
Ameliorative effects of *Curcuma longa* on methotrexate and meloxicam-induced hepatotoxicity and nephrotoxicity in rats

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Abstract

Methotrexate is widely used as chemotherapeutic agent in the treatment of malignancies and inflammatory diseases. Meloxicam is an analgesic and antipyretic drug. This study was carried out to determine if *Curcuma longa* has an ameliorative effect against Methotrexate and Meloxicam-induced toxicity in different organs in rats. Sprague-Dawley rats weighing 170 ± 20 gm were divided into six groups. Methotrexate and Meloxicam significantly increased serum AST, ALT, urea, and creatinine, liver & kidney body weight ratio as well as malondehyde and significant decrease in glutathione and superoxide dismutase when compared with control group. Administration of curcumin one hour before Methotrexate and Meloxicam significantly improved all the parameters mentioned before. The histopathological alterations confirmed the biochemical results. The data revealed that curcumin administration improved the liver and kidney histopathological lesions in rats treated with Methotrexate and Meloxicam. This study concluded that curcumin has a partial protective effect against the toxicity of Methotrexate and Meloxicam.

Key Words: Methotrexate, Meloxicam, *Curcuma longa*, Hepatorenal Toxicity, ROS.

Introduction

Methotrexate (MTX), a folic acid antagonist, is widely used as a cytotoxic chemotherapeutic agent in the treatment of various malignancies such as acute lymphoblastic leukaemia as well as in the treatment of various inflammatory diseases (Uzar et al., 2006). MTX has many serious adverse effects, such as myelosuppression, hepatic, renal and pulmonary disorders (Al-Quteimat et al., 2014 and Burukoglu et al., 2014). It is considered by many rheumatologists to be the most important and useful for DMARD disease modifying anti-rheumatic drug) and is often a component of the primary therapeutic protocol (Amy and Wasserman, 2011 and Dipiro et al., 2008). Methotrexate does have adverse effects and its toxicity includes gastrointestinal, hematologic, pulmonary, and hepatic (Chris et al., 2009).

Meloxicam (Mel), a new non-steroidal anti-inflammatory drug (NSAID) is an analgesic and antipyretic drug. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flure-medies. Acute overdoses of Mel can cause

potentially fatal liver damage kidney failure. It is a COX-2 (cyclo-oxygenase) inhibitor at its lowest therapeutic dose and is an anti-inflammatory by inhibiting prostanoid synthesis in inflammatory cells (Fleischmann et al., 2002). Mel is reported to be after as it produce significantly lower incidence of gastrointestinal adverse effects as compared to diclofenac sodium (Hawkey et al., 1998). It is metabolized to four biologically inactive main metabolites, which are exerted in both urine and faces.

Curcumin is the active constituent of *Curcuma longa*. It is widely used as a food coloring and flavoring agent and has wide range of pharmacological activities and therapeutic effects which include anti-inflammatory, antioxidative and anti-angiogenic properties (Sharma et al., 2005) Curcumin administration has been shown to be having hepatoprotective activity in hepatotoxicity caused by CCl₄, trichloroethylene, thioacetamide, endotoxin and ethanol (Rukkumani et al., 2004 and Shapiro et al., 2006). Curcumin has also shown to have anti-microbial activity and to protective in adverse diseases such as atherosclerosis, Ischemia

reperfusion injury, cystic fibrosis and diabetes mellitus (Vetrivelan et al., 2012). One of the major mechanism underlying *Curcuma Longa* disease-modifying effects is its pleiotropic anti-oxidant activity. It scavenges and prevents formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). In addition curcumin effects on cellular enzymes such as cyclooxygenase, glutathione-S-transferase and catalase include a potent antioxidant capacity at neutral and acidic PH that inhibits several cell signaling pathways. Also inhibits nuclear binding of hepatic nuclear factor kappa B in the rat model and inducing apoptosis in tumor cells (Anto et al., 2000 and Pillai et al., 2004). It has been reported that curcumin caused the apoptosis of tumor cells through various pathways (Mishra et al., 2005).

Aim of the work: The present study aimed at evaluating that if meloxicam will increase the toxicity of methotrexate and the possible biochemical, antioxidant and histopathological protective effects of *Curcumin* against the hepatorenal toxicity of MTX and meloxicam in rats

MATERIAL & METHODS:

Animals:

Male adult Sprague-Dawely rats of (170±20gm body weight) were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Egypt. Animals were maintained on a cages with free accesses to food and tap water. All animals were treated daily at fixed time as described in the experimental groups. Rats were randomly divided into groups of eight rats each.

The used dosages:

- Methotrexate 2.5 mg/kg, orally according to Jeffrey et al. (2010), Meloxicam 10mg/kg, orally according to Koc et al., (2005), *Curcuma longa* (Sigma) 100 mg/kg, p.o. according to Singh et al., (2012).

The experimental groups:

1) +ve control, received tween-80 vehicle 1 ml/kg /day x 7 days, 2) Received curcumin 100mg /kg, x 7 days, 3) Received MTX (2.5 mg/kg) x 7 days, 4) Received Mel (10 mg/kg) X 7days, 5) Received MTX (2.5 mg/kg) X 7days and Mel (10 mg/kg), 6) Received curcumin (100mg/kg) 1 h before MTX (2.5 mg/kg) and Mel (10 mg/kg) X 7days. Twenty four hours after the last treatment the animals were re-weighed and anesthetized using phenobarbital. Blood sample was collected from the retro-orbital plexus for biochemical assay. The animals were sacrificed; liver and kidney were removed, washed thoroughly with ice-cold saline (0.9% sodium chloride) and weighed. Finally, they were divided into 2 parts, one preserved in 10% formalin solution for the histological assessments and the other part was preserved in- 80°C refrigerator for detection of reactive oxygen species.

1- Liver and kidney Body weight Ratio:

Each animal body weight was recorded before sacrificing, and then its liver and kidney were removed and weighed then calculated according to the equation:

$$\text{Organ weight} / \text{Body weight} \times 100$$

2-Biochemical assays:

ALT and AST in serum was determined colourimetrically by the method described by Reitman and Frankel, (1957). Urea in serum was determined colourimetrically by the method described by Patton and Crouch, (1977). Creatinine in serum was determined colourimetrically by the method described by Husdan et al., (1968). Determination of MDA in liver and kidney tissues was carried out according to the method of Buege et al., (1978), with a slight modification in the incubation period according to the method of Deniz et al., (1997). GSH in liver and kidney tissues was determined according to the modified method of Beutler et al., (1963). SOD in liver and kidney tissues was measured according to the pyrogallol method of Markuland and Markuland, (1974).

3- Histopathological Examination:

Liver and kidney samples were removed rinsed in 10% formaline, dehydrated, cleared, impregnated, blocked and embedded in paraffin according to the standard histological techniques. Six micrometer-thick sections were cut through the liver (n=5). Sections were stained with hematoxyline and eosine for light microscopic examination (Broun, 1969).

4- Statistical analysis:

The results were expressed as mean±SE. Data were statistically analyzed using Neman-Kelus test to evaluate the comparisons between means at P<0.05 (Raslan, 2007).

Results:

Oral administration of MTX and its combination with Mel lead to significantly increase the liver and kidney body weight ratio when compared with control one (Table 1). The AST, ALT, urea and creatinine were significantly increased when compared with control one (Table 2). The ROS and antioxidant were also affected by MTX and its combination with Mel lead to significantly increase of MDA and significantly decrease of GSH and SOD in liver and kidney, when compared to the control group (Table 3). On the other hand, administration of *Curcuma longa* 1 hour before MTX or the combination decrease the liver and kidney body weight ratio, decrease the levels of all the parameters measured AST, ALT, urea, creatinine and improve the levels of MDA, GSH and SOD in liver and kidney when compared to

MTX or its combination with Mel (Tables, 1,2,3). The histological changes showed that Fig. (1): (A) –ve control, (B) curcumin showing normal histological structure of the central vein (cv) and surrounding hepatocytes (h). Administration of MTX and Mel (C) Liver showing cholangitis with severe inflammation in central vein. and (D) rat receiving Cur+MTX+MLX showing focal hepatic necrosis associated with inflammatory cells infiltration. Fig. (2): showing -ve Control kidney (A) and (B) rat receiving curcumin showing normal glomerulus (g), Administration of MTX and Mel (C) showing hypertrophy(h), vacuolation of glomerular tuft (vgt) and extravasated RBCs and (D) rat receiving Cur+MTX+MLX showing interstitial nephritis (In).

Table (1): Effect of Curcumin on the combination of MTX and mel-induced toxicity in rats.

Groups	Organ weight / Body weight X 100	
	Liver	Kidney
Control	3.47±0.10	0.75±0.05
Curcumin	3.44±0.09	0.74±0.05
MTX	4.60±0.17 ^a	0.92±0.02 ^a
% of change	32.56%	22.6
Mel	4.23±0.22 ^a	0.80±0.02 ^a
% of change	21.9%	6.7%
MTX+Mel	5.05±0.24 ^a	1.10±0.05 ^a
% of change	45.5%	46.6%
Cur+MTX+Mel	4.37±0.16 ^{abc}	0.96±0.03 ^{abc}
% of change	25.9%	28%

a:Significant with control and curcumin at p<0.05.

b:Significant with MTX at p<0.05

c: Significant with Mel at p<0.05

d: Significant with MTX+Mel at p<0.05

Discussion:

MTX administration for 7 consecutive days lead to significantly increased the organ/body weight ratio of liver and kidney. This findings agree with Raslan et al., (2007) and Raslan et al., (2010), who mentioned that the antitumor drug cisplatin and cyclophosphamide increase the liver and kidney body weight ratio. Also, significantly increased the AST & ALT and histopathological findings supported this conclusion. This findings agree with Verdi et al., (2010). Moreover, ceatinine and urea are increased due to MTX 15 mg/kg/ week and this findings agree with Seideman et al., 1993; Walker et al., 2000; Sener et al., (2006b) and Uraz et al., (2008). The results of the studies indicate that MTX causes oxidative stress damage by increasing the level of MDA in the liver and kidney and decreases the enzyme activities of SOD and GSH capacity this findings are agree with several reports demonstrating that MTX induces oxidative stress in tissues as demonstrated by increasing MDA levels and decreasing SOD and GSH levels in the liver and kidney (Jahovic et al., 2003; Cetinkaya et al.,

2006 and Cetin et al., (2008). Administration of Mel alone significantly increases the liver and kidney body weight ratio. The increased AST and ALT in this study are agree with Inal et al., (2014) who mention that liver injury usually ranges from mild elevations of liver enzymes to occasionally severe hepatic failure on using NSAIDs (Mel). The kidney function (urea, & creatinine) with no changes. This finding is agree with (Bevis et al., 1996) who mention that there is no changes in serum creatinine, serum urea or serum potassium in patients receive 15mg/kg Mel for consecutive 28 days. In this study there is a slightly increase in the MDA and slightly decrease of GSH and SOD but not significant. This findings are agree with (Villegas et al., 2000) who found that MDA, GSH and SOD are unaffected by meloxicam (3.75-30 mg/kg). and these results are in accordance with histopathology results.

In this work, administration of MTX and Mel together lead to significant changes in liver-kidney functions, significantly increase MDA and decrease of GSH and SOD. These changes are more or less like effects of the MTX alone, means that the toxicity of the combination is due to MTX dosage. These finding is agree with (Hubner et al., 1997 and Xie et al., 2003) who mention that the combination of Mel with MTX did not lead to increased MTX toxicity. Curcumin given 1 hr before the combination of MTX and Mel application provided significant decrease of liver and kidney functions. Also, significantly decrease MDA and increase of GSH and SOD. These result are similar to the finding of Rayes-Gordillo et al., (2007) and Sadzuka et al., (2012) that curcumin protect against hepatotoxic and nephrotoxic drugs (CC14 and Doxorubsin). The histopathological alteration of liver and kidney showing amelioration when curcumin administered before the combination of MTX and Mel.

We concluded that, MTX and Mel lead to hepatotoxic and nephrotoxic if administered alone, and in its combination. Meloxicam did not increase the toxicity of MTX. Curcumin was diminish oxidative stress induced by both MTX or Mel and its combination via protecting cells against free-radical damage and providing a partial protection against MTX- Mel induced hepatotoxicity and nephrotoxicity. We recommended that patients with rheumatoid arthritis and receive MTX alone or with Mel must check their liver- kidney functions and advised to take antioxidants supplementation like curcumin.

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Table (2): Effect of Curcumin on the combination of MTX and mel-induced toxicity in rats.

Groups	Liver Function		Kidney Function	
	AST	ALT	Urea	Creatinine
Control	51.0±2.4	42.5±2.1	27.5±2.4	0.32±0.02
Curcumin	52.0±3.3	41.50±2.9	28.5±2.6	0.31±0.02
MTX	91.50±4.2 ^{ac}	75.0±3.6 ^{ac}	48.75±3.1 ^{ace}	0.57±0.03 ^{ac}
% of change	79.4%	76.4%	77.2%	78.1%
Mel	85.33±3.9 ^{ad}	69.50±4.8 ^{ade}	36.1±2.7 ^{abd}	0.38±0.05 ^{bc}
% of change	67.3%	63.5%	31.2%	18.75%
MTX+Mel	98.0±3.3 ^{ace}	81.0±4.6 ^{ace}	50.1±2.7 ^{ace}	0.58±0.04 ^{ad}
% of change	92.0%	90.5%	82.1%	81.3%
Cur+MTX+Mel	80.83±4.2 ^{abd}	60.50±3.7 ^{abcd}	38.5±1.8 ^{abd}	0.47±0.03 ^{abcd}
% of change	58.5%	42.4%	38.9%	46.9%

a:Significant with control and curcumin at p<0.05.

b:Significant with MTX at p<0.05; c: Significant with Mel at p<0.05

d: Significant with MTX+Mel at p<0.05; e: Significant with Cur+MTX+Mel at p<0.05

Table (3): Effect of Curcumin on the combination of MTX and mel-induced toxicity in rats.

Groups	Liver			Kidney		
	MDA (nmol/g wet tissue)	GSH (µg/g wet tissue)	SOD (µg/g wet tissue)	MDA (nmol/g wet tissue)	GSH (µg/g wet tissue)	SOD (µg/g wet tissue)
Control	35.0±2.4	17.5±0.8	59.0±1.8	29.0±1.7	11.2±0.5	75.0±1.8
Curcumin	37.0±2.5	16.8±0.73	57.5±1.9	30.5±1.7	11.5±0.6	74.0±1.9
MTX	58.2±2.5 ^{ace}	8.8±0.6 ^{ace}	39.5±1.8 ^{ace}	50.5±1.9 ^{ace}	6.5±0.3 ^{ace}	42.0±1.7 ^{abde}
% of change	55.3%	49.7%	33.1%	74%	44.6%	44%
Mel	40.5±2.0 ^{bd}	14.5±0.6 ^{acde}	56.0±1.4 ^{bde}	32.5±1.6 ^{bde}	10.5±0.5 ^{bd}	67.5±1.5 ^{abde}
% of change	15.7%	17.1%	5.1%	15.5%	6.25%	10%
MTX+Mel	59.0±2.4 ^{ace}	9.0±0.5 ^{ace}	42.5±1.8 ^{abde}	51.3.3±2.3 ^{ace}	7.2±0.5 ^{ace}	44.0±1.5 ^{ace}
% of change	59.5%	48.5%	27.9%	76.8%	38.4%	41.3%
Cur+MTX+Mel	43.3±1.9 ^{bd}	13.0±0.6 ^{abcd}	51.0±1.9 ^{abcd}	40.2±2.8 ^{abde}	10.2±0.4 ^{bd}	61.7±2.1 ^{abcd}
% of change	23.7%	25.7%	13.5%	48.8%	8.9%	17.8%

a:Significant with control and curcumin at p<0.05.

b:Significant with MTX at p<0.05; c: Significant with Mel at p<0.05

d: Significant with MTX+Mel at p<0.05; e: Significant with Cur+MTX+Mel at p<0.05

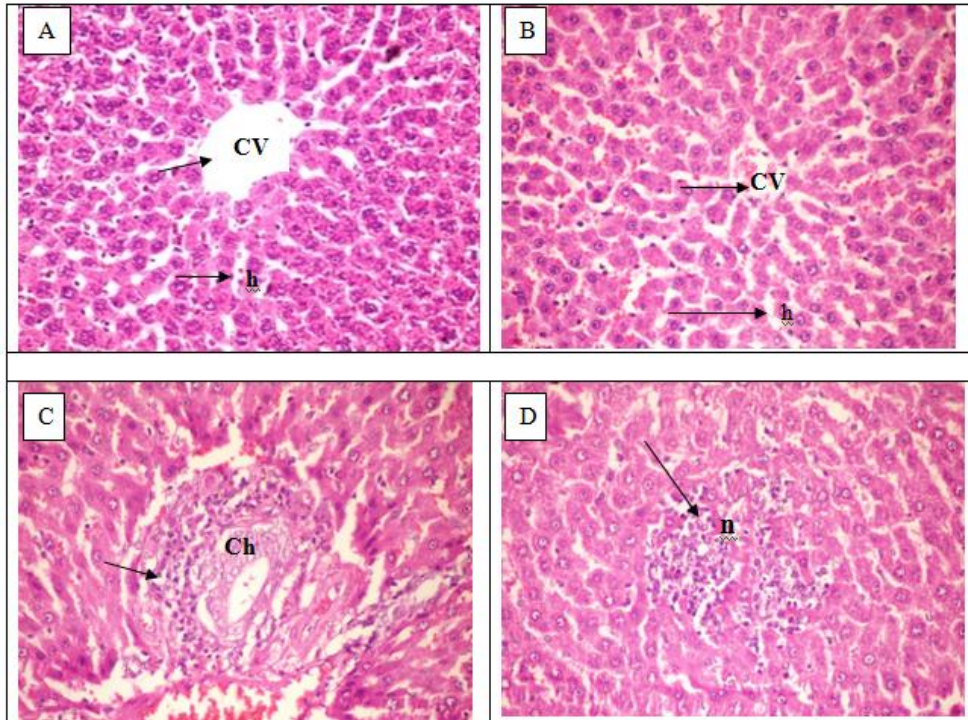


Fig.(1): Liver of rat (A) -ve control, (B) curcumin showing normal histological structure of the central vein (cv) and surrounding hepatocytes (h), rat receiving MTX and MLX. (C) showing cholangitis(ch) with sever inflammation in central vein (arrow), and (D) rat receiving Cur+MTX+MLX showing focal hepatic necrosis (n) associated with inflammatory cells infiltration (arrow) (H&E stain x40).

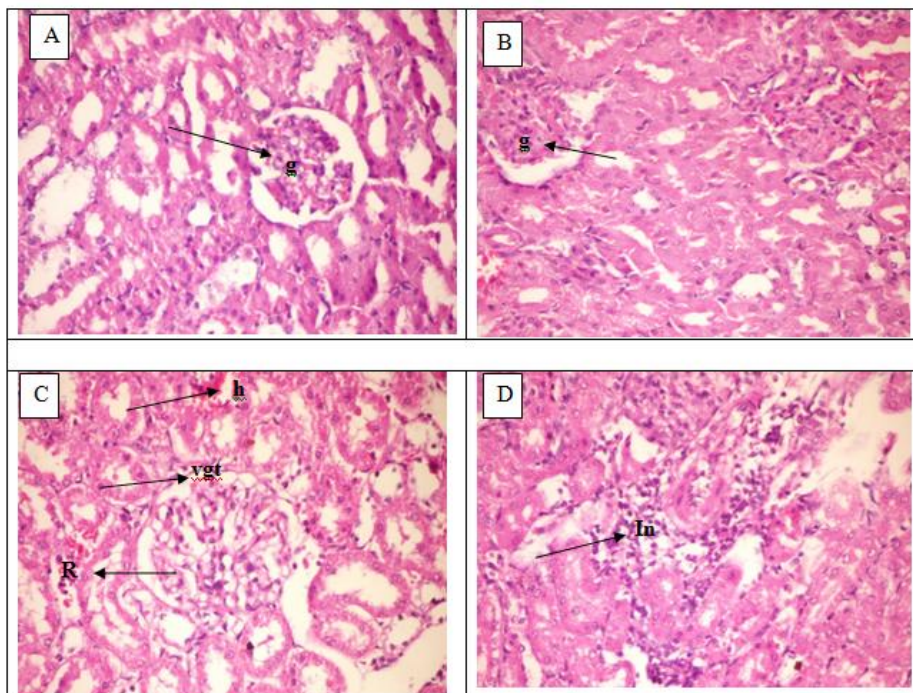


Fig. (2): Kidney of rat (A) -ve control kidney and (B) rat receiving curcumin showing normal glomerulus (g), (C) rat receiving MTX and MLX showing hypertrophy(h), vacuolation of glomerular tuft (vgt) and extravasated RBCs and (D) rat receiving Cur+MTX+MLX showing interstitial nephritis (In) (H&E stain x40).

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