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Assessment the Genotoxic Potential of Fluoxetine and Amitriptyline at Maximum Therapeutic Doses for Four-Week Treatment in Experimental Male Rats

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Abstract

At any moment, the continuous usage of medications can be accompanied by DNA damage and the accumulation of such damages can cause serious consequences. Antidepressants are long-term used drugs and the incidence of their genotoxic impacts cannot be excluded. Therefore, this work was designed to investigate the possible genotoxic effects of the commonly used antidepressants (fluoxetine and amitriptyline) in adult male rats. A total of 24 Swiss albino adult male rats were used in this study; animals were randomly allocated into three groups of 8 rats each: **Group I** - rats orally-administered distilled water via gavage tube for four weeks as a negative control. **Group II** - rats orally-treated with fluoxetine hydrochloride solution (7.2mg/kg/day) via gavage tube for four weeks. **Group III -** rats orally-treated with amitriptyline hydrochloride solution (27mg/kg/day) via gavage tube for four weeks.

At the end of experiment, the rats were sacrificed and the samples collected for detection of DNA damage in individual cells that have been assessed by means of comet and micronucleus assays in three different cell populations *i.e.* liver, testis and bone marrow tissues.

The results showed that both drugs (**Group II and Group III)** induced the same extent of DNA damage, as evidenced by significantly higher DNA fragmentation in liver and testis tissues with increased frequencies of micronuclei formation in bone marrow tissues as compared with the negative control (**Group I)**.

These findings indicate that both fluoxetine and amitriptyline have genotoxic potentials and can induce the same extent of cytogenetic damage in rats. Special precautions and medical supervision should be taken into consideration with their uses.

Keywords: Genotoxicity, Fluoxetine, Amitriptyline, Comet assay, Micronucleus assay.

تقييم السمية الجينية المحتملة من علاج اربعة اسابيع لجرعات علاجية قصوى من الفلوكسيتين والاميتريبتيلين في ذكور الجرذان

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الخلاصة

في أي لحظة ، يمكن أن يرافق الاستخدام المستمر للأدوية ضررا في الحمض النووي وقد يؤدي تراكم هذه الأضرار إلى عواقب وخيمة. تعد مضادات الأكتئاب من الأدوية التي تستخدم على المدى الطويل وأن أمكانية حدوث السمية الجينية المرافقة لأستخدامها شيء لايمكن أستبعاده. لذلك تم تصميم هذه الدراسة لتقييم السمية الجينية المحتملة لمضادات الأكتئاب الشائعة الأستخدام (الفلوكستين والأميتريبتيلين) في ذكور الجرذان البالغة. تم تقييم الكشف عن تلف الحمض النووي في الخلايا الفردية من خلال فحوصات المذنب والنوى الصغيرة في ثلاث مجموعات مختلفة من الخلايا، متمثلة بأنسجة الكبد والخصية والنخاع العظمي لـ ٢٤ من الجرذان الذكور البالغين البيضاء. تم توزيع الحيوانات بشكل عشوائي في ثلاث مجموعات, ٨ جرذان لكل منها: **المجموعة الأولى** - الجرذان التي تناولت الماء المقطر عن طريق الفم لمدة أربعة أسابيع كمجموعة سيطرة سالبة. **المجموعة الثانية** - الجرذان التي تم معالجتها بمحلول فلوكستين هايدروكلوريد (٧,٢ ملغ / كغ / يوم) عن طريق الفم لمدة أربعة أسابيع. **المجموعة الثالثة** - الجرذان التي تم معالجتها بمحلول أميتريبتلين هايدروكلوريد (٢٧ ملغ / كغ / يوم) عن طريق الفم لمدة أربعة أسابيع. أظهرت النتائج أن كلا العقارين (المجموعة الثانية والمجموعة الثالثة) تسببا بنفس المدى من الضرر الحاصل في الحمض النووي متمثلة بأرتفاعا معنويا ملحوظا في تكسر الحمض النووي في أنسجة الكبد والخصية مع زيادة ملحوظة بتواتر تكوين النوى الصغيرة في أنسجة نخاع العظام بالمقارنة مع مجموعة السيطرة. تشير هذه النتائج إلى أن كل من الفلوكستين والأميتريبتيلين لهما إمكانيات سمية جينية ويمكنهما إحداث نفس المدى من الضرر الجيني الخلوي في الجرذان. يجب الأخذ بعين الاعتبار الاحتياطات الخاصة والإشراف الطبي مع استخدام هذين العقارين.

الكلمات المفتاحية: السمية الجينية , فلوكستين , أميتريبتيلين , فحص المذنب , فحص النوى الصغيرة.

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Introduction

Depression and anxiety disorders are common growing problems in public health [(1)]. Depression affects approximately 350 million people worldwide; constituting a major portion of mental health disorders [(2)]. Regarding the prevalence of mental disorders in Iraq, the national Iraq Mental Health Survey (IMHS) conducted in 2007, with 4332 respondents, showed that anxiety disorders were the most common class (13·8%) and major depressive disorder was the most common disorder (7·2%) [(3)]. The World Health Organization (WHO) indicated that depression will be the disorder striking worldwide within the next decade, and is predicted to be the second largest burden to ischemic heart disease in the International Community of Health by 2020 [(4)]. Thus, Antidepressant drugs become commonly prescribed nowadays, and also their use becomes increasing throughout the world [(5)]. Substantial international studies on antidepressants prescribing patterns, showed that fluoxetine and amitriptyline are two of the most commonly prescribed antidepressants belonging to selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) groups, respectively [(6 , 7)].

Fluoxetine is a widely-marketed (SSRI) commonly used for treatment of major depressive disorder, obsessive compulsive disorder, panic disorder, bulimia nervosa and premenstrual dysphoric disorder [(8)]. Fluoxetine act by blocking serotonin (5-hydroxytryptamine) neurotransmitter reuptake into the presynaptic cells by binding to serotonin transporters, thus increasing such neurotransmitter in the synaptic cleft [(9)]. In spite of being an important antidepressant, fluoxetine may induce several unwanted effects, including anxiety, sexual dysfunction, insomnia, and GI problems [(10)].

While amitriptyline is a (TCA), used in the treatment of several psychiatric disorders, including major depression, obsessive compulsive, panic attacks, generalized anxiety, post-traumatic stress and bulimia, in addition to its different off-label uses, including migraine prevention, neuropathic pain management, fibromyalgia, and enuresis [(11)]. It is known to inhibit the presynaptic reuptake of serotonin (5-HT) and norepinephrine (NE) and thus increase the concentrations of both neurotransmitters at the synaptic cleft [(12)]. Some of the side effects for amitriptyline include anticholinergic effects such as constipation, dizziness, dry mouth, blurred vision and urinary retention, besides weight gain, sexual dysfunction, orthostatic hypotension and cardiotoxicity [(13)(14)].

Unfortunately, several studies showed that the fluoxetine or amitriptyline administration *in vivo* was accompanied by cytotoxic and genotoxic effects, evidenced by DNA fragmentations, sister-chromatid exchanges and chromosomal aberrations [(15 - 18)].

As long as the criteria for genotoxicity assessment suggests that no single assay can fully detect all genotoxic aspects [(19)]. Thus, combining the *in vivo* comet and micronucleus (MN) assays in the present investigation has been considered to be a valuable methodology for evaluating genetic damage, since the Comet assay can determine the short-lived DNA damage, while the MN assay detects the structural and numerical chromosomal damage [(20)]. Moreover, the antidepressants are medications that can be consumed regularly for 6 months or more, with a potential recurrence of the treatment [(21)]. Therefore, the aim of this study was to investigate the possible genotoxic effects of the commonly used antidepressants (fluoxetine and amitriptyline) in adult male rats.

Materials and Methods

Chemicals and drugs

Fluoxetine and amitriptyline as hydrochloride powders were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. All other chemicals used were of analytical grade.

Preparations of drugs treatment solutions

Fluoxetine and amitriptyline hydrochloride solutions were freshly-prepared every day by dissolving the required amount of each of drug powder in sterile distilled water to get a final concentration (7.2 mg/kg and 27 mg/kg B.wt per day) of fluoxetine and amitriptyline, respectively. The doses of fluoxetine and amitriptyline were calculated by extrapolating the human recommended maximum therapeutic doses to rat doses, according to the conversion table of Paget and Barnes [(22)].

Experimental animals

The study was performed on 24 healthy experimental Swiss Albino adult male rats, weighing (200-300 g), in accordance with the guidelines of the Biochemical and Research Ethical Committee; and approved by the Scientific Committee at the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. The animals were supplied by and kept in the Animal House of the College of Pharmacy, University of Baghdad – Iraq. All animals were housed within plastic cages and maintained under standard laboratory conditions at temperature 22-24°C under a 12-h light/dark cycle, and offered free access to food (commercial rat pellets) and water *ad libitum*. After 3 days of acclimation, experimental rats were randomly allocated into three groups of 8 rats each, as follows: **Group 1**: Rats orally-

administered distilled water (DW) daily via gavage tube for four weeks. This group served as a negative control. **Group 2:** Rats orally-administered a maximum therapeutic dose of fluoxetine hydrochloride solution (7.2mg/kg/day) via gavage tube for four weeks. **Group3**: Rats orally-administered a maximum therapeutic dose of amitriptyline hydrochloride solution (27mg/kg/day) via gavage tube for four weeks. After 24 hrs. of the end of the treatment duration (i.e. at day 29), rats were euthanized by diethyl ether and sacrificed by cervical dislocation. Livers and testes were excised, weighed and washed with normal saline 0.9%. The bone marrow samples were aspirated from the femur bone. A small piece of liver about 2 grams, the left testis and the bone marrow aspirate were preserved in chilled phosphate buffer saline (1X PBS) and kept frozen until further analysis.

Alkaline comet assay (single cell gel electrophoresis assay)

The comet assay (or a single cell Gel Electrophoresis) is a highly sensitive (accurate and reliable) method to detect low levels of DNA damage. The alkaline comet assay is the most commonly used version and widely accepted to detect a wide variety of DNA lesions such as single and double-strand breaks. Under an electrophoretic field, damaged cellular DNA is separated from intact DNA, yielding a classic "comet tail" shape under the microscope [23]. The alkaline comet assay was performed by using a commercial OxiSelect™ comet assay kit (Cell Biolabs, Inc., USA) for detecting DNA damage in individual cells, according to the method described by Singh *et al* (1988) [24] with modifications.

The DNA damage was manually quantified according to the method described by Collins *et al* (1995) [25]. One hundred cells (comets) selected at random from each slide were scored visually into 4 categories according to tail intensity (the extent of DNA migration), given a value from (0 to 3) as follows, 0 = no damage (no visible tail); 1= low level damage (short tail); 2= medium level damage (an obvious tail); 3= high level damage (head of a comet very small with long diffused tail). Thus, the total comet score (TCS) for 100 comets could range from 0 (all undamaged) to 300 (all maximally damaged) as arbitrary units [26]. The parameter "total comet score" (TCS) was calculated according to this formula [27]:

(Percentage of cells in class 0) × 0 + (percentage of cells in class 1) × 1 + (percentage of cells in class 2) × 2 + (percentage of cells in class 3) × 3.

Micronucleus assay (MN)

Micronucleus assay as an index of cytogenetic damage has been widely used to evaluate *in vivo* genotoxicity, evidenced by an increase in the frequency of micronucleated polychromatic erythrocyte (MNPCE) as a reflection of induced structural and/or numerical chromosomal damage [28].

The *in vivo* micronucleus assay was done according to the method described by Schmid (1976) [29] with slight modifications. The femur bone was taken and cleaned from the adhering tissues and muscles. After cutting both ends, the femur gapped from the middle with forceps in a vertical position over the edge of a test tube. By a sterile syringe (1-2ml) of PBS was injected in the bone cavity, to flush out and drop the bone marrow in the test tube. Then 1ml fresh fetal bovine serum was added into each test tube. The test tubes were centrifuged at speed of 1000 rpm for (5min). The supernatant was removed, and the cells were resuspended with (2ml) fetal bovine serum. Again the test tubes were centrifuged at speed of 1000 rpm for (5min). The supernatant was removed, and the cells pellet was resuspended with (170 µL) fetal bovine serum. A small amount of cells suspension was dropped on the end of microscopic slide to make a smear. The slides were kept at room temperature allowed to air dry for 24 hours. The slides were fixed with absolute methanol for 5min, then stained with Giemsa stain for 15min and then washed with distilled water and left to dry. The slide was examined under oil immersion lens (100X), two slides for each animal were prepared for micronucleus test.

A total of 1000 cells (including the polychromatic erythrocytes PCE and normochromatic erythrocyte NCE) were randomly examined for the formation of micronuclei, and the micronucleus index was calculated using the following equation [30]:

Micronucleus Index % =

$$\left(\frac{Number\ of\ (MNPCE)}{Total\ Count\ of\ (PCE+NCE)}\right) x\ 100$$

MNPCE: micro-nucleated polychromatic erythrocytes; **PCE**:polychromatic erythrocytes; **NCE**: normochromatic erythrocyte.

Results and Discussion

Statistical analysis of data was performed using SAS (Statistical Analysis System-version 9.1). Descriptive statistics for the numerical data were formulated as mean and standard deviation (mean ±SD). One way and two ways Analysis of Variance (ANOVA) and Least significant difference post-hoc test were used to assess the significant differences among groups. $P< 0.05$ is considered as statistically significant [31].

Comet assay in the hepatic and testicular tissue homogenate.

The results in (Table 1) and (Figure 1) demonstrate the score means in both tissues (liver and testis) among the three groups (fluoxetine, amitriptyline, and control). The analysis of data with a two-way

ANOVA test revealed that the comet score in liver and testis was significantly higher ($P<0.05$) in animals treated with fluoxetine and amitriptyline each compared to the control animals. On the other hand, there was a non-significant difference ($P>0.05$) in comet score between fluoxetine and amitriptyline-treated animals.

The DNA damage was quantified by measuring the total comet score (TCS) as seen in (Figure 2); where the extent of DNA damage was evaluated by visual scoring, and the comets were classified and assigned to four classes: (A) No damage (spheres with no visible tail); (B) Low damage (short tail); (C) Medium damage (an obvious tail); (D) High damage (small head of comet with long diffused tail).

Firstly, concerning fluoxetine, there were no previous *in vivo* studies that have been addressed the evaluation of fluoxetine-induced hepatic genotoxicity by comet assay; except few published articles regarding the genotoxicity of fluoxetine in liver. Thus, results of the current study could be interpreted in view of the research of Djordjevic *et al* (2011) [32], who showed an increase in DNA fragmentation accompanied by significant up-regulation of apoptotic Bax and down-regulation of antiapoptotic Bcl-2 proteins, obviously seen in hepatocytes undergoing apoptosis after 21-day period in fluoxetine-treated rats; and authors attributed their findings as a consequence of oxidative stress generation caused by the free radicals formation, which is a well-known molecular event in the activation of mitochondrial pathway of apoptosis.

Similar findings were recently reported in the study of Elgebaly *et al* (2018) [33], who conclude that olive oil and leaf extract prevented fluoxetine-induced apoptosis in the liver of rats as evidenced by decreased expression of apoptotic Bax and caspase-3, and up-regulated expression of antiapoptotic Bcl-2 proteins.

Addressing this problem, it is important to highlight the study of Souza *et al*. (1994) [34], who found that fluoxetine and its metabolite, norfluoxetine potentially exerted toxic impacts on energy metabolism in rats' liver mitochondria at high doses. Authors described that these effects seem to be a consequence of the solubilization of the drug and/or its metabolites in the inner mitochondrial membrane.

The present study demonstrated that fluoxetine exerted a pronounced DNA damage in testicular tissues (**Group 2**) compared to the negative control (**Group 1**) rats, as represented by comet scores in (Table 1) and (Figure 1).

Testicular or germ cells are important target in reproductive toxicology, which seems to be an easier and logical choice for DNA damage assessment and reproductive genotoxicity research by comet assay [35]; where, a recent study by Câmara *et al* (2019) [36] demonstrated that the effect of short-term treatment with fluoxetine on the adult rat testes caused a significant increase of ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) isoenzyme in the damaged seminiferous tubules associated with high incidence of cell death, since the ubiquitination minimizes DNA damage when spermatogonia are exposed to stress. The authors described that such isoenzyme seems to control spermatogenesis, as well as it involved in the molecular regulation of germ cells apoptosis.

In another study, Alzahrani (2012) [15] reported that a dose-dependent effect produced by fluoxetine administration for 5 days to mice showed a significant increase in sperm shape abnormalities and a significant decrease in both sperm motility and count in male mice.

Several explanations have been proposed for the testicular DNA damage induced by fluoxetine; where, researchers have reported that morphological abnormalities of sperm may be a marker of genetic mutations and a reflection of sperm DNA damage arising during spermatogenesis [37][38]; and these studies supporting the previously mentioned findings of Alzahrani (2012) [15], which is consistent with the results of the present work.

While other authors attributed such testicular genetic damage that mediated by fluoxetine to serotonin's capability of causing DNA strand cleavage, as a result of the elevated level of 5-HT during SSRI treatment, through an oxidative mechanism in the presence of cupric ions (Cu^{+2}), which can be reduced to cuprous ion (Cu^{+1}) by 5-HT with subsequent generation of ROS, such as the hydroxyl radical **(˙OH)**. Since copper is an essential component of chromatin; and the formation of a ternary complex of (serotonin-Cu^{+2}-DNA) was proposed to be the probable mechanism of DNA damage with 5-HT [39].

In contrast, Bendele *et al* (1992) [40] concluded that, fluoxetine is neither a complete carcinogen nor a tumor promoter after a long-term carcinogenicity study in rats and mice. In such study, fluoxetine was administered to the animals for 24 months at dietary doses of 0.5 to 10.0 mg/Kg B.wt in rats and 1.0 to 10.0 mg/Kg in mice, via continuously available mash diet. The authors examined multiple organs, among them liver and testes, and there was no evidence of an increased incidence of any type of neoplasm in either rats or mice.

Concerning amitriptyline, in the current study, the comet assay successfully detected the genetic damage induced by such drug in liver and testis tissues; where, amitriptyline (**Group 3**) caused a significant increase ($P<0.05$) in DNA fragmentation detected by comet assay in liver tissues compared to the negative control (**Group 1**) rats, as represented by comet scores in (Table 1) and (Figure 1).

Up to date, there are no previous *in vivo* studies that have been addressed the evaluation of amitriptyline-

induced hepatic genotoxicity by comet assay; except few articles were published regarding the genotoxicity of amitriptyline in such organ. Thus, results of the current study could be interpreted in view of the *in vitro* study of Taziki *et al* (2015) [41], who showed that amitriptyline-induced hepatotoxicity was associated with mitochondrial membrane potential collapse in isolated rat hepatocytes. The authors attributed their findings as a consequence of mitochondrial depolarization targeted by amitriptyline, which can lead to energy crisis and releasing of apoptotic signaling molecules, then progressively to cell death.

Similar findings were reported in the *in vitro* study of Villanueva-Paz *et al* (2016) [42], who found that amitriptyline-induced mitochondria dysfunction and oxidative stress that precedes apoptosis in human hepatic cancer cell line (HepG2), which provide some assurance about amitriptyline cytotoxicity.

In addition, a compendium of reports about DNA intercalative potential and genotoxicity assays performed on marketed drugs, among them amitriptyline, have been discussed by Snyder *et al* (2006) [43], who concluded that positive *in vitro* cytogenetics findings for amitriptyline might likely to be due to DNA intercalation (DNA groove-binding).

Researchers reported that the testicular genotoxicity, is an essential safety endpoint and a challenging issue in drug development and risk assessment [44]. The present study demonstrated that amitriptyline (**Group 3**) exerted a pronounced DNA damage in testicular tissues compared to the negative control (**Group 1**) rats, as represented by comet scores in (Table 1) and (Figure 1).

In agreement with these findings, Hassanane *et al* (2012) [17] have showed that the dose-dependent effect produced by the orally-administered amitriptyline-induced structural and numerical chromosomal abnormalities with a significant decrease in both sperm motility and count in germ cells (spermatocytes) of mice. Authors added that the sperm-head abnormalities shown in that study could be considered as a reflection of DNA content alteration caused by amitriptyline treatment.

Another study by Tousson *et al* (2018) [45], who demonstrated that amitriptyline-induced testicular tissue damage was associated with sperm morphological abnormalities and a significant expression of *P53* protein in the testis and epididymis of rats.

The *p53* protein was described as "the guardian of the genome", referring to its role in preserving genetic material stability. It has been well - documented that DNA damage or other cellular stress signals may trigger the expression of *p53* proteins, which have three major functions: growth arrest, DNA repair and apoptosis (cell death) induction [46].

Moreover, similar findings were reported in the study of Chowdary and Rao (1987) [18], who examined the cytogenetic impact of amitriptyline in germ cells of mice. Authors found that the orally given-amitriptyline also showed a highly significant number of chromosomal aberrations in spermatocytes at meiotic metaphase, and suggested that such genetic damage could be extended up to 3 generations.

In addition, the comet score in animals treated with fluoxetine was significantly higher ($P<0.05$) in liver tissues than in testis. On the other hand, there was a non-significant difference ($P>0.05$) in comet score between liver and testis tissues in amitriptyline-treated animals, as seen in (Table 1) and (Figure 1).

Varying degrees of DNA damage induced by fluoxetine was expected between liver and testis, because such differential tissue damage can give a clear explanation about enantio- and stereoselective aspects of fluoxetine, since fluoxetine has a chiral carbon center in its structure, and as a result, it exists as a racemic mixture with two enantiomeric forms as (S)-fluoxetine and (R)-fluoxetine [8]. Similarly, norfluoxetine, the main metabolite of fluoxetine, also exists in two enantiomeric forms as (S)-norfluoxetine and (R)-norfluoxetine, and the metabolism of both fluoxetine and norfluoxetine is stereoselectively catalyzed [47].

It has been well-documented that chiral medications can differ in their biological actions, potency and toxicity, since they undergo stereoselective mechanisms controlling their pharmacokinetic and pharmacodynamics properties, such as distribution, metabolism and excretion, as these processes usually favor one enantiomer over the other, due to stereoselective interactions of enantiomers with active biological systems [48].

Unfortunately, the enantioselective aspects of fluoxetine in animals have still not been thoroughly investigated, despite the evidence of stereoselective disposition of fluoxetine isomers that have been observed in humans and sheep [49][50].

Furthermore, it has been reported that the accumulative dosing of fluoxetine results in fluctuated blood levels and pharmacokinetics of the parent drug and its metabolite, than acute dosing, since fluoxetine and norfluoxetine can inhibit their own metabolism through interactions with the cytochrome P450 liver enzymes [51].

Micronucleus (MN) Formation in bone marrow (BM) samples.

The mean values of micronucleated polychromatic erythrocytes were shown in (Table 2) and (Figure 3); where, there was a significant increase ($P<0.05$) in the frequencies of MN formation in animals' bone marrows treated with fluoxetine and amitriptyline each compared to the control animals; while, there was a non-significant difference ($P>0.05$) in MN formation frequencies

between the two drugs as shown in (Table 2), (Figure 3), and (Figure 4).

Firstly, concerning fluoxetine, the present findings are in accordance with the results gathered from Alzahrani (2012) [15], who also examined sister-chromatid exchanges in BM cells of mice treated with fluoxetine for 5 consecutive days. The author reported that the highest tested dose of fluoxetine showed about two times increase in sister-chromatid exchanges than control levels.

In contrast, Düsman *et al* (2014) [52] demonstrated that orally- administered fluoxetine at doses of 0.5 to 2.0 mg/100 g B.wt./day failed to show any sister-chromatid exchanges in BM of Wistar rats after 7 days of treatment.

While for amitriptyline, The present findings are in accordance with the results gathered from Hassanane *et al* (2012) [17], who also reported that the highest tested dose of amitriptyline-induced significant chromosomal aberrations with a marked decline in both mitotic index and meiotic activity in BM cells of mice. Authors concluded that amitriptyline could interact with spindle fibers, as evidenced by the disruption of the centromeric apparatus during mitosis that has been observed in their results.

In agreement with these findings, Chowdary and Rao (1987) [18] have also revealed that amitriptyline significantly increased the frequency of micronuclei formation in BM cells of mice. Authors indicated that such chromosomal damage during late S and early G1 phases of the cell cycle might be due to the clastogenic and/or spindle disruption effects of the drug.

In contrast, an *in vitro* study by Saxena and Ahuja (1988) [53] was performed to evaluate amitriptyline and imipramine genotoxicity on cultured human lymphocytes; where, authors concluded that amitriptyline was non-genotoxic but such drug caused chromosomal aberrations and sister chromatid exchanges at concentrations significantly greater than those attained under normal therapy in humans.

In support of these facts, it seems that the chemical structure of the antidepressants plays a role in their genotoxic and carcinogenic potentials. Brambilla *et al* (2007) [54] reported that fluoxetine and amitriptyline are two of the nitrosatable drugs due to the presence of amine group in their structures, which by reacting with nitrite in the gastric environment, or even in other sites, can give arise to the formation of N-nitroso compounds or other reactive species; where, authors mentioned that the N-nitroso compounds have been found to produce genotoxic effects and to cause tumor development in laboratory animals. Furthermore, authors added that the exposure to the genotoxic-carcinogenic drug nitrosation products might be of great risk that required a concomitant consumption of antioxidants such as ascorbic acid.

Table 1. Comet score values in liver and testis tissues of rats.

Groups	Liver mean Comet Score	Testis mean Comet Score
Control	65.30±0.45 ^{bA}	61.18±2.30 ^{bA}
Fluoxetine	96.52±3.01 ^{aA}	90.91±3.94 ^{aB}
Amitriptyline	95.76±6.33 ^{aA}	91.48±4.31 ^{aA}

- Data are expressed as (mean ± SD); n=8 animals in each group;
- Means with a different small letters superscripts (a, b) in the same column are significantly different (P<0.05);
- Means with a different capital letters superscripts (A, B) in the same row are significantly different (P<0.05).

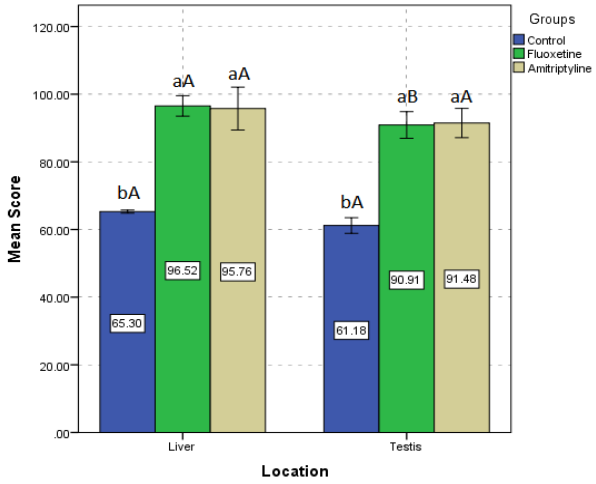

Figure 1 .Histogram of comet score values (mean ± SD) in liver and testis tissues. Mean values with different small letters are significantly different (P<0.05) among groups. Mean values with different capital letters are significantly different (P<0.05) among tissues.

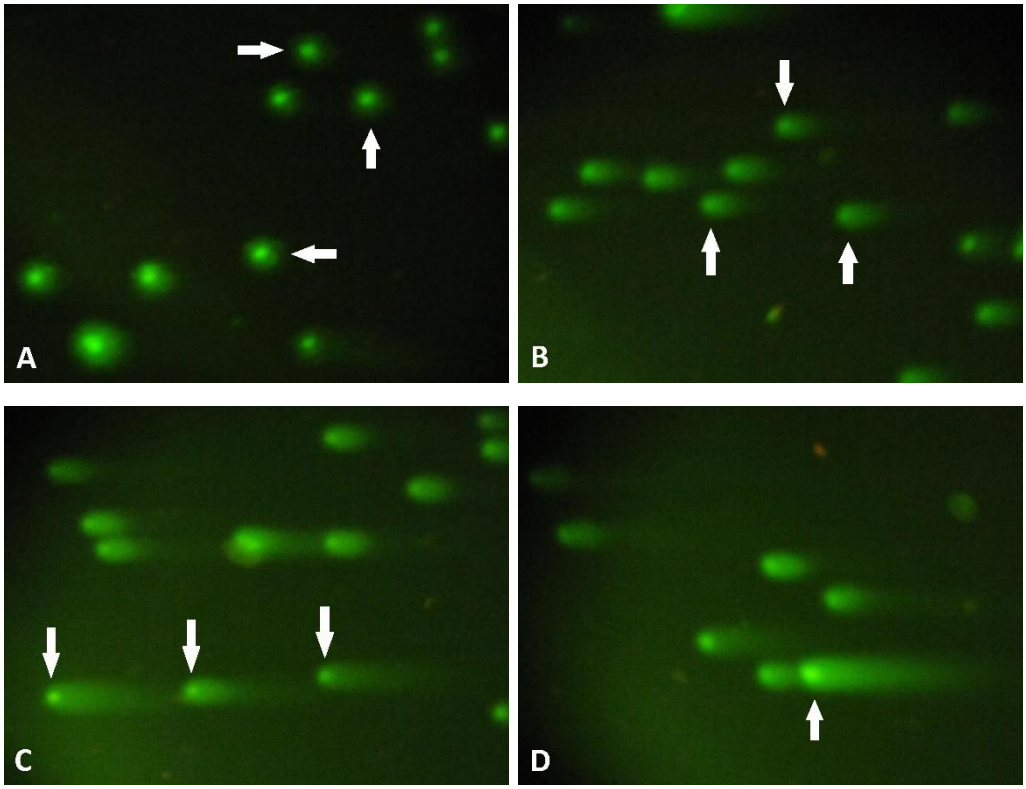

Figure 2. Classes of DNA damage as detected by the comet assay in liver and testis tissues of treated animals (fluoxetine- and amitriptyline-treated groups) examined by florescent microscope (400X). (A) No damage (spheres with no visible tail); (B) Low damage (short tail); (C) Medium damage (an obvious tail); (D) High damage (small head of comet with long diffused tail).

Table 2. Frequencies of micronucleated polychromatic erythrocytes in bone marrow of rats.

Groups	MN%
Control	2.36±0.36 [b]
Fluoxetine	4.94±0.54 [a]
Amitriptyline	4.75±0.62 [a]

- Data are expressed as (mean ± SD); n=8 animals in each group;
- Means with a different small letters superscripts (a, b) in the same column are significantly different (P<0.05).

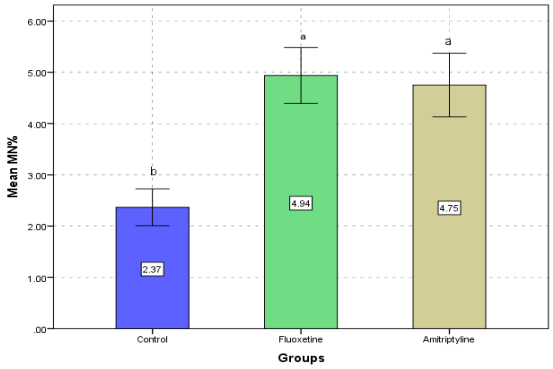

Figure 3. Histogram showing the Frequencies (mean ± SD) of micronucleated polychromatic erythrocytes in bone marrow. Mean values with different small letters are significantly different (P<0.05) among groups.

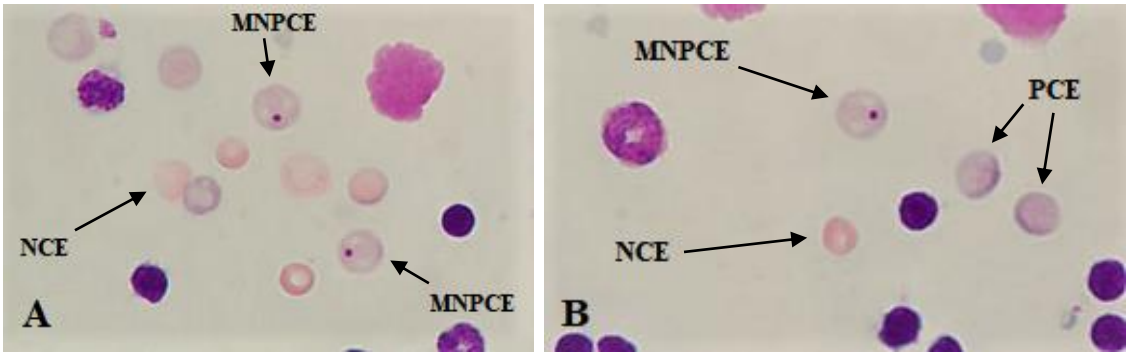

Figure 4. Bone marrow smears of rats treated with fluoxetine and amitriptyline (A and B), respectively; showing micronucleus induction as well as enucleated cells. **PCE: Polychromatic erythrocytes, NCE: Normochromatic erythrocyte, MNPCE: Micronucleated polychromatic erythrocyte.**

Conclusion

The present study concludes that fluoxetine and amitriptyline have genotoxic potentials and can induce the same extent of cytogenetic damage in liver, testis and bone marrow tissues of adult male rats, as evidenced by DNA fragmentations and induction of micronuclei assessed by comet and micronucleus assays.

Therefore, both drugs must be prescribed under careful medical supervision, and a concomitant administration of suitable exogenous antioxidant agent is recommended to minimize the risks of their toxicities by enhancing the antioxidant defenses system. Further studies should be performed on toxicities of fluoxetine and amitriptyline at different doses with longer treatment periods, to determine their safe doses and durations. More light needed to be shed on the exact molecular mechanisms behind their genotoxic potentials.

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