AIMS Medical Science

AIMS Medical Science, 10(1): 69–79.
DOI: 10.3934/medsci.2023007
Received: 27 July 2022
Revised: 01 March 2023
Accepted: 12 March 2023
Published: 30 March 2023

http://www.aimspress.com/journal/medicalScience

Research article

Fluoxetine induces oxidative stress-dependent DNA damage in human hepatoma cells

Running title: Fluoxetine-induced DNA damage in HepG2 cells

Somayeh Boshtam[1], Mohammad Shokrzadeh[2,*] and Nasrin Ghassemi-Barghi[1]

[1] Student Research Committee, Pharmacy School, Mazandaran University of Medical Sciences, Sari, Iran
[2] Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences, Sari, Iran

* **Correspondence:** Email: m.shokrzadeh@mazums.ac.ir, mslamuk@yahoo.com; Tel: +989111263448

Abstract: Fluoxetine is a selective serotonin reuptake inhibitor that is a commonly used drug for the treatment of depression and obsessive-compulsive disorders. Despite the positive effects of this drug, it seems to be associated with various side effects. Genotoxicity or DNA damage is an important side effect of some kinds of drugs. To date, the genotoxicity and cytotoxicity of fluoxetine are partially unknown. In the present study, some oxidative stress methods were used, such as ROS, MDA and GSH evaluation methods in HepG2 cells treated with fluoxetine (1–10 μM). A comet assay was used to evaluate the genotoxic effects of fluoxetine, and flow cytometry was used for apoptosis detection in these hepatic cells. Our data have shown that fluoxetine increased MDA and intracellular concentration of ROS significantly ($P < 0.001$), while the amount of GSH was reduced significantly ($P < 0.001$). Our results also indicated that fluoxetine increased the DNA damage of HepG2 cells. The tail percentage of DNA for control cells was 4%, but this percentage was 19%, 28% and 32% for 1, 5 and 10 μM of fluoxetine concentration, respectively ($P < 0.01$ and $P < 0.001$). The flow cytometry results have also shown increases in early and late apoptosis for fluoxetine (13.31% and 9.54%, respectively). In conclusion, the present study has shown that fluoxetine is able to induce oxidative stress-dependent DNA damage. Anyway, more studies are needed to accurately explore the molecular and cellular aspects of fluoxetine.

Keywords: fluoxetine; genotoxicity; DNA damage; depression; ROS; apoptosis

1. Introduction

Depression is a common problem and disease in many developed countries, and it is related to lifestyle [1]. There are many therapeutics which have been introduced up to now for some types of depression disorders, such as the tricyclic anti-depressants (TCAs) imipiramine and amtryptiline, which are old drugs with some adverse effects, and selective serotonin reuptake inhibitors (SSRIs), which are newer than TCAs, with fewer adverse effects and better results for anxiety disorders [1]. Monoamine oxidase inhibitors such as tranylcypromine, which is used for some cases of major depression (MD); serotonin-norepinephrine reuptake inhibitors such as venlafaxine, which has almost the same application as SSRIs, with some differences; and some also herbal medicines, which have been applied for depression [1]. Fluoxetine is the most common SSRI, and it serves as the first-line drug for the treatment of MD. MD seems to be a major challenge in medical practice also it can be a social and economic challenge [1]. Due to the high prevalence of MD in human society (10–15% of the population worldwide), fluoxetine is one of the most prescribed drugs [2]. However, its effectiveness is different in different patients with MD. For instance, it has been reported that 60–70% of patients do not experience remission after fluoxetine treatment. However, this type of medication does not show a significant response in 30–40% of patients [3,4].

It is generally accepted that SSRIs, and particularly, fluoxetine, are associated with a large spectrum of side effects. For instance, it has been suggested that, in some cases, fluoxetine treatment causes blurred vision and increased pupil dilation with unknown mechanisms of action [5]. In addition, prolonged treatment with fluoxetine may also increase suicidal tendencies [6]. Moreover, other related studies reported that fluoxetine has unfavorable side effects on the gastrointestinal and central nervous systems [7].

Genotoxicity is generally defined as any type of damage to the whole genome of the organism. Genotoxicity and oxidative stress may affect the regulation and normal activity of cells, contributing to a wide variety of disorders such as malignancy, as well as neurodegenerative diseases [8,9]. For instance, it has been shown that patients who took genotoxic drugs have a higher prevalence of some cancers. A growing body of studies is in support of the ability of genotoxic agents to induce DNA damage through the excess accumulation of reactive oxygen species, i.e., oxidative stress [10]. In oxidative stress circumstances, biological molecules such as DNA, proteins and lipids are damaged. Reactive oxygen species (ROS) generally affects DNA to break its strands. Prolonged exposure of DNA to ROS leads to a double-strand break and DNA lesion. DNA damage, as one of the most dangerous events of oxidative stress, is detected by sensor proteins such as the MRE11–RAD50–NBS1 complex that transmits the information to some signaling cascade, which eventually induces apoptosis. Lipid oxidation results in the accumulation of malondialdehyde (MDA). Therefore, MDA is served as a biomarker of oxidative stress-induced lipid damage that eventually induces apoptosis [11]. Biological defense against oxidative stress involves increasing of some peptides and proteins as glutathione (GSH) which appears to be increased during oxidative stress.

There are currently few studies on the genotoxicity of fluoxetine in humans, and most of them show inconclusive results. The current study was designed to determine the cytotoxicity and genotoxicity of fluoxetine in HepG2 cells.

2. Materials and methods

2.1. Cell cultures

The human hepatoma cell line (HepG2) was provided by Pasture, Iran. These cells were cultured and incubated in DMEM (Bioidea, Iran) supplemented with 50 mg/L of Pen-strept and 10% fetal bovine serum (Invitrogen, Massachusetts, USA). The cells were also maintained in a humidified incubator containing 5% CO_2 at 37 ℃. The culture medium was changed every 48 h, and the cells were also subcultured when their confluence reached 80%.

2.2. DNA damage assessment (comet assay)

The single-cell gel electrophoresis was performed according to the previous study [12]. Briefly, 50,000 cells in each well were cultured for 24 h, followed by treatment with different concentrations of fluoxetine. After 24 h of incubation, the HepG2 cells were harvested by centrifugation at 1,200 rpm at 4 ℃ for 5 min; then they were re-suspended again one time. The cell pellets were finally re-suspended in phosphate buffer saline in a cold room for the comet assay. The samples which showed cell viability of more than 70% were further processed for the comet assay. To achieve this goal, 20,000 cells were mixed with 80 µl of 0.5% low-melting-point agarose and added to a glass slide precoated with normal agarose (1%). The samples were then covered by 100-µl low-melting-point agarose, followed by solidification of the gel. The slides were then immersed in lysing solution for 10 h at 4 ℃. Horizontal gel electrophoresis was performed by using a fresh cold alkaline electrophoresis buffer. Electrophoresis was performed at 4 ℃ for 20 min at 15 V (0.8 V/cm) and 300 mA. The slides were then neutralized with 0.4 M Tris buffer at pH 7.5, followed by staining them with ethidium bromide. Each slide was prepared in triplicate and 100 cells per slide were scored randomly and analyzed by using an image analyzing method (Komet 5.0, Kinetic Imaging, Liverpool, UK). The parameters, such as the DNA tail percentage (% tail DNA = 100 − % head DNA), tail length and tail moment were selected for DNA damage assessment by using Comet software (Kinetic Imaging, Liverpool, UK).

2.3. Intracellular ROS determination

The ROS concentration was evaluated by using 20, 70-dichlorfluorescein-diacetate (DCFH-DA) similar to a previous study [13]. In this study, the cells were treated with different concentrations of fluoxetine, and after the indicated incubation time, 10 mM of DCFH-DA was added to each sample, followed by incubation at 37 ℃ for 1 h. The samples were then washed with Phosphate buffer saline (PBS) and their fluorescence intensity was measured via fluorescence spectroscopy (excitation at 485 nm and emission at 530 nm).

2.4. Intracellular GSH levels assessment

The treated and untreated cells were incubated with monochlorobimane (mBCI, 40 µM) in a staining solution containing 5 mM glucose, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$ and 5 mg/ml Bovine serum albumin (BSA) for 30 min at 37 ℃ under dark conditions. mBCI is a nonfluorescent probe,

but it converts to a stable fluorescent adduct with GSH catalyzing the GSH S-transferases. The fluorescent intensity of samples was evaluated at λ = 380 nm for excitation and λ = 460 nm for emission. The fluorescent intensity was calculated as a fold change of control [14].

2.5. Lipid peroxidation assessment

Lipid peroxidation was evaluated according to the spectrophotometric measurement of the product of the reaction of thiobarbituric acid (TBA) and MDA [13]. Briefly, after the indicated treatment, the cells were mixed with 0.5 ml of tricholoroacetic acid (10%, w/v) solution, followed by heating on in a boiling water bath for 20 min. The cells were harvested, and then 1 ml of TBA solution was added to the samples, followed by heating again in boiling water. Finally, the absorbance of the samples was evaluated at 532 nm and the content of MDA was calculated as a fold change of control.

2.6. Flow cytometry assessment of apoptosis

Apoptosis was evaluated by performing annexin V and Propidium iodid (PI) staining as described previously [15]. The cells were cultured at a density of 3×10^5 per well in a six-well plate and incubated with different indicated concentrations of fluoxetine. The cells were washed twice with PBS and stained for 15 min at room temperature with annexin V-FITC and PI. The positive cells for each strain were measured by using the FACS Calibur flow cytometer (Tristar, CA, USA). In flow cytometry analysis, the quadrant quantification is an important issue. Early apoptotic/primary apoptotic cells were annexin V-positive and PI-negative. Late apoptotic cells were determined as both annexin V- and PI-positive; finally, necrotic cells were annexin V-negative and PI-positive. The analysis was performed by using Flow Jo software version 7.6.1 (Tristar, CA, USA).

2.7. Statistical analysis

The tail moment, tail length and percent of DNA in the tail are commonly used in DNA damage assessment. Here, we used these parameters for statistical analysis. One-way analysis of variance (ANOVA) and Tukey's multiple-comparison post hoc tests were done to compare the results of all assays. A P value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Fluoxetine induces genotoxicity

Genotoxicity was measured as the percent tail of DNA and olive tail in treated and untreated cells. The cells were exposed to various concentrations of fluoxetine. As indicated in Figure 1, increasing DNA damage was observed in the HepG2 cells in a dose-dependent manner. The tail percent of DNA for untreated cells was 4%; however, this percentage was 19%, 28% and 32% for 1, 5 and 10 μM of fluoxetine, respectively ($P < 0.01$ and $P < 0.001$). Similarly, the tail moment of DNA was 1, 2, 28 and 35 for the incubation of HepG2 cells with 0, 1, 5 and 10 μM of fluoxetine, respectively.

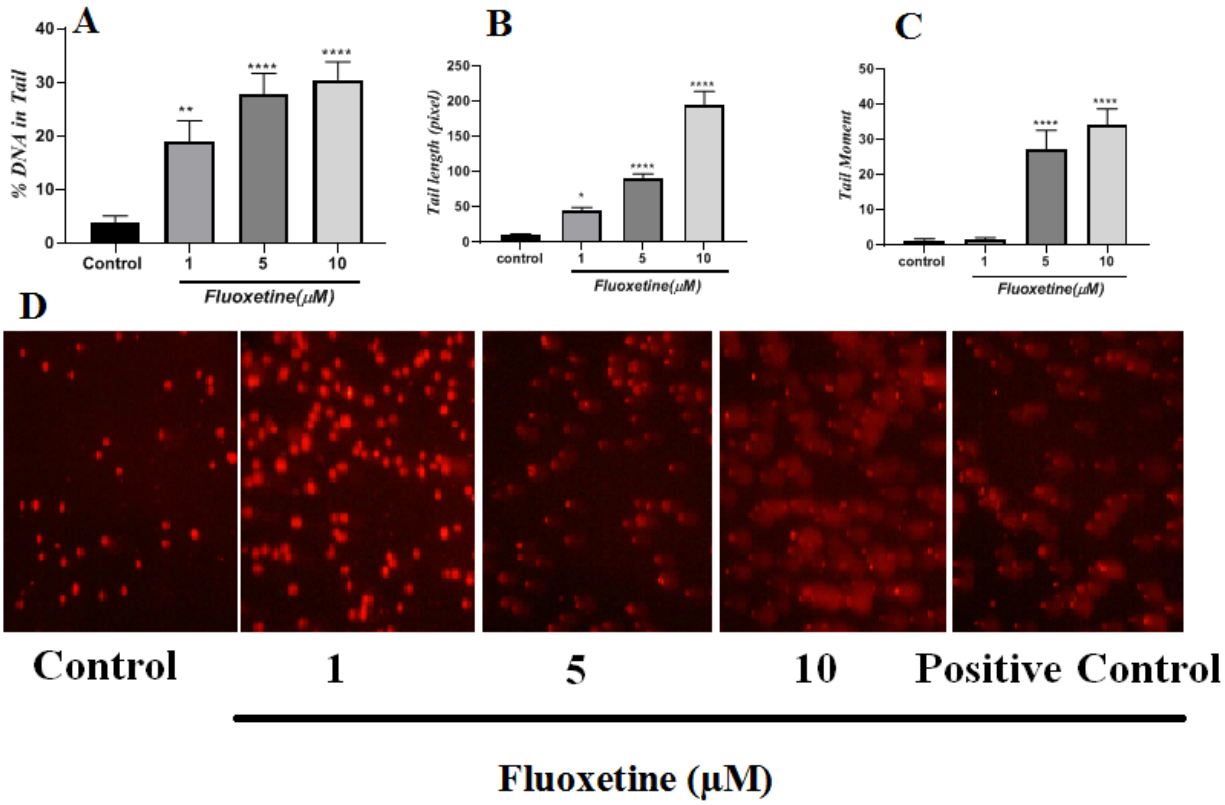

Figure 1. DNA damage in HepG2 cells in response to different concentrations of fluoxetine. A: percentage of tail DNA. B: tail length. C: tail moment. D: representative image of exposed cells. Each value was obtained from three different experiments (mean ± SD). ** and **** were considered as statistically different $P < 0.001$.

3.2. Fluoxetine induces ROS generation

To evaluate the role of ROS in fluoxetine-induced genotoxicity, here, DCFH-DA was used to measure the ROS generation in HepG2 cells in response to fluoxetine stimulation. Incubation with fluoxetine (1–10 μM) for 1 h showed catastrophic increases in oxidant-induced 2', 7'-dichlorofluorescein fluorescence in the HepG2 cells (Figure 2). H_2O_2-mediated fluorescence emission occurred 1 h after incubation with fluoxetine in the HepG2 cells ($P < 0.0001$), suggesting the involvement of oxidative stress in a concentration-dependent manner.

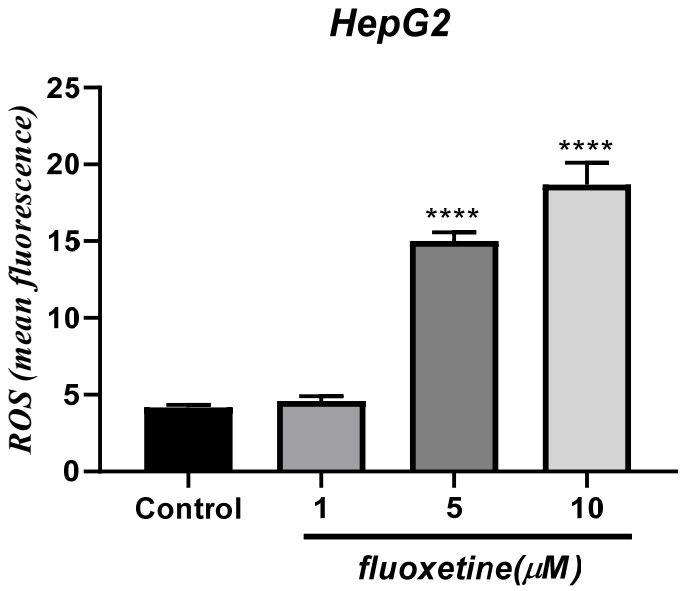

Figure 2. Effect of fluoxetine on ROS generation in HepG2 cells. (****) shows significantly increased results ($P < 0.0001$) as compared to the control group.

3.3. Fluoxetine reduces GSH level

As described in Figure 3, 1 h after treatment with fluoxetine, the intracellular levels of GSH were decreased significantly ($P < 0.001$). This finding was eventually confirmed by an enzymatic assay.

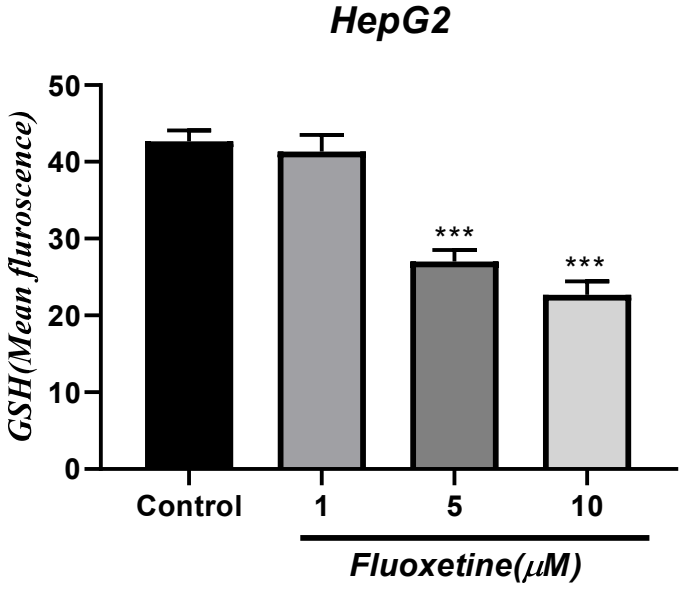

Figure 3. Effect of fluoxetine on the level of intracellular GSH. ANOVA results revealed that fluoxetine significantly decreased the level of GSH. *** stands for statistically significant difference ($P < 0.001$) as compared to the control group.

3.4. Fluoxetine reduces lipid peroxidation

The product of TBA reaction with peroxidized lipid was evaluated. This experiment measures the level of MDA, which is a major product of lipid peroxidation.

As indicated in Figure 4, fluoxetine treatment of HepG2 cells dose-dependently increased the concentration of MDA ($P < 0.0001$) compared to the control group.

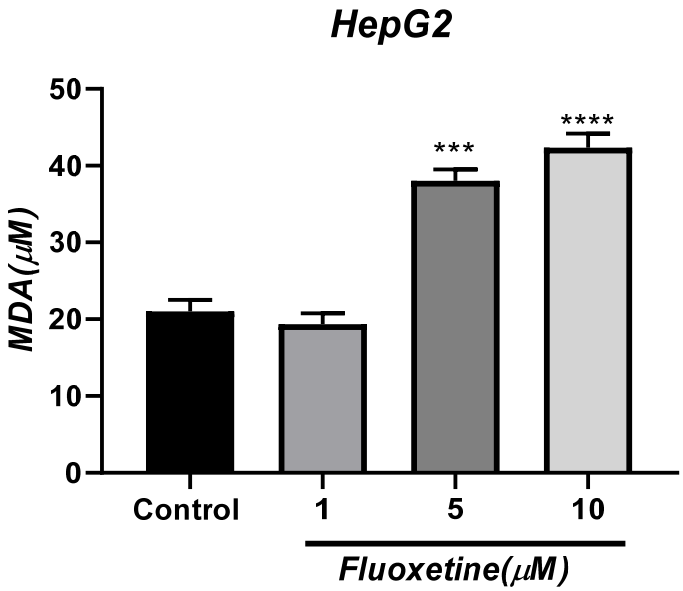

Figure 4. Effect of fluoxetine on MDA levels in HepG2 cells. *** and **** denote statistically significant difference ($P < 0.001$ and $P < 0.0001$, respectively) compared to the control group.

3.5. Fluoxetine induces apoptosis

Due to the morphological change assessment of HepG2 in response to incubation with fluoxetine, flow cytometry analysis was performed. Incubation with three concentrations of fluoxetine (1, 5 and 10 μM) for 24 h resulted in apoptosis induction (Figure 5). As described here, a concentration of 1 μM led to increases in the cells in early and late apoptosis (13.31% and 9.54%, respectively). For the concentration of 5 μM, the percentages of cells in early and late apoptosis were 40.25% and 40.52%, respectively. Finally, for 10 μM, the increases in early and late apoptosis were 25.49% and 48.75%, respectively. The obtained finding confirmed that the death induced by fluoxetine was apoptosis.

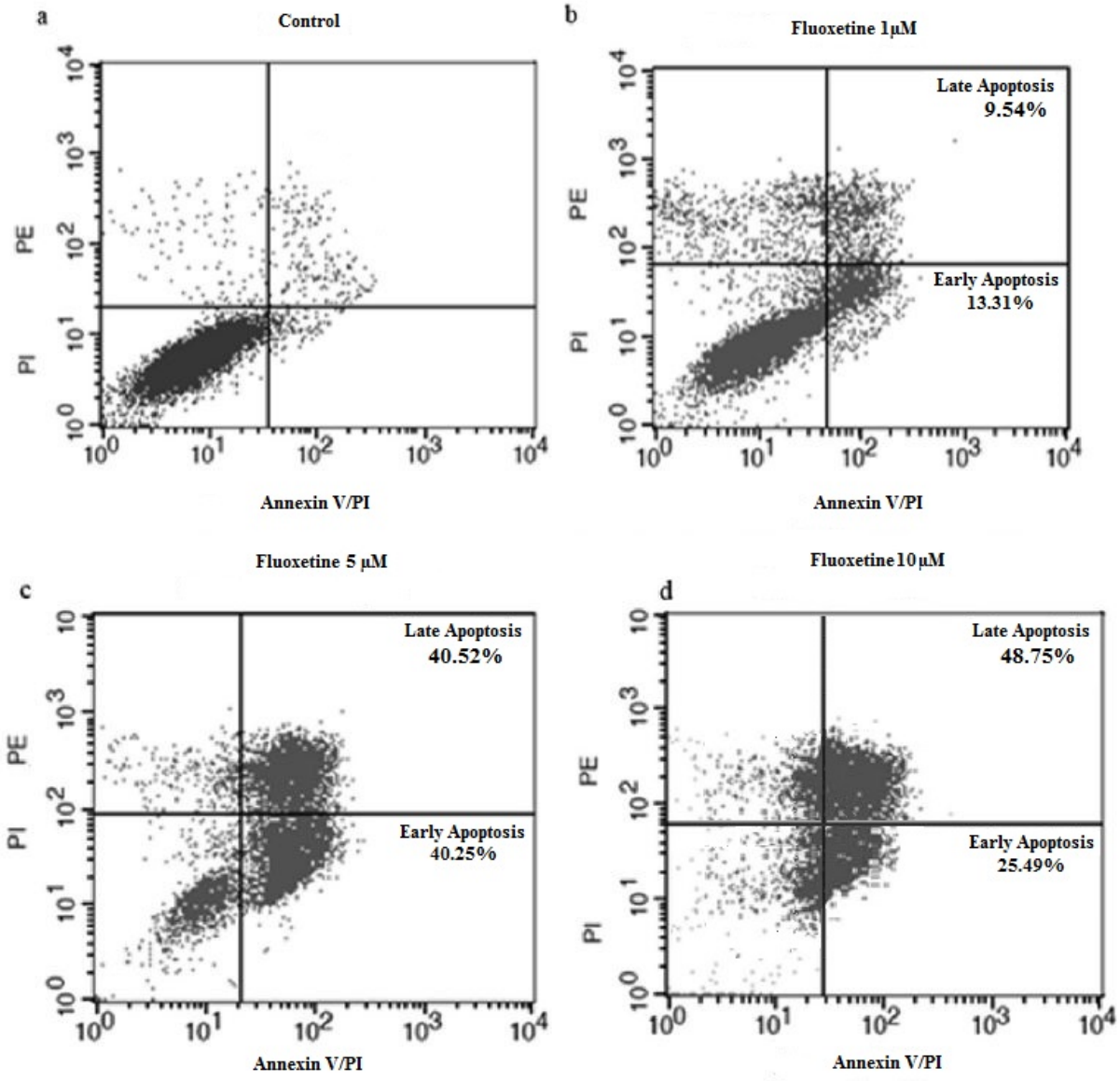

Figure 5. HepG2 cell flow cytometry analysis. (a) Untreated HepG2 cells. (b) HepG2 cells treated with 1 μM of fluoxetine. (c) HepG2 cells treated with 5 μM of fluoxetine. (d) HepG2 cells treated with 10 μM of fluoxetine.

4. Discussion

Fluoxetine is a commonly used pharmaceutical agent purposed to improve the symptoms of some diseases, such as depression, obsessive-compulsive disorder, panic attacks and social phobias. The exact details underlying the fluoxetine mechanism is partially unknown; however, fluoxetine appears to selectively block the reuptake of serotonin in the pre-synaptic space. Although this drug is considered to be safe in adults, some studies indicate that prolonged consumption of fluoxetine may result in endothelial reticulum (ER) stress [16]. Genotoxicity, as one of the main results of ER stress, is a common event in the life of a cell, and it can cause mutation and impaired apoptosis

regulation [17,18]. Disruption of the apoptotic pathway is more likely to be involved in a wide variety of cancers and other diseases [19].

Here, our data clearly indicate that fluoxetine treatment resulted in the accumulation of ROS. Given the available knowledge about oxidative stress, we hypothesized that fluoxetine incubation would lead to oxidative stress-induced cell damage. Therefore, oxidative damage to biological molecules such as lipids and DNA were determined. In this regard, our findings also revealed that MDA contents were increased significantly in response to fluoxetine treatment (in a concentration-dependent manner). A large body of studies has indicated that, under oxidative stress conditions, cells recruit all defense mechanisms. One of the best-studied natural antioxidant systems is GSH [20]. This tripeptide not only acts as an antioxidant, but it is also involved in the metabolic pathway, redox homeostasis and signaling cascades [21]. For instance, it has been indicated that tert-butyl hydroperoxide exposure of HepG2 cells resulted in a reduction of GSH levels [22]. Our results also showed that fluoxetine induces DNA damage in a concentration-dependent manner.

Previously, Zlatković and coworkers showed that chronic administration of fluoxetine (15 mg/kg/day) induces liver injury. They showed that carbonyl content and MDA increased. However, GSH was decreased significantly, suggesting a potential link between drugs and hepatic oxidative stress [23]. In addition, a human study also showed that MDA and superoxide dismutase were increased after 24 weeks of fluoxetine administration in patients with depression [24]. However, another study revealed that treatment with fluoxetine partially reverses the adverse oxidative stress effects [25,26]. On the other hand, with a glance at the literature, Choi and colleagues indicated that fluoxetine induces ER stress and mitochondrial dysfunction [16]. In addition, Bowie et al. also reported that fluoxetine is an inducer of ER stress and autophagy in triple-negative breast cancer cells [27]. Moreover, recently, it has been further confirmed that fluoxetine synergizes with temozolomide to induce ER stress in glioma cells [28]. Other related studies highlighted that ER stress either directly or indirectly resulted in DNA damage and genotoxicity [29,30]. Overall, our study is consistent with previous studies that indicated that fluoxetine, either via oxidative stress or ER stress, induces DNA damage and apoptosis. However, due to a large number of limitations, we could not further investigate the mechanism of action underlying the fluoxetine effects.

5. Conclusions

This study has shown that fluoxetine could induce oxidative stress-dependent DNA damage. So, in clinical practice, more consideration should be given regarding the long use of this drug. However, further precise studies are necessary to further confirm these results.

Conflict of interest

All authors declare no conflict of interest regarding the publication of this paper.

References

1. Gheysarzadeh A, Sadeghifard N, Afraidooni L, et al. (2018) Serum-based microRNA biomarkers for major depression: MiR-16, miR-135a, and miR-1202. *J Res Med Sci* 23: 69. https://doi.org/10.4103/jrms.JRMS_879_17

2. Alboni S, Van Dijk RM, Poggini S, et al. (2017) Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. *Mol Psychiatry* 22: 552–561. https://doi.org/10.1038/mp.2015.142
3. Trivedi MH, Rush AJ, Wisniewski SR, et al. (2006) Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry* 163: 28–40. https://doi.org/10.1176/appi.ajp.163.1.28
4. Kirsch I, Deacon BJ, Huedo-Medina TB, et al. (2008) Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. *PLoS Med* 5: e45. https://doi.org/10.1371/journal.pmed.0050045
5. Hughes S, Foster RG, Peirson SN, et al. (2016) Inhibitory effects of fluoxetine on photosensitive retinal ganglion cells. *Investig Ophthalmol Vis Sci* 57: 4660.
6. Kaur G, Invally M, Sanzagiri R, et al. (2015) Evaluation of the antidepressant activity of Moringa oleifera alone and in combination with fluoxetine. *J Ayurveda Integr Med* 6: 273–279. https://doi.org/10.4103/0975-9476.172384
7. Sandanapitchai V, Ramasamy S, Pichai E (2016) Prospective study to evaluate the efficacy of fluoxetine in comparison with amitriptyline in patients with depression. *Int J Basic Clin Pharmacol* 5: 2552–2555. https://doi.org/10.18203/2319-2003.ijbcp20164122
8. Gheysarzadeh A, Yazdanparast R (2015) STAT5 reactivation by catechin modulates H_2O_2-induced apoptosis through miR-182/FOXO1 pathway in SK-N-MC cells. *Cell Biochem Biophys* 71: 649–656. https://doi.org/10.1007/s12013-014-0244-6
9. Mohammadi M, Yazdanparast R (2011) Modulation of H_2O_2-induced mitogen-activated protein kinases activation and cell death in SK-N-MC cells by EUK134, a salen derivative. *Basic Clin Pharmacol Toxicol* 108: 378–384. https://doi.org/10.1111/j.1742-7843.2010.00664.x
10. Sies H (2000) What is oxidative stress? *Oxidative stress and vascular disease*. Boston: Springer, 1–8. https://doi.org/10.1007/978-1-4615-4649-8_1
11. Ji C, Rouzer CA, Marnett LJ, et al. (1998) Induction of cell cycle arrest by the endogenous product of lipid peroxidation, malondialdehyde. *Carcinogenesis* 19: 1275–1283. https://doi.org/10.1093/carcin/19.7.1275
12. Alarifi S, Ali D, Alkahtani S, et al. (2014) Iron oxide nanoparticles induce oxidative stress, DNA damage, and caspase activation in the human breast cancer cell line. *Biol Trace Elem Res* 159: 416–424. https://doi.org/10.1007/s12011-014-9972-0
13. Gheysarzadeh A, Yazdanparast R (2012) Inhibition of H_2O_2-induced cell death through FOXO1 modulation by EUK-172 in SK-N-MC cells. *Eur J Pharmacol* 697: 47–52. https://doi.org/10.1016/j.ejphar.2012.09.036
14. Ghassemi-Barghi N, Varshosaz J, Etebari M, et al. (2016) Role of recombinant human erythropoietin loading chitosan-tripolyphosphate nanoparticles in busulfan-induced genotoxicity: analysis of DNA fragmentation via comet assay in cultured HepG2 cells. *Toxicol In Vitro* 36: 46–52. https://doi.org/10.1016/j.tiv.2016.07.001
15. Maleki F, Sadeghifard N, Hosseini HM, et al. (2019) Growth-inhibitory effects of TGFαL3-SEB chimeric protein on colon cancer cell line. *Biomed Pharmacother* 110: 190–196. https://doi.org/10.1016/j.biopha.2018.11.025
16. Zheng Y, Chang X, Huang Y, et al. (2023) The application of antidepressant drugs in cancer treatment. *Biomed Pharmacother* 157: 113985. https://doi.org/10.1016/j.biopha.2022.113985

17. Szegezdi E, Logue SE, Gorman AM, et al. (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep* 7: 880–885. https://doi.org/10.1038/sj.embor.7400779
18. Rasheva VI, Domingos PM (2009) Cellular responses to endoplasmic reticulum stress and apoptosis. *Apoptosis* 14: 996–1007. https://doi.org/10.1007/s10495-009-0341-y
19. Carson DA, Ribeiro JM (1993) Apoptosis and disease. *Lancet* 341: 1251–1254. https://doi.org/10.1016/0140-6736(93)91154-E
20. Birben E, Sahiner UM, Sackesen C, et al. (2012) Oxidative stress and antioxidant defense. *World Allergy Organ J* 5: 9–19. https://doi.org/10.1097/WOX.0b013e3182439613
21. Laborde E (2010) Glutathione transferases as mediators of signaling pathways involved in cell proliferation and cell death. *Cell Death Differ* 17: 1373–1380. https://doi.org/10.1038/cdd.2010.80
22. Lima CF, Fernandes-Ferreira M, Pereira-Wilson C (2006) Phenolic compounds protect HepG2 cells from oxidative damage: relevance of glutathione levels. *Life Sci* 79: 2056–2068. https://doi.org/10.1016/j.lfs.2006.06.042
23. Zlatković J, Todorović N, Tomanović N, et al. (2014) Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: a histopathological study. *Eur J Pharm Sci* 59: 20–30. https://doi.org/10.1016/j.ejps.2014.04.010
24. Kotan VO, Sarandol E, Kirhan E, et al. (2011) Effects of long-term antidepressant treatment on oxidative status in major depressive disorder: a 24-week follow-up study. *Prog Neuropsychopharmacol Biol Psychiatry* 35: 1284–1290. https://doi.org/10.1016/j.pnpbp.2011.03.021
25. Novío S, Núñez MJ, Amigo G, et al. (2011) Effects of fluoxetine on the oxidative status of peripheral blood leucocytes of restraint-stressed mice. *Basic Clin Pharmacol Toxicol* 109: 365–371. https://doi.org/10.1111/j.1742-7843.2011.00736.x
26. Zafir A, Banu N (2007) Antioxidant potential of fluoxetine in comparison to Curcuma longa in restraint-stressed rats. *Eur J Pharmacol* 572: 23–31. https://doi.org/10.1016/j.ejphar.2007.05.062
27. Bowie M, Pilie P, Wulfkuhle J, et al. (2015) Fluoxetine induces cytotoxic endoplasmic reticulum stress and autophagy in triple negative breast cancer. *World J Clin Oncol* 6: 299–311. https://doi.org/10.5306/wjco.v6.i6.299
28. Ma J, Yang YR, Chen W, et al. (2016) Fluoxetine synergizes with temozolomide to induce the CHOP-dependent endoplasmic reticulum stress-related apoptosis pathway in glioma cells. *Oncol Rep* 36: 676–684. https://doi.org/10.3892/or.2016.4860
29. Dicks N, Gutierrez K, Michalak M, et al. (2015) Endoplasmic reticulum stress, genome damage, and cancer. *Front Oncol* 5: 11. https://doi.org/10.3389/fonc.2015.00011
30. Corcoran CA, Luo X, He Q, et al. (2005) Genotoxic and endoplasmic reticulum stresses differentially regulate TRB3 expression. *Cancer Biol Ther* 4: 1063–1067. https://doi.org/10.4161/cbt.4.10.2205

AIMS Press