAS No. 59-05-2; Batch No A1283L14) marketed by IPCA laboratories Mumbai, as Folitrax was used for the experiment. Colchicine (C_{22}H_{25}NO_{6}; CAS No.64-86-8; Batch No. T 823279) was purchased from SRL Ltd, Mumbai. Rutin trihydrate (CAS 250249-75-3; Batch No.000020566) was obtained from Himedia, India. All other chemicals were procured from Merck, SRL, Himedia, India.

Animals: Swiss albino mice belonging to the Mus musculus species inbred and maintained in the institutional animal house were used for the experiments. The animal experiments were conducted after obtaining the approval from the Institutional Animal Ethical Committee (IAEC) of Mangalore University. Care of the animals and experiments were conducted as per the guidelines of CPCSEA, (Committee for the Purpose of Control and Supervision of Experimentation on Animals) India. Animals were housed in polypropylene shoe box cages bedded with clean, dry paddy husk and kept in air-conditioned room at a temperature of 22± 2° C and relative humidity.
50±15%. They were fed with a standard pelleted diet and water ad libitum. The 8-10 weeks old animals of both the sexes with an average body weight of 23±0.5 g were used for the experiments. In each experimental and control group, five animals were maintained.

**Dose and treatment schedule:** The LD$_{50}$ value of methotrexate (MTX) for intra-peritoneal use has been reported as 50 mg/kg bw. in mice (datasheets.scbt.com/sc-3507.pdf). For the present study, we selected 20 mg/kg bw, dissolved in double distilled water and administered as a single dose in 0.2 ml quantity through intraperitoneal route. Double distilled water was used as a control. Rutin suspended in distilled water at different doses was orally administrated. MTX was i.p injected only once on the 4th day of rutin treatment (The Study design is shown in table 1.)

**Bone Marrow Micronucleus Test (MN Test):** the bone marrow MN preparations were made from different groups (Group 1 to VIII) of experimental animals, following the modified method of Schmid (1973) (39). Rutin with Methotrexate groups (Groups VI, VII and VIII) were sacrificed after 24hr of Rutin treatment(48 h post MTX dosing). The bone marrow cells from thigh bones were flushed with 5% BSA into a centrifuge tube using a syringe and thoroughly mixed. The suspension was centrifuged at 1000 rpm for 15 minutes. The supernatant was discarded and a drop of fresh 5% BSA was added to the pellet. A thin smear was prepared in clean grease free slides. The slides were air dried and soon fixed in methanol for 10 minutes and stained with buffered (pH 6.8) May-Grunwald-Giemsa stain. Both the stains were filtered through a Whatman filter paper No. 1 (pore size 1.00 μ). Two thousand PCEs/animal were screened for MN and the corresponding normochromatic erythrocytes (NCEs) in the field were also scored to determine the MN frequency. PCEs are younger and NCEs are older erythrocytes. PCEs stain bluish and NCEs stain reddish orange in colour Fig.1.

**Peripheral blood MN assay:** The peripheral blood MN assay was done by using the method of Schlegel and MacGregor (40). The blood was drawn from the tail vein on the day of animal sacrifice(48h post MTX dosing) and thin smears were prepared on clean grease free slides. They were fixed in absolute methanol for 10 minutes. The slides were then stained with buffered 10 % Giemsa (pH-6.8) taken in vertical couplin jars. About 2000 NCE per animal were scanned for the presence of MN. The number of PCE corresponding to 2000 NCE was also determined (41).

**Chromosomal Aberration (CA) Test:** The chromosomal preparations were made following the method of Tjio and Whang (42). The condition of rutin and MTX administrations were the same as those used for the MN test. The animals were sacrificed 24 h post rutin treatment (48h after MTX dosing) The experimental animals were intraperitoneally injected with colchicine solution (2 mg|kgbw), before 1 h of sacrifice by cervical dislocation. The marrow cells were flushed from femur and tibia bones with 0.56% potassium chloride (KCl). The marrow suspension was thoroughly mixed with the hypotonic solution and left at room temperature for 30 minutes. After this, the suspension was centrifuged at 1000 rpm for 10 minutes and the pellet obtained was resuspended in 2 ml of 1:3 acetic-methanol fixative. The suspension was kept in room temperature for 45 minutes and centrifuged. The supernatant was discarded, and the fixative was again added, mixed thoroughly, incubated at room temperature for ten minutes and centrifuged. This step was repeated thrice. Finally, the pellet was suspended in appropriate amount of fixative and thoroughly mixed. 2-3 drops of suspension were dropped from a height of about 3 feet on clean, pre-chilled slides and flame dried. The slides were stained with buffered Giemsa (pH 6.8) and observed under microscope (Olympus BX51). 100 well-spread metaphases were screened from each animal for the presence of several types of chromosomal aberrations (Fig.2). From each animal, a total of 2000 cells were scored for the presence of dividing and non-dividing cells to determine the mitotic index values. 

Mitotic Index (MI) = \[
\frac{\text{Number of dividing cells} \times 100}{\text{Total number of bone marrow cells counted}}
\]
Percentage reduction (%R) in the tests was calculated using the formula of Waters et al. (43,44): 
\[
% R = \left( \frac{\text{mean in A - mean B}}{\text{mean in A} - \text{mean in C}} \right) \times 100,
\]
where A is the group treated with MTX20, B is the group treated with different doses of rutin plus MTX20 and C represents the control group.

**Statistical Analysis:** The data were expressed as mean ± S.D. The micronucleus induction data and chromosomal aberration data were analyzed statistically by analysis of variance (ANOVA). In cases in which p<0.05, the Tukey’s test compared treatment means. All data were processed using the statistical package SPSS 20.0 for Windows (IBM Corporation, Armonk, NY).

**Results**

The results of bone marrow micronucleus test are presented in table 2. The polychromatic erythrocytes (PCE) appear blue in colour and they are larger than the normochromatic erythrocytes (NCE). The NCEs stain orange red in colour (Fig.1B). In the control (Group I), the frequency of MN in PCEs was 0.15 ±0.07. In MTX treated animals it was significantly higher i.e 1.19±0.16 (p<0.05). The Rutin alone group (group II, III and IV) did not show any statistically significant difference in MN induction in PCEs compared to control group. The proportion of PCEs to the total erythrocytes were also like that of control group (Table.2). This indicates that the doses of rutin selected and the route of dosing in the present study do not pose any clastogenic threat to the bone marrow in the Swiss albino mice. MN in PCE was observed at all combinations, with the percent of MN reduction ranging between 17-75% (Table.2). The pre-treatment of rutin 100 mg/kg body weight showed Higher reduction (75%) in MNPCE when compared other doses of rutin against the MTX 20 group (p<0.05).

In the peripheral blood micronucleus test, the frequency of MN NCE (Table.3 and Fig. 1-C,D) was increased in MTX treated mice compared to the control (distilled water) group (p<0.05). The percentage frequency of PCE in MTX treated group was significantly decreased compared to control and rutin group. Pre-treatment with rutin has shown improvement in the PCE production. The % PCE has reached near a value of 2.

The results of chromosomal aberration test (CA) are presented in table 4 and 5. Significant increase in the percent aberrant cells was observed in MTX20 group (41.80±3.70) compared to the control group (4.6±2.30) (Table.5). Several types of aberrations such as gaps, breaks, exchanges, fragments, multiple aberrations, centromeric separation, centromeric associations, stickiness and pulverization has been observed. Metaphase plates containing two or more diverse types of aberrations were included under multiple aberrations. In our study high frequency of breaks were also observed and gaps, breaks and exchange aberrations are interlinked. These are the conventional type of aberrations which are included in every cytogenetic analysis. In the present study, MTX also produced significant rings (Fig.2. B; Fig. 3) and centric fusions (Fig. 2.D). Ring chromosomes as unusual circular chromosomes generally result from breaks at the ends of both chromosome arms with subsequent fusion of the broken ends to produce a continuous ring. In addition, significant centric fusion (Robertsonian translocation) (Fig.2.C) observed in the MTX treated groups, probably

![Figure 1. Photomicrograph showing micronucleus in erythrocytes.](image)

A- micronucleus in a NCE in the bone marrow smear preparation.
B- PCE and NCE in the bone marrow smear preparation;
C- micronucleus in a PCE in blood smear preparation;
D- micronucleus in a NCE in blood smear preparation.

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originated from the fusion of two acrocentric chromosomes, due to action the drug.

The association of the two acrocentric chromosomes giving rise to a meta or submetacentric one by the fusion of centromere region is referred to as centric fusion or Robertsonian translocation. This phenomenon seems to be accenuated by exposure to MTX. The pre-treatment of rutin for five days, orally has shown some protection from the clastogenic action of MTX. When calculated, the % reduction of chromosomal aberrations in the combination group ranged from 20 -33 %(Table.5). Here, the 100 mg per kg dose has shown better reduction compared to other two doses.

The mitotic index (Fig.4) values of MTX alone were significantly (p<0.05) reduced when compared to the control group. This indicates the myelosuppression brought by the MTX in the haematopoietic stem cells. The three doses of

Fig. 2. Photograph (400X) showing diverse types of chromosomal aberrations induced by Methotrexate in the bone marrow cells of Swiss albino mouse. A. Normal Mataphase; B. Ring chromosome; C. Robertsonian translocation and D. Centromeric Association.

Fig. 3. Bar diagram depicting % ring chromosomes in rutin alone, Methotrexate alone and in combined groups of these two. Ring chromosome occurrence was more in the present in vivo study.

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rutin tested showed some effect on mitotic index. The rutin 100 mg per kg dose has shown increased value of mitotic index than the control group, whereas the other two doses, i.e., 50 mg per kg and 150 mg per kg showed some type of cytotoxicity as evidenced in the reduced mitotic index values. In combination group, all the three doses of rutin tested showed improved mitotic index values when compared to MTX alone group. Rutin 100 mg in combination with MTX showed a higher MI, suggesting mitogenic property.

Discussion

Despite of the development of diagnosis and therapy in medicine, many cancers are incurable. Only long-term treatment with harmful agents is available for these patients, such as MTX. MTX is a folate antagonist drug, and it is a structural analogue of folic acid. Therefore, it competes with folic acid (FA), the normal substrate for binding site on dihydrofolate reductase (DHFR), the key enzyme involved in the synthesis of DNA precursors. This will affect the nucleotide pool leading to perturbation in the DNA synthesis and cell proliferation (21). This may be the reason for the genotoxic damages such as chromosomal aberrations and micronucleus induction. The tissues with high cellular turnover are thus the most sensitive to the cytotoxic impact of MTX, which is responsible for its effectiveness as a chemotherapeutic agent, but also for many of its side effects such as mucositis, hair loss and cytopenias (45). The mutagenic effects of MTX have been attributed to a substantial proportion of cancer chemotherapeutic agents (46). In most cases, their cellular response is pleiotropic, making it challenging to develop these agents efficiently for potential therapeutic benefit (47). There is also absolute need to investigate for antimutagenic and anticarcinogenic potential of substances which could counteract the harmful chemotherapeutic agents. There are various plant-derived compounds improve the efficiency of cytotoxic agents, decrease their resistance, lower and alleviate toxic side effects. The interactions between dietary agents and chemotherapy drugs were studied using either in vitro cell systems or in vivo animal systems (48,49). Thus the concept of chemoprevention with natural or synthetic compounds to block, reverse or prevent the development of cancer has great appeal (50-52).

Fig. 4. The Bar diagram showing Mitotic Index in bone marrow of Methotrexate and rutin treated Swiss albino mice. MTX= Methotrexate (20mg/kg/ bw). RUT50-rutin 50mg/kg bw; RUT100-Rutin 100mg/kg bw; RUT150-Rutin 150mg/kg bw
Table 1. Description of in vivo study design opted in the present study.

<table>
<thead>
<tr>
<th>Test group</th>
<th>Chemical</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (CONTROL):</td>
<td>double distilled</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>water</td>
<td></td>
</tr>
<tr>
<td>Group II (RUT50)</td>
<td>rutin</td>
<td>rutin 50 mg/kg bw for five consecutive days.</td>
</tr>
<tr>
<td>Group III (RUT100)</td>
<td>rutin</td>
<td>rutin 100 mg/kg bw for five consecutive days.</td>
</tr>
<tr>
<td>Group IV (RUT150)</td>
<td>rutin</td>
<td>rutin 150 mg/kg bw for five consecutive days.</td>
</tr>
<tr>
<td>Group V (MTX20)</td>
<td>Methotrexate</td>
<td>20 mg/kg bw dose of methotrexate (single i.p.)</td>
</tr>
<tr>
<td>Group VI (MTX20+RUT50)</td>
<td>Methotrexate + rutin</td>
<td>rutin 50 mg/kg bw for five successive days + MTX 20 mg</td>
</tr>
<tr>
<td>Group VII (MTX20+RUT100)</td>
<td>Methotrexate + rutin</td>
<td>rutin 100 mg/kg bw for five successive days + MTX 20 mg</td>
</tr>
<tr>
<td>Group VIII (MTX20+RUT150)</td>
<td>Methotrexate + rutin</td>
<td>rutin 150 mg/kg bw for five successive days + MTX 20 mg</td>
</tr>
</tbody>
</table>

Table 2. Frequency of micronucleus and total MN in bone marrow cells of animals treated with different doses of rutin and MTX and their respective controls. a-compared with the control group (p < 0.05); b-compared with the MTX20 group (p < 0.05) RUT50-rutin 50mg/kg bw; RUT100-Rutin 100mg/kg bw; RUT150-Rutin 150mg/kg bw; MTX20-Methotrexate 20mg/kg bw

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MNPE ± SD (%)</th>
<th>% Reduction</th>
<th>Total MN ± SD (%)</th>
<th>% Reduction</th>
<th>P/NS ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>0.15 ±0.07</td>
<td></td>
<td>0.14±0.05</td>
<td>b</td>
<td>0.94±0.03 b</td>
</tr>
<tr>
<td>RUT50</td>
<td>0.14 ±0.08</td>
<td></td>
<td>0.14±0.02</td>
<td>b</td>
<td>0.97±0.44 b</td>
</tr>
<tr>
<td>RUT100</td>
<td>0.28 ±0.05</td>
<td></td>
<td>0.24±0.01</td>
<td>b</td>
<td>0.92±0.05 b</td>
</tr>
<tr>
<td>RUT150</td>
<td>0.40±0.11</td>
<td></td>
<td>0.30±0.08</td>
<td>b</td>
<td>0.88±0.06 b</td>
</tr>
<tr>
<td>MTX20</td>
<td>1.19±0.16</td>
<td></td>
<td>0.78±0.10</td>
<td>a b</td>
<td>0.51±0.03 a</td>
</tr>
<tr>
<td>MTX20+RUT50</td>
<td>0.62 ±0.16</td>
<td>54.8</td>
<td>0.32±0.07</td>
<td>a b</td>
<td>71.81</td>
</tr>
<tr>
<td></td>
<td>0.41±0.09 b</td>
<td></td>
<td>0.47±0.07</td>
<td>a b</td>
<td>48.44</td>
</tr>
<tr>
<td></td>
<td>1.01±0.34 a</td>
<td></td>
<td>0.69±0.19</td>
<td>a</td>
<td>14.06</td>
</tr>
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</table>

Antigenotoxic effects of rutin against methotrexate
In the present study, we investigated anticlastogenic effect against genotoxicity of MTX, by the flavonoid rutin. Our result showed MTX 20mg/kg bw of induced significant total aberrant cells and MN in mouse bone marrow and peripheral blood erythrocytes at 48 h harvesting indicating it’s genotoxic effects maintained for longer duration. Hall et al., (53) conducted in vivo clastogenicity and carcinogenicity assays in Sprague-Dawley with MTX. They reported that MTX did not induce conventional chromosome aberrations such as breaks, deletions, exchanges etc., and MTX significantly reduced the mitotic index values at higher doses which agreed with our observations. Hassanane et al., (54) also reported the anti genotoxicity of curcumin against methotrexate (10 mg/kg bw) in albino rats and observed significant chromosomal aberrations in MTX group and were similar to our present result. The methotrexate is reported to cause genotoxicity in vivo as well as in vitro systems (55). Keshava et al., (55) showed the chromosome damaging effects of MTX in V79 cells. They observed the aberrations such as gaps, breaks, fragments etc and the reduction in the mitotic index values in the MTX treated cells. The chromosome damage caused by the Methotrexate(Amethopterin) was found in cell cultures of patients who had been treated (56). Cytogenetic effects of MTX have also been reported in patients undergoing treatment for rheumatoid arthritis (57).

The MTX-induced micronuclei formation might be explained by the intracellular accumulation of the drug resulting in a continuous inhibition of deoxyribonucleotide triphosphate (dNTPs) synthesis, subsequently causing DNA lesions due to the inhibition of DNA repair. However, because insufficient dNTPs remain, DNA lesions induced by MTX genotoxicity present themselves as micronuclei (58-60).

Considering the toxicity of MTX in the therapy, the search is on for the substances which will help the patients to improve their health. Folinic acid(59)(Shahin, 2001), caffeic acid phenethyl

<table>
<thead>
<tr>
<th>Treatment/dose (mg/kg b w)</th>
<th>Mean%NCE±SD</th>
<th>Mean% PCE±SD</th>
<th>Mean% MN in NCE ±SD</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>97.92±0.28</td>
<td>2.08±0.28</td>
<td>0.05±0.03</td>
<td></td>
</tr>
<tr>
<td>RUT50</td>
<td>97.48±0.32</td>
<td>2.51±0.32</td>
<td>0.06±0.04</td>
<td></td>
</tr>
<tr>
<td>RUT100</td>
<td>97.59±0.24</td>
<td>2.41±0.27</td>
<td>0.10±0.03</td>
<td></td>
</tr>
<tr>
<td>RUT150</td>
<td>97.90±0.19</td>
<td>2.09±0.19</td>
<td>0.09±0.04</td>
<td></td>
</tr>
<tr>
<td>MTX20</td>
<td>98.93±0.12</td>
<td>1.06±0.12</td>
<td>0.25±0.09</td>
<td></td>
</tr>
<tr>
<td>RUT50+MTX20</td>
<td>98.57±0.07</td>
<td>1.43±0.07</td>
<td>0.17±0.02</td>
<td>40.00</td>
</tr>
<tr>
<td>RUT100+MTX20</td>
<td>97.94±0.20</td>
<td>2.06±0.20</td>
<td>0.14±0.05</td>
<td>55.00</td>
</tr>
<tr>
<td>RUT150+MTX20</td>
<td>97.90±0.15</td>
<td>2.09±0.15</td>
<td>0.15±0.07</td>
<td>50.00</td>
</tr>
</tbody>
</table>

Table 3. Peripheral blood MN test in animals treated with different doses of rutin and MTX. RUT50-rutin 50mg/kg bw; RUT100-Rutin 100mg/kg b. w; RUT150-Rutin 150mg/kg bw; MTX20 - Methotrexate 20 mg/kg bw. a-compared with the control group (p <0.05); b- compared with the MTX20 group (p <0.05)
### Table 4. The Effects of rutin on the frequency of different types of chromosomal aberrations induced by methotrexate in bone marrow cells of Swiss albino mice and controls at 48 hrs.

<table>
<thead>
<tr>
<th>Treatment (mg/kg bw)</th>
<th>Chromosomal aberration types</th>
<th>Mean±SD</th>
<th>Total % * ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gs</td>
<td>Bs</td>
<td>Ex</td>
</tr>
<tr>
<td>CONTROL</td>
<td>0.60±0.54 b</td>
<td>0.40±0.89</td>
<td>0.40±0.54 b</td>
</tr>
<tr>
<td>RUT50</td>
<td>1.60±0.89 b</td>
<td>1.40±1.14</td>
<td>2.20±1.30 b</td>
</tr>
<tr>
<td>RUT100</td>
<td>0.60±0.89 b</td>
<td>1.20±1.64</td>
<td>1.80±1.30 b</td>
</tr>
<tr>
<td>RUT150</td>
<td>1.2±1.83 b</td>
<td>2.00±2.00</td>
<td>2.20±1.92 b</td>
</tr>
<tr>
<td>MTX20</td>
<td>9.0±3.67 b</td>
<td>2.00±2.00</td>
<td>7.00±3.08 b</td>
</tr>
<tr>
<td>MTX20+ RUT50</td>
<td>2.60±1.81 b</td>
<td>0.40±0.54</td>
<td>3.60±1.67</td>
</tr>
<tr>
<td>MTX20+ RUT100</td>
<td>3.60±1.94 b</td>
<td>1.00±1.73</td>
<td>4.40±1.94 b</td>
</tr>
<tr>
<td>MTX20+ RUT150</td>
<td>5.40±2.60 a</td>
<td>5.80±1.78 a</td>
<td>4.00±1.58</td>
</tr>
</tbody>
</table>

* From 100 metaphases/animal; 5 animals/group.

* - compared with the control group (p < 0.05); b - compared with the MTX20 group (p < 0.05).
ester (61), vitamin A (62) and leucovorin (63), α-lipoic acid (64), silybinin(65), ambrex, a polyherbal formulation (66), Tinosporacordifolia (67), phloridzin (68), gamma-irradiated basil(69),Vanillin and chlorophyllin (70), curcumin (71), vitamin E(72), berberine (73) are a few of the substances. In a recent experimentation, methotrexate induced genotoxicity was evaluated in combination of polyphenol extracts of Asteracanthalongifolia Nees. and Piperbetle Linn. (74) in Heteropneustes fossilis (fish) and observed an induction of MN highest in the MTX treated fishes after 21 days.

The flavonoid rutin is known for its many biological phenomena. It is also available for us through common diet components. Many research reports have shown beneficial effects of this flavonoid. Pre-treatment of rutin prevented deteriorative effects induced by cisplatin through a protective mechanism that involved reduction of increased oxidative stress as well as caspase-3, TNF-α and NFκB protein expression levels. Arjumand et al., 2011 found pre-treatment with rutin restoring the histopathological changes produced by cisplatin(75). Rutin attenuated gentamicin-induced renal damage by reducing oxidative stress, inflammation, apoptosis, and autophagy in rats (32). In our study, 100mg per kg dose showed on pre treatment ameliorates the MTX induced micronuclei formation in bone marrow and peripheral blood system. Further rutin also reduced the chromosomal aberrations and increased the mitotic index compared to MTX induced.

**Conclusion**

The MTX is a widely used anticancer drug and it is also being used in the treatment of various other ailments. Present study showed the protective role of rutin against the MTX induced genotoxicity using a mouse in vivo system. In the light of these observations, it is very essential to use MTX drug judiciously for human applications. Our study suggests that When it becomes very essential to use MTX drug, rutin can be supplied as protective supplementary agents may be included in the therapeutic regime to prevent the harmful effects of the drug. Rutin has a weak clastogenic effect, and in combination with MTX, it can enhance MTX’s inhibitory effect on the MI and reduce CAs in bone marrow cells. This finding may direct attention to the beneficial effects of using rutin in chemotherapeutic approaches. In addition to its natural presence in the foods, rutin is also available as a supplement in the market.

<p>| Table 5. The Effect of different doses of rutin on percentage reduction of chromosomal aberrations in Swiss albino mice. For each treatment 500 metaphase plates were analysed to obtain the mean % chromosomal aberrations and respective % reduction. RUT50-rutin 50mg/kg bw; RUT100-Rutin 100mg/kg bw; RUT150-Rutin 150mg/kg bw; MTX20 - Methotrexate 20 mg/kg bw. a-compared with the control group (p &lt;0.05); b-compared with the MTX20 group (p &lt;0.05) |</p>
<table>
<thead>
<tr>
<th>Treatment/dose (mg/kg bw.)</th>
<th>% Aberrant cells (Mean ± SD)</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>4.6±2.30b</td>
<td>-</td>
</tr>
<tr>
<td>RUT50</td>
<td>11±2.82b</td>
<td>-</td>
</tr>
<tr>
<td>RUT100</td>
<td>12.2±4.49b</td>
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</tr>
<tr>
<td>RUT150</td>
<td>15.6±4.56b</td>
<td>-</td>
</tr>
<tr>
<td>MTX20</td>
<td>41.8±3.70a</td>
<td>-</td>
</tr>
<tr>
<td>MTX20+ RUT50</td>
<td>33.4±8.87a</td>
<td>22.58</td>
</tr>
<tr>
<td>MTX20+ RUT100</td>
<td>29.2±5.89 a,b</td>
<td>33.87</td>
</tr>
<tr>
<td>MTX20+ RUT150</td>
<td>34.2±9.52 a</td>
<td>20.43</td>
</tr>
</tbody>
</table>

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Acknowledgement

The first author is very much thankful to his research supervisor (Late) Dr. K.K. Vijayalaxmi for her incessant support and blessings given in the true spirit of professional recognition through the work. The authors thank SAP-UGC, Department of Applied Zoology and Mangalore University for facilities provided throughout the work.

References


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