Assiut University web-site: www.aun.edu.eg

HEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS INDUCED BY TRAMADOL ADMINISTRATION IN MALE ALBINO RATS

AZIZA A.M. EL-SHAFEY; MOSHIRA M.E. SELIEM; DOAA S. IBRAHIM AND HAITHAM A.S. IBRAHIM

Department of Zoology, Faculty of Science, Benha University, Egypt.

Received: 11 March 2019; Accepted: 20 March 2019

ABSTRACT

The use of analgesics to alleviate the severe pains associated with many diseases is necessary. It is therefore important to study the effects of administration these analgesics to determine the side effects for their use. Hence we decided to study the hematological and biochemical effects of tramadol and tramadol withdrawal on male albino rats. Rats were divided into four control rat groups (7 rats each), and four tramadol rat groups (7 rats each); two groups administrated a daily oral dose of tramadol (20 mg/kg b.wt) for 30 and 60 days and two groups also administrated a daily oral dose of tramadol for 30 and 60 days then tramadol withdrawal for 7 days. Hemoglobin (Hb) content, red blood cells (RBCs) count, hematocrit (Hct) value, and lipid profile decreased, while white blood cells (WBCs) and platelets counts, liver function enzymes, and kidney function parameters increased after tramadol administration. Most parameters improved after tramadol withdrawal. The study showed the harmful effects of tramadol on the hematological and biochemical parameters and the effects of improvement on rats after tramadol withdrawal from them, especially after a short period.

Key words: Tramadol; hematological parameters; liver function enzymes; kidney function parameters; lipid profile.

INTRODUCTION

Analgesics are a group of drugs that act in different ways on the central nervous system to analgesia and relief from pain (Kumar *et al*., 2010). In moderate and severe pain cases, opioid analgesic drugs are mainly used (El Shal and Selim, 2015). Opioid analgesic drugs might be obtained from natural sources (e.g. morphine, codeine) or from synthetic sources (as Tramadol, heroin, Hussein *et al*., 2017a).

Nowadays, tramadol is widely used as an analgesic drug in human medicine (Azari *et al*., 2014). Its mechanism of action is based on the inhibition of ascending pain to the central nervous system by its binding to μ- opiate receptors and inhibit the reuptake of norepinephrine and serotonin (Aldalou *et al*., 2014). Continuous tramadol administration leads to the appearance of its toxic effects on various organs of the body (Shadnia *et al*., 2008). Tramadol has toxic effects on the structure and function of hepatic, renal and testicular tissues of male albino rats (Youssef and Zidan, 2016). In addition, the neurotoxicity of tramadol has been reported by Hussein *et al*. (2017b). High doses of tramadol cause neuronal degeneration in the rat brain (Atici *et al*., 2005) and alter brain neurotransmitter levels (Bloms-Funke *et al*., 2011).

This study aims to investigate the effects of administration a daily oral dose (20 mg/kg b.wt) of tramadol for 30 and 60 days on the hematological and biochemical parameters and the tramadol withdrawal effects.

MATERIALS AND METHODS

Tramadol

Tramadol tablets were obtained from Sigma Pharmaceuticals Industries Company (Quesna, El Mnoufia, Egypt). It was orally administered daily (20 mg/kg b.wt) as previously used by El-Baky and Hafez (2017).

Animals

Rats were divided into four control rat groups (7 rats each), and four tramadol rat groups (7 rats each); two groups administrated tramadol for 30 and 60

Corresponding author: Dr. DOAA S. IBRAHIM
E-mail address: Doaa.mohamed@fsc.bu.edu.eg
Present address: Department of Zoology, Faculty of Science, Benha University, Egypt.

days and two groups administrated tramadol for 30 and 60 days then stayed in the experimental conditions for 7 days.

Blood sampling

After the experiment period, all fasted overnight rats were anesthetized. Then, rats blood samples were collected from posterior vena cava in two groups of tubes, the first tubes group were containing EDTA as an anticoagulant for determination of the hematological parameters, while the blood in the second tubes group was allowed to clot without using any anticoagulants for 1-2 h at 37˚C then centrifuged at 3000 rpm for 15 minutes. Sera were separated and stored at –20 ºC for determination of the biochemical parameters.

Determination of hematological parameters

A complete blood count performed using an automated hematology cell counter (Mindray BC-2800).

Determination of biochemical parameters:

Using a semi-automated blood chemistry analyzer (Mindray BA-88A) the following biochemical parameters were performed.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined kinetically according to Tietz (1976) by using BioMed Company kit.

Serum urea and creatinine levels were determined spectrophotometrically according to Tabacco *et al*. (1979) and Murray (1984), respectively by using Diamond Company kit.

Serum triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) concentrations were measured spectrophotometrically according to Bucolo and David (1973), Meiattini *et al*. (1978) and Grove (1979), respectively by using Spinreact Company kits. The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated by the equations of Friedewald *et al*. (1972).

Statistical analysis

All parameter values were expressed as the mean ± standard deviation. Statistical analysis was conducted by one-way ANOVA using the computer program Statistical Package for Social Science (SPSS, Version 20.00, Chicago, USA) followed by Least Significant Difference (LSD) test.

RESULTS

Hemoglobin (Hb) contents in all tramadol groups significantly decreased ($p<0.05$) in comparison with their control groups. In addition, tramadol withdrawal from the group that administrated tramadol for 60 days showed significant improvement in Hb content. Also, the count of red blood cells (RBCs) in all tramadol groups except in the group that administrated tramadol for 30 days decreased compared to their control groups. Both the group that administrated tramadol for 60 days and its withdrawal group showed low hematocrit (Hct) values. The count of white blood cells (WBCs) in all tramadol groups except in the group that administrated tramadol for 30 days increased compared to their control groups. In addition, tramadol withdrawal from the group that administrated tramadol for 30 days showed a significant increase in WBCs content. Blood platelets count significantly increased in the group that administrated tramadol for 30 days (Table 1).

All tramadol groups except in the group that administrated tramadol for 30 days have high activities of ALT compared to their control groups. Also, all tramadol groups except in the tramadol withdrawal group (60 + 7 days) have high activities of AST compared to their control groups. Activities of ALT and AST significantly decreased in the two tramadol withdrawal groups (Table 2).

Serum urea levels were high in all tramadol groups except in the group that administrated tramadol for 60 days compared to their control groups. The level of serum urea significantly decreased after tramadol withdrawal period from the group that administrated tramadol for 30 days. In addition, both tramadol administration groups have high levels of serum creatinine compared to their control groups. After the tramadol withdrawal period, serum creatinine levels significantly decreased in the two tramadol withdrawal groups (Table 3).

After 60 days of tramadol administration, serum triglycerides (TG) level significantly decreased ($p<0.05$) compared to its control group. Tramadol withdrawal from the group that administrated tramadol for 30 days decreased the TG level. Both tramadol administration groups have low levels of TC and LDL-C compared to their control groups. While the levels of TC and LDL-C significantly increased after tramadol withdrawal from the group that administrated tramadol for 30 days. Both the group that administrated tramadol for 30 days and the tramadol withdrawal group (60 + 7 days) showed low HDL-C levels compared to their control groups. Tramadol withdrawal from the group that administrated tramadol for 30 days increased the HDL-C level (Table 4).

Table 1: Effects of daily oral administrations of tramadol (20 mg/kg b.wt.) for 30 and 60 days and after 7 days of withdrawal on hematological parameters in male rats.

Parameters	Groups	Administration period		(Administration + Withdrawal) period	
		30 days	60 days	30 + 7 days	60 + 7 days
Hb (g/dl)	Control	15.03 ± 0.15	15.13 ± 0.48	15.35 ± 0.64	16.03 ± 0.30
	Tramadol	13.48 ± 0.17*	12.10 ± 0.39*	13.62 ± 0.62*	12.83 ± 0.29^{*B}
RBCs (10^6/mm^3)	Control	7.30 ± 0.06	7.65 ± 0.44	7.71 ± 0.40	7.75 ± 0.31
	Tramadol	7.20 ± 0.14	6.05 ± 0.13*	7.29 ± 0.24*	6.28 ± 0.28*
Hct (%)	Control	43.15 ± 0.90	45.75 ± 3.50	43.24 ± 1.08	46.50 ± 1.29
	Tramadol	43.05 ± 0.61	36.25 ± 2.63*	43.11 ± 1.55	37.50 ± 1.30*
WBCs (10^3/mm^3)	Control	9.92 ± 0.87	8.23 ± 0.34	8.90 ± 1.44	9.1 ± 1.17
	Tramadol	10.80 ± 0.42	12.78 ± 0.70*	12.60 ± 0.65^{*A}	13.43 ± 1.46*
Platelets (10^3/mm^3)	Control	742.00 ± 16.40	616.50 ±36.5	801.50 ± 59.05	675.5 ± 47.06
	Tramadol	857.25 ± 27.20*	663.50 ± 41.5	808.00 ±44.92	642.0 ± 59.83

Values are expressed as means ± standard deviation for seven rats.
*= Significant difference between control and tramadol groups at P< 0.05.
^{A}= Significant difference between tramadol administration (30 days) and tramadol withdrawal (30 + 7 days) groups at P< 0.05.
^{B}= Significant difference between tramadol administration (60 days) and tramadol withdrawal (60 + 7 days) groups at P< 0.05.

Table 2: Effects of daily oral administrations of tramadol (20 mg/kg b.wt.) for 30 and 60 days and after 7 days of withdrawal on liver function enzymes in male rats.

Parameters	Groups	Administration period		(Administration + Withdrawal) period	
		30 days	60 days	30 + 7 days	60 + 7 days
ALT (U/L)	Control	48.00 ± 3.16	68.50 ± 2.08	41.75 ± 3.09	51.75 ± 2.22
	Tramadol	51.75 ± 0.50	92.00 ± 4.08*	45.25 ± 0.50^{*A}	86.25 ± 3.86^{*B}
AST (U/L)	Control	143.00 ± 7.78	147.00 ± 2.94	136.00 ± 5.48	130.15 ± 4.27
	Tramadol	196.00 ± 17.14*	195.50 ± 4.20*	139.50 ± 5.80^{A}	140.75 ± 3.09^{*B}

Values are expressed as means ± standard deviation for seven rats.
*= Significant difference between control and tramadol groups at P< 0.05.
^{A}= Significant difference between tramadol administration (30 days) and tramadol withdrawal (30 + 7 days) groups at P< 0.05.
^{B}= Significant difference between tramadol administration (60 days) and tramadol withdrawal (60 + 7 days) groups at P< 0.05.

Table 3: Effects of daily oral administrations of tramadol (20 mg/kg b.wt.) for 30 and 60 days and after 7 days of withdrawal on kidney function parameters in male rats.

Parameters	Groups	Administration period		(Administration + Withdrawal) period	
		30 days	60 days	30 + 7 days	60 + 7 days
Urea (mg/dl)	Control	35.50 ± 1.29	46.75 ± 3.40	44.50 ± 4.04	38.00 ± 2.94
	Tramadol	62.75 ± 5.38*	50.00 ± 2.16	56.75 ± 2.99^{*A}	47.25 ± 2.50*
Creatinine (mg/dl)	Control	0.82 ± 0.08	0.80 ± 0.07	0.89 ± 0.03	0.88 ± 0.09
	Tramadol	1.27 ± 0.10*	1.23 ± 0.10*	0.94 ± 0.02^{A}	0.92 ± 0.09^{B}

Values are expressed as means ± standard deviation for seven rats.
*= Significant difference between control and tramadol groups at P< 0.05.
^{A}= Significant difference between tramadol administration (30 days) and tramadol withdrawal (30 + 7 days) groups at P< 0.05.
^{B}= Significant difference between tramadol administration (60 days) and tramadol withdrawal (60 + 7 days) groups at P< 0.05.

Table 4: Effects of daily oral administrations of tramadol (20 mg/kg b.wt.) for 30 and 60 days and after 7 days of withdrawal on the lipid profile parameters in male rats.

Parameters	Groups	Administration period		(Administration + Withdrawal) period	
		30 days	60 days	30 + 7 days	60 + 7 days
TG (mg/dl)	Control	112.25 ± 7.85	106.50 ± 4.79	99.75 ± 4.79	93.50 ± 3.69
	Tramadol	106.00 ± 6.68	94.50 ± 2.08*	95.00 ± 1.41A	91.75 ± 3.50
TC (mg/dl)	Control	96.00 ± 4.24	117.75 ± 4.35	110.25 ± 3.77	120.75 ± 2.98
	Tramadol	84.25 ± 6.13*	92.50 ± 3.11*	106.25 ± 5.12A	117.50 ± 2.65
HDL-C (mg/dl)	Control	58.25 ± 2.50	30.75 ± 1.71	59.75 ± 3.09	38.50 ± 3.11
	Tramadol	38.75 ± 0.96*	30.00 ± 2.16	55.25 ± 3.09A	34.25 ± 2.75*
LDL-C (mg/dl)	Control	27.75 ± 2.50	34.25 ± 2.50	29.00 ± 2.58	30.25 ± 1.70
	Tramadol	21.35 ± 4.27*	24.50 ± 1.29*	27.15 ± 3.59A	28.90 ± 3.56

Values are expressed as means ± standard deviation for seven rats.
*= Significant difference between control and tramadol groups at P< 0.05.
A= Significant difference between tramadol administration (30 days) and tramadol withdrawal (30 + 7 days) groups at P< 0.05.
B= Significant difference between tramadol administration (60 days) and tramadol withdrawal (60 + 7 days) groups at P< 0.05.

DISCUSSION

Our results showed decreases in Hb content, RBCs count, and Hct value after administration of tramadol (20 mg/kg b.wt.) for 30 and 60 days. Our findings agreed with those of Aldalou *et al*. (2014), who found that administration of sildenafil (1.40 mg/kg/day) and tramadol (4 mg/kg/day) for 25 days to rabbits led to significant decreases in RBCs count and Hb content. The decrease in the RBCs count may be due to the inhibitory effect of tramadol on erythropoiesis (Aldiwan *et al*., 2015). In addition, the WBCs count increased after administration of tramadol to rats. Aldiwan *et al*. (2015) explained the elevation in the WBCs count by the activation of defense immune system. While Aldalou *et al*. (2014) reported that high lymphocyte percentage in male rats injected by tramadol (100 mg/kg) for 30 days was the reason behind the WBCs count elevation.

Our data confirm that tramadol administration increased the activities of liver function enzymes (ALT and AST). The high levels of the liver enzymes in circulation indicated the severity of the liver damage (Simeon and Abbey, 2018). Also, tramadol causes severe cellular toxicity and liver failure (Elmanama *et al*., 2015). Aldalou *et al*. (2014), observed that sildenafil and tramadol administration might be responsible for impaired liver function. Tramadol administration was associated with hepatic congestion, hemorrhage and necrosis in the male rats (Loughrey *et al*., 2003). After tramadol withdrawal, activities of ALT and AST decreased which is a marker for improving liver functions. Nna *et al*. (2015) observed that the hepatotoxicity of tramadol (2 mg/100 g b.w., for 8 weeks) is reversed after withdrawal it for 8 weeks.

Our results showed elevations of serum urea and creatinine after tramadol administration. Nephrotoxicity induced by tramadol might be responsible for kidney dysfunction (Aldiwan *et al*., 2015). The high blood urea after tramadol treatment might be due to the destruction of RBCs (Aldalou *et al*., 2014). Also, a histopathological study reported the toxicity effect of tramadol on kidney tissue that represented in glomerular hemorrhage, atrophied glomeruli, wide Bowman's space, degenerated tubules and cellular infiltration (Youssef and Zidan, 2016).

Lipid profile parameters were decreased significantly in response to tramadol administration and increased after its withdrawal. The previous result agreed with Ahmed and Kurkar (2014) who demonstrated that tramadol reduced serum concentration of total cholesterol. The explanation of this observed may be based on the action of tramadol on lipid metabolism or lipid peroxidation (Aldalou *et al*., 2014). Tramadol may inhibit cholesterol synthesis (Nna *et al*., 2016).

CONCLUSION

Administration a daily oral dose (20 mg/kg b.wt) of tramadol for 30 and 60 days caused harmful effects on the hematological and biochemical parameters. Improvement of these parameters appeared after tramadol withdrawal, especially after a short period.

REFERENCES

Ahmed, M.A. and Kurkar, A. (2014): Effects of opioid (tramadol) treatment on testicular functions in adult male rats: The role of nitric oxide and oxidative stress. Clin. Exp. Pharmacol. Physiol., 41: 317-323.

Aldalou, A.R.; Abdel-Aziz, I. and Shahwan, O. (2014): Impact of giving sildenafil (viagra) / tramadol (tramal) combination on the blood of domestic rabbits. J. Sci., 4 (3): 162-169.

Aldiwan, M.A.; Alzobidy, A.M.H. and Younis, M.A. (2015): The effect of Tramadol on some blood and biochemical parameters of male rats (Rattus norvegicus). Baghdad Sci. J., 12(3): 496-502.

Atici, S.; Cinel, I.; Cinel, L.; Doruk, N.; Eskandari, G. and Oral, U. (2005): Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model. J. Biosci., 30(2): 245–252.

Azari, O.; Emadi, L.; Kheirandish, R.; Bafti, H.S.; Nejad, M.R.E. and Faroghi, F. (2014): The effects of long-term administration of tramadol on epididymal sperm quality and testicular tissue in mice. Iranian J. Veteran. Surg., 9 (1): 23-30.

Bloms-Funke, P.; Dremencov, E.; Cremers, T.I. and Tzschentke, T.M. (2011): Tramadol increases extracellular levels of serotonin and noradrenaline as measured by in vivo microdialysis in the ventral hippocampus of freely-moving rats. Neurosci. Lett., 490 (3): 191-195.

Bucolo, G. and David, M. (1973): Quantitative determination of serum triglycerides by use of enzymes. Clin. Chem., 19: 476-482.

El Shal, E.B. and Selim, M.M.H. (2015): The effect of tramadol treatment on rat testes and the possible protective role of selenium (light and electron microscopic study. Al-Azhar Assiut Med. J., 13 (1): 127-137.

El-Baky, A.E.A. and Hafez, M.M. (2017): NOS expression in oxidative stress, neurodegeneration and male infertility induced by the abuse of tramadol. Biochem. Pharmacol., 6: 223. doi: 10.4172/2167-0501.1000223.

Elmanama, A.A.; Abu-Tayyem, N.E.S.; Essawaf, N.N. and Hmaid, I.M. (2015): Tramadol induced liver and kidney toxicity among abusers in gaza strip, palestine. Jordan J. Biol. Sci., 8: 133-137.

Friedewald, W.T.; Levy, R.I. and Fredrickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Grove, TH. (1979): Effect of reagent pH on determination of high density lipoprotein cholesterol by precipitation with sodium phosphotungstate- magnesium. Clin. Chem., 25: 560-564.

Hussein, S.A.; Abdel Aal, S.A. and Ismail, H.K. (2017a): Effect of tramadol drug on some biochemical and immunological parameters in albino male rats; evaluation of possible reversal following its withdrawal. Benha Vet. Med. J., 33 (2): 418-429.

Hussein, S.A.; Abdel Aal, S.A. and Ismail, H.K. (2017b): Neurodegeneration and oxidative stress induced by tramadol administration in male rats: The effect of its withdrawal Benha Vet. Med. J., 33 (2): 149-159.

Kumar, M.; Shete, A. and Akbar, Z. (2010): A Review on analgesic: from natural sources. Int. J. Pharm. Biol. Sci. Arch., 1 (2): 95-100.

Loughrey, M.B.; Loughrey, C.M.; Johnston, S. and Rourke, D. (2003): Fatal hepatic failure following accidental tramadol overdose. Forensic Sci. Int., 134: 232-233.

Meiattini, F.; Prencipe, L. and Bardelli, F. (1978): The 4-hydroxy-benzoate/4-aminophenazone chromogenic system used in the enzymatic determination of serum cholesterol. Clin. Chem., 24: 2161–2165.

Murray, R.L. (1984): Creatinine. Kaplan A et al. The C.V. Mosby Company St Louis. Toronto. Princeton, Clin. Chem., 1261-1266.

Nna, V.U.; Akpan, U.P. and Osim, E.E. (2016): Hyperprolactinemia contributes to reproductive deficit in male rats chronically administered PDE5 inhibitors (sildenafil and tadalafil) and opioid (tramadol). Asian Pac. J. Reprod., 5 (5): 381–386.

Nna, V.U.; Akpan, U.P.; Okon, V.E. and Atangwho, I.J. (2015): Hepatotoxicity following separate administration of two phosphodiesterase-5 inhibitors (sildenafil and tadalafil) and opioid (tramadol); evaluation of possible reversal following their withdrawal. J. Applied Pharma. Sci., 5 (8):105-113.

Shadnia, S.; Soltaninejad, K.; Heydari, K.; Sasanian, G. and Abdollahi, M. (2008): Tramadol intoxication: a review of 114 cases. Hum. Exp. Toxicol., 27: 201-205.

Simeon, G.G. and Abbey, S.T. (2018): Some marker enzymes and histological alteration on the administration of tramadol hydrochloride on rat liver. Mod. Res. Inflamm., 7: 9-20.

Tabacco, A.; Meiattini, F.; Moda, E. and Tarli, P. (1979): Simplified enzymic/colorimetric serum urea nitrogen determination. Clin. Chem., 25(2): 336-337.

Tietz, N.W. (1976): Fundamentals of clinical chemistry Philadelphia, W.B., Saunders, Pp. 923-929.

Youssef, S.H. and Zidan, A.H.M. (2016): Histopathological and biochemical effects of acute and chronic tramadol drug toxicity on liver, kidney and testicular function in adult male albino rats. J. Med. Toxicol. Clin. Forensic Med., 1: 1-7.

التغيرات الدموية والبيوكيميائية لتعاطى الترامادول على ذكور الجرذان البيضاء

عزيزة عبد الصمد محمد الشافعى ، مشيرة محمد عزت سليم ، دعاء صبرى ابراهيم ، هيثم عبد الله صديق

E-mail: Doaa.mohamed@fsc.bu.edu.eg Assiut University web-site: www.aun.edu.eg

إن استخدام المسكنات لتخفيف الآلام الشديدة المرتبطة بالعديد من الأمراض يعد من الأمور الضرورية واللازمة لتخفيف آلام المرضى. ولذلك فمن المهم دراسة تأثير تناول هذه المسكنات لتحديد الآثار الجانبية لإستخدمها. ومن ثم فقد استهدفت هذه الدراسة استقراء التغيرات الدموية والبيوكيميائية لتعاطى الترامادول وكذلك انسحابه على ذكور الجرذان البيضاء. تم تقسيم الجرذان إلى أربع مجموعات جرذان ضابطة (٧ جرذان لكل منها) ، وأربعة مجموعات جرذان تتعاطى الترامادول (٧ جرذان لكل منها) ؛ مجموعتين تعاطت الترامادول (٢٠ ملجم / كجم من وزن الجسم) لمدة ٣٠ و٦٠ يومًا ومجموعتين تعاطت الترامادول لمدة ٣٠ و ٦٠ يومًا ثم تُترك فى ظروف التجربة لمدة ٧ أيام دون إعطائها أى مادة (فترة انسحاب الدواء). أظهرت النتائج انخفاض محتوى الهيموجلوبين (Hb) ، وعدد خلايا الدم الحمراء (RBCs) ، وقيمة الهيماتوكريت (Hct) ، وقياسات صورة الدهون ، في حين أن عدد خلايا الدم البيضاء (WBCs) والصفائح الدموية ، وإنزيمات وظائف الكبد ، ومؤشرات وظائف الكلى قد زادت بعد إعطاء الترامادول. وأظهرت الدراسة الآثار الضارة للترامادول على المؤشرات الدموية والبيوكيميائية وآثار التحسن على الجرذان بعد سحب الترامادول منها، خاصة بعد فترة قصيرة.