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The Effects of Tramadol Hydrochloride Administration on the Hematological and Biochemical Profiles of domestic male Rabbits.

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Abstract: The present work was conducted to assess the Hematological and biochemical toxicity profiles of tramadol during therapeutic use. Male adult domestic rabbits (weighting 1000-1300g) were divided into six groups. Group one received vehicle (saline), group two, three and four injected intramuscularly with doses of tramadol equal to 40 mg / kg body weight / day for 10, 20 and 30 days respectively followed by 10 days recovery period for group three and four. Hematological and Biochemical measurements were carried out every 10 days. Control and treated rabbits were sacrificed at the end of 10, 20, 30, and 40 days respectively. Blood was collected in dry centrifuge tubes. Sera were separated and kept at -20ºC until analysis.

Administration of tramadol at a dose of 40mg/kg. body weight for 30 days increased serum glucose, cholesterol, triglycerides, electrolytes (Na^+ and Ca^{2+}) and cholinesterase values. Theses increments were 21.53%, 9.64%, 3.39%, 2.14%, 6.67 and 20% respectively, compared to control level. Also there was a significant increase in kidney function indices. The effect was more pronounced on creatinine. Activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin were increased following tramadol injection to rabbits for 30 days. Concerning hematological parameters, the more obvious changes were observed in the increment of white blood cells (WBCs), lymphocyte and MCV value and the decrease in hematocrit, hemoglobin(Hb), red blood cells (RBCs) count, MCH, MCHC, and platelets (PLT) count in response to the administration of tramadol. In general recovery periods after tramadol abstinence, showed signs of improvement of blood indices.

Conclusion: Tramadol toxic effects should be avoided during long term therapy specially in large doses.

Key words: Tramadol, Gaza Strip, rabbits, blood indices, enzymes.

تأثير إعطاء الترامادول على بعض القياسات الدموية والبيوكيميائية في الأرانب المحلية

ملخص: يهدف هذا البحث إلى دراسة بعض القياسات البيوكيميائية والدموية في الأرانب نتيجة لتأثير اعطاء عقار الترامادول. حيث استخدم في هذا البحث ذكورالأرانب المحلية البالغة والتي يتراوح وزنها ما بين 1000-1300جرام وأعطيت جرعة يومية من الترامادول قدرها 40 ملجرام/كجم من وزن الجسم لمدة ثلاثين يوماً. أدى إعطاء الترامادول إلى ارتفاع في تركيز الجلوكوز والكولسترول الكلي والدهون الثلاثية والصوديوم والكالسيوم والكولينستيريز، حيث وصلت نسبة الزيادة إلى 21.53% و9.64% و3.39% و2.14% و6.67% و 20.24% على التوالي مقارنة بالمجموعة الضابطة كذلك ارتفع مستوى البولينا وحمض البوليك والكرياتينين وكان ارتفاع الكرياتينين أكثر وضوحا وأظهرت أيضا زيادة في نشاط الأنزيمات الناقلة لمجموعة الأمين ALT, AST والفوسفاتيز القاعدي والبيليروبين بينما انخفضت البروتينات الكلية والزلال والجلوبيولين. وأظهرت صورة الدم الكاملة زيادة في عدد كرات الدم البيضاء الكلي والخلايا الليمفاوية وMCV بينما نقص معدل الهيماتوكريت والهيموجلوبين وعدد كرات الدم الحمراء و MCHو MCHCوعدد الصفائح الدموية مقارنة بمجموعة الحيوانات الضابطة نتيجة إعطاء الترامادول. أما في مجموعات الاستشفاء فقد تحسنت معظم القياسات السابقة.

الكلمات المفتاحية: الترامادول - الأرانب - قياسات الدم - قطاع غزه- فلسطين- الانزيمات.

Introduction

Tramadol is a centrally acting analgesic agent with activity at μ-opioid, adrenergic and 5-hydroxytryptamine (5-HT) receptors. Its analgesic effect is a result of its dual mechanism of action, that is, as a re-uptake inhibitor of norepinephrine and serotonin and agonist of the μ-opioid receptor (Bamigbade et al., 1997; Halfpenny et al., 1999; Ide et al., 2006). Tramadol has been in clinical use for the relief of mild to moderate pain in human and veterinary medicine (Buback et al., 1996; Yaddanapudi et al., 2000; Ozcengiz et al., 2001; Mastrocinque and Fantoni, 2003; Pypendop and Ilkiw, 2008). Tramadol is also used perioperatively in veterinary anesthesia as it significantly reduces the requirements of volatile anesthetics and opioid agents (Wordliczek et al., 2002; Seddighi et al., 2009).

Although tramadol has relatively effective analgesic effects, a higher tramadol infusion rate was needed to reduce sevoflurane requirements in dogs (Seddighi et al., (2009). Furthermore, recent results showed that tramadol exhibits different metabolic rates between species: tramadol is metabolized quickly to inactive metabolites in goats, horses and dogs (KuKanich and Papich, 2004; de Sousa et al., 2008; Shilo et al., 2008;

Giorgi et al., 2009b), in contrast with cats and camels (Elghazali et al., 2008; Pypendop and Ilkiw, 2008).

Tramadol has a dose- dependent analgesic efficacy that lies between that of codeine and morphine, with a parenteral potency comparable to that of pethidine, i.e. about 10- 20 % of the standard morphine (Wilder- Smith *et al.*, 1999; Pang *et al*., 2003).

The central role of liver and kidney in drug metabolism predisposes them to toxic injury. Every drug has been associated with hepatotoxicity almost certainly due to the pivotal role of the liver in drug metabolism. Hepatic metabolism is, first and foremost, a mechanism that converts drugs and other compounds into products that are more easily excreted and that usually have a lower pharmacologic activity than the portent compound (Poppers 1980; Tolman 1998). A metabolite may have higher activity and/or greater toxicity than the original drug. Metabolites of the drugs that are excreted from kidneys may also cause cellular damage leading to kidney dysfunction (Singhal et al., 1998).

Tramadol is also thought to have some NMDA-type (N-methyl-D-aspartate) antagonist effects, which has given it a potential application in neuropathic pain states (Lintz et al., 1998). Increasing serotonin and norepinephrine may also reduce inflammatory cytokines which are released by the brain in response to stress. The inflammatory cytokines would have slowed down recovery from a workout or illness - impairing one's immune system and healing, thus tramadol may have an anti-inflammatory effect (Bianchi et al., 1999; Barkin, 2008).Tramadol carries all possible risks known from other opiates (Cicero et al., 2005; Adams et al., 2006). Side effects include dizziness, headache, somnolence, nausea, constipation, sweating, pruritus, and central nervous system stimulation (Reig, 2002; Kabel and van Puijenbroek, 2005). Tramadol causes respiratory depression, although usually weaker than that seen with other opiates and opioids (Senay et al., 2003).

Thus, the present study aims to investigate the hematological and biochemical effects of tramadol in rabbits.

Materials and methods

Experimental animals and dosing

The used adult rabbits in the present study were weighting 1000-1300g. They were purchased from local markets. Rabbits were left in the animals house for 1 week before experimentation to adapt to laboratory condition. They were kept in plastic cages with wire mesh covers and maintained under the following conditions: temperature (20ºC– 21ºC), relative humidity (40% - 60%) and a light /dark cycle of 14 and 10 hours. The cages were

freshly spread by wood saw to absorb urine of animals. Rabbits were given free access to commercial balanced diet and water *ad libitum* all over the experimental period. Animals were divide into five groups, as follows:
The first group was comprised 10 rabbits, served as control and injected intramuscularly with 1ml of saline solution for a month. The second, third and fourth groups, each comprised 6 rabbits, were administered intramuscular doses of 40 mg /Kg b. wt. /day tramadol HCl for a 10,20 and 30 days respectively (according to Sebnem et al., 2005). Six rabbits from third and fourth tramadol groups were left ten days more without any additional treatment as a recovery period after exposure.
All chemicals used were of analytical grade and were procured from Sigma Chemical Company Germany. Tramadol HCl (Tramal Ampoule) was purchased from Pharmacare Pharmaceutical company, Ramallah, West Bank .

Blood sampling and processing

Control and treated rabbits were decapitated at the end of 10, 20, 30, and 40 days, respectively. Blood was collected in dry centrifuge tubes. Sera were separated and kept at -20ºC until analysis. However, determinations of enzyme activities were carried out on fresh serum samples, Moreover, about 2 mL of blood samples were collected into a tube containing dipotasium ethylene diamine tetra acetate (EDTA) for the hematological tests.

Measurement of biochemical blood indices

Serum glucose, triglycerides and total cholesterol were determined using the method described by Trinder,(1969) Fossati and Prencipe (1980) and Allain et al., (1974) respectively. Serum urea measurement was based on the cleavage of urea with urease (Berthelot's reaction) according to Faweett and Scott(1960), serum uric acid was determined following the method described by Fossati et al.,(1982), serum creatinine was measured without protein precipitation according to Bartels et al., (1972). Serum total protein was determined according to biuret reaction ass designed by Armstrong and Carr, (1963).
The kits were purchased from Biotech laboratories, UK. Serum albumin was determined using RANDOX reagent kits, following their instruction manual according to the method of Doumas et al., (1971).
The concentrations of globulins (g/dL) were equal to Total protein – Albumin. The activities of serum AST and ALT were determined according to the method of Reitman and Frankel, (1957) The measurement of serum ALP and bilirubin activity was based on the method of Bessey et al.(1946) and Perry et al.(1983). Serum cholinesterase activity was measured by kinetic photometric test, according to the recommendation of German

Society of Clinical chemistry (DGKC), the method described by Ellman and his colleagues (Ellman, 1961) using Diasys reagent Kits. Estimation of serum Na was conducted by Flame Photometric method (Tietz,1990). Estimation of serum Ca was done by Gitel man(1967) method.

Hematological parameters

Determination of hematological parameters was carried out using an 18 automated parameter hematology analyzer. ABX Micros 60 from Horiba ABX. France.

Data analysis

Data were computer analyzed using SPSS version 11.0 for windows (Statistical Package for the Social Sciences lnc. Chicago, Illinois, USA). Means were compared by independent-samples test , $p<0.05$ was considered as significant. Percentage change was also calculated.

Table (1):Effect of Tramadol (40 mg/kg/day) Administration on Glucose, Cholesterol, Triglycerides and Bilirubin of rabbits.

parameters	Experimental period					
	control n=10	**10 days n=6**	**20 days n=6**	**30 days n=6**	**Recovery 20 days n=6**	**Recovery 30 days n=6**
Glucose (mg/dl)	90.1±0.36	94.67±0.35.07	105.33±0.4016.90	109.50±0.3021.53	90.5±0.360.44	104.5±0.2115.98
% change		>0.05	<0.05	<0.01	>0.05	<0.05
P value						
Cholesterol (mg/dl)	200.19±0.41	205±0.202.40	218.67±0.199.23	219.5±0.159.64	200.5±0.290.15	205±0.362.40
% change		>0.05	>0.05	>0.05	>0.05	>0.05
P value						
Triglycerides (mg/dl)	100.10±0.22	102±0.121.90	106±0.225.89	99.0±0.313.39	107.5±0.20-1.10	107±0.306.89
% change		>0.05	>0.05	>0.05	>0.05	>0.05
P value						
Bilirubin (mg/100ml)	0.21±0.05	0.50±0.06138.09	0.60±0.05185.74	0.65±0.03209.52	0.40±0.0690.48	0.5±0.03138.09
% change		<0.01	<0.01	<0.01	<0.01	<0.01
P value						

All values were expressed as mean ± S.E; P<0.05 significant; P<0.01 highly significant

Table (2): Effect of Tramadol (40 mg/kg/day) Administration on Protein Profile of rabbits.

parameters	Experimental period					
	control n=10	**10 days n=6**	**20 days n=6**	**30 days n=6**	**Recovery 20 days n=6**	**Recovery 30 days n=6**
Total protein (mg/dl)	7.99±0.29	6.98±0.26	6.69±0.22	6.80±0.30	6.19±0.33	6.96±0.20
% change		-12.64	-16.27	-14.89	-22.53	-12.89
P value		<0.05	<0.05	<0.05	<0.01	<0.05
Total albumin (mg/dl)	3.95±0.12	3.2±0.2	3.05±0.19	3.45±0.11	2.9±0.31	3.60±0.34
% change		-18.99	-22.78	-12.66	-26.58	-8.86
P value		<0.01	<0.01	<0.05	<0.01	>0.05
Total globulin (mg/dl)	4.04±0.12	3.78±0.15	3.64±0.13	3.35±0.11	3.29±0.01	3.30±0.13
% change		-6.44	-9.9	-17.08	-18.56	-18.32
P value		>0.05	>0.05	<0.01	<0.01	<0.01
urea (mg/dl)	34.19±0.70	35.18±0.80	44.11±0.16	46.5±0.31	34.5±0.12	34.90±0.13
% change		2.90	29.01	36.00	0.90	2.08
P value		>0.05	<0.01	<0.05	>0.05	>0.05
Uric acid (mg/dl)	4.10±0.1	4.20±0.11	4.33±0.12	5.5±0.13	3.99±.012	4.6±0.05
% change		2.44	5.61	34.15	-2.68	12.20
P value		>0.05	>0.05	<0.05	>0.05	<0.05
Creatinine(mg/dl)	0.98±0.05	1.10±0.06	1.45±0.03	1.55±0.05	1.0±0.04	1.15±0.06
% change		12.24	47.95	58.16	2.04	17.35
P value		<0.05	<0.01	<0.01	>0.05	>0.05

All values were expressed as mean±S.E; P<0.05 significant; P<0.01 highly significant

Table (3): Effect of Tramadol (40 mg/kg/day) Administration on Enzymes Activity and Electrolytes of rabbits.

parameters	Experimental period					
	control n=10	**10 days n=6**	**20 days n=6**	**30 days n=6**	**Recovery 20 days n=6**	**Recovery 30 days n=6**
Cholinesterase(U/L)	4200±149.11	4866.7±155.23	6166.67±172.66	5050±181.9	6450±191.60	5450±169.77
% change		15.86	46.81	20.24	53.57	29.76
P value		<0.05	<0.01	<0.01	<0.01	<0.01
ALP (U/L)	50.3±0.30	83.1±0.29	92.7±0.33	94.88±0.26	66.2±0.36	67.51±0.25
% change		65.21	84.29	88.63	31.61	34.21
P value		<0.01	<0.01	<0.01	<0.01	<0.01
ALT (U/L)	35.6±0.19	46.9±0.36	48.11±0.61	49.61±0.31	72.91±0.17	37.65±0.28
% change		31.74	35.14			

The Effects of Tramadol Hydrochloride Administration on

P value		<0.01	<0.01	39.35 <0.01	20.53 <0.01	5.76 >0.05
AST (U/L) **% change** **P value**	31.79±0.26	39.63±0.30 24.66 <0.01	41.39±0.35 30.20 <0.01	42.8±0.31 34.63 <0.01	40.1±0.22 26.14 <0.01	37.76±0.17 18.78 <0.01
Sodium meq/l **% change** **P value**	140±0.31	142.3±0.1 10.64 <0.05	155±0.3 10.71 <0.05	143±0.5 2.14 >0.05	164±0.25 17.5 <0.01	143.5±0.21 2.5
Calcium mg/dl **% change** **P value**	9.0±0.1	9.1±0.2 1.11 >0.05	9.3±0.1 3.33 >0.05	9.6±0.1 6.67 >0.05	10.75±0.2 19.44 <0.01	10.45±0.2 16.11 <0.05

All values were expressed as mean ± S.E; P<0.05 significant; P<0.01 highly significant

Table(4) effect of Tramadol (40 mg/kg/day) Administration on Total blood counts of rabbits.

Blood counts	Experimental period					
	control n=10	**10days n=6**	**20 days n=6**	**30days n=6**	**Recovery 20 days n=6**	**Recovery 30 days n=6**
WBC count ($x10^3$cell/ul) **% change** **P value**	7.51±0.18	14.75±0.16 96.40 <0.01	17.36±0.21 131.16 <0.01	19.4±0.22 158.32 <0.01	9.85±0.31 31.16 <0.01	10.3±0.16 37.15 <0.01
Lymphocyte **% change** **P value**	51.6±0.81	54.6±0.70 5.81 >0.05	60.2±0.71 16.67 <0.05	6.9±0.80 29.65 <0.01	58.6±0.19 13.57 <0.05	63.65±0.18 23.35 <0.01
RBC count ($x10^6$ cell/ul) **% change** **P value**	5.65±0.22	5.18±0.16 -8.32 >0.05	4.0±0.18 -29.20 <0.01	3.185±0.17 -43.63 <0.01	5.20±0.15 -7.96 >0.05	5.07±0.10 -10.27 >0.05
Hb (g/dl) **% change** **P value**	11.89±0.14	11.03±0.10 -7.23 >0.05	10.20±0.16 -14.21 <0.05	9.5±0.36 -20.10 <0.01	11.0±0.11 -7.49 >0.05	9.8±0.29 -17.58 <0.01
Hematocrit (%) **% change** **P value**	37.50±0.21	36.0±0.19 -4.0 >0.05	32.0±0.15 -14.67 <0.05	31.6±0.14 -15.73 <0.05	36.1±0.13 -3.73 >0.05	32.3±0.17 -13.87 <0.05
MCV (fi) **% change** **P value**	66.4±0.31	69.5±0.41 4.67 >0.05	82.0±0.30 23.49 <0.01	82.08±0.29 23.61 <0.01	69.42±0.35 4.59 >0.05	63.71±0.37 -4.05 >0.05
MCH (pg) **% change** **P value**	21.04±0.21	21.29±0.23 1.19 >0.05	25.5±0.19 21.20 <0.01	24.68±0.16 17.30 <0.05	21.15±0.17 0.52 >0.05	19.33±0.20 -8.13 >0.05
MCHC (g/dl) **% change** **P value**	31.71±0.25	30.61±0.25 -3.47 >0.05	31.10±0.17 -1.92 >0.05	30.06±0.19 -5.20 >0.05	30.47±9.19 -3.91 >0.05	30.34±0.16 -4.32 >0.05
Platelets($x10^3$ cell/ul) **% change** **P value**	386.0±25.90	307±30.91 -20.47 <0.01	298±22.61 -22.80 <0.01	286±29.70 -25.91 <0.01	369.5±30.69 -4.27 >0.05	374.0±26.29 -3.11 >0.05

All values were expressed as mean ± S.E; P<0.05 significant; P<0.01 highly significant

Results

Serum glucose, triglycerides and total cholesterol, mean values of rabbits affected by tramadol with/ without recovery are summarized in table (1). The data revealed that administration of tramadol for 30 days increased serum glucose level by 21.53% compared the control level. But after withdrawal of the drug the previous increment of serum glucose was reduced to 0.44% and 15.98% respectively, compared to the control level. while, triglycerides increased gradually and non-significantly from the first ten days treatment till one month ($p > 0.05$). On the other hand, mean values of serum cholesterol was non-significantly increased by 9.64 % in tramadol treated rabbits for 30 days compared to the control. Also, there was gradual higher significant increase in bilirubin observed at 10,20 and 30 days ($p< 0.01$) . The recovery group was noted to be significantly elevated over corresponding control group.

Table (2) showed that administration of 40 mg tramadol /Kg body wt./day significantly decreased serum total protein by -12.64, - 16.27 and -14.89 % at 10, 20 and 30 days respectively ($p< 0.01$). Also, serum Albumin concentration under the influence of tramadol for 10,20 and 30 days decreased by 18.99, 22.78 and 12.66% respectively. Moreover, table (2) shows a non-significant decrease in globulin concentration after tramadol treatment for 10 and 20 days($p> 0.05$), and highly significant decrease at 30 days ($p< 0.01$). Values of the recovery group was noted to be significantly decreased over corresponding control group.

Non protein nitrogenous constituents concentration in rabbits serum after administration of tramadol and the therapeutic action of recovery are tabulated in table (2). In general, intramuscular administration of tramadol increased urea, uric acid, and creatinine concentration compared to control level. The effect of tramadol was more pronounced on creatinine. However withdrawal of donates tramadol lowered these elevated values.

Table (3) indicated that administration of 40 mg tramadol /Kg body wt./day increased significantly the level of cholinesterase, ALT, AST and ALP at 10, 20 and 30 days of treatment ($p< 0.01$). The ten days recovery group remained significantly different compared to control group. In general, administration of tramadol increased sodium and calcium compared to control level at the different intervals of the experiment (table 3).

Table (4) shows blood indices in rabbits after administration of 40 mg tramadol /Kg body wt./day. The more obvious changes that resulted from the administration of tramadol were an increase in WBC count, lymphocyte, MCH and MCV, but there was a decrease in RBC count, hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), hematocrit and PLT.

Nevertheless, the recovery groups parameters showed signs of improvement.

Discussion

Data revealed a general increase in serum glucose levels in rabbits in response to tramadol administration. Tramadol may indirectly, play a specific role in carbohydrate metabolism probably due to enhancing gluconeogenesis and glucose mobilization to the blood (Berne and Levy 1998 and Bishop et al., 2005). The change observed in serum triglycerides and cholesterol content in response to treatment by tramadol drug; take place in the liver due to imbalance between the normal rabbits of lipid synthesis, utilization and secretion (Glasser and Mager,1972). The increment in cholesterol and triglyceride content agreed with that reported by Parker et al., (1984).The possible explanation of these observed increments may be explained on indirect action of tramadol on lipid metabolism or lipid peroxidation (Berne and Levy, 1998).

Urea is the principal end product of protein catabolism an accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated level of urea. The increment in blood urea, might be also due to the destruction of RBCs during the treatment. The presence of some toxic compounds might increase blood urea and decrease plasma protein (Varely 1976). On the other hand the elevation of blood urea might suggest that animals experienced hemoconcentration due to mild dehydration (Debra Manzell, 2008).

Moreover, the serum uric acid levels exhibited an increase in the treated rabbits for the experimental duration. This may be due to high degradation of purines or an increase of uric acid level by inability of its excretion by urinary system (Wolf et al., 1972). Hemolysis of red blood cells may cause damage of functioning nephrons and impairers renal function (Bishop et al., 2005).

On the other hand, current results indicated an increase in creatinine levels in rabbits which received 40 mg /Kg tramadol after 30 days treatment. These results are in accordance with Atici et al. (2005) who reported an increase in BUN (Blood Urea Nitrogen) and creatinine levels in rats receiving morphine for a month. The significant decreased levels of total protein in tramadol treated rabbits could be attributed to an increase in amino acids deamination. This decrease in serum total protein may be due to lowered synthesis of albumen and globulin in the liver in response to tramadol intake. It was reported that albumin levels are decreased in liver disease (Nyblom et al.,2004). The decrease in these blood proteins of the

rabbit may be due to usage of different amino acids in the production of antibodies in response to tramadol administration.

The significant increase in serum cholinesterase observation in the present work may be attributed to the idea that tramadol binds to acetylcholine receptors, leading to increase acetylcholine levels. The activity of cholinesterase enzyme leads to breaks down the excess acetylcholine i.e. increase cholinesterase activity recorded in the present work (Ides et al., 2006). After re repeated oral administration of tramadol in humans (Marthinesen et al., 1998), the main signs of intoxication include central nervous symptoms, from vomiting to convulsions or eventually anoxic brain damage (Spiller et al., 1997).

Serum transaminases (AST&ALT) and bilirubin exhibited a highly significant increase in tramadol treated rabbits compared to the control. As a liver specific enzyme ALT only significantly elevated in hepatobiliary disease. Increase in AST level, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma (Moss and Handerson 1999) and Vozarova et al., (2002).

The liver and kidney are responsible for tramadol metabolism and excretion. It may cause hepatotoxicity and nephrotoxicity during its metabolism (Wu et al., 2001; Janssen- Ortho Inc., 2005). Borzelleca et al. (1994) reported increased in ALT, AST and LDH activities in rats after long- term usage of morphine-like agent; levo-alpha-acetylmethadol HCl (LAAM). Also, a significant increase in the levels of ALT, LDH and lipid peroxides was reported among chronic heroin users (Panchenko et al., 1999). Similarly, the present data showed significant increase in the levels of ALT and AST among rabbits received doses of tramadol (40 mg /Kg body wt.) . Consequently, elevated ALT and AST activities observed in the current study in response to tramadol administration could be a common sign of impaired liver function. ALP present in cell surface in most human tissues. The highest concentration are found in the intestine, liver bone, spleen and kidney (Gitnick et al., 1992) and Moss and Handerson (1999) . The specific location of the enzyme with both sinusoidal and bile canalicular membranes accounts for the more predominant elevations in certain disorders as observed in the present study with tramadol administration. Impaired secretion of hepatic ALP of liver cell origin may be accompanied by acute cell necrosis, so liberation of ALP in the circulation is elevated. The cellular injury may still persist as indicated by increased AST, ALT and ALP activities. The findings of the current investigation were in agreement with the findings of Sebnem et al., (2005) who reported that the levels of

ALT, AST, LDH and Blood urea nitrogen (BUN), and creatinine were significantly higher in morphine grouped compared to the control group.
The findings also revealed a general increase in calcium and sodium levels in rabbits in response to tramadol administration. Serum level of electrolytes: (Na^{+}), (K^{+}), (Ca^{2+}) and (Po_4^{3+}) may be affected as a result of tissue damage due to hypoxia and asthmatic medication (Kolski et al 1988).
The present findings also showed significant increase of total WBCs count and lymphocyte. This significant increase in WBCs count indicated the activation of defense mechanism and immune system of rabbit. This induction of white blood cells is a positive response for survival due to cell mediated immune response of animals (Kollar and Roan, 1980). Leukocytosis was manifested by lymphocytosis, which was the main features of the differential leukocytic count.
The red blood cells(RBCs) count showed a general decrease in response to tramadol administration. This finding may be explained on the basis of inhibitory effect of tramadol on histogenesis. The decreased in RBC count and hemoglobin (Hb) lowered the oxygen supply to different tissues thus resulting in low energy production. Decrease in Hb contained MCH can be explained due to decreased size of RBC or impaired biosynthesis of heme in bone marrow. These findings are in agreement with the reported decrease in RBC count and Hb content after treatment with tramadol (Goeringer et al., 1997).

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