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Effect of Metamizole (Dipyrone) on Blood and Histological Pictures of Liver and Spleen in Rats

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WE used in our study Twenty rats ,divided into two groups .the first group consist of 10 rats considered as a control group, while the second group treated with 0.05 ml\body weight\day of dipyron injected for 30 days duration. The toxic effect of dipyrone was obvious in the different tissue and haematotoxic effects . The histological changes were obvious in the hepatic cell whichof liver were characterized by atrophy ,irregular hepatic cells ,RBC cells in the blood sinusoid. However,the spleen tissue contains nodule (white pulpe).The bony tissue have osteocytes large bony vacuoles and the blood vessel were congested.

Keywords: Metamizole, Dipyrone, Rats

Introduction

Metamizole (dipyrone) is a compound has important effects such as, analgesic, antipyretic and spasmolytic effects. Moreover, has a small anti-inflammatory activity [1]. it used in both human and veterinary medicine, also considered strongest non-opioid analgesic [2]. Metamizole mechanism acts relies on the inhibition of a central cyclooxygenase-3 (COX-3) interfere with prostaglandin synthesis [3]. Chemically, metamizole is sodium N-(2, 3dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)-N- methyl amino methane sulphonate [4]. It is a water soluble pyrazolone derivative available in oral, rectal and injectable forms. Metamizole has also been widely used by equine practitioners to treat equine colic .and other conditions of gastrointestinal spasm in both small and large animal [5]. World Health Organization [WHO] name: metamizole; American and British name: dipyrone)[6]. It was taken off the Dutch market because as a result of reports of agranulocytosis after metamizole used [7].

The aim is to prove the undesirable side effect of drug on blood picture and some organs (spleen and liver) because lacks of any data on haematotoxic effects attributed to administration of metamizole in animals .

Material and Methods

Animals

We used 20 Wistar rats (9 weeks old, 180-200 g body weight) in this trials were divided into two groups (control and experimental group) each group contains 10 rats. Rats were acclimatized

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and maintained in the college of veterinary medicine –Tikrit University under laboratory conditions in group cages. Standard diet pellets and water was provided to rats *ad libitum*.

Drug

We used in this experiment dipyrone drug and gave intramuscular (IM) injection with dose 0.05 ml\body weight [8]. dipyrone 500mg\100 ml (uvedipyrone) supplied from uvedco Jordan .

Experimental design

Treatment was last for thirty days. The animals were killed at the end of period after the last dose under intensive dose of chloroform.

Sample collection and histological technique

Spleen and livers of the animals were rapidly removed and micro dissected to obtain tissue samples for histological examination. Blocks of tissues were immediately fixed in 10% neutral buffered formalin, dehydrated with graded series of ethyl alcohol and embedded in paraffin. Sections of 5 microns were cut and stained with eosin and hemotoxylin then Photomicrographs of the slides were taken using digital camera attached to light microscope. The whole photomicrographs were compared with those of liver and kidneys of control group, all Samples were prepared for histological examination by following steps: 1. Fixation, 2. Washing , 3. Dehydration , 4. Clearing , 5. Infiltration, 6. Embedding , 7. Sectioning , 8. Staining and 9. Screening and imaging according to Luna, [6].

Hematological studies

Blood samples were collected from animals in each group after submitted of all animals to anesthetise conditions with diethyl-ether and anesthesia and then puncture corner of eye by using specialised anticoagulant capillary tube . Whole blood samples were transported directly to laboratory to measure Red blood cell (RBC) count, total white blood cell (WBC) count, and haemoglobin by using automated cell counter.

Statistical Analysis

The results were analyzed using the SPSS program for values representing the Mean and Standard Error and analyzed the data using the ANOVA Analysis of variance One Way. The differences between the groups were determined using the Duncan multiple range test. At probability level ($p \leq 0.05$).

TABLE 1. Effect of dipyrone on some blood parameters after 30 days of administration in rats compering with control group

Parameter	Control group	Treated group
WBC (10^3 cell/ml^3)	9.16±0.253 a	5.55±0.327 b
RBC (10^6 cell/ml^3)	8.68±0.411 a	6.43±0.352 b
Hb (gm/ml)	12.73±0.501 a	9.74±0.542 b

The difference was significant at (P≤0.05) Values are given as mean ± SD
Differences in the letters in the row is indicator to the difference

Results

Histological study

liver

We can notice in the studied slides The parenchyma of liver was suffered from atrophy and degeneration in all of studied sections, appeared a small groups between network of blood sinusoid that contained kupffer cells (Fig.3). The sever a trophy of liver cells were well demonstrated, and most of these cells contains degenerated cytoplasm with vacuolation and the blood sinusoid was wide channels with number of kupffer cells inside it (Fig.4). The central vein in the central hepatic lobule was engorged with haemolysed blood (Fig. 5).

Spleen

Figures (6,7) showing severe hyperemia in the red pulps and sinusoids with distorted lymphoid nodules of the white pulp.

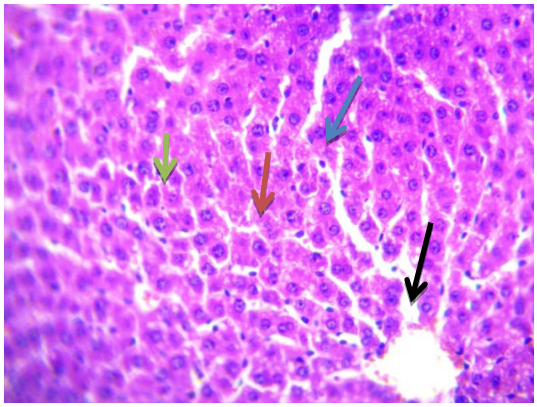

Fig.1. Hepatic lobule, central vein (black arrow). liver cells (red arrow).blood (blue arrow) kupffer cells (green arrow)(H&EX20).

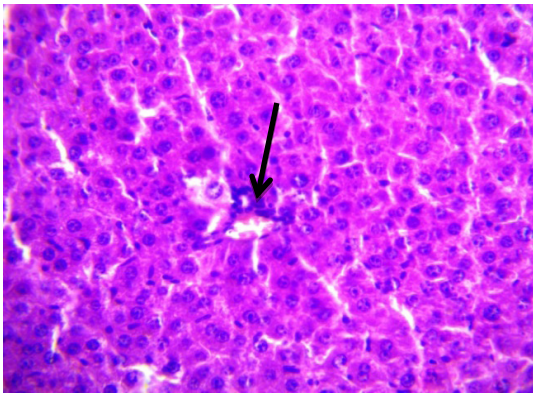

Fig.2. Portal area of liver (H&EX40)

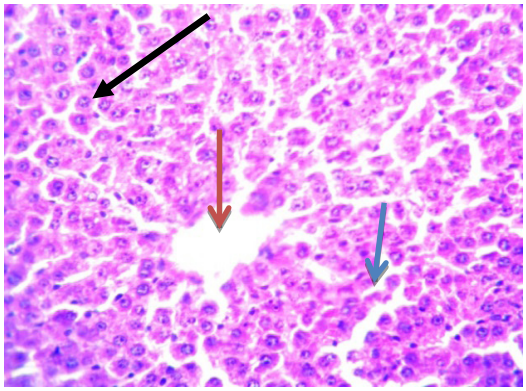

Fig.3. The parenchyme of the liver degenerating cells (black arrow).central vein (red arrow). blood sinusoid (blue arrow) (H&E40X)

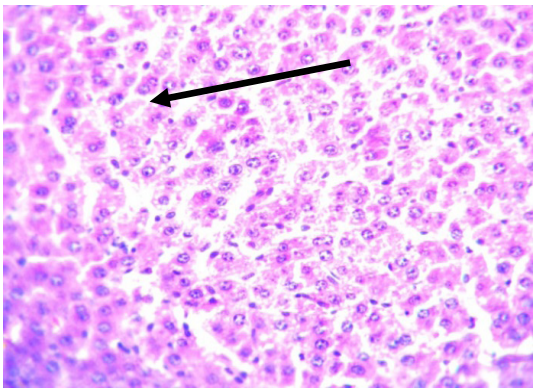

Fig.4. Extensive degeneration of liver cells (H&E40X)

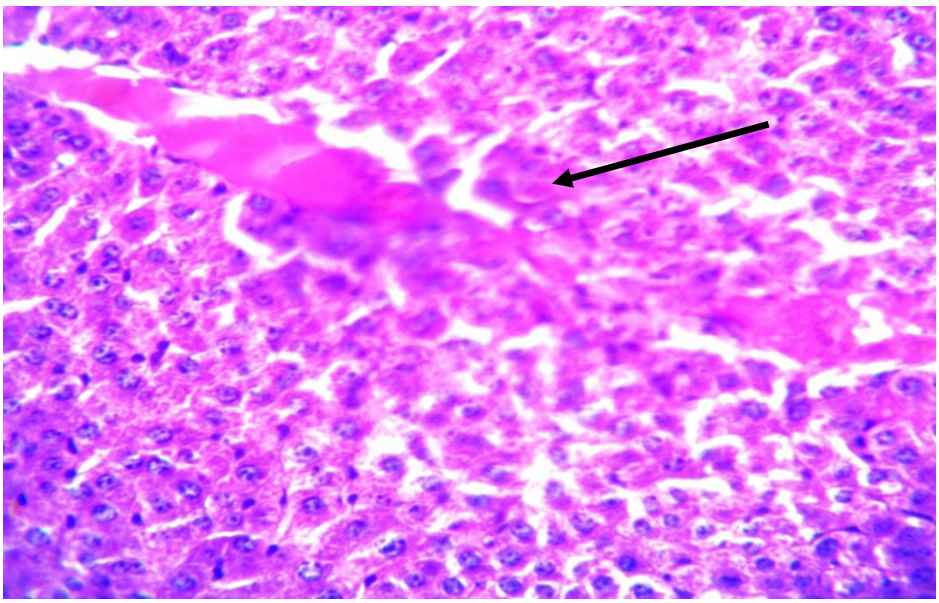

Fig. 5. Central engorged with haemolysis blood (H&E40X)

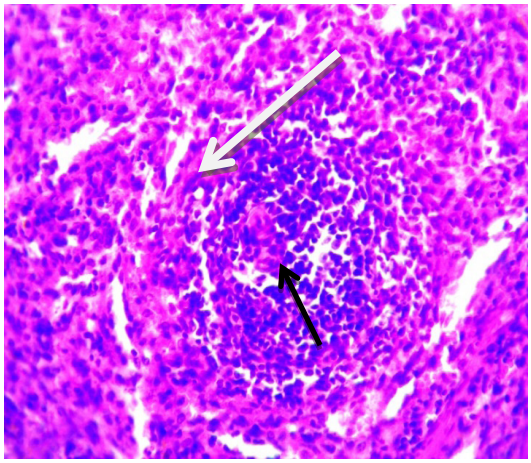

Fig.6. Parenchyma of spleen contain nodule (white pulpe)(black arrow).germinal centre (white arrow(H&EX40)

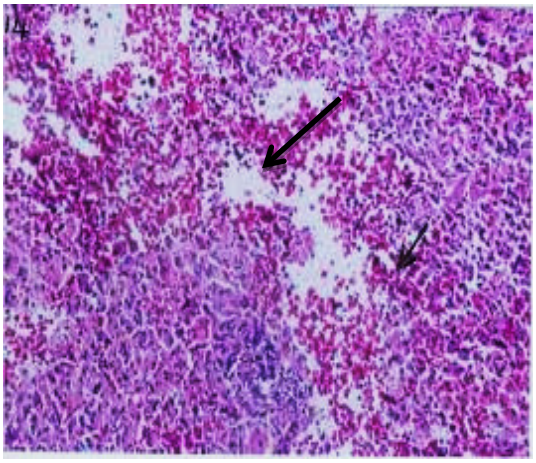

Fig.7. Red pulpe of spleen with blood sinuses and lumphocytes aggregation (H&E40X)

Discussion

In this study we were focusing on some blood parameters and two organs (liver and spleen) our data confirm that the use of dipyrone (metamizol) is leading to blood disorder and this finding can be attributed to its effects on bone marrow and particularly damaging in bone marrow, the mechanism may be due to the suppression of the growth of myeloid progenitors, primitive multipotential progenitors and erythroid progenitors as reported by Redondo-Pachon et al. [9]. rather than our results explained that continuous using of this drug leads to bad effects on vital organs like (spleen and liver) that we were studied it. The explanation of this fact may be due to this drug dipyron is able to cause toxic effects on organs and the mechanism may directly involve the bone marrow and other organs and these findings agreed with Cristian, et al.[10]. Hedenmalm and Spigset 2002 [11] suggested that after the prolonged administration of this drug might cause damaging on precursors of blood components and some disorders will occur because of over dose of this drug like leukopenia, agranulocytosis and even aplasticanaemia.

Dipyrone increase the risk of hepatotoxic because the inhibition the producing of COX enzyme or insufficiently [12]. Our findings also agreed with Anne et al. [13] which revealed in his study that Metamizole cause some hepatotoxic effects .One of the researchers [14] confirmed that the using of this drug lead to direct damages of the myeloid precursor and repeating use of this drug metamizole might be consider as a dangerous factor due to its direct toxic effects on granulocyte precursors. The mechanism of metamizole-induced agranulocytosis has not been completely clarified [15]. The hypotheses that explain this findings may be due to the reactions with neutrophils after oxidation of these compounds into a reactive oxygen species and free radicals Mathias, [16]. We can conclude from this study that over using of this drug lead to harmful effects on both blood constituents and more vital organs of the body

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Conflict of interest: Authors declare no conflict of interest.

Ethical consideration: The study was conducted according to the ethical standards and institutional guides that recorded in Instructions of the Ministry of Higher education and scientific research .

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تأثير الميتاميزول (ديبيرون) على صورة الدم والشكل الهستولوجى لانسجة الكبد والطحال في الفئران

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استخدمنا في دراستنا عشرون فئران ، مقسمة إلى مجموعتين. المجموعة الأولى تتكون من ١٠ فئران تعتبر مجموعة مراقبة ، في حين أن المجموعة الثانية عولجت بـ ٠,٠٥ مل \ وزن الجسم \ يوم من الديبيرون الذي تم حقنه لمدة ٣٠ يومًا. كان التأثير السام للديبيرون واضحا في الأنسجة المختلفة والآثار السامة للدم. كانت التغيرات النسيجية واضحة في الخلية الكبدية التي تميز الكبد بها ضمور ، خلايا الكبد غير النظامية ، خلايا RBC في الجيوب الأنفية في الدم. ومع ذلك ، يحتوي نسيج الطحال على عقيدة (عجينة بيضاء). تحتوي الأنسجة العظمية على عظميات كبيرة فجوات عظمية واحتقان الوعاء الدموي.

الكلمات الدالة: Metamizole، Dipyrone ، الفئران.