Hematological and Biochemical Effects of Meloxicam in Male Albino Rats

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ABSTRACT

The present study was planned to investigate the hemato-biochemical alterations induced by Meloxicam, a new non-steroidal anti-inflammatory drug, in albino rats and to determine the modulatory action of vitamin C against the adverse effects of Meloxicam. Seventy-eight male albino rats (140-160 g) were divided into five groups. The first group (G1) was considered as control group, in which rats were given 1 ml/kg of saline solution. The second group (G2) was given orally the therapeutic (low) dose of Meloxicam (0.2mg/kg) alone. The third group (G3) was given three-fold the therapeutic (high) dose of Meloxicam (0.6 mg/kg). The forth group (G4) was treated with the low dose of Meloxicam along with vitamin C orally (200 mg/Kg). The fifth group (G5) was treated with the high dose of Meloxicam along with vitamin C (200 mg/Kg). At the end of the experimental period (four weeks), the obtained results showed that Meloxicam at both doses did not alter the levels of RBCs, PCV% and total protein compared with the control group, while, the low and high doses of Meloxicam induced significant increases in total WBCs counts, blood Hb, platelets, serum creatinine, total cholesterol, LDL-C, AST, ALT and MDA and decreases in serum HDL-C, and SOD. Administration of vitamin C along with Meloxicam intoxication led to decrease the severe biochemical and hematological alterations induced by Meloxicam by preventing the rise in the levels of MDA, ALT, AST, total cholesterol, LDL-C, creatinine and the values of WBCs, platelets and Hb, and ameliorated the decline in the levels of SOD and HDL-C. We could conclude that Meloxicam at both dose levels causes hepatotoxicity, nephrotoxicity and produces lesion in hematological parameters. Supplementation with vitamin C could counteract the toxic effects of Meloxicam.

Key words: Meloxicam, Vitamin C, hemato-biochemical parameters, Albino rats.

Introduction

A wide variety of pharmacological and chemical agents is known to produce a range of acute or chronic liver and kidney diseases and hematological alterations. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used drugs in both veterinary and human medicine for various inflammatory conditions. NSAIDs act both centrally (eg, analgesic, antipyretic actions) and peripherally (eg, analgesic, antithrombic actions) and are one of the best therapeutic choices to prevent and treat postoperative pain (Adams, 2001). These drugs are used to relieve acute visceral and musculoskeletal signs of pain (including those associated with trauma and chronic signs of pain (from conditions such as arthritis) and to decrease inflammation and central nervous sensitization associated with surgery (Burian and Geisslinger, 2005 and Carroll and Simonson, 2005).

The recent introduction of NSAIDs with selective COX-2 inhibitory effect is a major pharmacologic milestone in therapeutics. They are a new version of NSAIDs. Meloxicam is a new and very popular anti-inflammatory and analgesic drug related to this group. It is a member of the enolic class of NSAIDs that, at low doses, exerts its effect via selective inhibition of the cycloxygenase-2 (COX-2), thereby, preventing prostaglandin synthesis, which can lead to pain, fever and inflammation. At high doses, meloxicam may also inhibit the COX-1 pathway, resulting in decreased production of the physiologically important and protective prostaglandins (Gassel *et al.*, 2005 and Kirchgessner, 2006). Meloxicam, as NSAID, either alone or with antimicrobial drugs, is indicated for use in ruminants for the treatment of pneumonia, pleuritis, laminitis, myositis, mastitis,

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prolapse of uterus, premature labour etc. The principle side effect of Meloxicam is gastrointestinal irritation (Vane and Botting, 1997 and Lees *et al.*, 2004).

The desired effects of this group and their clinical benefits are related to inhibition of COX-2 whereas inhibition of COX-1 activity by these drugs has been associated with serious adverse effects including gastrointestinal mucosal injury and renal dysfunction. These side effects limited their chronic use in spite of their good efficacy. However, animal studies have revealed a protective role for the COX-2 enzyme in the stomach, kidney, heart, vasculature and reproductive system. Therefore, COX-2 selective inhibitors might be expected to produce effects in these tissues similar to non-selective NSAIDs. In addition, recent observations by other authors have implicated COX-2 as an integral component in the maintenance of physiologic homeostasis. They found that COX-2 is widely distributed in the kidney and it is present in a constitutive fashion in vasculature, glomerulus, tubular segments and interstitium. The constitutive COX-2 in renal tissues has important implications with respect to understanding the impact of NSAIDs especially the COX-2 inhibitors on the renal structure, function and the occurrence of adverse renal effects. Therefore, the effects of COX2 selective inhibitors on these vital organs need to be reassessed.

Vitamin C or ascorbic acid, the most abundant and effective antioxidant in the human body, is a potent water-soluble antioxidant that scavenges reactive oxygen and nitrogen species. It is necessary for collagen formation, adequate functioning of the immune system, and as a tissue antioxidant. Vitamin C decreases oxidative stress and lipid peroxidation, thereby preventing many damaging processes in cells (Mackay and Miller, 2003). Vitamin C also indirectly protects the cell membranes and lipid-based structures along with Vitamin E. It is an electron donor and therefore a reducing agent (Bashandy and Alwasel, 2011).

Relatively, little is known about Meloxicam adverse effects and presently literature on vitamin C interaction on Meloxicam toxicity is scanty. Because of the approved protective effects of vitamin C dietary supplementation against various pathologies, the present study aimed to investigate its effects in counteracting the Meloxicam induced hematological and biochemical alterations and to emphasize the role of this agent in modulating or preventing the toxicity of Meloxicam in high and low doses in experimental rats.

Materials and Methods

Experimental animals:

A total of seventy-eight male albino rats of Sprague dawley strain weighing (140-160 g) were used in this study. The rats were obtained from Crops Department, Faculty of Agriculture, Minia, Egypt. The study protocol was approved by the animal Ethics Committee of the Zoology Department at the Faculty of Science, Minia University according to Helsinki principles. The guide for the care and use of laboratory animals was followed. The rats were housed in well aerated cages under hygienic condition and were provided commercial rodent diet and water ad libitum for two weeks for adaptation and housed in temperature controlled room (25°C) with constant humidity and 12h/12h light/dark cycle.

Chemicals:

Meloxicam was purchased from "Outpatient Pharmacy and 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 the group dosed with therapeutic dose of Meloxicam (0.2mg/kg.BW) and 0.6ml / 100gm.BW for animals dosed with three fold the therapeutic dose (0.6mg/ kg.BW) of meloxicam (Carpenter *et al*, 2009). Vitamin C (100 mg tablet), was purchased from "Outpatient Pharmacy and was prepared in distilled water to make 10% stock solution. The concentration that was prepared for the experiment was 200 mg/kg (Aksoy *et al*, 2005).

All the other chemical reagents and kits used in the study were of standard analytical grades and purchased from Sigma Chemical Co. (St. Louis, O, USA).

Experimental design:

After the adaptation period, the rats were divided into five groups (n=6 in each group) as following:

Group 1: considered as the control group of six rats which were given 1 ml/kg of saline solution (0.9 % Nacl) orally and once daily for twenty-eight days.

Group 2: served as the low Meloxicam group in which rats were given orally therapeutic dose of Meloxicam (0.2mg/kg) once daily for twenty- eight days.

Group 3: represented the high Meloxicam group in which rats were given three-fold therapeutic dose (high) of Meloxicam (0.6mg/kg) once daily and orally for twenty-eight days.

Group 4: this group was treated orally with the low dose of Meloxicam (0.2mg/kg) along with vitamin C (200 mg/Kg) in aqueous suspension for twenty-eight days.

Group 5: that received orally the high dose of Meloxicam (0.6mg/kg) along with vitamin C (200 mg/Kg) in aqueous suspension for twenty-eight days.

Hematological and biochemical analysis:

At the end of the experimental period, animals were fasted overnight but allowed free access to water. Animals were sacrificed under anesthesia with diethyl ether, and two blood samples were immediately collected. The first sample was collected in heparinized tubes (2.25µ heparin / 5 ml blood) for hematological studies. The second sample was collected in non–heparinized tubes for separation of serum. The tubes were centrifuged for 15 minutes at 3000 rpm for estimation of some biochemical parameters. Malondialdehyde (MDA) and Superoxide dismutase (SOD) were measured in liver homogenate. The liver was separated and one gram of the liver was homogenized in 0.1M phosphate buffer (pH 7.4) on ice and cleared by centrifugation at 3000 rpm at 4∘C for 15 minutes.

Hematological parameters were determined using an automated cell counter Micros ABX (Roche Diagnostic System, Montpellier, France) (Casacó *et al*, 2010). The following hematological parameters were analyzed: hemoglobin, packed cell volume, erythrocyte count, platelet count and leukocyte count. Serum alanine and aspartate aminotransferase (ALT&AST) levels were estimated according to the method of (Reitman and Frankel, 1957). High-density lipoprotein cholesterol (HDL-C) was determined by using enzymatic colorimetric methods described by (Kostener, 1977). Total cholesterol was determined spectroscopically in serum by using the method of (Allain *et al*., 1974). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the method of (Fruchart, 1982). Serum total protein was determined by using the method described by (Bishop *et al*., 2000). Serum creatinine was determined according to the method described by (Schirmeister, 1964). MDA in liver tissue was determined according to the method of Uchiyama and Mihara, 1978 and SOD level was measured following the method of Marklund and Marklund (1974).

Statistical analysis:

Statistical analysis of the present data was performed throughout one-way analysis of variance (ANOVA test). Student "t" test was used for significance according to Artimage and Berry, 1987. The results were expressed and drawn using the mean± standard error (M± SE) and differences were considered to be significant at ($P \leq 0.05$), highly significant at ($P \leq 0.01$) and very highly significant at ($P \leq 0.001$).

Results

Effects of Meloxicam on hematological parameters:

Hematological parameters like total leucocyte count (WBCs), total erythrocyte count (RBCs), hemoglobin content (Hb), packed cell volume (PCV%) and platelets count were estimated in control and treatment groups and are presented in Table (1) and Fig.(1). There were no significant changes in both RBCs count and PCV percentage between rats of low and high Meloxicam treated groups in comparison with that of the control one. The values of total WBCs and platelets were increased significantly ($p < 0.001$) in rats given low dose of Meloxicam (0.2 mg/kg b. w.) as compared with the control rats. Administration of vitamin C along with the low dose of Meloxicam decreased significantly the WBCs and platelets counts ($p < 0.001$) as compared to low Meloxicam alone treated group. Rats treated with Meloxicam in high dose (0.6 mg/kg b. w.) had significantly higher WBCs ($p < 0.001$) and platelets counts ($p < 0.05$), as compared to the controls. In addition, Co-administration of high dose Meloxicam and vitamin C decreased significantly WBCs ($p < 0.01$) and platelets counts ($p < 0.001$) as compared to the high dose Meloxicam group alone. Hb content increased significantly ($p < 0.001$) in rats given low and high doses of Meloxicam as compared to the control group. Administration of vitamin C along with the low dose of Meloxicam had no significant effect on Hb content compared with the low dose of meloxicam alone. While, vitamin C plus high dose of meloxicam showed a significant decreasing effect ($p < 0.01$) in Hb content as compared to high Meloxicam alone treated group.

Table 1: Effects of low and high doses of Meloxicam with or without vitamin C on hematological parameters in male albino rats (means ± SE).

Parameters		Group 1 (Control)	Group 2 Low MLX	Group 4 Low MLX +vitamin	Group 3 High MLX	Group 5 High MLX +vitamin
WBCs	($\times 10^9$ / L)	8.5±0.0	14 ±1.2 ***	7.9 ±0.8 ###	33 ±0.8 ***	14.2 ±2.5 ##
RBCs	($\times 10^{12}$ / L)	8.09±0.5	8.7 ±0.4	8.96 ±0.4	8.6 ±0.08	8.4 ± 0.3
Hb	(g / L)	12.6±0.0	15.9 ±0.3 ***	15.7 ±0.2	16.2 ±0.1 ***	14.9 ±0.15##
PCV	(%)	44.3±1.1	48.6 ±0.8	50.3 ± 2.3	47.9 ±0.16	47.5 ± 1.7
Platelets	($\times 10^9$ / L)	264.7±70	855 ±270***	663 ±112 #	984 ±0.44 ***	676.8 ± 5o ###

Significant increase or decrease compared with the control group (P<0.05, ** P<0.01, *** P<0.001).*
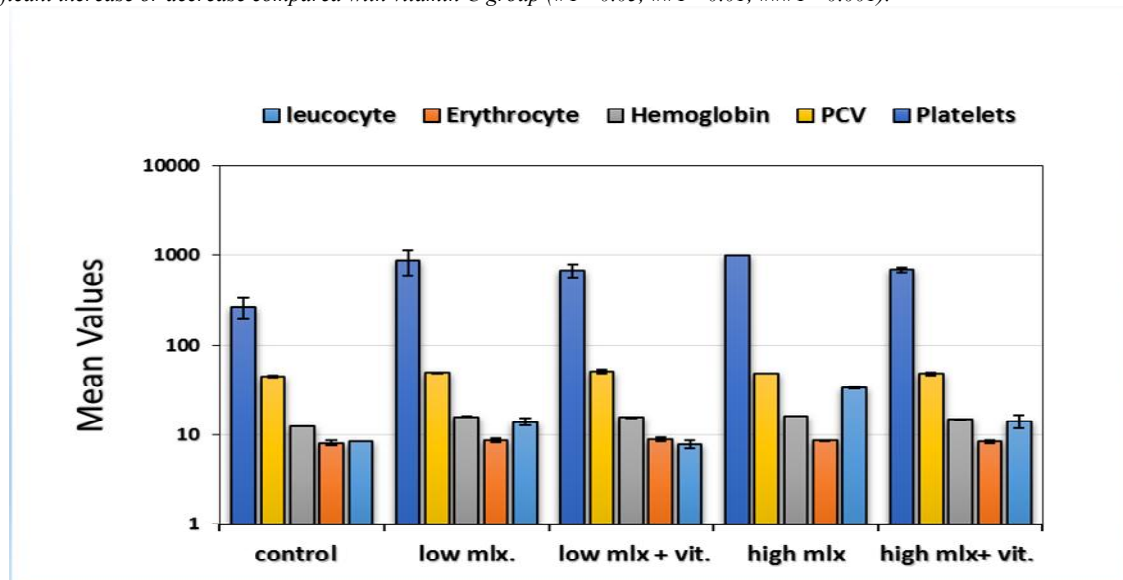*Significant increase or decrease compared with vitamin C group (# P<0.05, ## P<0.01, ### P<0.001).*

Fig. 1: Effects of low and high doses of Meloxicam alone or in combination with vitamin C on total leucocyte count, total erythrocyte count, hemoglobin content, packed cell volume and platelets count of male albino rats.

Effects of Meloxicam on some biochemical parameters:

The results of the biochemical parameters of this study are presented in table (2) and figure (2). Data reveals that serum creatinine level (as renal function marker) increased significantly ($P < 0.01$) in rats treated with low (0.2mg/kg b. w.) and high (0.6mg/kg b. w.) doses of Meloxicam in groups 2 and 3 as compared to the rats in the control group. On the other hand, administration of vitamin C along with Meloxicam significantly decreased the rise in creatinine levels in groups 4 and 5 ($p<0.05$ and $P < 0.001$, respectively) as compared to low and high Meloxicam alone groups.

Data presented in table (2) and figure (2) reveals that rats treatment with Meloxicam in low (0.2mg/kg b. w.) and high (0.6mg/kg b. w.) doses showed significant increase in total cholesterol ($P < 0.01$ and $P < 0.001$, respectively) as compared to control rats. However, administration of vitamin C along with Meloxicam in low and high doses significantly ($P < 0.05$) decreased the total cholesterol content as compared to low and high Meloxicam alone groups. Also, treatment with low and high doses of Meloxicam significantly increased LDL-C levels (($p<0.01$ and $P < 0.001$, respectively) as compared with the control group. While, vitamin C significantly (($p<0.01$ and $P < 0.05$, respectively) decreased LDL-C levels in group 4 and group 5. Administration of low and high doses of Meloxicam significantly decreased HDL-C levels (($p<0.01$ and $P < 0.001$, respectively) as compared with the control group. While, vitamin C significantly (($p<0.01$) increased HDL-C levels in group 4 and group 5. The results of the present study showed that rats treatment with Meloxicam in low (0.2mg/kg b. w.) and high (0.6mg/kg b. w.) doses caused significant increase in serum AST ($P < 0.01$ and $P < 0.05$, respectively) as compared to control rats, while, administration of vitamin C along with Meloxicam in low and high doses significantly ($P < 0.001$) decreased AST content as compared to low and high Meloxicam alone groups. Also, the low and high doses of Meloxicam increased significantly serum ALT content ($P < 0.01$ and $P < 0.001$, respectively) as compared to control rats, but, administration of vitamin C along with Meloxicam in low and high doses significantly ($P < 0.01$ and $P < 0.05$, respectively) decreased ALT content as compared to low and high Meloxicam alone groups.

In the present study, in spite of the increase in the means of total proteins under the effects of the low and high Meloxicam levels, there were no significant changes in total protein content between low and high Meloxicam groups in comparison with the control one. As a marker of oxidative stress, MDA levels increased significantly in the low and high Meloxicam groups ($P < 0.05$ and $P < 0.01$, respectively) as compared to control group. Moreover, SOD level decreased significantly in the low and high Meloxicam groups ($P < 0.05$ and $P < 0.001$, respectively) as compared with the control group. Although vitamin C did not show any significant effect on MDA levels in the present study, administration of vitamin C along with Meloxicam in low and high doses significantly ($P < 0.05$) increased SOD levels as compared to low and high Meloxicam alone groups.

Table 2: *Effects of low and high doses of Meloxicam with or without vitamin C on some biochemical parameters in male albino rats (means ± SE).*

Parameters	Group 1 (Control)	Group 2 Low MLX	Group 4 Low MLX +vitamin	Group 3 High MLX	Group 5 High MLX +vitamin
Creatinine (Mg/dl)	1±0.0	2.7 ± 0.17 **	2.18 ± 0.23 #	2.9 ± 0.1 **	2 ± 0.08 ###
TC (Mg/dl)	66.5±0.0	106 ± 5.5 **	79.8 ± 8.1 #	111 ± 0.5 ***	75 ± 6.4 #
HDL-C (Mg/dl)	18±0.0	7 ± 1.6 **	17 ± 1.9 ##	15.5± 0.03 ***	20.6 ± 0.82 ##
LDL-C (Mg/dl)	50±0.0	77 ± 2.6 **	62 ± 1.1 ##	83 ± 0.07 ***	51 ± 5.5 #
AST (u / l)	88±0.0	152 ± 7.7 **	80 ± 4.4###	118 ± 4 *	91.2 ± 4.5 ###
ALT (u / l)	44±0.0	87 ± 5.1 **	47 ± 7.8 ##	71 ± 1 ***	62 ± 6.7 #
TP (g/dl)	4.85±0.9	7.6 ± 1.1	4.5 ± 0.5	5.8 ± 1.6	4 ± 0.7
MDA (Nmol /ml)	1.48±0.0	2.3 ± 0.14 *	2.9 ± 0.4	2.2 ± 0.02 **	2.6 ± 0.04
SOD (U / ml)	4.8±0.0	2.9 ± 0.1*	3.36 ± 0.1#	2.8 ± 0.03***	3.19 ± 0.04 #

Significant increase or decrease compared with the control group (P<0.05, ** P<0.01, *** P<0.001).*
Significant increase or decrease compared with vitamin C group (# P<0.05, ## P<0.01, ### P<0.001).
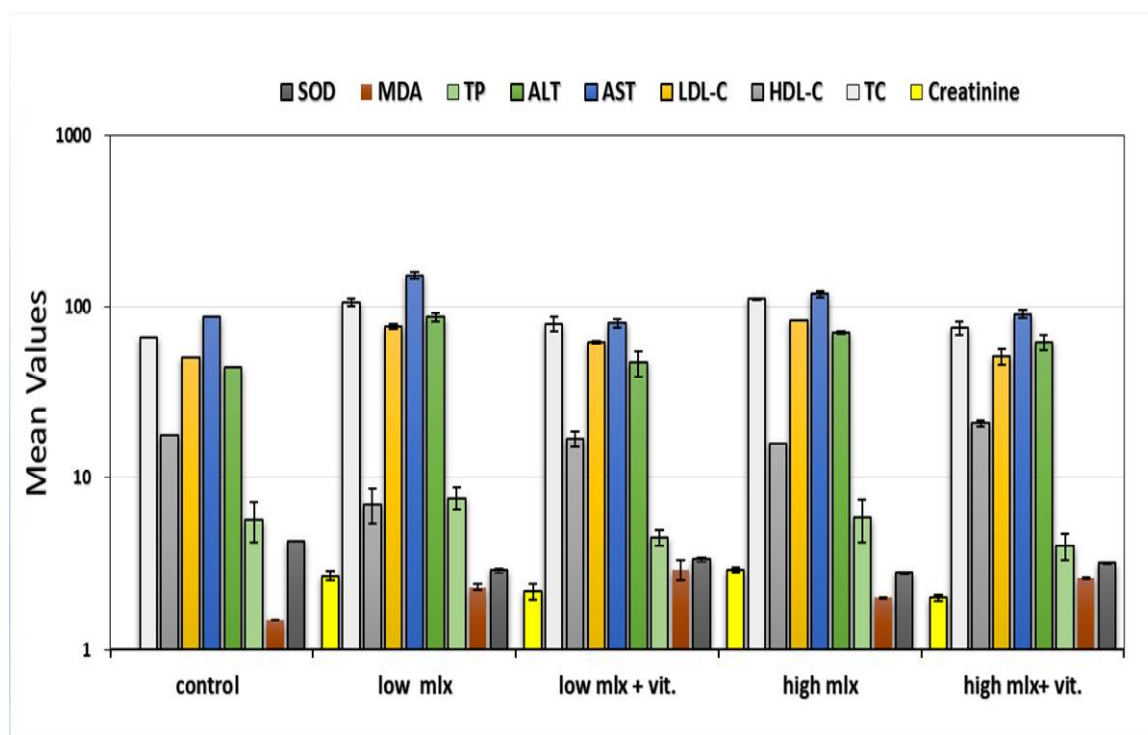
Fig. 2: Effect of low or high doses of Meloxicam alone and in combination with vitamin C on serum creatinine, total cholesterol, HDL-C, LDL-C, AST, ALT total protein, MDA and SOD level of male albino rats.

Discussion:

Because of the approved protective effects of vitamin C dietary supplementation against various pathologies, this work focused on its effects in counteracting the Meloxicam induced alterations. Interestingly, the observed results, herein, show that vitamin C re-establish the disordered conditions (Ergul *et al.*, 2010).

Even though many previous studies observed the toxicity of Meloxicam, the basic mechanism of the toxicity is not understood thoroughly (Bhadja, 2007). The non –steroidal anti –inflammatory drugs inhibit cyclo-oxygenase and therefore the biosynthesis of prostaglandins and it has been proposed that this biochemical intervention is the basis not only of their therapeutic activity but also contribute to their side effects.

In the literature, Meloxicam-induced hematological disorders are well documented, and the results of the present study are in accordance with this (Pachathundikandi and Varghese, 2006 and El-Sharaky *et al.*, 2010). Hematological parameters like Hb concentration, WBCs, PCV, RBCs and platelets were estimated in control and treatment groups and are presented in Table (1) and Figure (1). There were no significant changes in both RBCs count and PCV% between animals of low and high Meloxicam groups in comparison with that of control one, which may be due to the highly inhibition of cyclooxygenase enzymes.

Hemopoietic and leukocytic are two dynamic systems, which react quickly to chemical intoxications, and condition the maintenance of homeostasis by an organism. However, the observed effects of Meloxicam, which are represented by increased levels of WBCs, Hb, and platelets are generally in agreement with the results of several investigations on the animals treated with different chemical factors (Rafatullah *et al.*, 1991; Al-Attar, 2007; Krishna and Ramachandran, 2009; Salah *et al.*, 2009; Al-Attar and Al-Taisan, 2010).

When Meloxicam was fed orally at 2 mg/kg for 16 days, Alencar, *et al.* (2003) reported anemia in Meloxicam intoxicated dogs. Similar result was observed in rats treated with Meloxicam (Bhadja, 2007) and mice treated with aspirin (Merchant *et al.*, 2004) and monkey treated with lornoxicam (Atzpodien *et al.*, 1997). No results substantiated the findings of the present experiment as a contrary

report was given by our data, which demonstrated increased levels of Hb in Meloxicam groups compared with the control group. The increase in Hb values as observed in the present study may be due to the effect of Meloxicam, which may increase the bone marrow activity. Moreover, the increase in Hb may be due to the increase of its biosynthesis by increasing the succinyl pool as well as glycine pool. Both are required in the initial stage of the heme biosynthesis. Simultaneous treatment with vitamin C decreased the toxic effect of Meloxicam by inducing significant decreasing effect on hemoglobin content. This may be due to ameliorating the bone marrow activity.

In this study, the results showed significant increase (P<0.01) in platelets count of the two Meloxicam groups in comparison with the control group. The increase in platelet count occurred may be due to increased production or decreased consumption of them. Also, there was marked elevation in WBCs count of rats from G2, and G3 groups. The increase in leucocyte count recorded in the present study could be attributed to ulcerogenic effect of Meloxicam as observed in present study and as reported earlier (Alencar *et al*, 2003). Leucocytes are known to increase sharply when infection occurs, as one of the first line of defense of the body (Ganong 2001). Significant increase in WBC values may be due to enhanced bone marrow effect of Meloxicam on leucopoiesis (Merchant, 2004). Similar finding were observed in dog administered with NSAID (Sharma *et al*., 2002) and buffalo calves treated with Meloxicam (Magarwadiya *et al*., 2002). This observation supports the findings that NSAID stimulate immune functions in rats (Bjarnsholt *et al* 2005).

The serum biochemical parameters studied during the present experiment are presented in Table (2) and Figure (2). The observations revealed that the rats treated with the low and high doses of Meloxicam resulted in a significant elevation of AST and ALT values, serum creatinine, serum total cholesterol and LDL cholesterol and insignificant increase in total protein on comparing with the control group. The groups G4 and G5 receiving Meloxicam and vitamin C showed relatively reduced values as compared to groups treated with Meloxicam alone. The elevated levels indicate disturbances in protein and lipid metabolism induced by Meloxicam intoxication and suggest hepatotoxic, and nephrotoxic effects of Meloxicam. These results came in agreement with other study conducted by Al-Rekabi *et al*., 2009. Similar serum biochemical changes in case of a female patient with rheumatoid arthritis who developed acute cytolytic hepatitis due to Meloxicam was reported earlier (Staerkel and Horsmans, 1999).

The increase in total protein noticed in the present study are not in agreement with other findings that showed reduced levels of total protein induced by Meloxicam intoxication which was attributed to the disruption of lysosomal membranes under the effect of NSAID toxicity leading to the liberation of their hydrolytic enzymes in the cytoplasm resulting in marked lysis and dissolution of the target material (Ebaid *et al*., 2007). Similar value also reported in monkey treated with lornoxicam (Atzpodien *et al*., 1997), rats treated with Meloxicam (Bhadja, 2007), mice treated with piroxicam (Ebaid *et al*., 2007). In the present study, the alterations in the level of serum proteins after Meloxicam administration might be an outcome of both hepatic and renal damage, where it causes alteration in a number of enzymes and could disturb protein synthesis in hepatocytes. Moreover, increased serum protein levels could be attributed to increased hepatic DNA and RNA synthesis, which in turn affects utilization of free amino acids for protein synthesis (Aprioku *et al*., 2014, Lewis *et al*., 1983 and Doutremepuich *et al*., 2012).

The increase in serum levels of ALT and AST is considered as initial step in detecting liver damage due to viral, alcoholic and drug-induced hepatocyte disorder. In this study, a significant increase was observed in AST, ALT levels in Meloxicam -treated rats. These results are in accordance with many investigations on Meloxicam-induced liver damage in experimental animals (Yousef *et al*., 1999). The increase in the activities of these enzymes may be due to the increase in the secretory activities of the hepatocyte cell which were in accordance with the findings reported in sheep (Abdulaziz and Hristev, 1996). The disturbance in the transport function of the hepatocytes because of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane (Fan *et al*., 2009). The increased levels of serum enzymes indicate an enhancement of permeability, damage or necrosis of hepatocytes. These results were confirmed by previous histopathological findings that showed high concentration of Meloxicam in tissue of liver and kidney after multiple oral dose of Meloxicam 1mg/Kg.BW/day for 5days in male and female black hooded rats and revealed variable lesions ranged from extensive necrosis with mild lymphocytic infiltration (Ulrich *et al*., 1998). In the present study, Meloxicam-induced liver toxicity is obvious, as liver is the major target

organ for drug metabolism and hepatic biotransformation reactions are known to induce apoptosis of hepatocytes (Taiwo, 2008 and Aprioku *et al.*, 2014). However, vitamin C in the present study and other studies prevented the increase in the activities of these enzymes, which is the primary evidence of its hepatoprotective activity (Ergul *et al.*, 2010).

A change in serum creatinine level is an important indicator of kidney function. Therefore, in the present study serum creatinine was determined to find out the effect of Meloxicam on renal functional markers. In this study, serum creatinine of the low and high Meloxicam rats was found to be more than that in control group. Moreover, dogs treated with meloxicam at 1 mg and 2-mg/kg body weight had elevation in serum creatinine and is in agreement with the present findings (Alencar *et al.*, 2002). Increased serum creatinine levels reflect the nephrotoxic effects of Meloxicam (Farombi, 2006) and may be attributed to compromise of the renal functional capacity. Meloxicam might have either interfered with creatinine meta- bolism leading to increased synthesis or the tissues might have compromised all or part of its functional capacity of tubular excretion or it may be due to an adverse effect on the renal function which might be due to oxidative stress induced by Meloxicam on the renal tissue (Farombi, 2006). Adverse effects following Meloxicam administration in normal rats might be an outcome of NSAIDs induced inhibition of prostaglandin synthesis, which leads to renal vasoconstriction, and decreased renal perfusion, which is responsible for acute renal abnormalities Purohit, and Daradka, 1999, Matzke, 1996 and Huerta *et al.*, 2005).

As shown in Table (2) and Figure (2), Meloxicam in low and high doses caused significant elevation in the level of malondialdehyde (MDA) compared to control group. This indicates that Meloxicam induces oxidative stress since MDA, which is the last product in lipid peroxidation process, is considered as oxidative stress marker (Ayala *et al.*, 2014). However, similar elevation in MDA level in response to Meloxicam treatment was recorded in previous studies (Villegas *et al.*, 2001). This increase in MDA concentration was definitely accompanied by enhanced lipid peroxidation, DNA damage, altered calcium and sulfydryl homeostasis as well as marked disturbances in antioxidant defense system occurred (Mahaprabhu *et al.*, 2011). With respect to SOD, Meloxicam has been found to cause significant decrease in the level of SOD, while a significant increase was detected in the Meloxicam plus vitamin C groups in comparison to Meloxicam alone. So, vitamin C was found to be effective in prevention of oxidative damage induced by Meloxicam by significant increasing in SOD levels.

In this study, the observed increase in the levels of total cholesterol and LDL-cholesterol which was associated with decrease in HDL cholesterol, in the rats exposed to Meloxicam may be a potential indicator for fatty acid metabolism, and implicitly of a possible membrane lipid peroxidation. Our results showed an important levelling up of MDA, indicating lipid peroxidation resulting from exposure to Meloxiam. Thus, it becomes conceivable that the observed alteration in circulating cholesterol levels may be a consequence of membrane lipid peroxidation and free radical release (Ergul *et al.*, 2010).

The possible mechanism of vitamin C as hepatoprotective factor may be due to its antioxidant effect, which impairs the activation of Meloxicam into the reactive form. In addition, ascorbic acid is a potent scavenger of reactive oxygen species in plasma and extracellular compartments of the liver (Inoue, 2001). Ascorbic acid scavenges and destroys free radicals in combination with vitamin E and glutathione. George (2003) reported that the drastic decrease of ascorbic acid in dimethylnitrosamine (DMN)-induced hepatic fibrosis may indicate increased oxidative stress, free radical formation, and simultaneous damage of the liver plasma membrane lipid bilayers. After scavenging the reactive oxygen species, an amount of ascorbic acid could regenerate either enzymatically using monodehydroascorbate reductase, or nonenzymatically by spontaneous dismutation. The enzymatic regeneration of the ascorbic acid may occur principally intracellularly at the expense of reduced glutathione. The highly reduced concentrations of glutathione in liver diseases (Bianchi *et al.*, 1997; Jain *et al.*, 2002) may affect the enzymatic regeneration of the ascorbic acid, which results in the sacrifice of ascorbic acid during scavenging of reactive oxygen species in liver diseases. Under physiologic conditions, the regenerating system of ascorbic acid may function normally, but during extreme necrosis of the liver, it may be impaired, which contributes to a decreased ascorbic acid concentration in both liver and circulating system (George, 2003).

Conclusion: We can conclude that treatment with low and high doses of Meloxicam causes haematological, hepatic and renal toxicities. From the present study, it is obviously that the

administration of vitamin C along with Meloxicam intoxication led to decrease the severe biochemical and hematological alterations by preventing the rise in the levels of MDA, ALT, AST, total cholesterol, LDL-cholesterol, creatinine and the values of WBCs, platelets and Hb, and ameliorated the decline in the levels of SOD and HDL-cholesterol. So, the vitamin C supply is a putative protector against the mentioned parameters and was ascertained to reduce their harmful effects. Further, it was observed that vitamin C could counteract untoward effect of Meloxicam. A further detailed study at molecular level is needed to know the exact mechanism of Meloxicam toxicity, and the protective mechanism of vitamin C could be explored.

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