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 subchronic 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≤0.01) of both treated groups and significant increase clotting time of T2 group (P≤0.01) in comparison with the T1 group and control one. The results of differential count of white blood cells registered significant decrease(P≤0.05) in neutrophils and significant increases (P≤0.01) 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≤0.01) 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, karyorrhexis of hepatocytes.

Keywords: Meloxicam, Hematological, Biochemical, Histopathological, Rat.

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Tأثير التعرض شبه المزمن للملوكسيكام على بعض المتغيرات الدموية والكيميائية والنسيجية في أكباد الجرذان

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الخلاصة

تولت هذه التجربة للدراسة تأثير التعرض شبه المزمن للملوكسيكام على الصورة الدموية والكيميائية والتغيرات النسيجية المرضية لأكباد أثاثية عشر من ذكور الجرذان المفاه. قسمت الحيوانات بالتشابه إلى مجموعتين علاج: T1 تمت الجرعة العلاجية المرضية لأكباد عشرة عشر من ذكور الجرذان المفاه. قسمت الحيوانات بالتشابه إلى مجموعتين علاج 0.2 ملمع/كم و T2 تمت ثلاثة أضعاف الجرعة العلاجية 0.6 ملمع كم، فيما جرعت مجموعتان تعديل للعنوان. 2) دراسة صحة الدم و🎊، إذ تحدث تغييرات في النسب والخصائص الكيميائية والكيميائية للخلايا، وتظهر تعديلات في نسبة نسب تفاعلات الأنزيمات الكيميائية بالدم و込みات العناصر الكيميائية (C) والفيرونتير (C), وتدور نتائج التجربة على الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتائج في الأداء النتاء
among the most widely prescribed drugs worldwide, being chronic exposure of Meloxicam at different doses on (11,12). This experiment was amid to study the effect of resulting from cases, such as shock or drug toxicity on cells. If the liver is injured, the liver cells spill the enzymes aminotransferases. They include aspartate aminotransferase (SGPT). These enzymes are normally contained within liver cells count, leucopoenia and thrombocytopenia (9). More frequent than 1%: anemia between 0.1 and 1%: disturbances of blood count, including differential white cell count, leucopenia 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) cm³ 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 (microhematocrit 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:

$$[T - C / S - B] \times 0.4 \times 1 \times 30 \times 1000 \times 0.1 \quad (ALT)$$

$$[T - C / S - B] \times 0.4 \times 1 \times 60 \times 1000 \times 0.1 \quad (AST)$$

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 antipyrene 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 ($P \leq 0.05$) and ($P \leq 0.01$) to compare the data of different groups throughout the period of experiment (15).

Results and discussion
Hematology
RBC count, WBC count and platelets count

<table>
<thead>
<tr>
<th>Group</th>
<th>RBCs Cu / mm M ± SE</th>
<th>WBCs Cu / mm M ± SE</th>
<th>Platelets Cu/mm M ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 n=6</td>
<td>$6.78x10^6 \pm 134.1x10^3$ a</td>
<td>$6.983x10^7 \pm 1186$ a</td>
<td>$41.33x10^3 \pm 2.23x10^4$ a</td>
</tr>
<tr>
<td>T2 n=6</td>
<td>$6.903x10^6 \pm 603.33x10^3$ a</td>
<td>$6.763x10^3 \pm 703$ a</td>
<td>$41.50x10^3 \pm 3.73x10^3$ a</td>
</tr>
<tr>
<td>C n=6</td>
<td>$8.09x10^6 \pm 388x10^3$ a</td>
<td>$8.583x10^3 \pm 140$ a</td>
<td>$124.5x10^3 \pm 20.76x10^3$ c</td>
</tr>
</tbody>
</table>

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 ($P \leq 0.01$).
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.

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

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Neutrophils % M ± SE</th>
<th>Eosinophils % M ± SE</th>
<th>Basophiles % M ± SE</th>
<th>Lymphocytes % M ± SE</th>
<th>Monocytes % M ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 1 N = 6</td>
<td>15.5 ± 6.32 a</td>
<td>0.6 ± 0.33 a</td>
<td>0.6 ± 0.51 a</td>
<td>79.1 ± 1.95 a</td>
<td>4 ± 1.5 a</td>
</tr>
<tr>
<td>T 2 N = 6</td>
<td>11.33 ± 0.55 b</td>
<td>1.6 ± 0.6 a</td>
<td>0.6 ± 0.33 a</td>
<td>79.5 ± 1.87 a</td>
<td>4.3 ± 0.66 a</td>
</tr>
<tr>
<td>C N=6</td>
<td>31.3 ± 0.76 b</td>
<td>1.16 ± 0.3 a</td>
<td>0.3 ± 0.2 a</td>
<td>66.3 ± 1.02 b</td>
<td>0.8 ± 0.3 b</td>
</tr>
</tbody>
</table>

T1= sub acute exposure to therapeutic dose (T.D) 0.2 mg/kg BW, T2= sub acute exposure to threefold 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).

Biochemical markers

Urea

There 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 Aspartateaminotransferase of both two treated animals groups T1 and T2 revealed 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 and drug-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 severe 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 male and 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>UREA mmoL / L (M ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 n=6</td>
<td>13.45 ± 1.46 a</td>
</tr>
<tr>
<td>T2 n=6</td>
<td>15.2 ± 6.9 a</td>
</tr>
<tr>
<td>C n=6</td>
<td>7.87 ± 0.36 a</td>
</tr>
</tbody>
</table>

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 (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.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT U/ L</th>
<th>AST U/ L</th>
<th>AP U/ L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M ± S E</td>
<td>M ± S E</td>
<td>M ± S E</td>
</tr>
<tr>
<td>T1 n=6</td>
<td>111.6 ± 2.7 a</td>
<td>151.6 ± 0.95 a</td>
<td>39.4 ± 4.8 a</td>
</tr>
<tr>
<td>T2 n=6</td>
<td>115.6 ± 5.9 a</td>
<td>154.5 ± 2.93 a</td>
<td>75.8 ± 16.4 b</td>
</tr>
<tr>
<td>C n=6</td>
<td>19.8 ± 8.09 b</td>
<td>64.6 ± 6.5 b</td>
<td>21.5 ± 2.5 a</td>
</tr>
</tbody>
</table>

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).
References

10. EMEA. Meloxicam Extrapolation to rabbits and goats. 2006.