

The investigation of the *in vivo* cytogenetic effects of psychotropic drugs in human lymphocyte cultures

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Abstract: The connection of nearly all current antipsychotic drugs to their *in vivo* cytogenetic activity has not been yet fully investigated. Fluvoxamine, Valproic acid (VA) and Haloperidol (HLP) are three universally common consumed psychotic drugs whereas used to treat several psychiatric disorders. This study aims to investigate the cytogenetic effects of these three psychotropic drugs by evaluating the frequency of Sister Chromatid Exchanges (SCEs) and the Proliferation Rate Index (PRI) in cultured lymphocytes. Fifteen patients with psychiatric disorders (i.e. depression, bipolar and schizophrenia) consisting of smokers and non-smokers were included. Estimation of SCEs was used as a sensitive biomarker of the potential cytotoxicity, while PRI was used as a valuable marker of cytostatic activity. A significant increase of SCEs in the cultured lymphocyte of the smoker controls ($P = 0.013$) was found in compared to the non-smoker controls. This study found that there is no difference in the average of SCEs values in lymphocytes isolated from the smoker and non-smoker patients treated with Fluvoxamine, Valproic acid and Haloperidol ($P > 0.05$). A significant difference of PRI ($P = 0.036$) in the lymphocytes of smoker controls compared to those of the non-smoker controls were detected. This study also found a significant difference with respect to PRI between the three patient groups ($P = 0.017$). These results illustrated that treatment (monotherapy) of psychiatric patients with Fluvoxamine, Valproic acid, and Haloperidol exerts a significant cytostatic but not cytotoxic effect on their lymphocytes whereas these effects are intensified by smoking.

Keywords: Cytogenetic activity, Fluvoxamine, Valproic acid, Haloperidol, Sister Chromatid Exchange.

INTRODUCTION

Mental illness is defined as a condition that influences a person's reasoning, feelings, or mindset. Such conditions may influence someone's capacity to relate to others and their capacity to function on a day-to-day basis. According to the WHO, in low and low-middle income countries like Jordan, up to 75% of people who need mental care do not get it. It is estimated that 20% of the Jordanian population needs mental care (Maayeh, 2008; WHO, 2008). This percentage was increased by 30% between 2003 and 2008, raising the number of patients who admitted to the public psychiatric hospital in Fuheis to 2,090 (Maayeh, 2008).

Fluvoxamine, Valproic acid (VA) and Haloperidol (HLP) are three universally consumed psychotic drugs. Fluvoxamine is recommended as a first-line treatment for several psychiatric disorders, but it is mainly used for the treatment of major depressive disorder (MDD) (Figgitt and McClellan, 2000). Fluvoxamine is a powerful selective serotonin reuptake inhibitor (SSRI) which has been in use since 1983 (Burton, 1991). In the previous decade, the SSRI class of drugs has been noted to be associated with a variety of side effects demonstrated by *in vivo* studies (Galal *et al.*, 2016).

Valproic acid (VA) is mainly used in a clinical context for the treatment of bipolar disorder (Hu Karapidaki *et al.*, 2011; Hu *et al.*, 1990). Valproic acid is known to affect the role of the Gamma-aminobutyric Acid (GABA) neurotransmitter in two ways: indirectly by acting as a GABA agonist through the inhibition of GABA transaminase, or directly by reversing the transamination process to form GABA (Rosenberg, 2007). Unlike Fluvoxamine, Valproic acid has been tested for cytotoxicity and genotoxicity in various systems, but the results have been contradictory. Haloperidol (HLP) is used mainly to manage the symptoms of psychotic disorders such as violent behavior in schizophrenic patients because its character as a dopamine antagonist gives it selectivity for the D2-like receptors (Katzung *et al.*, 2001; Powney *et al.*, 2012). *In vitro* study reported that HLP was able to induce cytostatic and genotoxic effects in the tested lymphocyte cells (Gajski *et al.*, 2014). However, another study found that HLP may not be clastogenic *in vitro* (at plasma concentrations), but it was clastogenic *in vivo* (Ahuja *et al.*, 1984). For these well-known and widely used psychotic drugs, their cytotoxic and cytostatic effects on the lymphocytes of psychiatric patients must be investigated, especially for patients who undergo long-term treatment (i.e. treatment for more than three months) and those who smoke.

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Tobacco smoking is estimated to contain over 3800 chemicals. Studies of the genotoxic effect of mainstream cigarette smoke and cigarette smoke condensate revealed an increase in the formation of micronuclei and chromatid exchange in lymphocytes, as the micronucleus test is one of the less invasive methods to measure DNA damage in humans. Besides, several studies have demonstrated the relationship of cytogenetic abnormalities with cigarette smoking in acute myelogenous leukemia patients. Most striking is the reports frequency about the abnormalities of chromosome 8, especially trisomy 8 and t(8;21) (Crane *et al.*, 1996; Davico *et al.*, 1998; Moorman *et al.*, 2002) and of chromosome 7 (Sandler *et al.*, 1993; Bjork *et al.*, 2000, 2001). One report found an association of acute myelogenous leukemia with chromosome 5 abnormalities in patients who smoked cigarettes (Crane *et al.*, 1996). All those findings increased the interest to study the role of smoking in intensifying the cytotoxic and the cytostatic effects in patients treated with Fluvoxamine, Valproic acid, and Haloperidol. The main objective of this study is to give more insight into the cytotoxic and cytostatic effects of these prevalent antipsychotic drugs to improve the treatment strategies of the patients, especially those who require long-term treatment for better healthcare outcomes.

MATERIALS AND METHODS

The methodology applied in this research is adopted from Constatinos *et al.*, 2015, in which blood samples were taken from patients undergoing therapy with psychotic drugs.

Study design and participants

In this case-control study, the cases were divided into three groups, with each one consisting of five in-patient males who were diagnosed with depression, bipolar disorder, or schizophrenia by a psychologist from the Department of Psychiatry at King Abdullah University Hospital. Patients were undergoing monotherapy with Fluvoxamine, Valproic acid, and Haloperidol. Each group also included smokers and non-smokers as well as patients undergoing long-term and recently-initiated (i.e. less than 3 months) drug treatment. The Control group comprises five healthy males who are not receiving any drug recruited from King Abdullah University Hospital-Hematology lab. The control group also contains smokers and non-smokers (Tables 1 and 2).

Blood from case and control groups was used to culture lymphocytes for the Sister Chromatid Exchanges (SCEs) and Proliferation Rate Index (PRI) assays. Patients and healthy donors were between 30 to 40 years old, have no history of alcohol abuse, and have not been suffering from any kind of infection in the last 15 days before sample collection. All donors were asked to give back their written informed consent according to the Institutional

Review Board of Jordan University of Science and Technology (JUST-IRB) guidelines, and they were informed that they were free to abstain or to withdraw from participation at any time of the study. Whole blood samples were obtained using heparin tubes and they were cultured within a few hours of sampling. Samples were labeled with an ID number for each participant so that the investigator and the data analyst are all unaware of sample identity and group (triple-blinded study) to enhance the objectivity of measurements and avoid bias.

Sister chromatid exchange and proliferation rate index tests

Human Blood Cell Culture

For the SCEs and PRI analysis, blood cultures were carried out by inoculating 1 ml of freshly drawn blood and filtered bromodeoxyuridine (BrdU) (30μ of 0.01g/1ml) into a 15 ml polypropylene screw-capped tube containing 9 ml of PB-Max medium (complete media) which is composed of RPMI 1640 medium with 1% penicillin-streptomycin, 15% fetal bovine serum and 3% of phytohaemagglutinin (PHA) in order to stimulate the lymphocyte culture (Rooney *et al.*, 2001; M'Bemba-Meka *et al.*, 2007). The cultures were incubated at 37°C for 72h in the dark to minimize the photolysis of BrdU (Kaya *et al.*, 2007).

Harvesting and metaphase slides preparation

Harvesting and metaphase preparation were followed based on a published protocol by Rooney (2001) and M'Bemba-Meka *et al* (2007). Harvesting involves obtaining the isolated cells from the culture mixtures followed with Metaphase slide preparation then metaphase slide staining by Hoechst dye, which prepares the chromosomes for reading and scoring the SCEs and PRI.

Analysis of sister chromatid exchanges and proliferation rate index

For the SCEs evaluation, all metaphases were scored on a high-resolution light microscope (Nikon, Japan) with 1000X final magnification. SCE slides were scored using the second division cells (M2) phase. Fifty suitably spread lymphocyte cells that only contain 42-46 chromosomes from each person are examined by calculating the number of SCEs in each lymphocyte cell then the average of SCEs was calculated (Wolff *et al.*, 1996). PRI is estimated according to the following formula: $PRI = \frac{M1 + 2M2 + 3M3 + \dots}{100}$, where M1, M2, M3, etc. are the percentage values of cells in the first, second, third and higher divisions, respectively (Constantinos *et al.*, 2015). All of the previous procedures are applied randomly for both control and case samples by writing the available case ID down on papers, mixing them, and finally, drawn at random to test the SCE and PRI. In this way bias and measurement errors were minimized.

Table 1: Demographic information of controls.

Control	Age (Years)	Smoker	Non-smoker
C1	31		*
C2	32		*
C3	39	*	
C4	36	*	
C5	38	*	

Table 2: Clinical characteristics of patients treated with Fluvoxamine, Valproic Acid and Haloperidol.

Patients	Age (Years)	Dose of the drug	Duration of treatment	Smoker	Non-smoker
F1	40	150 mg	11 years	*	
F2	33	150 mg	2 years	*	
F3	30	100 mg	5 years		*
F4	37	25 mg	2 weeks	*	
F5	32	50 mg	1.5 months		*
V1	38	125 mg	7 months	*	
V2	35	250 mg	1 year	*	
V3	39	500 mg	18 year		*
V4	31	100 mg	3 weeks	*	
V5	33	100 mg	2 months		*
H1	35	50 mg	9 months	*	
H2	38	20 mg	1 year	*	
H3	40	50 mg	3 years		*
H4	32	0.5 mg	1 weeks	*	
H5	30	2 mg	3 weeks		*

*F: Patient treated with Fluvoxamine, *V: Patient treated with valproic acid, *H: Patient treated with Haloperidol

Table 3: Effect of smoking on the average SCEs values in cultured lymphocytes from the control group.

Control	N*	M±SD**	t	P**
Non-smoker	100	1.17941 ± 0.11794	-2.500	0.013
Smoker	150	1.33079 ± 0.10902		

Table 4: Effect of smoking on the Proliferation Rate Index (PRI) in the control group.

Control	N*	M±SD**	P**
Smoker	150	3.4000 ± 0.06245	0.03606
Non smoker	100	3.9950 ± 0.21920	0.15500

N*: Total number of checked lymphocyte cells, M±SD**: Mean ± standard deviation, P***: P-value<0.05 is considered significant.

STATISTICAL ANALYSIS

The statically analysis is performed using SPSS (Version 16, USA). Data is expressed as mean ± standard error (SE). The comparison of parameters is performed using T-Test. Differences regarded as significant at $P < 0.05$ (by using one-way ANOVA test).

RESULTS

The average of sister chromatids exchange (SCEs) & proliferation rate index (PRI) scores in controls

The average score of SCEs for both, smoker and non-smoker controls are shown in table 3, which shows the effect of smoking on SCEs in cultured lymphocytes from five controls with a total of 250 lymphocyte cells in M2

phase. One-way ANOVA analysis for the overall effect of smoking on SCEs showed a significant change to SCEs values in smokers within the control group ($P = 0.013$).

Difference in the average scores of SCEs between smokers and non-smokers within the control group with the smoker group having a higher mean score of SCEs compared to non-smokers; a higher score of SCEs indicates more cytotoxicity (fig. 1). Additionally, fig. 2 shows the percentage of SCEs distribution in the smokers and non-smokers of the control group. Most cell populations for the non-smoker controls are located in the normal range of SCEs which is less than 3. A lower percentage of cells occupied the higher average of SCEs, while the cells in smoker controls are shifting toward a higher average of SCEs (fig. 2).

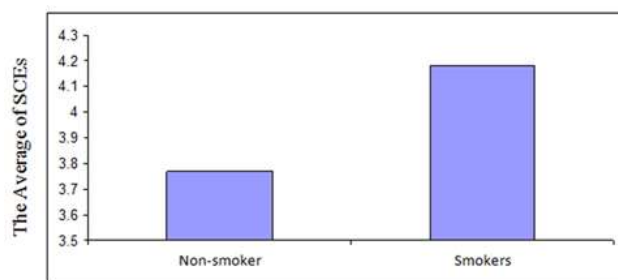


Fig. 1: The difference in the average score of SCEs between smokers and non-smokers within the control group.

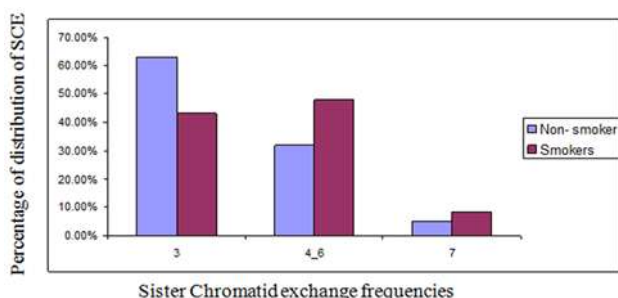


Fig. 2: The percentage of SCEs distribution in the control group. Most cell populations of the non-smoker controls are located within the normal range of SCEs (<3). A lower percentage of cells occupied the higher average of SCEs, while the cells in smoker controls are shifting toward a higher average of SCEs.

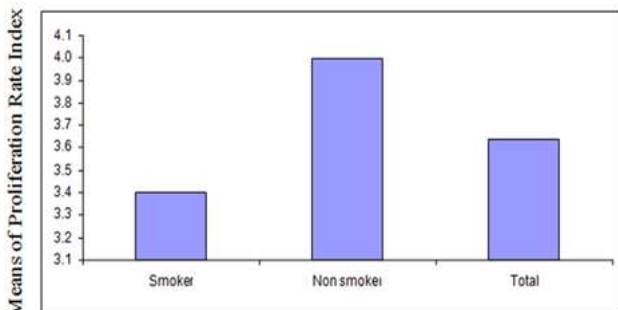


Fig. 3: The difference in the means of the PRI values between smoker and non-smoker controls.

Table 4 shows the effect of smoking on the PRI in smokers and non-smokers in the control group. Smoking appears to have a significant effect on the PRI of the smoker control group, as it shows a significant increase ($P=0.036$). Fig. 3 represents the difference in the means of PRI between smoker and non-smoker controls, with non-smoker controls having a higher PRI mean compared to smokers, which indicates that smokers have a low growth rate or suppression in the cell cycle kinetics (fig. 3).

The average of sister chromatid exchange (SCEs) scores & proliferation index (PRI) values in Cases

SCEs frequencies and their distributions in the control, Fluvoxamine, Valproic acid, and Haloperidol patient

groups are shown in figs. 4, 5, and 6. Chromosomes profiling of different phases M1 (Fig. 7 represents two chromatids that are uniformly and darkly stained), M2 (fig. 8 represents differently stained chromatids where one is lightly stained and the other is darkly stained), M3 (fig. 9 represents a mixture of lightly stained, darkly stained and differentially stained chromatids) and M4 (fig. 10 represents uniformly and lightly-stained chromatids) in control, Fluvoxamine, Valproic acid, and haloperidol treated patients.

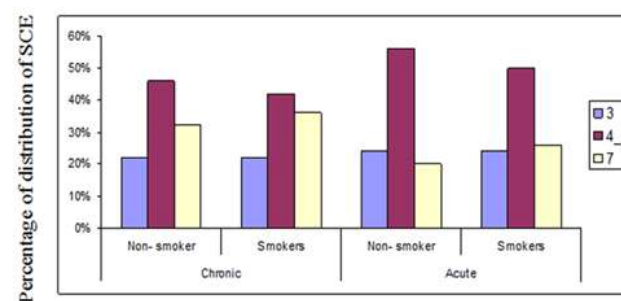


Fig. 4: The percentage of SCEs distribution in Fluvoxamine-treated patients. Most cell populations for acute non-smoker patients are located in the middle range of SCEs (4-6), a similar observation for acute smokers. In acute patients, a lower percentage of cells are occupying the higher distribution of the average of SCEs (>7). In contrast, the cells in chronic patients (smoker and non-smokers) are shifting towards a higher distribution of SCEs average values (>7).

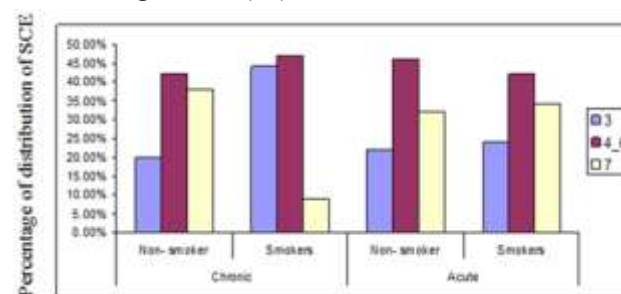


Fig. 5: The percentage of SCEs distribution in Valproic-acid-treated patients. Most cell populations for the acute non-smoker patients are located in the middle range of SCEs values (4-6), and a similar observation was made for acute smokers. In acute patients, a lower percentage of cells are occupying the higher distribution of average SCEs values (<7). While most cells in patients who are smokers and undergoing long-term treatment occupy a low (3) and middle (4-6) distribution of SCEs percentages, non-smokers undergoing long-term treatment show a high percentage of cells that occupy a higher range of SCEs (<7).

To further investigate the effect of smoking on the average values of SCEs in lymphocytes isolated from Fluvoxamine, Valproic acid, and Haloperidol treated patients, one-way ANOVA analysis was conducted between and within the aforementioned groups to show

Table 5: The differences between average SCEs values in lymphocytes isolated from Fluvoxamine-treated, Valproic-acid-treated acid and Haloperidol-treated patients (stratified for smoking).

Category	Subcategory	N*	M±SD**	P***
Fluvoxamine	Non smoker	100	4.8800 ± 0.16834	0.808
	Smoker	150	4.9333 ± 0.13988	
Valproic Acid	Non smoker	100	5.3300 ± 0.18371	0.632
	Smoker	150	5.4467 ± 0.15674	
Haloperidol	Non smoker	100	5.4400 ± 0.11917	0.619
	Smoker	150	5.5667 ± 0.19349	

Table 6: The differences between average SCE values in lymphocytes isolated from Fluvoxamine-treated, Valproic-acid-treated and Haloperidol-treated patients (stratified for treatment duration).

Category	Subcategory	N*	M±SD**	P***
Fluvoxamine	Chronic	150	4.9801 ± 0.13857	0.434
	Acute	100	4.8081 ± 0.17029	
Valproic Acid	Chronic	150	5.4768 ± 0.15719	0.427
	Acute	100	5.2828 ± 0.18220	
Haloperidol	Chronic	150	5.6424 ± 0.15621	0.211
	Acute	100	5.3232 ± 0.20498	

N*: Total number of checked lymphocyte cells, M±SD**: Mean ± standard deviation, P***: *P*-value < 0.05 is considered significant.

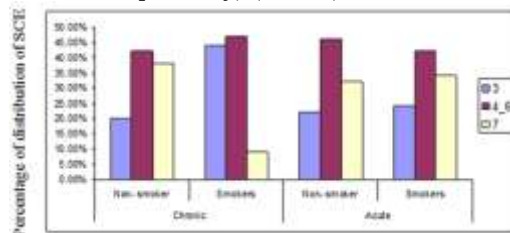
Table 7: The differences in PRI values between the three groups of lymphocytes that were isolated from Fluvoxamine-treated, Valproic-acid-treated, and haloperidol-treated patients.

Category	Sum of PRI	DF*	Mean	F	P**
Between Groups of Patients	0.425	1	0.425	22.820	0.017
Within Groups of Patients	0.056	3	0.019		
Total	0.481	4			

*D: Degree of Freedom, P**: *P*-value < 0.05 is considered significant.

the differences between smoker and non-smoker SCEs values. According to table 5, no significant difference was observed within or between the 3 patient groups (*P*= 0.808, *P*= 0.632, and *P*= 0.619, respectively).

On the other hand, to investigate the effect of treatment duration on the average SCEs values in lymphocytes isolated from the three patient groups, the analysis was conducted between and within the groups for patients undergoing both long- and short-term treatments. Interestingly, no significant difference was observed within or between the patient groups (*P*= 0.434, *P*= 0.427, and *P*= 0.211, respectively) (table 6).

**Fig. 6:** The percentage of SCEs distribution in Haloperidol-treated patients. Most cell populations for the smoking and non-smoking patients undergoing short-term therapy is located in the middle range of SCEs (4-6). A

lower percentage of cells in patients undergoing short-term therapy occupy the higher distribution of average SCEs (>7). While most cells in smoking patients undergoing long-term therapy occupies a low (3) and middle (4- 6) distribution of SCEs percentages, the non-smokers undergoing long-term therapy show a high percentage of cells occupying a higher range of SCEs (>7).

Table 7 shows a significant difference between the average PRI values in the cultured lymphocytes of the 3 patient groups (*P*= 0.017).

DISCUSSION

The present study revealed that Fluvoxamine, Valproic acid, and Haloperidol showed no cytotoxic effects, but they illustrated cytostatic effects *in vivo*. In particular, smoking was affecting SCEs and PRI in the smoker control group, which showed a higher SCEs score indicating a cytotoxic effect and a lower PRI value with slower growth than the non-smoker controls. This suggested a synergistic effect of smoking and cigarette products on DNA integrity. Moreover, smoking appears to affect the kinetics of the cell cycle by suppressing PRI

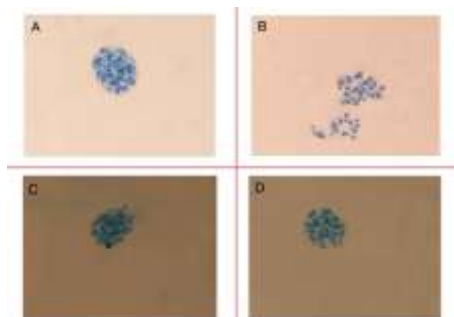


Fig. 7: Chromosomes profiling of the M1 phase (two chromatids are uniformly and darkly stained) using a high-resolution microscope (Nikon/Japan) with a total magnification of 1000X.

- A) Chromosomes from a Control
- B) Chromosomes from a Fluvoxamine-treated patient
- C) Chromosomes from a Valproic-acid-treated patient
- D) Chromosomes from a Haloperidol-treated patient

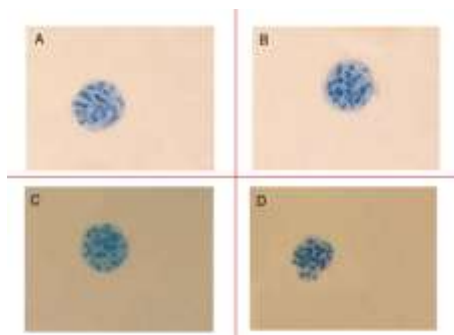


Fig. 9: Chromosomes profiling of M3 phase -containing a mixture of lightly stained, darkly stained and differentially stained chromatids using high resolution microscope (Nikon/Japan) with a total magnification of 1000X

- A) Chromosomes from a Control
- B) Chromosomes from a Fluvoxamine treated patient
- C) Chromosomes from a Valproic treated patient
- D) Chromosomes from a Haloperidol treated patient

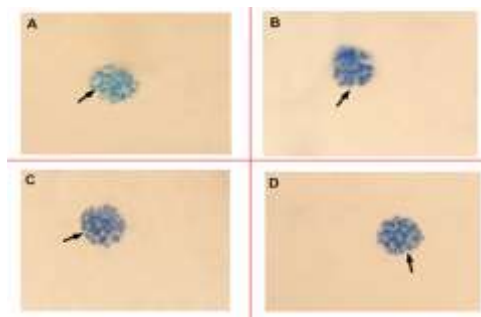


Fig. 8: Chromosomes profiling of the M2 phase (they have differently stained chromatids where one is lightly stained and the other is darkly stained) using a high-resolution microscope (Nikon/Japan) with a total magnification of 1000X.

- A) Chromosomes from a Control
- B) Chromosomes from a Fluvoxamine-treated patient
- C) Chromosomes from a Valproic-acid-treated patient
- D) Chromosomes from a Haloperidol-treated patient

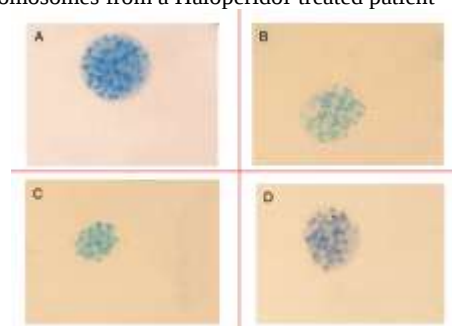


Fig. 10: Chromosomes profiling of the M4 phase (having uniformly and lightly-stained chromatids) using a high-resolution microscope (Nikon/Japan) with a total magnification of 1000X

- A) Chromosomes from a Control
- B) Chromosomes from a Fluvoxamine-treated patient
- C) Chromosomes from a Valproic-acid-treated patient
- D) Chromosomes from a Haloperidol-treated patient

for the lymphocytes of both, the control and patient groups. This is consistent with an earlier study showing that the incidence of SCEs in the lymphocytes of heavy smokers is significantly greater than that of non-smokers (Stahl, 2008). Constantinos *et al.*, study showed that SCEs were significantly increased in smoking controls. On the other hand, the same study later showed that there is no difference between smoking and non-smoking controls regarding the PRI (Constantinos *et al.*, 2015), which is in contrast with our findings on the PRI. The percentage of SCEs distribution in the control group was distributed normally, and as expected, most cell percentages occupy the normal distribution of the SCEs values which is less than 3 (≤ 3) and between 4 to 6 (4-6). The remaining small cell percentages are in a higher distribution of SCEs greater than or equal to seven (≥ 7). The SCEs have been mainly used as a sensitive indicator of DNA damage and

of subsequent effectiveness in DNA repair, recognition and signaling (Akritopoulou *et al.*, 2009). The high values of SCEs in lymphocytes from smoker controls could be either due to many DNA damages or inability to repair damages. The changes that were observed in the control cell cycle kinetics (which were indicated by PRI suppression in lymphocytes from smoker controls) have been proven to be an important and very sensitive marker of the cytostatic action resulting from different medications and environmental agents (Mourelatos, 1996).

The results here suggest that the treatment of depressed patients with Fluvoxamine as a monotherapy exerts no significant cytotoxic effect, but a significant cytostatic effect on their lymphocytes after taking into consideration smoking and treatment duration. To the best of our

knowledge, there were little or no studies on the cytogenetic activities of fluvoxamine, especially studies that were considered the PRI values. In depressed patients treated with Fluvoxamine, most cell percentages of the SCEs are located in the middle range of the SCEs (4-6), for both smokers and non-smokers undergoing short-term treatments. A lower cell percentage of patients undergoing short-term treatments were occupied the higher distribution of SCEs average values (>7). However, cells in patients undergoing long-term treatments (smoker and non-smokers) shift towards a higher distribution of average SCEs values which are greater than 7. This is something to be expected as a result of treatment with high doses of Fluvoxamine (100 and 150 mg) for long periods of time.

This study indicated that the treatment of bipolar patients with monotherapy of Valproic acid exerts no significant cytotoxic effects on their lymphocytes considering smoking and treatment duration. This is in accordance with a preliminary *in vivo* study showing no difference in the SCEs frequency between Valproic-Acid-treated and untreated epileptic patients (Schaumann *et al.*, 1989; Taneja *et al.*, 1992). However, a significant cytostatic difference in the PRI was detected within the cultured lymphocytes from patients treated with Valproic Acid, indicated suppression in the cell cycle kinetics. On the other hand, previous studies showed that SCEs frequency in the peripheral lymphocytes of epileptic children treated using Valproic acid monotherapy was significantly higher compared to the control group ($P<0.01$) (Hu *et al.*, 1990). Additionally, a significant increase in the SCEs of lymphocytes in healthy children exposed to sodium valproate *in vitro* (Hu *et al.*, 1990). Another *in vitro* study reported that lymphocyte cultures treated with Valproic Acid monotherapy showed a significant increase in the frequency of SCE and a decrease in the PRI values ($P<0.001$) (Karapidaki *et al.*, 2011). Such contrast in the findings between the current *in vivo* study and previous *in vitro* studies was expected. Most cell populations for smokers and non-smoker undergoing long-term Valproic Acid treatment are in the middle range of the SCEs (4-6). A lower cell percentage of patients undergoing short-term treatment occupies a higher distribution of average SCEs values which is greater than 7, suggesting an effect of Valproic acid on the SCEs. However, most cells in smoking patients undergoing long-term therapy occupy a low and middle distribution of SCEs percentages if compared to the non-smoking patients undergoing identical treatment. A possible explanation could be that two out of the three selected smoking patients undergoing long-term treatment were not receiving high doses of the drug (125 mg and 250 mg), or the treatment duration was not long enough.

Finally, the effects of Haloperidol were also investigated by measuring its cytotoxic and cytostatic effects on

cultured lymphocytes isolated from patients treated with the drug (monotherapy). No significant difference in the SCEs values was observed within and between lymphocytes groups, indicating that this drug has no cytotoxic effects on the patients. However, it shows a significant cytostatic difference in the PRI values. It was reported in a previous *in vitro* study that HLP was able to induce cyto/genotoxic effects in the tested lymphocyte cells (Gajski *et al.*, 2014), while another study has reported that HLP may not be clastogenic *in vitro* at plasma concentration, but it was clastogenic *in vivo* (Ahuja *et al.*, 1984). The significant difference in the PRI values of cultured lymphocytes from HLP-treated patients indicating an alteration in the cell cycle kinetics due to the metabolism of the drug or results of smoking. Regarding the distribution of the SCEs percentage in HLP-treated patients, most cell populations from smoking and non-smoking patients undergoing short-term therapy are located in the middle range of the SCEs values (4-6). While most cells in smoking patients undergoing long-term therapy occupy a low and middle distribution of the SCEs percentage could be explained by the fact that these patients receive intermediate doses and shorter treatment duration.

CONCLUSION

The main findings of the present study suggest cytostatic *in vivo* effects of Fluvoxamine, Valproic acid, and Haloperidol but not cytotoxic effects which are in contrast with previous studies. The cytostatic effects of these drugs were recognized as a significant decrease in the PRI and a cell cycle suppression by these drugs in the three patient groups. A cytostatic effect was also observed in the smoking control group, which revealed the serious effect of cigarette products on DNA integrity and cell cycle. Therefore, treatments with these drugs may be considered relatively safe in terms of cytotoxic effects, but the cytostatic effects of these drugs must be taken into consideration by psychiatrists in future prescriptions. Furthermore, in this case, treatments with those drugs may be considered relatively safe in terms of cytotoxic effects. In addition, this study showed how smoking intensifies the cytotoxic and cytostatic effects in the control group.

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