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Cytogenetic Evaluation of the Genotoxicity in Cultured Lymphocytes of Some Tramadol-Dependent Egyptians

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Abstract Tramadol is a widely used, synthetic opioid analgesic for the prevention and treatment of moderate to severe pain in acute or chronic conditions. Several studies have been performed to evaluate its safety. Among the various possible toxic effects for this drug overdose is its potential genotoxicity. The purpose of the present study was to assess the possible genotoxic effects of tramadol dependence in humans using the chromosomal aberration (CA), sister chromatid exchange (SCE), and the micronucleus (MN) assays. This work was carried out on 30 adult male subjects ranged in age from 20 to 35 years; 10 of them were used as controls, 10 subjects had orally taken tramadol for 2-3 years, and another 10 subjects had orally taken tramadol for 4-5 years. Those poisoned subjects (No=20) were admitted to Tanta Poison Center in the period from June 2011 to January 2012 and were diagnosed as tramadol overdose on top of dependence. The results showed elevation in the frequencies of chromosomal aberrations, SCEs and micronuclei in cultured lymphocytes from tramadol-dependent patients which were related to the duration of drug intake.

Keywords Tramadol, genotoxicity, chromosomal aberrations, sister chromatid exchange, micronucleus

Introduction

Opioid analgesics are pharmaceuticals used for pathological conditions that often require their long-term administration. Tramadol is a centrally acting drug that has been used as an analgesic agent for moderate to severe pain in acute or chronic conditions. It was first introduced in Germany in 1977 and has become the most prescribed opioid worldwide. It has been approved for use in the United States since 1995 and in France since 1997 (Shipton, 2000).

As with all other analgesic drugs, the dose of tramadol should be adjusted according to the severity of pain and the individual sensitivity. Unless otherwise prescribed by the physician, the therapeutic dose ranges from 50–100 mg of tramadol three to four times daily and the total daily dose should not exceed 400 mg (Raffa et al., 1993; Williams, 1997).

It was found that tramadol has a low affinity to opioid receptors. It inhibits the reuptake of biogenic amines especially norepinephrine and serotonin producing an analgesic action by blocking nociceptive impulses in the spine (Clarot et al., 2003; Lesani et al. 2010). In humans, parenteral tramadol is approximately one tenth as potent as morphine in producing analgesia (Gutstein and Akil, 2001).

Tramadol is rapidly absorbed orally and has a distribution volume of 3 l/kg. Elimination is slow and is characterized by an elimination half-life of 5–6 h. Tramadol is metabolized to an active desmethyl derivative (O-desmethyltramadol) and several inactive compounds. O-Desmethyltramadol shows higher affinity for the μ-opioid receptors and has twice the analgesic potency of the parent drug. It has been shown that the hepatic O-demethylation of tramadol is carried out by the isoenzyme cytochrome P4502D6 (Clarot et al., 2003).

Tramadol is generally said to be devoid of many serious adverse effects of traditional opioids, such as respiratory depression and drug dependence (Klotz, 2003). Based on this fact, the abuse potential of tramadol is considered to be low or absent in contrast to other opioids (Grond and Sablotzki, 2004; Cicero et al., 2005; McDiarmid et al., 2005). Tramadol abuse has been reported in post-marketing surveillance and in case reports (Epstein et al., 2006).

Despite evidence that repeated use of tramadol may lead to physical dependence, no well-controlled studies indicated the ability of tramadol to produce opioid physical dependence in humans. There

have been case reports of patients with apparent physical dependence on tramadol, as manifested by withdrawal upon cessation of tramadol use (Yates et al., 2001; Barsotti et al., 2003; Senay et al., 2003).

It was reported that the potential risk of genotoxic effects of tramadol to humans is among the various toxic effects to this drug. In order to assess the potential risk of genotoxic effects to humans, the regulatory authorities of Europe, USA and Japan recommend that genotoxicity study is performed before the application for marketing approval of pharmaceuticals (CDER, 1996 & 1997; Müller et al., 1999). Chromosome aberration analysis and chromosome stability assay remain the best approach to genotoxicity hazard identification (Muller and Kasper, 2000).

The aim of the present study was to evaluate the genotoxic effect of tramadol among some tramadol-dependent Egyptians using the chromosomal aberration assay, SCE and micronucleus test.

Subjects and methods

This study was carried out on thirty adult males (ranging from 20-35 years old). All participants were asked to complete a face-to-face questionnaire, which included standard demographic data (age, address, occupation, marital state) as well as medical history and lifestyle. Exclusion criteria included smoking, alcoholism, other substance abuse, and history of medical disease (e.g. diabetes, hypertension), chemotherapy, or recent exposure to radiation for at least 12 months before sample collection. An informed consent was taken from each participant in the study after giving detailed information about the nature of the research work. The subjects were then divided into three equal groups (10 individuals each) as follows:

- **Group I (Control group):** included 10 individuals who were apparently healthy with no history of tramadol intake of comparable age, marital state and occupations.
- **Group II:** included 10 individuals who had orally taken tramadol (average dose 350-500 mg) for 2-3 years.
- **Group III:** included 10 individuals who had orally taken tramadol (average dose 350-500 mg) for 4-5 years.

The poisoned groups (Groups II and III, n=20) were admitted to Tanta Poison Center in Tanta Emergency University Hospital within the period between June 2011 and January 2012 and were diagnosed as tramadol overdose on top of its dependence. All patients tested positive for tramadol on urine analysis. Glasgow Coma Scale (GCS), vital signs recordings and occurrence of seizure were documented at the time of admission.

Laboratory parameters

1- Toxicological analysis of urine samples

Twenty ml urine was obtained from each patient at the time of admission and before receiving any treatment. Any turbid samples or those containing blood were excluded from the study. Catheterization was done if the patient was unable to void urine. Each sample was collected in a clean dry labeled container with patient's name, serial number and time of taking the sample.

The sample was subjected to rapid screening test: 10 ml of each urine sample was used for rapid qualitative screening by ACON® tramadol one step test card for detection of tramadol in human urine. The kits utilize Enzyme Immunoassay (EIA) technique by competitive binding. Drugs, which may be present in the urine sample, compete against their respective drug conjugate for binding sites on their specific antibody. The measured reaction is directly proportional to the amount of drug of interest and/or its metabolites in the specimen. A drug-positive urine sample was followed by multi drug panel ACON® DOA™ kits to exclude cannabinoids, opiates, benzodiazepines, barbiturates and amphetamines in human urine (John and Ronald, 2006).

2- Cytogenetic analysis of blood samples

Freshly heparinized peripheral venous blood samples (2 ml each) were collected from each subject. Whole blood culture was done to increase the number of the cells and to visualize the metaphases stage easily according to the standard protocol (Gersen and Keagle, 1999). Each sample (0.5 ml of heparinized blood) was grown and maintained in 5 ml of aqueous growth media (RPMI 1640, Gibco) supplemented with 2% of L-glutamine, 12.5% of fetal calf serum (Biochrom), 100 units/ml of penicillin, 100 ug/ml of streptomycin (Biochrom) and phytohaemagglutinin (0.2ml/5ml) (PHA, Biochrom). The culture media were prepared under complete aseptic conditions in a biological safety cabinet (laminar air flow) at Cytogenetic and Tissue Culture Lab, Histology Department, Faculty of Medicine, Tanta University. Culture tubes were incubated at 37˚C in slanting position for 72 hours. The following assays were performed for each culture:

A) The Mitotic Index (MI) and structural chromosomal aberrations

In the present work, blood samples were collected from tramadol-dependent patients to study the possible chromosomal aberrations and changes in the mitotic index in human lymphocyte cultures using the solid staining technique as a method for the study according to the standard protocol of Watt and Stephen (1987). Two hours before harvesting, the cells were arrested in metaphase by the addition of 0.1 ug/ml of colcemid (colchicine) solution to each culture tube. Cells were collected by centrifugation at 1000 rpm for 10 minutes, re-suspended in a pre-warmed hypotonic potassium chloride solution (0.075M of KCl, 0.45%) for 15 minutes and were then fixed in glacial acetic acid-methanol (1:3, v/v) for three times. Few drops were dropped on wet cool slides, air-dried, and stained with 10% Giemsa for 15 minutes. Slides were examined using the oil immersion lens.

The mitotic index was determined by examination of 1000 lymphocytes from each culture and the percentages of dividing cells were measured. Moreover, 50 well spread metaphases from each individual were examined for chromosomal aberrations and the percentages of the recorded aberrations were

scored. The chi-square test (X2 test) was used for statistical evaluation of the data (Preston et al., 1987).

All aberrations such as gaps, breaks, dicentric chromosome, deletions and fragments were recorded. In this study, gaps were recorded and the mean values of total chromosomal aberrations were evaluated both excluding and including gaps as gaps were not considered as true discontinuities of the chromosome structure by some authors such as Tang et al. (1990).

B) Sister Chromatid Exchange technique (SCE)

For this technique, blood samples were cultured as those used for chromosomal aberration according to Saplakoglu and Iscan (1998). Thymidine analogue (bromodeoxyuridine 10 uM) was added 24 hours after culture initiation. Cells were then grown and harvested by the usual technique. The slides were stained using fluorescence plus Giemsa staining technique by staining the dried slides with diluted Hoechst (bisbenzimide 5 mg/ml, diluted 1:200). After rinsing the slides, they were immersed in sodium salt citrate and were irradiated with ultraviolet rays (254 nm for 60 minutes). The slides were stained with10% Giemsa for 15 minutes. The numbers of SCEs were scored in 50 complete and well spread metaphases in the second cycle of division (M2) per group and the mean number of SCEs per cell was calculated. The second division was identified by the dark staining of one chromatid and light staining of its sister chromatid (harlequin chromosome).

The data were analyzed using the SPSS 16.0 program (SPSS Inc, Chicago, Illinois, USA). The results were recorded as means and standard deviation for each group. Statistical analysis was done to compare the exposed groups with the control group using the Student`s t-test.

C) The micronucleus test

This technique was applied according to Surralles et al. (1992) by culturing the blood samples using the usual technique. Then, cytochalasin-B was added (6 ug/ml culture medium) at 44 hours from culture initiation. It was used to arrest cytokinesis of the dividing cells. Cultures were harvested at 72 hours by centrifugation. Short hypotonic treatment was carried out with 0.45% KCl for 3 minutes. Cells were then fixed by a fixative composed of 5 parts of methanol and 1 part of glacial acetic acid. This step was repeated twice. Finally, the centrifuged fixed cells were dropped on to clean and dry slides and stained with 10% Giemsa for 7 minutes.

The number of micronuclei was calculated by scoring 1000 binucleated cells with well preserved cytoplasm from each culture.

The results were recorded as the mean frequencies of the percentage for each group and then statistically analyzed using the chi-square test (X2 test). $P<0.05$ was considered statistically significant and $P<0.001$ was highly significant.

Results

Data were collected for a total of 30 male subjects aged 20-35 years (mean 28 years). Twelve cases of them (40%) were married and their occupations were different manual work-shops workers (e.g. Carpenter, plumper, painter,….), while nine cases of them (30%) were married highly educated personnel, followed by another six unmarried students (20%), then, three cases (10%) were unmarried highly educated personnel. At presentation of 20 male tramadol-dependent subjects, Glasgow Coma Scale (GCS) of (80% of them) ranged 7-13 (mean was 10±3). Only four patients of them (20%) had a GCS of less than 7 on admission. Meanwhile, fifteen patients from group II & III (75%) had experienced seizures (Figs. 1 and 2).

Results of genotoxicity study

A) The mitotic index (MI) and structural chromosomal aberrations

The control samples showed normal chromosomal pattern regarding number and structure (Fig. 3). However, a very low percentage of structural chromosomal aberrations were scored (1.8% including gaps and 0.5% excluding gaps). The scored aberrations were in the form of gaps and breaks. The mitotic index for this group was 8.9%.

Regarding specimens obtained from subjects exposed to tramadol for 2-3 years (Group II), there was a non-significant increase ($P> 0.05$) in the percentages of total chromosomal aberrations both including gaps (3.15%) and excluding gaps (1.4%). The scored aberrations were mostly in the form of gaps (Fig. 4) and breaks (Fig. 5). There was also a non-significant reduction in the mitotic index that was measured as 7.8%.

On the other hand, samples obtained from patients exposed to tramadol for 4-5 years (Group III) showed a highly significant increase ($P< 0.001$) in the percentages of total chromosomal aberrations both including gaps (8.4%) and excluding gaps (5.8%) compared with the control. The scored chromosomal aberrations were in the form of gaps, breaks, acentric fragments and other types of aberrations such as deletions, dicentric, and ring chromosomes (Figs. 6, 7, 8 & 9). Moreover, there was a marked proliferative inhibition with a statisitically significant decrease ($P<0.05$) in the mitotic index to become 6.2% compared with the control (Table 1).

B) Sister chromatid exchange (SCE)

By examination of cells in the second mitotic division (M2), it was found that the mean number of SCEs per cell was 4.2 ± 1.3 in the control group (Fig. 10). Regarding groups II & III, there was a highly significant increase in the mean number of SCEs per cell to become (7.4±1.5) & (9.6±1.4), respectively when compared with the control group (Figs.11 & 12; Table 2).

C) The micronucleus test

It was noted that the percentage of micronuclei in binucleated cells was 1.2% in control groups (Fig. 13). In group II, there was a significant ($P<0.05$) increase in

the mean frequencies of micronuclei in binucleated lymphocytes with a percentage of 3.5% compared with the control group (Fig. 14). Regarding group III, there was a highly significant (P<0.001) increase in the mean frequencies of micronuclei in binucleated cells with a percentage of 5.1% compared with the control (Fig. 15 & Table 3).

Table (1): The percentage of the total chromosomal aberrations and mitotic index in human lymphocyte cultures (number of metaphases/individual=50) from different studied groups (each n=10).

Groups	% of total chromosomal aberrations		Mitotic index
	Including gaps	Excluding gaps	
Group I (control)	1.8%	0.5%	8.9%
Group II	3.15%	1.4%	7.8%
Group III	8.4% **	5.8% **	6.2% *

**= significant compared with control, **= highly significant compared with control*

Table (2): The mean number of SCEs per cell in human lymphocyte cultures (number of metaphases /individual=50) from different studied groups (each n=10).

Groups	Number of subjects	Number of scored metaphases in M2/individual	Mean number SCEs/cell ± standard deviation
Group I (control)	10	50	4.2 ± 1.3
Group II	10	50	7.4 ± 1.5 **
Group III	10	50	9.6 ± 1.4 **

***= highly significant compared with control*

Table (3): The percentage of micronuclei in binucleated lymphocytes (number of scored binucleated cells /individual=1000) from different studied groups (each n=10).

Groups	% of micronuclei in binucleated cells
Group I (control)	1.2%
Group II	3.5% *
Group III	5.1% **

**= significant compared with control, **= highly significant compared with control*

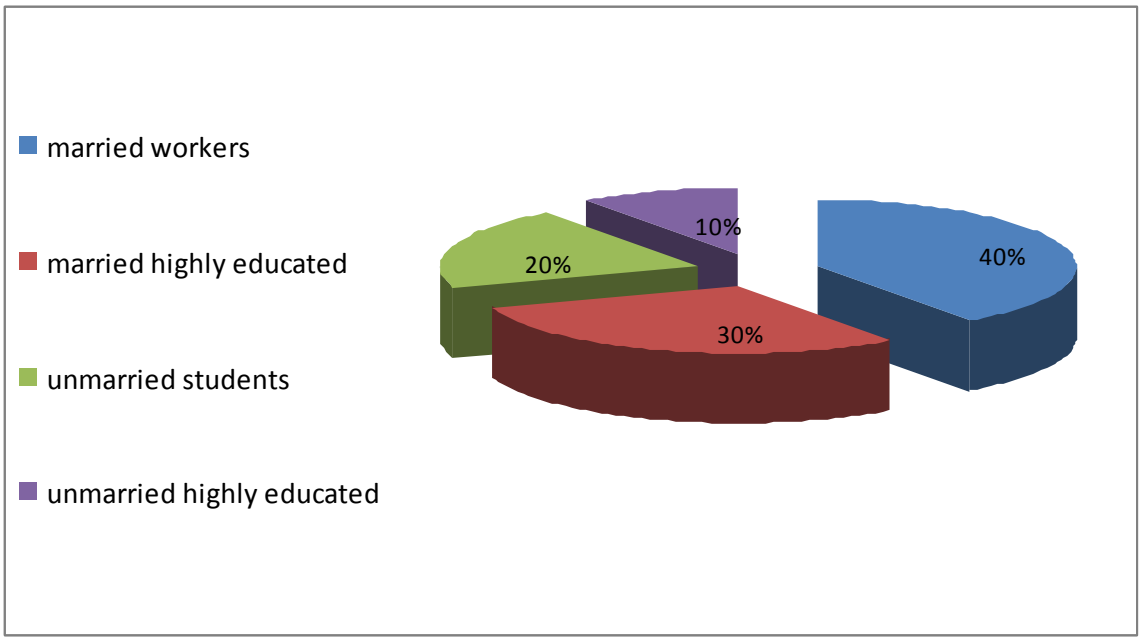

Figure (1): Pie chart showing the percentage of marital state and occupation of 30 male subjects from different studied groups (each n=10).

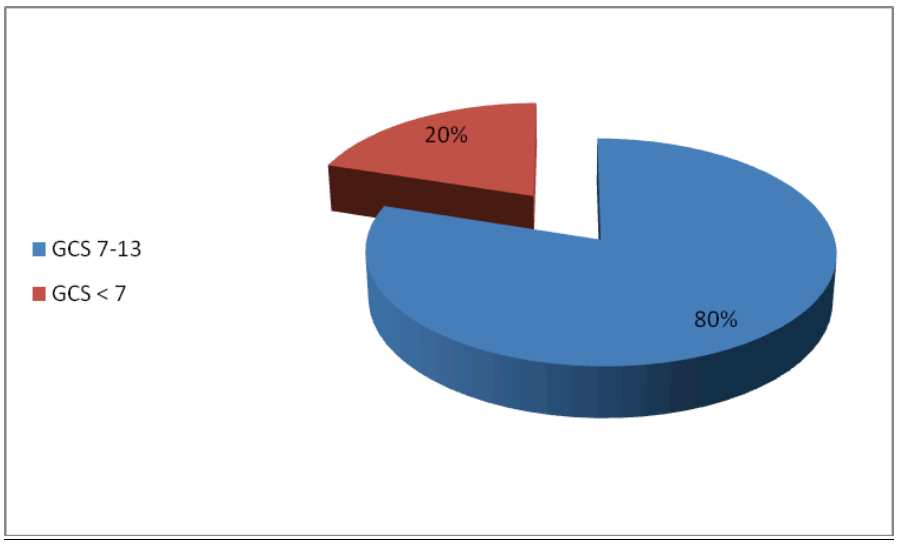

Figure (2): Pie chart showing the percentage of Glasgow Coma Scale (GCS) at presentation of Groups II & III tramadol-dependent subjects (each n=10).

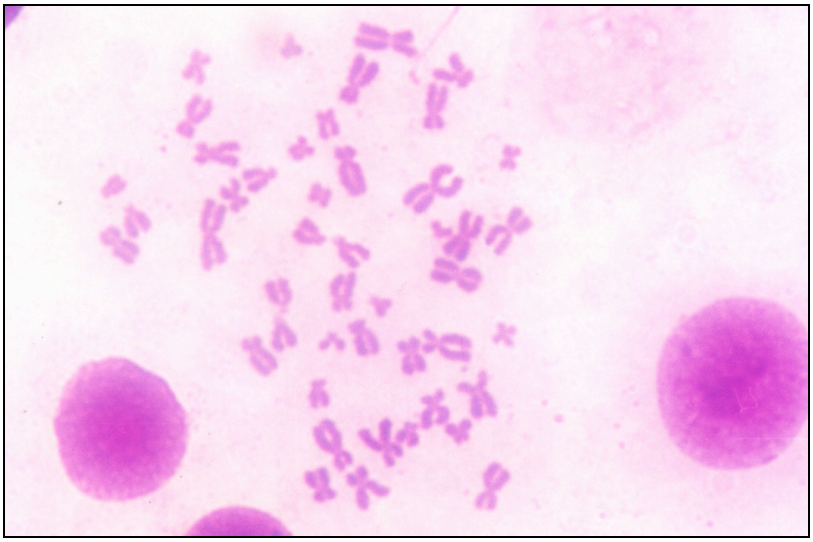

Figure (3): A photomicrograph of a metaphase spread of lymphocytic cultures from control group showing normal chromosomal pattern (Giemsa stain x1000).

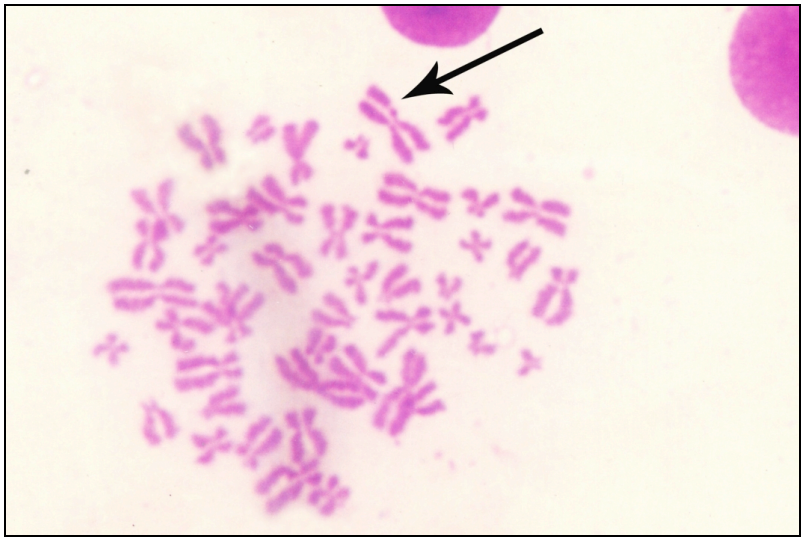

Figure (4): A photomicrograph of a metaphase spread of lymphocytic cultures from Group II showing a gap in one chromosome (→) (Giemsa stain x1000).

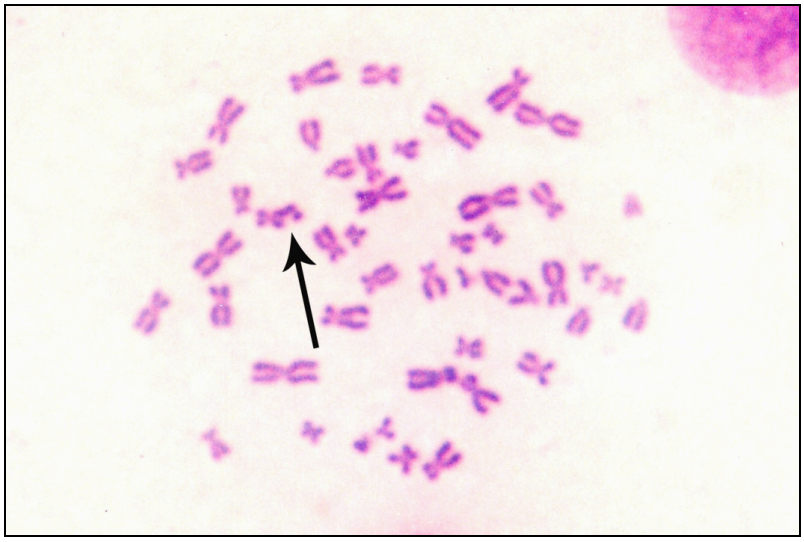

Figure (5): A photomicrograph of a metaphase spread of lymphocytic cultures from Group II showing a break in one chromosome (→) (Giemsa stain x1000).

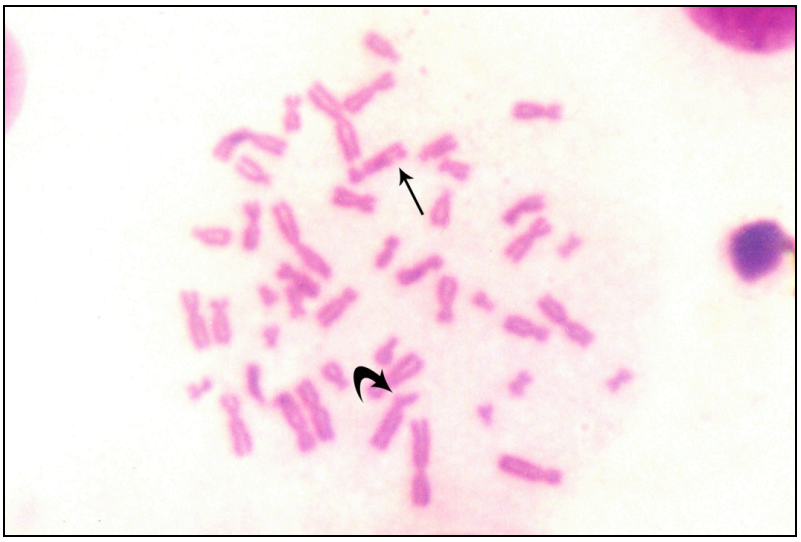

Figure (6): A photomicrograph of a metaphase spread of lymphocytic cultures from Group III showing a gap in one chromosome (→) and a deletion in another chromosome (curved arrow) (Giemsa stain x1000).

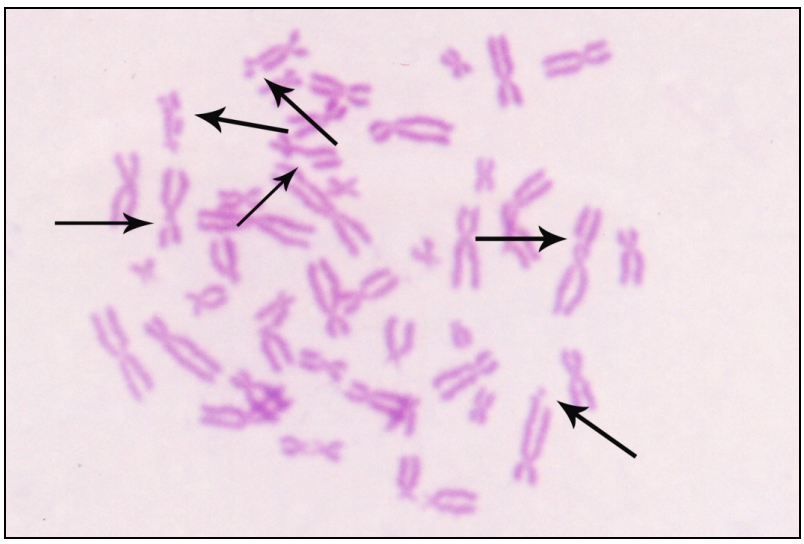

Figure (7): A photomicrograph of a metaphase spread of lymphocytic cultures from Group III showing multiple chromosomal breaks (→) (Giemsa stain x1000).

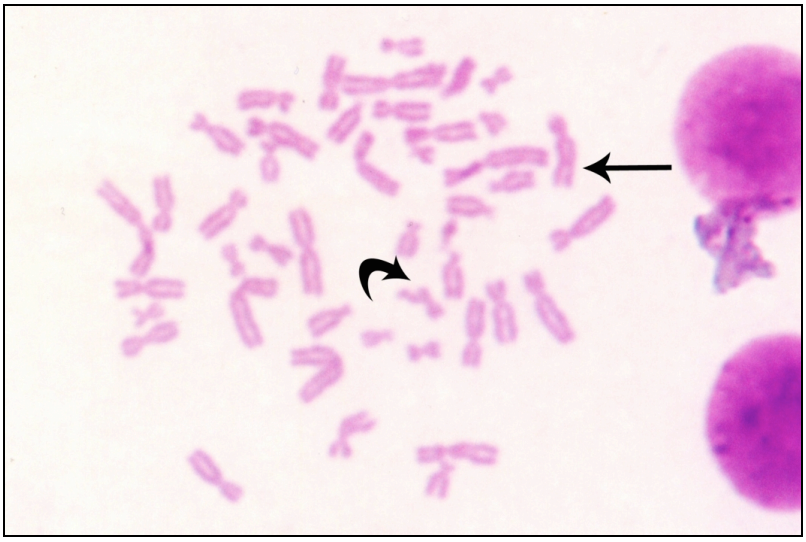

Figure (8): A photomicrograph of a metaphase spread of lymphocytic cultures from Group III showing a deletion in one chromosome (curved arrow) and a dicentric chromosome (→) (Giemsa stain x1000).

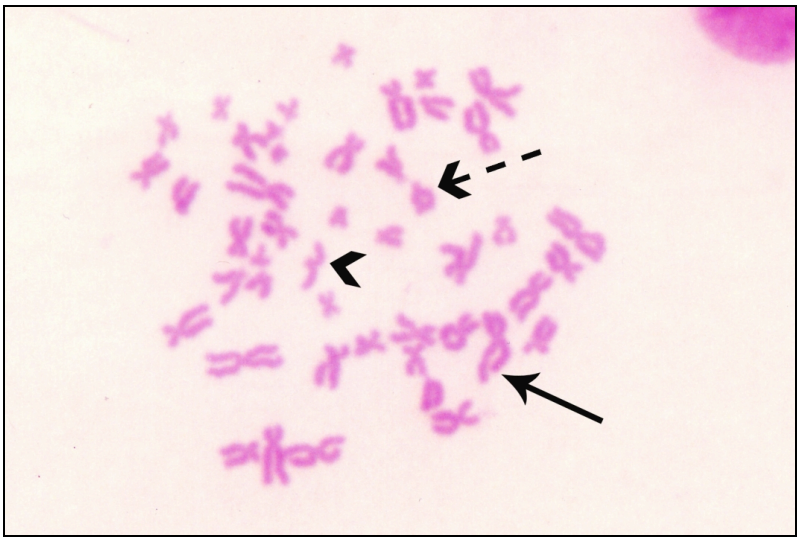

Figure (9): A photomicrograph of a metaphase spread of lymphocytic cultures from Group III showing a deletion in one chromosome (→), acentric fragment (>) and a ring chromosome (- - >) (Giemsa stain x1000).

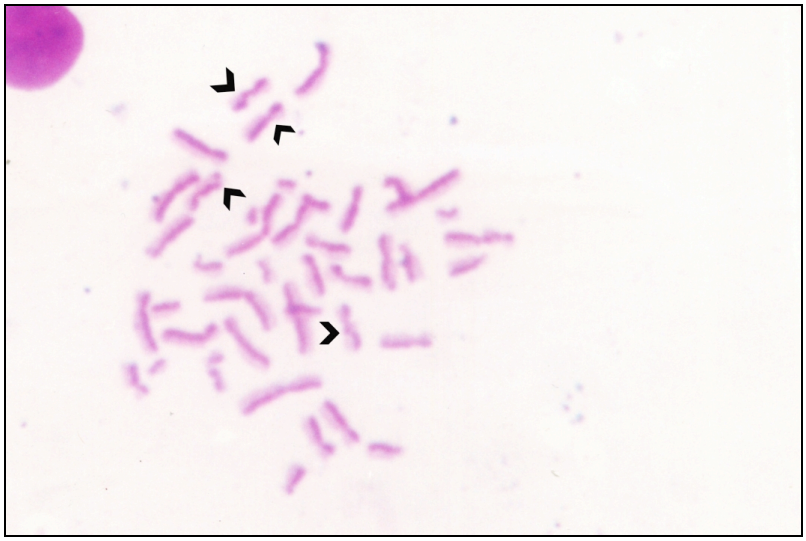

Figure (10): A photomicrograph of a metaphase spread of lymphocytic cultures from control group showing 4 exchanges between sister chromatids (>) (Fluorescent plus Giemsa stain x1000).

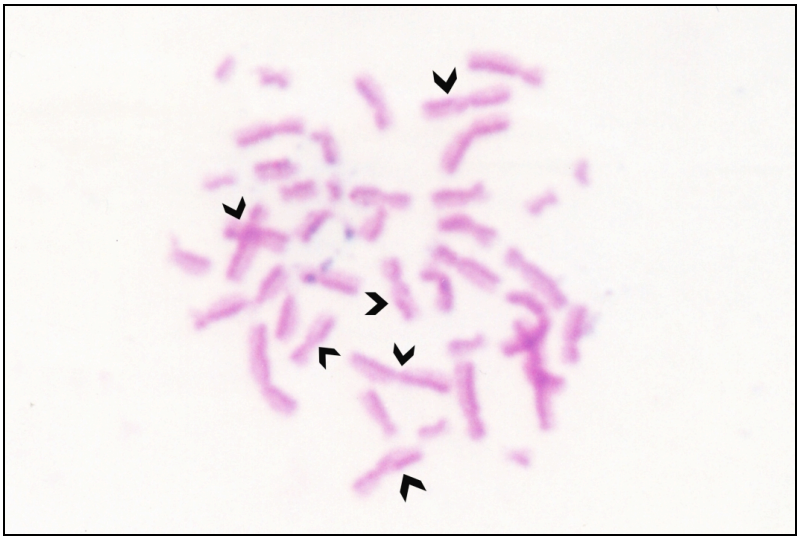

Figure (11): A photomicrograph of a metaphase spread of lymphocytic cultures from Group II showing 6 exchanges between sister chromatids (>) (Fluorescent plus Giemsa stain x1000).

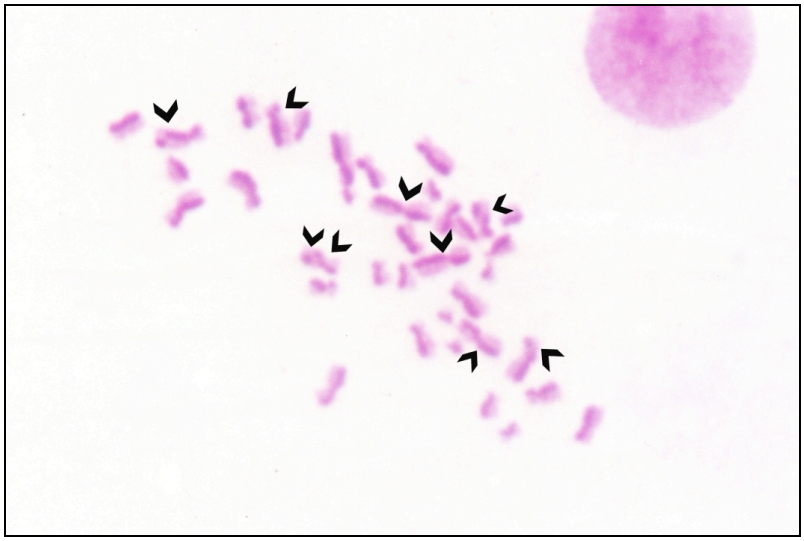

Figure (12): A photomicrograph of a metaphase spread of lymphocytic cultures from Group III showing 9 exchanges between sister chromatids (>) (Fluorescent plus Giemsa stain x1000).

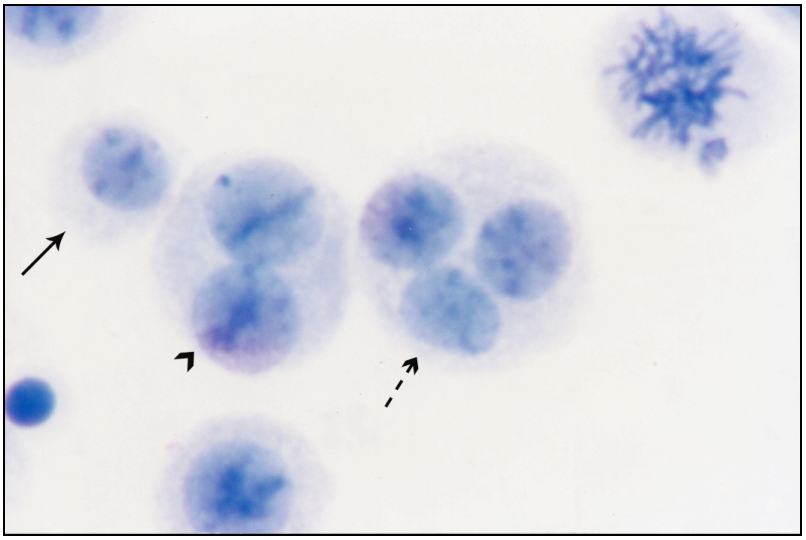

Figure (13): A photomicrograph of a human lymphocyte culture from the control group showing normal binucleated (>), mononucleated (→), and tri-nucleated cells (- - >) (Giemsa stain x1000).

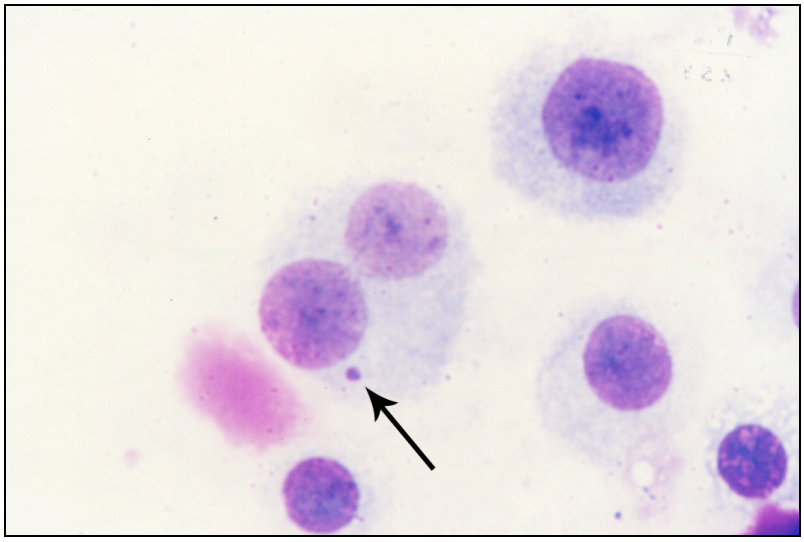

Figure (14): A photomicrograph of a human lymphocyte culture from group II showing a binucleated cell with a micronucleus (→) (Giemsa stain x1000).

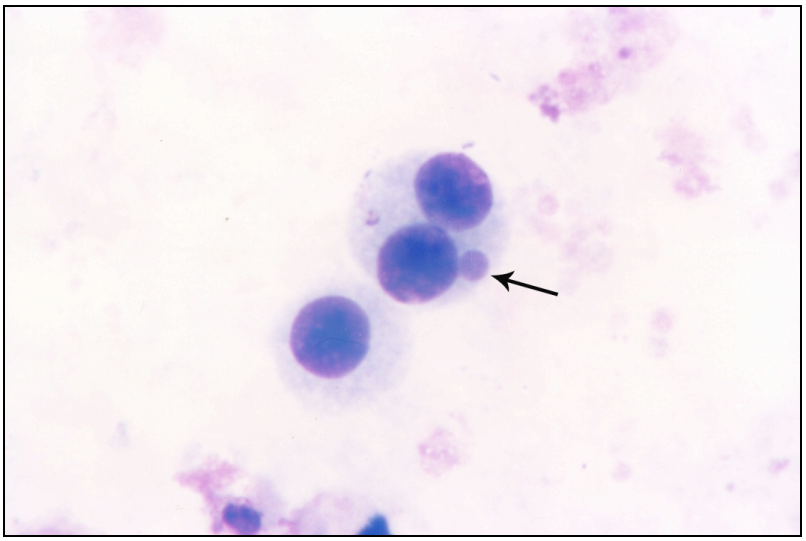

Figure (15): A photomicrograph of a human lymphocyte culture from group III showing a binucleated cell with a micronucleus (→) (Giemsa stain x1000).

Discussion

Tramadol is a synthetic opioid; its exact mechanism of action is unclear but thought to be the result of low binding affinity at μ-opioid receptors through an active metabolite (O-desmethyltramadol) with a possibly shorter half-life. It also inhibits the reuptake of norepinephrine and serotonin. Furthermore, tramadol as opposed to typical opioids, has not been associated with significant opioid side effects (e.g., respiratory depression, sedation, constipation) (Lesani et al., 2010). Several studies have indicated that long-term use of tramadol can precipitate tolerance, physical dependence and withdrawal symptoms due to augmented glutamate release and activation of the ionotropic N-methyl-D-aspartate (NMDA) subtype of glutamate receptors which result from the subsequent activation of nitric oxide synthase (NO synthase) and the formation of nitric oxide (NO) (Miranda and Pinardi, 1998; Abdel-Zaher et al., 2011).

In the current study, the participants were adult males (ranging from 20-35 years old). Seventy percentages of cases were married, while 30% of them were unmarried. This result coincided with the result obtained by Bar-Or et al., (2012). This could be explained by the report that tramadol is an effective treatment for premature ejaculation, resulting in a significant prolongation of intravaginal ejaculation latency time (IELT) that showed clinical improvement in satisfaction with sexual intercourse and control over ejaculation, and decreases in ejaculation-related personal distress and interpersonal difficulty. Tramadol acts as a mild μ-opioid agonist and, N-methyl-D-aspartate receptor antagonist, 5-hydroxytryptamine type 2C receptor antagonist, 5 nicotinic acetylcholine receptor antagonist, M1 and M3 muscarinic acetylcholine receptor antagonist, and a serotonin and norepinephrine modulator. It is possible that one or a

combination of these effects leads to a delay in ejaculation (Bar-Or et al., 2012).

In the current study, Glasgow Coma Scale (GCS) of most patients at presentation ranged 7-13 (mean was 10±3). Only, four patients of them (20%) had a GCS of less than 7 on admission. GCS was considered as a tool for recording the level of consciousness at a particular moment, whatever the cause of impairment, systematically and reproducibly. Repeated recordings can give an impression of deterioration or improvement. It has gained acceptance in the management of poisoned patients presenting to the Emergency Department (ED). It has also been said that a GCS of 8 or less is a useful guide for the requirement of endotracheal intubation where the cause of coma is poisoning (Isbister et al., 2004; Duncan and Thakore, 2009). This could be explained by the report that during the whole clinical course of oral tramadol overdose alone, a variety of poisoning symptoms were observed, including respiratory depression, seizures, hyper-reflexia, tachycardia, coma or unconsciousness and lastly, multiple organ dysfunction syndrome (Decker et al., 2008).

The current work also, demonstrated that 75% of admitted patients had experienced seizures. As opioid analgesics can induce an anticonvulsant or proconvulsant effect, depending on the dose of opioid and the seizure model, whereas animal studies have indicated that convulsions occur only at high, toxic doses (Potschka et al., 2000). Tramadol mediates its seizurogenic effect by overactivation of opioid receptors (Rehni et al., 2008). In contrast, it has been reported that tramadol induces its anticonvulsant effect in maximal electroshock seizures in mice and also in rats. It is known that tramadol slightly suppresses the severity of seizures at clinically relevant doses; however, at high doses, tramadol paradoxically induces seizures both in monotherapy and in concomitant therapy with other drugs. It has been demonstrated that the κ-opioid receptor subtype, alone or independently with the GABA receptor, plays a role in the mediation of the anticonvulsant effect of tramadol on maximal electroshock in mice. Other data has shown that tramadol exerts a seizurogenic effect on mice treated intraperitoneally with pentylenetetrazole (PTZ) through an opioid-dependent GABA-inhibitory pathway. Tramadol also exerts anticonvulsant activity at doses within the analgesic range in the kindling model, but the mechanisms involved are not known (Lesani et al., 2010).

The present work showed evident genotoxicity of tramadol as revealed by significant elevation in the frequencies of chromosomal aberrations, SCEs and micronuclei in cultured lymphocytes from tramadol-dependent patients which were related to the duration of dependence. In agreement with this finding, it has been reported that heroin addicts have elevated frequencies of chromosomal aberrations in peripheral blood lymphocytes compared to the general population (Falek et al., 1987; Sawant et al., 2001). In addition, the genotoxicity of morphine following acute in vivo administration of the drug has been demonstrated in a variety of test systems, including the induction of micronuclei in murine reticulocytes and splenocytes (Das and Swain, 1982; Sawant et al., 2001). Moreover, administration of morphine to mice produced dose-related increases in the frequencies of micronucleated cells and sister chromatid exchanges in bone marrow cells in vivo, suggesting its genotoxic effect. The damage appears to arise by an indirect mechanism, as incubation of isolated human or mouse lymphocytes with morphine in vitro does not increase the frequency of chromosomal aberrations or micronucleated cells indicating that the genotoxic response is opioid receptor-mediated (Sawant and Couch., 1995). Moreover, results in the mouse lymphoma assay indicated an in vitro genotoxic activity for tramadol at toxic doses. These findings are suggestive of chromosomal effects secondary to the high level of induced cytotoxicity (Matthiesena et al., 1998). An alternative explanation for the *in vivo* micronucleus effect is that it is a consequence of hypothermia, which is caused in rodents by morphine (Baker and Meert., 2002).

In the present work, a highly significant increase in the mean number of SCEs per cell was observed in all groups of tramadol-dependent patients compared with the control group. It was stated that SCE represents the reciprocal interchange of DNA between chromatids at homologous loci, which does not result in an overall alteration of chromosome morphology. The exchange process presumably involves DNA breakage and reunion. Mutagens can induce SCEs at concentrations much lower than those which induce chromosomal aberrations, so SCE has been considered to be an extremely sensitive indicator for assessing potential mutagens at subtoxic doses and short exposure time (Ishidate et al., 1988; Duydu and Süzen, 2003). It has been suggested that SCE may be directly related to point mutation or to misreplication of a damaged DNA (Li and Heflich, 1991).

Micronuclei were identified by Karmakar et al., (1998) as small chromatin-containing bodies arising from acentric chromosome fragments or whole chromosomes that were not incorporated into daughter nuclei following mitosis. Therefore, the micronucleus test was considered as a biomarker of either chromosomal breaks or damage of the mitotic apparatus resulting in chromosome loss.

This genotoxic effect of tramadol could be explained by activation of the hypothalamo–pituitary–adrenal (HPA) axis, resulting in increase in serum corticosterone levels. Several results indicated that steroids are involved in the production of micronuclei by morphine, but it could not be concluded that

activation of the HPA axis is required or that steroids are directly responsible for the genotoxic response. Moreover, morphine has been shown to inhibit UV-induced unscheduled DNA synthesis and to increase the frequency of UV-induced sister chromatid exchanges in human lymphocytes, and it is possible that it may inhibit the repair of genetic damage induced by endogenous glucocorticosteroids as well (Sawant et al., 2001; Aardema et al., 2008). Morphine was also shown to induce DNA strand breaks in the comet assay in cultured human lymphocytes (Shafer et al., 1994). This fact was supported by the observation that morphine has induced DNA fragmentation and apoptosis in human peripheral lymphocytes (Singhal et al., 2001).

In contrast, a number of genotoxicity studies on the natural opioid morphine, have reported negative results in limited bacterial mutation assays (Friesen et al., 1985) and in vitro clastogenicity assays using both Chinese hamster ovary CHO cells (Ishidate and Harnois, 1987) and human lymphocytes (Falek and Hollingsworth, 1980). Some studies showed that morphine produced no evidence of chromosomal aberrations when incubated with murine splenocytes (Tweats, et al., 2007).

It is possible that the differences in drug sensitivity between subject populations may account in part for the discrepancy in different studies. The difference in doses may be an issue in which tramadol did not produce opiate-like effects in opiate abusers (Cami et al., 1994). Another possibility is related to the differential pharmacokinetics of tramadol when administered orally versus parenterally. When administered orally, first-pass O-demethylation metabolism of tramadol produces an active metabolite (O-desmethyltramadol) which is thought to be primarily responsible for the opioidergic effects of tramadol (Raffa and Friderichs, 1996). Because of the first-pass effect, more O-desmethyltramadol is produced following oral administration of tramadol than if the drug were given parenterally. This may be why opiate-like effects were obtained in the Jasinski et al., (1993) study but not in the studies in which tramadol was administered parenterally (Cami et al., 1994). Moreover, the metabolism of tramadol to its opioidergic active metabolite is slow in humans (Grond and Sablotzki, 2004), as measured by the time it takes to reach maximum plasma concentrations (Nobilis et al., 2002). This slow rate of metabolism could explain the slower onset of opioid-like effects of tramadol relative to other mu opioid agonists, as noted in Knaggs et al., (2004) studies.

Conclusions

The present work showed elevation in the frequencies of chromosomal aberrations, SCEs and micronuclei in cultured lymphocytes from tramadol-dependent patients which were related to the duration of dependence.

Recommendations

We recommend further studies on animals and/or human to study the protective and preventive effects of different anti-oxidants, also to know if this geno-toxic effect is reversible or not. Further studies are recommended to verify the reversibility of the genotoxicity after withdrawal of tramadol.

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الملخص العربي

التقييم الوراثي الخلوي للسمية الجينية في الخلايا الليمفاوية المستنبتة من بعض المصريين مدمني الترامادول

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الترامادول هو مسكن صناعي أفيوني المفعول واسع الاستخدام للوقاية والعلاج من الآلام المتوسطة والشديدة في الحالات الحادة أو المزمنة، وقد أجريت العديد من الدراسات لتقييم مدى أمان استخدامه ومن بين التفاعلات الضارة المختلفة والمحتملة للجرعة الزائدة من هذا الدواء هي السمية الجينية المحتملة. وقد كان الغرض من هذه الدراسة تقييم التأثيرات السمية الجينية المحتملة لإدمان الترامادول في البشر باستخدام دراسة تشوهات الكروموسومات، التبادل الكروماتيدي الشقيقي و الأنوية الدقيقة. وقد أجري هذا البحث على 30 شخصًا من الذكور البالغين تراوحت أعمارهم بين 20 و35 عامًا، واستخدم 10 أشخاص كمجموعة ضابطة، 10 أشخاص كانوا قد تناولوا الترامادول بالفم لمدة 2 3 سنوات، و 10 أشخاص آخرين كانوا قد تناولوا الترامادول بالفم لمدة 4 5 سنوات، وقد كان الأشخاص السابقين قد أدخلوا إلى مركز طنطا للسموم في الفترة من يونيو 2011 إلى يناير 2012 وقد تم تشخيصهم على أنهم مدمنين للترامادول قد تعاطوا جرعة مفرطة منه. وأظهرت النتائج زيادة بنسبة تشوهات الكروموسومات، التبادل الكروماتيدي الشقيقي، و الأنوية الدقيقة في الخلايا الليمفاوية المزروعة من المرضى المدمنين للترامادول والتي كانت مرتبطة بمدة تعاطي الترامادول.

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