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Renal Effects of Tramadol Addiction and Cannabinoid Abuse.

El-Safty IAM [1], Mohy El-Deen IE [2], El-Rasheed AH[3], and El-Ashmawy MFM [4]*.

[1]Biochemistry Section, Chemistry Department, Faculty of Education, Ain-Shams University, Cairo, Egypt.
[2]Head of Chemistry Department, Faculty of Science, Port-said University, Egypt.
[3]Institute of Psychiatry, Faculty of Medicine, Ain-shams University, Cairo, Egypt.
[4]Chemistry Department, Faculty Of Science, Port-Saed University, Egypt.

ABSTRACT

This study investigates early changes in function and structure of glomeruli and proximal tubules due to tramadol addiction alone and in combination with cannabinoid. This included 72 males (G_1 = 23 controls, G_2 = 21 tramadol addicts and G_3 = 28 tramadol coabused with cannabinoid addicts). We measured urinary parameters; urinary total protein (U.TP), urinary microalbumin (U.μ-alb), urinary alpha-1-microglobulin (U.α_1-m), urinary leucine aminopeptidase (U.LAP), urinary N-acetyl-β-D-glucosaminidase (U.NAG). Urinary tramadol (U.Tr) was measured in G_2 and G_3, while urinary cannabinoid (U.THC) was measured in G_3. In G_2, levels of U.TP and U.μ-alb were decreased while U.α_1-m, U.LAP and U.NAG were increased in comparison with G_1. These changes were insignificant. In G_3, all parameters were increased insignificantly when compared with each of G_1 and G_2. In addition, U.THC was significantly correlated with U.α_1-m ($r = 0.507$, $P < 0.01$) and U.LAP ($r = 0.888$, $P < 0.01$) in G_3, while U.Tr did not show any correlation with any parameter in G_2 or G_3. Tramadol addiction may affect only proximal tubules, while tramadol addition coabused with cannabinoid may cause glomerular functional impairment and increase the proximal tubular dysfunction than tramadol addition alone.

Keywords: Tramadol, cannabinoid, Microalbumin, Alpha-1-microglobulin, Leucine aminopeptidase, N-acetyl-beta-D-glucosaminidase

Corresponding author

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INTRODUCTION

In Egypt, drug addiction is considered one of the serious problems that worry both people and the government. It affects young people within their productive years. It may lead to many problems such as social maladaptation, decreased work productivity and job loss [1].

Tramadol (Tr), (2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol), is a synthetic opioid analgesic of the aminocyclohexanol type [2]. It was first synthesised in 1962. It was registered in 1977 in Germany [3], 1994 in the UK, and 1995 in the US [4]. Recently, it was reported that the synthetic analgesic Tr is a true natural product which occurs in the roots of the *nauclea latifolia Sm.* (Rubiaceae) plant, commonly known as African peach or pincushion tree [5]. Also, the three major mammalian metabolites mono-O-demethyl-tramadol (M1), mono-N-demethyl-tramadol (M2) and 4-hydroxycyclohexyltramadol in the roots of *N. latifolia* and five other plant species, and also in soil and local water bodies only in the Far North region of Cameroon [6]. Clinical and experimental studies demonstrated that Tr did not induce tolerance and dependence on repeated administration [7,8]. On the other hand, results of other studies suggest that Tr may have abuse liability under some conditions or in certain populations [9,10]. Post-marketing surveillance studies consistently showed that the abuse and diversion of Tr was relatively low [11]. However, a significant finding was that for the cases of Tr abuse, 97% of the drug addicts used Tr in combination with other drugs or they had a previous history of addiction to substance of abuse [12], other studies suggest that Tr with no history of substance abuse has a high risk of producing dependence potential under a long period and/or high doses [13]. Tr undergoes extensive and complex metabolism in the liver via cytochrome P450 system, with 23 metabolites identified: 11 phase I identified and 12 phase II conjugates [14]. Around 90% of the Tr was excreted in the urine, the residual appears in the feces [15]. 30 % of the Tr is excreted through the kidneys unmetabolised, while the remaining is metabolised by O-and N-demethylation, followed by conjugation with glucuronic acid and sulphates [16,17].

Bango is the name of cannabis leaves used in Egypt and North Africa. There has been noticeable increase in consumption of cannabis and its products among teenagers and adults [18]. The crude drug derived from the plant *cannabis sativa* is called marijuana which contains more than 489 compounds, of which 77 are defined as cannabinoids [19], based on their typical 21-carbon structure [20]. Among the 66 different cannabinoids, Delta-9-tetrahydrocannabinol (Δ^9-THC) were first extracted from cannabis in 1942 [21]. Δ^9-THC is major psychoactive constituent of marijuana, it was isolated in pure form and its structure was elucidated by Gaoni and Mechoulam [22], is rapidly hydroxylated to an active metabolite by the hepatic cytochrome P450 enzyme system [23], to active 11-hydroxy-delta-9-tetrahydrocannabinol, which is intermediate for further metabolism to inactive 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and its glucuronide and sulfate conjugates. THC-COOH is the major metabolite of Δ^9-THC in urine [24]. Only negligible amounts of cannabinoid are excreted as unchanged drug [25]. Cannabis use, despite being the most wide-spread of the illicit substances, caused very few deaths due to its low toxicity. Six deaths due to acute cardiac problems [26], and several reports of renal infarctions associated with cannabis addiction [27, 28] have been described.

Metabolites of the drugs that are excreted from kidneys may cause cellular damage leading to kidney dysfunction. Various urinary parameters of the kidney such as µ-alb and α_1-m were proved useful to assess functional integrity of glomeruli and proximal tubules respectively, whereas urinary kidney-specific enzymes such as brush-border LAP and lysosomal NAG are indicators for structural integrity of proximal tubules [29].

The present work is a preliminary study to investigate the effect of Tr addiction among Egyptian drug addicts on some aspects of glomerular and proximal tubular functions as well as structural integrity of proximal tubules by measuring urinary parameters as indicators of early alterations of the kidney function. In addition, the study is extended to evaluate the effect of cannabinoid addiction when it is co-abused with Tr.

MATERIAL AND METHODS

Preparation

Male drug addicted participated in the present study were recruited on voluntary bases from those attended the out-patient clinic, Institute of Psychiatry, Ain-Shams University, Cairo, for treatment of drug addiction. All participants were subjected to interview using a questionnaire designed to obtain information on

previous medical and occupational history, medication intake, actual health status, and subjective symptoms. All subjects underwent a routine clinical examination and a routine urinalysis. The interview and clinical examination were performed by the clinic physicians under the supervision of one of the authors. The drug addicts were excluded from the present study if they had; a history of kidney disease or any disease likely to impair renal function or affect the urinary excretion of the investigated parameters (e. g. diabetes mellitus, hypertension, urinary tract disease), a previous or present exposure to agents capable of damaging the kidney (heavy metals such as lead, cadmium and other nephrotoxins such as organic solvents), Regular and prolonged treatment by drugs affecting the kidney (e.g. aminoglycosides), Dental mercury amalgam fillings as it may affect the kidney.

Urine sampling & Chemicals

Morning urine sample was suggested as the best sample for detecting early kidney abnormalities [30]. Spot morning urine sample was collected by each participant who was instructed to void the urine sample directly into 100 ml sterilized plastic container and centrifuged at 4500 rpm for 5 minutes, and then the clear supernatant was distributed in polyethylene vials (1.5 ml capacity). One vial was used on the same day of urine was collected for measuring main metabolites of Tr (M1, M2) and cannabinoid (THC-COOH) in urine using Immunalysis Tramadol EIA kit (Immunalysis Corporation, USA), DRI® Cannabinoid assay kit (Microgenics, USA), respectively, and the instrument Biolis 24i Premium (Tokyo Boeki Medical System, Japan). According to the manufacturer of the kits, both methods have 100% correlation with GC/MS when 200 ng/ml and 50 ng/ml cutoff calibrator are used, respectively. U.THC was measured in the same day of urine collection to reduce its adsorption onto the surface of the plastic container and possibility of its degradation during storage [31]. The rest of the vials were stored at -20^{0}C without preservatives until analyzed within 2 weeks for the assessment of Glomerular function by measuring U.TP using dye-binding method kit (Stanbio Laboratory, USA), U.μ-alb using ELISA method kit (Orgentec Diagnostika GmbH, Germany), Proximal tubular function by measuring U.α_1-m using ELISA method kit (Assaypro, USA), and the instrument Plate Reader 8 channel ELISA photometer (das srl, Italy), While proximal tubular structural integrity by measuring urinary activities of U.LAP by Colorimetric method kit (Randox UK) and U.NAG by Colorimetric method kit (Diazyme USA). And measuring U.Cr using kit (Greiner Diagnostic GmbH, Germany), and the instrument Analyzer 90 photometer (das srl, Italy).

Statistical Analysis

Data were presented as mean ± SD. Student t-test and ANOVA were used to compare between the means of parametric data, while Mann-Whitney test and Kruskall-Wallis were used for non-parametric data. Correlation coefficient (r) was calculated to test the association between two quantitative variables. P-values < 0.05 were considered statistically significant. SPSS version 15.0 was used.

RESULTS

Seventy two males were included in the study. G_2 was comprised of 21 males (age: 18 - 40 y, mean ± SD: 28.71 ± 6.64, addiction duration: 6 months – 17 y, mean ± SD: 4.41 ± 4.12), and G_3 was comprised of 28 males (age: 16 - 40 y, means ± SD: 26.82 ± 5.45, addiction duration: 3 - 20 y, mean ± SD: 7.86 ± 4.83). Another 23 G_1 (age: 19 - 38 y, mean ± SD: 25.44 ± 5.25) were recruited from relatives of the addicted participants after applying the same exclusion criteria and clinical examination.

Spot urine measurements were used because it has been shown that U.TP/U.Cr [32], and U.μ-alb/U.Cr [33], as well as enzyme activity/creatinine [34], in a random urine sample correlate with 24-hour urinary excretion and eliminate variations caused by changing rates of urine output and provide a measure independent of urine concentration.

Table 1: Correlation Coefficient (r) between age and urinary parameters of G_1

	U.TP/U.Cr	U.μ-alb/U.Cr	U.NAG/U Cr	U.LAP/U Cr	U.α_1-m/U.Cr
Age	0.185	-0.149	0.057	0.492*	0.521*

*r is statistically significant as (P < 0.05).

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Table 2: Comparison (Mean ± SD) between variables of the different studied groups

Parameter	G_1 (N=23)	G_2 (N=21)	G_3 (N=28)	P
A) Age (years)	25.44 ± 5.25	28.71 ± 6.64	26.82 ± 5.45	> 0.05
B) Glomerular Functional integrity				
U.TP/U.Cr (mg/mg cr)	98.59 ± 84.66	80.65 ± 70.18	124.50 ± 155.88	> 0.05
U.μ-alb/U.Cr (μg/mg cr)	17.85 ± 19.16	11.38 ± 8.11	22.21 ± 40.72	> 0.05
C) Tubular Functional integrity				
U.α_1-m/U.Cr (μg/ mg cr)	7.70 ± 5.18	10.42 ± 11.89	10.94 ± 12.00	> 0.05
D) Tubular Structural integrity				
U.NAG/U.Cr (U/mg cr)	9.15 ± 6.21	11.35 ± 9.57	13.10 ± 14.76	> 0.05
U.LAP/U.Cr (U/mg cr)	5.52 ± 3.68	7.45 ± 14.03	9.65 ± 14.76	> 0.05

N (Number of volunteer).

Table 3: Correlation coefficient (r) between different urinary parameters and levels of Urinary tramadol addicted groups

Uninary parameter	G_2	G_3	
	U.Tr/U.Cr	U.Tr/U.Cr	U.THC/U.Cr
U.TP/U.Cr	-0.121	-0.157	0.360
U.μ-alb/U.Cr	-0.124	-0.154	-0.185
U.α_1-m/U.Cr	-0.252	-0.239	0.507**
U.NAG/U.Cr	0.104	-0.208	0.211
U.LAP/U.Cr	0.095	-0.178	0.888**

** r is highly significant as (P < 0.01), U. Tr/U. Cr (Urinary Tramadol urinary creatinine ratio), U.TP/U.Cr (Urinary total protein urinary creatinine ratio), U.μ-alb/U.Cr (Urinary microalbumin urinary creatinine ratio), U.α_1-m/U.Cr (Urinary alpha-1-microglobulin urinary creatinine ratio), U.LAP/U.Cr (Urinary Leucine-aminopeptidase urinary creatinine ratio), U.THC/U.Cr (Urinary cannabinoid urinary creatinine ratio), U.NAG/U.Cr (Urinary N-acetyl-β-D-glucosaminidase urinary creatinine ratio), G_1 (Controls group), G_2 (Tramadol addicts group), G_3 (Tramadol coabused with cannabinoid addicts group).

DISCUSSION

Data of the present study (Table 1) showed among G_1 a positive correlation between age and each of U.LAP (r= 0.492. P= 0.017) and U.α_1-m (r= 0.521, P= 0.011). Also, regarding the age, the present results (Table 2) showed insignificant difference (P > 0.05) between G_1 and each of G_2 and G_3. Urinary parameters of glomerular function were decreased in G_2 as compared to G_1 while function and structure of proximal tubules was increased (Table 2). These changes were statistically insignificant (P > 0.05). The results (Table 2) revealed insignificant increase (P > 0.05) in the urinary parameters among subjects in G_3 as compared to G_1 or G_2. Furthermore, there was a significant correlation between U.THC/U.Cr and each of U.α_1-m/U.Cr and U.LAP/U.Cr in G_3, while U.Tr/U.Cr showed no correlation with any of the measured urinary parameters in G_2 or G_3 (Table 3).

Results of the present study (Tables 1 and 2) demonstrated that structural and functional integrity of proximal tubules are deteriorated with age, supporting the report that structural and physiological changes in the kidney are associated with aging [35], and that G_1 matched with both G_2 and G_3 to avoid the effect of age on the measured urinary parameters. Substances with the potential to be abused may have direct or indirect effects on physiologic mechanisms that lead to organ system dysfunction and disease. A multitude of renal diseases are associated with drug abuse because of many different substances used with widely varying pharmacologic effects. Such drugs have been associated with several renal syndromes by varied mechanisms [36].

Albumin is the major plasma protein, and protein uptake is via a constitutive reabsorption pathway in the proximal tubule cells [37]. The structural and functional changes to the proximal tubule cells are a key contributing factor to the development of excessive albumin loss in urine (albuminuria). Albumin present in the urine is often the first indicator of glomerular damage [38], and a decline in renal function. Results of the present study (Table 2) for the effect of Tr addiction on glomerular function showed insignificant decrease in

U.TP and U.μ-alb in G_2 as compared to G_1, suggesting no glomerular damage. This result is an accordance with finding of other investigators who reported that no histological changes in the glomeruli were observed in experimental rats given Tr intraperitoneally at doses of 20, 40 and 80 mg/kg/day in the first, second and the third ten days of the study, respectively [39].

It was reported that THC is the principle constituent of cannabinoid [40], and the concentration of THC from autopsy tissues (liver, kidney, spleen, stomach and intestine) was highest in the kidney followed by the liver [41]. Subjects used cannabinoid in combination with Tr showed increased levels of U.TP and U.μ-alb in G_3, and this may be due to increase glomerular filteration of these substances in comparison with G_1 and G_2, but the difference of this increase was statistically insignificant (Table 2). This suggestion is supported by the report that heavy marijuana use caused membranous glomerulonephritis due to granular deposits of immunoglobulin G and C9 along the outer surface of the capillary wall in all glomeruli [42]. Also, other investigator s showed that histopathological examination of kidney tissues of experimental animals given the extract of cannabis leaves showed destruction of some of the renal corpuscles which are formed of a glomerulus and Bowman's capsule [43].

Although U.TP provides information of severity of proteinuria, it is protein type that renders a more specific picture of protein composition of urine. Data of the present study (Table 2) for the effect of Tr addiction on proximal tubular function showed an increase in the urinary excretion of α_1-m in G_2, suggesting impairment in renal proximal tubular reabsorption function, but the increased level was insignificant when compared with G_1. This suggestion is supported by other investigators who reported a histopathological changes in renal tubules due to Tr alone in animal experiments [39], and in human post-mortem microscopy examination of a young patient who died of fatal Tr intoxicaton due to acute tubular necrosis of the kidney [44].

Regarding the effect of cannabinoid use in combination with Tr addiction on tubular function, data of the present study (Table 2) showed an increase in urinary excretion of α_1-m in G_3 more than in G_2, suggesting an increase in impairment of reabsorption capacity of proximal tubules due to cannabinoid use, but the increase of U.α_1-m in G_3 was insignificant when compared with each of G_1 and G_2. This suggestion is supported by the results of the present study (Table 3) which showed a positive correlation between U.THC and U.α_1-m ($r = 0.507$, $P = 0.006$) in G_3. This suggestion is also supported by a report that all of the pathological findings among 101 addicts (opiates 18, barbiturates 9, benzodiazepines 22, methaqualone 10, cannabis 42) pointed to tubular damage in the proximal region, and they concluded with high probability that tubular dysfunction is frequent in addicts [45]. Moreover, a reported showed that histopathological examination of kidney tissues from experimental animals given the extract of cannabis leaves demonstrated congestion of the peritubular blood vessels and dilated as well as swollen tubules [43].

Many enzymes have been detected in urine and a few appear to possess diagnostic relevance in recognition of renal injury. Choice of investigated urinary enzymes in this study was made on the basis of site specificity. Data of the present study (Table 2) for the effect of Tr addiction on the tubular structure showed an increase in the U.NAG and U.LAP excretion, but the increase was insignificant when compared with G_1. The increased leakage of enzymes characteristic of these cells results from tubular damage, suggesting the possibility of a nephrotoxic effect of Tr addiction. This suggestion is supported by other studies elsewhere that revealed proximal tubular histopathological effect due to Tr alone in human post-mortem [44], and experimental studies in animals [39].

Results of the present study (Table 2) concerning the effect of cannabinoid use in combination with Tr addiction on tubular structure showed an increased excretion of U.LAP and U.NAG in G_3 more than in G_2, suggesting the possibility of more damage to proximal tubules in G_3 due to cannabinoid. The increased in U.LAP and U.NAG levels in G_3 were insignificant when compared with those in G_1 and G_2. This suggestion is supported by the results of the present study (Table 3) which showed a positive correlation between U.THC and U.LAP in G_3 and report of renal biopsy that heavy marijuana use caused an acute tubular necrosis [44].

The effects of cannabis depend upon the dose received, the mode of administration, the user's prior experience with cannabis, any concurrent drug use, the user's expectations, attitudes towards the effect of cannabis, their mood state and the social setting in which it is used [46].

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CONCLUSIONS

Tramadol addiction may affect only the function and structure of proximal tubules, although U.Tr level showed no correlation with any of the measured urinary parameters of proximal tubules. Cannabinoid use in combination with Tr addiction may cause glomerular damage and increase the proximal tubular dysfunction which caused by Tr addiction and this may be due to the synergetic effect of cannabinoid to Tr. Therefore, another group of cannabis alone should be included to best analyze more accurately the synergistic effect of Tr and cannabis. Also, the effect of longer addiction duration on the function and structure of both the glomeruli and proximal tubules needs further study.

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REFERENCES

[1] El-Akabawi AS. World Psychiatric Association Series 2001, pp. 143-150.
[2] Paar WD, Frankus P, Dengler HJ. Clin Investig 1992;70(8):708-10.
[3] Schenck EG, Arend I. Arzneimittelforschung 1978;28(1a):209-12.
[4] Grond S, Sablotzki A. Clin Pharmacokinet 2004;43(13):879-923..
[5] Boumendjel A, Sotoing Taïwe G, Ngo Bum E, Chabrol T, Beney C, Sinniger V, et al. Angew Chem Int Ed Engl 2013;52(45):11780-4.
[6] Kusari S, Tatsimo SJ, Zühlke S, Talontsi FM, Kouam SF, Spiteller M. Angew Chem Int Ed Engl 2014;53(45):12073-6.
[7] Miranda HF, Pinardi G. Pharmacol Biochem Behav 1998;61(4):357-60.
[8] Kitahara M, Kojima K, Hanada M, Kuriyama Y, Ohmura A. Masui 2009;58(8):971-5.
[9] Tjäderborn M, Jönsson AK, Ahlner J, Hägg S. Pharmacoepidemiol Drug Saf 2009;18(12):1192-8.
[10] Lanier RK, Lofwall MR, Mintzer MZ, Bigelow GE, Strain EC. Psychopharmacology (Berl) 2010;211(4):457-66.
[11] Knisely JS, Campbell ED, Dawson KS, Schnoll SH. Drug Alcohol Depend 2002;68(1):15-22.
[12] Cicero TJ, Adams EH, Geller A, Inciardi JA, Muñoz A, Schnoll SH, et al. Drug Alcohol Depend 1999;57(1):7-22.
[13] Zhang H, Liu Z. Biomed Res Int 2013;2013:283425.
[14] Wu WN, McKown LA, Liao S. Xenobiotica 2002;32(5):411-25.
[15] Lintz W, Erlaçin S, Frankus E, Uragg H. Arzneimittelforschung 1981;31(11):1932-43.
[16] Gutustein HB, Akil H. New York: McGraw-Hill. 2001; 10th ed. pp. 569-620.
[17] Leppert W, Mikolajczak P. Curr Pharm Biotechnol 2011;12(2):306-12.
[18] European Monitoring Centre for Drugs and Drug Addiction. Office for Official Publications of the European Communities, Luxembourg. 2004, p. 28-30.
[19] Elsohly MA, Slade D. Life Sci 2005;78(5):539-48.
[20] Mechoulam R. Science 1970;168(3936):1159-66.
[21] Wollner HJ, Matchett JR, Levine J, Loewe S. J Am Chem Soc 1942;64(1):26-9.
[22] Gaoni Y, Mechoulam R. J Am Chem Soc 1964;86(8):1646-7.
[23] Krishna DR, Klotz U. Clin Pharmacokinet 1994; 26(2):144-60.
[24] Jones AB, ElSohly HN, Arafat ES, ElSohly MA. J Anal Toxicol 1984;8(6):249-51.
[25] Wall ME, Sadler BM, Brine D, Taylor H, Perez-Reyes M. Clin Pharmacol Ther. 1983;34(3):352-63.
[26] Bachs L, Mørland H. Forensic Sci Int 2001;124(2-3):200-3.
[27] Lambrecht GL, Malbrain ML, Coremans P, Verbist L, Verhaegen H. Nephron 1995;70(4):494-6.
[28] Le Guen PY, Gestin S, Plat E, Quéhé P, Bressollette L. J Mal Vasc 2011;36(1):41-4.
[29] Mueller PW, Lash LH, Price RG, Stolte H, Gelpi E, Maack T, et al. Ren Fail 1997;19(4):505-21.
[30] Zuppi C, Baroni S, Scribano D, Di Salvo S, Musumeci V. Ann Clin Biochem 1995;32(Pt 4):373-8.
[31] Molnar A, Lewis J, Fu S. Forensic Sci Int. 2013; 227(1-3):69-73.
[32] Lemann J Jr, Doumas BT. Clin Chem 1987;33(2 Pt 1):297-9.
[33] Woolerton J, Jury DR, Dunn PJ, Speed JF. N Z Med J 1987;100(819):130-4.

[34] Jung K. Eur J Clin Chem Clin Biochem 1991;29(11):725-9.
[35] Musso CG, Oreopoulos DG. Nephron Physiol. 2011;119 (S1):1-5.
[36] Kimmel PL, Alam S, Lew SQ. Renal disease in patients with substance abuse. In: Schena FP editor. Nephrology. London: McGraw-Hill. 2001; pp. 237-43.
[37] Christensen EI, Birn H, Rippe B, Maunsbach AB. Kidney Int. 2007;72(10):1192-4.
[38] Thrailkill KM, Nimmo T, Bunn RC, Cockrell GE, Moreau CS, Mackintosh S, et al. Diabetes Care 2009;32(7):1266-8.
[39] Atici S, Cinel I, Cinel L, Doruk N, Eskandari G, Oral U. J Biosci 2005;30(2):245-52.
[40] Pertwee RG. Addict Biol. 2008;13(2):147-59.
[41] Tewari SN, Sharma JD. Toxicol Lett 1980;5(3-4):279-81.
[42] Bohatyrewicz M, Urasinska E, Rozanski J, Ciechanowski K. Transplant Proc 2007;39(10):3054-6.
[43] Yassa HA, Dawood Ael W, Shehata MM, Abdel-Hady RH, Aal KM. Hum Exp Toxicol 2010;29(1):37-47.
[44] De Decker K, Cordonnier J, Jacobs W, Coucke V, Schepens P, Jorens PG. Forensic Sci Int. 2008;175(1):79-82.
[45] Sommer GL, Schmid R, Lubec G. Drug Alcohol Depend 1985;16(3):287-9.
[46] MacPhee D. Effects of marijuana on cell nuclei: a review of the literature relating to the genotoxicity of cannabis. In: Kalant H, Corrigal W, Hall W, Smart R, editors. The health effects of cannabis. Toronto Center for Addiction and Mental Health. 1st ed. 1999; p. 293-300.