ABSTRACT Aim: Sertraline is an active antidepressant drug used in the treatment of psychiatric disorders for long term use. The genotoxic effects of this drug. Sufficient studies reporting this drug’s genotoxic and carcinogenic effects have not been reported yet. This study aimed to assess the frequency of chromosomal aberrations on human lymphocytes in vitro. Material and Methods: In this study, the in vitro genotoxic effects of Sertraline have been determined in human peripheral blood lymphocytes by using chromosomal aberrations: 5; 10; 25; 50.00; 75 and 100mg/mL concentrations of Sertraline was used. Sertraline significantly increased the frequency of chromosomal aberrations in high doses compared with the control. The two highest concentrations (75 and 100mg/mL) of Sertraline were significantly increased chromosomal aberrational s compared to recommended concentration (50mg/mL). In addition, a significantly higher frequency of chromosomal aberrations (p<0.05) was determined in treated cultures. Conclusion: Sertraline is the genotoxic active ingredient in human lymphocytes in vitro. However, this genotoxic effect should be supported by in vivo studies.

KEYWORDS Antidepressant, Sertraline, chromosomal aberrations, cytogenetics

Introduction
Depression is a complex disorder characterized by anxiety, irritability, agitation, fatigue, psychomotor changes, guilt, and low self-esteem. The most common treatment for this condition is with antidepressants [1,2].

Antidepressants are drugs that are widely used as standard treatments to relieve the symptoms of depression. They may improve depressive symptoms by acting on emotional neural systems. These medications were developed for the first time in the 1950s and regularly have been used since that time [3].

The neuropsychological mechanism by which antidepressants relieve depression symptoms is unknown. Antidepressants, for example, are not clear whether they affect emotional states by reducing negative emotional brain processes, increasing positive emotional neural processes, or both. Depressed patients tend to pay less attention to positive emotions [4]. Many patients take Antidepressants for long periods, and there are numerous studies in the literature indicating genotoxic effects. Therefore, some drug tests are needed before it goes on sale, including a genotoxicity test. Genotoxicity studies are determined whether the DNA damage is caused by a chemical [5,6,7].

Today, there are mostly 30 types of antidepressants, and four main types are Monoamine oxidase inhibitors (MAOIs), tricyclics, selective serotonin reuptake inhibitors (SSRIs), and serotonin and norepinephrine reuptake inhibitors (SNRIs) [8].

Because they are safer and as effective as other antidepressants, the SSRIs antidepressants are used to treat various mental problems, including depression, obsessive-compulsive disorder, panic attacks, and social phobias [9]. Sertraline was chosen as the model of SSRIs because it is the most commonly prescribed antidepressant by psychiatrists in Duhok, Iraq’s Kurdistan region.

Sertraline is an antidepressant that belongs to the selective serotonin reuptake inhibitor class of drugs that are frequently used to treat depression and obsessive-compulsive disorder, panic attacks, and post-traumatic stress disorder [10,11].
the most commonly used psychiatric medication because it aids in the improvement of mood, appetite, and energy levels and reduces anxiety and insomnia associated with depression. Sertraline’s mechanism of action is that it aids in the restoration of the normal balance of the brain’s natural chemical serotonin [12,13].

Nowadays, Antidepressants are long-term medications that are given to a large number of patients. When evaluating these drugs, keep in mind that the occurrence of a genotoxic and carcinogenic effect cannot be ruled out among the various adverse reactions that these drugs may cause. Furthermore, due to the rapid increase in the use of antidepressants, it has become critical to determine whether these drugs have negative effects on genetic structure [7,14].

This DNA damage can present in the form of single or double-stranded breaks, losses during excision repair, cross-links, alkaline labile regions, point mutations, and structural and numerical chromosomal aberrations [15,16]. In the present study, experiments are performed to investigate the cytogenetic effects of Sertraline in peripheral blood lymphocytes of patients who have used this medication for more than three years as well as cultured human lymphocytes by using the in vitro cytogenetic assay.

**Material and Methods**

**Ethical Approval**

This study was approved by the Scientific Research Committee of the College of Medicine/ University of Duhok and the Ethics Committee of the Directorate of Health. Oral and written consent forms were obtained from all participants.

**Preparation of Insecticide concentrations**

The test substances Sertraline tablet (brand name Zoloft) was obtained from the local pharmacy market for research. Sertraline was dissolved in distilled water and different concentration of Sertraline under and above the recommended concentration. The recommended concentration was (50 µg/mL), and (5; 10; 25; 75; and 100µg/mL) concentrations were prepared and tested on the culture containing human blood lymphocytes, with three replicates. The chemical structure and formula of Sertraline are shown in Figure 1.

**Sample**

The study was carried out under sterile conditions using Human peripheral venous blood samples. From 30 participants dividing into two main groups:

- **Group One:** 15 Treated culture of peripheral blood lymphocytes from healthy participants in vitro by adding different concentrations of Sertraline.

- **Group Two:** 15 Untreated cultures of peripheral blood lymphocytes from healthy participants in vitro without adding different concentrations of Sertraline.

All participants were non-smokers, non-drinkers, non-taking drug therapy at least for the last six months, and had no recent history of exposure to mutagenic agents. The ages of enrolled participants were ranged from 18–36 years old (11 male and 19 female), and they selected randomly from a rural area of Duhok province. Informed consent was obtained from all donors, and the study was carried out according to the local ethics committee.

**Chromosomal aberration Assay**

Cytogenetics technique for the preparation of chromosomes from the cultured lymphocytes of peripheral blood was carried out according to the method described by Rooney (2001) with some modification [18].

For each group of participants, two chromosomal cultures were carried out by adding 0.9 ml of peripheral blood in sodium heparinized tube, then added to culture tubes that containing culture media (10ml of RPMI 1640L-glutamine, 2ml of fetal bovine serum, 200µg of Phytohemagglutinin (10µg/ml), and 200µg penicillin-streptomycin solution (10µg/ml). After 24 hours of culturing, 200µg of each concentration of Sertraline was added to the culture tubes of the First group and cultured for the remaining 48 hours.

After 72 hours of culturing, 200µg of Colcemid was added to each culture tube to stop the cell division in metaphase and incubated at 37°C for one hour. Then, tubes were centrifuged at 1500 rpm for 7 min. Then, 10 ml of 0.075M KCl solution was added, mixed, and incubated at 37°C for 30 min. After centrifugation at 1500 rpm for 7 min, the cells were treated with hypotonic solution (0.4 % KCl). Then, 10 ml of cold, fresh fixative solution (3:1 Methanol: Glacial acetic acid) was added drop by drop for the first 2 ml to the cell pellet. Centrifugation was done afterwards, the supernatant was removed, and the last step was repeated until a clear pellet was obtained.

Finally, microscope slides were prepared by dropping cell samples, air drying and staining with 5% Giemsa solution at pH 6.8 for 5 min. They were finally washed in distilled water and dried at room temperature. For each concentration and control, chromosomal abnormalities of 25 cells at the metaphase stage were counted, analyzed and compared with control.

**Statistical Analysis**

All the statistical analyses were performed, and the mean frequencies of chromosomal aberrations of each group were compared using Student’s t-test. (P ≤ 0.05) was considered statisti-
cally significant. Twenty-five cells were scored per participant to determine the frequency of various chromosomal aberrations.

**Results**

A total of 30 volunteers, the peripheral blood lymphocyte from 15, was used as a treated culture with different concentrations of Sertraline. In contrast, the peripheral blood lymphocyte from the other 15 healthy donors was used as negative control culture, and no concentration of Sertraline was added, who were non-smokers, non-alcohol drinkers and with no history of taking any drug or medication for at least the last six months were randomly selected from different rural areas of Duhok province. The age of the enrolled participants ranged from 18 days to 36 years with a mean (26.59 ±66) years. Of these participants, 11 (36.6%) were males, and 19 (63.4%) were females with a male-to-female ratio of 1:1.7. The Demographic characteristics of all participants are shown in Table 1.

Table 1: Demographic characteristics of participants.

| Variable                  | Treated cultures | Untreated cultures |
|---------------------------|------------------|--------------------|
| Number of Participants    | 15               | 15                 |
| Age (Mean±St)             | 26.12±30         | 27.25±66           |
| Taking other medication   | 0                | 0                  |
| Alcohol consumption (Person) | 0              | 0                  |
| No. of Cigarette per day  | 1-2              | 0                  |

The frequency of chromosomal abnormalities was determined in human lymphocytes of both treated cultures with various doses of Sertraline (5; 10; 25; 50; 75; and 100 µg/mL) and treated cultures negative controls (0.0 µg/mL). The effect of Sertraline on chromosomes of the human peripheral lymphocytes for the two groups are represented in Table (2).

Table 2: Chromosomal aberrations in both treated and untreated cultures.

| Type of abnormality      | Treated cultures | Untreated cultures |
|--------------------------|------------------|--------------------|
| Chromatid break          | 3                | 4                  |
| Chromatid gap            | 0                | 0                  |
| Dicentric                | 0                | 0                  |
| Acentric                 | 1                | 1                  |
| Interchange              | 3                | 5                  |
| Total                    | 7                | 9                  |

For each concentration, the data of 25 metaphases per cell were scored in three replicates. The results revealed significant differences (P<0.05) in chromosomes aberrations in treated groups compared with the control group. Furthermore, the effects constantly increased with the increase of Sertraline concentrations.

The present study revealed no numerical chromosomal aberrations in blood lymphocytes in any treated concentrations, even in the high dose of (100 µg/mL). At the same time, the results revealed that Sertraline caused six types of structural chromosomal aberrations in treated cultures: Ring chromosome, chromatid break, chromatid gap, dicentric chromosome, acentric chromosome and interchange chromosomes, as shown in Figure 3.

In all these tested systems, data showed that Sertraline induces genotoxicity at almost all concentrations and induced a significant increase in the frequency of chromosomal aberrations in all concentrations compared to the negative control. For example, numbers of chromatid breaks increased as the concentration of Sertraline increased: 5µg/mL=3, 10µg/mL=4, 25µg/mL=25, 50µg/mL=41, 75µg/mL=53 and 100µg/mL=98. Total chromosomal aberrations in each concentration are shown in Figure 2.

**Discussion**

Depression is the most frequent complicated psychiatric disorder in the world, and it leads to significant patient dysfunction. This soreness gets worse with each passing day and frequently worsens with relapses. Due to stress and cardiovascular issues, the World Health Organization estimates that depression will become the second greatest cause of death in the future [19, 20].
According to epidemiological studies in Iraq [21], a major depressive episode is a prevalent mental condition in Iraq, affecting roughly 475,000 Iraqi adults annually, with 46% being severe or very severe. According to epidemiological research in Turkey, the prevalence of clinical depression in the general population is roughly 10%, with depression becoming chronic in about one-third of patients. Depression affects around 21% of the world’s population [22].

Antidepressants are long-term medicinal products given to several patients, and a large number of literature studies have reported genotoxic and carcinogenic effects [23, 24]. In other important ways, the literature contains controversial literature reports about the use and association of antidepressants with cancer. Certain authors suggest that antidepressants are associated with increased cancer risk. The use of antidepressants has increased over the years, making this potential combination an important issue for discussion. Cancer is a potentially fatal disease. It’s also important to note that antidepressants can help cancer patients with depression [25,26,27].

Sertraline was chosen as an SSRI model because it is the most commonly used antidepressant in the Duhok province of Iraq’s Kurdistan region. In addition, the possible genotoxic effect of Sertraline on chromosomal aberrations in peripheral blood lymphocytes in vitro was investigated in this study, which is a reliable assay for evaluating the impact of mutagenicity and carcinogenicity, as most tumours are of epithelial origin and detecting human cancer risks.

There have been few studies on the effects of antidepressants on invertebrates, with the majority of them focusing on the direct effect on biological functions such as reproduction and growth. However, in-depth molecular responses of antioxidant defences against SSRI-mediated oxidative stress concerning in-vivo effects of SSRIs, such as sertraline, remains questionable cancer [28].

Sertraline was developed and intended to treat neurological disorders such as epilepsy and depression. However, it is now prescribed by the medical profession for various other purposes. It selectively inhibits serotonin reuptake in presynapses. In adults, their safety profiles are thought to be superior to those of other antidepressants, particularly in the treatment of depression in people with cardiovascular disease [29]. In general, several techniques and assays are used to assess the genotoxicity and cytotoxicity of drugs and medications, including the Chromosomal aberration assay, the Micronucleus assay, and the Comet assay. These techniques have been classified as indicators of the early biological effects of carcinogen exposure. They are also well-known biomarkers of genotoxic and mutagenic effects. In this study, we used an in vitro chromosomal aberration assay to assess different concentrations of Sertraline in human peripheral blood lymphocytes.

Compared to the control group, the current study found a significant frequency of chromosomal aberrations in human lymphocytes as genotoxic indicators. Sertraline produced a series of genotoxic effects on peripheral blood lymphocytes after 24 hours of treatment with different doses, represented by six types of chromosomal aberrations: Ring chromosome, Chromatid break, Centromeric gap, Dicentric chromosome, Acentric chromosome, and Interchange. With increasing Sertraline concentration in higher doses, there was a significant increase in the total number of chromosomal abnormalities, dose-dependent. The total number of abnormalities increased from 6 in untreated cells (0µg/mL) of Sertraline to 182 in cells treated with (100µg/mL) of Sertraline. This increase in chromosomal aberrations may be due to the accumulation of Sertraline’s chemical substance in blood tissues, which causes DNA damage as a result of treatment.

The current findings on the frequency of chromosomal aberrations in human peripheral blood lymphocytes are consistent with previous studies done by Draz et al. (2009), who reported that Sertraline induced a significant increase in the release of fragments of DNA in the male group at 400bp (69.12±13.84) and female group (37.56±6.92) at optical density 200bp when compared to controls. Furthermore, at optical density 400 and 200bp, the optical density values of the release of DNA fragments in male patients were significantly higher than those in female patients [30].

Some other medicines are available, but the genotoxicity of their medicines has not been proved in vivo. Trazodone and milnacipran are some of these commonly used drugs. A trazodone study showed that it was not Carcinogenic in rats for a long-term carcinogenesis assay. In the same vein, milnacipran could not be found to increase tumour incidence in lifetime carcinogenicity studies [31,32]. More researchers also reported in vitro their genotoxicity. They reported that increased chromosome aberration levels in lymphocytes significantly increased the risk of cancer development. The results of this research suggest that the frequency of chromosomal aberrations in human lymphocytes increased significantly between trazodone and milnacipran. Chromosomes and chromatid breaks in both treatment groups are the most frequent aberrations [33]. The results of this study show that at some levels of human lymphocytes cultivated, trazodone and milnacipran can have clastogenic or mutagenic potential.

Some studies indicate that some antidepressant drugs do not have a genotoxic effect. According to Bozkurt et al. (2004), there was no statistically significant difference in chromosomal aberrations between patients receiving (sertraline was provided orally at 50mg daily for 10 months to 1 year) and those who did not get sertraline medication [34]. Peripheral blood lymphocytes from sertraline-treated Wistar albino rats (10, 40, and 80mg/kg) were used in another study. In terms of DNA damage, there was no statistically significant difference between the sertraline-treated and control groups.

Total chromosomal aberrations were significantly higher in cultures of recommended concentration (50µg/mL) of the drug than in negative control or lower concentration doses. However, this high aberration does not indicate potential genotoxicity of the drug because there is no enzymatic regulation in the culture tubes to minimize the effects of the drug as happens in; as a result, the genotoxicity of this drug must be assessed in vivo using other genotoxicity methods.

Conclusion

Antidepressants are drugs of long-term use and administered to numerous patients. For this reason, evaluation of the benefit and the occurrence of a genotoxic effect should be considered. In conclusion, Sertraline is the genotoxic active ingredient in human lymphocytes in vitro. However, this genotoxic effect should be supported by in vivo studies. To boot, the mechanisms of the detrimental effects of these substances need to be clarified by more elaborate subjects.
Funding
This work did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest
There are no conflicts of interest to declare by any of the authors of this study.

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