Protective Effect of Dimethyl Fumarate on Doxorubicin-induced Genotoxicity in Rats

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Abstract

Background and Purpose Doxorubicin is a broad-spectrum antineoplastic agent; however, however, its genotoxic/cytotoxic effects limit its clinical application. Dimethyl fumarate (DMF) is an FDA-approved oral drug shown to have antioxidant, anti-inflammatory and antimutagenic effects via activating Nrf2 antioxidant pathway. The present study aimed to investigate the possible protective effect of DMF against doxorubicin-induced chromosomal and DNA damage in rat bone marrow cells. Experimental Approach Wistar Albino rats of both sexes were administered DMF orally (15mg/kg once daily for 14 days) alone or with doxorubicin which was injected as a single dose (90 mg/kg at day 14) to induce toxicity. The blood samples were collected 24 hours after doxorubicin's injection from all groups to measure the serum levels of MDA, GSH, SOD, and GPx1 and bone marrow was harvested to assess chromosomal aberration, micronucleus, and comet assays. Key Results The rats in the doxorubicin-only group exhibited a significant decrease in mitotic index and depleted GSH and antioxidants enzymes serum levels with a significant elevation in MDA serum level, % DNA in Tail, micronucleus appearance and chromosomal aberrations compared to the control group; DMF pretreatment prior to doxorubicin exposure, significantly-reduced % DNA in Tail, micronucleus appearance, and chromosomal aberrations, improved mitotic index, restored GSH level and antioxidant enzymes activity compared doxorubicin-only group. Conclusion and Implication This study revealed that DMF alone has no DNA-damaging or clastogenic activities; DMF has protective effects against the genotoxicity induced by doxorubicin; thus, DMF might be a potential chemoprotective agent against doxorubicin-induced toxicity in cancer chemotherapy

Protective Effect of Dimethyl Fumarate on Doxorubicin-induced Genotoxicity in Rats

Running title "DMF Alleviate DOX-Related Genotoxicity"

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Author contribution statement

All authors contributed to the study's concept and design; SA contributed to the experimental design, conducted the experiments, analysed the data, and wrote the original manuscript. NA contributed to the experimental design, confirmed the authenticity of all raw data, and participated in revising the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors report no conflicts of interest.

Ethics statements

The animal study was reviewed and approved by The Graduate Studies and Ethics Committees of the College of Pharmacy, University of Baghdad.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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Background and Purpose

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Experimental Approach

Wistar Albino rats of both sexes were administered DMF orally (15mg/kg once daily for 14 days) alone or with doxorubicin which was injected as a single dose (90 mg/kg at day 14) to induce toxicity. The blood samples were collected 24 hours after doxorubicin's injection from all groups to measure the serum levels of MDA, GSH, SOD, and GPx1 and bone marrow was harvested to assess chromosomal aberration, micronucleus, and comet assays.

Key Results

The rats in the doxorubicin-only group exhibited a significant decrease in mitotic index and depleted GSH and antioxidants enzymes serum levels with a significant elevation in MDA serum level, % DNA in Tail, micronucleus appearance and chromosomal aberrations compared to the control group; DMF pretreatment prior to doxorubicin exposure, significantly-reduced % DNA in Tail, micronucleus appearance, and chromosomal aberrations, improved mitotic index, restored GSH level and antioxidant enzymes activity compared doxorubicin-only group.

Conclusion and Implication

This study revealed that DMF alone has no DNA-damaging or clastogenic activities; DMF has protective effects against the genotoxicity induced by doxorubicin; thus, DMF might be a potential chemoprotective agent against doxorubicin-induced toxicity in cancer chemotherapy.

Keywords: Doxorubicin; Dimethyl Fumarate; Genotoxicity; Chromosomal Aberration; Micronucleus assay; Comet assay.

What is already known

DOX therapy is associated with myelosuppression and genotoxicity, which may lead to secondary malignancy.

DOX-induced genotoxicity/cytotoxicity due to the increased level of oxidative stress and DNA damage in normal cells.

What does this study add

Oxidative stress inhibition by dimethyl fumarate pretreatment ameliorates DOX-related genotoxic adverse effects in rats' bone marrow cells.

What is the clinical significance

DMF has promising chemoprotective effects against DOX-related oxidative stress-induced genotoxic/cytotoxic effects, which might allow the use of DMF as an adjuvant in chemotherapy

1. Introduction

Doxorubicin (DOX), also known as adriamycin, is an important member of the anthracyclines group of chemotherapeutic drugs; and it has a broad anti-tumour spectrum; where it is used alone or in combination with other chemotherapeutic agents worldwide in the treatment of haematological malignancies, solid tumours, soft tissue sarcomas, small-cell lung, and breast carcinoma; moreover, doxorubicin is also the principal component in the management of Hodgkin's disease and lymphomas (1). However, the dose-dependent response relation of doxorubicin in many anticancer regimens has been well-defined; an increase in its dose restricts its use due to the development of severe cardiotoxicity, in addition to other cytotoxic effects on normal cells and a substantial negative impact on patient's health, which poses a significant hurdle in doxorubicin clinical application (2)(3).

The anti-tumour activity of DOX is mediated through its direct intercalating with deoxyribonucleic acid (DNA) and by interfering with the function of many enzymes that are necessary for DNA replication, including topoisomerase-II; where it stabilises the DNA-topoisomerase-II intermediate complex and this, in turn, leads to the distortion of DNA repairing, which consequently results in DNA double-stranded breakage and nuclei fragmentation with condensed chromatin (4)(5).

In addition, oxidative stress (OS) and overproduction of free radicals is an essential part of the doxorubicin mechanism of action, where the metabolism of DOX in the body is mediated by NADPH-dependent cytochrome P-450 that generates free radicals such as semiquinone, quinone, hydrogen peroxide (H_2O_2), superoxide anion (O_2^{*-}), and hydroxyl radical ($OH^{@}$) which can deplete glutathione and exhaust antioxidants enzymes, increase lipid, protein and nucleic acid peroxidation (6). Furthermore, the lipid peroxidation product malondialdehyde (MDA) can interact with the DNA; this consequently can cause inhibition of DNA replication and chromosomal damage through the formation of DNA adducts; these cytotoxic actions not only affect cancer cells but also can affect normal cells triggering mutation and chromosomal abnormalities including chromosomal aberrations and DNA damage (7)(8). Therefore, enhancing the cellular antioxidant response could reduce doxorubicin-induced oxidative damage.

Dimethyl fumarate (DMF) is a fumaric acid-derived small molecule that exhibits potent antioxidant and anti-inflammatory properties; DMF is a disease-modifying agent under the brand name "Tecfidera" that has been FDA-approved to be used to treat patients with severe psoriasis and relapsing-remitting multiple sclerosis (RRMS) (9). DMF's antioxidant and anti-inflammatory mechanism of action is thought to involve the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2), which controls the expression of various genes that regulates antioxidant and detoxification processes (10). The activation of the Nrf2 pathway by DMF has been demonstrated in several studies; DMF treatment showed increased Nrf2 protein levels and gene expression, which was accompanied by increased expression of downstream target genes, including heme oxygenase 1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (NQO1), reduced Nf-kB, TGF-β signalling and cell senescence (11)(12)(13)(14). The present study was designed to test the possible protective effect of DMF against doxorubicin-induced genotoxicity in rats through the assessment of the extent of chromosomal aberrations (CAs), micronucleus appearance (MN) and mitotic index (MI) in addition to the utilisation of the comet assay a sensitive technique to measure the extent of oxidative DNA damage.

2. Materials and methods

2.1. Chemicals

DMF (Purity >97%, CAS no.624-49-7) and Colchicine (Purity >95%, CAS no.64-86-8) were acquired from Sigma-Aldrich (St. Louis, MO 63103, USA). Doxorubicin (Doxorubicin HCl 50mg powder for injection, Khandelwal Labs, India) was purchased from local pharmacies. Fetal calf serum (FCS) was obtained from Capricon Scientific GmbH, South America, Giemsa stain (Sigma Chemicals, USA). Polysorbate 20 (Tween-20) was from Sinopharm chemical reagent Co., Ltd, China. All solvents and chemicals used were of analytical grade.

2.2. Dose selection, preparation, and mode of administration

Doxorubicin was dissolved in 0.9% normal saline, and a single dose of 90mg/kg body weight (BW) was intraperitoneally (IP) injected based on its success in inducing chromosomal damage in Wister rats (15).

In addition, DMF was prepared as a suspension using 5% tween-20 in double distilled water (DDW); where it was freshly prepared each day just before treatment and orally administered to rats by the utilisation of oral gavage as a single dose of 15mg/kg/day which was selected based on the previously-shown protective effect (16).

2.3. Animals and experimental design

The Graduate Studies and the Ethical Committees of the College of Pharmacy, University of Baghdad, approved the study protocol.

Wistar Albino experimental rats of both sexes aged six weeks with an average weight of 150gm were utilized in this study; since the animals were acquired and maintained in the College of Pharmacy Experimental Animal House, University of Baghdad, Iraq; in addition, the experimental rats were acclimatized for one week prior to starting the experiment; the rats were housed under controlled conditions of a light/dark cycle (12hours), temperature at (23±2°C) and humidity (50±5%); and had free access to a standard commercial diet, which was purchased from the local market, and tap water *ad libitum* .

The experimental animals (32 Rats) were randomly assigned into four groups (n=8) as follows:

Group I : Each rat was orally administered vehicle only (5% tween in DDW) *via* oral gavage for 14 consecutive days. Then a single dose of normal saline (NaCl) (0.9%) was IP injected 1 hour after the last vehicle administration on day 14. This group served as the control group.

Group II : Each rat was orally administered DMF only (15mg/kg/day) for 14 consecutive days.

Group III : Each rat was orally-administered vehicle (5% tween in DDW) only *via* oral gavage for 14 consecutive days. Then a single dose of DOX (90mg/kg) was IP injected 1hour after the last vehicle administration on day 14. This group served as the model group.

Group IV : Each rat was orally administered DMF (15mg/kg/day) for 14 consecutive days, then a single dose of DOX (90mg/kg) was IP -injected 1 hour after the last DMF treatment on day 14.

Twenty-four hours after DOX injection (i.e., at day 15), rats were anaesthetised using diethyl ether, blood samples were collected from the Jugular vein in non-heparinized tubes and were left to clot at room temperature, then centrifuged for 20 min at 4000 rpm to obtain serum and stored at -20°C for biochemical analysis; the animals were sacrificed by cervical dislocation, and the rats' femoral bone marrow (BM) was harvested and processed for genotoxicity evaluations (17).

2.4. Assessment of Oxidative stress parameters

The obtained serum was used to assess the oxidative/antioxidant status by measuring malondialdehyde (MDA), Glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels using commercially available ELISA kits obtained from MyBioSource (San Diego, US).

2.5. Preparation of bone marrow (BM) cells for the genotoxic evaluations

2.5.1. Evaluation of the Chromosomal aberrations (CAs) and the Mitotic Index (MI)

BM cells were prepared according to the colchicine-hypotonic citrate technique for CAs. Briefly, 2 hours before sacrifice, rats were IP-injected with colchicine (2mg/kg BW); the femur bone of each animal was taken and cleaned from tissues and muscles, and then the femoral marrow was flushed out using (0.075M) potassium chloride into a centrifuge tube and centrifuged at 2000rpm for 10minutes. Additionally, the cells pellets were fixed in (1:3) acetic acid/methanol (v/v), which was repeated three times; then the cell suspension was dropped on coded and sterile cleaned frosted slides and then dried followed by staining with 10% Giemsa stain; and finally, the slides were examined under a light microscope (Japan, Meiji). The frequency of CAs was scored in at least 100 meta-phase plates per animal; moreover, the chromatid -gaps and -break chromosome gaps and breaks, ring, deletion and exchanges were recorded; and the MI was obtained by counting at least 1000 cells per animal for dividing cells (18) (19).

2.5.2. Evaluation of the Micronuclei (MN) Appearance

The bone marrow cells were prepared following the method of Schmid (20) with certain adjustments described by Bhilwade et al. (2004) (21). Each animal's femur was taken, and the excess tissues and muscles were removed. The femoral marrow was flushed out using fetal bovine serum (FBS) into a centrifuge tube and centrifuged at 1500 rpm for 10 min to obtain cells pellet, which was thoroughly mixed, then smeared on coded and cleaned frosted slides, air-dried and fixed with absolute methanol. The slides were stained in May–Gruenwald for 5min, then with 10% Giemsa for 10 min, followed by thorough washing with DW. The slides were dried and examined under a light microscope. At least 1000 cells/animal were screened for scoring the frequency of micronucleated polychromatic erythrocytes (MNPCEs) (22).

2.6. Single-cell gel electrophoresis (SCGE) /Comet assay

The comet assay was performed following the method described by Dhawan*et al.* (23) based on the original procedure developed by Singh et al. (24). Concisely, the BM was flushed out with the chilled Hanks' Balanced Salt Solution (HBSS) buffer into a microcentrifuge tube; then 5µl was mixed with 75µl of 0.5% low melting agarose solution prepared in 0.9% normal saline and transferred onto frosted slides, which were kept in lysis buffer (20 mM EDTA, 10% DMSO and 0.1% Triton X-100) for 2 hours at 4 °C. Then the slides were removed from the lysis buffer and placed on a horizontal electrophoresis gel box; then the slides were kept in freshly prepared alkaline buffer (Electrophoresis Buffer) with pH>13 for 20 min to unwind the DNA strands. Electrophoresis was carried out for 30 min at 24 volts (˜0.74 V/cm), 300 milliamperes. The slides were gently washed in a neutralising buffer for 5min, which was repeated twice to remove the alkaline buffer and then dried. The slides were stained with 80µL 1X Ethidium Bromide, and a minimum of 50 cells/slide was captured using a 40x objective on a fluorescent microscope. The comet images were analysed using "Open Comet" digital imaging software. The percent (%) DNA in Tail, which is the fraction of DNA in the comet tail divided by the total amount of DNA associated with a cell multiplied by 100, was measured to assess the extent of oxidative DNA damage.

2.7. Statistical Analysis

The data are demonstrated as Mean ± Standard deviation (SD), and the statistical significance among groups was determined using one-way analysis of variance (ANOVA) test followed by Tukey's post-hoc test for multiple comparisons using GraphPad Prism version 9.5.0. The$P$ values<0.05 were regarded as statistically significant.

3. Results

3.1. Effect of DMF on oxidative stress parameters

The antioxidant activity of DMF was assessed in rats following acute DOX exposure, as oxidative stress plays a vital role in DOX-induced cytotoxicity. Administration of DOX alone **(Group III)** caused a highly significant increase ($p < 0.0001$) in the MDA level in comparison to the control group **(Group I)** ; However,

pre-treatment with DMF **(Group IV)** significantly ameliorated the increase in MDA serum level **Fig. 1A** . Additionally, DOX alone**(Group III)** led to significant GSH depletion, as well as SOD and GPx-1 exhaustion, compared to the control group **(Group I).**In contrast, pre-treatment with DMF prior to DOX exposure **(Group IV)** significantly increased the levels of GSH and restored SOD and GPx-1 activity levels in comparison to the DOX group **(Group III),** as shown in **Fig. 1B-D.** Furthermore, DMF alone**(Group II)** caused no significant difference in MDA level ($p > 0.05$) **Fig. 1A** and significantly increased SOD serum level ($p < 0.001$) when compared to the control group

(Group I) Fig. 1C.

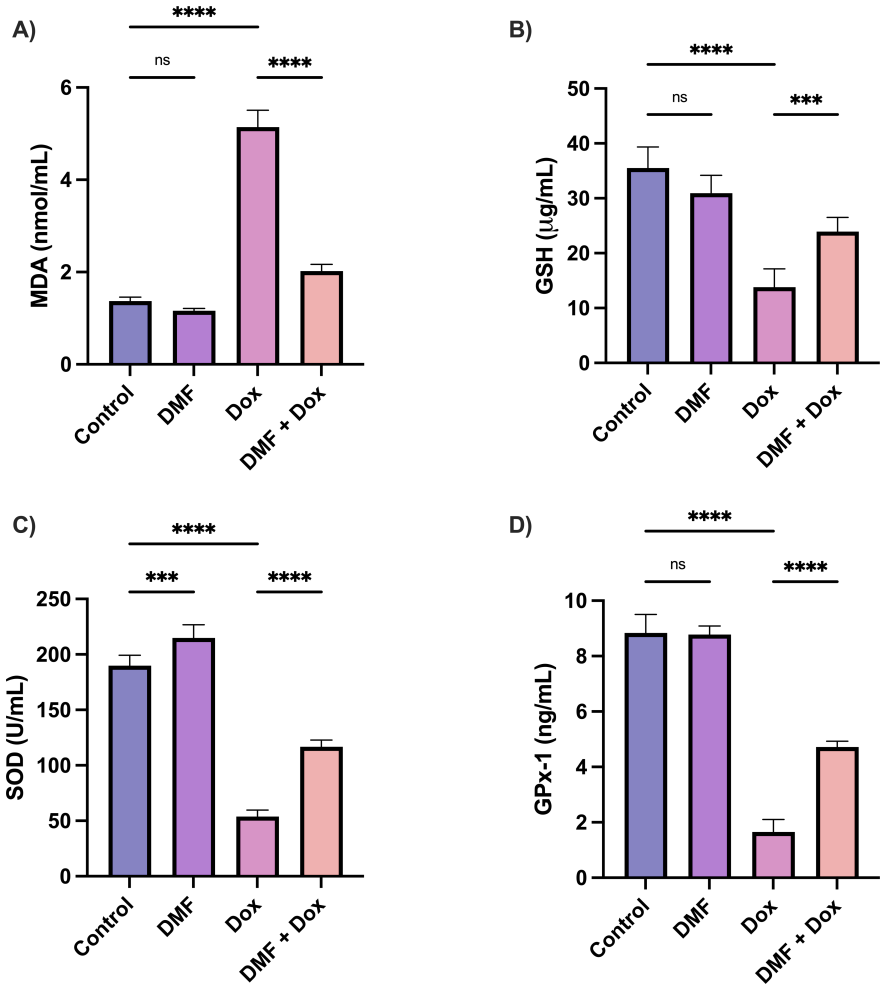

Figure 1. Effect of DMF on Oxidative Stress markers in Wister rats. DMF, Dimethyl fumarate; DOX, doxorubicin. A) serum level of MDA, B) serum level of GSH, C) serum level of SOD, D) serum level of GPx1; Data are expressed as (mean±SD), (n=8); *** ($p < 0.001$), **** ($p < 0.0001$) vs DOX-only group; ns ($P>0.05$), no significant difference.

3.2. Effect of DMF on Mitotic index (MI) and Chromosomal Aberrations (CAs)

Table 1 showed that in **Group II** [rats orally administered DMF alone (15mg/kg/day)], there was a non-significant difference in the frequency of TCAs ($P > 0.05$) in compassion to**Group I** (control).

Furthermore, rats IP injected with a single dose of DOX (90mg/kg) (**Group III**) exhibited a significant

increase*(P* <0.05) in the frequency of all structural CAs types, total chromosome aberrations, and abnormal metaphases compared to the control (**Group I)** rats.

However, in **Group IV** (DMF pre-treatment prior to DOX (90mg/kg) exposure), there were significant reductions *(P* <0.05) in the frequency of TCA, chromatid break, ring formation, and chromosomal break compared to such frequency values in **Group III** , with no significant change in the chromatid and chromosomal gap appearance, chromosomal fragment deletion, acentric and dicentric chromosome when compared to (**Group III**) rats.

Concerning the mitotic index (MI), table 1 showed that there was a non-significant difference in the percentage of MI (*P* >0.05) in **Group II** rats (DMF alone) when compared to the corresponding index in control (**Group I**). Moreover, in rats IP-injected with a single dose of DOX (**Group III**) there was a significant decrease *(P* <0.05) in the MI value compared to the corresponding index in control (**Group I**).

However, in **Group IV** (DMF pre-treatment prior to DOX exposure), there was a significant increase *(P* <0.05) in MI compared to the corresponding index in **Group III (** DOX only).

Table 1. Effect of DMF on the mitotic index (MI) and structural chromosomal aberrations (CAs) in Wister rats' bone marrow cells

Groups	**MI %**	**Structural type of aberration** Chromatid Break	**Structural type of aberration** Chromatid gap	**Structural type** Acentric
Control	8.5 ± 0.58	0.06 ± 0.008	0.07 ± 0.008	0.25 ± 0.032
DMF	8.6 ± 0.45	0.06 ± 0.008	0.06 ± 0.01	0.22 ± 0.06
Dox	5.4 ± 0.98 *	0.06 ± 0.09 *	0.12 ± 0.04 *	0.31 ± 0.07
DMF + Dox	6.7 ± 1.03 #	0.07 ± 0.01#	0.08 ± 0.02	0.26 ± 0.03

Data are expressed as (mean±SD), (n=8). TCA, total chromosomal aberration. DMF, Dimethyl Fumarate; DOX, Doxorubicin

* $p < 0.05$ vs vehicle-only (control/Group I)

# $p < 0.05$ vs. doxorubicin-only (Group III)

3.3. Effect of DMF on Micronucleus (MN) appearance

Table 2 showed no significant difference in the appearance of micronucleated polychromatic erythrocytes (Mn-PCEs%) in Group II rats compared to the control group.

Furthermore, table 2 also showed that acute exposure to DOX (90mg/kg) (**Group III**) caused a significant increase*(P* <0.05) in the frequency of MN appearance in comparison to such appearance in control (**Group I**). in contrast, DMF pretreatment prior to DOX Exposure (**Group IV**) caused a significant decrease (*P* <0.05) in the appearance of Mn-PCEs when compared to **Group III** .

Table 2. Effect of Dimethyl Fumarate (DMF) on the frequency of micronucleated polychromatic erythrocytes (%Mn-PCEs) appearance in Wister rats' bone marrow cells

	Groups	**% Mn-PCEs**
I	**Control (vehicle-only)**	6.91 ± 0.64
II	**DMF-only**	6.58 ± 0.36
III	**Dox-only**	10.21 ± 0.66 *
IV	**DMF + Dox**	9.45 ± 0.39 #

Data are expressed as (mean±SD), (n=8); % Mn-Es: numbers of micronucleated cells/total erythrocytes scored.

* $p < 0.05$ vs the vehicle-only (control)

# $p < 0.05$ vs the doxorubicin-only/model (Group III)

3.4. Effect of DMF on oxidative DNA damage/comet assay

There was a non-significant difference in the appearance of the comet, which can be seen in the form of intact nuclei with supercoiled undamaged DNA without comet tail in **Group II** (DMF only) compared to that in **Group I** (control) **Fig. 2A** ; in addition, DMF alone produced non-statistically significant differences (P >0.05) in the DNA damage (% DNA in tail) compared to the control **fig. 2B** .

While acute exposure to DOX (**Group III)** showed damaged abnormal nuclei with DNA strand breaks in the form of comet tail emerging as a hollow area; moreover, there was a significant increase (P <0.0001) in % DNA in tail value in **Group III** compared to the control group **fig 2B** . However, the BM cells from the rats pretreated with DMF prior to DOX (**Group IV)**exhibited an improvement in the comet appearance; in addition, to a significant inhibition (P <0.0001) of DNA damage (i.e., a reduction in the value of the % DNA in tail) compared to**Group III** .

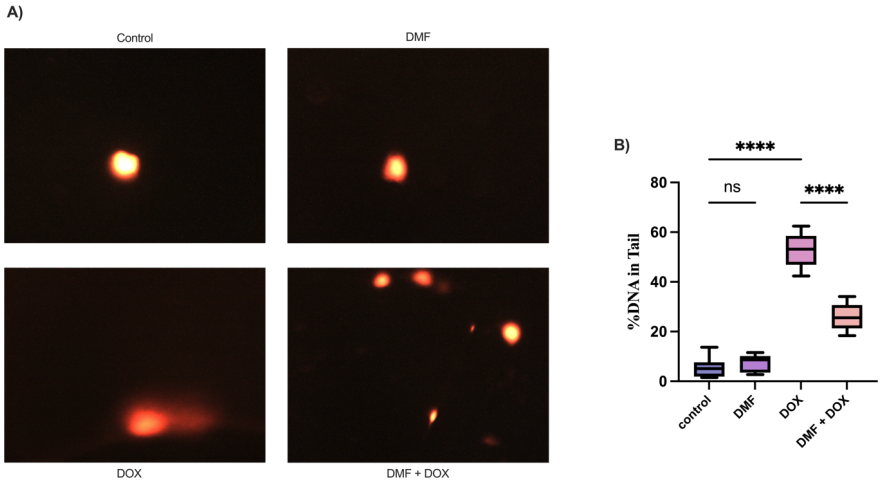

Figure 2. Effect of DMF on DNA damage in Wister rats. DMF, Dimethyl fumarate; DOX, doxorubicin.

A: Photomicrographs showing comet of rats' bone marrow stained with Ethidium Bromide.

B: DNA damage measured as % DNA in tail; Data are expressed as (mean±SD), (n=8); **** ($p < 0.0001$) vs DOX-only group; ns (P >0.05), no significant difference.

Discussion

Although DOX is used for treating a broad range of solid tumours, nevertheless, its use is associated with severe adverse effects such as cardiotoxicity and myelosuppression (2)(3). The reduced efficacy of DOX in treating cancer and the significant genotoxicity was reported to be related to the direct DNA damage caused by such chemotherapeutic drug and its ability to interact with the DNA molecule and interfering with the activity of topoisomerase-II enzyme, thus, interfering with DNA replication and repair; furthermore, DOX may have an indirect effect through the generation of free radicals, and this consequently leads to the depletion of the antioxidants, increasing lipid, protein and nucleic acid peroxidation, DNA double-strand breakage and chromosomal aberration in normal cells (4)(7). Therefore, reducing the DOX induced unwanted effects on normal cells would enable broader use in chemotherapeutic regimens and improve outcomes in cancer patients.

In the current study, the data showed that acute exposure to DOX caused a significant increase in the serum level of MDA, depleted GSH and antioxidant enzymes, induced structural chromosomal aberration and increased micronuclei appearance in addition to stimulated DNA damage compared to those in control (**Group I**) as shown in Fig 1A, tables 1 and 2, and figure 2 A&B, respectively. These results are agreeable with previous studies stating that DOX caused oxidative stress, clastogenic changes and DNA damage (25)(26)(27). In addition, DOX has a noticeable inhibitory effect on the cell division and the mitotic index value, which is agreeable with the earlier findings reported in previous studies (15)(28)(29). Furthermore, the results revealed that DMF (15mg/kg/day) alone caused a non-significant difference in TCAs, MN appearance and the % DNA in Tail of the comet; this might indicate that DMF may have no clastogenic or DNA damaging effects on rats' BM *in vivo* .

However, DMF (15mg/kg/day) orally administered for 14 days to rats prior to doxorubicin (90mg/kg) exposure (**Group IV**) significantly reduced MDA level, restored GSH level and antioxidant enzymes activity compared to **Group III** which supports its antioxidant role. Moreover, in rats of **Group IV** , DMF significantly reduced (P <0.05) TCAs, MN appearance and % DNA in Tail compared to those in the control group; thus, it efficiently protected against DOX-induced genotoxic effects in rats' BM [Fig. 1B-D, Tables 1 and 2 and Fig. 2B].

There are no previous studies regarding the protective effect of DMF against genotoxicity induced by DOX *in vivo* . Thus, the present study possibly is the first that demonstrates the modulatory effect of DMF pre-treatment on DOX-induced chromosomal and DNA damage effects in rats' BM *in vivo* .

The mechanism underlying DMF anticlastogenic effect against DOX could be related to its antioxidant activity, as it might reduce the DOX-mediated free radicals' generation, inhibited the formation of DNA adduct, and reduced DNA and chromosomal damage since DOX is well-renowned for inducing cellular oxidative stress (OS) and subsequent DNA damage; what's more, DMF showed to have an anticarcinogenic/antimutagenic activity in animal models by modulating NrF2 axis (30)(31); furthermore, DMF was previously-reported to show a protective activity against myocardial Ischemic/Reperfusion which is related to DMF mediated improved cellular viability, reduced oxidative stress and enhanced the expression of Nrf2-regulated antioxidative genes; in addition, DMF was reported to prevent apoptosis, increase the survival rate and proliferation of human adipose-derived mesenchymal stem cells (hASCs) against oxidative stress which is mediated by upregulation of HO-1 and NQO-1 expression (32). Similarly, DMF has been shown to induce apoptosis in cancer and damaged cells, which may facilitate the reduction in the appearance of chromosomal damage (33).

Conclusion

According to results obtained from this study, it can be concluded that oral administration of DMF (15mg/kg/day) alone has no DNA damaging effect or clastogenic activity. Its administration prior to IP injection of DOX improved the mitotic index and reduced the extent of DNA damage and chromosomal damage, and MN appearance in Wistar rats BM cells under the present experimental conditions; thus, DMF might be a potential chemoprotective agent against doxorubicin-induced adverse effect in cancer chemotherapy; consequently, DMF might help prevent reproductive abnormalities, secondary malignancy development and myelosuppression in cancer patients.

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Figures legend

Figure 1. Effect of DMF on Oxidative Stress markers in Wister rats. DMF, Dimethyl fumarate; DOX, doxorubicin.

serum level of MDA, B) serum level of GSH, C) serum level of SOD, D) serum level of GPx1.

Data are expressed as (mean±SD), (n=8); *** ($p < 0.001$), **** ($p < 0.0001$) vs DOX-only group; ns ($P>0.05$), no significant difference.

Figure 2. Effect of DMF on DNA damage in Wister rats. DMF, Dimethyl fumarate; DOX, doxorubicin.

A: Photomicrographs showing comet of rats' bone marrow stained with Ethidium Bromide.

B: DNA damage measured as % DNA in tail.

Data are expressed as (mean±SD), (n=8); **** ($p < 0.0001$) vs DOX-only group; ns ($P >0.05$), no significant difference.