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Effects of subchronic exposure to meloxicam on some hematological, biochemical and liver histopathological parameters in rats

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Abstract

This experiment was conducted to study the effects of sub chronic exposure of Meloxicam in male albino rats by measuring Hematological, Biochemical and Histopathological changes of Livers in (18) rats divided equally into two treatment groups, T1 which dosed with 0.2 mg/KG.BW as therapeutic dose and T2 which dosed with 0.6 mg/KG.BW as three fold dose, while the other (6) animals considered as control group dosed with distilled water by stomach tube for duration (2) months. Evaluation of complete blood picture (RBCs and WBCs counts, differential WBCs count, platelets count, PCV and Hb), clotting time and serum level of liver enzyme function, Blood urea (BU) and histopathological examination of liver was performed at the end of experiment. The results revealed significant decrease in platelets count ($P\leq0.01$) of both treated groups and significant increase clotting time of T2 group ($P\leq0.01$) in comparison with the T1 group and control one. The results of differential count of white blood cells registered significant decrease($P\leq0.05$) in neutrophils and significant increases ($P\leq0.01$) in Monocytes and Lymphocytes of both treated groups in comparison with control group. The results of serum level of liver enzymes function revealed only significant increase ($P\leq0.01$) in AST of both treated groups in comparison with the control one, while the histopathological study of liver showed lesions was vary between vasodilatation, vasocongestion and necrosis, karryorrhexis of hepatocytes.

Keywords: Meloxicam, Hematological, Biochemical, Histopathological, Rat.
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تأثير التعرض شبه المزمن للميلوكسيكام على بعض المتغيرات الدموية والكيموحيوية والنسيجية في أكباد الجرذان

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الخلاصة

اجريت هذه التجربة لدراسة تأثير التعرض شبه المزمن للميلوكسيكام على الصورة الدموية والكيموحيوية والتغيرات النسيجية المرضية لأكباد ثمانية عشر من ذكور الجرذان المهقاء. قسمت الحيوانات بالتساوي إلى مجموعتي علاج، T1 تمثل الجرعة العلاجية ٠,٢ ملغم/كغم و T2 تمثل ثلاثة أضعاف الجرعة العلاجية ٠,٦ ملغم /كغم فيما جرعت مجموعة السيطرة بالماء المقطر يوميا لمدة شهرين باستعمال أنبوب اللي المعدي. تم فحص الصورة الدموية (عدد كريات الدم الحمر والبيضاء والعد التفريقي لكريات الدم البيضاء وعدد الأقراص الدموية وتحديد مستوى الهيموغلوبين بالدم وحجم الخلايا المرصوص (وحساب الزمن اللازم لتجلط الدم و تقدير مستويات الخمائر السريرية في مصل الدم (AP,ALT,AST) ويوريا الدم والفحص النسيجي المرضي لأكباد حيوانات مجاميع التجربة في نهايتها وأشارت نتائج هذه الدراسة إلى نقصان معنوي ي في عدد الأقراص الدموية على مستوى ($P\leq0.01$) لكلتي مجموعتي المعالجة مع زيادة معنوية في الوقت اللازم لتجلط الدم على مستوى ($P\leq0.01$) في المجموعة الثانية (T_2) مقارنة بمجموعتي المعالجة الأولى (T_1) والسيطرة (C). وقد أظهرت نتائج العد التفريقي لخلايا الدم البيضاء نقصانا معنويا في الخلايا العدلة على مستوى ($P\leq0.05$) وزيادة معنوية في كل من الخلايا وحيدة النواة والخلايا اللمفاوية على مستوى ($P\leq0.01$) في مجموعتي المعالج بالمقارنة مع مجموعة السيطرة،

بينما أشارت مستويات أنزيمات وظائف الكبد في مصل الدم إلى إلى زيادة معنوية على مستوى ($P\leq0.01$) في أنزيم (AST) لكلتا مجموعتي المعالجة (T_1،T_2) مقارنة بمجموعة السيطرة وأظهرت الدراسة النسيجية المرضية لأكباد مجموعتي المعالجة تراوحت بين توسع واحتقان الأوعية الدموية وتنكس وموت واضمحلال التركيبة البنيوية للخلايا.

Introduction

Nonsteroid anti-inflammatory drugs (NSAIDs) are among the most widely prescribed drugs worldwide, being the drugs of first choice in the treatment of rheumatic disorders and other degenerative inflammatory joint diseases. Inhibition of acyclooxygenase (COX), and therefore prostaglandin production, is the common mechanism of action of the NSAIDs (1). NSAIDs derive much of their anti-inflammatory properties from their capacity to inhibit the synthesis of prostaglandins (2). Meloxicam, an oxicam derivative, is a member of the enolic acid group of nonsteroidal anti-inflammatory drugs (NSAIDs), it is act by inhibiting Cyclooxygenase enzyme(COX) (3,4). Both COX-1 and COX-2 are affected by Meloxicam (5). Meloxicam has been found to be effective in the treatment of rheumatoid arthritis, osteoarthritis and degenerative joint diseases (6). Meloxicam is used in the control of acute musculoskeletal inflammation and pain in cat and also used in the alleviation of chronic pain and inflammation; however, additional research would more clearly define dosage recommendations for cats. (7). Gastrointestinal adverse effects are most commonly associated with meloxicam therapy (26.6%). These include abdominal pain (2.6%), constipation (1.2%), diarrhea (2.7%), dyspepsia (7.4%), flatulence (0.4%), nausea (4.7%), and vomiting (0.8%) (8). More frequent than 1%: anemia between 0.1 and 1%: disturbances of blood count, including differential white cell count, leucopoenia and thrombocytopenia (9). Meloxicam doses in rats, mice were in the dose range of 0.2 to 10 mg/kg BW. (10). dogs receiving 0.3 mg/kg a day and 0.5 mg/kg a day for six weeks developed renal enlargement. When the kidneys were examined microscopically, degeneration or slight necrosis at the tip of the papilla was noted in three dogs receiving 0.5 mg/kg a day (8). Among the most sensitive and widely used of liver enzymes are the aminotransferases. They include aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). These enzymes are normally contained within liver cells. If the liver is injured, the liver cells spill the enzymes into blood, The level is increased in cases of liver cell death resulting from cases, such as shock or drug toxicity. (11,12). This experiment was amid to study the effect of sub chronic exposure of Meloxicam at different doses on Hematology, Biochemistry as well as Histopathological changes.

Materials and methods

Total number of eighteen (18) Albino Wibster rats weighed (250-300) grams were raised and bred in the animal house of college of medicine-kufa University where the research was done. The animals were kept in cages of (20x30x50) cm^3 dimensions in average of three rats in each cage one month before study for acclimatization in optimum conditions of breeding at (22±3) ºC with a(14 /10) Hours (Light/Dark) cycle. Standard Pellet was provided ad-libitum. The animals divided equally into two treatment group T1, T2 and control group (C). Dosing solution of meloxicam (Boehringer Ingelheim, Germany) was prepared by dissolving one tablet of 7.5 mg in 75 milliliters of distilled water to prepare concentration of 0.1 mg/ mL that used for dosing all animals of treatment groups at following volume dose rate 0.2ml / 100gm.BW for T 1 group dosed with therapeutic dose of meloxicam (0.2mg/kg.BW). 0.6ml / 100gm.BW for T 2 animals dosed with three fold dose (0.6mg/ kg.BW) for (60) days, while the animals of control group dosed with distilled water**.** Animals anesthetized by chloroform and the blood collected from the heart divided for both hematological and biochemical tests. certain hematological tests which included red Blood cells counting according to, differential white Blood cells counting, Determination of Packed cells Volume (PCV***)*** according to microhematocrit method determination of Clotting time by non heparinized Capillary tubes (micro-hematocrit tubes) were used, after blood was drawn from tail and filled the tube with blood, timing is beginning when a fibrin strand appears a piece of the tube breaks off once every 60 seconds till the blood has clotted, so calculate the time of clotting by number of broken pieces. Determination of hemoglobin**:-**by converting Hemoglobin to mithemoglobin by drabkin reagent and read by Hb-meter method. Platelet count when blood delivered with out frothing into a tube containing the anticoagulant dipotassium EDTA then: according to Lucas and Jamroz method. Biochemical tests for evaluation liver functions through estimation of liver enzymes such as Alanine amino-transferase (ALT) (Randox, United Kingdom), Aspartate amino- transferase (AST) (Randox, United Kingdom), the pyruvate which produces by transaminase of (ALT) react with 2,4-Dinitrophenyl hydrazine (NAPH) to give colored hydrozones, while oxaloacetate which produces by (AST) decarboxylates spontaneously to pyruvate, in both reaction measured colorimeter at 510 nm, then apply the following equations:

[T-C / S-B] × 0.4 × 1 / 30 × 1000 / 0.1 (ALT)

[T-C / S-B] × 0.4 × 1 / 60 × 1000 / 0.1 (AST)

T = test, C = control, S = standard, B = blank, 0.4 = normality of Na OH, 30, 60 = time of pyruvate formation, 1000 = pyruvate formed per litter.

And Alkaline Phosphates (AP) (Randox, United Kingdom) estimation through colorimeter determination of librates phenol in the presence of 4-amino antipyrine and potassium ferricyanide (13), also blood urea by urea – kit (Randox, United Kingdom) enables end point enzymatic determination of urea concentration (Conc.) (urease - modified Benhelot reaction) in serum, urease hydrolyzes urea by producing ammonium which is formed green color indophenol in an alkaline medium when reacts with salicylate and hydrochloride, the color intensity is proportional to the urea Conc. in sample (14). Biopsy of livers and kidneys from all treated and Control animals sent to histopathological study presented in 10 % formalin.

Statistical analysis

Analysis of variance (ANOVA) one way and least significant differences (LSD) at significant level of (P≤0.05) and (P≤ 0.01) to compare the data of different groups through out the period of experiment (15).

Results and discussion

Hematology

RBC count, WBC count and platelets count

There were no significant changes in both RBCs and WBCs count between animals of (T1, T2) groups in comparison with that of control one (C), which is may be due to the highly inhibition of Cyclooxygenase enzymes. The results showed significant decreases (P≤0.01) in platelets count of both treated groups animals in comparison with the control group, which was positively proportional with the dose. Table (1) the administration of 7.5 mg and 15 mg of Meloxicam caused dose-dependent reduction in platelet COX-1 activity by 25% and 35% respectively.

Packed cell volume (PCV), Hemoglobin (Hb) and Clotting Time

The results of packed cell volume showed significant decrease in PCV (P≤0.01) of both treated groups T1 and T2 in comparison with animals of the control group (C). which may be due to the non significant decrease of both RBCes and WBCes count in one hand and decrease in platelets count of both groups in the other hand, table (2). Hemoglobin level showed significant decrease (P≤0.05) only in animals of T2 group in comparison with hemoglobin of control group animals as listed in table (2). This decrease might be due to high dose of Meloxicam penetration into the red blood cells less than 10% after oral administration of (16). The event of hemoglobin decrease in the present study was in agreement with (16) who found that the immune-mediated anemia in dog during two field studies with initial subcutaneous injection of meloxicam (0.2mg/Kg.BW) on the first day followed by (0.1mg/Kg.BW) orally once a day for 13 days. Clotting time values showed significant increases (P≤0.01) of treatment groups T1 and T2 in comparison with the animals of control group. The effect was positively proportional with the dose as listed in table (2) Meloxicam with high dose has been associated with inhibition of platelets aggregation and with potential bleeding through minimize TXA2 (17). We could say that the significant decrease in platelets count resulted increase in clotting time.

Table (1): The effects of sub chronic exposure of meloxicam in two different doses on red blood corpuscles count (RBCs count), white blood cells count (WBCs count) and Platelets count in male albino rats.

Group	RBCs Cu / mm M ± SE	WBCs Cu / mm M ± SE	Platelets Cu/mm M ± SE
T1 n=6	$6.78x10^6 \pm 134.1x10^3$ a	$6.983x10^3 \pm 1186$ a	$41.333x10^3 \pm 2.231x10^3$ a
T2 n=6	$6.903x10^6 \pm 603.33x10^3$ a	$6.763x10^3 \pm 703$ a	$41.500x10^3 \pm 3.730x10^3$ b
C n=6	$8.09x10^6 \pm 388x10^3$ a	$8.583x10^3 \pm 140$ a	$124.5x10^3 \pm 20.762x10^3$ c

T1= sub acute exposure to therapeutic dose (T.D) 0.2 mg/kg BW, T2= sub acute exposure to three folded dose (3 FD) 0.6mg/Kg BW, C= control group dosed distilled water (D.W), N= number of animals, Different letters mean significant changes between groups at level (P≤0.01).

Table (2): The effects of sub chronic exposure of meloxicam in two different doses on packed cells volume (PCV), hemoglobin (Hb) and clotting time in male albino rats.

Group	PCV% M ± SE	Hb g/100 ml M ± SE	Clotting time/minute M ± SE
T1 n=6	36.9 ± 0.68 a	11.63 ± 0.39 a	5.4 ± 0.23 a
T2 n=6	31.4 ± 3.1 a	9.61 ± 0.96 b	5.5 ± 0.22 a
C n=6	44.3 ± 1.84 b	12.6 ± 0.41 a	3.58 ± 0.23 b

T1= sub acute exposure to therapeutic dose (T.D) 0.2 mg/kg BW, T2= sub acute exposure to three folded dose (3 FD) 0.6mg/Kg BW, C= control group dosed distilled water (D.W), N= number of animals, Different letters mean significant changes between groups at level (P≤0.01).

Differential count of WBC

The results of differential count of white blood cells showed significant decrease (P≤0.05) in neutrophils percent of the treated groups animals (T1; T2) in comparison with that of control one, table (3) which was nearly resemble to the results of (9) who found that more frequent than 1% of disturbance in blood counting include differential white cells, leucopenia and thrombocytopenia. Eosinophils and Basophiles of both treated groups (T1; T2) showed no significant changes in comparison with that of the control group, table (3). Lymphocytes and. monocytes of both treated groups (T1; T2) showed significant increases (P≤0.01) in comparison with that of the control one Table (3). This might be due to the inhibitory effect of meloxicam on monocyte COX-2 as reported by (10) who administered orally 7.5 and 15 mg of meloxicam daily for 7 consecutive days caused dose-dependent reduction in monocytes COX-2 activity by 51% and 70% respectively and confirmed by detection the reduce in prostaglandin E2 in plasma as an index of Monocyte activity.

Table (3): The effect of sub chronic exposure of meloxicam at different two doses o differential counts of white blood Cells in Male Albino rats.

Group	Parameter				
	Neutrophils % M ± SE	Eosinophils % M ± SE	Basophiles % M ± SE	Lymphocytes % M ± SE	Monocytes % M ± SE
T 1	15.5 ± 6.32	0.6 ± 0.33	0.6 ± 0.51	79.1 ± 1.95	4 ± 1.5
N = 6	a	a	a	a	a
T 2	11.33 ± 0.55	1.6 ± 0.6	0.6 ± 0.33	79.5 ± 1.87	4.3 ± 0.66
N = 6	b	a	a	a	a
C	31.3 ± 0.76	1.16 ± 0.3	0.3 ± 0.2	66.3 ± 1.02	0.8 ± 0.3
N = 6	c	a	a	b	b

T1= sub acute exposure to therapeutic dose (T.D) 0.2 mg/kg BW, T2= sub acute exposure to three folded dose (3 FD) 0.6mg/Kg BW, C= control group dosed distilled water (D.W), N= number of animals, Different letters of neutrophils mean significant decrease (P≤0.05), Different letters mean significant changes between groups at level (P≤0.01).

Biochemical markers

Urea

Their was no significant changes in blood urea of both treated groups (T1, T 2) animals in comparison with the animals of the control one table (4).

AST, ALT and AP

The serum level of Alanineaminotransferase and Aspartareaminotrasferase of both two treated animals groups T1 and T2 reveled significant increases (P ≤ 0.01) in comparison with the animals of control one (C). While only the serum level of Alkaline phosphates of T 2 animals group showed significant increase (P ≤ 0.01) in comparison with the animals of the control group. The increases in enzyme level were accordingly with the dose Table (5).

The increase in serum levels of ALT and AST and AP considered as initial step in detecting liver damage due to viral, alcoholic anddrug-induced hepatocyte damage. These significant increases in serum levels of AST and ALT and AP of treated groups could be confirmed by our histopathological findings in livers which were revealed variable lesions ranged from extensive necrosis with mild

lymphocytic infiltration in T2 group, Figure (1), while the liver of (T2) treatment group revealed lymphocytic infiltration, hemorrhagic areas and sever necrosis, Figure (2). The marked extensive histopathological changes which observed in livers of affected groups might be due to the highest tissue concentration of Meloxicam in liver similar to that observed by (18) who found the high concentration of meloxicam in tissue of liver and kidney after multiple oral dose of {C14} meloxicam 1mg/Kg.BW/day for 5days in maleand female black hooded rats.

Table (4): The effect of sub acute exposure of Meloxicam at two different doses on Blood urea (B U) level (mmoL / L) in male albino rats.

Group	UREA mmoL / L (M ± SE)	
T1 n=6	13.45 ± 1.46	a
T2 n=6	15.2 ± 6.9	a
C n=6	7.87 ± 0,36	a

T1= sub acute exposure to therapeutic dose (T.D) 0.2 mg/kg BW, T2= sub acute exposure to three folded dose (3 FD) 0.6mg/Kg BW, C= control group dosed distilled water (D.W), N= number of animals.

Table (5): The effects of sub chronic exposure of two different doses of meloxicam on enzymes of liver function (ALT, AST and AP) in male albino rats.

Group	A L T. U/ L M ± S E	A S T. U/ L M ± S E	A P. U / L M ± S E
T1 n=6	111. 6 ± 2. 7 a	151. 6 ± 0.95 a	39. 4 ± 4. 8 a
T2 n=6	115.6 ± 5,9 a	154.5 ± 2. 93 a	75. 8 ± 16 4 b
C n=6	19.8 ± 8.09 b	64.6 ± 6,5 b	21.5 ± 2.5 a

T1= sub acute exposure to therapeutic dose (T.D) 0.2 mg/kg BW, T2= sub acute exposure to three folded dose (3 FD) 0.6mg/Kg BW, C= control group dosed distilled water (D.W), N= number of animals, Different letters mean significant changes between groups at level (P≤0.01).

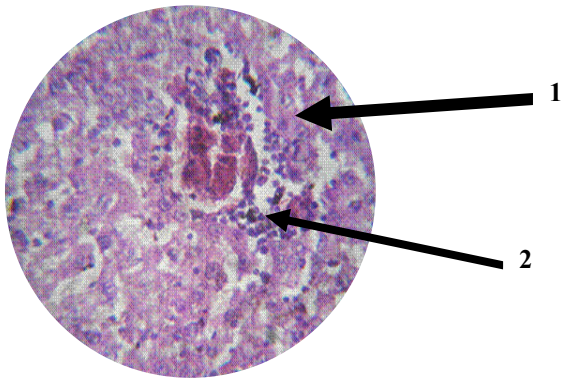

Figure (1): Cross section in liver of (T1) male rat received therapeutic dose of meloxicam for two months, observe,1- sever necrosis.2- mild lymphocytic infiltration. H & E (X 40).

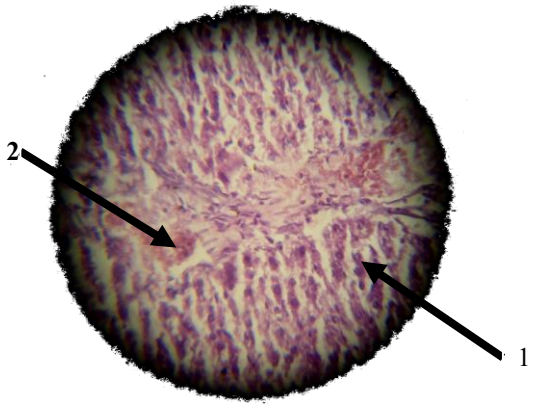

Figure (2): Cross section of liver of (T2) male rat received 3fd of meloxicam for 2 months, observe 1- sever necrosis, 2- area of hemorrhages. H & E (X 40).

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