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# Ameliorative effect of grapefruit juice on amiodarone-induced cytogenetic and testicular damage in albino rats

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## ARTICLE INFO

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## Comments

This is a very good study in which the authors evaluated the ameliorative role of grapefruit juice on the cytogenetic damage induced by the antiarrhythmic drug amiodarone in albino rats. The results are interesting and suggested that the ameliorative effect of grapefruit juice against amiodarone toxicity may be attributed to its antioxidant activity.

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## ABSTRACT

**Objective:** To evaluate the ameliorative role of grapefruit juice on the cytogenetic and testicular damage induced by the antiarrhythmic drug amiodarone in albino rats. **Methods:** Animals were divided into four groups. Group I was considered as control. Group II was given grapefruit juice at a dose level of 27 mL/kg body weight. Group III was orally administered amiodarone (18 mg/kg body weight) daily for 5 weeks. Animals were sacrificed after 5 weeks of treatment. Bone marrow was collected from the femurs for analysis of chromosomal aberrations and mitotic indices. Testes were removed and stained with H&E for histological examination. Sperms were collected from epididymis for detection of sperm head abnormalities. Comet assay was used to detect DNA damage. **Results:** Amiodarone treatment caused a significant increase in the percentage of chromosomal aberrations, decreased the mitotic index and increased DNA damage. The testis showed many histopathological alterations, inhibition of spermatogenesis and morphometric changes. The number of sperm head abnormalities was increased. Treating animals with amiodarone and grapefruit juice caused a reduction in chromosomal aberrations, mitotic index, DNA damage and testicular alterations caused by amiodarone. **Conclusions:** The results of this study indicated that grapefruit juice ameliorates the cytotoxicity and testicular alterations induced by amiodarone in albino rats and this may be due to the potent antioxidant effects of its components.

## KEYWORDS

Amiodarone, Grapefruit, Chromosome, Comet assay, Testis

## 1. Introduction

Amiodarone is a member of antiarrhythmic drugs which is used in a wide variety of cardiac arrhythmias resistant to other treatments. It showed beta-blocker like and potassium channel blocker like actions, increasing the refractory period via sodium and potassium channel effect, besides slowing intera-cardiac conduction of the cardiac action potential via sodium channel effect<sup>[1]</sup>. On the other hand, the drug has many extracardiac side effects, including corneal microdeposits, photosensitivity, and hypo- or hyper-thyroidism<sup>[2]</sup>. It was reported that 15%–40% of patients taking amiodarone develop an asymptomatic hepatic dysfunction, which is dose dependent and reversible on stopping treatment<sup>[3]</sup>. Amiodarone may accumulate within

many organs including the testis and was associated with epididymitis<sup>[4,5]</sup>. It induced apoptosis in H9c2 cardiac cells and chromosomal aberrations in rats<sup>[6,7]</sup>.

Plants and plant derived pure chemicals are now used for treatment of many diseases. Grapefruit is a subtropical citrus and is an excellent source of many nutrients and phytochemicals, able to contribute to a healthy diet<sup>[8]</sup>. The pink and red hues contain the beneficial antioxidant lycopene<sup>[9]</sup>. Grapefruit seed extract has been claimed to have strong antimicrobial properties<sup>[10,11]</sup> and cholesterol reduction ability<sup>[12]</sup>. There is an evidence of anticancer properties of grapefruit. Miller *et al.* reported that naringin and naringenin, two flavonoids found in high concentrations in grapefruit, may be able to inhibit the development of oral carcinogenesis<sup>[13]</sup>. Naringin, abioflonoid predominant

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in grape fruit and other citrus fruits, has been found to scavenge free radicals that reduce induced damage such as reduction of aberrant cells and chromosomal aberrations[14]. Consumption of grapefruit juice was found to be beneficial for human health, including protection against the DNA damage[15]. The present work aims to study the ameliorative effect of grapefruit juice on amiodarone-induced cytogenetic and testicular alterations in albino rats.

## 2. Materials and methods

### 2.1. Rats and treatments

Two hundred healthy adult male albino rats (*Rattus norvegicus*), approximately 3 months old and weighing (130±5) g, were used. The animals were kept in individual special rodents cages in the laboratory under constant condition of temperature (25±3) °C with a reverse natural dark–light cycle 12/12 h. Animals were maintained on a standard rodent diet, obtained from Egyptian company of Oils and Soap Kafr-Elzayat Egypt, manufactured especially for laboratory purposes. The diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch. Water was available *ad libitum*. Maintenance of animals and experimental procedures were approved by the animal ethical committee in accordance with the guide for care and use of laboratory animals of Menoufia University, Egypt.

Animals were divided into four groups. The rats in Group I served as controls. The rats in Group II were orally given grapefruit juice. Juice of grapefruit (*Citrus Paradise*) was prepared by squeezing the fresh fruit. The rats were regularly orally administered with grapefruit juice at a dose level of 27 mL/kg body weight[16]. The rats in Group III were treated with amiodarone. Tablets of amiodarone were ground and dissolved in distilled water. It is used at a dose level of 18 mg/kg body weight modified according to therapeutic dose of human, and each animal was orally given 0.5 mL containing the desired dose daily for 5 weeks. The rats in Group IV were given amiodarone (18 mg/kg body weight) followed by grapefruit juice (27 mL/kg body weight) daily for 5 weeks.

### 2.2. Chromosomal preparation

Animals were sacrificed after 5 weeks of treatment. Bone marrow cell preparations for analysis of chromosomal aberrations and mitotic indices were conducted by colchicines–hypotonic technique. After completion of the treatment period, five animals from each group were intraperitoneally injected, 2 h before sacrifice, with 0.5 mL colchicine (3 mg/kg body weight), to increase the number of metaphase spreads. Bone marrow cells were collected from the femurs in isotonic NaCl solution and bone marrow smears were prepared[17]. For each group, slides were stained with Giemsa and mounted in DPX. For each animal, 50 metaphase spreads were scored for chromosomal aberrations. The mitotic index was obtained by counting the number of mitotic cells in 1 000 cells/animal.

### 2.3. Comet assay

This technique is widely used for detection of single stranded breaks[18]. Eukaryotic cells are embedded in

agarose gel on a microscopic slide, lysed by detergents and high salt at pH 10, and then electrophoresed for damage display which shows increased migration of the DNA from the nucleus towards the anode. This technique permits the detection and an evaluation of single-stranded DNA breaks. Low-melting temperature agarose and ultra pure agarose, Triton X-100, sodium sarcosinate, ethylene-diamine–tetra acetic acid disodium salt (Na<sub>2</sub>-EDTA), Tizma base and ethidium bromide were obtained from Sigma chemical company. Phosphate buffered saline (PBS) without calcium and magnesium, RBMI 1640 medium (Gibco), Ficoll separating solution and trypan-blue were used in comet assay. Examination was done with a fluorescent microscope (Olympus BX 41, Japan) equipped with an excitation filter of 510 nm and barrier filter of 590 nm. The migration was evaluated by observing and measuring the nuclear DNA, and 500 spots of DNA were examined and classified into three types: (i) normal spots with round shape; (ii) damaged spots in which the length of the migrated fragments was less than or equal to the diameter of the basal nuclear DNA; and (iii) strongly damaged spots where the length of the comet was greater than the diameter of the basal nuclear DNA.

### 2.4. Histological studies

For light microscopic studies, animals were dissected and their testes were removed and fixed in 10% neutral formalin for 24 h, washed in running tap water for 24 h and transferred to 70% ethyl alcohol. Tissues were dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded in wax. Sections of 5 µm thickness were cut using rotary microtome and mounted on clean slides without using any adhesive medium. Sections were stained with Ehrlich's haematoxylin, counterstained with eosin and photographed.

### 2.5. Sperm head abnormalities test

The sperm suspension was obtained from animals by cutting the caudal epididymis of a testis in few drops of mammalian saline. The sperm suspension was spread on clean glass slides. Sperm smears were dried in air and incubated at oven at 50 °C overnight. The sperms were fixed in methyl alcohol and stained with haematoxylin and eosin. A total of 1000 sperms were examined for each animal directly under microscope to detect the morphological abnormalities in head region.

### 2.6. Statistical analysis

In the present work, the results are represented in Tables as mean number ± standard error. The significance of difference between the treated and normal groups was calculated using chi-square and student's *t*-test.

## 3. Results

### 3.1. Chromosomal aberrations and mitotic index

Both structural and numerical aberrations were resulted from the treatment with amiodarone. Structural aberrations including chromatid deletion, chromatid fragment, chromosomal ring, centromeric attenuation of chromosomes,

centric fusion of chromosomes, end to end association, chromatid gaps and chromatide breaks were observed (Table 1). Numerical aberrations such as monosomy, trisomy, tetrasomy and polyploidy were also recorded (Table 2). A significant increase in these aberrations was observed after the treatment with amiodarone when compared with the control animals, while the animals given grapefruit juice showed a decrease in these aberrations. The animals treated with amiodarone showed a decrease in the mitotic index when compared with control group and increased after grapefruit juice administration.

3.2. Detection of DNA damage by comet assay

Detection of DNA damage by comet assay in blood lymphocytes cells showed a significant increase in DNA fragmentation in the animals treated with amiodarone appeared as damaged and strongly damaged spots, while the animals given grapefruit juice showed an improvement in DNA by decreasing the number of damaged spots (Figure 1, Table 3).

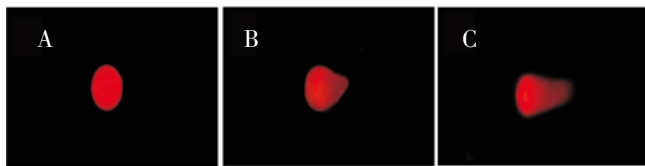


Figure 1. Photomicrograph showing single strand breaks of DNA of rat lymphocytes.

A: Normal DNA spots (no migration); B: Damaged DNA spots (migration towards the anode); C: Strongly damaged DNA spots (more migration towards the anode).

3.3. Histological observations

Sections in testis of control rats showed typical structure of normal seminiferous tubules, spermatogenic cells, intertubular connective tissue and spermatozoa (Figure 2). The animals administrated grapefruit juice for 5 weeks showed the same histological structure as those served as controls. Sections of testis of animals given amiodarone for 5 weeks showed a histological difference when compared with the control ones. The outlines of the seminiferous tubules appeared irregular and sometimes were with thick basement and irregular membranes.

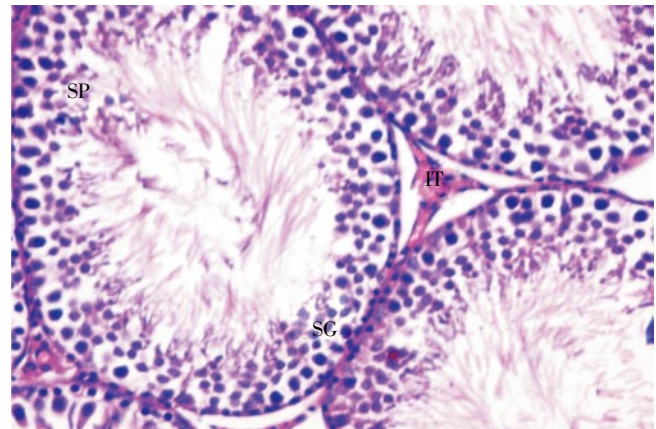


Figure 2. Section in testis of a control rat showing normal seminiferous tubules (H&E×400).

SG: spermatogonia; SP: spermatozoa; IT: intertubular connective tissue.

Table 1

Average of structural chromosomal abnormalities observed in bone marrow cells of male rats treated with amiodarone and grapefruit juice (mean±SE).

Animal group	Deletion	Chromatid fragment	Centromeric attenuation	End to end association	Centric fusion	Chromosomal ring	Gaps	Breaks	Total
Normal	10.00±0.44	4.20±0.37	0.60±0.24	0.60±0.24	0.00±0.00	16.00±0.70	0.00±0.00	0.00±0.00	31.40±1.99
Grapefruit juice	9.60±2.27	16.00±1.12	0.00±0.00	1.20±0.49	1.20±0.97	16.20±3.30	0.60±0.24	0.00±0.00	44.80±8.39
Amiodarone	36.60±3.80	16.00±1.18	2.40±0.74	1.80±0.73	2.60±0.67	30.80±2.47	2.80±0.37	1.60±0.40	94.60±10.36*
Amiodarone+grapefruit juice	17.00±2.55	13.80±1.62	1.40±0.24	1.40±0.40	2.40±0.24	11.00±1.00	0.00±0.00	0.20±0.20	47.20±6.25

\*Significant at P<0.05.

Table 2

Average of numerical chromosomal abnormalities observed in bonemarrow cells of male rats treated with amiodarone and grapefruit juice (mean±SE).

Animal group	Monosomy	Trisomy	Tetrasomy	Polyploidy	Total
Control	4.0±0.3	0.0±0.0	0.0±0.0	0.4±0.2	4.4±0.6
Grapefruit juice	6.0±1.1	0.6±0.2	0.4±0.2	1.0±0.3	8.0±2.0
Amiodarone	13.0±1.0	8.2±1.9	1.2±0.3	1.4±0.5	23.8±3.9*
Amiodarone+grapefruit juice	7.6±1.1	0.0±0.0	0.2±0.2	0.2±0.2	8.0±1.5

\*P<0.05 compared with contral.

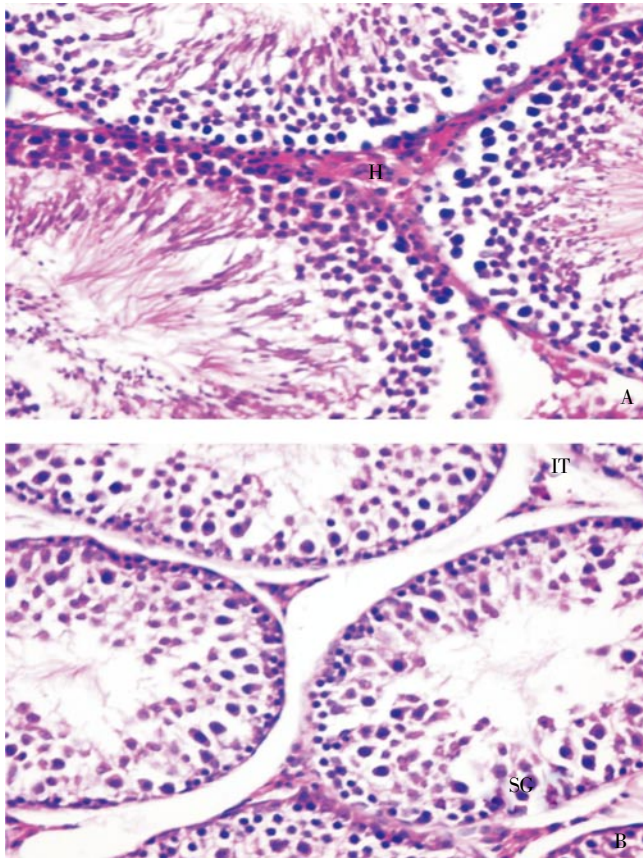
Table 3

Mean value of DNA damage detected by comet assay in rat lymphocytes treated with amiodarone and grapefruit juice (mean±SE).

Animal group	Normal cells	Damaged cells	Strongly damaged cells	Total damaged cells
Control	97±0.83	3±0.83	0±0.00	3±0.83
Grapefruit juice	95±1.09	5±1.09	0±0.00	5±1.09*
Amiodarone	80±2.32*#	18±1.64*#	2±0.70#	20±2.32*#
Amiodarone+grapefruit juice	89±1.34*#°	10±0.83*#°	1±0.54	11±1.34*#°

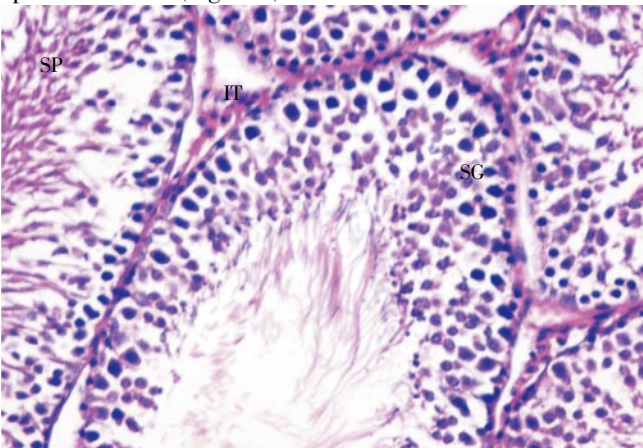
\*Statistically significant in comparison with control; #Statistically significant in comparison with grapefruit; °Statistically significant in comparison with treatment with cordarone.

The interstitial tissue was degenerated and intertubular hemorrhage was observed (Figure 3A). The spermatogenic cells decreased in number, degenerated and appeared with pyknotic nuclei (Figure 3B).



**Figure 3.** Sections in testes of rats treated with amiodarone for 5 weeks (×400). A: Irregular seminiferous tubules and intertubular hemorrhage (H); B: Degenerated interstitial tissue (IT) and degenerated spermatogonia (SG) with pyknotic nuclei and loss of spermatozoa.

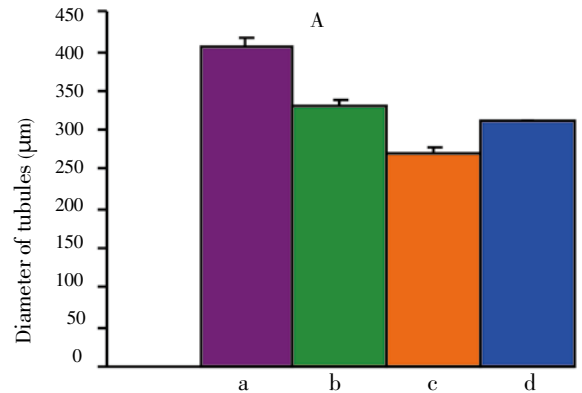
Few sperms were present and scattered randomly in the tubules. Sections of animals given amiodarone with grapefruit juice showed improvement in the histopathological alterations appeared in animals given amiodarone only. The spermatogenic layers increased with appearance of somewhat normal cells with increase of sperms bundles (Figure 4).



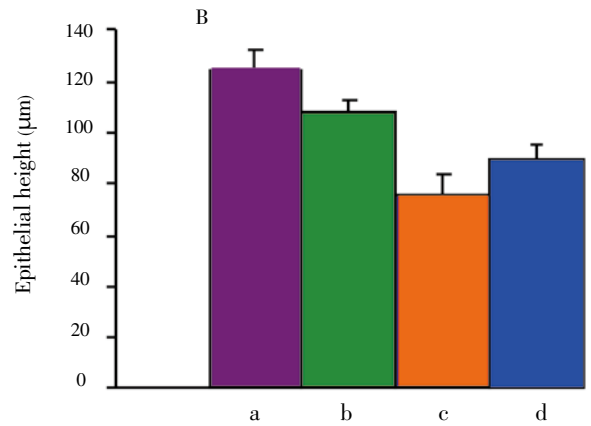
**Figure 4.** Section in testis of a rat given amiodarone and grapefruit juice showing an improvement in histological appearance and seminiferous tubules showing different stages of spermatogenic cells (×400). SG: spermatogonia; IT: interstitial tissue; SP: sperms.

### 3.4. Morphometric results

Treating animals with amiodarone caused atrophy of the seminiferous tubules. A decrease in diameter of seminiferous tubules and germ cell height of seminiferous tubules was recorded in comparison with normal ones (Figure 5). Animals treated with amiodarone and grapefruit juice showed marked improvement in the mean tubular diameter and germ cell height in comparison with amiodarone treated animals.



a Control; b Grapefruit; c Amiodarone; d Amiodarone+grapefruit

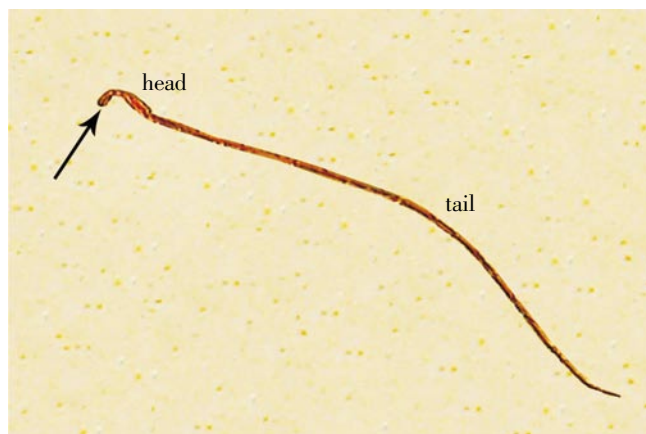


a Control; b Grapefruit; c Amiodarone; d Amiodarone+grapefruit

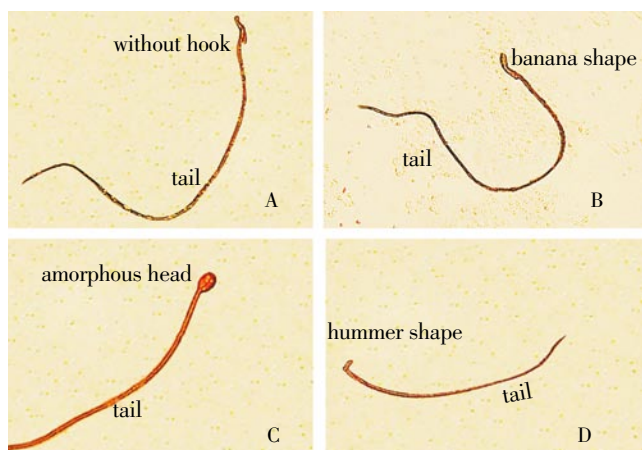
**Figure 5.** Changes of seminiferous tubules in different animal groups.

### 3.5. Sperm head abnormalities

Figure 6 shows a sperm with normal head and tail. Figure 7 shows sperm head abnormalities (without hook, banana type, amorphous head and hummer shape). The rats treated with amiodarone caused a significant increase in the mean number of sperm head abnormalities. On the other hand, the animals given amiodarone and grapefruit juice showed a significant decrease in these abnormalities (Table 4).



**Figure 6.** Sperm with normal head, tail and hook (arrow) (×1000).



**Figure 7.** Sperms with abnormal heads (×1000). A: Without hook; B: Banana type; C: Amorphous head; D: Hammer shape.

#### 4. Discussion

The present study showed that treating rats with amiodarone caused an increase in the mean number of structural chromosomal aberrations including chromatide deletion, fragments, chromosomal ring, centromeric attenuation of chromosomes, centric fusion, end to end association of chromosomes, chromatid gaps and breaks. Numerical aberrations (monoploidy, triploidy, tetraploidy and polyploidy) were also increased. On the other hand, mitotic index decreased by the treatment with this drug. Similarly, Almeida *et al.* reported that amiodarone caused an increase in chromosomal aberrations and abnormal metaphase in bone marrow cells of Wistar–Kyoto rats[7].

Telez *et al.* showed that the antihypertensive drug atenolol was found to induce chromosome loss[19].

DNA damage was recorded after treatment with amiodarone. Isomoto *et al.* reported that amiodarone induced DNA fragmentation (apoptosis) in culture H9c2 cells[6]. Choi *et al.* demonstrated that L–132 human lung cell line treated with amiodarone exhibited several features of apoptosis and increase of mRNA levels of bax and caspase–3[20]. Amiodarone was found to cause testicular damage, inhibit spermatogenesis and increase sperm head abnormalities. Dobs *et al.* reported that atrophic testes were more commonly observed in amiodarone–treated men[21]. They added that amiodarone–treated men had higher serum follicle–stimulating hormone and luteinizing hormone concentrations compared with control subjects. Ward *et al.* reported a case of epididymitis associated with amiodarone treatment and a high concentration of desethylamiodarone in the semen[5]. Khan *et al.* reported that patients taking beta–blockers experience sexual dysfunction[22]. El–Sayed *et al.* reported that atenolol, metoprolol and propranolol have a toxic effect on male fertility and induced significant decrease in percent of progressive motility of sperm besides increase in sperm head and tail abnormalities and cause a significant decrease in the level of testosterone hormone[23].

It was suggested that free radicals are produced during the metabolism of amiodarone and involved in the mechanism of the drug's side effects. Vereckei *et al.* verified that amiodarone generated free radicals *in vitro* by chemiluminometric method and caused a significant increase of NADPH and  $Fe^{3+}$  induced lipid peroxidation in the liver microsomal fraction[24]. On the other hand, oxidative stress was not involved in the pathogenesis of amiodarone toxicity[25,26]. The potential mechanisms of amiodarone toxicity include direct and indirect cytotoxicity. In this concern, Agoston *et al.* reported that amiodarone treatment increased lysosomal phospholipidosis in liver of male Fischer 344 rats[27]. The role played by oxidative stress in amiodarone–induced mitochondrial toxicity was investigated by Serviddio *et al.*[28]. They reported that amiodarone increased mitochondrial  $H_2O_2$  synthesis, which in turn induced cardiolipin peroxidation. Moreover, amiodarone inhibited Complex I activity and uncoupled oxidative phosphorylation, leading to a reduction in the hepatic ATP content. One or more of these mechanisms may be responsible for amiodarone toxicity recorded in the present work.

Treatments involving antioxidants have been used successfully to decrease oxidative stress related injuries in

**Table 4**

Effect of amiodarone and/or grapefruit juice on sperm head abnormalities.

Animal group	Normal	Without hook	Banana shape	Amorphous head	Hammer shape
Control	994.40±0.50	1.20±0.37	1.20±0.20	0.00±0.00	0.00±0.00
Grapefruit juice	992.20±1.39	2.60±0.81	1.80±0.37	0.60±0.24	0.00±0.00
Amiodarone	972.60±5.78	16.40±2.71*	5.80±1.20	1.80±0.37	1.20±0.37
Amiodarone+grapefruit juice	985.80±1.85	9.80±0.66	4.40±0.74	1.20±0.20	0.00±0.00

\* $P<0.05$ .

different organs. Administrating rats with grapefruit juice ameliorated the cytogenetic and testicular abnormalities induced by amiodarone. The protective effects of grapefruit were studied by many investigators. Attia reported that naringin, a grapefruit flavonone, showed anti-mutagenic effects against lomefloxacin-induced genomic instability *in vivo* in mouse bone marrow cells and decreased chromosomal aberration[29]. Miyata *et al.* stated that rats allowed free access to grapefruit juice for 5 days prior to AFB1 administration resulted in clearly reduced DNA damage in liver[30]. Alvarez-González *et al.* reported that the consumption of grapefruit juice has been associated with various activities potentially beneficial for human health, including protection against the DNA damage produced by dounoubicin[15]. Alvarez-González *et al.* found that grapefruit juice inhibited the rate of micronucleated polychromatic erythrocytes (MNPE) and sister chromatid exchanges induced by the antineoplastic alkylating agent ifosfamide[31]. Liu *et al.* reported that quercetin has protective effect against lead-induced hepatic apoptosis[32]. Miyata *et al.* studied the influence of grapefruit juice intake on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced colon DNA damage and found that grapefruit juice suppresses PhIP-induced colon DNA damage by a mechanism independent of PhIP absorption in the intestine[33]. Jagetia *et al.* reported that naringin, a bioflavonoid predominant in grape fruit and other citrus fruits, has been found to scavenge free radicals; and it reduced radiation induced damage such as reduction of aberrant cells and chromosomal aberrations like acentric fragments, chromatid and chromosome breaks, centric rings, dicentric and exchange[14].

The antioxidant activity of grapefruit components was studied. Girenavar *et al.* isolated two bioactive compounds from grapefruit juice and grapefruit peel oil[34]. Structures of the compounds were identified as bergaptol and geranyl coumarin. Bergaptol showed a very good radical scavenging activity at all the tested concentrations. Furthermore, these compounds were found to be potent inhibitors of debenzoylation activity of CYP3A4 enzyme. Muthukumaran *et al.* investigated the protective effect of quercetin (QN) against nicotine-induced prooxidant and antioxidant imbalance in circulation, lung, liver and kidney of experimental rats[35]. The extent of DNA damage (evaluated by comet assay) was significantly increased in circulatory blood of nicotine-treated rats, which was effectively brought down by QN treatment. The data suggested that QN exerts its protective effect by modulating the extent of lipid peroxidation and augmenting antioxidant defense system and thus protects the DNA in experimental animals. Thus, it is concluded from the present work that the ameliorative effect of grapefruit juice against amiodarone toxicity may be attributed to its antioxidant activity.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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### Comments

#### Background

Amiodarone is a member of antiarrhythmic drugs which is used in a wide variety of cardiac arrhythmias resistant to other treatments. The drug has many extracardiac side effects, including corneal microdeposits, photosensitivity, and hypo- or hyper-thyroidism. As recorded, 15%–40% of patients taking amiodarone develop an asymptomatic hepatic dysfunction, which is dose dependent and reversible on stopping treatment. Amiodarone may accumulate within many organs including the testis.

#### Research frontiers

The present work aims to study the ameliorative effect of grapefruit juice on amiodarone-induced cytogenetic and testicular alterations in albino rats.

#### Related reports

Grapefruit may be able to inhibit the development of oral carcinogenesis. Naringin, bioflavonoid predominant in grape fruit and other citrus fruits, has been found to scavenge free radicals that reduce induced damage such as reduction of aberrant cells and chromosomal aberrations. Consumption of grapefruit juice was found to be beneficial for human health, including protection against the DNA damage.

#### Innovations and breakthroughs

Data in this present study showed that treating rats with amiodarone caused an increase in the mean number of structural chromosomal aberrations including chromatid deletion, fragments, chromosomal ring, centromeric attenuation of chromosomes, centric fusion, end to end association of chromosomes, chromatid gaps and breaks. Numerical aberrations (monoploidy, triploidy, tetraploidy and polyploidy) were also increased. On the other hand, mitotic index decreased by the treatment with this drug.

#### Applications

It may be significant to know the effect of treatment with grapefruit juice on the cytogenetic damage induced by the antiarrhythmic drug amiodarone in albino rats.

#### Peer review

This is a very good study in which the authors evaluated the ameliorative role of grapefruit juice on the cytogenetic damage induced by the antiarrhythmic drug amiodarone in albino rats. The results are interesting and suggested that the ameliorative effect of grapefruit juice against amiodarone toxicity may be attributed to its antioxidant activity.

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