Is Lysosomal Enzymes Changes Important In the Pathogenesis of Liver And Kidney Injury Induced By Short and Long Term Administration of Some NSAID' Drugs in Rats?

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Abstract: Moderate lysosomal membrane permeabilization is an important inducer of apoptosis. The objective of this study was to investigate the harmful hepatotoxicity and nephrotoxicity effects induced by short and long term administration of paracetamol, nimesulide, and lornoxicam drugs in rats. Results revealed that liver and kidney lysosomal enzymes activities (ACP, β-NAG and β-GAL) were significantly increased by paracetamol followed by nimesulide in acute study as compared with chronic study, while no changes were observed in lysosomal enzymes of lornoxicam group in both studies. Serum liver enzymes activities (AST, ALT and γ-GT) and (urea and creatinine) levels were significantly increased by paracetamol followed by nimesulide and lornoxicam in chronic study as compared with acute study. Liver and kidney GSH levels and antioxidant enzymes activities were significantly decreased by paracetamol followed by nimesulide in acute study as compared with chronic study, while no changes were observed by lornoxicam in both organs either in acute or chronic studies. Liver and kidney MDA levels were significantly increased by paracetamol followed by nimesulide in acute study as compared to chronic study. These results demonstrated that liver and kidney functions were affected by oxidative stress greatly by paracetamol than nimesulide or lornoxicam in both studies.

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1. Introduction

Hepatotoxicity is the potential complication of most drug therapies prevalent in patients treated with nonsteroidal anti- inflammatory drugs (NSAIDs) (Bjornsson, 2010). NSAIDs are the most frequently prescribed therapeutic agents. Due to its over use, pronounced side effects occur, which range from mild, transient elevations in serum transaminases to pronounced hepatocellular and/or cholestatic injury sometimes leading to fatal fulminant hepatitis. Cases of reported hepatotoxicity are one of the important reasons resulting in withdrawal of some of the popular NSAIDs (Han *et al.,* 2010).

Paracetamol is a widely used analgesic and antipyretic drug and is safe at therapeutic doses but accidental or intentional overdose causes acute liver and kidney failure (Boutis and Shannon. 2001and Larson *et al.,* 2005). Because of its clinical importance Paracetamol -induced acute toxicity has become an indispensable model for studying drug-induced liver and kidney injury. At therapeutic doses, PAR is metabolized via glucuronidation and sulfuration reactions occurring primarily in the liver, and results in water-soluble metabolites that are excreted via the kidney.

Nimesulide (N-(4-nitro-2-phenoxyphenyl) methanesulfonamide), an NSAID with anti-inflammatory, anti-pyretic and analgesic properties, is one of the most prescribed drug with largest market as compared to other NSAIDs (Kalra *et al.,* 2009). Reactive oxygen species (ROS) and resultant mitochondrial dysfunction has been implicated as a general mechanism in the toxicity of many NSAIDs (Han *et al.,* 2010). Several groups have suggested that intracellular ROS generation may also constitute a conserved apoptotic event and cite ROS production as a critical determinant of toxicity associated with exposure to such drugs (Zhang *et al.,* 2010). As mitochondria are the major site of ROS production, hence they may create adequate oxidative stress which may lead to organelle dysfunction, ultimately leading to cell death. Mitochondria have also been found to be frequently involved in the toxicity of many drugs and other xenobiotics and have been the subject of excellent reviews (Han *et al.,* 2010).

Lornoxicam, a new oxicam derivative, is a strong anti-inflammatory agent. Studies have shown that lornoxicam inhibits polymorphonuclear (PMN) leukocyte migration, superoxide release from human PMN leukocytes and nitric oxide release from

macrophages (Pruss *et al.,* 1990; Berg *et al.,* 1999 and Radhofer-Welte and Rabasseda, 2000).

In chronic health problems where use of NSAIDs becomes imperative, it is logical to search for alternative/complimentary medicines which can reduce its toxicity. In cases of liver injury, antioxidants hold promise.

The aim of the present study was undertaken to investigate the comparative biochemical changes in liver and kidney of rats induced by NSAIDs *in vivo*, and also to identify the drugs most commonly responsible for lysosomal enzymes dysfunctions *in vitro*.

2. Materials and Methods

Animals

The animal studies were carried out in compliance with policies outlined in the 'Guide for the Care and Use of Laboratory Animals', published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). Experiments were carried out on adult male Albino rats aged 10-12 weeks and weighing 150-200 g obtained from the animal house of the National Research Centre (Giza, Egypt). Rats were housed in groups of five per cage under controlled environmental conditions of temperature and humidity and exposed to a 12-h light/dark cycle. Unless otherwise indicated, animals were fed on normal laboratory chow and given tap water *ad libitum* throughout the experimental work.

Experimental protocol

Animals were divided into two main groups acute (4 days) and chronic (4 weeks), then, each main group was further subdivided into 4 subgroups as follows: I) Normal control (C) group: received 1% tween 80 in distilled water as a vehicle. II) Paracetamol-treated group (PAR): received paracetamol in a dose of 500mg/kg b.wt./day (Garba *et al.,* 2009). III) Nimesulide- treated group (NIM): received nimesulide in a dose of 18mg/kg b.wt./day (Singh *et al.,* 2003). IV) Lornoxicam- treated group (LOR): received lornoxicam in a dose of 1.4mg/kg/day (Ayan *et al.,* 2008). All groups were administered the different drugs for four days (acute study) and four weeks for the (chronic) one.

Drugs and Chemicals

Drugs: Paracetamol (N-acetyl p-amino phenol) obtained from Amiriya pharmaceutical industries; nimesulide (4-Nitro-2-phenoxymethane sulphonanilide) obtained from Sigma pharmaceutical industries; lornoxicam (Chlorotenoxicam) obtained from Delta pharmaceutical industries, Egypt. All chemicals for which source is not noted were of analytical grade from Sigma, Fluka, BDH, Park, and Riedel-de Haën.

Assays

At the end of the experimental period, the animals were fasted overnight and were then sacrificed by cervical decapitation (between 9:00 and 11:00 am), 24 h after the last dose application. Blood samples were obtained from the Retro-orbital venous plexus (by means of fine capillary glass tube), in clean and dry heparinized centrifuge tubes (Becton Dickinson Co., Rutherford, NJ) and centrifuged (800 x g, 15 min, 4 ^{o}C). The separated plasma was used for the colorimetric assay of Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Reitman and Frankel, 1957), while plasma γ-glutamyl aminotransferase (γ-GT) was determined by kinetic colorimetric method according to Szasz and Persijn (1974). Plasma urea and creatinine concentrations were measured according to Mackcy and Mackay (1982) and Bartles and Bohmer (1972) respectively.

Immediately after decapitation, rats were dissected for isolation of liver and both kidneys. The separated liver and kidneys were rinsed with ice-cold saline, blotted dry, weighed, and homogenized in ice cold normal saline using a Potter Elvehjem Homogenizer fitted with a Teflon plunger. Homogenates were sonicated at a low frequency (10 kHz), centrifuged at 3,000 rpm for 15 minutes at 4^{o}C to remove the nuclear debris using (Sigma Lab. Centrifuge 3K30), fractions of the resultant supernatant were used for the assays of reduced glutathione (GSH) according to the method of Ellman (1979) as modified by Ahmed *et al*. (1991), malondialdehyde (MDA) by the method of Buege and Aust (1978), the activity of catalase (CAT) was determined by the method of Takahara *et al.*(1960) and protein content (Lowry *et al.,* 1951).

Cytosolic fractions of liver and kidney are prepared by centrifuging homogenates (30,000 r.p.m, 15 min, 4^{o}C), using (Sigma Lab. Centrifuge 3K30) and the cytosolic fractions were separated and used for superoxide dismutase (SOD) determination (Marklund and Marklund, 1974).

Preparation of liver lysosomal fraction:

Liver lysosomal fraction was prepared according to the method of Tanaka and Iisuka (1968). 1 gm of liver tissue was homogenized in 3 ml of 0.25 M sucrose buffer (pH 7.4). After homogenization the volume were adjusted to 6 ml with sucrose buffer. The homogenates were then centrifuged (820 xg, 15 min, 4^{o}C). The supernatant was separated and the sediment washed and recentrifuged under the same condition, then the supernatant was separated and added to the first supernatant. The whole lysosomal fractions were prepared by centrifuging the combined supernatant (14,000 xg, 15 min, 4^{o}C). The sediment was washed and resuspended in 0.25 M sucrose

buffer and this step was repeated three times for isolating pure lysosomal fraction. After washing and purification, the sediment was resuspended in 0.25 M sucrose buffer to give 1 g liver weight per 1.25 ml sucrose buffer.

Preparation of kidney lysosomal fraction:

Kidney lysosomal fraction was prepared according to the method of Shibco and Tappel (1965) and Meisner (1981). After the capsule and fat were removed, the kidneys were longitudinally divided, and then the cortex separated from the remainder of the kidney. The renal (cortex 0.4) gm was then homogenized in 3 ml 0.25 M sucrose buffer and centrifuged (2000 rpm, 5 min, at 4^{o}C). The resulting supernatant fraction was centrifuged (9000 rpm, 15 min at 4^{o}C), the obtained supernatant was stored at 4^{o}C. The lysosomal pellets of every gram of kidney used were then resuspended in 2 ml of 0.25 M sucrose buffer pH 7.4 (Ngha and Ogunleye, 1983).

The activity of the three lysosomal enzymes acid phosphatase (ACP), N-acetyl-β-glucosaminidase (β-NAG) and β-galactosidase (β-GAL) has been measured in both liver and kidney according to the method described by Van Hoof and Hers (1968) as modified by Younan and Roseleff (1974).

Statistical Analysis

The results were expressed as the mean ± SE and analyzed for statistical significance by one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons, using SPSS program version 10. Unless otherwise indicated, values were considered statistically significant at $p< 0.01$ and <0.05.

3.Results

Serum markers of liver damage AST, ALT and γ-GT activities:

Table (1) shows that the activities of serum enzymes AST, ALT and γ-GT were markedly increased by the administration of PAR, NIM and LOR in rats either after acute or chronic periods when compared with their control groups. The elevation of liver biomarkers was more pronounced in chronic study than acute one. Moreover, PAR-administration induces liver biomarkers enzymes more than NIM or LOR-administration in both studies.

Serum creatinine and urea levels

Serum creatinine and urea levels were markedly increased after administration of PAR, NIM or LOR after 4 days (acute) and 4 weeks (chronic) periods when compared with their control groups. Another important observation was that the increases in these parameters were found to be higher in chronic than in acute study (Fig.1).

Hepatic GSH, MDA levels, catalase and SOD activities

Rats administered with either PAR or NIM resulted in significant reduction of the activities of CAT and SOD and the level of GSH, and significant elevation of the hepatic MDA levels compared to their control groups throughout 4 days and 4 weeks administration (PAR>NIM); and these changes were marked in acute than in chronic study. In contrast, no significant changes were observed in hepatic GSH and MDA levels, Catalase and SOD activities of LOR-administered groups either in acute or chronic periods when compared with their control groups (Fig. 2).

Hepatic lysosomal enzymes (ACP, β-NAG and β-GAL) activities

Table (2) reveals that the activities of hepatic lysosomal enzymes ACP, β-NAG and β-GAL were significantly increased in PAR-administered groups followed by NIM-administered groups when compared to either acute or chronic control groups. More pronounced changes in these parameters were obtained in the acute study when compared chronic one. However, no changes in the activities of hepatic lysosomal enzymes were observed in LOR-administered groups in both studies when compared with their control groups.

Renal GSH, MDA levels, catalase and SOD activities

As indicated in figure (3) PAR, NIM or LOR administration to rats significantly reduced renal GSH level and the activities of the antioxidant enzymes along with the increased levels of renal MDA compared to normal controls in acute and chronic studies. However, LOR-administered group showed no significant alterations in renal in the studied parameters when compared to control groups in acute or chronic studies.

Renal lysosomal enzymes (ACP, β -NAG and β -GAL) activities

Renal activities of lysosomal enzymes ACP, β-NAG and β -GAL were significantly increased after the administration of either PAR or NIM to rats when compared to their control groups throughout 4 days and 4 weeks treatment periods. A significant rise in renal activities of enzymes ACP, β-NAG and β -GAL were observed after PAR-administration in comparison with NIM-administration, in addition, PAR or NIM administration to animals was found to induce significant increase in these parameters in acute than in chronic study. However, the activities of renal lysosomal enzymes were not significantly altered after LOR-administration in both studies when compared with their control groups (Table 3).

Table (1): Effect of daily administration of paracetamol (PAR) (500mg/kg), nimesulide (NIM) (18mg/kg) and lornoxicam (LOR) (1.4mg/kg) for 4 days (acute) and 4 weeks (chronic) on AST, ALT and γ-GT activities in (U/L) in rat serum in comparison with their normal controls (NC).

	Acute				Chronic			
Groups / parameter	NC	PAR	NIM	LOR	NC	PAR	NIM	LOR
AST	38.34±1.9	64.01**± 2.7	63.89**± 2.7	57.97**± 2.4	38.28± 1.9	95.25**± 2.9	83.69**± 3.1	77.99**± 2.6
ALT	32.33±1.2	56.15**± 2.1	53.09**± 2.6	50.66**± 1.9	35.18± 1.7	79.52**± 2.9	72.04**± 2.4	68.8**± 3.7
γ-GT	24.9± 1.3	63.71**± 3.1	58.94**± 2.4	42.43**± 2.5	23.5± 1.7	82.5**± 4.2	81.03**± 3.9	70.5**± 3.6

Table (2): Effect of daily administration of paracetamol (PAR) (500mg/kg), nimesulide (NIM) (18mg/kg) and lornoxicam (LOR) (1.4mg/kg) for 4 days (acute) and 4 weeks (chronic) on ACP, β-NAG and β-GAL activities in (nmol/ml/h) in rat liver homogenates in comparison with normal controls (NC).

	Acute				Chronic			
Groups / parameter	NC	PAR	NIM	LOR	NC	PAR	NIM	LOR
ACP	1243.85±7 3.2	7274.05**± 406.7	5698.63**± 414.8	1321.2± 101.2	1275.2± 57.2	3843.57**± 164.9	3217.28**± 139.8	1296± 49.7
β-NAG	415.19± 18.8	1146.59**± 50.1	842.88**± 58.6	543.91± 23.7	452.81± 15.9	840.6**± 56.8	791.23**± 61.01	494.24± 21.5
β -GAL	238.31± 10.9	779.32**± 42. 5	766.55**± 49.2	250.94± 13. 5	283.51± 12.9	742.57**± 32.2	711.62**± 31.4	326.87± 14.6

Table (3): Effect of daily administration of paracetamol (500mg/kg), nimesulide (18mg/kg) and lornoxicam (1.4mg/kg) for 4 days (acute) and 4 weeks (chronic) on ACP, β-NAG and β-GAL activities in (nmol/ml/h) in rat kidney homogenates in comparison with their normal controls (NC).

	Acute				Chronic			
Groups / parameter	NC	PAR	NIM	LOR	NC	PAR	NIM	LOR
ACP	1309.91± 91.4	2695.53**± 199.8	2151.2**± 153.7	1522.37± 117.28	1337.14± 76.6	1741.73**± 76.5	1631.37*± 75.5	1480.00± 64.8
β-NAG	387.89± 20.2	1532.95**± 66.4	934.92**± 40.7	494.07± 33.6	379.71± 16.8	994.23**± 61.6	787.98**± 65.6	449.94± 19.9
β -GAL	317.94± 13.8	705.13**± 30.9	615.6**± 26.2	336.72± 19.9	306.11± 12.8	476.878**± 37.8	454.38**± 23.9	322.62± 13.1

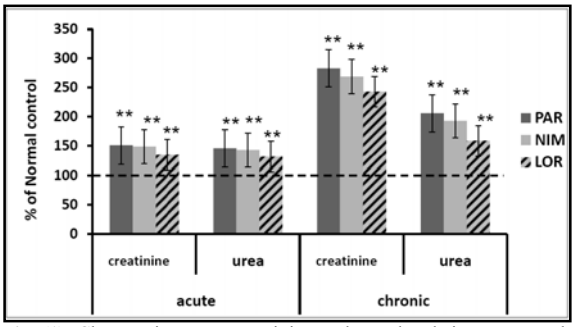

Fig. (1). Changes in serum creatinine and urea levels in rats treated with either paracetamol (PAR), nimesulide (NIM) or lornoxicam (LOR) for 4 days (acute) and 4 weeks (chronic).
The values presented as percentage of normal control ±SEM of eight rats in each group.
(*) significantly different from normal control at p<0.05.
(**) significantly different from normal control at p<0.01.

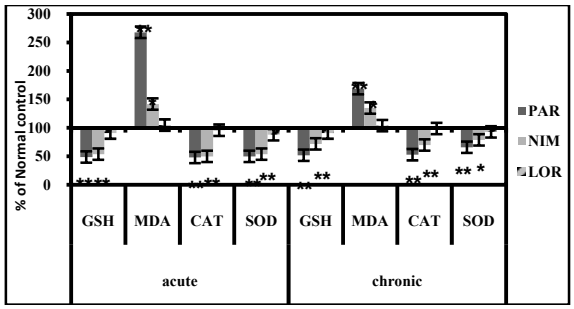

Fig. (2). Changes in hepatic content of reduced glutathione (GSH), malondialdhyde (MDA) and the activities of catalase (CAT), superoxide dismutase (SOD) in rats treated with either paracetamol (PAR), nimesulide (NIM) or lornoxicam (LOR) for 4 days (acute) and 4 weeks (chronic).
The values presented as percentage of normal control ±SEM of eight rats in each group.
(*) significantly different from normal control at p<0.05.
(**) significantly different from normal control at p<0.01.

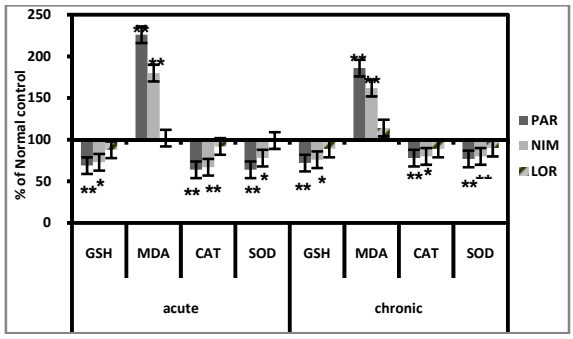

Fig. (3). Changes in renal content of reduced glutathione (GSH), malondialdhyde (MDA) and the activities of catalase (CAT), superoxide dismutase (SOD) in rats treated with either paracetamol (PAR), nimesulide (NIM) or lornoxicam (LOR) for 4 days (acute) and 4 weeks (chronic).
The values presented as percentage of normal control ±SEM of eight rats in each group.
(*) significantly different from normal control at p<0.05.
(**) significantly different from normal control at p<0.01.

4.Disscusion

Hepatotoxicity is a well known reason for drug withdrawal, or delay in the development of safe therapeutics. A number of researches elucidated that NSAIDs are highly liver specific, causing irreversible or reversible toxicity depending on the severity, in humans and animal models (Wallace et al., 2010). The present study showed that the activities of serum enzymes AST, ALT and γ-GT were markedly increased by the administration of PAR, NIM and LOR in rats either after acute or chronic periods when compared with their control groups. The elevation of liver biomarkers was more pronounced in chronic study than acute one. Moreover, PAR-administration induces liver biomarkers enzymes more than NIM or LOR-administration in both studies.

The safety of the chronic use of PAR at therapeutic dose has generated a lot of hot debate (Watkins et al., 2006). At therapeutic doses, PAR is metabolized via glucuronidation and sulfuration reactions occurring primarily in the liver, and results in water-soluble metabolites that are excreted via the kidney. As a result of the metabolic conversion of PAR by the microsomal CYP-450 enzyme system, a highly reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI) is produced (Dahlin et al., 1984). NAPQI directly reacts with glutathione (GSH) and at overdoses of PAR, the depletion of cellular GSH occurs which is coincide with our results. This allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to hepatic injury (Dahlin et al., 1984; Kanbur et al., 2009 and Wilhelm et al., 2009) and renal [31] injury, which subsequently alters the liver function tests (LFTs) (Nirmala et al., 2012; Sabir et al., 2012 and Salminen et al., 2012).

The current study showed that NIM induced elevated levels of serum activities of AST, ALT and γ-GT associated with adverse reactions in the liver, including hepatocellular necrosis, and/or intrahepatic cholestasis (Boelsterli, 2002a). Licata et al. (2010) found that 70% of the patients had liver damage attributable to NIM. Moreover, the number of patients with elevated aminotransferases during treatment with NIM is, however, increasing, and cases of fulminant and subacute hepatitis, sometimes fatal, have been documented. The formation of nitroso- or hydroxylamine reactive metabolites of NIM has been suggested to be responsible for the liver damage from the drug (Boelsterli, 2002b), like that of reactive metabolite injury from diclofenac, paracetamol and other hepatotoxins (Boelsterli, 2002a). However, there is no evidence to support this reactive metabolite hypothesis of cell injury by NIM. Ong et al. (2006) data confirm and extend earlier studies demonstrating a clear mitochondrial hazard

precipitated by NIM (Moreno-Sanchez et al., 1999; Mingatto et al., 2000 & 2002; and Moreno et al., 2007). Reduction in mitochondrial ATP and other functions has been observed with NIM following administration of high doses of the drug to rats. This phenomenon is related to uncoupling of oxidative phosphorylation like that observed with acidic NSAIDs and might account for the development of liver injury by these drugs. Reduction in ATP may initiate apoptosis by these drugs (Rainsford, 2006 and Tripathi et al., 2010 & 2011).

Our results of elevation of serum levels of aminotransferases by NIM are in accordance with Borku et al. (2008) a case report in a male kitten and many clinical studies of Betrosian et al. (2009).

The current study showed that the increase in serum transaminases levels (AST, ALT and γ-GT) with treatment by LOR may be explained due to LOR has been approved to be eliminated predominantly by hepatic biotransformation. Its major metabolite is the pharmacologically inactive 5V-hydroxylornoxicam (Bonnabry et al., 1996), which accounted for up to 95% of total intrinsic lornoxicam clearance and cytochrome P450 2C9 (CYP2C9) has been proved to be the principal CYP isozyme involved in the main metabolic pathway (Balfour et al., 1996 and Radhofer-Welte and Rabasseda, 2000). This might account for alteration of liver function tests by treatment with LOR.

In the present study, we assessed the nephrotoxic effects caused by acute and chronic administration of different NSAIDs. Our results showed that serum creatinine and urea levels were markedly increased after administration of PAR, NIM or LOR after acute and chronic periods when compared with their control groups, and the rise in these parameters were found to be higher in chronic than in acute study (Fig.2).

Plasma urea and creatinine levels were significantly increased in groups treated with PAR, demonstrating the deterioration of the renal function, in comparison with those of the control group. These findings are consistent with the results of a previous study in which PAR was administered to rats (Fouad et al., 2009 and Ghosh et al., 2010). The mechanisms involved in PAR-induced cell death in nephrotoxicity may differ from hepatotoxicity, as suggested by the fact that N-acetylcysteine can prevent in vivo PAR-induced hepatic damage (Flanagan and Meredith, 1991 and Kozer and Koren, 2001) but did not prevent renal cell death. PAR drug-induced nephrotoxicities are often associated with marked elevations in blood urea nitrogen, serum creatinine and acute tubular necrosis (Verpooten et al., 1998). These findings could be attributed to the ability of PAR overdose to induce an inflammatory reactions with increased production of α-tumor necrosis factor (TNF-α) in the renal tissue (Fouad et al., 2009 and Ghosh et al., 2010). This is in agreement with a number of previous studies which showed that other nephrotoxic drugs can induce renal inflammation with increased generation of (TNF-α) (Kuhad et al., 2007 and Zager, 2007). Moreover, PAR-induced nephrotoxicity may be explained due to this metabolic activation of PAR to the reactive metabolite, NAPQI (Hart et al., 1994).

Nimesulide treated groups showed significantly elevated serum levels of urea and creatinine which were higher in chronic than in acute study, in the same time they were lower than in PAR groups in both studies when compared with their control groups. Our results are consistent with Borku et al. (2008), who reported a case of nimesulide-induced reversible acute biliary injury and renal failure in a three month old kitten. The results revealed high serum levels of urea and creatinine. Renal adverse effects to this drug may be attributed to the inhibition of prostaglandin synthesis, an increase in renal vascular resistance with a concomitant decrease in diuresis, golmerular filtration rate (GFR), and renal blood flow acute reversible renal failure (Apostolou et al., 1997; Balasubramaniam, 2000 and Prevot et al., 2004).

The results of the current study revealed a remarkable increase in serum levels of urea and creatinine of LOR- treated groups in both studies (acute and chronic), which were found higher in chronic than in acute study, but still lower than PAR and NIM-treated groups when compared with their control groups. LOR had high therapeutic potency and less gastrointestinal side effect when compared to naproxen (Radhofer-Welte and Rabasseda, 2000). Pohlmeyer-Esch et al. (1997) investigated different doses of LOR (such as 0.06, 0.16 or 0.40 mg/kg/day) for chronic toxicity in rats. In their study, drug related and dose dependent toxicity of lornoxicam mainly included mortality, reduced body weight gain, some clinicopathological changes (such as anaemia resulting from blood loss), renal damage (renal papillary necrosis) and gastrointestinal mucosal lesions but none of these changes was present after the recovery period. Our study differs from Pohlmeyer-Esch et al. (1997) study by administration of higher doses of LOR by intraperitoneal route but not orally. We preferred the dose of 1.4 mg/kg for LOR since it was found to be fully effective to prevent hyperalgesia in rats (Bianchi and Panerai, 2002). According to Sen et al. (2006) the use of LOR alone caused deleterious effects on gastric and renal systems on the fifteenth day which were indicated by histological lesions, where as the administration of lornoxicam and nitroglycerin together prevented these side effects.

The present results indicated in figures 3&5 cleared that PAR-treatment to rats produces a significant elevation of hepatic and renal MDA levels in both studies which is more obvious in acute than chronic study. These findings are consistent with a previous study (Wu et al., 2010). Previous studies have shown that the elevation of hepatic and renal MDA levels can be attributed in part to NAPQI which is the reactive metabolite product caused by PAR-induced hepatotoxicity and nephrotoxicity, leads to GSH depletion and covalently binds to cysteine residues on proteins, which results in lipid peroxidation reaction (Dahlin et al., 1984). Moreover, the Increase in the levels of MDA in the PAR-treated group is in agreement with the findings of other researchers (Olaleye and Rocha, 2008; Sabir and Rocha, 2008; Bhadauria and Kumar, 2009; Kanbur et al., 2009; Wilhelm et al., 2009; Fouad and Jresat, 2012 and Sabir et al., 2012). Manimaran et al. (2010) stated that PAR reduced hepatic GSH by 60–90%, indicating inefficient detoxification of NAPQI and its eventual availability for interaction with the cellular macromolecules. Treatment with PAR caused a statistically significant decrease in hepatic and renal SOD activities. Reduction in the activity of SOD is likely to be a result of futile cycling of P450, caused by NAPQI which utilized reducing equivalent of NADPH with concomitant reduction of molecular superoxide anion radical ($O^{2\cdot -}$), hence there will be a reduction in superoxide dismutase activity (Bessems and Vermeulen, 2001 and Phil et al., 2012). Moreover, the present results agree with Fouad et al. (2009) who also indicate that increased production of TNF-α, the pro-inflammatory cytokine, in renal tissue that was observed in their study indicates that inflammation is also involved in the pathogenesis of PAR-induced nephrotoxicity and also agree with Cekmen et al. (2009) ,Ghosh et al. (2010) and Ahmad et al. (2012).

Catalase is a crucial enzyme in cellular antioxidative defense mechanisms and efficiently degrades endogenously produced hydrogen peroxide. Catalase activity was found to be significantly decreased after a toxic PAR dose. Our results are in accordance with Mirochnitchenko et al. (1999), and Rajesh and Parames (2006) who reported that catalase activities were significantly diminished following toxic PAR dose (Fouad and Jresat 2012 and Sabir et al., 2012). This would allow for the accumulation of reactive oxygen species and hydrogen peroxide, which can exacerbate the hepatocellular damage initiated by NAPQI. Also increased reactive oxygen species (ROS) production, impairs mitochondrial respiration, causes ATP depletion, opens the mitochondrial permeability transition pore and makes the mitochondrial inner membrane abruptly permeable to solutes up to 1500 Da (James et al., 2003 and Jaeschke and Bajt, 2006). These events lead to onset of the mitochondrial permeability transition (MPT), which is a common pathway leading to both necrotic and apoptotic cell death (Yang and Salminen, 2011).

The present results of repeated dosing of PAR-treatment to rats decreases their sensitivity to its hepatotoxic effects, which are associated with oxidative stress and glutathione depletion are in accordance with P J O'Brien (2000).

Resistance to PAR hepatotoxicity and nephrotoxicity produced by repeated exposure to PAR is partially attributable to upregulation of hepatic glucose-6-dehydrogenase (G6PD) and glutathione reductase (GR) activity as an adaptive and protective response to oxidative stress and glutathione depletion. Ghanem et al. (2009) indicates that chronic ingestion of the drug (several tablets per day, for weeks) leads to development of tolerance to the toxic effects of PAR, usually delaying the onset of liver injury. Tolerance to PAR toxicity also occurs in experimental animals such as mice and rats and the mechanism is likely multifactorial (Shayiq et al., 1999; Dalhoff et al., 2001 and Aleksunes et al., 2008). As we mentioned before that PAR is metabolized in the liver mainly by glucuronidation and sulfation, thus generating the non toxic metabolites PAR-glucuronide (PAR-glu) and PAR-sulfate (Thomas, 1993). PAR-glu excretion in bile is mediated by the canalicular multidrug resistance-associated protein 2 (Mrp2) (Xiong et al., 2000). Basolateral efflux of PAR-glu in liver may also occur and has been linked to the expression of Mrp3 (Manautou et al., 2004), an ATP-dependent transporter expressed on the basolateral domain of the hepatocyte (Crocenzi et al., 2004). Ghanem et al. (2005 & 2009) demonstrated that pretreatment with PAR led to a marked increase in the hepatic expression and activity of Mrp3 that was correlated with significant shift from canaclicular to basolateral efflux of PAR-glu and a decrease in its enterohepatic recirculation. Thus, increased expression of the basolateral Mrp3 transporter relative to the canalicular Mrp2 transporter is associated with a shift from biliary to basolateral and thereby urinary excretion of PAR-glu. This decrease in enterohepatic recirculation is postulated to contribute to PAR-decreased hepatotoxicity and nephrotoxicity by minimizing exposure to the liver and kidney and PAR activation to the toxic reactive metabolite.

These alterations may contribute to explain the resistance to liver toxicity of the drug, in addition to other well characterized mechanisms (Shayiq et al., 1999; Dalhoff et al., 2001 and Aleksunes et al., 2008).

Rats treated with NIM showed a significant increase in hepatic and renal MDA levels in both studies which is obvious in acute more than chronic study. However, the hepatic and renal MDA levels still lower than PAR-treated groups. Also, our experimental result here showed significant decrease in hepatic and renal GSH level, SOD and CAT activities in acute study which increase gradually in chronic treatment but still significant lower than PAR-treated group when compared with their control groups. This finding is in agreement with Tripathi et al. (2010) (in vitro study) who stated that mitochondria are known to be a major source of intracellular ROS generation and are particularly vulnerable to oxidative stress. We suggest that oxidative damage synchronized with mitochondrial damage and subsequent apoptosis is the main cause of NIM-induced cytotoxicity in hepatic cells. Tripathi et al. (2011) indicated that NIM exposure caused significant alterations in the antioxidant level leading to oxidative stress. Roig et al. (2002) showed that NIM (5 mg/kg/ day) for 8 days reduced renal blood flow in dogs. Nimesulide injected acutely was shown to reduce renal blood flow in newborn rabbits (Prevot et al., 2004). In Al Suleimani et al. (2010) study, a combination of nimesulide and cisplatin (CP) exaggerated renal tissue damage, as demonstrated by histopathology in rats.

LOR-treated groups showed insignificant changes in hepatic MDA levels, GSH content as well as hepatic SOD and CAT activities in both studies which indicate that LOR may cause liver damage without oxidative stress. These findings are in accordance with Ayan et al. (2008) which demonstrated that LOR can inhibit oxidative tissue damage in the trachea and the lungs induced with direct intratracheal application of peroxynitrite and with Sen et al. (2006) who also reported that CAT and GSH levels increased in LOR-treated group compared to those in control groups. It was demonstrated that oxicams are more reactive against ROS than NIM and ibuprofen in rats (Van Antwerpen, 2004) and moreover LOR appears to be a significantly better antioxidant than tenoxicam. It was stated that the antioxidant properties of lornoxicam might be related to its chemical structure. Bulbuloglu et al. (2005) concluded that the use of lornoxicam was effective in decreasing the oxidative stress of tissue during peritonitis. Based on these data, it is assumed that although LOR may cause liver and kidney damage but without ROS formation. According to Baliga et al. (2010) study, it was shown that LOR in a dose of 16 mg/day had an early onset of action and a better tolerability profile as compared to diclofenac 150 mg/day in the treatment of adult Indian patients with osteoarthritis. It could be therefore a safer and alternative option in the symptomatic treatment of patients with osteoarthritis with lesser dosing frequency. They believe that this effect of LOR is not only a result of cyclo-oxygenase inhibition but also is attributable to its nitric oxide (NO) and superoxide inhibitory properties as mentioned in previous studies (Pruss et al., 1990 and Radhofer-Welte and Rabasseda, 2000).

Our results in figures 4&6 concerning lysosomal enzymes are consistent with Khandkar et al. (1996) which suggest that PAR toxicity specifically induced the synthesis of the lysosomal enzymes and moreover, Bhadauria and Kumar (2009) suggested that PAR toxicity specifically induced the synthesis of the lysosomal enzymes in both organs (liver and kidney).The present results also showed NIM-treated groups produce significant increase in hepatic and renal lysosomal enzymes activities (ACP, β-NAG and β-GAL) in both studies but in acute more than chronic, on the other hand, NIM-treated groups showed lesser significant lysosomal enzymes activities than PAR-treated groups when compared with their normal controls. As we discussed above that lysosomal enzymes may increase under pathological conditions and under the effects of some xenobiotics (e.g. drugs) which leads to labialization of the lysosomal membrane which leads to leakage of lysosomal enzymes (Khandkar et al., 1996 and Sabir and Rocha, 2008).

However, LOR-treated groups in this study showed insignificant changes in hepatic lysosomal enzymes activities (ACP, β-NAG and β-GAL) in both studies when compared with their control groups.The reason for this normalcy may be attributed to the anti-inflammatory effect of the drug, probably due to stabilization of the lysosomal membrane (Poonguzhali et al., 1998).

Poonguzhali et al. (1998) showed that tenoxicam, which is a member of oxicams family produce stabilization of lysosomal membrane which may be attributed to the anti-inflammatory effect of the drug, probably. These findings indicate that LOR may exert the same effects as tenoxicam on liver and kidney lysosomal membranes. Also as shown above in the present results, LOR-treated groups showed insignificant changes in the antioxidant system which indicate that LOR does not produce ROS that may alters the stability of lysosomal membranes as well as the cell membranes. These alterations in lysosomal enzymes may contribute to explain the hepatotoxicity and nephrotoxicity under the effects of some NSAIDS drugs which leads to labiliazation of the lysosomal membrane which leads to leakage of lysosomal enzymes (Khandkar et al., 1996 and Bhadauria and Kumar, 2009).

Lornoxicam treated groups in this study showed insignificant changes in hepatic lysosomal enzymes activities (ACP, β-NAG and β-GAL) in both studies when compared with their control groups.

The results concerning repeated exposure to PAR and NIM were consistent with those of Ghanem et al. (2005 & 2009) studies which indicate that the alterations in the antioxidant system in both studies (acute and chronic) may contribute to explain the resistance to liver and kidney toxicity of both drugs (PAR and NIM).

Excessive lysosomal enzymes synthesis may be attributing to the membrane lipids damage. In the present study, a rise was found in the liver and renal lysosomal enzymes activity which appears to be a cause of increased membrane lysis and cell damage. This effect is exaggerated with changes in the antioxidant system in liver and kidney.

Conclusions

In conclusions, the present study showed that paracetamol is the most hepatotoxic and nephrotoxic drug than nimesulide and lornoxicam. Resistance to xenobiotic hepatotoxicity and nephrotoxicity produced by repeated exposure (chronic) is partially attributable to upregulation of antioxidant defense system activity as an adaptive and protective response to oxidative stress. Also, these results indicate that lornoxicam may induce hepatotoxicity and nephrotoxicity but not associated with oxidative stress. Among the treated groups studied, only lornoxicam showed that the least changes in most of the parameters measured in the current study. These findings suggest that LOR may hold potential as promising approaches for treatment of human with the least side effects than other NSAIDs.

Abbreviations:

ACP: acid phosphatase
ANOVA: Analysis of variance
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ATP: Adenosine triphosphate
CAT: catalase
G6PD: Glucose-6-dehydrogenase
GFR: Glomelular filtration rate
GR: Glutathione reductase
GSH: Reduced Glutathione (L-γ -glutamyl-L-cysteinyl glycine)
LFTs: liver function test
LOR: lornoxicam
MDA: Malondialdehyde
MPT: Mitochondrial permeability transition
Mrp: Multidrug resistance associated protein
NAPQI: N-acetyl-p-benzoquinoneimine
NO: nitric oxide
NSAIDs: Non-steroidal anti-inflammatory drugs
PAR: paraceamol
PMN: Polymorphnuclear
ROS: reactive oxygen species
rpm: rotation per minute
SE: standard error
SOD: superoxide dismutase
TNF-α: Tumor necrosis factor-α
β-GAL: β-Galactosidase
β-NAG: N-Acetyl β-D-Glucosaminidase
γ-GT: Gamma-glutamyltransferase

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