THE EFFECTS OF SUB ACUTE EXPOSURE OF MELOXICAM AT DIFFERENT DOSES ON HEMATOLOGY , BIOCHEMISTERY AND LIVER HISTOPATHOLOGY IN MALE ALBINO RATS.

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ABSTRACT

This experiment was conducted to study the effects of sub acute 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 was dosed with 0.2 mg/kg.BW as therapeutic dose and T2 which was dosed with 0.6mg/kg.BW as three fold the therapeutic dose, while the other (6) animals were considered as a control group and was dosed with distilled water by stomach tube for duration of (4) weeks. Evaluation of complete blood picture (RBCs and WBCs counts, differential WBCs count, platelets count, PCV and Hb), clotting time and serum level of liver enzymes, Blood urea (BU) and histopathological examination of liver was performed at the end of the experiment. The results revealed a significant decrease in platelet count ($p \leq 0.01$) of both treated groups and significant increase in clotting time of T2 group ($p \leq 0.01$) in comparison with theT1 group and control one.The results of differential count of white blood cells showed a significant decrease ($P \leq 0.05$) in neutrophils and a significant increases ($P \leq 0.01$) in monocytes and lymphocytes of both treated groups in comparison to control group. The results of serum level of liver enzymes revealed only a significant increase ($P \leq 0.01$) in AST of both treated groups in comparison to the control one, while the histopathological study of liver showed lesions which varied in severity according to meloxicam dose and included vasodilatation , vasocongestion , necrosis and ,karryorrhexis of hepatocytes .

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(Dhikav *et al* .,2002). Inhibition of cyclooxygenase (COX), and therefore prostaglandin production, is the common mechanism of action of the NSAIDs (Vane, 1971). NSAIDs derive much of their anti-inflammatory properties from their capacity to inhibit the synthesis of

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prostaglandins(Abransom and Weissman 1989). Meloxicam, an oxicam derivative, is a member of the enolic acid group of nonsteroidal anti-inflammatory drugs (NSAIDs), it acts by inhibiting cyclooxygenase enzyme(COX) (Churchill *et al*., 1996 .,Engelhardt *et al*., 1996). Both COX-1 and COX-2 are affected by Meloxicam (Patrignani *et al*., 1997 ., Riendeau *et al.*, 1997). Gastrointestinal adverse effects are most commonly associated with meloxicam therapy (26.6%). They include abdominal pain (2.6%), constipation (1.2%), diarrhea (2.7%), dyspepsia (7.4%), flatulence (0.4%), nausea (4.7%), and vomiting (0.8%) (Boehringer, 2001).Others including anemia , disturbances of blood count, leucopoenia and thrombocytopenia. .(Furno,2001) . Meloxicam doses in rats and mice range from 0.2 to 10 mg/kg BW(EMEA, 2006). 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 (Boehringer, 2003). Among the most sensitive and widely used in human and animals of liver enzymes are the aminotransferases. Their level is increased in cases of liver cell death resulting from cases , such as shock or drug toxicity where monitoring of liver function tests (LFTs) is recommended (Hutchinson *et al* ; 2002 .,Sally *et al.* ; 2002) . This experiment was aimed to study the effect of sub acute exposure of meloxicam at different doses on Hematological , Biochemical 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 (20Hx30Wx50L) 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 (Hafes,1970).Standard pellet was provided *ad-libitum* .The animals were divided equally into two treatment group T 1 , T 2 and control group(C) . Meloxicam* was dosed orally at a dose of 0.2 mg/kg.BW for T1group representing normal therapeutic dose(by divided the maximum daily therapeutic dose 15 mg to the mean body weight of human 70 kg), and 0.6 mg/kg.BW for T2group representing three fold therapeutic dose(because the toxicity exposure study needs at least two different level of exposure) . While the animals of control group were dosed with distilled water. Dosing continued for four weeks . Animals were anesthetized by chloroform and blood was collected from the heart for both hematological and biochemical tests. Certain hematological tests which included red blood cells counting according to (Dacie and Lewis, 1984), differential white Blood cells counting , and determination of packed cells Volume (PCV***)*** (Coles, 1986), Determination of clotting time by non

heparinized capillary tubes (micro-hematocrit tubes) were used ,After blood was drawn from the tail and filled the tube with blood. When a fibrin strand appears a piece of the tube breaks off once every 60 seconds till the blood clots. The time of clotting was calculating by number of broken pieces (Coles, 1986). Determination of hemoglobin was made by converting hemoglobin to methemoglobin by drabkin reagent and read by Hb-meter (Coles , 1986). Platelet count **,** blood was delivered without frothing into a tube containing the anticoagulant dipotassium EDTA then:calculated according to method of (Archer, 1965).Biochemical tests for evaluating liver functions through estimation of liver enzymes such as Alanine amino-transferase (ALT) **, Aspartate amino- transferase (AST) **, were measured by using colorimeter determination (Reitmans, and Frankel, 1957), and Alkaline Phosphates (AP)* * was estimated by colorimeter determination (Kind and King, 1954) . also blood urea by urea – kit** (Wills and Savory, 1981). Biopsy of livers and kidneys from all treated and control animals were sent for histopathological study in 10 % formalin.

S***tatistical Analysis :-***

Analysis of variance (ANOVA) one way and least significant differences (LSD) at a significant level of ($p \leq 0.05$) and ($p \leq 0.01$) to compare the data of different groups through out the period of experiment.(Al–Mohammed, *et al.*;1986).

RESULTS AND DISCUSSION

1-Hematology

- RBC ,WBC and platelets counts :- There were no significant changes($p \leq 0.05$) in both RBC and WBC count between animals of (T1, T2)groups in comparison with that of control one (C) . The results showed significant decreases ($P \leq 0.01$) in platelets count of both treated groups in comparison with the control group, which was positively proportional with the dose. Table (1). Immune thrombocytopenia reported by Boehringer (2003) in dogs during two field studies with initial subcutaneous injection of Meloxicam 0,2mg/kg.BWon the first day followed by 0.1mg/Kg.BW orally once a day for 13 days, while Maria *et al* (1999) found that administration of 7.5 mg and 15 mg of Meloxicam caused dose-dependent reduction in human platelet COX-1 activity by 25% and 35% respectively.

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Table 1. *The effects of sub acute exposure of meloxicam in two different doses on red blood cell count(RBCcount) , white blood cell count (WBC count) & Platelets count in male albino rats* **.**

Group	RBC cu / mm M ± SE	WBC cu / mm M ± SE	Platelets cu/mm M ± SE
Control C n=6	$8.09x10^{6} \pm 388.00x10^{3}$ **A**	$8.58x10^{3} \pm 140$ **A**	$124.50x10^{3} \pm 20.76x10^{3}$ **C**
T1 n=6	$7.54 \times 10^{6} \pm 112.75 \times 10^{3}$ **A**	$7.25x10^{3} \pm 832$ **A**	$96.80x10^{3} \pm 11.93x10^{3}$ **A**
T2 n=6	$7 .37x10^{6} \pm 372.40x10^{3}$ **A**	$7.01x10^{3} \pm 926$ A	$52.16x10^{3} \pm 3.71x10^{3}$ **B**

- T1= sub acute exposure to therapeutic dose(T.D) 0.2 mg/kg.BW .
-T2= sub acute exposure to three fold 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 ($P \leq 0.01$

- Packed cell volume(PCV),Hemoglobin(Hb) and Clotting Time:- The results of packed cell volume (PCV %) and hemoglobin (Hb) of T1 and T2 groups didn't show any significant changes in comparisons with the control one, table (2). Clotting time values showed significant increases ($P \leq 0.01$) in T2 group in comparison with T1 and control group , because meloxicam in high doses was reported to be associated with inhibition of platelets aggregation and with potential bleeding through inhibition Thromboxane (TXA2) (Mathews *et al*, *2001*). The animals of T1 group showed no significant change($p \leq 0.05$) in clotting time in comparison with the animals of control group .

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

parameter / Group	PCV% M ± SE	Hb g/100 ml M ± SE	Clotting time(minutes) M ± SE
Control C n= 6	44.30 ± 1.84 **A**	12.60 ± 0.41 **A**	3.58 ± 0.23 **A**
T 1 n = 6	**44.16 ± 1.55** **A**	**12.28 ± 1.15** **A**	3.91 ± 0.20 **A**
T2 n = 6	**42.50 ± 2.75** **A**	**12.08 ± 1.11** **A**	5.40 ± 0.23 **B**

- T1= sub acute exposure to therapeutic dose(T.D) 0.2 mg/kg.BW .
-T2= sub acute exposure to three fold 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 ($P \leq 0.01$).

- Differential count of WBC :- The results of differential count of white blood cells showed significant decrease ($P \leq 0.05$) in neutrophils percent of the treated animals (T 1 ; T 2) in comparison with that of control , which was similar to the results of Furno,(2001)who found that more frequent than 1% of disturbance in blood counting include differential white cells, leucopenia and thrombocytopenia.. Eosinophils & basophiles of both treated groups (T 1,T 2) showed no significant changes in comparison with that of the control group. Lymphocytes and monocytes of both treated groups (T 1 ,T 2)showed significant increases ($P \leq 0.01$) in comparison with that of the control one . This might be due to the inhibitory effect of meloxicam on monocyte COX-2 as reported by EMEA(2006) who administered orally 7.5 & 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 the reduction in prostaglandinE2 in plasma as an index of monocyte activity ,Table(3).

Table 3. *the effect of subacute exposure of meloxicam at different two doses o differential counts of white blood Cells in Male Albino rats.*

parameter / Group	Neutrophils % M ± SE	Eosinophils % M ± SE	Basophiles % M ± SE	Lymphocytes % M ± SE	Monocytes % M ± SE
Control C n = 6	31.3 ± 0.76 C	1.16 ± 0.3 A	0.3 ± 0.2 A	66.3 ± 1.02 B	0.8 ± 0.33 B
T1 n = 6	16.1 ± 0.79 A	0.5 ± 0.34 A	0.83 ± 0.54 A	78.6 ± 1.17 A	3 ± 0.44 A
T2 n = 6	16.83 ± 2.21 B	0.5 ± 0.22 A	0.6 ± 0.21 A	78.8 ± 2.13 A	4 ± 0.63 A

- T1= sub acute exposure to therapeutic dose(T.D) 0.2 mg/kg.BW . .
-T2= sub acute exposure to three fold 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 ($P \leq 0.05$).
- Different letters of lymphocytes & monocytes mean significant increase ($P \leq 0.01$)

2- Biochemical markers:-

- Urea :-Their was no significant change ($p \leq 0.05$) in blood urea of both treated groups (T1, T 2) in comparison with the animals of the control one table (4) .

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**
Control C n=6	7.87 ± 0,36 **A**
T1 n=6	8.69 ± 2.10 **A**
T2 n=6	12.57 ± 3.90 **A**

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

- AST, ALT and ALP

There were no significant differences in the serum level of both alanine aminotransferase (ALT) and alkaline phosohatase(AP) between both treated groups
(T 1, T 2) in comparison with that of the control one,. The serum level of Aspartateaminotrasferase(AST) of both treated animals T 1 , T 2 showed significant increases ($P \leq 0.01$) between them and in comparison with the control one. The increase in enzyme level was according to the dose.(table 5) .The increase in serum levels of ALT&AST &AP is considered as initial step in detecting liver damage due to viral, alcoholic &drug-induced hepatocyte damage (Hochadel; 2004). These significant increases in serum levels of AST and non significant increases in both ALT & AP of treated groups could be confirmed by our histopathological findings in livers which revealed variable lesions ranged from mild to severe necrosis and loss of tissue architecture and also loss cellular outline .The liver of (T 1) treatment group revealed picnotic nuclei which was prominent indicator for necrosis , perivascular lymphocytic infiltration , congestion of portal vein , vasodilatation , congestion of branch of hepatic artery , cytoplasmic vacculation Figure(1) , while the Liver of (T 2) treatment group showed severe coagulative necrosis with loss of tissue architecture , loss of cell outline and karryorrhexis with cytoplasmic vacculation , and picnotic nuclei (Figure 2) .

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

parameter / GROUP	A L T . U/ L M ± S E	A S T . U/ L M ± S E	A LP . U / L M ± S E
Control © **n = 6**	19.8 ± 8.09 **A**	64.6 ± 6,50 **C**	21.5 ± 2.50 A
T 1 **n = 6**	**2 7.0 ± 6 . 90** **A**	**92.0 ± 7.70** **A**	33.8 ± 8.60 **A**
T2 **n = 6**	**44.3 ± 10.40** **A**	**127.0 ± 4 . 60** **B**	38.9 ± 7 . 70 **A**

- T1= sub acute exposure to therapeutic dose(T.D) 0.2 mg/chg. .
-T2= sub acute exposure to three folde dose (3 FD) 0.6mg/Kg.BW.
- n= number of animals..
- Different letters mean significant increase ($P \leq 0.01$).

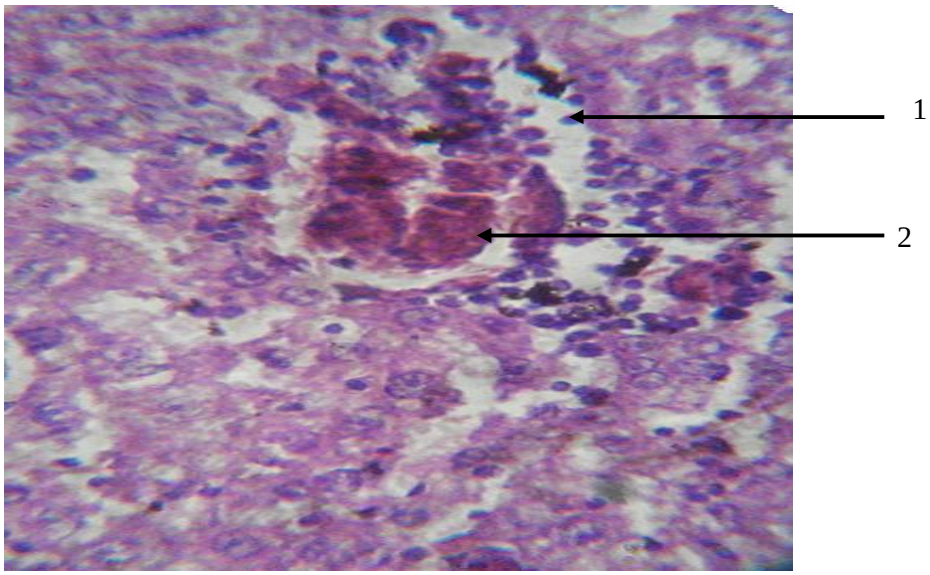

Figure 1. *Cross section of liver of male rat (T1) receiving therapeutic dose of meloxicam for one month , 1- perivascular lymphocytic infiltration & 2- congestion . H&E (X40)*

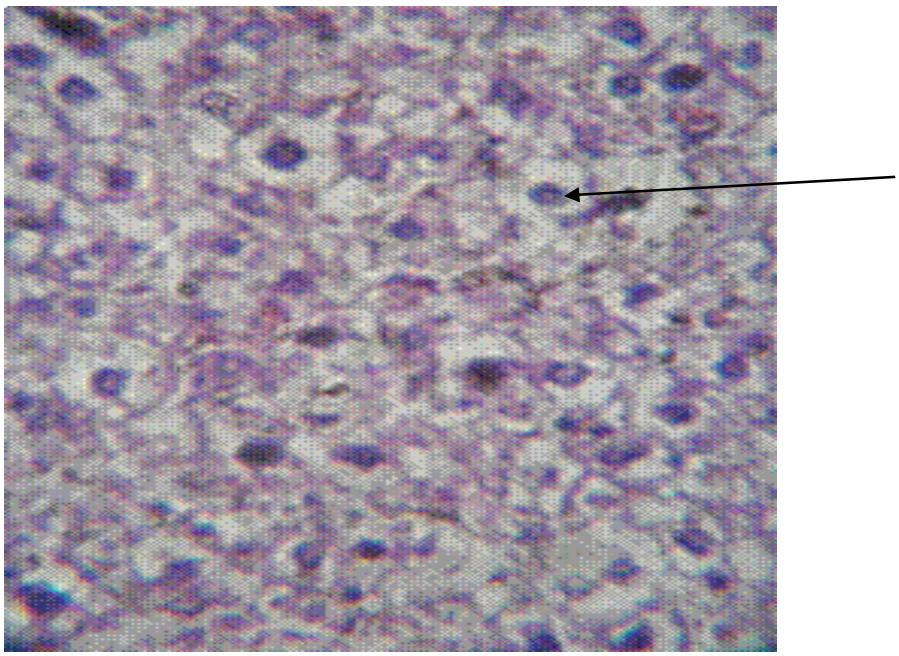

Figure 2. *Cross section of liver of(T2) male rat receiving 0,6 mg/kg.BW of meloxicam for one month , showing picnotic nuclei(arrow) H & E (X 40) .*

CONCLUSION

Hematological , biochemical results and histopathological changes were registered in liver and kidney of treated animal with meloxicam revealed that they are dose dependent .

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تأثير التعرض تحت الحاد للميلوكسيكام بجرع مختلفة على الصورة الدموية والكيموحيوية والنسيجية في أكباد ذكور الجرذان المهقاء.

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الخلاصة

أجريت هذه التجربة لدراسة تأثير التعرض شبه الحاد 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 العلاجية0.6 ملغم /كغم فيما جرعت مجموعة السيطرة بالماء المقطر يوميا لمدة أربعة أسابيع 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 ($P\leq 0.01$) لكلتي مجموعتي المعالجة مع زياد'ة معنوية في الوقت اللازم لتجلط الدم على مستوى ($P\leq 0.01$)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 ($P\leq 0.05$)وزيادة معنوية في كل من الخلايا وحيدة النواة والخلايا اللمفاوية على مستوى ($P\leq 0.01$) في مجموعتي المعالجة بالمقارنة مع مجموعة السيطرة ، بينما أشارت مستويات خمائر وظائف الكبد في مصل الدم إلى زيادة معنوية على مستوى ($P\leq 0.01$) في خميرة (AST) لكلتا

مجموعتي المعالجة (T_1،T_2) مقارنة بمجموعة السيطرة C). أظهرت الدراسة النسيجية المرضية لأكباد مجموعتي المعالجة تغيرات تراوحت بشدتها حسب الجرعة بين توسع واحتقان الأوعية الدموية وتنكس وموت واضمحلال التركيبة البنيوية للخلايا .