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Antimutagenic effect of grape seed extracted oil on diazinon induced genotoxicity in albino mice

Mustafa S. Al-Attar

University of Salahaddin-Erbil, College of Science, Environmental Science Department

Abstract:

Background: Human health hazard due to exposure to chemical pollutants is a global and chronic problem. The pesticides constitute the primary toxic chemicals in the environment. The present study explored the effects of grape seed oil supplementation on diazinon (DZN) induced chromosomal aberrations and sperm abnormalities in male mice.

Materials and methods: The tests were carried out on 35 adult male albino mice (16-20 weeks, weighed 30-35 g). Animals were categorized into seven groups each with five mice; the first group was treated with phos¬phate buffer saline (PBS) as negative control group. The second and third groups were treated daily and orally with two different doses of the DZN (60 and 90 mg/kg body weight) respectively dissolved in corn oil. Animals of fourth, fifth and sixth groups were orally administered grape seed oil at a dose of 2 g/kg body weight, after 6 hours the fourth and fifth groups subjected to DZN at the same dose given to second and third groups. Animals of the seventh group were supplied with corn oil. The treatments were continued for five weeks.

Results: The results showed that DZN increased the frequency of both chromatid and chromosome aberrations in bone marrow cells and sperm abnormalities. Treatment with grape seed oil extract showed a significant decrease ($P<0.01$) on both total abnormal chromosomes and sperms, and there was no significant difference between these groups and negative control in most analyzed parameters. The dose of grape seed oil has significantly minimized the effect of first dose of DZN concerning chromosome structure while it worked in the second dose regarding sperm abnormalities.

Conclusions: This finding suggests the protective action of grape seed oil against DZN induced clastogenecity and sperm abnormalities.

Key words: *chromosomal aberrations, sperm abnormalities, diazinon, grape seed oil.*

Introduction:

The pollution of the environment plays a great role in the occurrence of many diseases affecting humans, animals, and plants. One of the major factors causing contamination of the environment is the wrong use of organophosphorus pesticides [1]. The contact with organophosphorus pesticides is a serious health problem for agricultural workers [2].

Diazinon (DZN) is a widespread used organophosphorus insecticide. It has been used since 1956 for the control of pests and soil insects, on fruits, and on flourish plants, vegetables and field crops [3]. Its residential uses have been revoked since 2004 by US Environmental Protection Agency, but its agricultural application is still very common [4]. It is used to control flies, greenhouses, animal facilities, places and other institutions and public places where food or animal decays might be collected [5]. DZN can be highly toxic to human and animals [6, 7]. The mechanism of action of DZN is acetylcholinesterase enzyme inhibition [8], resulting in cholinergic overstimulation [9]. However, it may cause an imbalance in the free radicals production /elimination processes with the indirect reason of cellular damage [8, 10, 11, 12].

The plant products continuously play the basic role in treatments of about 80–85% of the world's population. Although the trends of chemistry and molecular biology providing fast growth of synthesized de novo drugs, plants remain a regular origin of medicinal compounds; up to 40% of new drugs may directly or indirectly be correlated to natural compounds [13].

Grape (Vitis vinifera) is one of the world's abundant fruit crops, and grape seed extract (GSE) is a complex matrix containing 16% oil, 11% proteins, 40% fiber, and 7% complex phenols including tannins, in addition to mineral salts and sugars [14]. It is a rich source of one of the most beneficial groups of plant flavonoids and proanthocyanidins oligomers [15]. GSE

Corresponding address:
Mustafa S. Al-Attar
University of Salahaddin-Erbil, College of Science, Environmental Science Department
Email: mustafa.mustafa@su.edu.krd

contains mainly flavonoids, which involved in reduction the oxidative stress in vitro and in vivo [16].

[17] Stated that GSE considered as a powerful antioxidant nutritive supplement that prevents premature aging and diseases. Oil of grape seeds regarded as a high potent antioxidant because of its rich source of polyphenolics [18].

[18, 19] showed that extracts obtained from grape seed have antimutagenic and anticarcinogenic effects by inhibiting enzymes of free radicals productions. The consumption of grape seed extracts may be useful in decreasing the side effects of chemotherapeutic agents in cancer treatment [20].

The present study was aimed to investigate the protective role of grape seed oil supplementation on chromosomal aberrations and sperm abnormalities induced by DZN toxicity in male mice.

Materials and methods:

The present study was carried out at Salahaddin University/ college of science/ Department of biology. The grape seed extracted oil was provided by Basso Fedele and Figli srl company (Italy).The experiments were carried out on 35 adult male albino mice (16-20 weeks, weighed 30-35 g), and were kept under con¬stant environmental conditions with a 12:12 light-dark cycle. The animals were provided standard granulated chow. In the present work, animals were arranged into seven groups each with five mice; the first group was treated daily and orally with phos¬phate buffer saline (PBS) as negative control group. The second and third groups were treated daily and orally with two different doses of the DZN (60, 90 mg/kg body weight) in corn oil for five weeks. Animals of fourth and fifth groups were orally administered grape seed oil at a dose of 2 g/kg body weight (0.1 ml/animal/day) and after 6 hours subjected to DZN at the same dose given to second and third groups, daily for five weeks. Animals of the sixth group were treated with grape seed oil at the same dose given to groups 4 and 5. Animals of the seventh group were supplemented with 0.1 ml of corn oil.

Then at the time of data harvesting chromosomal preparations were done by the standard method of [21]. Sperm was taken from epididymis using the method of [22] and [23].

Statistical analysis:

All data are expressed as means ± standard error (M±SE) and statistical analysis were carried out using SPSS version 21. Comparisons between groups were made using one-way analysis of variance (ANOVA) with performing Duncan t-test as post hoc analysis. P values ≤ 0.05 were considered as significant difference.

Results:

Table (1) summarizes the treatment effect of grape seed oil doses against DZN induced chromosomal aberrations.

A significant induction ($p<0.01$) was found in two different doses of DZN on the total abnormal chromosome. Most types of aberrations were increased when compared with negative control group, while the acentric fragment was the only abnormality recorded there was non-significant effect in the second dose of DZN (90 mg/kg/day) (Figure 1).

The centromeric gap (8.60±0.24) in second dose of DZN (90 mg/kg/day) and (4.80±0.37) in first dose of DZN (60 mg/kg/day) showed the highest values of aberrant types respectively, while polyploidy (1.40±0.24) in the first dose and acentric fragment (1.00±0.00) in the second dose recorded the lowest values.

Treatment with grape seed oil extract showed significant protection ($P<0.01$) for total abnormal metaphase including most studied aberrations when compared with positive control. The frequency of aberrations was not significant with negative control. It was apparent from the Table (1) that the dose of grape seed oil was have minimized the effect of DZN on chromosome structure, the most protective effects of grape seed oil was worked with the first dose of DZN.

Table1. Effects of grape seed oil extract against diazinon induced chromosomal aberrations in male albino mice. (Mean ± SE) ($P<0.01$).

	Total normal Chromosome	Total abnormal Chromosome	Centromeric gap	Centromeric break	Chromatid gap	Chromatid break	Ring chromosome	Dicentric chromosome	Pulverization	Polyploidy	Acentric fragment
Control	89.80 ± 0.58 ^{d}	10.20 ± 0.58 ^{a}	2.60 ± 0.40 ^{a}	1.60 ± 0.24 ^{a}	1.20 ± 0.20 ^{a}	0.40 ± 0.24 ^{a}	1.60 ± 0.24 ^{a}	0.40 ± 0.40 ^{a}	1.00 ± 0.31 ^{a}	0.20 ± 0.20 ^{a}	1.20 ± 0.20 ^{a}
DZN 60 mg/ Kg/day	77.40 ± 0.92 ^{b}	22.60 ± 0.92 ^{c}	4.80 ± 0.37 ^{b}	2.60 ± 0.24 ^{ab}	2.40 ± 0.40 ^{bc}	2.40 ± 0.24 ^{b}	2.80 ± 0.20 ^{b}	2.00 ± 0.00 ^{b}	2.00 ± 0.31 ^{ab}	1.40 ± 0.24 ^{bc}	2.20 ± 0.37 ^{b}
DZN 90 mg/ Kg/day	74.20 ± 1.42 ^{a}	25.80 ± 1.42 ^{d}	8.60 ± 0.24 ^{c}	3.00 ± 0.63 ^{b}	2.60 ± 0.24 ^{c}	2.20 ±0.58 ^{b}	2.60 ± 0.24 ^{b}	1.80 ± 0.37 ^{b}	2.00 ± 0.31 ^{ab}	2.00 ± 0.44 ^{c}	1.00 ± 0.00 ^{a}
DZN 60 mg/ Kg/day + Grape oil (2g/ kg/day)	82.00 ± 1.30 ^{c}	18.00 ± 1.30 ^{b}	3.00 ± 0.44 ^{a}	2.00 ± 0.31 ^{ab}	1.60 ± 0.24 ^{ab}	0.80 ±0.20 ^{a}	2.80 ± 0.20 ^{b}	2.00 ± 0.00 ^{b}	2.20 ± 0.20 ^{b}	1.60 ± 0.24 ^{bc}	2.00 ± 0.54 ^{ab}

DZN 90 mg/ Kg/day + Grape oil (2g/ kg/day)	79.80 ± 1.39 bc	20.20 ± 1.39 bc	3.60 ± 0.24 a	2.60 ± 0.24 ab	3.00 ± 0.54 c	2.40 ±0.24 b	3.00 ± 0.54 b	1.60 ± 0.67 b	1.40 ± 0.24 ab	0.80 ± 0.37 ab	1.80 ± 0.37 ab
Grape oil (2g/ kg/day)	89.60 ± 0.50 d	10.40 ± 0.50 a	2.80 ± 0.20 a	2.00 ± 0.31 ab	1.20 ± 0.20 a	0.00 ±0.00 a	1.60 ± 0.40 a	0.20 ± 0.20 a	1.20 ± 0.37 ab	0.20 ± 0.20 a	1.20 ± 0.20 a
Corn oil (2g/ kg/day)	89.00 ± 0.54 d	11.00 ± 0.54 a	3.40 ± 0.24 a	1.80 ± 0.20 a	1.20 ± 0.20 a	0.40 ±0.24 a	1.40 ± 0.24 a	0.40 ± 0.40 a	1.20 ± 0.37 ab	0.20 ± 0.20 a	1.00 ± 0.00 a

Note: Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.

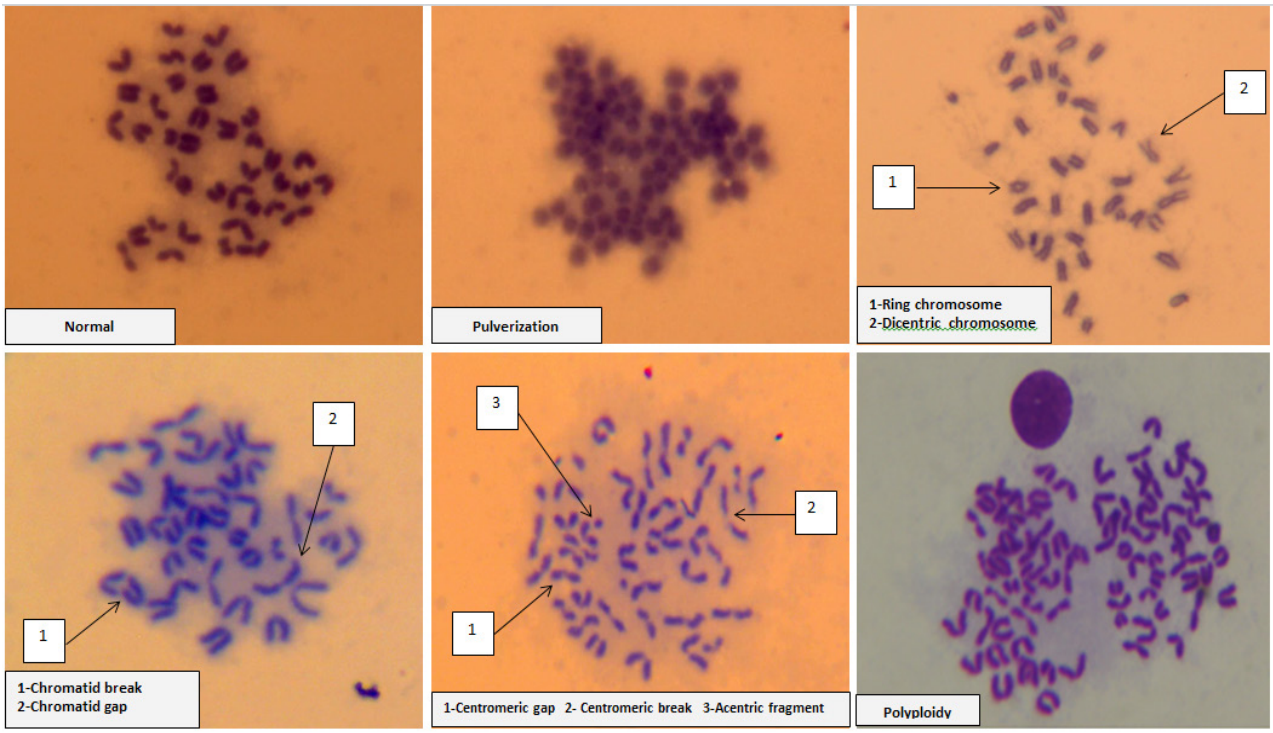

Figure 1. Types of structural and numerical chromosomal aberrations induced by diazinon (1000 X).

Table (2) showed the effect of grape seed oil treatment against DZN induced sperm abnormalities. Highly significant increase at ($P<0.01$) were found in both DZN-treated groups (positive control) regarding the total abnormal sperms including (sperm without head, sperm without tail, sperm without hook, swollen head sperm, defective head sperm, and blunt hook sperm) when compared with negative control (PBS), but double head sperm was showed non-significant difference ($P<0.01$) in the first treated group (Figure 2).

The highest value of abnormality type was sperm without tail (10.80±1.24), (7.80±0.96) in the first and second dose of DZN respectively, while the lowest value was double tail sperm (0.40±0.24) in the first dose and double head sperm (2.40±0.50) in the second dose respectively. Treatment with grape seed oil extract showed the significant decrease ($P<0.01$) in total abnormal sperm including all studied sperm abnormalities compared to the positive control, while there was no significant difference between grape treated groups and negative control in most analyzed parameters. From the Table (2) it is clear that the dose of grape seed extract decreased the effect of DZN on sperm morphology, the most protective effects of grape seed oil were worked with the second dose of DZN.

Table2. Effects of grape seed oil extract against diazinon induced sperm abnormalities in male albino mice. (Mean ± SE) (P<0.01).

	Total normal sperm	Total abnormal sperm	Sperm without tail	Sperm without head	Sperm without hook	Double head sperm	Double tail sperm	Swollen head sperm	Defective head sperm	Blunt hook sperm
Control	± 89.20 ^{e} 0.73	± 10.80 ^{a} 0.73	0.40 ± 2.60 ^{a}	0.50 ± 2.40 ^{ab}	± 1.80 ^{a} 0.20	± 0.20 ^{a} 0.20	± 0.00 ^{a} 0.00	± 1.60 ^{a} 0.24	0.20 ± 1.20 ^{a}	0.00 ± 1.00 ^{ab}
DZN 60 mg/ Kg/day	± 63.80 ^{a} 2.03	± 36.20 ^{e} 2.03	± 10.80 ^{e} 1.24	1.06 ± 7.20 ^{c}	0.81 ± 3.60 ^{b}	± 1.00 ^{a} 0.31	0.24 ± 0.40 ^{ab}	± 4.00 ^{b} 0.31	0.37 ± 4.80 ^{c}	0.40 ± 4.40 ^{d}
DZN 90 mg/ Kg/day	± 68.40 ^{b} 1.02	± 31.60 ^{d} 1.02	0.96 ± 7.80 ^{d}	0.37 ± 4.20 ^{b}	0.37 ± 3.80 ^{b}	± 2.40 ^{b} 0.50	± 2.60 ^{d} 0.40	± 4.60 ^{b} 0.60	0.40 ± 3.60 ^{bc}	^{c} 0.4 ± 2.60
DZN 60 mg/ Kg/day + Grape oil (2g/ (kg/day	± 80.40 ^{d} 1.20	± 19.60 ^{b} 1.20	0.54 ± 5.00 ^{bc}	0.37 ± 3.80 ^{ab}	0.24 ± 2.60 ^{ab}	± 1.40 ^{ab} 0.74	0.58 ± 1.20 ^{bc}	± 2.40 ^{a} 0.60	0.24 ± 2.40 ^{ab}	0.20 ± 0.80 ^{a}
DZN 90 mg/ Kg/day + Grape oil (2g/ (kg/day	± 73.60 ^{c} 1.40	± 26.40 ^{c} 1.43	0.24 ± 5.60 ^{c}	0.31 ± 3.00 ^{b}	0.40 ± 3.60 ^{b}	± 2.40 ^{b} 0.24	0.20 ± 1.80 ^{cd}	± 4.40 ^{b} 0.67	0.67 ± 3.60 ^{bc}	0.70 ± 2.00 ^{bc}
Grape oil (2g/ (kg/day	± 89.60 ^{e} 0.50	± 10.40 ^{a} 0.50	0.24 ± 2.60 ^{a}	0.54 ± 2.00 ^{a}	± 1.40 ^{a} 0.24	± 0.20 ^{a} 0.20	± 0.00 ^{a} 0.00	± 1.40 ^{a} 0.24	0.24 ± 1.60 ^{a}	0.20 ± 1.20 ^{ab}
Corn oil (2g/ (kg/day	± 86.60 ^{e} 1.63	± 13.40 ^{a} 1.63	0.58 ± 3.20 ^{ab}	0.60 ± 2.60 ^{ab}	± 2.20 ^{a} 0.20	± 0.20 ^{a} 0.20	± 0.00 ^{a} 0.00	± 1.80 ^{a} 0.20	0.44 ± 2.00 ^{a}	0.24 ± 1.40 ^{ab}

Note: Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.

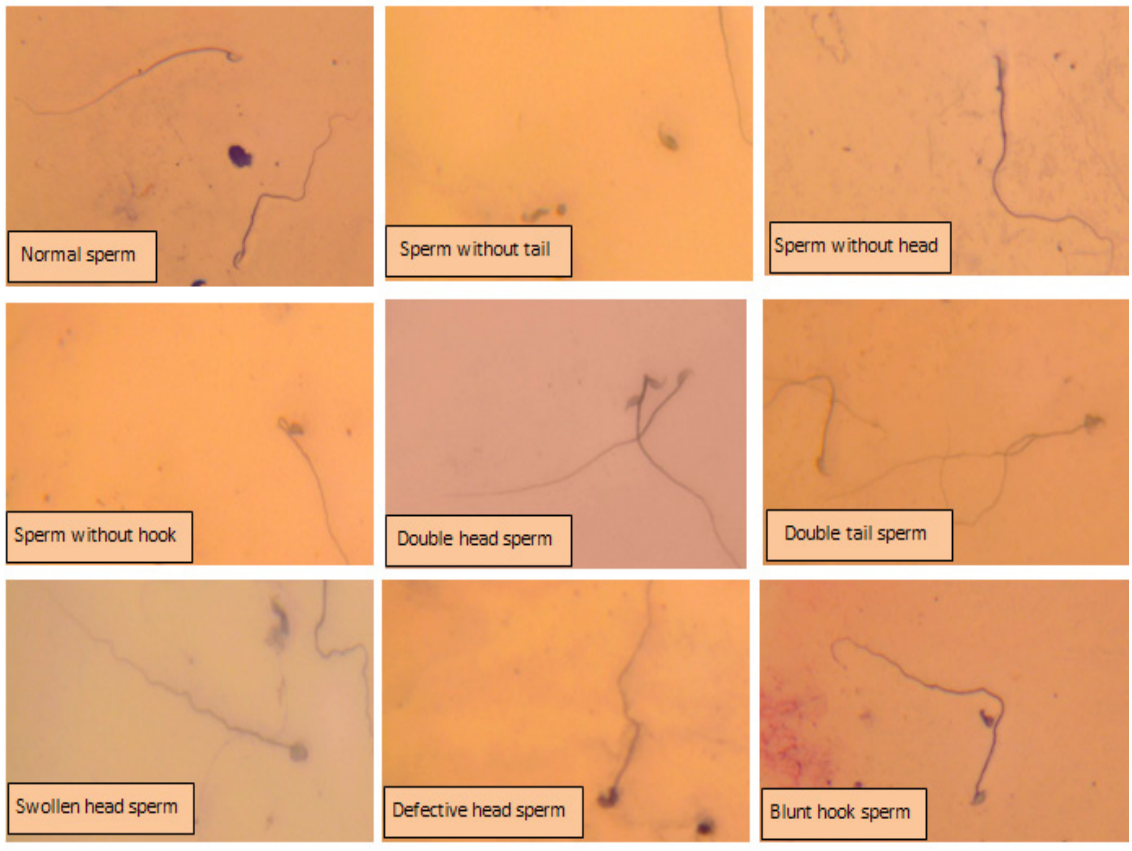

Figure 2. Types of sperm abnormalities on mice induced by diazinon (1000 X).

Discussion:

The undesired effects of pesticides have been identified as a serious public health concern during the past decades. [3].

The present study was conducted to examine the possible protective activity of orally administered grape seed oil against DZN induced cytotoxicity and genotoxicity towards mice somatic cells and sperm morphology in vivo.

Pretreatment of mice with grape seed oil for five weeks and simultaneously with doses of DZN significantly decreased the frequency of chromosomal aberrations in bone marrow cells and sperm abnormalities. These results agree with Abd El-Rahim and Hafiz (2009) [24] whom evaluated the protection conferred by grape seed oil and linseed oil against cyclophosphamide-induced bone marrow chromosomal aberrations and sperm abnormalities in adult Swiss albino mice. Who concluded that each of grape seed and linseed oils serves as an excellent antioxidant that limited genotoxicity of bone marrow cells and sperm abnormalities. Grape seed oil and wheat germ oil supplementation significantly decrease the toxic effects of chlorpyrifos-induced oxidative stress and caused a significant change in different biochemical parameters in the liver of male albino rats [25]. The administration of grape seed oil before gamma-radiation exposure may be a promising attempt in attenuating the extent of oxidative damage accompanying radiotherapy [26].

Additionally, [27] concluded that the grape seed oil plays a significant role in decreasing the effects of acrylamide intoxication in male rat genital organs especially in testes, epididymis, prostate glands and seminal vesicles that lead to infertility.

The exposure to pesticides including DZN produced testicular damage, which resulted in the spermatogenic arrest [3, 28, 29, 30].

Diazinon (DZN) caused a significant decrease in sperm counts and spermatogenic, Leydig and Sertoli cells and a reduction in serum testosterone concentration. Also, a significant decrease was recognized in diameter and weight of testes after DZN administration [31].

[32] Suggested that the direct effects of the DZN induced sever necrosis in the germinal cells and remarkable germinal cells degeneration lowered the sperm quality and quantity. Therefore the high content of pressured germinal cells, elevated abnormal, immature, death sperms and high infiltration of immune cells.

Grape seed extract acts as a potent antioxidant prevented genotoxicity of bone marrow cells by reducing a total number of aberrant cells and different types of structural chromosomal aberrations caused by mutagen [33]. Also, [34] reported that grape seed inhibits the mutagenic effects of cyclophosphamide and/or other mutagens in rats and mice, both in vitro and/or in vivo.

Grape seed extracts prevented DNA oxidative damage in various tissues and DNA fragmentation induced by many agents [33, 35, 36].

[37] Proposed that in vivo protection of DNA by grape seed extracts might be due to detoxification of cytotoxic radicals and considered the contribution to DNA repair. The cause of the antigenotoxic effect of the grape seed extracts is the presence of a lot of biologically active compounds in it, mainly antioxidants. From a cellular perspective, one of the most advantageous features of proanthocyanidins oligomers free radical scavenging activity is chemical structure; it is incorporated into cell membranes. This physical characteristic along with its ability to protect against both water and fat-soluble free radicals provides unbelievable protection to the cells against free radical alterations on chromosomes [38].

Many researchers found the straight relations between genotoxicity and chromosomal instability induced by many agents with the parameters of oxidative stress [39, 40].

The elevated oxidative stress due to treatment with cispla¬tin might be the cause of chromosomal aberration and sperm shape abnormalities. Extract mixtures prepared from a polar and non-polar fraction of red grape, coriander, roselle, and fen¬nel were shown to be efficient in lowering chromosomal aberration and abnormal sperm induced by cisplatin [41].

The presence of antioxidants and anti-inflammatory bioactive constituent in these extract mixtures might be the cause of reduction of such chromosomal aberration and sperm shape abnormalities. Stability of genome might be afforded by treatment with antioxidant, anti-mutagenic, anti-carci¬nogenic and anti-inflammatory bioactive constituent such as phenolic compounds [42]. Grape seed extract (GSE) significantly protected mice bone marrow chromosomes from gentamicin-induced genotoxicity by re¬ducing different types of structural chromosomal aberrations and the total number of aberrant cells. So, the grape extracts mixture, acted as a potent antioxidant preventing kidney damage and genotoxicity of bone marrow cells, which agreed with a study of [33].

GSE significantly preserved mice bone marrow chro¬mosomes from doxorubicin-induced genotoxicity by reducing the frequency of structural chromosomal aberrations and the total aberrant metaphases [43].

Pretreatment of mice with GSE significantly elevated the level of sperm motility reduced by the low dose of cisplatin. At the higher dose of cisplatin, the effect of GSE was not statistically significant comparing with the cisplatin group alone. The cause of the antigenotoxic effect of the GSE is the presence of a lot of biologically active compounds in it, mainly antioxidants that protect DNA through detoxification of free radicals. Many vegetables and fruits are known to prevent chromosomally and DNA damage in animals [44, 45]. Also [46] demonstrated that grape seed proanthocyanidins (GSPs) could improve functional activation of the immune system, and the antitumor effects of GSPs were achieved by immunostimulating properties.

Conclusion and recommendation:

The antioxidative effects of grape seed extracted oil may play an important role in cell protection from genotoxic effects of DZN. Considering the present results, it can be concluded that this study shows that the grape seed extracted oil is beneficial in lowering the clastogenecity and sperm abnor-

malities induced by DZN exposure in mice. Finally, further investigations are needed to explore the mechanism action of grape seed extracted oil against DZN toxicity.

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التأثيرات المضادة للتطفير لمستخلص زيت بذور العنب على مبيد الديازينون في الفئران المختبرية

مصطفى صابر مصطفى العطار

جامعة صلاح الدين- أربيل/ كلية العلوم- قسم العلوم البيئية

الخلاصه:

تعد المخاطر على صحة الإنسان بسبب التعرض للملوثات الكيميائية من المشاكل العالمية المزمنة. وتشكل المبيدات المواد الكيميائية السامة الهامة في البيئة. بحثت الدراسة الحالية تأثير مستخلص زيت بذور العنب على الديازينون (DZN) المسبب للتغيرات الكروموسومية وتشوهات الحيوانات المنوية في ذكور الفئران المختبريه. تم إجراء الاختبارات على 35 من ذكور الفئران المختبرية البيضاء (بعمر 16-20 أسبوعا و وزن 30-35 غم). قسمت الحيوانات إلى سبعة مجاميع لكل منها خمسة فئران. اعطيت المجموعة الأولى المحلول المتعادل (PBS) كمجموعة مقارنة سلبية. واعطيت المجموعتين الثانية والثالثة يوميا وعن طريق الفم الجرعتين من مبيد الديازينون 60 و 90 ملغم / كغم من وزن الجسم) المذاب في زيت الذرة على التوالي. الحيوانات في المجموعات الرابعة والخامسة والسادسة اعطيت وعن طريق الفم زيت بذور العنب بتركيز 2 غرام / كغم من وزن الجسم، وبعد 6 ساعات المجموعتين الرابعة والخامسة اعطيت DZN بنفس الجرعات المعطاه المجموعتين الثانية والثالثة. تم معاملة الحيوانات في المجموعة السابعة بزيت الذرة. واستمر التجربة لمدة خمسة أسابيع.

وأظهرت النتائج أن المبيد DZN يعمل على زيادة وتيرة التغيرات الكروماتيدية والانحرافات الكروموسومية في خلايا نخاع العظام وتشوهات الحيوانات المنوية. وأظهرت المعاملة مع مستخلص زيت بذور العنب انخفاضا معنويا ($P < 0.01$) في كل من إجمالي الكروموسومات غير الطبيعية والحيوانات المنوية، وكان هناك فرق كبير بين هذه المجاميع ومجموعة المقارنة السلبية في معظم المعاملات المدروسة. ان جرعة زيت بذور العنب ادت الى تقليل معنوي في تأثير الجرعة الأولى من المبيد على التغيرات الكروموسومية, بينما ادت جرعة زيت بذور العنب الى تقليل معنوي في تأثير الجرعة الثانية للمبيد فى تشوهات الحيوانات المنوية. وتقترح هذه النتيجة بأن زيت بذور العنب له تأثير وقائي ضد مبيد الديازينون DZN المسبب للتغيرات الكروموسومية وتشوهات الحيوانات المنوية في ذكور الفئران المختبرية.