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Original Article

In Vivo Comparative Studies on Antigenotoxicity of Date Palm (*Phoenix Dactylifera L.*) Pits Extract Against DNA Damage Induced by N-Nitroso-N-methylurea in Mice

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ABSTRACT

Aqueous extract of the date palm *(Phoenix dactylifera L.)* pits was prepared and its antigenotoxic activity was evaluated against N-Nitroso-N-methylurea (NMU) induced mutagenic effect in mice, using chromosome aberration (CA), micronuclei (MN) and DNA fragmentation assays as experimental end points in male mice. Date pits extract (DPE) was given orally to mice at the dose 25 mg/25 g mouse for successive five days in a week up to four consecutive weeks. NMU was used as mutagen and was given intraperitoneal (i.p) injection at single dose 80 mg/kg b.w., 24 hr after last dose of DPE in pre-treatment regimen and 24 hr before the first dose of DPE in the post-treatment regimen. Mice were scarified after one, two and seven days after the end of treatment. The results have shown that pre-and post-treatment regimens of DPE were significantly restored the DNA damage induced by NMU, as revealed by lowering of the occurrence of CAs and MN in bone marrow cells and inhibition of hepatic DNA fragmentation. These findings suggested that DPE produced their inhibitory activity either by desmutagenic or bioantimutagenic manner in pre-and post-treatment regimens respectively.

Key words: Chromosome aberration assay, date palm pits, DNA fragmentation assay, micronucleus assay, N-Nitro-N-methylurea

INTRODUCTION

Fruit seeds have not generally received much attention as antioxidant sources and this could be due to their lack of popularity and lack of commercial applications. However, there are considerably higher ratios of by-products arising from fruit-processing plants and derived products have experienced growing worldwide popularity.[1]

The date palm (*Phoenix dactylifera L.*) has been an important crop in Egypt and Middle Eastern countries. As per recent information, date production of Egypt alone represented about 20% of the total world production for 2008.[2,3] In ancient Egypt the pollen grains of the date palm have been used to improve fertility in women. For Muslims all over the world date are of religious importance and are mentioned in many places in the Holy Quran. They are customarily used to break the day long fast during the holy month of Ramadan.[4] In Egypt 2006; productive date palms were estimated at 11,888,020 million trees and produced 1,328,720 tons of dates.[2] Therefore, utilization of date by-products, particular seeds, is very important to date cultivation and to increase the national income to this sector which supports over one million people. Recently, date pit powders are marketed and are a source of choice to people preferring a non-caffeinated coffee with coffee-related flavor.[5]

Experimental studies have shown that feeding rats with the aqueous extract of date flesh or pits exhibit gastroprotective,

Access this article online	
Quick Response Code:	Website: www.toxicologyinternational.com
	DOI: 10.4103/0971-6580.103669

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hepatoprotective and nephroprotective effects in rodents.[6-8] The date kernels have also been reported to exhibit anti-aging properties and significantly reduce skin wrinkles.[9] What's more, date pits were grind and added to the feed of domesticated animals to enhance growth and this was ascribed to an increase in the plasma level of estrogens or testosterone.[5,10,11]

N-Nitroso compounds (NOC) represent a major class of important chemical carcinogens and mutagens which have been implicated as a hazard to human health.[12,13] The presence of these compounds and their precursors in the environment, in certain occupational settings, in diet and also due to the use of tobacco products, cosmetics, pharmaceutical products as well as their endogenous formation in the human body from dietary components, may be potential risk factors in cancer.[14] NOC are divided into two categories: nitrosamines, which require activation to exert their genotoxic effect, and nitrosamide include N-Nitro-N-methylurea (NMU) which spontaneously decompose to form alkylating agent.[15]

NMU induces various cancers in animal models include: squamous cell carcinomas of the forestomach, sarcomas and gliomas of the brain, adeno-carcinomas of the pancreas, leukemia, and lymphomas.[16] The presence of characteristic ras mutations might be a possible fingerprint of nitroso compounds.[17] In 30% of rat colon carcinomas, NMU was reported to induce G to A transitions in codons 12 and 13 of K-ras.[18] Besides, exposure to NMU during pre-implantation, post-implantation, organogenesis or by paternal exposure results in cleft palate and skeletal defects, increased fetal resorption, and fetal growth retardation.[19,20] The increasing appreciation of the importance of NOC as potential human carcinogens stimulated intense research on protective dietary factors in chemical carcinogenesis.

To the best of our knowledge, there are no published data on antigenotoxic activity of date palm pits in mammalian systems. Accordingly, the present investigation is a novel attempt to spotlight on the efficacy of the aqueous date pits extract for reducing DNA damage induced by NMU in male mice.

MATERIALS AND METHODS

Experimental animals

All experiments were performed with male Swiss mice aged 10-14 weeks and weighing 25-30 g. Animals were supplied by Animal House of the National Research Center, Dokki, Cairo, Egypt. Mice acclimated for a period of one week before the beginning of the experiment. The animals were housed in stainless steel polypropylene hanging cages with wood chip bedding, maintained in an experimental room under controlled conditions of temperature, humidity and light. The animals were kept on standard solid pellet diet and water *ad libitum*.

Chemicals

N-Nitroso-N-methyl urea (NMU; CAS 684-93-5) was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) dissolve in distilled water. Mice received a single intraperitoneal dose of 80 mg NMU/kg b.wt.

Preparation of date pits extract (DPE)

Date fruits were purchased from a local market in Dokki, Cairo, Egypt. The pits were manually separated from the flesh rip fruit and washed clear of any flesh by water. The dried date pits were oven dried for seven days at 60°C and then finely ground into powder using a stainless-steel blender. The water extract of the date pit of 100 g was made by adding distilled water to coarsely pounded date pit (1:2 ratio, weight to volume), and kept in water bath 100°C for 6 h, repeatedly for five times. The resulting brownish and dark extract were filtered and then stored in fridge in dark place.

Mode of administration of DPE

Mice were given DPE at the dose of 25 mg/25 g mouse by oral gavage for successive five days in a week up to four consecutive four weeks.

Treatment schedule

Two kinds of treatment pre- and post-treatment were given to mice on the basis of the time of administration of DPE to evaluate its preventive and therapeutic potential respectively. Mice were randomly assigned to five main experimental groups as shown below in Table 1.

Table 1: Experimental design of the current study

Treatment groups	Treatment schedule	Harvest periods (day)	No. of mice
I- Negative control	No treatment (distilled water)	-	5
II-Negative extract (DPE)	Mice were given only DPE	1	5
		2	5
		7	5
III-Pre-treatment (DPE+NMU)	To evaluate preventive potential of DPE, mice were given first DPE, then NMU treatment after 24 hr from the end of treatment of DPE.	1	5
		2	5
		7	5
IV-Post-treatment (NMU+DPE)	To evaluate therapeutic potential of DPE, mice were given first NMU, then DPE treatment after 24hr from the end of injection with NMU.	1	5
		2	5
		7	5
V-Positive control (NMU)	Mice were given NMU only	1	5
		2	5
		7	5

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At the end one, two and seven day of the last treatment schedule, all animals were injected with colchicine 2h before killing by cervical dislocation. Then all animals were dissected and the samples of bone marrow and liver tissues of each animal were collected for different analysis.

Chromosome aberrations (CAs) assay

Bone marrow preparation for metaphase cells was made according to the standard technique.[21] In brief, mice were i.p injected with colchicine 2 h before scheduled killing by cervical dislocation. Bone marrow cells were collected from both femurs by flushing with 0.56% KCl and incubated for 20 min at 37°C. Cells were centrifuged and pellet was dispersed in fresh and chilled Carnoy's fixation. The slides were prepared by dropping the cells on clean chilled slides; air dried to stain in 5% buffered Giemsa (pH. 6.8). A total 100 well spread metaphase per animal were examined by light microscope at 2500 X magnification for chromosome aberrations.

Micronucleus (MN) assay

The assay was performed according to the standard protocols described by Schmid.[22] Briefly, bone marrow cells were gently flushed out in fetal calf serum, centrifuged at 1000 rpm for 5 min and the supernatant was decanted. Cell pellets were dispersed in 0.25 ml, smeared on clean slides, fixed in 70% methanol, air dried and stained with May-Grunwald/Giemsa protocol. The combination of May–Grunwald and Giemsa was stained PCEs bluish in color. Micronuclei are generally round or almonds shaped, have sharp borders and are generally 5-20% the size of the PCE. For the determination of the frequency of micronucleated polychromatic erythrocytes (MNPCEs), a total 2000 polychromatic erythrocytes (PCE) per animal were analyzed by light microscopy at 1000 X magnification.

Diphenylamine (DPA) assay

DNA fragmentation in liver tissue was carried out according to Wu *et al.*[23] with some modification. Briefly, about 50 mg of liver tissue were homogenized in lysis buffer pH 8.0 (10mM Tris base, 1mM EDTA and 0.2% triton X-100) and incubated on ice for 20 min. The cell lysate were centrifuged at 12.000 rpm for 30 min at 4°C. The supernatant containing small DNA fragments was separated. The supernatant and pellet were re-suspended in1N and 0.5N of perchloric acid respectively. Then samples heated at 90°C for 20 min and centrifuged at 11.000 rpm for 10 min to remove proteins. Supernatant fractions were reacted with diphenylamine (DPA) for 16-20 hr at room temperature and the developing blue color was measured at 600 nm. DNA fragmentation in samples [(fragmented DNA in supernatant)/fragmented DNA in supernatant + intact DNA in pellet) ×100] were expressed as percentage of total DNA appearing in the supernatant fraction.

Statistical analysis

The results were expressed as means%±standard Error. All data were computerized using the Statistical Package for Social Sciences (SPSS, version 11). The results were statistically analyzed by using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) for comparison between different treatment groups. Statistical significance was set at $P < 0.05$.

The suppression of mutagenicity was calculated according to Hu *et al.*[24] using the following formula

Reduction (%) =[(% aberrant cells in group A–% aberrant cells in group B)/(% aberrant cells in group A)]×100

A: represents groups treated with NMU alone
B: represents groups treated with NMU + DPE

RESULTS

Chromosome aberrations (CAs) assay

Data tabulated in Table 2 and Figure 1 pointed up that

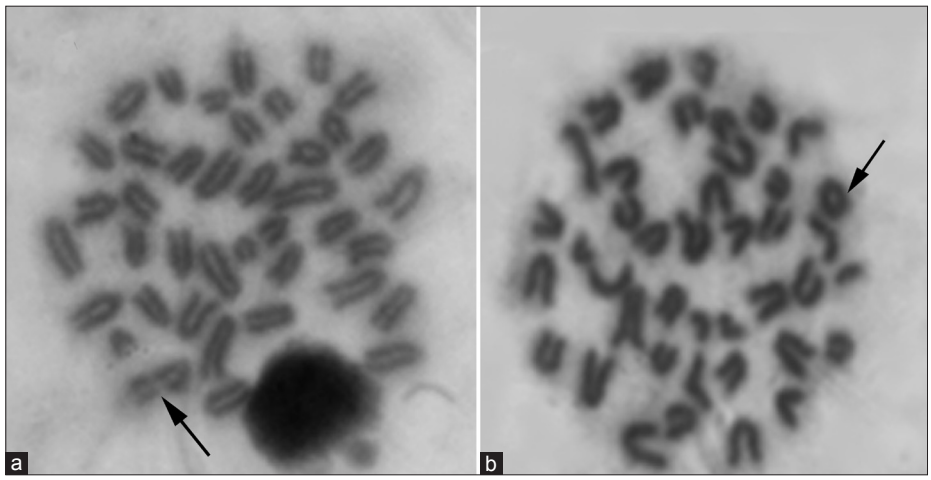


Figure 1: Metaphases plates from mouse bone marrow cells after i.p. treatment with NMU showing a) break, b) Aneuploidy with ring chromosome.

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Table 2: Preventive and therapeutic effect of DPE on NMU induced chromosomal aberrations (CAs) in mouse bone marrow cells

Treatment	Harvest time (day)	No. and % of metaphases with different types of C.A							Abnormal metaphases		Reduction index (%)
		Gap	B and/or Fr	Del.	Ring	M.A	Ane	Poly.	Including gaps Mean %±S.E.	Excluding gaps Mean%± S. E.	
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)			
I-Negative control	-	5 (1.00)	4 (0.80)	6 (1.20)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3.00±0.71^{ab}	2.00±0.55^{a}	
	1	5 (1.00)	9 (1.80)	5 (1.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3.80±0.80^{ab}	2.80±0.37^{a}	
DPE	2	2 (0.40)	9 (1.80)	4 (0.80)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.20)	3.20±0.49^{ab}	2.80±0.37^{a}	
	7	2 (0.40)	4 (0.80)	6 (1.20)	0 (0.00)	1 (0.20)	0 (0.00)	1 (0.20)	2.80±0.37^{a}	2.40±0.24^{a}	
Pre-treatment DEP+NMU	1	11 (2.20)	51 (10.20)	6 (1.20)	1 (0.20)	11 (2.20)	0 (0.00)	1 (0.20)	16.20±0.49^{f}	14.00±0.98^{e}	26.4 23.1
	2	3 (0.60)	23 (4.60)	9 (1.80)	0 (0.00)	4 (0.80)	0 (0.00)	2 (0.40)	8.20±0.49^{c}	7.60±0.60^{c}	43.1 42.4
	7	1 (0.20)	7 (1.40)	8 (1.60)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.60)	3.80±0.49^{ab}	3.60±0.40^{ab}	64.8 51.4
Post-treatment NUM+DEP	1	1 (0.20)	7 (1.40)	12 (2.40)	0 (0.00)	3 (0.60)	0 (0.00)	2 (0.40)	5.00±0.32^{b}	4.80±0.37^{b}	77.3 73.6
	2	0 (0.00)	5 (1.00)	10 (2.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.60)	3.60±0.60^{ab}	3.60±0.60^{ab}	75.0 72.7
	7	0 (0.00)	7 (1.40)	6 (1.20)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.40)	3.00±0.63^{ab}	3.00±0.63^{ab}	72.2 59.5
Positive control MNU	1	19 (3.80)	57 (11.40)	11 (2.20)	3 (0.60)	14 (2.80)	3 (0.60)	3 (0.60)	22.0±0.70^{g}	18.20±0.86^{f}	
	2	6 (1.20)	31 (6.20)	18 (3.60)	2 (0.40)	7 (1.40)	2 (0.40)	6 (1.20)	14.40±0.60^{e}	13.20±0.20^{d}	
	7	17 (3.40)	18 (3.60)	11 (2.20)	3 (0.60)	2 (0.40)	1 (0.20)	2 (0.40)	10.80±1.07^{d}	7.40 ±0.93^{c}	

500 metaphase were examined in 5 mice per each experimental group. Aneuploidy and polyploidy were calculated with or without structural aberrations. Values within the same column follow by different superscript letter are significantly different from one another ($P<0.05$). Ane = Aneuploidy was calculated with or without structural aberration; Br = Break; Del = Deletion; Fr = Fragment; M.A. = metaphases with more than one type of aberrations; Poly = polyploidy

NMU induced different types of structural and numerical CAs in mouse bone marrow cells. The percentage of CAs reached 22.00, 14.40 and 10.80% after one, two and seven day post-treatment with NMU respectively compared with the control group (3.00%). Such values represent 7.3, 4.8 and 3.5 fold increases as compared with that value of the control group after three treatment periods respectively. This finding implies that, NMU is mutagenic agent in somatic cells. However, mice administrated DPE alone show no significant change in the percentage of CAs at one, two and seven days and their values were near normal value.

In view of DPE pretreatment experiments, the results illustrated that DPE was significantly minimized the incidence of CAs at three treatment periods even after excluding gap compared with that observed in the corresponding groups treated with NMU only. The suppression rate reached 26.4, 43.1 and 64.8% after one, two and seven day from the end of the treatment, respectively indicating that DPE had moderate protective effect.

On the other hand, the results of DPE post-treatment experiments showed that the frequency of CAs was significantly lowered after one, two and seven days from the end of treatment with DPE compared with the corresponding groups treated NMU only. The reduction rate reached 77.3, 75.0 and 72.2% at the three treatment periods respectively suggesting that DPE had potential therapeutic effect.

Micronucleus (MN) assay

A remarkable and statistically significant increase in the frequencies of MNPCE was found in groups of mice treated with NMU alone at the three treatment periods compared with the negative control [Table 3, Figure 2].

Nevertheless, no significant difference in the induction of micronuclei was observed between the groups treated with DPE alone and the negative control. Its percentage reached 0.06±0.04, 0.57±0.05, 0.50±0.04 at the three tested periods one, two and seven days respectively compared with 0.47±0.03 for the control group.

As shown in Table 3, the mean percentage of MNPCE reached 1.11±0.09, 0.83±0.05 and 0.66±0.17 in pre-treatment groups and reached 0.97±0.08, 0.66±0.03 and 0.51±0.02 in post-treatment groups at the three treatment periods one, two and seven day, respectively compared with 2.26±0.18, 2.26±0.18 and 1.60±0.22 for the corresponding groups treated with the NMU only. These results imply that, DPE was significantly minimized the frequency of MNPCE induced by NMU.

DNA fragmentation assay

The pre-and post-treatment effect of DPE against NMU-

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Table 3: Preventive and therapeutic effect of DPE on NMU induced Micronucleated polychromatic erythrocytes (MNPCE) in mouse bone marrow cells

Treatment	Harvest time (day)	Micronucleated PCE (MNPCE) MNPCE/2000 PCE		Reduction (%)
		No.	Mean%± S.E.	
I-Negative control	-	47	0.47±0.03[a]	
	1	60	0.06±0.04[a]	
DPE	2	57	0.57±0.05[a]	
	7	50	0.50±0.04[a]	
Pre-treatment DEP+NMU	1	111	1.11±0.09[b]	50.9
	2	83	0.83±0.05[ab]	59.7
	7	66	0.66±0.17[ab]	58.8
Post-treatment NUM+DEP	1	97	0.97±0.08[b]	54.9
	2	62	0.66±0.03[a]	69.9
	7	51	0.51±0.02[a]	68.1
Positive control MNU	1	226	2.26±0.18[e]	
	2	206	2.06±0.18[d]	
	7	160	1.60±0.22[d]	

10000 Polychromatic erythrocytes (PEC) were examined in 5 mice per each experimental group. Values within the same column follow by different superscript letter are significantly different from one another (P<0.05)

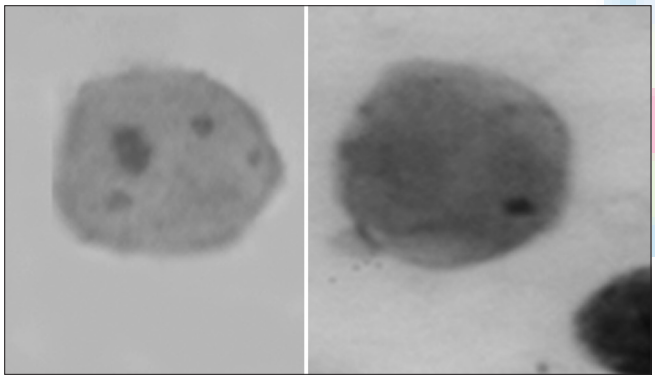


Figure 2: Difference in size of micronuclei from mouse bone marrow cells after i.p. treatment with NMU

induced DNA damage was evaluated by measuring the level of genomic DNA fragmentation using the diphenylamine assay [Table 4]. NMU treatment only caused a significant increase in the percentage of liver genomic DNA fragmentation which reached 21.29, 19.46 and 17.35% at three treatment periods one, two and seven days, respectively compared with the control group (6.09%). However, there was no significant change in liver genomic DNA fragmentation in DPE groups compared with the control group.

The administration of DPE prior or after NMU was effective in blunting liver genomic DNA fragmentation to as low as 13.8, 24.8 and 34.5% in pre-treatment and 27.1, 36.8 and 43.7% in post-treatment groups at three treatment periods one, two and seven days, respectively.

Table 4: Preventive and therapeutic effect of DPE on NMU induced genomic DNA fragmentation in mouse hepatocytes

Treatment	Harvest time (day)	% DNA fragmentation Mean%±S.E.	
		Mean%±S.E.	Reduction (%)
I-Negative control	-	6.09±0.76a	
DPE	1	7.23±0.49[a]	
	2	6.96±0.87[a]	
	7	6.57±0.58[a]	
Pre-treatment DEP+NMU	1	18.30±0.79[f]	13.8
	2	14.60±0.35[d]	24.8
	7	11.66±0.45[bc]	34.5
Post-treatment NUM+DEP	1	15.52±0.93[de]	27.1
	2	12.27±0.62[c]	36.8
	7	9.77±0.57[b]	43.7
Positive control MNU	1	21.29±0.82[g]	
	2	19.46±0.66 [fg]	
	7	17.35±0.96[ef]	

Values within the same column follow by different superscript letter are significantly different from one another (P<0.05)

DISCUSSION

NMU is a well-known direct action mutagen and inflicts DNA damage.[25] Several studies have shown that NMU is genetoxic "*in vitro*" and "*in vivo*" through induction of sister chromatid exchange, micronuclei, structural chromosomal aberration, aneuploidy and comet assay.[25-29] Our results confirmed the genotoxicity of NMU by showing an increase in the incidence of CAs, MNPCE and DNA fragmentation over the vehicle control group. In fact, NMU has a half life of <1 h under physiological conditions and its genotoxic effect occurs within a very short period of its mechanism.[30] To further understand mechanism of NMU, Stephanou *et al.*[26] reported that NMU is alkylating agent able to react with DNA and other biomolecules in most tissues. This reaction takes place by a transfer of methyl group to nitrogen bases of DNA resulting in potential altered bas pairing. Methylation at the O6 position of guanine is considered to be an important lesion, especially G→A transition during DNA replication, with significant role in mutagenesis and carcinogenesis.[31,32] O6-methylguanine (O6MeG) base lesion is known to be signal for apoptosis.[33] Frequency of O6MeG is well correlated with the induction of different mutations in *E. coli,* mammalian cell cultures as well as in murine germ cells.[34] Apart from methylating DNA bases, NMU enhances peroxidation of lipids, Thereby, generate clastogenic free radical species.[35]

With regard to the genotoxicity of date pits extract (DPE), the results illustrated that DPE by itself didn't induce any significant difference in the percentage of all tested assays as compared to control suggesting absence of its genotoxic effect. Similar findings were obtained by Vayalil[36] who

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proven that fruit extract possessed antimutagenic properties in the Ames mutagenicity assay. The extract caused a dose-dependent inhibition of benzo (a) pyrene-induced mutagenecity on *Salmonella* tester strains TA-98 and TA-100 with metabolic activation.

Analysis of CAs was reflected that efficacy of DPE in reducing the frequency of different types of CAs induced by MNU in bone marrow cells in particular aneuploidy and ring chromosome. This finding implies that DPE have antimutagenic and protect the cells from the aneugenic effect of MNU.

The micronucleus (MN) test is able to assess both the clastogenic and aneugenic properties of a test compound. Micronuclei are cytoplasmic chromatin-containing bodies formed when a centric chromosome fragments or intact chromosomes lag during anaphase and fail to become incorporated into daughter cell nucleus during cell division.[37] In the present study, micronuclei analysis showed that NMU induced micronuclei in mouse bone marrow cells. This is due to clastogenic and aneugenic effect of NMU. These activities are largely documented in previous reports.[25,26,29] On the other hand, DPE have proven an efficacy in reduction of number of micronuclei induced by NMU. This reduction is marker of enhanced DNA repair in the cells or due to cell death or apoptosis of heavy DNA damage.[38]

Apoptosis is a normal event that occurs both during and after development. This phenomenon occurs in cells injured by certain levels of toxic agents.[39] The diphenylamine assay is a very useful method for measuring apoptosis by determining the percentage of fragmentation of DNA into oligosomal-sized fragments. Measure of soluble DNA released from apoptotic nuclei into the cytoplasm constitutes a quantitative measure of cellular response.[40] Hepatocytes were chosen as indicators of apoptosis because of their high metabolic capacity which enables a highly sensitive test system for antimutagenicity and detoxification properties.[41]

The present findings show that, DPE reduced the DNA fragmentation in mouse hepatoctes indicating its hepatoprotective effect. These results are consistent with Al-Qarawi *et al.*[6] who reported that pre- and post-treatment with aqueous extract of date flesh or pits significantly reduced CCl4 induced hepatoxicity in mice by elevation of bilirubin concentration, hepatic marker enzymes and concomitantly increased the level of antioxidant enzymes.

Basically, there are two main groups of protective mechanisms for DNA, desmutagenic and bio-antimutagenic according to mode of action. Desmutagenesis could be detected with pre-treatment, while bio-antimutagenicity could be better detected with post-treatment.[42] The two regimens treatment used in the present study could lead to a better manner of understanding the antimutagenicity mechanisms of DPE.

Our data have shown that pretreatment regimen was significantly minimized the genotoxicity of NMU in all examined assays however the genotoxicity was not recovered to the normal control value. These findings indicated that DPE interacted extracellulary with NMU prior to attack DNA in desmutagenic manner. This view supported by De Flora *et al.*[43] who reported that desmutagenic agents are compounds that act directly on mutagens, or on their precursors, in order to inactivate them; they can bind to the mutagenic compound in an irreversible way, inactivating it chemically through a direct link or inhibiting activation by the modulation of the phase I and II enzymes.

It is well documented that, the mutagenic and carcinogenic actions of alkylating agents may be related to their ability to form O6-methylguanine (O6MeG).[26] O6MeG is repaired by protein termed O6-methylguanine DNA methyltransferase (MGMT).[44] MGMT directly repair O6MeG lesion in DNA by transferring methyl group from a base lesion to a cysteine residue, thereby restoring the integrity of the DNA without creating additional DNA damage,[45] cells which unable to repair O6MeG because of lack of expression of MGMT are highly sensitive to O6MeG agents compared to MGMT competent cells.[46] In contract, over expression of MGMT has been shown to render tissues more resistant to the carcinogenic effects of alkylating agents. Post-treatment experiments illustrated that DPE has more pronounced effect in reduction genotoxic effect induced by NMU in all examined assays but the genotoxicity was nearly recovered to the normal control value. These results indicated DPE acts intracellulary by the DNA repair damage induced by NMU in a bioantimutagenic manner. This means that DPE have the ability of anti-alkylation damage and improves the activity of MGMT. This view supported by De Flora[43] who reported that the bioantimutagenic agents act on the physiological mechanisms of DNA protection and repair, reverting the mutagenic effects and preventing the fixation of mutations.

It is noteworthy; two regimens of DPE treatment were resulted in an earlier recovery of bone marrow cells and hepatocytes compared to mice treated with NMU only. This reflected that DPE had effect on the acceleration of cell recovery by restitution of damage parts of DNA. Nevertheless, DPE had more pronounced inhibitory rate in post-treatment compared with pre-treatment regimens in all examined assay.

The mechanism by which date pits enables to suppress genotoxic effect of NMU is not certain. However, the earlier studies reported that date pits contain higher quantity of protein (5.1 g/100 g) and fat (9.0 g/100 g)

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such as oleic, linoleic, lauric, palmitic, myristic and stearic when compared to date flesh. It is also high in dietary fiber (73.1g/100 g), phenolics (3942 mg/100 g), antioxidants (80,400 μmol/100 g) and trace elements such as potassium, manganese, calcium, phosphorus, sodium, iron, aluminum, cadmium, chloride, lead, sulfur, and fluoride in various proportions.[47,48] It is logical to assume that cumulatively the presence of all these components in the date pits may have been responsible for reduction of DNA binding and methylation with NMU. It is a well know that 1-alkane diazotic acid is the central inactive metabolite of NMU, which is formed directly from hydrolysis of NMU[28] and is transformed to a free radical through the microsomal cytochrome P-450-dependent enzymes, resulting in activation of NMU toxicity.[35] Therefore, the antigenotoxicity of date pits is due to its ability to scavenge the alkyl radical or inhibit the aromatase activity of cytochrome P-450 or blocking the reaction between methane diazonium ion and DNA. Indeed, "*in vitro*" studies have shown that the aqueous extract of date fruit was a potent scavenger of superoxide and hydroxyl radicals and to inhibit iron-induced lipid peroxidation and protein oxidation in the rat brain homogenate in a concentration-dependent manner.[36] Animal studies have also shown that oral feeding of p-coumaric acid present in date increases the expression of antioxidant enzyme genes in rat cardiac tissue.[49]

CONCLUSIONS

Our data illustrated that DPE produced their inhibitory activity either by pre-treatment or by post-treatment. These results suggested that DPE interacted with intact NMU extracellulary, in a desmutagenic manner, and act intracellulary by affect the DNA repair damage induced by NMU in a bioantimutagenic manner. The observed antimutagenic activity may be attributed to the presence of mineral and various photochemical of diverse chemical structure. Also, these findings suggested that date by-products are considered as an inexpensive source of natural antioxidant and can be exploited economical in pharmaceutical industries.

Therefore, further studies should be identifying the active compounds of DPE and the application other "*in vivo*"" and "*in vitro*" genotoxic end-points to contribute to understanding of mechanism of action of the chemopreventive strategies of DPE.

ACKNOWLEDGMENT

This work is supported by National Research Center, Dokki, Cairo, Egypt. The authors thank Prof. Dr. Aida I. El-makawy for critical reading of the manuscript and her helpful suggestions.

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How to cite this article: Diab K, Aboul-Ela EI. In vivo comparative studies on antigenotoxicity of date palm (Phoenix dactylifera l.) pits extract against DNA damage induced by N-Nitroso-N-methylurea in mice. Toxicol Int 2012;19:279-86.

Source of Support: Nil. **Conflict of Interest:** None declared.