Effect of acetaminofen versus lornoxicam administration on oxidative stress in rat hepatic and renal tissues

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Abstract

Background: The aim of study was to assess the oxidative status in rat hepatic and renal tissues after intraperitoneal administration of acetaminophen (AP) versus lornoxicam (L).

Materials and Methods: For this purpose 18 Wistar Albino rats were randomly divided into 3 groups; each group consists of 6 rats. Group Control (Group C) remain untreated, comprises healthy rats. Group AP received AP (100 mg kg-1) and Group L received L (1.3 mg kg-1) intraperitoneally. Oxidative status was evaluated by MDA, SOD, GST and CAT in hepatic and renal tissues. Furthermore histopathalogical evaluation was performed in both tissues.

Results: The lornoxicam received rats (Group L) showed significantly increased level of MDA and GST [(p=0.015), (p=0.048) respectively],decreased level of SOD (p=0.02)in liver tissue. Renal tissue MDA, SOD and GST activity and CAT levels were similar in groups [(p=0.168), (p=0.270), (p=0.686) respectively]. Histopathalogically Group AP and Group L more damaged than in Group C. Hepatic injury was moderate level in two groups. Minimal injury was observed in group AP. Renal injury in group L more than the Group AP.

Conclusion: The results suggest that hepatotoxic effects of lornoxicam more than the AP while no remarkable difference nephrotoxicity.

Key words: Oxidative Stress, Acetaminophen, Lornoxicam, Rat, Hepatic, Renal

Introduction

Nonsteroidal anti-inflammatory drug (NSAID)'s and acetaminophen (AP) have been used as an analgesic (1,2). Acetaminophen is normally metabolized in the liver and kidney by cytochrome P450 enzymes which differ somewhat in character between the liver and kidney.

In spite of no toxicity is observed with therapeutic doses of AP both clinical and experimental studies revealed that even much lower doses can produce renal damage (2). Nephrotoxicity is a major complication of AP exerts acute and chronic nephrotoxic effects. AP toxicity is dependent on the bioactivation by CYT enzymes to N-acetyl p-benzoquinoneimine (NAPQI) depletion of GSH, adduct formation to target proteins and oxidative stress. Although the sequence of biochemical changes associated with AP toxicity is believed to be proportional to the degree of covalent binding of AP to target proteins.

The cytotoxicity of NAPQI can be dependent on its metabolism via one-electron reduction followed by reoxidation. This redox cycle reaction can reduce molecular oxygen to superoxide anion with a consequent formation of hydrogen peroxide and hydroxyl radical. In addition to reactive oxygen species, AP toxicity can increase reactive nitrogen species generation, and these oxidative species can induce lipid peroxidation, protein oxidation, and DNA fragmentation; hence are a potential cause of cell death. Renal tissue is the secondary while liver the first target organ of AP toxicity. Oxidative stress is an important component of the AP hepatotoxicity (2,3). AP produces necrosis of the centrilobular cells of the liver and often causes liver failure when taken in overdose (3). Non-steroidal anti-inflammatory drug may cause to reduce the renal blood flow, glomerular filtration rate, retention of water and sodium and may also cause hyperkalemia (4) the administration of single dose of lornoxicam on rats in our previous

study, revealed that liver and renal blood flow was decreased but this was not statistically significant. On the other hand paracetamol did not cause any alterations (5).

Recent reports described liver injures in association with cycloxygenase ranging from acute liver failure to varying degree of transient cholestatic liver injury (6).

Lornoxicam (chlorotenoxicam), a NSAID drug of the oxicam class with analgesic, anti-inflammatory and antioxidant properties, is available in oral and parenteral formulations (7). Anti-inflammatory and analgesic properties of lornoxicam have greater potency (8).

Many other drugs in addition to AP such as L are known to induce oxidative stress and the cause side effects during their metabolism in the liver. Although side effects of lornoxicam have beenwell documented in previous studies (9,10), comparison with lornoxicam and AP has not been investigated to the best of our knowledge. The purpose of this study was to determine the effects of intraperitoneally administered single dose AP versus L on oxidative stress in rat renal and hepatic tissue in terms of MDA, SOD, GST activity, CAT levels and tissue histopathalogy

Material and method

Animals and study Design

This study was conducted in the Physiology laboratory of Kırıkkale University upon the consent of the Experimental Animals Ethics Committee of Kırıkkale University. The experiments were conducted in accordance with ethical guidelines for investigations in laboratory animals.

In the study, 18 male Wistar Albino Rats 250-300 gr in weight, raised under the same enviromental conditions, were used. The rats were kept under 20-21 oC at cycles of 12-hour daylight and 12-hour darkness and had free access to food until 2 hours before the anaesthesia procedure.

Three groups of rats were formed as the study and control groups. Randomized 6 rats were grouped as control and no surgical procedure was performed (Group C, n=6). The study groups were administered lornoxicam (Xefo® Abi İbrahim İlaç San ve Tic A.Ş, İstanbul, Turkey) 1.3 mg kg-1 intraperitoneally (Group L) and another study groups were administered i.v. paracetamol (Perfalgan®Bristol-Myers Squibb Pharmaceuticals Ltd, UK) 100 mg kg-1 intraperitoneally (Group AP).

Thirty minutes after lornoxicam and iv paracetamol administration, the rats were weighed and then anaesthetized with ketamine (Ketalar®100 mg mL-1, Pfizer, İstanbul, Turkey), and the euthanasia via intraabdominal blood uptake was performed.

Histopathological evaluation

The semi-qualitative evaluation technique, which was used by Abdel-Wahhab et al (11) and Sen et al (10) was employed for the evaluation of structural changes in control and experimental groups. The slides for control and experimental groups were examined and assigned semi-qualitatively for severity of changes using scores on a scale of none (-), mild (+), moderate (++) and severe (+++) damage.

Biochemical Analysis

The liver and renal tissues were first washed with cold deionized water to discard blood contamination and then homogenized in a homogenizator. Measurements on cell contest require an initial preparation of the tissues. The preparation procedure may involve grinding of the tissue in a ground glass tissue blender using a rotor driven by a simple electric motor. The homogenizator as a tissue blender similar to the typical kitchen blender is used to emulsify and pulverize the tissue (Heidolph Instruments GMBH&CO KGDiax 900 Germany®) at 1000 U for about 3 min. After centrifugation at 10 000 g for about 60 min, the upper clear layer was taken.

MDA levels were determined using the method of Van Ye et al. (12) based on the reaction of MDA with thiobarbituric acid (TBA). In the TBA test reaction, MDA and TBA react in acid pH to form a pink pigment with an absorption maximum at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3-tetraethoxypropane). Results were expressed as nmol/mg.protein.

Part of the homogenate was extracted in ethanol/chloroform mixture (5/3 v/v) to discard the lipid fraction, which caused interferences in the activity measurements of T-SOD, CAT and GST activities. After centrifugation at 10.000 x g for 60 min, the upper clear layer was removed and used for the T-SOD, CAT, GST analyses.

In the upper clear layer, T-SOD, CAT and GST enzyme activities were measured as described Durak, et al (13), Aebi (14) and Habig et al (15), methods respectively. One unit of SOD activity was defined as the enzyme protein amount causing 50% inhibition in NBTH2 reduction rate and result were expressed in U/mg protein. The CAT activity method is based on the measurement of absorbance decrease due to H2O2 consumption at 240 nm. The GST activity method is based on the measurement of absorbance changes at 340 nm due to formation of GSH-CDNB complex. PON1 activity toward

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paraoxon was assessed by adding samples to 2 mL Tris/HCl buffer containing CaCl2, paraoxon (O,O-diethyl-O-p- nitrophenylphosphate), and NaCl. Rate of generation of p-nitrophenol was determined at 405 nm and 25 °C in a spectrophotometer.

Increases in absorbance were recorded at 30-sec intervals during 5 min after 30 sec of initial pre-incubation. Enzymatic activity was calculated from molar extinction coefficient (17,000 L/mol.cm).

Statistical analysis

All variables were expressed as median and range. Differences between groups were evaluated by Kruskal–Wallis variance analysis followed by a post-hoc Mann–Whitney U-test. P-values <0.05 were considered statistically significant. All data were entered into and processed by SPSS 20.0 for Windows statistical package

Results

In present study hepatic tissue MDA, SOD, GST enzyme activity was showed significant difference in groups [(p=0.015), (p=0.002), (p=0.048) respectively]. MDA and GST enzyme activity is higher in Group L than in Group AP [(p=0.014), (p=0.048) respectively, SOD activity was lowest in Group AP, and lower in Group L than in Group C [(p=0.011), (p=0.003), (Table 1)]. But CAT and GST enzyme level in hepatic tissue was similar to that of in Group AP and Group L (Table 1).

Renal tissue MDA, SOD, GST activity and CAT levels were similar in groups [(p=0.168), (p=0.270), (p=0.673), (p=0.686) respectively (Table 2)].

Histopathological results

Liver

In the control group, the histopathological evaluation of the liver tissue showed hepatocytes in a radial pattern extending along vena centralis and a normal architecture of sinusoids.

Hepatocyte cordons had a homogenous distribution pattern. Congestion in vena centralis, sinusoidal dilatation and minimal cellular changes were observed. In addition mild non-specific inflammatory cells and mild dilatation of sinusoids were appearent in portal area (Figure 1, Table 3).

Histological sections obtained from lornoxicam showed a significant hepatic degeneration, sinusoidal dilatation, picnotic and hyperchromatic cells, focal necrosis sites and mononuclear cell infiltration, Kuppfer cell hyperplasia, inflammation, congestion and ballooning of peripheral hepatocytes.

Necrotic and apoptotic appearance, degenerative changes, vasocongestion and bleeding areas were evident/appearent on hepatocytes around vena centralis. (Figure 2a, 2b, Table 3).

In the paracetamol group the alignment of the sinusoids were irregular. The size of the hepatocytes and hepatocyte cordons around vena centralis were similar with the control group. Vacuoles were noted around sinusoids adjacent to paranchimal cells.

Congestion in vena centralis and dilatation of sinusoids were observed as well as minimal cellular changes. In some areas mild nonspecific inflammatory cells, mild dilatation of sinusoids and mild hydrophilic degeneration of hepatocytes were identified. There were basophilic areas with an irregular structures in the cytoplasm of hepatocytes. (Figure 3, Table 3).

Kidney

The semislides obtained from the control group revealed no abnormal changes from the normal histological structures. (Figure 4).

In the lornoxicam group the most noticeable findings were the vacualizations in proximal tubular epithelium and the reduction in brush border. Tubules comprising vacuoles in basal localization and transparent tubules were also remarkable.

Browman distance and enlargement and congestion in blood vessels between tubules were observed. Abundant increase in mesangial matrix, glomerular hyperthrophy, thickening of basement membrane were not detected. Dilated tubules were mostly seen in distal tubules Bleeding areas, inflammatory cells, disseminated tubular cells were also observed. Glucogenic vacuolization (Armani-Ebstein lesions), degeneration and detachment of brush border of tubular epithelium, particles in tubular epithelial cell cytoplasm were detected in proximal tubules. (Figure 5).

In the paracetamol group loss of brush border and microvillus and focal vacualizations were identified. Infiltrations, vasocongestion, asymmetrical proximal tubules, bleeding areas, thickened basal membrane and shortening of brush border were also observed.

Dilatation of tubules was rare. Tubular degeneration was seen in some certain tubulus and was not common. The tubules which doesn't have a certain vacuolization had a normal structure. Narrowing in browmann distance, increase in mesangial matrix and glomerular hypertrophy were not observed. (Figure 6).

Table 1. Oxidative status parameters in rat hepatic tissue [Mean±SD]

	Group C (n=6)	Group AP (n=6)	Group L (n=6)	P**
MDA (nmol/mg prot)	2,28±1,00	4,35±2,33	6,05±2,33*	0,015
SOD(U/mg protein)	383,35±91,27	238,63±58,48*	258,89±42,14*	0,002
GST (Liver) (IU/mg prot)	17,75±5,15	25,73±8,69*	26,73±4,58*	0,048
CAT (IU/mg prot)	5920,67±1700,42	6463,86±1932,57	7458,86±1010,15	0,234

P**: p< 0.05 (with Kruskal Wallis test)
*p<0.05: Comparison with Group C

Table 2. Oxidative status parameters in rat kidney tissues [Mean±SD]

	Group C (n=6)	Group AP (n=6)	Group L (n=6)	P**
MDA (nmol/mg prot)	7,82±2,58	9,43±1,66	10,42±2,88	0,168
SOD(U/mg protein)	95,48±40,03	62,94±32,87	82,40±33,16	0,270
GST (IU/mg prot)	1,14±0,27	1,00±0,31	1,07±0,25	0,673

P**: Kruskal Wallis test

Table 3. Comparison of histological changes in rat liver by means of semi-qualitative evaluation

	Group C (n=6)	Group AP (n=6)	Group L (n=6)
Hepatocyte degeneration	-	+	+++
Sinusoidal dilatation	+	++	++
Pcynotic nucleus	-	+	++
Cell pre necrosis	-	+	++
MN cellular infiltration in the parenchyme	+	++	+++
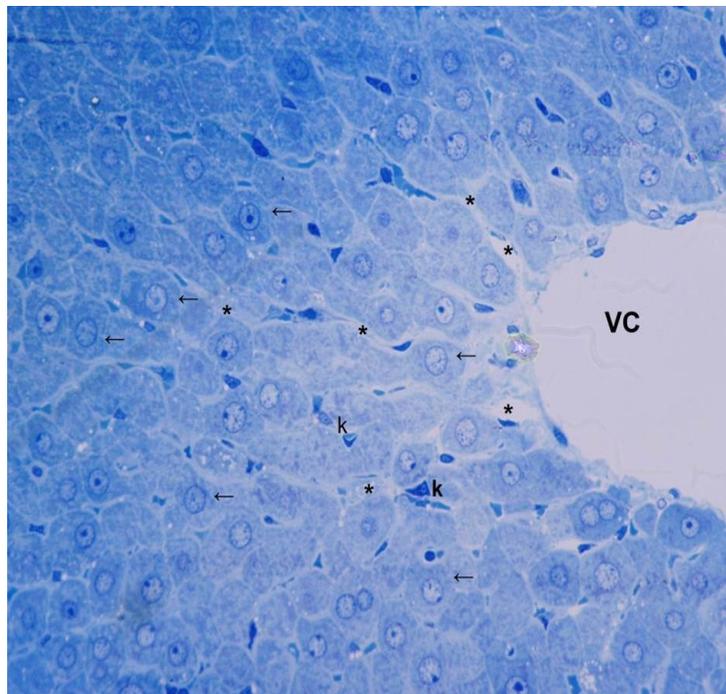
Figure 1: A slide of liver tissue obtained from the control group (vc: vena centralis, * dilated sinusoids Toluidine blue.x40)
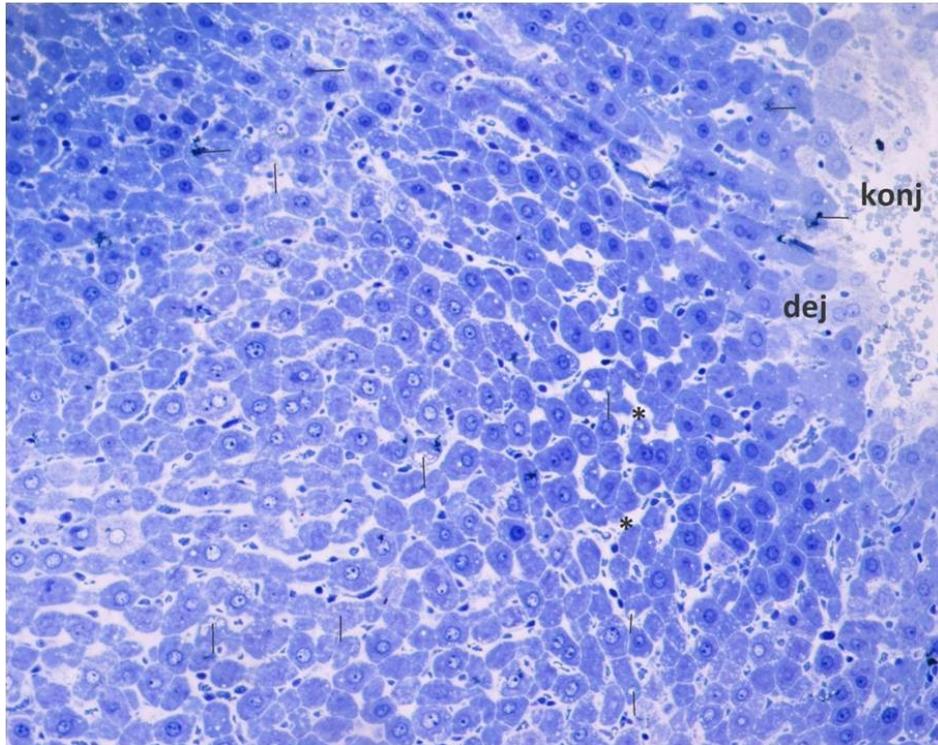
Figure 2a: Hepatoycte and vena centralis degeneration in lornoxicam group)(centrolobular damage)sinusoidal dilatation, (*) Picnotic and hyperchromatic nüclei(←) Vacuolar degeneration (↑)(Toluidine blue.x20)
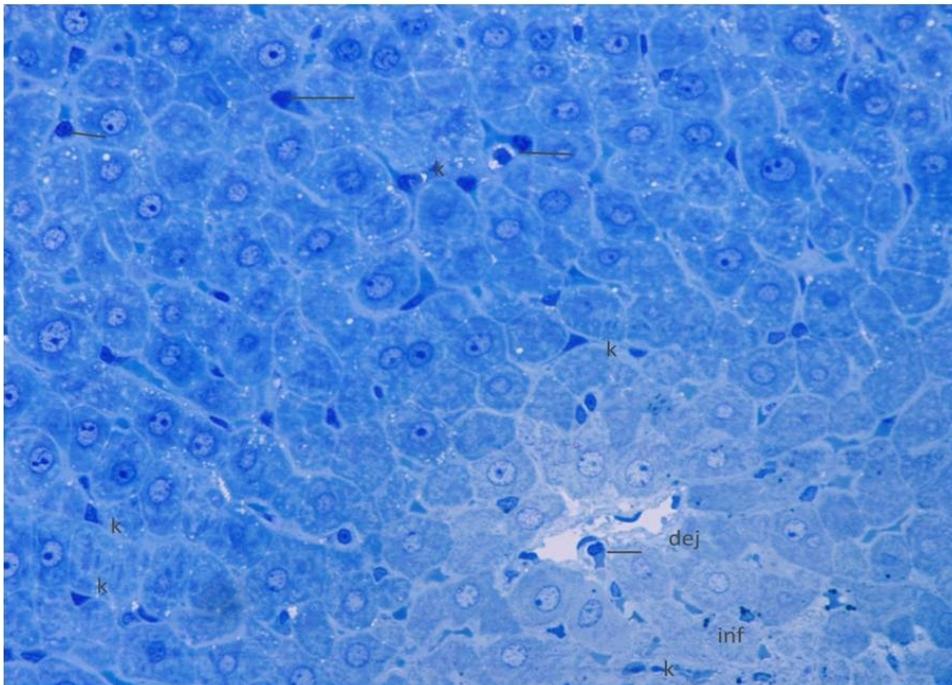

Figure 2b: Necrotic and apoptotic appearance of hepatocytes around vena centralis in the semislides obtained from the lornoxicam group (nec), Kupffer cell hyperplasia (k), Picnotic and hyperchromatic nuclei (←), sinusoidal dilatation (*)Toluidine blue.x40)
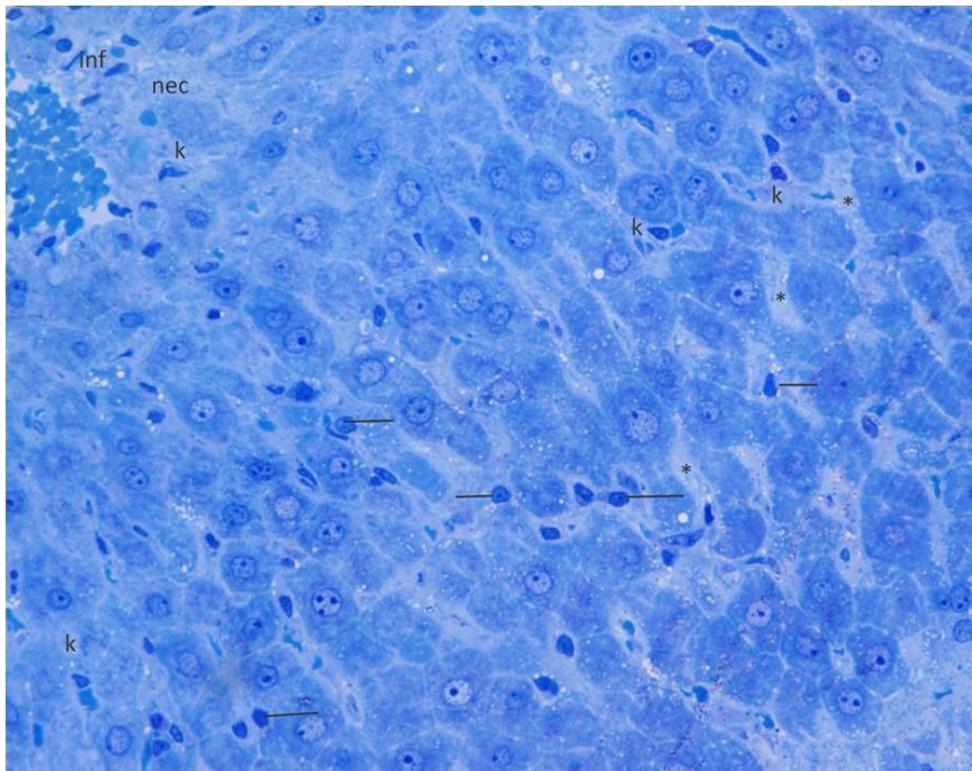
Figure 3: (Hydrophylic degeneration of hepatocytes around vena centralis in acetaminophen group, necrotic and apoptotic appearance, Kuppfer cell hyperplasia(k), picnotic and hyperchromatic nuclei(←), sinusoidal dilatation(*)Toluidine blue.x40)
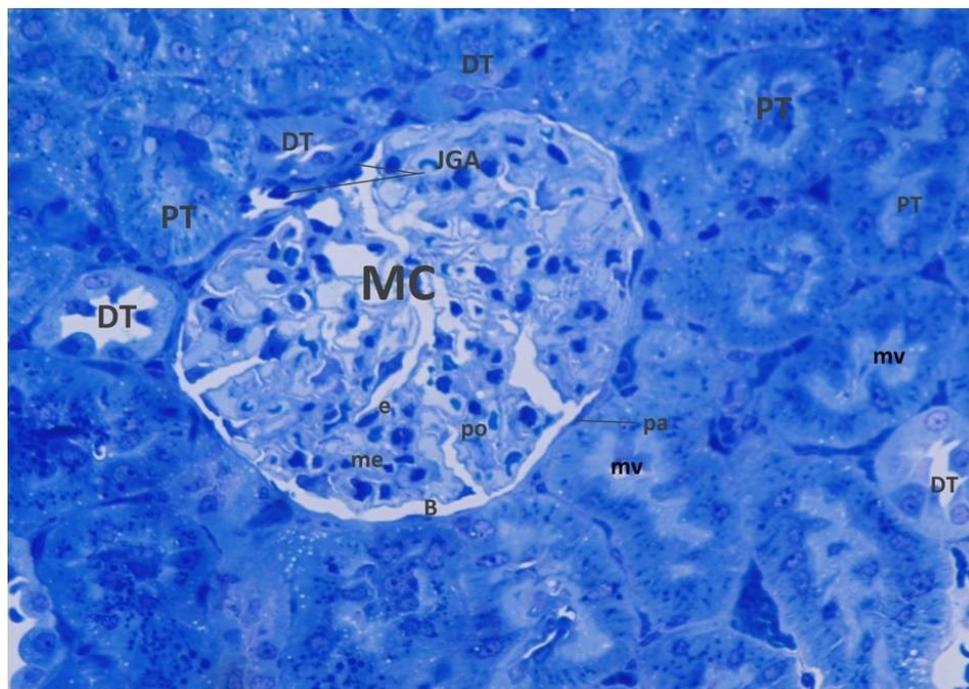

Figure 4: (Proximal and distal tubules of kidney tissue in control group MC: malpighian corpuscle, B: Bowman's gap, pa:parietal fascia, po: podocyte nucleus, e:endothelium, me: mesangial cell, mv: brush border of the proximal tubule, JGA: juxta glomerular apparatus) (Toluidine blue.x40)
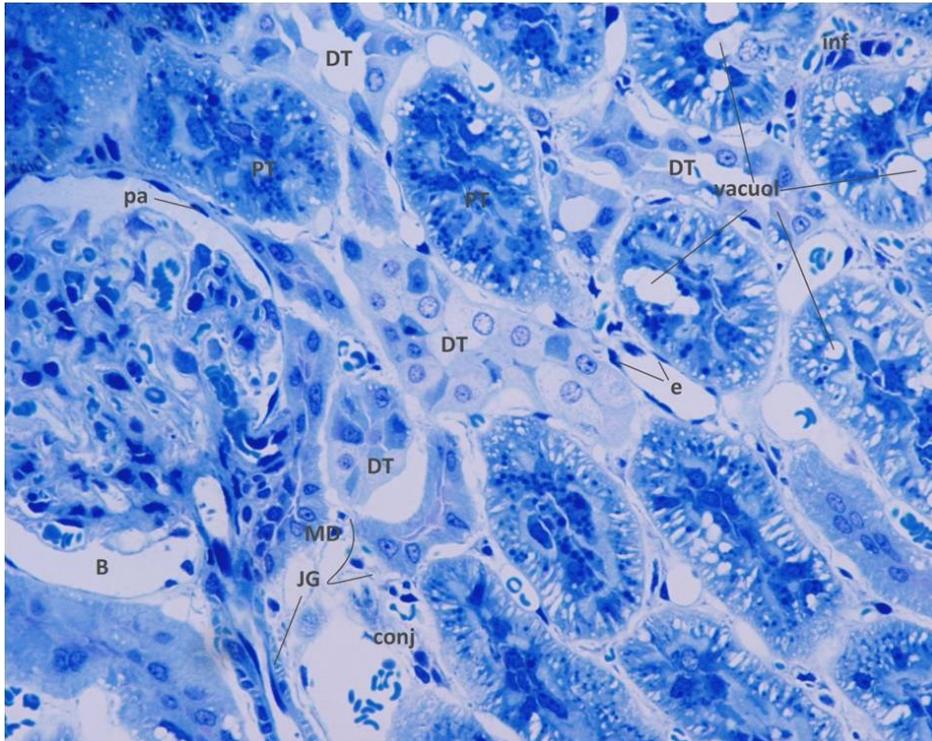
Figure 5: Malpighi corpuscule in the semislide of lornoxicam group, proximal and distal tubules. PT: Proximal tubule, DT: Distal tubule, B: Bowman's gap, pa: squamous epithelium of parietal fascia, e: endothelium, MD: macula densa and JG: juxtaglomerular cells, conj: congestion, inf: inflammation, vacuole: diffuse proximal tubule vacuolization (increase in the number and size of vacuoles) (Toluidine blue.x40)
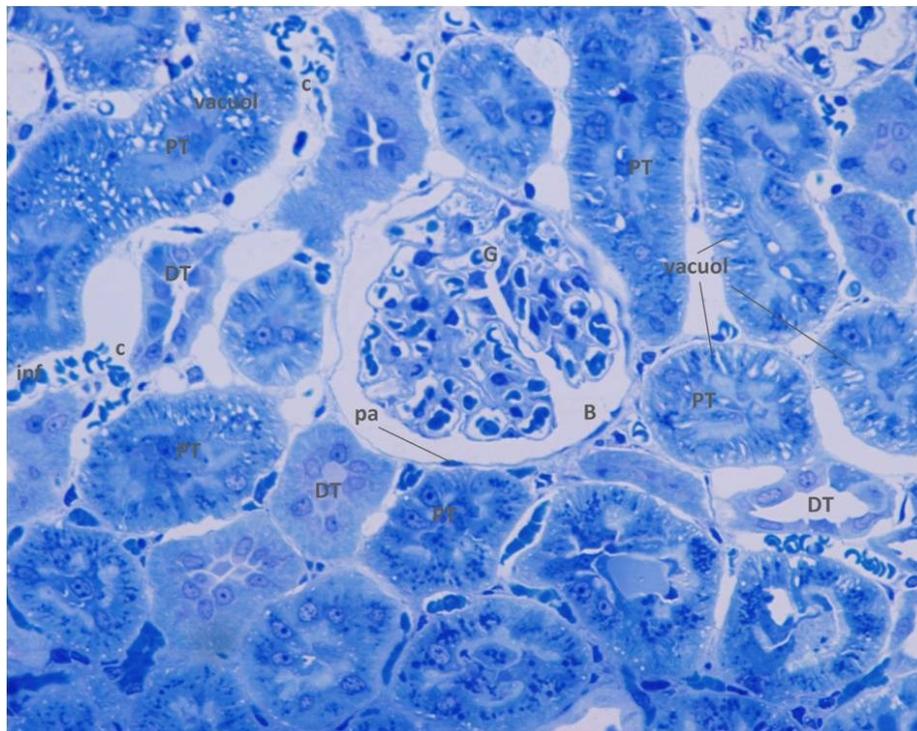

Figure 6: Renal cortex including malpighi corpuscule, proximal and distal tubules are shown in the semislide obtained from acetaminophen group. G: glomerulus, PT: proximal tubule, DT: distal tubule, B: Bowman's gap, pa: squamous epithelium of parietal fascia, c: congestion, inf: inflammation, vacuole: vacuolization of focal proximal tubule) (Toluidine blue.x40)

Discussion

Acetaminophen-Liver

Acetaminophen is the most commonly used medication worldwide because of its efficacy and safety profile. There have been several reports in the literature that suggest that acetaminophen leads to liver damage and failure (16-18). It is important to note that the doses leading to liver failure are within therapeutic range (19).

Acetaminophen produces hepatic necrosis due to the chemical reactions and interactions among hepatocytes. Acetaminophen is regarded as hepatotoxin (20). The toxicity occurs by a complex sequence of events. These include prothein, oxidative stress, calcium imbalance, changes in transcription ways, signals leading to inflammation and apoptosis. (21)

The studies on lipid peroxidation, antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) have been found to be of great importance in the assessment of liver damage (22).

Oxidative stress is reported to constitute a major mechanism in the pathogenesis of PCM-induced liver andrenal damage in experimental animals (23).

Oxidative damage has been reported as a mechanism contributing to acetaminophen toxicity (24). Koçak et al (24) reported that there was no statistically significant difference in terms of lipid peroxidation levels under low doses of acetaminophen(5, 10 ve 20 mg/kg) in comparison with control group. A slight increase was found in MDA levels in high dose of acetaminophen administration (100, 200 ve 500 mg/kg) in comparison with control and low dose groups. However this finding was reported not to be statistically significant. SOD values were also found to be similar.

Koçak et al. (24) concluded that intraperitoneal administration of acetaminophen (5, 10 ve 20 mg/kg) had no acute toxicity on liver.

Lores et al (25) found no significant change in SOD efficiacy following 375 mg/kg acetaminophen administration, while a 40-53% decrease ws reported in GSH-Px efficacy.

In an experimental study by Ranivce et al a significant increase was reported in GPx levels following paracetamol use (26).

Lipid peroxidation in acute acetaminophen toxicity is controversial (27). In the present study a mild increase in MDA levels were observed, whereas there was no significant difference among groups. Our results correlate with the findings of Knight et al (28), who reported no significant change following 300 mg/kg paracetamol use. Paracetamol-treated animals showed alterations in the antioxidant status of the tissues, which is manifested as an abnormal histopathology like cloudy swelling, centrilobular fatty changes, steatosis, fatty vacuolization and individual hepatocytic necrosis of hepatic cells (26).

Koçak et al. (24) demonstrated mononuclear cell infiltration around portal vein, picnotic and hyperchromatic cells and granular and vacuolar degeneration in hepatocytes following administration of 100 mg/kg acetaminophen use.

In the present study vacuolar structures were in the parenchymal cells adjacent to the sinusoids. Congestion of vena centralis, sinusoidal dilatation and minimal cellular changes were also evident in some areas. Mild non-specific inflammatory cells on portal areas, mild dilatation of sinusoids and mild hydrophilic degeneration of hepatocytes were observed.

Acetaminophen-Kidney

Renal failure secondary to analgesic and antipyretic drug use has been frequently reported in the literature. However renal failure following paracetamol and flurbiprophen administration in therapeutic doses has rarely been reported (17,18).

Gökçeoğlu et al (19) reported four cases of acute tubulointerstitial nephritis. The findings of the biopsy specimen obtained from the first case revealed moderate interstitial inflammation, lenfocyte infiltration, moderate interstiyel fibrosis, tubular necrosis, mild tubular atrophy. Mild interstitial inflammation, lenfocyte and eosinofil infiltration, mild interstitial fibrozis, tubular atrophy were reported for the second biopsy specimen of the second patient. Immunofluorescent evaluation was reported to be negative for both cases.

In these two cases the paracetamol dosage was in therapeutic limits and the usage period was short. However the medical history of the patients included antibiotic usage combined with paracetamol use (19).

In the present study the slides of paracetamol group revealed infiltration centers, vasocongestion, asymmetrical proximal tubules, bleeding areas, thickened basement membrane and shortening of brush border. Tubular dilatation was rare. New tubulus formations were present. Tubular degeneration was observed at some tubules and was not common. The tubules presenting cloudy vacuolization were in normal architecture. Narrowing of Browman distance, increase in mesangial matrix and glomerular hyperthrophy were not observed.

Linares et al. (29) reported that, during kidney injury, superoxide radicals are generated at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide

radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism.

Availability of paracetamol as an over counter medication alone and in combination with other prescription creates a situation that may lead to exposure to excessive quantities of the drug (30). Insensitive individuals, such as persons with renal insufficiency, therapeutic doses of paracetamol have also been implicated in kidney damage (31).

In the present study Significant increases were detected in SOD and CAT levels in rat kidney tissues. However there was no significant difference among groups.

Lornoxicam-Liver

Kathleen and Michael (32) reported adverse effects with NASIDs used in dog and rats which include gastrointestinal bleeding, ulceration and platelet dysfunction nephrotoxicity, hepatotoxicity. Which results in fulminant hepatic failure and acute tubular necrosis (10).

As a possible mechanism, production of cytochrome p450-mediated metabolic activation, un coupling of oxidative phosphorylation, mitochondrial permeability transition and generation of reactive oxygen species have been suggested (33).

Sen et al. (10) who also reported that CAT and GSH levels increased in LOR-treated group compared to those in control groups.

Hummdi et al. (34) reported that hepatocytes degradation and necrosis surrounding the portal area as well as lost their nuclei with cirrhosis of the space around the portal vein and increase the thickness of theartery and the expansion of the of lymph duct also observed.

LeBail et al. (35) investigations consistent with the current study, they attribute hypertrophy of Kupffer's cells to the defense activity of these cells in the phagocytosis of red blood cells infected and cellular debris. The hypertrophy of endothelial lining cells due to their important role in inflammatory reactions against injuries and damages tissue.

In the current study histological sections obtained from lornoxicam showed a significant hepatic degeneration, sinusoidal dilatation, picnotic and hyperchromatic cells, focal necrosis sites and mononuclear cell infiltration, Kuppfer cell hyperplasia, inflammation, congestion and ballooning of peripheral hepatocytes. Necrotic and apoptotic appearance, degenerative changes, vasocongestion and bleeding areas were appearent on hepatocytes around vena centralis.

Lornoxicam-Kidney

Rabab et al. (36) reported that the kidney showed congestion in tauft of glomuruli associated with the degeneration in the lining epithelium of the renal tubules. Focal inflammatory cell infiltration between the tubules with therapeutic dose.

Radhofer–Welte and Rabasseda (37) studied the clinico pathological changes of the kidney by effect oflornoxicam as renal papillary necrosis but the kidney associated changes were notcompletely reversible during recovery period. Aydin et al. (38) determined that th elesion of the kidney varied from degeneration and epithelial cell necrosis in epithelial linning of some tubules with mononuclear cell infiltration in the interstatium, eosinophilic secretion in the tubules lumen.

In the current study the lornoxicam group showed vacuolisations and decrease in brush border of proximal tubulus epithelium. Tubules comprising vacuolar in multiple sizes and transparent tubules were remarkable. Enlargement of browman distance and blood vessels and congestion of blood vessels were significant. Tubular dilatation was generally observed in distal tubules. Bleeding areas, inflammatory cells, and diffuse tubule cells were also observed

Conclusion

These results suggest that lornoxicam may cause unfavorable pathological changes in the liver and kidney more than acetaminophen, and that further studies are needed to identify the nephrotoxic and hepatotoxic effect in patient with renal or hepatic deficiency.

Lornoxicam and acetaminophen were cause to mild to moderate reversible injury in rat hepatic or renal tissue. Acetaminophen or lornoxicam induced oxidative stress in rat hepatic tissue more than in renal tissue. Further studies are required to understand toxic effect of acetaminophen or lornoxicamin renal or hepatic deficiency patients

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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