DOI: https://doi.org/10.31351/vol32issSuppl.pp16-25

Protective Effect of Cafestol on Doxorubicin-induced Genotoxicity in Rats[#]

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[#] 2nd Scientific Conference for Postgraduate Students Researches

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Abstract

Doxorubicin is an efficient antineoplastic agent that has a broad antitumour spectrum; however, its genotoxic adverse effects on normal cells can be produced through oxidative damage, and this limits its clinical application. Cafestol is a naturally-occurring diterpene in unfiltered coffee with noteworthy antioxidant, antimutagenic and anti-inflammatory activities. The present study aimed to investigate the possible protective effect of cafestol against doxorubicin-induced chromosomal and DNA damage in rat bone marrow cells. Forty-eight Wistar Albino rats of both sexes were divided randomly into **Group I** (negative control/vehicle only), **Group II** [cafestol-only (5mg/kg once daily by oral gavage for 14 consecutive days)], **Group III** [(DOX only/model) injected as a single dose 90 mg/kg intraperitoneally at day 14 to induce toxicity], and **Group IV** (Cafestol 5mg/kg once daily by oral gavage for 14 consecutive days then DOX was injected as a single dose 90 mg/kg intraperitoneally at day 14). The bone marrow was harvested 24 hours after doxorubicin's injection from all groups for the assessment of structural chromosomal aberration, micronucleus, and comet assays. The result showed that rats in the doxorubicin-only group exhibited a significant decrease (P<0.05) in mitotic index with a significant elevation (P<0.05) in the % DNA in Tail, micronucleus appearance and total structural chromosomal aberrations compared to those of the negative control group; while oral administration of cafestol 14 days prior to doxorubicin, significantly-reduced the % DNA in Tail, micronucleus appearance, and the total number of chromosomal aberrations (P<0.05), and improved the mitotic index compared to rats intraperitoneally-injected with doxorubicin alone. This study revealed that cafestol has protective effects against the genotoxicity induced by doxorubicin.

Keywords: Doxorubicin, Cafestol; Genotoxicity, Mitotic index, Chromosomal aberration, Micronucleus assay, Comet assay.

التأثير الوقائي للكافيستول ضد السمية الجينية المستحثة باستخدام الدوكسوروبيسين في الجرذان[#]

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الخلاصة

ان الدوكسوروبيسين علاج ذو طيف واسع فعال لعلاج للأورام السرطانية. رغم ذلك، فان استخدامه يسبب تأثيرات سامة على الجينات في الخلايا الطبيعية غير السرطانية، وهذا يحد من تطبيقه السريري.

ان الكافستول هو داي تيربين موجود بشكل طبيعي في القهوة غير المفلترة، له تأثيرات جديرة بالملاحظة حيث انه يعمل كمضاد -للأكسدة و-للجراثيم ومضاد للالتهابات.

تهدف الدراسة الحالية إلى التحقق من التأثير الوقائي المحتمل للكافستول ضد أضرار الكروموسومات التي يسببها الدوكسوروبسين وتلف الحمض النووي في خلايا نخاع عظام في الجرذان.

تم استخدام ثمانية واربعون جرذ من كلا الجنسين وتم تقسيمهم كالاتي **المجموعة الأولى** (مجموعة السيطرة السلبية)، **المجموعة الثانية** (تم اعطائها الكافستول لوحده ٥ ملغم/كجم مرة واحدة يوميًا لمدة ١٤ يومًا متتاليًا عن طريق الفم باستخدام انبوب التجريع)، **المجموعة الثالثة** (تم حقن الدوكسوروبسين لوحده كجرعة وحيدة ٩٠ ملغم / كغم داخل الصفاق في اليوم الرابع عشر (١٤) لاستحداث السمية)، **المجموعة الرابعة** (تم اعطائها الكافستول ٥ ملغم/كجم يوميًا لمدة ١٤ يومًا متتاليًا عن طريق الفم بعدها تم حقن الدوكسوروبسين ٩٠ ملغم / كغم داخل الصفاق في اليوم الرابع عشر). تم استخراج النخاع العظمي بعد ٢٤ ساعة من حقن دوكسوروبيسين في جميع المجموعات لتقييم التغيرات الشكلية في الكروموسومات وظهور النواة الصغيرة وقياس المذنب.

بينت النتائج أن الجرذان في مجموعة الدوكسوروبيسين لوحده ظهر عندها انخفاضًا ملحوظا في مؤشر الانقسام المغزلي بالإضافة الى ارتفاع كبير في النسبة المئوية للحمض النووي في ذيل المذنب مع ظهور النواة الدقيقة وارتفاع نسبه ظهور التغيرات الشكلية في الكروموسومات بالمقارنة مع تلك الموجودة في مجموعة السيطرة السلبية؛ بينما في الجرذان التي جرعت الكافستول عن طريق الفم لمده ١٤ يومًا قبل حقن الدوكسوروبيسين أدى إلى انخفاض كبير في نسبة تمركز الحمض النووي في ذيل المذنب وتقليل ظهور النواة الدقيقة ، بالإضافة الى تقليل النسبة الإجمالية للتغيرات الشكلية في الكروموسومات ، مع تحسن مؤشر الانقسام المغزلي مقارنة بالجرذان التي تم حقنها دوكسوروبيسين وحده.

الاستنتاج: كشفت هذه الدراسة أن الكافستول له تأثيرات وقائية ضد السمية الجينية التي يسببها الدوكسوروبيسي في الجرذان.

الكلمات المفتاحية : دوكسوروبيسين، كافيستول، السمية الجينية، مؤشر الانقسام، التغيرات الكروموسومية، فحص النواه الصغيرة، فحص المذنب.

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Received: 25/3 /2023

Accepted: 18/4 /2023

Iraqi Journal of Pharmaceutical Sciences

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 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)]. The dose-dependent response relation of doxorubicin in many anticancer regimens has been well-defined, and, 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 major 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 stabilizes 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 are an important part of 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 ($O_2^{\bullet-}$), and hydroxyl radical ($OH^{\bullet}$) which can -deplete antioxidants and glutathione, -increase lipid, protein and nucleic acid peroxidation [(6)]. 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; and these cytotoxic actions not only affect cancer cells but also can affect normal cells leading to mutation and chromosomal abnormalities including chromosomal aberrations (CAs) and DNA damage that characterized by micronuclei (MN) formation and decreased mitotic index (MI), Furthermore, researchers reported that the chemical mutagens and carcinogens can induce CAs, which is referred to be an any change either in the structure of a chromosome (structural CA) or in the number of chromosomes; where, the structural abnormalities may include deletions, ring chromosomes, dicentric chromosomes or acentric chromosomes [(7,8)].

Additionally, the MN are small extra-nuclei in the cytoplasm alone or in aggregation with other chromosomal fragments that are left out of the daughter nuclei during cellular division [(9)].

Cafestol is a natural diterpene that is extracted from the lipid fraction of the coffee bean which predominantly-exist in unfiltered coffee as a fatty ester [(10)]. Researchers mentioned that cafestol can counteract OS [(11)], has anticarcinogenic activity [(12)], induce apoptosis in malignant cells [(13)], and have anti-inflammatory effect [(14)(15)(16)]; furthermore, cafestol has an antimutagenic activity which can be related to its ability to modulate the expression of several detoxifying enzymes that are involved in detoxification and carcinogenesis; where, studies demonstrated that the expression of hepatic cytochrome P450s (CYP450s) phase I metabolizing enzymes that play an essential role in many carcinogens' activation was downregulated by cafestol; in addition, cafestol can also enhance the activity of phase II detoxifying enzymes; thereby, inhibiting the formation of oxidative and electrophilic metabolites produced from carcinogens activation thus prevent carcinogenesis [(17)(18)(19)]. Moreover, cafestol can enhance glutathione-S-transferase (GST) enzyme activity in the liver and the small bowel in the experimental animals; additionally, it can greatly-enhance the activity of quinine oxidoreductase1 and U5′-diphosphoglucuronosyl transferase phase II metabolizing enzymes [(20) (21)].

Furthermore, it has been reported that cafestol activated the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway, antioxidant proteins and phase II detoxifying enzymes; in addition it inhibited the expression of the inflammatory mediator through the activation of nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway [(14)].

Therefore, the present study is designed to investigate the modulatory effect of cafestol against doxorubicin-induced genotoxicity in rats by the assessment of CAs, MN and MI in addition to the utilization of the comet assay, which is a sensitive method to measure the extent of oxidative DNA damage.

Materials and methods

Chemicals

Cafestol (Purity >98%, CAS no.81760-48-7) 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.

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 [22].

In addition, cafestol 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 utilization of oral gavage as a single dose of 5mg/kg/day which was selected based on the previously-shown protective effect [11] and optimized in our preliminary experiment; since, the administration of cafestol suspension at a dose of 5mg/kg BW didn't cause any statistically-significant difference in the selected parameters in comparison with the control group, which received only the vehicle; thus, cafestol at the dose of 5mg/kg BW used in the current study is safe with no toxic effects.

Animals and experimental design

The study protocol was approved by the Graduate Studies and the Ethical Committees of the College of Pharmacy, University of Baghdad. Forty-eight (48) 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; since, 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 (48 Rats) were randomly assigned into 4 groups (n=6) in each assay, in which 24 animals were used to perform the Chromosomal aberration assay, and the other 24 animals were utilized to perform both the micronucleus assay and the Comet assay as follows:

Group I*:* Each rat was orally-administered vehicle only/(5% tween in DDW) *via* an 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 cafestol only (5mg/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 cafestol (5mg/kg/day) for 14 consecutive days, then a single dose of DOX (90mg/kg) was IP-injected-injected 1hour after the last cafestol treatment on day 14.

Twenty-four hours after DOX injection (i.e., at day 15), rats were anaesthetized using diethyl ether and then sacrificed by cervical dislocation and the rats' femoral bone marrow (BM) was harvested and processed for genotoxicity evaluations [23][24].

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

Preparation and the Evaluation of the Chromosomal Aberrations (CAs) and the Mitotic Index (MI)

The preparation of BM cells was done according to the colchicine-hypotonic citrate technique for CAs. Briefly, 2hours before sacrifice, rats were IP-injected with colchicine (2mg/kg BW); and 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; and 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 [25,26].

Preparation and the Evaluation of the Micronuclei (MN) Appearance

The micronuclei (MN) were prepared following the method of Schmid [27] with certain adjustments described by Bhilwade *et al* (2004) [28]. Briefly, the femur of each animal was taken, and the excess tissues and muscles were removed, then 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) [29].

Single-cell gel electrophoresis (SCGE) /Comet assay

The comet assay was carried out following the method described by Dhawan *et al* [30] based on the original work developed by Singh *et al* [31]. Briefly, the BM was flushed out with the chilled Hanks' Balanced Salt Solution (HBSS) buffer into a microcentrifuge tube; then 5µl were mixed with 75µl of 0.8% 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 neutralizing buffer (0.4 M Tris- HCl, pH 7.5) for 5min which was repeated two more times to remove the alkaline buffer and then dried. The slides were stained with 80µL 1X Ethidium Bromide and a minimum of 50 cells/slide were 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, that is the fraction of DNA in the 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.

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.

Results

Effect of Cafestol on Mitotic index and Chromosomal Aberrations (CAs)

Table 1 showed the frequency of CAs, where, in **Group II** rats [orally-administered cafestol alone (5mg/kg/day)], there was a non-significant difference in the frequency of TCAs (*P*>0.05) in compassion to that frequency in **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** [rats orally-administered cafestol prior to a single IP dose of DOX (90mg/kg)], there were significant reductions *(P*<0.05) in the frequency of TCAs, chromatid break and gap, ring formation, chromosomal fragment deletion, acentric and dicentric chromosome compared to such frequency values in **Group III** (model group). However, cafestol pretreatment caused no significant difference in the chromosomal break and gap appearance when compared to (**Group III**) rats.

Concerning MI, table 1 showed that there was a non-significant difference in the percentage of MI (*P*>0.05) in **Group II** rats (cafestol alone/orally-administered 5mg/kg/day for 14 consecutive days) compared to the corresponding index in the control (**Group I**). Moreover, in rats IP-injected with a single dose of DOX (**Group III**) at a dose of 90mg/kg, there was a significant decrease *(P*<0.05) in the MI value compared to the corresponding index in the control (**Group I**) rats.

Furthermore, table 1 showed that in **Group IV** [rats orally-administered cafestol prior to IP injection of a single dose of DOX (90mg/kg)] there was a significant increase *(P*<0.05) in mitotic index (MI) compared to the corresponding index in **Group III [**DOX only (model)].

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

Groups	MI %	Structural type of aberration								TCA %
		Chromatid Break	Chromatid gap	Acentric	Dicentric	Ring	Deletion	Chromosome break	Chromosome gap	
Control	8.5 ± 0.58	0.06 ± 0.008	0.07 ± 0.008	0.25 ± 0.03	0.21 ± 0.04	0.21 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.125 ± 0.008
Caf	9.05 ± 0.59	0.04 ± 0.01	0.05 ± 0.01	0.19 ± 0.06	0.18 ± 0.01	0.20 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.05 ± 0.008	0.118 ±0.008
Dox	5.4 ± 0.98 *	0.06 ± 0.09 *	0.12 ± 0.04*	0.31 ± 0.07*	0.23 ± 0.04*	0.28 ± 0.05*	0.07 ± 0.01*	0.10 ± 0.03*	0.11 ± 0.03*	0.166 ± 0.021 *
Caf + Dox	7.45 ± 0.60 #	0.05 ± 0.01#	0.06 ± 0.01#	0.21 ± 0.01#	0.20 ± 0.02#	0.23 ± 0.04#	0.04 ± 0.01#	0.07 ± 0.03	0.06 ± 0.04	0.118 ± 0.007 #

Data are expressed as (mean±SD), n=6. TCA, total chromosomal aberration.
* $p < 0.05$ vs. vehicle-only (control/Group I)
# $p < 0.05$ vs. doxorubicin-only (Group III)

Effect of cafestol on Micronucleus (MN) appearance

Table 2 showed that there was a significant decrease (P<0.05) in the appearance of micronucleated polychromatic erythrocytes (Mn-PCEs) in **Group II** rats in comparison to such appearance in **Group I** (control) rats.

Furthermore, table 2 also showed that a single IP dose of DOX (90mg/kg) to rats (**Group III**) caused a significant increase (P<0.05) in the frequency of MN appearance in comparison to such appearance in the control (**Group I**) rats. Moreover, in rats that orally-administered cafestol (5mg/kg/day) for 14 days prior to IP injection of a single dose of DOX (90mg/kg) (**Group IV**), there was a significant decrease (P<0.05) in the appearance of Mn-PCEs when compared to **Group III/** [model (doxorubicin only)] rats.

Table 2. Effect of cafestol on the frequency of micronucleated erythrocytes appearance in Wister rats' bone marrow cells

	Groups	%Mn
I	**Control (vehicle-only)**	6.91 ± 0.64
II	**Cafestol-only**	6.10 ± 0.22 *
III	**Doxorubicin-only**	10.21 ± 0.66 *
IV	**Cafestol + doxorubicin**	9.11 ± 0.35 #

Data are expressed as (mean±SD), n=6; %MN: 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)

Effect of cafestol on oxidative DNA damage/comet Assay

There was a comparable appearance of the comet in **Group II** rats/cafestol only compared to that in **Group I** (control) rats (**figure 1A**) in the form of intact nuclei with supercoiled undamaged DNA without comet tail; in addition, cafestol alone produced non-statistically significant differences (P>0.05) in the DNA damage (% DNA in tail) compared to the control (**Group I)** rats (**figure 1B**). While rats in **Group III** (model/DOX), **figure 1A** showed damaged abnormal nuclei with DNA strand breaks in the form of a comet with a tail emerging as a hollow area; moreover, there was a significant increase P<0.05 in the value of the % DNA in tail in **Group III** rats compared to the corresponding damage in vehicle control (**Group I)** rats **(figure 1B)**. However, the BM cells from the rats pretreated with cafestol prior to DOX (**Group IV**), **figure 1A** showed that there was an improvement in the comet appearance; in addition, a significant inhibition P<0.05 of DNA damage (i.e., a reduction in the value of the % DNA in tail) in rats of **Group IV** compared to such percentage in **Group III**/model (DOX) rats.

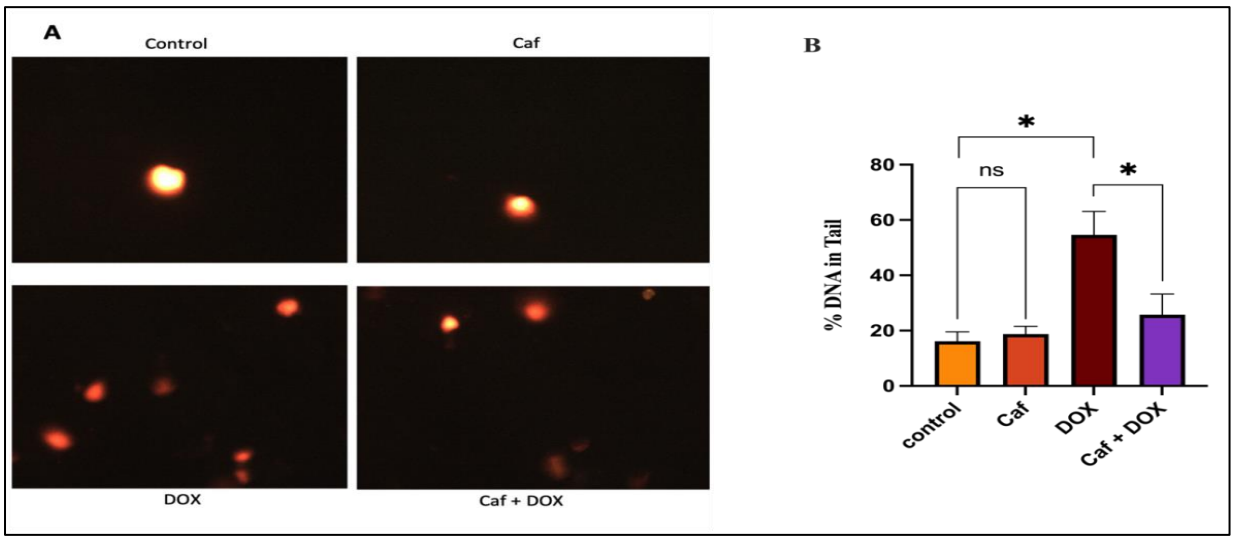

Figure 1. Effect of cafestol on DNA damage in Wister rats. Caf, cafestol; DOX, doxorubicin.

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=6); * (p < 0.05) vs. DOX-only group; ns (P>0.05), no significant difference.

Discussion

Researchers reported that although DOX is used for treating broad-range of solid tumours, but its use is associated with severe adverse effects such as cardiotoxicity, myelosuppression, and others [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 that is mediated by the generation of free radicals and this consequently lead to the depletion of the antioxidants, increasing lipid, protein and nucleic acid peroxidation, DNA double-strand breakage and chromosomal aberration in normal cells [32] [33]. Therefore, reducing the DOX induced-unwanted effects on normal cells would enable broader use of such drug in chemotherapeutic regimens and improve outcomes in cancer patients.

In the current study, the data showed that in rats IP-injected with a single dose of DOX (90mg/kg)/model (**Group III**) exhibited a significant increase in the appearance of structural CAs, the frequency of MN formation and the DNA damage compared to those in control (**Group I**) rats as shown in tables 1 and 2, and figure 1 A&B, respectively. These results are agreeable with other studies stating that DOX caused clastogenic activity and DNA damage in BM cells [31-33]. In addition, DOX has a noticeable inhibitory effect on the cell division and the MI value which can be related to its cytotoxic effects on BM cells; and these observations support the earlier findings reported in previous studies [22][34][35]. Furthermore, results of this study revealed that in **Group II** rats/orally administered cafestol (5mg/kg/day) alone, there was a non-significant difference in TCAs, MN appearance and in the % DNA in Tail, this may indicate that cafestol may have no clastogenic or DNA damaging effects on rats' BM *in vivo*.

Moreover, orally-administered cafestol (5mg/kg/day) for 14 days to rats prior to a single dose of DOX (90mg/kg) (**Group IV**) significantly increased the MI compared to such index in rats IP injected with a single dose of DOX 90mg/kg (**Group III/model**) rats (**Table 1**). However, in rats of **Group IV**, cafestol significantly reduced (P<0.05) TCAs, MN appearance and % DNA in Tail compared to those in control (**Group I**) rats; and thus efficiently protected against DOX-induced genotoxic effects in rats' BM [Tables 1 and 2 and figure 1 B].

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

The mechanism underlying cafestol anticlastogenic effect against DOX could be related to its antioxidant activity; since, it may reduce the DOX-mediated free radicals' generation, inhibited the formation of DNA adduct, and reduced chromosomal damage; since, DOX is well-recognized to induce cellular oxidative stress (OS) and subsequent DNA damage; furthermore, studies showed that cafestol, a coffee-specific diterpene has an anticarcinogenic/antimutagenic effects in animal models [13][36][37] which is related to cafestol's ability to enhance the expression of glutathione-S-transferases (GSTs) [29]; furthermore, cafestol in a mixture with kahweol was previously-reported to prevent genotoxicity induced by aflatoxin B1 through a dual mechanism that involved the modulation of xenobiotic activating enzymes expression that have the ability to activate potential carcinogens and the increase the expression of GST that in turn provides a chemoprotective effect; in addition, cafestol can activate the Nrf2/HO-1pathway resulting in the inhibition of the redox signaling [38][39]. Similarly, cafestol has been found to attenuate apoptosis and protect cells against OS and DNA damage induced by hydrogen peroxide [17][18]; in addition, cafestol has been shown to induce apoptosis in cancer and damaged cells, which may facilitate the reduction in the appearance of chromosomal damage [19][40].

Conclusion

According to results obtained from this study, it can be concluded that oral administration of cafestol (5mg/kg/day) alone has no DNA damaging effect or clastogenic activity, and 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; and thus, cafestol can be applicable for the reduction of DOX's adverse effect in cancer chemotherapy.

Acknowledgements

The authors are thankful to the College of Pharmacy, University of Baghdad, for providing the experimental animals.

Conflicts of interest

The authors declare that the research was conducted in the absence of any conflict of interest.

Funding

The research did not receive any financial support from an Institution.

Ethics Statements

The study protocol was approved by the Graduate Studies and Ethics Committees of the College of Pharmacy/ University of Baghdad.

Author Contribution

The authors confirm their contribution to the paper as follows: study conception and design: Sara A., Nada N.; data collection: Sara A.; analysis and interpretation of results: Sara A.; draft manuscript preparation: Sara A., Nada N. All authors reviewed the results and approved the final version of the manuscript.

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