Protective Effects of Ginger extract against Methotrexate induced cytotoxicity in mice

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Abstract

Ginger (*Zingiber officinalis*) is one of the frequently used spices in the world and medicinal plant, which has been used all over the world. This study was designed to investigate the role of aqueous ginger extract to inhibit the genotoxicity of methotrexate (MTX) in female albino mice by using these parameters: mitotic index (MI), chromosome aberrations (CA) and micronuclei formation (MNPCE) in somatic cells (bone marrow). The cytological protection of aqueous ginger extract (AGE) was performed at doses (25, 50) mg kg against MTX effects (0.5) mg kg for three weeks exposure. The results revealed the high inhibitory effects of MTX for cell division in addition to induction of chromosome aberration with micronuclei formation, the absence of cytotoxicity for AGE at tested doses, and the inhibitory efficiency of AGE against the toxicity and mutagenicity of MTX, specially at the dose 50 mg kg. From the results we can concluded that the AGE has a promising role in the protection of somatic cells from cytotoxic effects of methotrexate.

**Keywords: Zingiber officinalis, methotrexate, genotoxicity, bone marrow**

Introduction

The majority of antineoplastic drugs are especially designed to interfere with DNA molecule, cellular metabolism and cell division (Williams et al., 1985, Majeed & Jumaah, 2019). Due to this mode of action, these drugs are expected to cause mutations and cytogenetic abnormalities. Therefore, anticancer drugs are used as mutagens in most antimutagenic tests (Zignaigo et al., 1990). During recent years, considerable efforts have been focused on using antimutagens to modulate the genotoxic effects of the mutagenic antineoplastic drugs (AL-Sharif, 2011).

Methotrexate (MTX), a cytotoxic chemotherapeutic agent, which is used in the
treatment of acute lymphoblastic leukaemia, lymphoma, osteosarcoma, breast cancer, head and neck cancers and also in the therapy of non-oncologic disorders such as rheumatic diseases and psoriasis (Mikkelsen et al., 2011). Methotrexate restricts the synthesis of thymidilate and purine nucleotides by inhibiting dihydrofolate re-ductase and to a lesser extent, thymidilate synthatase. In cells treated with methotrexate, a progressive accumulation of strand break in mature DNA (post-replicated DNA) was detected by (Li and Kaminskas, 1985). Beside the therapeutic effects there are many toxic effects including gastrointestinal, central nervous system, hepatic, and bone marrow toxicity (Xin et al., 2019; Homady et al., 2018). Zingiber officinale Roscoe (family: Zingiberaceae), known as ginger, is a perennial, herbaceous plant of about 1 meter in height, with leaves developing from a branched rhizome (Van Wyk & Van Staden, 2002). The exact country of origin is uncertain, but was thought to be originally native of tropical South East Asia before it spread to Africa (Raji et al., 2002). The important active components of the ginger rhizome are volatile oils and pungent phenol compounds such as gingerols, shogaols, and zingerone (Zancan et al., 2002). The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry (Ali et al., 2008). Ginger rhizome is used medicinally that has anti-oxidant, cell-protective effects as antioxidant, anticoagulant, weight control, immunomodulatory, antigenotoxic, antimicrobial, antiarthritic (Yadav et al., 2016; Aziz et al., 2019). In this study, the effect of ginger as a protective agent against methotrexate toxicity using cytogenetic parameters were performed.

Methods and Materials

Preparation of extract

The rhizomes of Ginger (Z. officinale R.) were obtained from the market, cut into pieces and grinded into fine powder, then macerated (125g) of this fine powder in one liter of distilled water for (12 hr.) at 25°C and filtered. The filtrate was dried in the oven at 45°C, powdered and stored at 4°C (Kamtchoving et al., 2002).

Animals of Experiment:

Thirty-six of healthy adult males of albino mice aged 2-4 months (25-30 g) were used, provided with food and water ad libitum, and kept at 12 h: light 12 h dark cycles at room temperature at least 2 days before the experiment. This experiment was approved by the Central Committee for Bioethics in collage of Sciences/ Kufa, Iraq. Mice were randomly divided into six groups each group 6 animals:
Control group: mice were given 0.05 ml of distilled water.

**MTX group:** methotrexate (solved in distilled water) was given to mice orally (0.5mg/kg) of body weight, for three days weekly for three weeks.

**AGE groups (25mg/kg):** the aqueous extract of ginger (25 mg/kg) was given to mice orally (0.05 ml) for five days weekly for three weeks.

**AGE groups (50mg/kg):** the aqueous extract of ginger (50 mg/kg) were given to mice orally (0.05 ml) for five days weekly for three weeks.

**MTX+ AGE Group:** methotrexate (0.5mg/kg) was given to mice orally followed by the aqueous extract of ginger (25 mg/kg) for five days weekly for a three weeks.

**MTX+ AGE Group:** methotrexate (0.5mg/kg) was given to mice orally followed by the aqueous extract of ginger (50 mg/kg) for five days weekly for a three weeks.

The mice were sacrificed 24 hours after last gavage for all groups.

**Karyotype preparation**

The chromosomes preparations were done by using the direct method (Sharma& Sharma, 2014) with some modifications, as follow:

The mice were injected intraperitoneally with colchicine (4mg/kg), left for two hours, and were sacrificed. The femurs were removed and freed. The bone marrow was emptied from the inner portion of femur by injection of 3ml of hypotonic solution (KCl), and incubated in (water bath) at 37ºC for 20min. After that 1ml of very cold fixative solution was added, and centrifuged at 2000 rpm for 2 min with discarding of supernatant-this step was repeated twice-. At last 3 ml of cold fixative was added slowly, three to five drops of chromosome suspension were dropped in an appropriate height over cold slides, these slides were dried and stained with Giemsa stain.

Calculation of Mitotic index was determined by scoring at least 1000 cells from each animal, and using the following formula (Shubber &Juma, 1999):

\[
\text{Mitotic index (MI)} = \frac{\text{No. of mitotic division cells}}{\text{Total No. of cells scored}} \times 100
\]

**Micronuclei test**

Smears of the bone marrow method were carried out according to (Agarwal &Chauhan, 1999), then stained with Giemsa. The micronuclei frequency in the polychromatic (PCEs) and normochromatic (NCEs) of erythrocytes was determined and the PCEs/NCEs ratios were scored (1000 cells per animal).

Statistical analysis:
The data were analyzed by using (SPSS) system/ version 17, and the results were expressed as (mean ± S.E) .The analysis of variance (ANOVA) was used for this purpose.

**Results**

In the present study the mitotic index (MI) of bone marrow was decrease significantly (P<0.05) in MTX group when compared to the negative control. While
MTX + AGE group (50mg/kg) showed that there wasn’t any significant difference in MI compared to negative control as shown in table (1).

Also the group of mice treated with MTX exhibited a highly significant (P<0.05) increase in the mean frequency of chromosome aberrations compared to the control and AGE groups table (1). The types of structural chromosomal aberrations most frequently observed in the MTX group were breaks, fragments and ring chromosomes. Numerical aberrations were also recorded in all experimental groups, treatment with ginger extract at MTX+AGE (50mg/kg) significantly (P<0.05) reduced the number of chromosome aberration in cells table (1) compared to MTX alone.

Table (1) Mitotic index and chromosomes aberration means of bone marrow cells of mice (mean±S.E).

| Group            | (MI) | Chromosomes aberration | Polyploidy | Aneuploidy |
|------------------|------|------------------------|------------|------------|
|                  |      | Broken                 | Fragmented | Ring       |            |
| control          | 8.67±1.67a | 0.00±0.00a           | 1.33±0.49a | 1.00±0.37a | 0.00±0.00  |
| MTX              | 3.67±1.20b | 6.00±1.00b            | 4.67±0.49b | 6.00±1.46b | 2.83±0.61b |
| AGE(25mg/k)      | 9.00±0.57a | 0.00±0.00a           | 1.33±0.41a | 1.00±0.37a | 0.24±0.00  |
| AGE(50mg/k)      | 8.33±1.76a | 0.00±0.00a           | 1.00±0.26a | 0.83±0.33a | 0.67±0.33a |
| MTX+AGE (25mg/k) | 6.33±0.88ab | 3.68±1.45abc         | 1.67±0.67a | 5.67±1.12b | 2.00±0.52a |
| MTX+AGE (50mg/k) | 8.00±1.15a | 2.00±0.58ab          | 0.83±0.31a | 3.33±0.61b | 0.83±0.3a  |

Different letters: significant difference between groups, Similar letters: non-significant difference between groups, n=3, S.E: Standard error.

The Methotrexate clastogenicity has been tested using the bone marrow micronucleus assay which was positive in inducing significant increases (p<0.05) of micronucleated polychromatic erythrocytes (MNPCE) and decreasing in P/N ratio as well as % PCEs in comparison to AGE group, as well as negative control, while the incidence of micronuclei in polychromatic erythrocytes in MTX+AGE group showed that the high dose of AGE (50mg/kg) significantly reduced micronuclei frequency when compared to MTX group, table (2).

Table (2): Polychromatic erythrocytes with micronuclei (MNPCE) observed in bone marrow cells of experimental mice (mean ± S.E).
|                | MN (%) | PCEs (%) | NCEs (%) | PCEs/NCEs |
|----------------|--------|----------|----------|-----------|
| MTX            | 24.33 ± 1.45<sup>b</sup> | 40.17<sup>b</sup> | 59.83<sup>b</sup> | 0.67<sup>b</sup> |
| AGE (25 mg/kg) | 1.5 ± 0.33<sup>a</sup> | 50.98<sup>a</sup> | 49.02<sup>a</sup> | 1.01<sup>a</sup> |
| AGE (50 mg/kg) | 1.83 ± 0.3<sup>a</sup> | 51.53<sup>a</sup> | 48.57<sup>a</sup> | 1.06<sup>a</sup> |
| MTX+AGE (25 mg/kg) | 10.67 ± 1.82<sup>b</sup> | 47.47<sup>a</sup> | 5.53<sup>a</sup> | 0.91<sup>a</sup> |
| MTX+AGE (50 mg/kg) | 5.17 ± 0.88<sup>a</sup> | 49.11<sup>a</sup> | 50.89<sup>a</sup> | 0.96<sup>a</sup> |

%MN: micronucleated cell percentage; % PCEs: polychromatic erythrocytes percentage; % NCEs, normochromatic erythrocytes percentage; PCEs/NCEs ratio, the ratio of PCEs to NCEs. Different letters: significant difference between groups, Similar letters: non-significant difference between groups, n=3, S.E: Standard error.
Discussion

The present study evaluates the ginger extract protective effects against the genotoxicity of methotrexate (MTX) in mice. Many compounds and plant extract are known to produce antioxidant anti-genotoxic effect by reducing free radicals (Hadi et al., 2016; López-Romero et al., 2018). The present results of MTX administration resulted in a significant reduction in the mitotic index and that the incidence of the mean of various types of chromosomal aberrations were significantly increased (P<0.05), many In vivo and In vitro studies observed structural and numerical chromosomal aberrations in methotrexate treated groups (Delinassios & Talieri, 1987; Choudhury et al., 2001; El-Alfy et al., 2016). Moreover, the present study results showed that the treatment of mice with MTX produced a significant increase (P<0.05) in the frequency of MNPCEs, decrease in P/N ratio as well as % PCEs in comparison to AGE group (Table2). These results corroborate with that of (Ashoka & Vijayalaxmi, 2016) whom observed similar changes following MTX administration. In fact MTX has been proofed to be a generator of excess toxic reactive oxygen free radical and induce lipid peroxidation (Ali et al., 2017; De et al., 2015). As well as MTX is a folate antagonist drug, and it is a folic acid structural analogue. Therefore, it competes with folic acid (FA), the normal substrate for binding site on dihydrofolate reductase (DHFR), which considered as a key enzyme that involved in the synthesis of DNA precursors. This will affect the nucleotide pool leading to perturbation in the DNA synthesis. This may be the reason for the genotoxic damages such as chromosomal aberrations and micronucleus induction (Padmanabhan et al., 2009).

In the present study, the treatment of two doses of AGE were safe when compared to the control group, indicating the safety of the specific used dose, so Ginger is generally considered a safe herbal medicine of insignificant adverse/side effects.
Administration of 25 mg/kg and 50 mg/kg AGE for 21 days prior to MTX gavage, reversed the negative effects specially the higher dose (50 mg/kg) of AGE which returned the mitotic index changes, incidence chromosomal break, ring and other multiple aberrations induced with MTX to a significant rates when compared to that of MTX treated group alone, also this dose significantly reversed the frequency of MNPCEs, the alteration in %PCEs and P/N ratio on bone marrow of treated mice (Table1). These results are agreed with the observation of (Nirmala et al., 2007) who has proved the In vivo antimutagenic ability of ginger on formation and excretion of urinary mutagens in rats. Also this result is in agreement with the findings of (AL-Sharif, 2011) who reported that the Simultaneous treatment of ginger was effective in reducing the genotoxic effects induced by drug taxol especially in the total number of the chromosomal aberrations and the number of micronuclei, so this protective effect of AGE against MTX induced oxidative stress and subsequent nuclear damages may be belong to its strong antioxidant activity which is well documented in earlier studies (Adefegha 2010; Tohma, 2017). Moreover this anti-oxidant activity of ginger might be attributed to its constituents which contains active phenolic compounds, such as 6-gingerol (the main bioactive compound in fresh ginger) paradol and shogoal (Jeyakumar, 1999) and other antioxidant compounds such as vitamin C, vitamin E, beta-carotene, lutein, lycopene, quercetin, genistein, and tannin (de Lima et al., 2018; He et al., 2018). (Beg et al., 2008) proved that 6-Gingerol can be equally effective in reducing genotoxic damage at appropriate doses. While ascorbic acid in ginger suppresses peroxidation in both aqueous and lipid regions of cells (Dadheech et al., 2006), traps peroxyl radicals before they can provoke lipid peroxidation and aids in the regeneration of vitamin E (Chatterjee et al., 1991). In addition to that, earlier studies have confirmed that ginger extract protects against oxidative stress by enhancing superoxide dismutase (SOD) and catalase activity, increasing GSH content and decreasing MDA levels (Danwilai et al., 2017). At last we can concluded that ginger extract can have a promising role in the protection of somatic cells from cytotoxic effects of methotrexate especially (50 mg/kg) and this activity may be related to its strong antioxidant properties.

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