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The effect of Tramadol on some blood and biochemical parameters of male rats (*Rattus norvegicus*)

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Abstract:

The present study aimed to explain the dose-dependent possible deleterious effects of 30 day administration of Tramadol on some hematological and biochemical parameters of laboratory male rats (*Rattus norvegicus*), the study consisted of eighteen adult male rats randomly divided into three equal groups (each of six). Group 1 (control) were treated by intraperitoneal injection of normal saline solution (0.2 ml), group two (low dose) was treated by intraperitonealy (i.p) injection of Tramadol at a dose of 50 mg/kg/day, group three (high dose) was treated by intraperitonealy injection of Tramadol at a dose of 100 mg/kg/day for 30 days. At the end of experimental period, rats were sacrificed. Blood were collected by cardiac puncture to investigate blood film and biochemical parameters which include Aspartate transaminase (AST), Alanine transaminase (ALT), urea, and glucose. Results explained a significant reduction in hemoglobin (Hb), packed cell volume (PCV), and red blood cells count (RBC), in both treated group and significant elevation in WBC count which is clearly appeared in lymphocyte count, while the biochemical results showed a significant increased in ALT, blood urea, and decreased in blood glucose level in high dose treated group mostly.

Key words: Tramadol, Rats, blood film, liver enzymes.

Introduction:

Tramadol hydrochloride (TH), a synthetic opiod of the aminocycloh-exanol group, is a centrally acting analgesic that has been proved to be effective in both experimental and clinical pain treatment without causing serious cardiovascular or respiratory side effect [1]. Unlike traditional opiod receptor agonists, Tramadol hydrochloride has limited effects on respiratory or cardiovascular parameters as well as low abuse or dependence potentiality. Oral and parental Tramadol hydrochloride effectively relieves acute or chronic; moderate to severe pain condition [2]. Tramadol hydrochloride, a widely used opiod in recent years, is an effective analgesic agent for the treatment of moderately sever acute or chronic pain [3] its prolonged use in chronic cancer and non- cancer pain (e.g. lowback, osteoarthritis, neuropathicpathic, fibromyalgia, migraine …etc) is well established [4]. Also, it has been suggested that Tramadol hydrochloride could be effective for alleviating symptoms of depression, anxiety, and phobias [5]. Additionally, Tramadol hydrochloride seems to have a specific

role in the treatment of opiate withdrawal [6] and premature ejaculation [7]. The most frequent adverse effects of Tramadol hydrochloride include constipation, nausea, dizziness, headache, somnolence, and vomiting [8]. The most serious adverse reactions include confusion, hallucination, convulsions, serotonin syndrome and hypersensitivity reactions also, several reports of .Tramadol has a dose – dependent analgesic efficacy that lies between that of codeine and morphine, with parental potency comparable to that of pethidine, i.e. about 10—20% of standard morphine [9, 10]. Long term administration of an opiod drug for chronic non-cancer pain continues to be controversial [11, 12]. Also long-term effects of Tramadol at cellular level are not clearly understood [13]. So the present study was conducted to assess some blood and biochemical parameters of the (Tramadol hydrochloride) during 30 days treatment.

Materials and Methods:

The experiment was conducted at the animal house of Veterinary Medicine College / university of Basra. Where 18 adult male rats (Rattus norvegicus), age 8 weeks old and average body weight between (180-200 gr.) were selected randomly, they were maintained at standard experimental condition. The rats were housed in a quiet non-stressful environment for one week before the study. All rats were housed in plastic cages in a room with controlled temperature and humidity. They were kept under good hygienic conditions. Food and water were provided daily (*ad libitum*). Rats were maintained on a natural 12 h light- 12 h dark cycle. The general condition and behavior of rats were noticed. After the accommodation period, eighteen young male rats were randomly divided into three groups (6 in each group) as following: group one (control): in which the animals were injected normal saline solution (0.2ml) intraperitonealy for 30 days group two: the animals were injected with Tramadol drug intraperitonealy at a dose of 50 mg/kg/ day for 30 days. Group three: the animals were injected with Tramadol drug intraperitonealy at a dose of 100 mg/kg/day for 30 days. All experimental rats were sacrificed at the end of experiment period by anesthetized them with chloroform, after that abdominal cavity was opened by midline incision and take blood samples. Blood samples were collected via cardiac puncture by using 5 ml disposable syringe. The blood sample were collected in tube containing Ethline diamine tetracetate (EDTA) anticoagulant to study the blood parameters hemoglobin (Hb), packed cell volume (PCV), and differential white blood cell (WBC) in according to [14] and total WBC in according with [15]. While the second part of blood was collected into test tube free from anticoagulant to separate serum for estimation the biochemical parameters (AST,ALT, urea, blood glucose) by using chemistry autoanalyzer (Serial No.20628, Human Star, Germany). The device has 54 wells numbered from 1 to 54. The Samples were placed in each specific well of the device. The reagent was put in special container beside the wells.

The results of the present study were analyzed by univalent analysis of variance (ANOVA) by using computerized SPSS (Statistical Packages for the Social Sciences) V.13 program. $P<0.05$ was considered to be the limit of significance. The data were expressed as mean ± standard deviation (mean ± SD).Least significant difference test (LSD) was used to test the difference between groups (SPSS, 2001).

Results:

Table (1) showed that the Hb concentration, PCV, and RBC count were significantly decreased ($p \leq 0.05$) in mals rats injected with Tramadol hydrochloride 50 and 100 mg/kg BW as compared with control group.

Table (2) showed a significant increased ($p \leq 0.05$) in total WBC count in male rats injected Tramadol hydrochloride 50 and 100 mg/kg BW as compared with control group with non significant differences between treated group. As illustrated in results of this table that there were non significant differences in monocyte, neutrophil, basophile, and eosinophil, percentage in all treated group as compared with control group. Whereas There were significant increased ($p \leq 0.05$) in lymphocyte percentage in male rats injected Tramadol hydrochloride 100 mg/kg compared with control group with non significant differences between treated group, and no significant changes in rats treated with Tramadol 50 mg /kg as compared with control group.

Table (3) showed that the Alanine transaminase (ALT) activity significantly increased ($p \leq 0.05$) in male rats injected with Tramadol hydrochloride 50 and 100 mg/kg BW groups as compared with control group. Whereas there were no significant differences among treated animals groups. The blood glucose level was significantly decreased ($p \leq 0.05$) in Tramadol hydrochloride 100 mg/kg group and a significant increased ($p \leq 0.05$) in blood urea in the same group as compared with control group. There were no significant changes in Aspartate transaminase (AST), in male rats' injected Tramadol hydrochloride 50 and 100 mg/kg BW compared with controlled group.

Table (1) The effects of Tramadol on hemoglobin concentration (Hb) g/dl. Packed cell volume (PCV) % and red blood cell count (RBCC) $n \times 10^6$ (Mean ± SD)

Parameter / Group	Hb g/dl	PCV %	RBC n x 10^6
Control group	13.7 ± 0.1 $_a$	38.5 ± 0.8 $_a$	7.4 ±0.1 $_a$
Group 1 50 mg/kg	12.2 ±1.3 $_b$	35.9 ±1.2 $_b$	6.5 ± 0.8 $_b$
Group 2 100 mg/kg	9.0 ± 1.2 $_c$	30.7 ± 0.5 $_c$	4.9 ± 0.9 $_c$
LSD	1.32	1.12	0.90

Different letters represent significant difference at ($p \leq 0.05$)

Table (2) The effect of Tramadol hydrochloride on white blood cell (WBC) count and differential white blood cell count (DWBC) % count in male rats: (Mean ± SD)

Parameters / Group	Total WBC $n \times 10^3$	Differential WBC %				
		Lymphocyte	monocyte	neutrophil	basophil	eosinophil
Control group	6.89± 0.5$_a$	84.0± 1.1 $_a$	9.34± 0.7 $_a$	5.18± 0.6 $_a$	0.98± 0.6 $_a$	0.49± 0.4 $_a$
Group 1:- 50 mg /kg	7.5 ±0.7 $_{ab}$	85.4 ± 2.7 $_{ab}$	8.35 ± 1.7 $_a$	4.89 ± 1.0 $_a$	0.61 ± 0.5 $_a$	0.72 ± 0.2 $_a$
Group 2 :- 100 mg/kg	8.97 ± 2.0 $_b$	86.71 ± 2.2 $_b$	8.24 ± 1.6 $_a$	4.32 ± 0.7 $_a$	0.40 ±0.1 $_a$	0.33 ± 0.2 $_a$
LSD	1.55	2.64	1.77	1.06	0.64	0.41

Different letters represent significant difference at ($p \leq 0.05$).

Table (3) The effects of Tramadol on Aspartate transaminase (AST), Alanine transaminase (ALT), urea, and serum glucose level (mean ± SD).

Parameters / Groups	AST (GOT)u/l	ALT (GPT) u/l	Glucose mg/dl	Urea mg/dl
Control group	162 ± 32.2 a	34.1 ± 5.4 a	191.6 ± 48.6 a	30 ± 3.1 a
Group 1:- 50mg/kg	162.7 ± 42.7 a	57.1 ± 10.0 bc	177.3 ± 21.2 a	32 ± 3.1 a
Group 2:- 100mg/kg	169.7 ± 22.4 a	68.4 ± 23.6 c	150.3 ± 9.8 b	35.1 ± 4.3 b
LSD	41.26	18.66	38.37	4.41

Different letters represent significant difference at ($p \leq 0.05$)

Discussion:

The results of the present study explained that Tramadol hydrochloride at doses 50 mg and 100 mg/kg BW were significantly decreased in red blood cell (RBC) count, packed cell volume (PCV), and hemoglobin concentration (Hb) levels in male rats after 30 days injection. The present study also revealed that the total white blood cell (WBC) counts, and lymphocyte, were significantly elevated in 50 mg and 100 mg/kg BW. This significant increase in WBCs count indicated the activation of defense mechanism and immune system of rats. This induction of white blood cells is a positive response for survival due to cell mediated immune response of animals [16].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 erythropoiesis. The decreased in RBC count and hemoglobin (Hb) lowered the oxygen supply to different tissues thus resulting in low energy production. These findings are in agreement with the reported decrease in RBC count and Hb content after treatment with Tramadol [17].

These results were found a significant increased in the level of Alanine transaminase (ALT) among rats received both doses of Tramadol (50 mg and 100 mg/kg BW). Long-term use of Tramadol in rats was reported to significantly increase serum ALT level alone [18], whereas increased in Alanine transaminase(ALT) and Aspartate transaminase (AST) were observed in morphine following the same duration of administration [13]. Repeated Tramadol administration in patients might lead to the accumulation of toxic metabolites in the body, increase the risk for pharmacokinetic interactions, and/or decrease the clearance of Tramadol, thus increasing its potential for toxicity. In addition metabolites may have a higher activity and /or greater toxicity than the original drug. Therefore, metabolites of drugs that excreted via the kidneys may also cause cellular damage leading to kidney dysfunction [19]. The liver and kidney are responsible for Tramadol metabolism and excretion. It may cause hepatotoxicity and nephrotoxicty during its metabolism. Liver specific enzyme ALT is 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 [20].

The present results also revealed reduced levels of glucose in Tramadol 100 mg/kg BW group, these results supported by another study [21] which showed that Tramadol can decrease glucose in diabetic rats, via the activation of opiod μ- receptors,

suggesting a mechanism possibly related to those of dextro-propoxyphene. Moreover, Tramadol act as serotonin reuptake inhibitor and hypoglycemia has been described with some serotonin antidepressant sertraline [22]. The hypoglycemic effect of Tramacet (Tramadol containing product) has been also reported as a metabolic disorder that occurred as an incidence of less than 1 % in clinical trials [23]. Tramadol may indirectly, play a specific role in carbohydrate metabolism probably due to suppress gluconeogenesis and glucose mobilization to the blood [24,25].

On the other hand, current results indicated a slight increased in blood urea in rats received Tramadol 100 mg/kg BW. These are in accordance with other study [13] who reported an increased in blood urea and creatinine levels in rats receiving Tramadol for a 30 day and after long term use of levo-alpha-acetylmethadol HCl (LAAM) [26]. 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.

From the results of our study it is obvious that Tramadol hydrochloride has a toxic effect on the liver, kidney and blood parameter (Hb, PCV%, RBC, and WBC count) especially at large doses and during chronic and longe term use, therefore its use should be cautious and dose selection should be careful, also we recommended for further study on Tramadol hydrochloride in pregnant female to study the teratogenic effects and also female and fetus side effects.

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تاثير عقار الترامادول على بعض المعايير الدمويه والكيموحيويه في ذكور الجرذان المختبريه

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الخلاصة:

اجريت الدراسة الحالية لدراسة تاثير عقار الترامادول على بعض المعاييرالدموية والكيمياء حيويه في ذكور الجرذان المختبرية (Rattus norvegicus).

شملت التجربة استخدام 18 من ذكورالجرذان المختبرية وقسمت الى ثلاثة مجاميع (6 لكل مجموعة) .

المجموعة الاولى: وتعتبر مجموعة سيطرة تم حقنها في غشاء الجدار البطني بمحلول الملح الاعتيادي لمدة 30 يوم ،المجموعة الثانية :تم حقنها بعقار الترامادول بتركيز 50 ملغم /كغم في غشاء الجدار البطني(I.P) intraperitonial لمدة 30 يوم ،المجموعة الثالثة : تم حقنها بعقار الترامادول بتركيز 100 ملغم /كغم في غشاء الجار البطني(I.P) intraperitonial لمدة 30 يوم ،وتم اعطاء الحيوانات عليقة متوازنة من الغذاء وكذلك الماء عند الرغبة خلال فترة التجربة . في نهاية التجربة جمعت عينات الدم وحساب عدد كريات الدم البيض الكلي (WBC)والعدد التفريقي لكريات الدم البيض(DWBC)، عدد كريات الدم الحمر(RBC)، نسبة الهميموغاوبين (Hdb)، وحجم كريات الدم الحمر المرصوصة(PCV). اظهرت النتائج ان التعرض للترامادول لفترات طويلة نتج عنه نقصان في عدد كريات الدم الحمر ونسبة الهيموغلوبين في الدم وحجم كريات الدم المرصوص في كلا المجموعتين وكذلك نتج عنه زيادة في عدد كريات الدم البيض وكانت الزيادة واضحة في زيادة نسبة الخلايا اللمفاوية في كريات الدم البيض . وكذلك كان هناك زيادة في انزيمات الكبد (ALT) وزيادة في نسبة اليوريا في الدم وانخفاض نسبة السكر في الدم وكانت وكانت التغيرات في مجموعة الجرعة العالية اكثر وضوحا.

الكلمات المفتاحية : الترامادول، الجرذان، قراءات الدم، انزيمات الكبد.