

CHRONIC INTERACTION BETWEEN METFORMIN AND MELOXICAM IN MICE

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ABSTRACT**Objective:** This study is performed for investigation the chronic interaction between metformin and meloxicam from toxicological view.**Methods:** Lethal dose 50 after chronic exposure assessed in mice by up and down method. Their interaction analyzed by isobolographic analysis indicated that both medicines exhibited synergism. Assessment of the effects of repeated chronic dosing for 3 months of both medicines also performed on mice. Medicines in question administered orally as therapeutic doses of metformin 14 mg/kg. Body weight (BW) (G_1), meloxicam 0.2 mg/kg.BW (G_2), and combination of both medicines (G_3) while G_4 assigned control and dosed DW.**Results:** Both G_1 and G_3 showed significant $p < 0.05$ decrease in blood glucose and serum cholesterol levels. Meloxicam group (G_3) showed statistically significant $p < 0.05$ increase in triglycerides and alanine aminotransferase (ALT), and aspartate aminotransferase (AST), while group of combination showed statistically significant $p \leq 0.05$ decrease in both ALT and AST. Blood urea nitrogen, uric acid, and serum creatinine showed statistically significant $p < 0.05$ increase in group of meloxicam but decrease in G_3 and no changes in G_1 . Histopathological changes included variable lesions in kidney such as swelling and necrosis in renal tubules of metformin group, cortical vacuolar degeneration in renal tubules, and deterioration of most glomeruli in G_2 , while G_3 showed generalized cortical necrosis of renal tubules and interstitial nephritis. Liver lesions included central venous congestion (VC) and perivascular lymphocytic infiltration and marked necrosis in both groups of metformin and meloxicam, while there were severe VC and necrosis of hepatocytes in group of combination.**Conclusion:** Metformin administration with meloxicam may have beneficial important through preventing many deleterious effects of meloxicam after long-standing administration, but adjusting dosing regimen study might be recommended.**Keywords:** Chronic, Interaction, Metformin, Meloxicam, Mice.© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2019.v12i2.29947>**INTRODUCTION**

More than 50 years, metformin is a worldwide prescribed antidiabetic [1,2], it is a biguanide derivative [3] and considered the cornerstone and first-line defense against type II diabetes mellitus (is a most common metabolic disorder) through decrease hepatic insulin resistance, decrease amyloid deposits, and alteration in bile acid metabolism [4,5]. The antihyperglycemic effect of metformin was dose dependent and ranged from maximum effect dose of 2000 to minimum 1000 mg/day in most patients [6,7]. Metformin is primarily absorbed from the upper gastrointestinal tract [8]. The bioavailability of metformin is 50% [9]. It is delivered into liver in concentration more higher than in the blood, finely excreted unchanged through kidney [10,11]. Metformin alone is a relatively safe drug clinically with only mild side effects including gastrointestinal disturbances (diarrhea, nausea, and irritation of the abdomen) [2]. Although the fact that the efficacy of metformin offers wide range of benefits beyond its blood glucose control, physicians have always been well aware of its adverse effects [12,13]. Meloxicam is a nonsteroidal anti-inflammatory (NSAID) has analgesic and antipyretic effects through the selective inhibition of cyclooxygenase-2 (COX-2) [14,15]. It is recommended for treating rheumatoid arthritis, osteoarthritis (OA), postoperative pain and fever [16]. Meloxicam recommended at dose 15 mg/day in the treatment of rheumatoid arthritis [17], its bioavailability in mice is 94% after oral administration [18]. Meloxicam administration can result some gastrointestinal side effects such as intestinal bleeding and also serious cardiovascular problem, especially in persons are suffering from hypertension, hypercholesterolemia, and diabetes [19]. Liver and kidney toxicity both are adverse effects of meloxicam [20]. Prolonged uses of NSAIDs can produce severe adverse effects [21]. The ability of metformin to decrease pain severity and inflammation in

that contributed to the pathophysiology of OA, with no serious adverse effects, has been carried out in many animal model studies [22,23]. This can be indicated that metformin can be recommended as potential drug to treat inflammation-related disorders [22]. Several studies have demonstrated that inflammation correlates with incident diabetes [24]. The expression of COX-2 is increased in the peripheral tissues of diabetic neuropathy models [25]. NSAIDs are a double-edged sword in that their long-term use requires caution due to their well-known side effects [26,27]. Extensive studies are required to validate the safety and evaluation of some toxicological aspects of coadministration of metformin and meloxicam, so we are targeted one aspect which is represented by chronic interaction between these two medicines in mice.

METHODS**Medicines and chemicals**

Mobic® tablet contains 15 mg meloxicam obtained from Boehringer Ingelheim (Germany) and Glucophage® film-coated tablet contains 500 mg metformin from MERECK SERONO (France). Commercial Kits from Biosystems (Spain) used for the assessment of random blood glucose, cholesterol, triglycerides (TGs), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine, and serum uric acid.

Animals

Balb-C male mice, 3 months ago, purchased from Pharmaceutical quality control/Ministry of Health - Iraq. They were housed under optimum condition of temperature $25 \pm 1^\circ\text{C}$ with photoperiod followed dark: light cycle of 12:12 h along the period of experiment. Standard rodent pellet feed and drinking water provided *ad libitum*. They were remaining 2 weeks for acclimatization before any interference.

Experimental design

This experiment was carried out as approved by the Scientific Committee in Physiology, Biochemistry, and Pharmacology Department/College of Veterinary Medicine, University of Baghdad, accordance to ethical standard of working on laboratory animals.

Dosages and dosing

All medicines that involved were calculated according to the body weight (BW) of animal as mg/kg.BW. They were administered with dose volume 0.1 ml/10 g.BW of mice by calculating and fitting all their concentrations for all experiments.

Median lethal dose 50 (LD₅₀) assessment

Median LD₅₀ of meloxicam, metformin, and their combination had calculated after chronic orally administration by up and down method [28]. Regarding to the outcome of each dose whether the animal was dead or alive, the doses of both medicines were decreased and increased by 20% respectively. LD₅₀ for each medicine and their combination was calculated by the following equation:

$$LD_{50} = Xf + Kd$$

Where, Xf=the last dose

K=Constant

d= difference between doses.

Assessment of interaction of both medicines (isobolographic analysis)

The isobolography analysis used to determine the sort of interaction between and meloxicam (drug A) and metformin (drug B), if we denote the intercepts by A for the LD₅₀ of drug A and by B for the LD₅₀ of drug B, then the isobole is expressed by the simple linear equation:

$$\frac{a}{A} + \frac{b}{B}$$

Where, a is the LD₅₀ of drug A and b is of drug B when the two are administered together and the A and B are now the respective individual doses. The isobole expressed in equation above allows the assessment of the interactions whether are synergism, antagonism, or additive when actual combination doses are tested. If testing shows that the specified effect of a combination is achieved by a dose pair that plots as a point below the isobole, this means that the effect was attained with doses less than those on the line, a situation that denotes synergism. In contrast, an experiment may show that greater combination doses are needed to produce the specified effect, and therefore, this dose pair plots as a point above the isobole line denote antagonism. Dose pairs that experimentally lie on the line (or not significantly off the line) are termed additive [29].

Assessment of the effects of repeated chronic dosing of both medicines

A total of 20 male Balb-C mice divided equally into four groups, orally administered all medicines daily for 3 months and assigned as Group 1 (G₁) metformin 14 mg/kg.BW, Group 2 (G₂) meloxicam 0.2 mg/kg.BW, and Group 3 (G₃) combination of metformin plus meloxicam while the 4th group (C) dosed distilled water and considered control group.

Blood samples collected after 3 months of dosing through heart puncture technique, serum obtained by centrifugation with 3000 rpm

for 10 min for clinical chemistry. Blood glucose by spectrophotometry measured coupled colored complex quinoneimine [30]. Serum Cholesterol was measured by spectrophotometric method, briefly by converting cholesteryl ester to cholestenone [31]. TGs measured spectrophotometrically by converting them to glycerol [32]. AST estimated spectrophotometry through determination catalytic concentration of enzyme from the rate of decreased nicotinamide adenine dinucleotide [33]. Serum uric acid measured through coupled reaction which converts uric acid to allantoin in the presence of uricase and colored complex that can be measured by spectrophotometer [34,35]. BUN measured spectrophotometrically through coupled reaction which mediated by urease and glutamate dehydrogenase to form glutamate and NAD which could be measured [36,37]. Serum creatinine measured by reaction with picric acid in alkaline medium and forming colored complex which could be measured spectrophotometrically [38,39].

Statistical analysis was carried out by SPSS version 24.00. One- and two-way ANOVA employed to differentiate between means. p<0.05 was considered statistically significant. Least significant differences (LSD) used to compare between means.

RESULTS

Median LD₅₀

The results of median LD₅₀ of metformin, meloxicam alone, and their combination after chronic repeated administration in male mice are summarized in Table 1. Regarding to isobolography of two medicines metformin and meloxicam, simultaneously administrated in combination exhibited synergism with value of 0.77 (Diagram 1). Clinical observation of all mice subjected to both exposures for 24 h revealed restlessness or anxiety, raised tail, and grooming included rapid respiration, tremor, convulsions, laying down on one side, and finally death along 24 h of administration.

Clinical chemistry

Blood glucose, serum cholesterol, and TG

The both Groups 1 (metformin) and 3 (metformin+meloxicam) revealed statistically significant p<0.05 decrease in blood glucose level while Group 2 (meloxicam), showed non-significant change, in comparison with control group, Table 2. The serum cholesterol level of both Groups 1 and 3 showed statistically significant p<0.05 decrease while

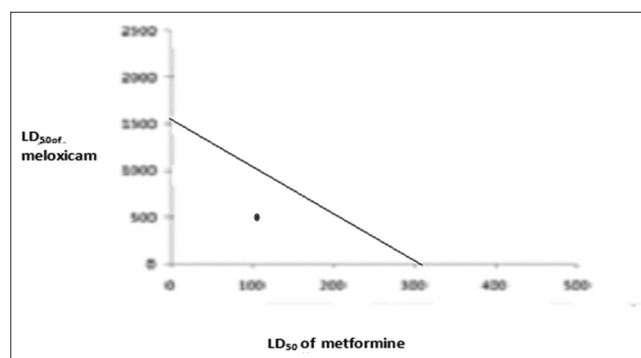


Diagram 1: Isobolographic of combination lethal dose 50 of metformin plus meloxicam in mice after orally chronic administration

Table 1: LD₅₀ of metformin, meloxicam, and their combination after chronic orally administration in male Balb-C mice

Medicine	Initial dose mg/kg.BW	Last dos mg/kg.BW	Number of animals	Outcome	Differences between doses	LD ₅₀ mg/kg.BW
Metformin	1000	1400	5	OXOOX	200	1575.6
Meloxicam	400	240	5	OXXXO	80	303
Metf+melox	755.5+171.5	755.5+171.5	5	XOXOX	200 metf, 80 melox	615.3+115.42

K of metformin=0.878, K of meloxicam=1.288, K of combination=-0.701. LD₅₀: Lethal dose 50, BW: Body weight

Table 2: Glucose mg/dl, cholesterol mg/dl, and TG mg/dl

Group	Glucose M±SE	Cholesterol M±SE	TG M±SE
G ₁ (metformin)	49.00±4.99C	46.00±1.81B	74.00±3.03B
G ₂ (meloxicam)	141.60±4.26A	75.60±1.96A	106.00±2.91A
G ₃ (metformin+meloxicam)	105.00±2.73B	46.40±3.90B	74.20±1.77B
G ₄ (DW) control	144.60±3.04A	94.60±19.04A	79.60±13.03B
LSD	9.23	24.22	17.05

Capital letters denote statistically significant differences $p < 0.05$ between groups. LSD: Least significant differences

Group 2 did not show any significant change in comparison with the control group, Table 2. The only serum TG level of Group 2 (meloxicam) exhibited statistically significant $p < 0.05$ increase, while other treated Groups 1 and 2 did not do when compared to control group, Table 2.

ALT and AST

The animals of treated group two (meloxicam) showed statistically significant $p < 0.05$ increase in both ALT and AST serum activity in comparison with both treated Groups 1 and 3 (metformin, combination) and control group. Furthermore, Group 3 revealed statistically significant $p < 0.05$ increase in both ALT and AST when compared to G₁ and control group, while Group 1 did not show any significant changes in both enzymes activity in comparison with the control, Table 3.

Serum uric acid, creatinine, and BUN

The animals of Group 2 (meloxicam) revealed statistically significant $p < 0.05$ increase in serum uric acid, creatinine, and BUN when compared to other treated groups and control, while the animals of Group 1 (metformin) showed non-significant changes in all these parameters in comparison with the control one. Group 3 (combination) showed statistically significant $p < 0.05$ decrease in uric acid, increase in serum creatinine in comparison to the control group, and there is no significant change in BUN, Table 4.

BW

There were statistically significant $p < 0.05$ differences in BW between all treated groups before treatment while after 3 months, the BWs revealed no significant differences. However, the results of BW within groups showed statistically significant $p < 0.05$ decrease after 3 months of treatment in comparison with their pretreatment BWs, Table 5.

Histopathology

Kidney

The renal sections of G₁ (metformin) showed acute generalize cortical cellular swelling with necrosis of renal tubules, and medullary vacuolar degeneration (Vd) and necrosis of tubules (Figs. 1 and 2). The renal histopathological lesions of G₂ (meloxicam) were included severe cortical Vd of proximal and distal renal tubules with deterioration of most glomeruli (Fig. 3); however, the renal medulla showed variable degrees of degenerative changes of collecting tubules with cast formation and necrosis (Fig. 4), while G₃ (metformin plus meloxicam) revealed generalize cortical necrosis of renal tubules and acute cellular swelling and interstitial nephritis in medulla (Fig. 5).

Liver

Liver section of both G₁ and G₂ showed similar histopathological lesions which represented by central venous congestion (VC), perivascular lymphocytic infiltration with marked necrosis of hepatocyte cords (Fig. 6). The lesion which was observed in liver of G₃ included severe central VC, disarranged of hepatocyte cords, and necrosis (Figs.7 and 8).

DISCUSSION

Drug interaction is a status when two or more than one medicine are taking simultaneously for caring certain disease. Mostly, in clinical application, physicians prescribe more than one medicine, so these medicines may interfere between each other through

Table 3: ALT and AST (U/L)

Group	ALT M±SE	AST M±SE
G ₁ (metformin)	38.60±3.48C	94.60±4.54C
G ₂ (meloxicam)	88.00±4.27A	167.80±9.02A
G ₃ (metformin+meloxicam)	57.00±8.22B	129.64±10.02B
G ₄ (DW) control	34.20±3.73C	100.20±7.67C
LSD	13.06	19.97

Capital letters denote statistically significant differences $p < 0.05$ between groups. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase. LSD: Least significant differences

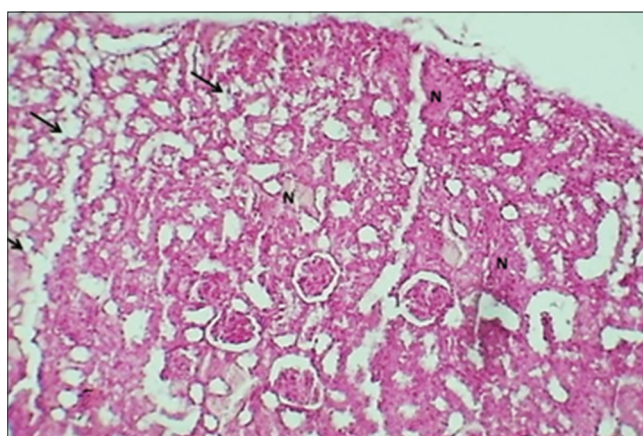


Fig. 1: Section of renal cortex (G₁) shows cellular swelling (arrows) and necrosis of renal tubules (N). Hematoxylin and eosin stain ×100

pharmacokinetic pathways or pharmacodynamically. Both pharmacological pathways consequence several sorts of interactions whether are beneficial or non-beneficial. The beneficial one is mostly therapeutically desirable while the non-beneficial one may be considered toxicological interaction and may harmful to the patient. Here, we conducted our study to review the interaction between hypoglycemic medicine metformin and analgesic, anti-inflammatory one meloxicam from some toxicological views, which included evaluation chronic interaction through measuring LD₅₀ of both two medicines when administrated separately and in combination, also some liver and kidney functions biochemical markers use as tools for this purpose. In such study, in mice, both medicines showed synergism and antagonism sort of interaction after acute and subchronic administration, respectively [40].

We thought the decrease in blood glucose level in the animals treated with metformin alone and in combination is a logical consequence due to hypoglycemic effect of metformin, meloxicam did not influence the hypoglycemic effect of metformin, and this is also evident by not affecting the level of blood glucose level in the mice that treated with meloxicam alone. It is noteworthy that the remaining 50% of metformin, which is unabsorbed, accumulates in the gut mucosa of the distal small intestine at concentrations 30–300-fold greater than in the plasma [41]. Moreover, ultimately is eliminated with feces. However, in humans, gut effect of metformin remains largely obscure, although several

Table 4: Uric acid, creatinine, and BUN

Group	Uric acid	Creatinine	BUN
	M±SE	M±SE	M±SE
G ₁ (metformin)	6.30±0.43 BC	0.42±0.03C	23.00±3.27B
G ₂ (Meloxicam)	14.80±1.71A	1.64±0.15A	40.40±3.31A
G ₃ (metformin+meloxicam)	5.28±0.28C	1.00±0.27B	23.60±2.11B
G ₄ (DW) control	9.82±2.50B	0.34±0.05C	24.80±1.49B
LSD	3.8	0.13	6.57

Capital letters denote statistically significant differences $p < 0.05$ between groups. LSD: Least significant differences, BUN: Blood urea nitrogen

Table 5: BW/kg

Group	Pretreatment M±SE	After 3 months of treatment M±SE
G ₁ (metformin)	27.05±1.26 B a	23.33±0.84 A b
G ₂ (meloxicam)	29.25±0.94 B a	23.33±0.98 A b
G ₃ (metformin+meloxicam)	32.50±6.44 A a	23.66±0.95 A b

LSD=2.48. Capital letters denote statistically significant differences $p < 0.05$ between groups. Small letters denote statistically significant differences $p < 0.05$ within groups. LSD: Least significant differences, BW: Body weight

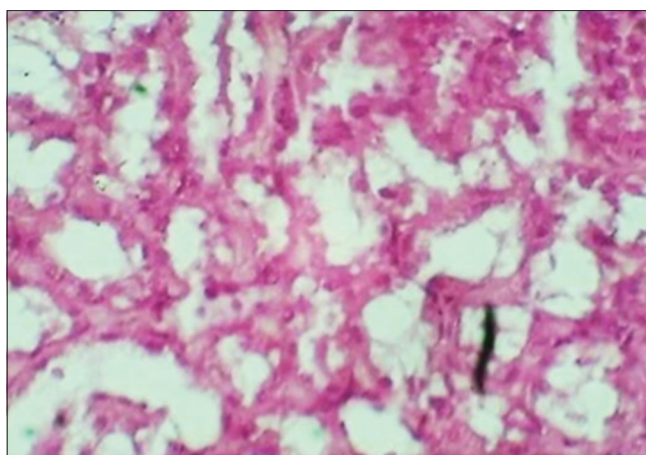


Fig. 2: Section of renal medulla (G₁) shows vacuolar degeneration and necrosis of tubules. Hematoxylin and eosin stain ×400

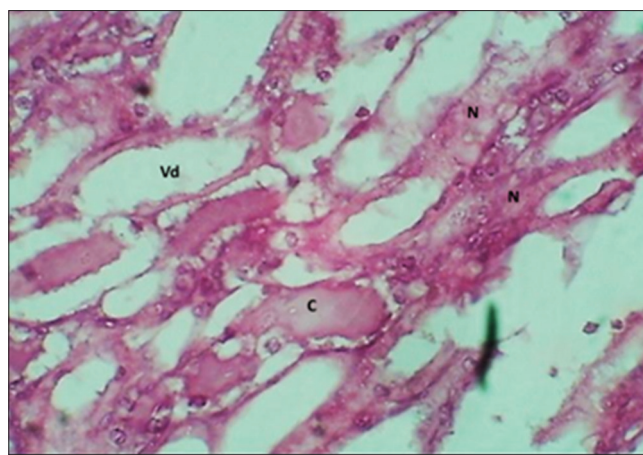


Fig. 4: Section of renal medulla (G₂) shows cast formation (C), vacuolar degeneration, and necrosis of collecting tubules (N). Hematoxylin and eosin stain ×400

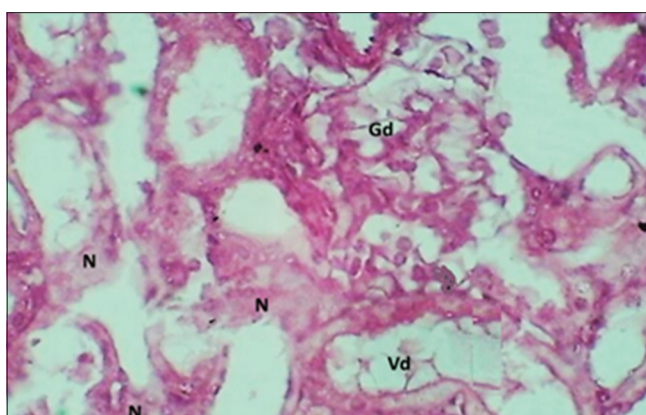


Fig. 3: Section of renal cortex (G₂) shows vacuolar degeneration and necrosis of collecting tubules (N). Hematoxylin and eosin stain ×400

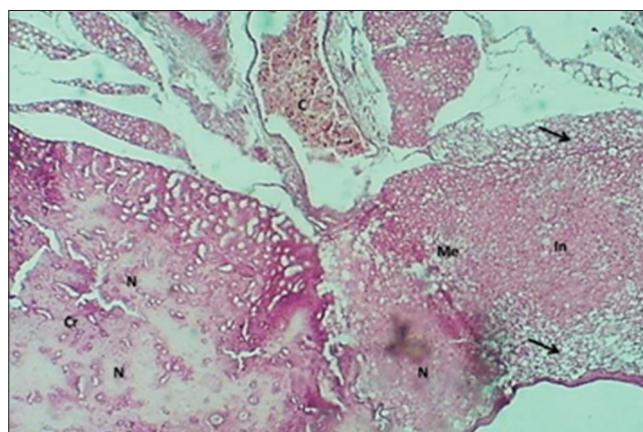


Fig. 5: Section of kidney (G₃) shows cortical region (Cr), necrosis (N), medulla region (Me) interstitial nephritis (In), and acute cellular swelling (arrows). Hematoxylin and eosin stain ×400

proposals have been suggested from animal experiments including delayed intestinal glucose absorption [2]. Metformin non-competitively inhibits the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase, resulting in an altered hepatocellular redox state, reduced conversion of lactate and glycerol to glucose, and decreased hepatic gluconeogenesis [42]. A certain study has demonstrated that the gut is the primary site of action [43].

Hyperlipidemia is an elevation of lipids in the blood. These include cholesterol, cholesterol esters, phospholipids, and TGs [44]. Measurement of TG and total blood cholesterol is important in the diagnosis and management of hyperlipidemia, hypertriglyceridemia refers to high TG levels in the blood [45,46]. Acute pancreatitis is an

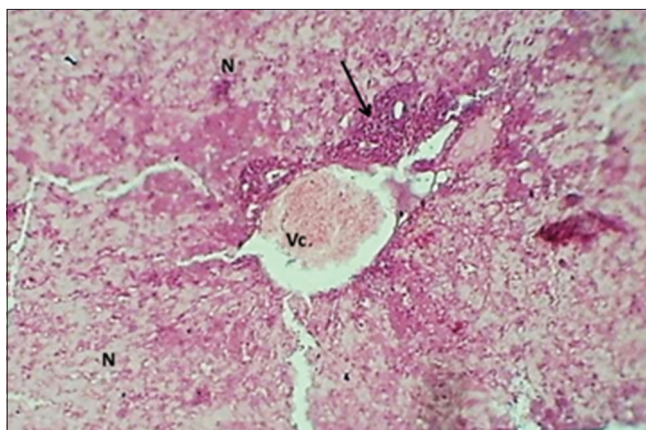


Fig. 6: Section of liver lobule shows, central venous congestion, lymphocytes infiltration (arrow), and necrosis (N). H and E stain $\times 400$

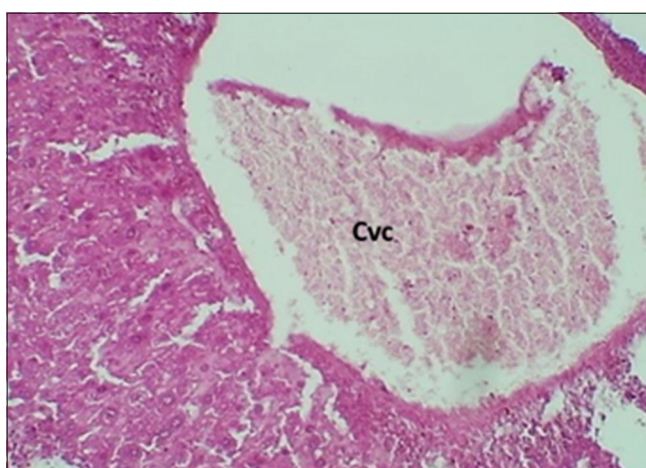


Fig. 7: Section of liver (G₃) shows congestion of central vein (Cvc). Hematoxylin and eosin stain $\times 100$

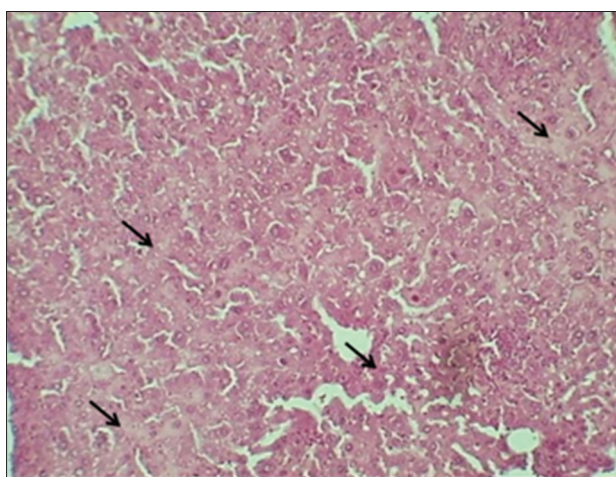


Fig. 8: Section of liver (G₃) shows narcosis of hepatocytes (arrows) and marked disarrangement of hepatic hexagon. Hematoxylin and eosin stain $\times 100$

inflammatory process of the pancreas with varying involvement of other regional tissues or remote organ systems [47]. Very high TG levels also increase the risk of acute pancreatitis [48]. Chronic pancreatitis may develop as a result of acute pancreatitis, chronic pancreatitis can

lead to diabetes or pancreatic cancer and unexplained weight loss may occur from a lack of pancreatic enzymes hindering digestion. We thought this is one of the relied perceptions of weight loss in mice of all experimental groups (Table 5), and the suspicion that meloxicam dosing is underlying in pancreatitis in mice especially of G₃. However, up to 2% of acute pancreatitis cases may be caused by drugs [49,50]. Potential mechanisms of drug-induced pancreatitis include hypersensitivity (onset after 4–8 weeks of use), accumulation of a toxic metabolite (onset after several months of use), and hypertriglyceridemia (onset after several months of use) [47]. Meloxicam is one of the drugs which is implicated in ≤ 10 cases of pancreatitis [49].

It has become increasingly apparent that the liver and kidney are also important targets for untoward clinical events of NSAIDs [51,52]. In general, the clinical manifestations of NSAID toxicity in liver can present as two distinct forms, mild hepatic changes and the more significant hepatic injuries. The mild hepatic changes, which are relatively frequent and usually observed in Phase III clinical trial before marketing, are evident as minor increases in liver enzymes in the plasma [53]. This is in agreement with our finding when we found mild increasing ALT activity in mice exposed to meloxicam for 3 months, Table 3; consequently, meloxicam caused mild hepatic change. However, orally administration both meloxicam and metformin to mice for 2 months had had no significant changes in serum TG, ALT, AST activity, and cholesterol [40]. On the other hand, the latter form of liver injury is rare but can have fatal outcome. In general, the mechanism is thought to be an idiosyncratic reaction (immunologic or metabolic) rather than an intrinsic toxicity of the agent. Although hepatotoxicity has been attributed to the entire therapeutic class of NSAIDs, the rates and types of injury often vary within and between chemical classes [54]. We thought that the mild hepatic histological lesion which represented by central VC, perivascular lymphocytic infiltration with necrosis of hepatocyte cords in G₃ mice (exposed to meloxicam) is not enough to being hepatotoxic. Generally, liver is highly regenerative organ which can overcome some injuries. One of the typical complications induced by COX-2 inhibitors was found, such as cardiovascular complications [54]. Somewhat, when we found increasing in AST activity in mice exposed to meloxicam only (Table 3), this is considered one of the several biomarkers referring cardiotoxicity. In a study by Al-Rekabi *et al.* [55], it was shown that administration of 0.2 and 0.6 mg/kg meloxicam to rats increased aspartate aminotransferase level.

COX-2 is also expressed constitutively in a few organs including the brain and kidney [56]. Basal levels of COX-2 have been located in the macula densa, thick ascending limbs, and papillary interstitial cells of rats and in the glomerular podocytes [57]. Despite extensive studies on the toxicity of NSAIDs on various organs, the mechanism of NSAID-induced renal injury has not been completely clarified said [53]. Obviously, the elevations in serum UA, creatinine, and BUN are clinically biomarker for renal impairment or toxicity; this is specifically proved in group received meloxicam only in our recent study, Table 4. Enzymatic substance determination in blood and sermi-optical test after Warburb. The thought that kidney susceptibility to any toxic injury is partially due to its high blood flow. Kidneys receive about 20–25% of the resting cardiac output, and hence, any drug in the systemic circulation will be delivered to this organ in relatively high amounts. The processes involved in forming concentrated urine also serve to concentrate potential toxicants in the tubular fluid [57]. In addition to the increase in the indices of renal toxicity due to the chronic exposure to meloxicam, there are also the severe histological changes observed in the kidneys of these mice, which were represented by severe cortical Vd of proximal and distal renal tubules with deterioration of most glomeruli. Since COX-2 is important in renal prostaglandin I₂ synthesis, it implies that COX-2-selective inhibitors would have the same effects on renal function as conventional NSAIDs. Indeed, two COX-2-selective inhibitors, celecoxib and rofecoxib, have been shown to cause sodium retention and decrease glomerular filtration rate to a similar extent as non-selective NSAIDs [58].

Despite meloxicam is one COX-2-selective NSAIDs do not seem to spare the kidneys from adverse side effects. In a certain case report, a 56-year-old male who kept under metformin 750 mg daily for long period, BUN, serum creatinine, and liver enzymes were in normal limit [59].

CONCLUSION

Metformin administration with meloxicam have may beneficial important through preventing many deleterious effects of meloxicam after long-standing administration, but adjusting dosing regimen study might be recommended.

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AUTHOR'S CONTRIBUTIONS

FMK Al-Rekabi designated the study and performed experiments as well analyzed the data.

CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

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