# Resveratrol Mediated Protection of Dacarbazine-Induced Mutagenicity in Mice

**Ramadan, A.M. Ali**

Zoology Dept., College for Girls for Science, Arts and Education, Ain-Shams Univ., Heliopolis, Cairo, Egypt
ramadanali27@gmail.com

**Abstract:** Dacarbazine (DTIC) is one of the most effective chemotherapeutic drugs that have been successfully applied to treat malignancy. *In vivo* studies revealed that DTIC induced oxidative DNA damage and cell cycle arrest. Resveratrol (RES) is a natural polyphenol (*trans*-3,5,4-trihydroxy stilbene) found in grapes and attracts public attention because it possesses diverse biochemical, anticancer and antigenotoxic actions. The present work was aimed to investigate the antigenotoxic activity of RES against DTIC-induced chromosomal aberrations (CA) and micronucleated polychromatic erythrocytes (Mn-PCEs) formation, micronucleated peripheral blood reticulocytes (Mn-Ret) development and DNA fragmentation in bone marrow cells of mice. The animals divided into 10 groups each with 6 animals. Each animal group received either a single dose of 0, 2.5, 5, 10 and 20 mg/kg b.w. DTIC or pretreated daily with 0, 50 mg/kg b.w. for 15 days with RES. Animals were sacrificed 24 h post treatment with DTIC. RES treatment for 15 days decreased the control base line of CA, Mn-PCEs, Mn-Ret incidences and DNA fragmentation and elevated the averages of mitotic indices and PCEs/NCEs. RES treatment significantly reduced the increased averages of CA, percentages of Mn-Ret and DNA fragmentation induced by DTIC. Moreover, RES significantly ($P< 0.001$) elevated the averages of PCEs/NCEs and percentages of mitotic index induced by DTIC. It is concluded that, administration of resveratrol improved the genotoxic effects of dacarbazine and the results of this work draw attention for application of a safer chemotherapeutic protocol for cancer treatment by using strong antioxidants concurrently with chemotherapeutic agents.

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**Key words:** Dacarbazine; Resveratrol; Micronucleus; Chromosomal aberrations; DNA fragmentation; Mouse; Bone marrow cells

## 1. Introduction

Resveratrol (RES) is a natural polyphenol phytoalexin (*trans*-3,5,4-trihydroxy stilbene) found in grapes, peanuts and cranberries and possesses diverse biochemical and physiological actions (**Wang *et al*., 2002**). RES ameliorates experimental autoimmune myocarditis (**Yoshida *et al*., 2007**), prevents estrogen-DNA adduct formation and neoplastic transformation in MCF-10F cells (**Lu *et al*., 2008**), attenuates the experimental neuroinflammation-mediated cognitive deficits in rats (**Gong *et al*., 2010**), inhibits the transcription of lytic genes and the lytic cycle of Epstein-Barr Virus to reduce the production of viral particles (**Yiu *et al*., 2010**) and reduced lipid peroxidation induced by tert-butyl hydroperoxide in human sperm and in rat spermatocytes and spermatids (**Collodel *et al*., 2011**). RES reduces the incidences of comets in lymphocytes induced by platinum compounds (**Olas *et al*., 2005**), protectes the arsenite induced DNA damage in normal mammalian V79 cells (**Roy *et al*., 2008**), inhibites the frequencies of micronuclei induced by hydrogen peroxide in astroglial cells (**Quincozes-Santos *et al*., 2010**) and protected against ethanol-induced oxidative DNA damage in human peripheral lymphocytes (**Yan *et al*., 2011**).

Dacarbazine (DTIC) [5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide] is a synthetic analogue of the naturally occurring purine precursor, 5-amino-IH-imidazole-4- carboxamide (**Rooseboom *et al*., 2004**). DTIC is alkylating cytostatic drug extensively used as a single agent or in combination with other drugs for treatment of malignant melanoma, soft tissue sarcoma, renal adenocarcinoma, solid tumors, osteogenic sarcoma, neuroblastomas and malignant lymphomas (**Yi *et al*., 2011**). A combination of mesan, adriamycin, ifosfamide and dacarbazine (MAID combination) is used against advanced soft tissue sarcomas which has been transform many incurable tumors to highly curable ones (**Verma *et al*., 2008**). Chronic oral and intraperitoneal administration of DTIC to mice induced mammary adenocarcinomas, thymic and splenic lymphomas, breast, brain and lung cancers (**Kakumanu *et al*., 2011**).

In mice, DTIC induced reduction in the mitotic index and increases the chromosomal aberrations in bone marrow cells (**Al-Saleh, 2001**), increased the micronuclei in peripheral blood cells (**Adler *et al*., 2002**) and induced defects in the spermatogenesis (**Adler *et al*., 2002**). **IARC (1981)** reported that, DTIC is mutagenic in mouse lymphoma cells *in vitro*. Moreover, DTIC induces teratogenicity in humans (**Aviles *et al*., 1991**), induced micronuclei in peripheral blood lymphocytes of patients treated against metastatic melanoma

(**Miele *et al*., 1998**), induced DNA damaged peripheral blood lymphocytes (**Yoshida *et al*., 2006**) and in human A375 melanoma cells (**Samulitis *et al*., 2011**).

The extensive use of DTIC in malignancy treatment and the associated harmful genotoxic side effects attracts attention to do a trial to reduce the genotoxicity of DTIC by resveratrol antioxidant for the application of a new and safer chemotherapeutic protocols.

## 2. Materials and Methods:

### 1- Chemicals:

Dacarbazine (DTIC) purchased from local pharmacies under the trade name DETICENE 200 mg vial provided with a vial containing 19.7 ml of sterile water for injection and produced by SANOFI AVENTIS-EGYPT. Resveratrol (purity 99%), purchased from Sigma (St. Louis, MO, U.S.A. CAS number 501-36-0). Fetal bovine serum was provided from Gibco BRL (Grand Island, NY, U.S.A.). Chemicals were dissolved in appropriate concentrations for animals to receive the injected volumes equivalent to 1.0 ml solution /100 g animal b.w.

### 2- Animals and treatments:

Total of 60 BALB/C adult male albino mice, aged 9-10 weeks and weighing 24-26 g purchased from The Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt were utilized in this work. Animals were acclimatized for one week prior to experimentation and housed in plastic cages (40 × 30 × 16 cm) at 22 ± $2^0$C and 30–70% relative humidity. The mice were accommodated 6 per cage with wooden chip bedding and given commercial food pellets and water *ad libitum*. All experimental procedures were performed according to the Institutional Animal Care and Experiment Committee of Ain-Shams University.

The doses were proportional to the dose rate used for human treatment. Animals were arranged in groups of 6 animals / experimental point. Animals divided into two main groups. Group one included 30 mice and subdivided into 5 subgroups; 6 mice each. One subgroup includes animals of the control group and received 0.2 ml dis H20. The 4 subgroups received either single dose of 0, 2.5, 5, 10 and 20 mg/kg b.w. DTIC for 24 h. Group two included 30 mice and subdivided into 5 subgroups; 6 mice each. One subgroup includes animals received oral dose of 50 mg/kg b.w. RES daily for 15 days. The 4 subgroups received oral daily doses of 50 mg/kg b.w. RES for 15 days. At day 14 of RES treatment, the animal subgroups are injected intraperitoneally with single doses of 2.5, 5, 10 and 20 mg/kg b.w DTIC. The animals were sacrificed 24 h post last treatment(s).

### 3- Bone marrow chromosomal aberration assay:

Chromosomes were prepared from bone marrow cells of mice according to the method previously postulated by **Preston *et al*. (1987)**. 200 well spread metaphases were examined per animal with oil immersion of Meiji microscope. The chromosomal aberrations were classified according to **Savage (1976)**. Mitotic indices were determined from scoring 1000 cells for each animal. The counts were carried out with the hand tally counter.

### 4- Bone marrow micronucleus (Mn) assay:

Animals were sacrificed by cervical dislocation and bone marrow cells smeared on glass slides, fixed with methanol. The slides were stained just before examination with 20.0 µL of the fluorescence dye acridine orange (1.0 mg/mL) and coverslipped (**Hayashi *et al*., 1983**). The slides examined by fluorescence microscopy, using blue light (488 nm) and an orange filter, with a 40 times objective. For each animal, 2000 PCEs scored for the presence of Mn. In addition to that, the frequencies of PCEs among 2000 bone marrow erythrocytes (NCEs + PCEs) calculated to determine the frequencies of bone marrow depression, and the values were expressed as PCE/NCE.

### 5- Micronucleus assay in peripheral blood:

The peripheral blood micronucleus assay carried out according to **Hayashi *et al.* (1990)**. A total of 2000 reticulocyte /animal were examined for statistical analysis. The reticulocytes (Ret) are characterized by their red fluoresces due to the presence of remnants of variable amounts of RNA, that related to the maturity of reticulocyte. The micronucleus appears as a small rounded body that has yellowish green fluoresces inside the Ret.

### 6- DNA fragmentation assay

The DNA fragmentation was assessed by agarose gel electrophoresis according to **Enari *et al.* (1998)** with some modifications. Bone marrow cells were suspended in 1 ml of hypotonic solution (5.6 g/L KCl) and incubated for 10 min at 37°C. The clear supernatant is discarded and the cell pellet is suspended in 500 µL of lysing buffer (50 mM NaCl, 1 mM Na EDTA, 0.5 % SDS, pH 8.3) and incubated at 37°C overnight with 1 mg/ml proteinase K. Cellular DNA was isolated by phenol extraction and the DNA samples were carefully loaded into the wells of a 2.0% agarose gel. Electrophoresis was carried out in TBE buffer at 50 V for 1 h and the DNA was visualized by ethidium bromide staining.

### 7-Statistics

All values are reported in the form of averages ± SD of the mean, except where indicated. The results of the different treatment groups were compared using Students'*t*-test (**Fowler *et al*., 1998**). Significance was indicated by *P* values <0.05. A set

of statistical calculations were carried out to compare between the results obtained from animals treated with DTIC with control untreated animals. Another set of comparisons was carried out between results of animals treated with DTIC alone and those treated with the same doses of DTIC and 50 mg/kg b.w. RES.

**3. Results**

As shown in table 1 and figure 1; DTIC (5, 10 and 20 mg/kg b.w.) induced significant ($P<0.001$) increases in both the averages of structural chromosomal aberrations and the percentages of aberrant cells. In addition, DTIC induced a significant inhibition in percentages of mitotic indices in a dose dependent and linear manner. Structural chromosomal aberrations appeared in the form of chromatid gap, chromatid break, acentric fragments, deletions, dicentric chromosomes and centric fusions. RES treatment induced significant inhibitions in the percentages of chromosomal aberrations and increases in mitotic indices. The aberrant metaphases showed a statistically significant decrease ($P<0.05$) after treatment with RES and DTIC in comparison with those induced by DTIC treatment alone. While, animals treated with RES showed a non-significant decrease in percentages of chromosomal aberrations or elevations in the mitotic indices in comparison to the control level.

As shown in table 2, figure 2 and figure 3; Bone marrow cells of mice treated with DTIC at dose levels of 0, 2.5, 5, 10 and 20 mg/kg showed high significant ($P<0.001$) increases in incidences of Mn-PCEs in comparison to the control level. The increases in Mn-PCEs was dose dependent and highly significant ($P< 0.001$). The PCEs were appeared as a progenitor of RBCs that have a reddish cytoplasm because of some RNA presence, that make them easily differentiated from mature normochromatic erythrocytes (NCEs) which has not stained. The Mn-PCEs appeared as PCEs that having small rounded bodies called the micronucleus which stained greenish yellow. Moreover, signs of bone marrow depression were detected in the form of a dose-related significant reduction in the percentages of PCEs. Animals treated daily with 50 mg/kg resveratrol for 15 consecutive days did not induce a significant elevation in Mn-PCEs than the control level. However, resveratrol could protect against DTIC induced Mn-PCEs. Treatment with 0, 2.5, 5, 10 and 20 mg/kg DTIC without and with 50 mg/kg resveratrol induced $1.83 \pm 0.75$, $6.83 \pm 13.67$, $23.67 \pm 1.63$, $43.83 \pm 3.97$ and $1.50 \pm 0.84$, $2.0 \pm 0.89$, $8.67 \pm 1.63$, $18.17 \pm 1.94$, $33.17 \pm 3.31$ Mn-PCEs per 2000 PCEs, respectively. RES significantly ($P< 0.001$) elevated the averages of PCEs/NCEs.

As shown in in table 2 and figure 4; peripheral blood Mn-Ret showed significant increases in micronuclei formation due to treatment with DTIC. In peripheral blood samples, Ret appears to have variable amounts of RNA that makes them easily observable from the mature RBCs presented in Fig. 5. While, Mn-Rets are having a small rounded body that fluoresces yellowish green this considered as an indicator for the presence of DNA.

The graded doses of 0, 2.5, 5, 10 and 20 mg/kg b.w. DTIC showed a fragmentation of DNA in the form of low molecular weight DNA fragments in comparison to the intact DNA of bone marrow cells derived from control untreated animals (Fig. 6). RES treatment could induce a reduction in DNA fragmentation induced by DTIC. Unfortunately, RES did not retain the DTIC-induced DNA fragmentation to the base line of the control level of damage.

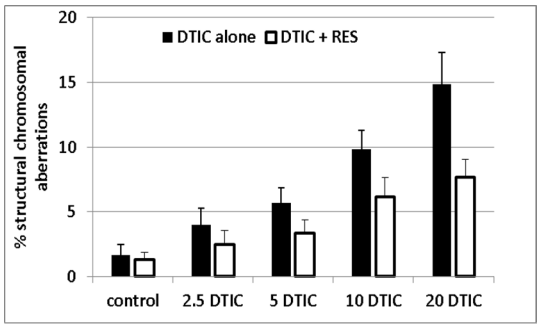


Fig.1. Percentages of chromosomal aberrations in mice treated with 0, 2.5, 5, 10 and 20 mg/kg b.w DTIC alone (black columns) or in combination with 0 and 50 mg/kg RES (white columns). DTIC induced significant elevations with all dose levels. RES could induce a statistically significant inhibition in DTIC-induced % chromosomal aberrations ($P<0.05$).

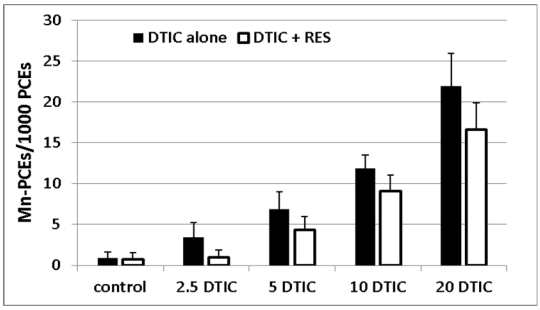


Fig. 2. Averages of Mn-PCEs in bone marrow samples of animals treated with 0, 2.5, 5, 10 and 20 mg/kg b.w. DTIC alone (Black columns) or in combination with 0 and 50 mg/kg b.w. RES (white columns). Note that, RES reduced the averrages of Mn-PCEs induced by 2.5 mg/kg b.w DTIC to the control level and significantly induced an inhibition in the averages of Mn-PCEs in comparison to those induced by 5, 10 and 20 mg/kg b.w. alone.

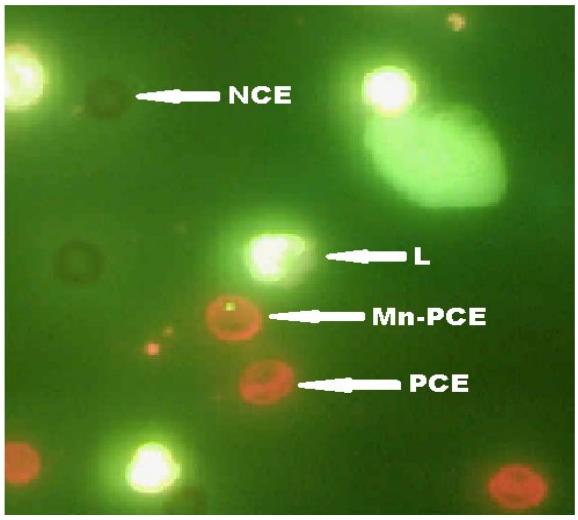


Fig. 3. Bone marrow smear showing Mn-PCE from femoral bone marrow of mouse. The arrows point out to Mn-PCE, the micronucleus is visualized as a small rounded body that has a yellowish green fluoresces due to the presence of DNA that stained green. The polychromatic erythrocyte (PCE) are characterized by their red fluoresces due to the presence of remnants of RNA, as shown in the figure the reticulocytes have variable amounts of RNA. The mature normochromatic erythrocyte (NCE) appears as an opaque unstained rounded bodies. Leucocytes (L) appear as brightly fluoresces green bodies.

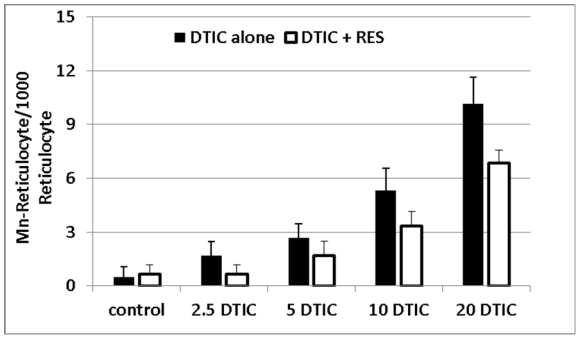


Fig. 4. Averages of Mn-Ret/1000 Ret in peripheral blood of animals treated with 0, 2.5, 5, 10 and 20 mg/kg b.w. DTIC alone (Black columns) or in combination with 0 and 50 mg/kg b.w. RES (white columns). Note that, RES reduced the averrages of Mn-Ret induced by 2.5 mg/kg b.w DTIC to the control level.

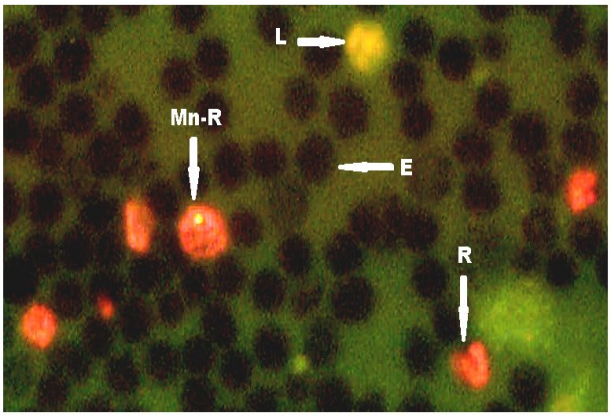


Fig. 5 : Peripheral blood Micronucleated-Reticulocyte (Mn-R) from caudal vein of mouse treated with 20 mg/Kg b.w. DTIC. The arrow point out to Mn-Reticulocyte (MN-R), the micronucleus is visualized as a small rounded body that has a yellowish green fluoresces. The reticulocytes (R) are characterized by their red fluoresces due to the presence of remnants of RNA, as shown in the figure the reticulocytes have variable amounts of RNA related to the maturity of reticulocyte. The mature erythrocyte (E) appear as an opaque unstained rounded bodies. Leucocytes (L) appear as brightly fluoresces green bodies.

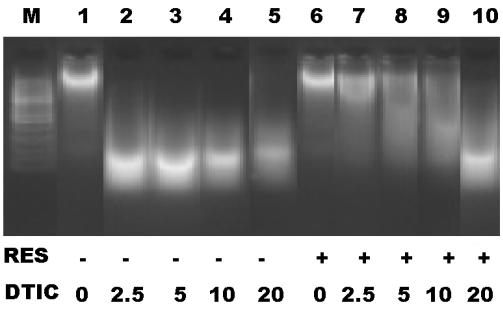


Fig. 6 : Resveratrol (RES) mediated protection of dacarbazine (DTIC) induced mutagenicity in mice bone marrow cells. Bone marrow cells were derived from animals treated for 24 h with 0, 2.5, 5, 10 and 20 mg/kg b.w. DTIC represented in lanes 1-5, respectively. The graded doses of DTIC (Lanes 2-5) showed a fragmentation of DNA in the form of low molecular weight DNA fragments in comparison to the intact DNA of bone marrow cells derived from control untreated animals (Lane 1). The lanes 6-10 represent the DNA fragmentation pattern in bone marrow cells derived from animals treated daily with 50 mg/kg b.w. resveratrol (RES) for 15 days, animals received 0, 2.5, 5, 10 and 20 mg/kg. b.w. dacarbazine (DTIC) concurrently with the last dose of RES. As shown in lanes 7-10 the effect of RES is reversibly with the increased doses of DTIC.

**Table (1). Effect of resveratrol (0, 50 mg/kg b.w. daily doses for 15 days) on the induction of mouse bone marrow chromosomal aberrations by dacrabazine (0, 2.5, 5, 10 and 20 mg/kg b.w. single dose concurrently administered with the last dose of resveratrol).**

| Treatment (mg/kg b.w.) | | Structural Chromosomal aberrations | | | | | | Aberrations/600 metaphases (Average ± S.D.) | Aberrant cells (%) | Mitotic Index (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Chromatid Gap | Break | Deletion | Acentric Fragment | Dicentric | Centric Fusion | | | |
| DTIC | RES | | | | | | | | | |
| 0 | 0 | 1 | 0 | 2 | 2 | 2 | 3 | 10 (1.67 ± 0.82)$^{a}$ | 1.50 ± 0.55$^{a}$ | 5.17 ± 1.17$^{a}$ |
| 2.5 | 0 | 4 | 4 | 5 | 3 | 4 | 4 | 24 (4.00 ± 1.26)$^{b*}$ | 3.17 ± 0.75$^{b*}$ | 4.67 ± 1.03$^{b}$ |
| 5 | 0 | 4 | 5 | 5 | 7 | 9 | 4 | 34 (5.67 ± 1.21)$^{c***}$ | 4.50 ± 0.55$^{c**}$ | 3.67 ± 0.82$^{c*}$ |
| 10 | 0 | 12 | 10 | 9 | 10 | 9 | 9 | 59 (9.83 ± 1.47)$^{d***}$ | 6.83 ± 0.98$^{d***}$ | 3.00 ± 0.63$^{d**}$ |
| 20 | 0 | 14 | 12 | 13 | 14 | 17 | 19 | 89 (14.83 ± 2.48)$^{e***}$ | 9.00 ± 1.41$^{e***}$ | 2.17 ± 0.75$^{e***}$ |
| 0 | 50 | 0 | 0 | 2 | 3 | 2 | 1 | 8 (1.33 ± 0.52)$^{f}$ | 1.30 ± 0.52$^{f}$ | 5.50 ± 1.05$^{f}$ |
| 2.5 | 50 | 2 | 2 | 1 | 2 | 4 | 4 | 15 (2.50 ± 1.05)$^{g**}$ | 2.30 ± 1.03$^{g}$ | 5.17 ± 1.17$^{g*}$ |
| 5 | 50 | 2 | 5 | 5 | 3 | 4 | 1 | 20 (3.33 ± 1.03)$^{h**}$ | 3.00 ± 0.89$^{h*}$ | 4.83 ± 0.75$^{h*}$ |
| 10 | 50 | 5 | 5 | 6 | 6 | 6 | 9 | 37 (6.17 ± 1.47)$^{i***}$ | 5.50 ± 1.05$^{i*}$ | 4.50 ± 0.66$^{i*}$ |
| 20 | 50 | 8 | 4 | 7 | 9 | 7 | 11 | 46 (7.67 ± 1.37)$^{j***}$ | 7.67 ± 0.89$^{j*}$ | 2.67 ± 0.82$^{j}$ |

$^{b}$, $^{c}$, $^{d}$, $^{e}$ compared to $^{a}$
$^{f}$ compared to $^{a}$
$^{g}$ compared to $^{b}$
$^{h}$ compared to $^{c}$
$^{i}$ compared to $^{d}$
$^{j}$ compared to $^{e}$
*$p$<0.05, **$p$<0.01, ***$p$<0.001

**Table (2). Effect of resveratrol (0, 50 mg/kg b.w. daily doses for 15 days) on the induction of mouse bone marrow and peripheral blood micronuclei by dacrabazine (0, 2.5, 5, 10 and 20 mg/kg b.w. single dose concurrently administered with the last dose of resveratrol).**

| Treatment (mg/kg b.w.) | | Bone marrow Mn-PCEs/2000 PCEs/ animal (Total, Average ± S.D.) | Bone marrow PCE/NCEs (Average ± S.D.) | Peripheral blood Mn-Ret/1000 Ret (Average ± S.D.) |
|---|---|---|---|---|
| DTIC | RES | | | |
| 0 | 0 | 1, 2, 2, 3, 1, 2 (11, 1.83 ± 0.75)$^{a}$ | 0.83 ± 0.09$^{a}$ | 0.50 ± 0.55$^{a}$ |
| 2.5 | 0 | 6, 9, 5, 5, 9, 7 (41, 6.83 ± 1.83)$^{b**}$ | 0.73 ± 0.05$^{b}$ | 1.67 ± 0.82$^{b}$ |
| 5 | 0 | 14, 13, 11, 17, 15, 12 (82, 13.67 ± 2.16)$^{c***}$ | 0.64 ± 0.06$^{c*}$ | 2.67 ± 0.82$^{c*}$ |
| 10 | 0 | 22, 23, 22, 26, 25, 24 (142, 23.67 ± 1.63)$^{d***}$ | 0.51 ± 0.04$^{d**}$ | 5.33 ± 1.21$^{d**}$ |
| 20 | 0 | 46, 41, 42, 39, 45, 50 (263, 43.83 ± 3.97)$^{e***}$ | 0.36 ± 0.07$^{e***}$ | 10.17 ± 1.47$^{e***}$ |
| 0 | 50 | 2, 1, 1, 1, 3, 1 (9, 1.50 ± 0.84)$^{f}$ | 1.11 ± 0.13$^{f*}$ | 0.67 ± 0.52$^{f}$ |
| 2.5 | 50 | 3, 2, 3, 1, 2, 1 (12, 2.00 ± 0.89)$^{g**}$ | 0.87 ± 0.08$^{g}$ | 0.67 ± 0.52$^{g*}$ |
| 5 | 50 | 9, 7, 7, 8, 10, 11 (52, 8.67 ± 1.63)$^{h**}$ | 0.79 ± 0.08$^{h*}$ | 1.67 ± 0.82$^{h*}$ |
| 10 | 50 | 17, 19, 15, 20, 18, 20 (109, 18.17 ± 1.94)$^{i***}$ | 0.71 ± 0.08$^{i*}$ | 3.33 ± 0.82$^{i*}$ |
| 20 | 50 | 35, 29, 32, 36, 37, 30 (199, 33.17 ± 3.31)$^{j***}$ | 0.62 ± 0.06$^{j**}$ | 6.83 ± 0.75$^{j**}$ |

$^{b}$, $^{c}$, $^{d}$, $^{e}$ compared to $^{a}$
$^{f}$ compared to $^{a}$
$^{g}$ compared to $^{b}$
$^{h}$ compared to $^{c}$
$^{i}$ compared to $^{d}$
$^{j}$ compared to $^{e}$
*$p$<0.05, **$p$<0.01, ***$p$<0.001

**Discussion**

To establish the antigenotoxic action of resveratrol on DTIC-induced chromosomal damage and micronuclei formation in bone marrow cells of mice; animals treated daily with 0 and 50 mg/kg b.w. RES for 15 days followed by a single dose of 0, 2.5, 5, 10 and 20 mg/kg b.w.

Induction of bone marrow chromosomal aberrations (CA) and micronucleated polychromatic erythrocytes (MN-PCEs) has been commonly used as sensitive biological indicator with high veracity in the mutagenic bioassays of the drugs (**Adekunle *et al*., 2009**).

In the present work, DTIC induced significant ($P < 0.001$) increases in averages of chromosomal aberrations. In addition to that, DTIC induced a significant ($P < 0.001$) elevations in the incidences of Mn-PCEs and Mn-Rets that having one or more micronuclei of the small type. Small type micronucleus has a diameter less than 1/5 the diameter of the containing cell. These Mn-PCEs and Mn-Rets are having small type micronucleus that most probably containing chromosomal fragments (**Norppa and Falck, 2003**). The production of small micronuclei in bone marrow cells induced by DTIC was previously reported (**Al- Hawary and Al- Saleh, 1989; Jeremice *et al*., 1996; Adler *et al*., 2002; Khan *et al*., 2010**). Moreover, DTIC induced cytotoxicity in the form of reduction of percentages of PCEs and in the mitotic indices (MI) which pointed out that there is an inhibition in mitotic processes (**Al-Hawary and Al-Saleh, 1989; Adler, et al., 2002**). Literatures showed that, DTIC induced sister-chromatid exchanges in bone marrow cells of CBA/Ca mice (**Varga *et al*., 1991**), cytokinesis-blocked micronuclei in murine SCCVII cell line (**Jeremic *et al*., 1996**), micronuclei containing whole chromosomes from peripheral blood derived from melanoma patients (**Miele *et al*., 1998**), bone marrow micronuclei of mice (**Adler *et al*., 2002**) and micronuclei in Chinese hamster V79 cells (**Kersten *et al*., 2002**).

The mechanism by which DTIC produces its genotoxic action is still unclear. However, the possible mechanism of genotoxicity exerts by DTIC is summarized as follows; DTIC is P450-activated prodrug. DTIC is activated by hydroxylation to produce 5-(3-hydroxymethyl-3-methyl-triazen-1-yl) imidazole-4-carboxamide (HMMTIC). Formaldehyde is subsequently eliminated from HMMTIC nonenzymatically, resulting in 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC), which rapidly decomposes to the genotoxic agents aminoimidazole carboxamide, $N_2$, and $CH_3^+$ (**Rooseboom *et al*., 2004; Basaran *et al*., 2010**). P450-mediated DTIC bioactivation induces apoptosis and mutagenicity via the formation of $O^6$-methylguanine-DNA adducts (**Kaina *et al*., 2007; Pourahmad *et al*., 2009**). The $O^6$-methylguanine preferentially pairs with thymine and leads to GC to AT base pair transitions (**Gerson, 2002**). Moreover, DTIC produces its cytotoxicity via another possible mechanism. DTIC induced interstrand DNA cross-links which are particularly cytotoxic because they block DNA replication and lysosomal breakdown with the release of DNase and alterations in the level of enzymes and co-enzymes (**Fulda and Debatin, 2006; Apraiz *et al*., 2011**).

Diet represents a major influence on the reduction of cancer and chemotherapeutic side effects (**Riso *et al*., 2009**). A micronutrient- equilibrated diet can contribute to genomic stability and hence cancers cure (Prado *et al*., 2010). In the present work, RES significantly attenuates the frequencies of CA, Mn-PCEs, Mn-Ret and DNA fragmentation induced by DTIC. As well as, RES significantly improved the averages of mitotic index and PCEs/NCEs induced by DTIC. Previous work of **Fusser *et al*. (2011)** showed that, application of RES either for 7 days per gavage (100 mg/kg body w.t.) or for 3–9 months in the diet (0.04% ad libitum), reduces the endogenous oxidative DNA base damage in the livers of the $Csb^{m/m}Ogg1^{-/-}$ mice by 20–30% ($P < 0.01$). Moreover, RES and its analogues protects against ethanol-induced oxidative DNA damage in human peripheral lymphocytes (**Yan *et al*., 2011**) and protects mouse embryonic stem cells from ionizing radiation by accelerating recovery from DNA strand breakage (**Denissova *et al*., 2011**).

Previous observations showed that, deficiencies in dietary microelements like vitamins and minerals in human diet are thought to generate DNA damage by enhancing the occurrence of breaks and oxidative lesions (**Brevik *et al*., 2011**). Since mutations are the key elements in neoplasic processes, there is a considerable amount of epidemiological evidence relating diets rich in fresh fruit and vegetables and a decrease in cancer incidence (**Chang *et al*., 2010; Brevik *et al*., 2011**).

Resveratrol protects DNA through redox state of cells so, it is indirectly enhances the integrity of genomic DNA and acts as inhibitor of tumor initiation (**Jang *et al*., 1997; Gatz and Wiesmuller, 2008**). The antioxidant activity of resveratrol was realized by *in vitro* experiments of **Leonard *et al*. (2003)** where, resveratrol at high doses can act as radical scavenger in hydroxyl and superoxide radical generating systems.

In conclusion dacarbazine is clastogenic to mice bone marrow cells causing severe damage at the level of DNA. The administration of resveratrol improved the genotoxic effects of dacarbazine. The

results of this work draw attention for application of a safer chemotherapeutic protocol for cancer treatment by using strong antioxidants with chemotherapeutic agents.

**Corresponding author**
Ramadan, A.M. Ali
Zoology Dept., College for Girls for Science, Arts and Education, Ain-Shams Univ., Heliopolis, Cairo, Egypt.
ramadanali27@gmail.com

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