BENHA VETERINARY MEDICAL JOURNAL, VOL. 36, NO. 1:361-372, March, 2019

Protective effect of vitamin E against hepato-renal toxicity induced by meloxicam in rat

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A B S T R A C T

In this research, we evaluated the effect of meloxicam, (NSAID) on antioxidant parameters and lipid peroxidation and liver, kidney histopathology in rats after oral administration for 21 days This study was conducted to evaluate the protective effects of vitamin E (100 mg/kg) on meloxicam possible adverse effects (0.27 mg/kg) for 21 consecutive days and its antioxidant scavenging capacity. Blood samples were collected at 1st ,7th, 14th, 21st days post-treatment and liver and kidney samples were collected at 7th,14th days post treatment to assess the protective effects of Vit. E. Our results indicated a significant increase in antioxidant enzymes CAT, SOD, GPX and a significant decrease in MDA. Also, a decrease in fatty changes of liver and a decrease in degenerative changes In hepatocytes caused by meloxicam administration, as demonstrated by hepatic histopathology. Also, showing a decrease in degenerative changes in renal tubular epithelium caused by meloxicam administration, as demonstrated by kidney histopathology

Key words Antioxidant activity, Histopathology, Meloxicam, Vitamin E.

(http://www.bvmj.bu.edu.eg) **BVMJ-36(1): 361-372, 2019)**

1. INTRODUCTION

Meloxicam, a non-steroidal anti-inflammatory drug (NSAID) is widely used in humans and animals. Meloxicam is a COX-2 (cyclo-oxygenase) inhibitor at its lowest therapeutic dose and is an anti-inflammatory by inhibiting prostanoid synthesis in firey cells (Fleischmann et al., 2002). Meloxicam repress COX-2 about 12 times more selectively than COX-1 (Ogino et al., 1996).

Meloxicam is NSAID that possesses potent anti-inflammatory, analgesic and antipyretic properties and is used clinically for the management of rheumatoid arthritis and osteoarthritis. In contrast to other currently used NSAIDs, meloxicam has been consistently shown to have a more inhibitory effect against COX-2. This selectivity on COX-2 has been demonstrated to be a reversible action both in vivo and in vitro (Engelhardt, 1996).

Selective inhibitors of COX-2 are drugs whose therapeutic effects are as strong as conventional NSAIDs but which lead to fewer side effects (Simon & Milis 1980). Meloxicam, a relatively selective COX-2 inhibitor, has a more potent inhibitory effect on COX-2 than on COX-1 (Schattenkirchner, 1997).

Meloxicam is very much endured by patients (Hawkey et al., 1998). It has been as viable as other nonsteroidal mitigating drugs in the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis and

was shown to have a better safety profile (Lund et al., 1998).

It has been reported that meloxicam inhibits leukocytes migration into inflamed sites (Engelhardt et al., 1996) and interferes with neutrophil function as well as with cytokine-mediated activation in leukocytes adhesion receptors (García-Vicuña et al., 1997). In addition, as inhibitors of endogenous prostaglandin synthesis, meloxicam can reduce the formation of singlet oxygen and other reactive oxidants that are produced during the transformation of PGG2 to PGH2 (Cadenas et al., 1983).

Regarding the effects of meloxicam on thiols and albumin plasma contents, it could be mediated via its inhibitory effects on free radical-mediated reactions, as observed in this study. In addition, as an anti-inflammatory drug, meloxicam could decrease the enhanced capillary permeability induced by inflammatory mediators (Kahn et al., 1976). and consequently, restore the plasma thiols and albumin levels.

Meloxicam typically rectify oxidative imbalance (Ozgocmen et al., 2005; Gunes et al., 2011; Edfawy et al., 2012) several NSAID have led to alteration in antioxidant levels, there by revealing oxidative stress as the mechanism of toxicity (Burak Cimen et al., 2003; Li et al., 2008).

The treatment of complicated infection requires weeks of anti-inflammatory course to manage associated inflammatory (Stevermer et al., 2000; Villegas et al., 2002; Lipsky et al., 2010) conditions as hyperthermia and pain.

Antioxidant defenses against the damage produced from oxidation (Oxidant by-products of normal metabolism cause extensive damage to DNA, protein, and lipid. This damage (the same as that produced by radiation) is a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts (Ames et al., 1993).

Vitamin E act as an antioxidant. It protects unsaturated fats in the body from oxidation by peroxides and free radicals (Dutta et al., 1994). Vit. E protects against lipid peroxidation so stabilize the cell membrane, maintaining its permeability and have an important role scavenging free radicals by reaction with them to form α-tocopherol radical which will be oxidized by a hydrogen donor and thus return to its reduced state (Herrera and Barbas, 2001). Vit. E is the master inhibitor of oxidation of the bad cholesterol LDL which is believed to the first step in atherosclerosis (Brigelius-Flohe, 1999).

Recorded that Vit. E reduced the lipid peroxidation (LPO) so protected the membrane against reactive oxygen species (ROS) and improved sperm motility and viability in vitro induced oxidative stress. The present study clarified the adverse effects of meloxicam and the protective effect of antioxidants (vitamin E) and its combination against its oxidative damage and nephrotoxicity and hepatotoxicity will be induced in rats by using meloxicam

2. MATERIALS AND METHODS

2.1. Drugs and chemicals

Meloxicam (MOBIC^{R} 15) 0.27 mg/kg rats according to (*Paget* and Barnes, 1964) was supplied by RAMEDA pharmaceutical industry. Meloxicam is dissolved in normal saline. Vitamin E (Vitamin E^{R}) (100 mg/ Kg) it was supplied by PHARCO pharmaceutical CO., Alex., Egypt. Vitamin E dissolved in corn oil.

2.2. Animals

The present study was carried on 80 adult male albino rats (Sprague dawely) weight 150-200g, obtained from Laboratory Animal Farm, Faculty of Veterinary Medicine, Zagazig University. All animals were kept under observation for two weeks for acclimation to the laboratory environment before starting the experiments. The animals were kept under hygienic condition in polypropylene cages and fed on food, water ad.lib.

2.3. Experimental design

The rats were allocated into four groups and each group Contain 20 rats.

- The 1st group: (control): Rats in this group were not medicated and left as control.
- The 2nd group : (meloxicam therapeutic dose): Rats in this group received repeated oral doses of meloxicam for successive 21 days, once daily.
- The 3rd group : (vitamin E): Rats in this group received repeated oral doses of vitamin E for successive 21 days as a standard antioxidant once daily.
- The 4th group : (meloxicam + Vit E): Rats in this group received repeated oral doses of meloxicam For successive 21 days plus (Vit. E) 100mg / kg b.wt once daily for 21 days.

2.4. Preparation of serum sample and tissue sample

At the end of experiment (24 hrs. after the last dose) rats were sacrificed and blood samples were collected in sterile Wasserman tube without anticoagulant from 5 rats/group at the 1st ,7th, 14th, 21st days post treatment. Blood was collected and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 rpm for 15 minutes for determination of antioxidant enzymes. The liver and the kidney of each rat were collected at 7th and 14th days post-treatment for histopathological evaluation .

2.5. Biochemical markers of antioxidant activity

Determination of catalase activity (CAT), superoxide dismutase activity (SOD), glutathione peroxidase activity (GPX) (Aebi,1984), (Nishikimi et al.,1972) and malondialdehyde conc. (MDA) (Paglia and Valentine 1967).

2.6. Hepatic and renal histopathological evaluation

Kidney and liver tissues were fixed in 10% neutral buffered formalin solutions for 24 hours. Then tissue processing and paraffin blocks preparation were done (Suvarna et al., 2013).

2.7. Statistical analysis

Statistical analyses were carried out by the one-way analysis of variance (ANOVA + Duncan test) (Tamhane and Dunlop 2000).

3. RESULTS

3.1. Effect of Vit. E on Biochemical markers

Effects of combination between Meloxicam (0.27 mg/kg, P.O. once daily), Vit. E (100 mg/kg, P.O. once daily) and their combination for 21 consecutive days on biochemical markers of antioxidant enzymes.

Effect of combination between meloxicam and Vit. E on CAT in the 1st day there was an increase in catalase activity (200.56b±5.66) compared with (187.91c±1.49) for the meloxicam group.

In the 7th day a significant increase in catalase enzyme was observed (229.50 ±1.01) compared with (214.58 ± 1.89) for meloxicam group. While, in the 14th day a significant increase in catalase activity (241.19 ± 1.20) compared with (227.55 ± 3.96) for meloxicam group. On the other hand, in the 21st day resulted in an increase in catalase activity (251.10 ± 0.81)compared with (241.96 ± 4.22) for meloxicam group. as shown in table 1 .

Effect of combination between meloxicam and vit. E on SOD in the 1st day there was an increase in SOD activity (15.41 ± 1.00) compared with (9.82 ± 0.48) for the meloxicam group

In the 7th day a significant increase in SOD enzyme was observed (20.60 ± 1.38) compared with (16.07 ± 1.51) for meloxicam group. While, in the 14th day a significant increase in SOD activity (21.86 ± 0.88) compared with (19.83 ± 0.44) for meloxicam group. On the other hand, in the 21st day resulted in an increase in SOD activity (23.19 ± 1.07) compared with (21.45 ± 0.27) for meloxicam group .as shown in table 1

Effect of combination between meloxicam and vit. E on GPX in the 1st day there was an increase in GPX activity (91.16 b ± 2.02) compared with (74.79 ± 2.42) for the meloxicam group .

In the 7th day a significant increase in GPX enzyme was observed (101.85 ± 5.94)

compared with (89.00 ± 2.57) for meloxicam group. While, in the 14th day a significant increase in GPX activity (105.79 ± 8.31) compared with (98.43 ± 1.50) for meloxicam group. On the other hand, in the 21st day resulted in an increase in GPX activity (115.63 ± 2.69) compared with (107.33 ± 2.86) for meloxicam group .

Effect of combination between meloxicam and vit. E on MDA in the 1st day there was a significant decrease in MDA activity (25.69 ± 2.77) compared with (39.33 ± 2.37) for the meloxicam group .

In the 7th day a significant decrease in MDA enzyme was observed (21.34 ± 2.15) compared with (28.77 ± 2.35) for meloxicam group. While, in the 14th day a significant decrease in MDA activity (14.45 ± 2.85) compared with (19.45 ± 4.24) for meloxicam group. On the other hand, in the 21st day resulted in a decrease in MDA activity (9.84 ± 0.76) compared with (11.98 ± 2.41) for meloxicam group .

Table (1) The effect of vitamin E (100 mg/kg, P.O. once daily), meloxicam (0.27 mg/kg, P.O. once daily) and their combination for 21 consecutive days on antioxidant enzyme activities of rats at 1st,7th,14th and 21st days post treatment (n=5,mean ± SE).

Time post-treatment	Groups	CAT (µ/L)	SOD (µ/ml)	GPX (µ/L)	MDA (nmol/ml)
1st Day	Control	242.86 ± 1.30	24.63 ^{a} ± 2.43	114.46 ^{a} ± 2.41	6.41 ^{c} ± 0.22
	Vitamin E	242.48 ^{a} ± 0.65	24.99 ^{a} ± 1.87	115.60 ^{a} ± 1.54	6.28 ^{c} ± 0.44
	Meloxicam	187.91 ^{c} ± 1.49	9.82 ^{c} ± 0.48	74.79 ^{c} ± 2.42	39.33 ^{a} ± 2.37
	Meloxicam + Vit. E	200.56 ^{b} ± 5.66	15.41 ^{b} ± 1.00	91.16 ^{b} ± 2.02	25.69 ^{b} ± 2.77
7th Day	Control	246.25 ^{a} ± 3.16	22.93 ^{a} ± 1.11	113.15 ^{a} ± 1.51	6.15 ^{c} ± 0.10
	Vitamin E	245.97 ^{a} ± 1.54	23.81 ^{a} ± 0.78	113.37 ^{a} ± 1.88	6.15 ^{c} ± 0.12
	Meloxicam	214.58 ^{c} ± 1.89	16.07 ^{b} ± 1.51	89.00 ^{b} ± 2.57	28.77 ^{a} ± 2.35
	Meloxicam + Vit. E	229.50 ^{b} ± 1.01	20.60 ^{a} ± 1.38	101.85 ^{a} ± 5.94	21.34 ^{b} ± 2.15
14th Day	Control	249.29 ^{a} ± 3.36	24.54 ^{a} ± 1.82	116.67 ^{a} ± 1.60	6.24 ^{b} ± 0.32
	Vitamin E	249.87 ^{a} ± 3.52	24.69 ^{a} ± 0.94	115.94 ^{a} ± 1.30	6.02 ^{b} ± 0.05
	Meloxicam	227.55 ^{b} ± 3.96	19.83 ^{b} ± 0.44	98.43 ^{b} ± 1.50	19.45 ^{a} ± 4.24
	Meloxicam + Vit. E	241.19 ^{a} ± 1.20	21.86 ^{a} ± 0.88	105.79 ^{a} ± 8.31	14.45 ^{ab} ± 2.85
21st Day	Control	253.14 ^{a} ± 5.59	23.67 ^{a} ± 0.58	119.93 ^{a} ± 1.20	6.54 ^{b} ± 0.39
	Vitamin E	254.39 ^{a} ± 2.12	24.40 ^{a} ± 0.82	117.84 ^{a} ± 4.10	6.70 ^{b} ± 0.49
	Meloxicam	241.96 ^{a} ± 4.22	21.45 ^{a} ± 0.27	107.33 ^{a} ± 2.86	11.98 ^{a} ± 2.41
	Meloxicam + Vit. E	251.10 ^{a} ± 0.81	23.19 ^{a} ± 1.07	115.63 ^{a} ± 2.69	9.84 ^{ab} ± 0.76

Mean within the same column superscripts are significant different at P ≤ 0.05

3.2. Histopathological findings

3.2.1. Meloxicam 7th day post treatment

3.2.1.1. Liver:

all examined sections showed normal liver histopathology with preserved lobular pattern ,portal and vascular structures and cords. Arrangements. The Kupffer cells were mildly hypertrophied.

3.2.1.2. Kidney

The renal blood vessels and inter tubular capillaries were moderately congested with perivascular edema and a few round cells infiltration. The cortex and medullary renal tubules showed various degenerative and necrotic changes and a moderate number of them were dilated. Some glomeruli were lobulated or shrinked. (Fig.1).

3.2.2. Meloxicam 14th day post treatment

3.2.2.1. liver:

Examined sections showed mild to moderate congestion of hepatic blood vessels specially in the portal area with mild perivascular edema. The bile ducts were mildly hyperplastic and the Kupffer cells were

mildly hypertrophied .Sinusoidal dilatation and mild degenerative changes in hepatocytes mainly cloudy swelling and hydropic degeneration were seen.

3.2.2.2. Kidney:

Characteristic congestion of the renal blood vessels and perivascular edema with round cells infiltration were outstanding. The renal tubular epithelium in most parts of the kidney was degenerated (cloudy swelling and hydropic degeneration), some with necrotic changes and other tubules were cystically dilated with presence of hyaline casts . The glomeruli in some parts were lobulated or shrinked with periglomerular fibrosis and mesangial hypertrophy. A diverticulum was seen evaginated in some renal papillae. (Fig.2).

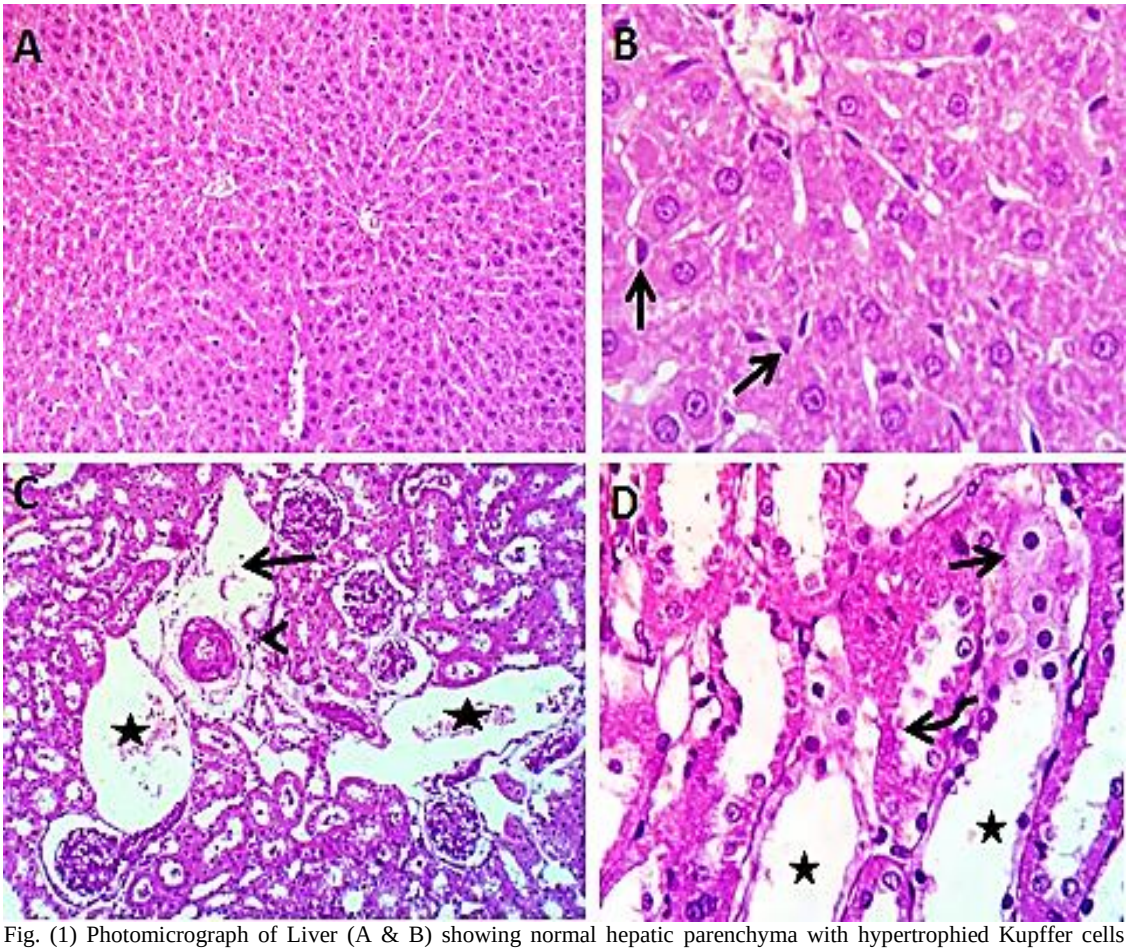

Fig. (1) Photomicrograph of Liver (A & B) showing normal hepatic parenchyma with hypertrophied Kupffer cells (arrows). Kidney (C) showing congested renal blood vessels (stars) with perivascular edema (arrow) and a few round cells infiltration (arrowhead). (D) The renal tubular epithelium showing various degenerative (arrow) and necrotic (curved arrow) changes with dilated some renal tubules (stars). H&E X 100, and 400.

3.2.3. Meloxicam + Vit. E 7th day post treatment

3.2.3.1. liver:

Some sections revealed apparently normal hepatic parenchyma with preserved lobular pattern ,portal structures and cord arrangement . Other sections showed mild to moderate congestion of hepatic blood vessels ,portal biliary hyperplastic changes which extended to the small interlobular branches. The Kupffer cells were mildly hypertrophied, most of the hepatocytes were apparently normal.

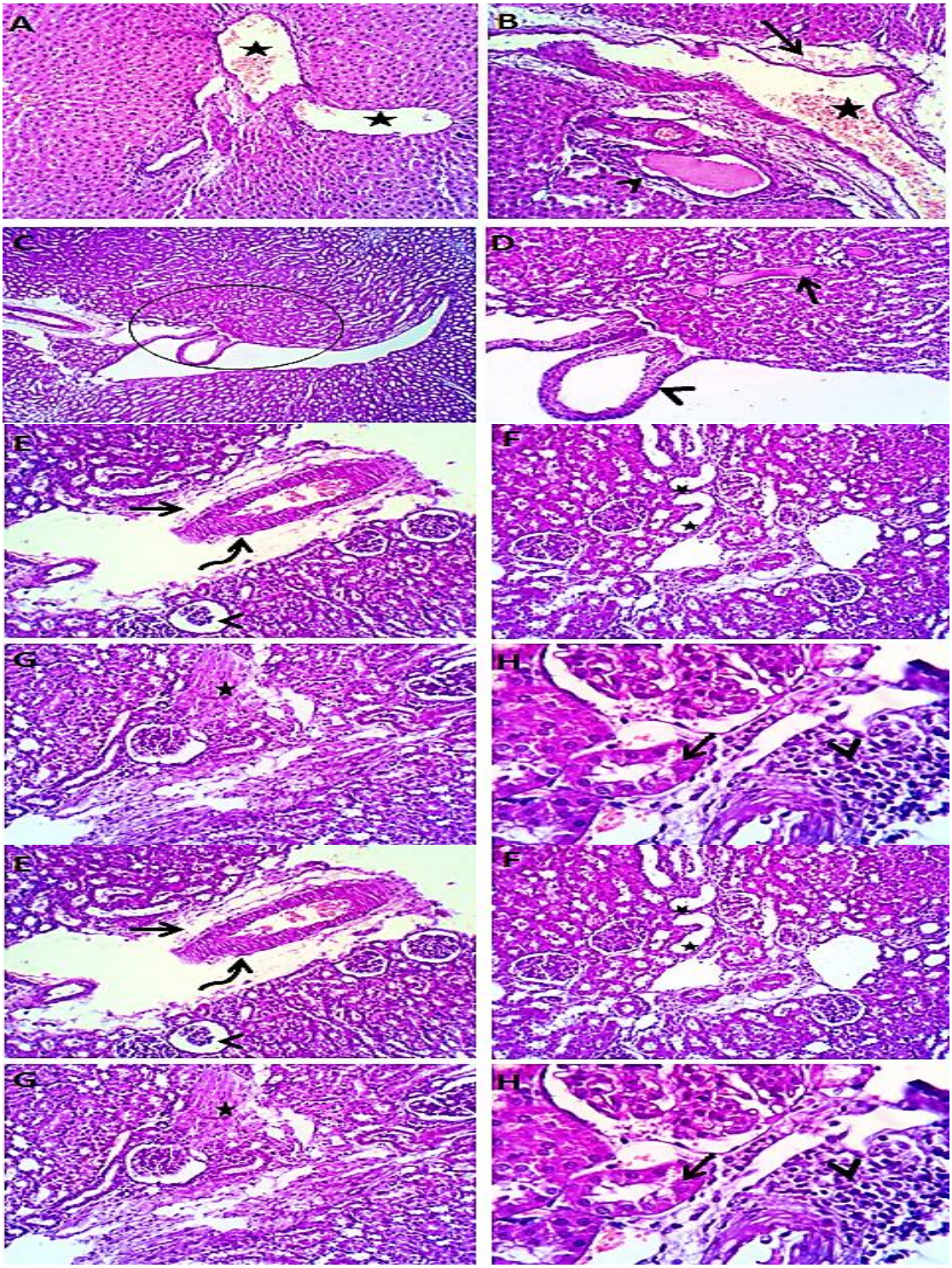

Fig. (2) Photomicrograph of liver (A&B) showing congestion of hepatic blood vessels (stars) with mild perivascular edema (arrow). the bile ducts appears mildly hyperplastic (arrowhead). Kidney (C&D) showing diverticulum evaginated in a renal papilla (arrowhead) with hyaline casts in some renal tubules (arrow). (E&F) congestion of the renal blood vessels (curved arrow), perivascular edema(arrow) with shrinked glomeruli (arrowhead) and dilated renal tubules (stars). (G&H) periglomerular fibrosis (star) with round cells infiltration (arrowhead) and degenerative changes in renal tubular epithelium (arrow). H&E X 100, and 400.

3.2.3.2. Kidney:
Mild to moderate congestion of renal blood vessels ,cloudy swelling in the tubular epithelium of proximal and distal convoluted tubules ,cystic dilatation in a mild to moderate number of collecting tubules especially in the medulla with flattened epithelial lining and intratubular hyaline casts were seen. Some glomeruli were lobulated or shrinked (Fig. 3).

3.2.4. Meloxicam + Vit. E 14th day post treatment
3.2.4.1. Liver:
Most of the examined sections revealed apparently normal hepatic parenchyma.

3.2.4.2. Kidney:
In some sections mild congestion of renal blood vessels with perivascular edema and degenerative changes mainly cloudy swelling in some cortical tubular epithelium with dilatation of some medullary collecting tubules were detected . In other sections most of renal parenchymal functional units were apparently normal. (Fig. 4).

4. DISCUSION

Meloxicam (Hayes and McLellan 1999) have been reported to deplete glutathione levels.

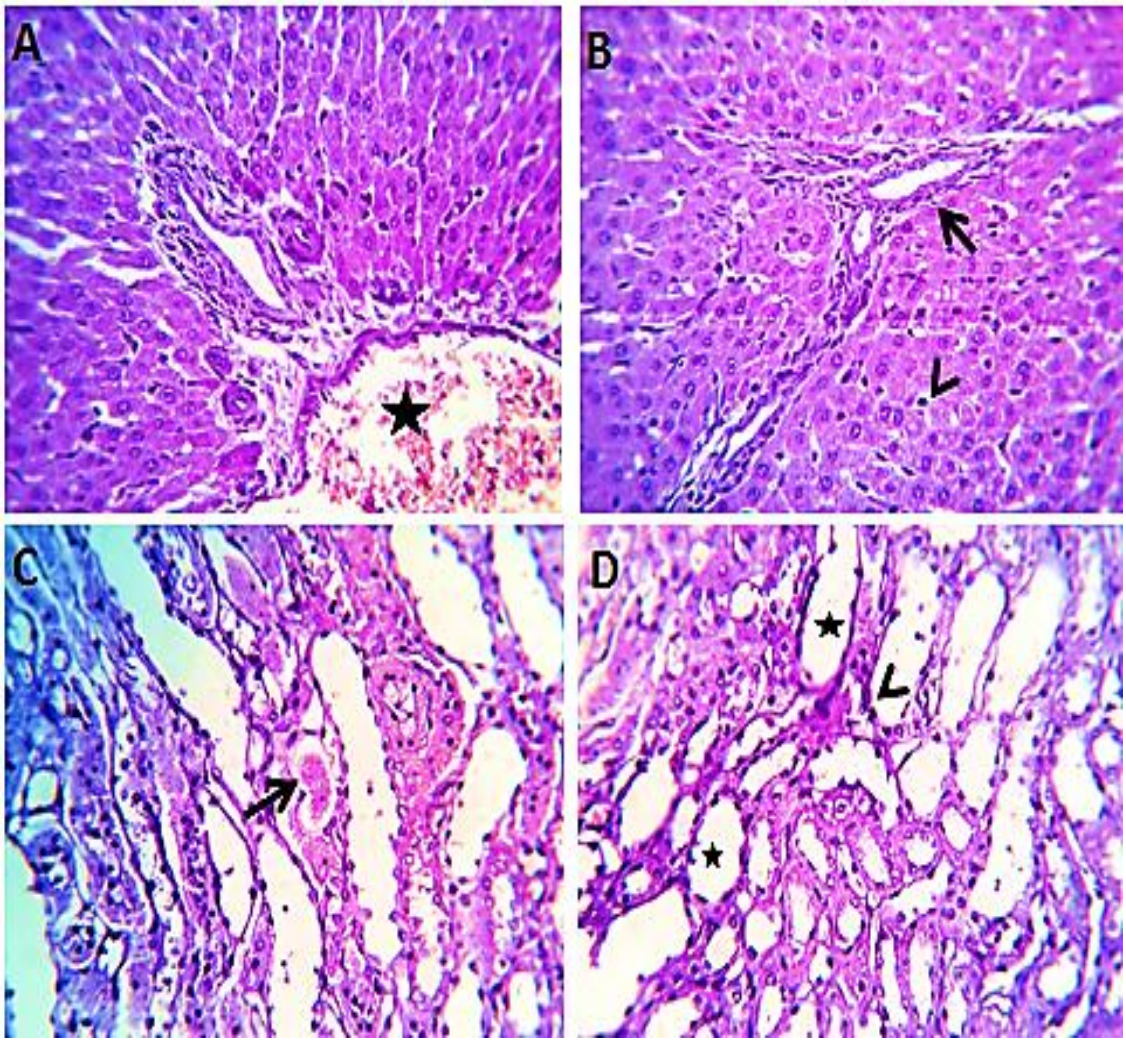

Fig. (3) Photomicrograph of liver (A&B) showing congestion of hepatic blood vessels (star), biliary hyperplastic changes (arrow) with hypertrophied Kupffer cells (arrowhead). Kidney (C&D) showing intratubular hyaline casts (arrow) and cystic dilatation of some tubules (stars) with flattened epithelial lining. H&E X 100, and 400.

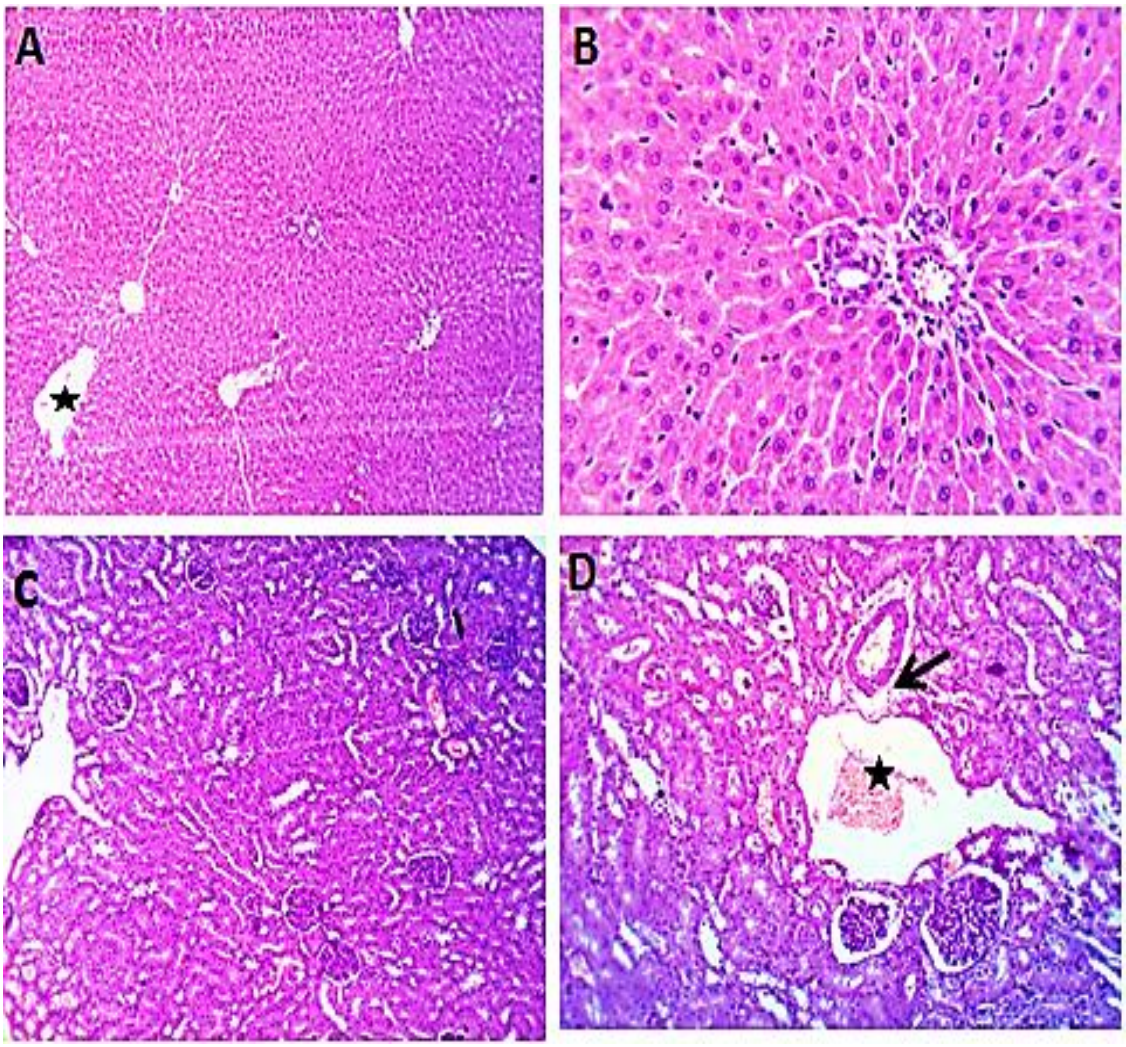

Fig. (4) Photomicrograph of liver (A &B) showing apparently normal hepatic parenchyma. Kidney (C &D) showing congested renal blood vessels (star) with mild perivascular edema (arrow).H&E X 100, and 400.

Glutathione is the primary in intracellular water-soluble antioxidant that participates in the detoxification of toxic peroxides, maintenance of protein SH groups, and conjugation of xenobiotics to enable their elimination (Bashan et al., 2009). Because glutathione depletion increases the susceptibility of cells and tissues to oxidative injury, it is considered an early hallmark in the progression of cell death in response to various apoptotic stimuli (Circu and Aw 2008; Franco et al., 2008; Dar et al., 2013).

SOD is the first line of defense against the action of O_2 and other ROS. (Rampal et al., 2008; Dubey et al., 2012) a nonsignificant change of GPx activity is reported with meloxicam (Hayes and McLellan 1999). GPx catalyzes the detoxification of a wide range of peroxides by using glutathione as reducing equivalent (Khan et al., 2013). Final detoxification of mitochondrial superoxide can occur by conversion of H2O2 to H2O by catalase at high H2O2 concentrations or by GPx at low H2O2 concentrations (Bashan et al., 2009).

An in vivo study revealed that NSAID may increase cardiovascular risk by inducing oxidative stress in the vasculature, with nonselective NSAID having a greater effect than do coxibs (Noeman et al., 2011). The extent of lipid peroxidation indirectly reflects the degree to which biomembrane lipids have

been attacked by free radicals. Antioxidant enzymes are inactivated by malondialdehyde crosslinking, which results in an increased accumulation of ROS and aggravation of macromolecular damage (Ristow and Schmeisser 2011). Oxidative stress can be regarded as the imbalance between pro- and antioxidant mechanisms. Simultaneous evaluation of the markers of oxidative stress, such as the endogenous antioxidant glutathione and various antioxidant enzymes, are considered to determine oxidative stress, (Grotto et al., 2009; Vora, 2009) due to the non-specificity of the malondialdehyde assay (Kovacic et al., 2005).

During energy transduction from the mitochondrial electron transport chain, a small number of electrons 'leak' prematurely to oxygen, forming O_2^- (Valko et al., 2004; Valko et al., 2007). This process is exacerbated with increased xenobiotics metabolism, an energy-expending process. The biologic outcome of mitochondrial ROS production and their potential involvement in physiologic (signal transduction) compared with pathologic (oxidative imbalance or stress) processes depends on the critical equilibrium between their production and detoxification (Valko et al., 2007). The response of the body to ROS is the expression of transcription factor Nrf2, which increases the expression of pro-antioxidant genes (Khan et al., 2013) increase activity of catalase in meloxicam-treated animals on day 14. However, the increased activity of these antioxidants was insufficient to completely prevent oxidative damage. Another probable reason for the significant lipid peroxidation in the meloxicam-treated groups is the depletion of an important antioxidant, glutathione. Both increases and decreases in the expression or activity of antioxidant enzymes are indicative of oxidative stress (Noeman et al., 2011) However, inhibiting the activity or sufficient synthesis of antioxidants by consistently increasing exposure to prooxidative xenobiotics overwhelms the antioxidant status, which predisposes cells to oxidative damage (Bashan et al., 2009).

5. CONCULOSIONS

It could be concluded that Vit. E has a protective effect against hepato-nephrotoxicity of meloxicam which might attributed to decrease the harmful effects of meloxicam by inhabiting free radical formation and by restoration of the antioxidant systems. The combination of Vit. E and meloxicam showed better results than meloxicam alone.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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