Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model[§]

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In this study, histopathological and biochemical changes due to chronic usage of morphine or tramadol in liver and kidney were assessed in rats. Thirty male Wistar rats (180–220 g) were included and divided into three groups. Normal saline (1 ml) was given intraperitoneally as placebo in the control group (n = 10). Morphine group (n = 10) received morphine intraperitoneally at a dose of 4, 8, 10 mg/kg/day in the first, second and the third ten days of the study, respectively. Tramadol group (n = 10), received the drug intraperitoneally at doses of 20, 40 and 80 mg/kg/day in the first, second and the third ten days of the study, respectively. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinin, blood urea nitrogen (BUN) and malondialdehyde (MDA) levels were measured in the serum. Liver and kidney specimens were evaluated by light microscopy. Serum ALT, AST, LDH, BUN and creatinin levels were significantly higher in morphine group compared to the control group. Serum LDH, BUN and creatinin levels were significantly increased in the morphine group compared to the tramadol group. The mean MDA level was significantly higher in morphine group compared to the tramadol and control groups ($P < 0{\cdot}05$). Light microscopy revealed severe centrolobular congestion and focal necrosis in the liver of morphine and tramadol groups, but perivenular necrosis was present only in the morphine group. The main histopathologic finding was vacuolization in tubular cells in morphine and tramadol groups. Our findings pointed out the risk of increased lipid peroxidation, hepatic and renal damage due to long term use of opioids, especially morphine. Although opioids are reported to be effective in pain management, their toxic effects should be kept in mind during chronic usage.

[Atici S, Cinel I, Cinel L, Doruk N, Eskandari G and Oral U 2005 Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model; *J. Biosci*. **30** 245–252]

1. Introduction

The central role of liver and kidney in drug metabolism predisposes them to toxic injury. Every drug has been associated with hepatotoxicity almost certainly due to the pivotal role of the liver in drug metabolism. Hepatic metabolism is, first and foremost, a mechanism that converts drugs and other compounds into products that are more easily excreted and that usually have a lower pharmacologic activity than the portent compound (Poppers 1980; Tolman 1998). A metabolite may have higher activity and/or greater toxicity than the original drug. Metabolites of the drugs that are excreted from kidneys may also cause cellular damage leading to kidney dysfunction (Singhal *et al* 1998).

Opioids are the most potent and effective analgesics available and have become accepted as appropriate treatment for acute, cancer and non-cancer chronic pain (Collet 2001; Quang-Cantagrel *et al* 2000; Bannwarth 1999). Morphine, which is commonly used for the treatment of severe pain, is metabolized essentially in the liver, gastrointestinal tract and kidneys (Pacifici *et al* 1986; Stain-Texier *et al* 1998).

Tramadol, a widely used opioid in recent years, is an effective analgesic agent for the treatment of moderately

Keywords. Kidney toxicity; liver toxicity; morphine; tramadol

[§]Presented at the 10th XX Annual ESRA Congress, 6–9 April 2002, Warsaw, Poland.

Abbreviations used: ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; LAAM, levo-alpha-acetylmethadol; LDH, lactate dehydrogenase; MDA, malondialdehyde.

severe acute or chronic pain (Lee *et al* 1993). It is converted in the liver to O-desmethyl-tramadol, which itself is an active substance and 2 to 4 times more potent than tramadol (Wu *et al* 2001; Tao *et al* 2002). Further, biotransformation results in inactive metabolites, which are excreted by kidneys (Lee *et al* 1993; Matthiessen *et al* 1998).

Long-term administration of an opioid drug for chronic non-cancer pain continues to be controversial (McCarberg and Barkin 2001; Brena and Sander 1991). In this study, histopathological and biochemical changes in liver and kidneys due to chronic usage of morphine or tramadol were assessed in rats.

2. Materials and methods

The experiments described in this manuscript were performed in adherence to the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals, and approval of the ethic committee of Mersin University School of Medicine was obtained before study.

2.1 *Experimental protocol*

Thirty male Wistar rats, weighing between 180–200 g were used. As a standard protocol, all the rats were housed in a quiet non-stressful environment for one week before the study. Wistar rats were divided into three groups. All rats were given 45–50 kcal/day normal rat chows during the experimental period. The first group (morphine group, $n = 10$) received morphine intraperitoneally at a dose of 4 mg/kg/day for the first 10 days, 8 mg/kg/day between 11–20 days and 12 mg/kg/day between 21–30 days. The second group (tramadol group, $n = 10$) received tramadol (Contramal, Grunenthal, Germany) intraperitoneally at doses of 20, 40 and 80 mg/kg/day on the first, second and third ten days of the study, respectively. All morphine and tramadol doses were delivered in a volume of 1 ml of normal saline. It was planned to return and continue with the previous dose if any complications, related to morphine or tramadol, such as apnea, convulsion, were observed. As placebo, normal saline (0·9% NaCl, 1 ml) was given intraperitoneally to the control group ($n = 10$).

All rats were anaesthetized with ketamine (75 mg/kg) on 31st day. Midline laparotomy was performed and liver and kidney specimens were obtained. Blood samples were drawn by cardiac puncture.

2.2 *Biochemical analysis*

Blood samples were taken into glass bottles with rubber caps, labelled and centrifuged at 4000 *g* for 10 min. Serum enzyme [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH)] activities and serum creatinin and blood urea nitrogen (BUN) levels were determined using Cobas Integra system by kinetic and kinetic Jaffe reaction, respectively (Roche Diagnostics, Mannheim, Germany).

2.3 *Determination of lipid peroxides*

Lipid peroxides in samples were determined by the method described by Ohkawa *et al* (1979). A 0·2 ml of 8·1% sodium dodecyl sulphate, 1·5 ml of 20% acetic acid and 1·5 ml of 0·8% thiobarbituric acid solutions were added to 0·1 ml of serum pipetted into a tube. The mixture was heated in a 95°C water bath for 30 min. After cooling, the colour was extracted into 5·0 ml of n-butanol-pyridine (with a ratio of 15 : 1) and absorbance was measured at 532 nm using a spectrophotometer (Carry 50 Bio, UV Visible Spectrophotometer, Varian, USA). The amounts of lipid peroxides were calculated as thiobarbituric acid reactive products of lipid peroxidation and reported as nmol of malondialdehyde (MDA) per ml of serum.

2.4 *Histopathologic examination*

The specimens were fixed in 10% formalin for 24 h, and standard dehydration and paraffin-wax embedding procedures were used. Sections (5 μm) were cut in a microtome, adhered to glass slides with polylysine. Hematoxylin and eosin-stained slides were prepared by using standard methods and evaluated by light microscopy.

2.5 *Statistical analysis*

Statistical analyses were performed using analysis of variance (ANOVA), followed by post-hoc (Bonfferroni) least significant difference testing where $P < 0·05$ on ANOVA. Data were presented as mean ± SD and $P < 0·05$ was considered statistically significant. All calculations were made using SPSS for Windows 10·0 program.

3. Results

3.1 *Biochemical analysis*

ALT, AST and LDH levels were significantly higher in morphine group compared to control group ($P < 0·05$, $P < 0·05$, $P < 0·01$ respectively). Although AST and LDH levels were not different between the tramadol and the control groups, only ALT level was significantly higher in the tramadol group ($P < 0·05$). LDH level was significantly higher in the morphine group compared to tramadol group ($P < 0·05$) (table 1).

BUN and creatinin levels were found to be significantly higher in the morphine group compared to the tramadol and the control groups (BUN; $P < 0·01$, $P < 0·01$, creatinin; $P < 0·01$, $P < 0·01$ respectively). However, no significant differences in BUN and creatinin levels were found between the tramadol and the control groups ($P > 0·05$) (table 1).

Serum MDA levels were significantly increased in the morphine group compared to control and the tramadol groups ($P < 0{\cdot}05$) (figure 1).

3.2 *Histopathologic examination*

3.2a *Liver*: Sinusoidal dilatation and congestion was observed in all rats in the morphine group. Hydropic degeneration (ballooning) was also observed in most of the rats in perivenular region (zone 3). Degeneration had proceeded to midzonal region (zone 2) in some rats. In addition to these findings perivenular necrosis and haemorrhage were found in three rats and focal microvesicular steatosis in four (figure 2B). The only pathologic finding was perivenular hydropic degeneration in tramadol group (figure 2C). No histopathologic abnormality was found in the control group (figure 2A).

3.2b *Kidney*: The main histopathologic finding was vacuolization in tubular cells in the morphine group. Additionally interstitial mononuclear cell infiltration was found in five rats and focal necrosis and haemorrhage in three (figure 3B). Interstitial mononuclear cell infiltration was present in two rats and minimal vacuolization was found in the tramadol group (figure 3C). Histopathological findings were normal except minimal congestion in the control group (figure 3A).

No glomerular damage was found in any of the groups.

Histopathologic changes of injury are summarized in table 2.

4. Discussion

Although opioids are being widely used since very long time, their long-term effects especially at cellular level, are not clearly understood.

Table 1. Effects of morphine and tramadol on liver and kidney functions in rats.

Groups	ALT (U/L)	AST (U/L)	LDH (U/L)	BUN (mg/dL)	Creatinin (mg/dL)
Control	35·2 ± 5·8	126·4 ± 13·4	559·7 ± 151·9	34·2 ± 6·7	0·4 ± 0·04
Morphine	43·5 ± 5·8[a]	211·4 ± 68·9[a]	1603·5 ± 660·2[b]	43·6 ± 5·8[b]	0·6 ± 0·08[b]
Tramadol	50·0 ± 5·0[a]	203·8 ± 50·0	804·3 ± 303·8[c]	29·4 ± 6·9[d]	0·4 ± 0·05[d]

Values are expressed as mean ± SD.
[a]$P < 0{\cdot}05$ compared with control group; [b]$P < 0{\cdot}01$ compared with control group; [c]$P < 0{\cdot}05$ compared with morphine group; [d]$P < 0{\cdot}05$ compared with morphine group.

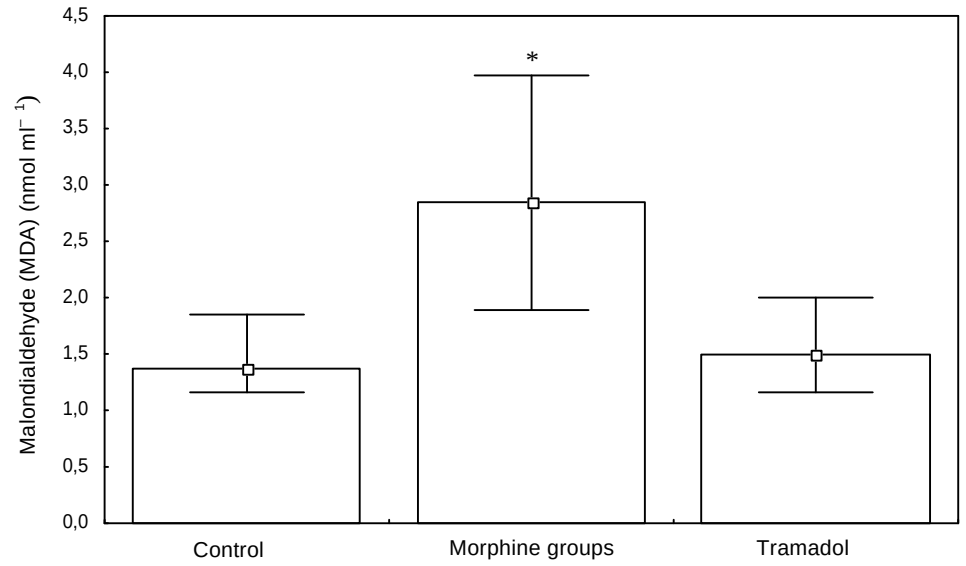
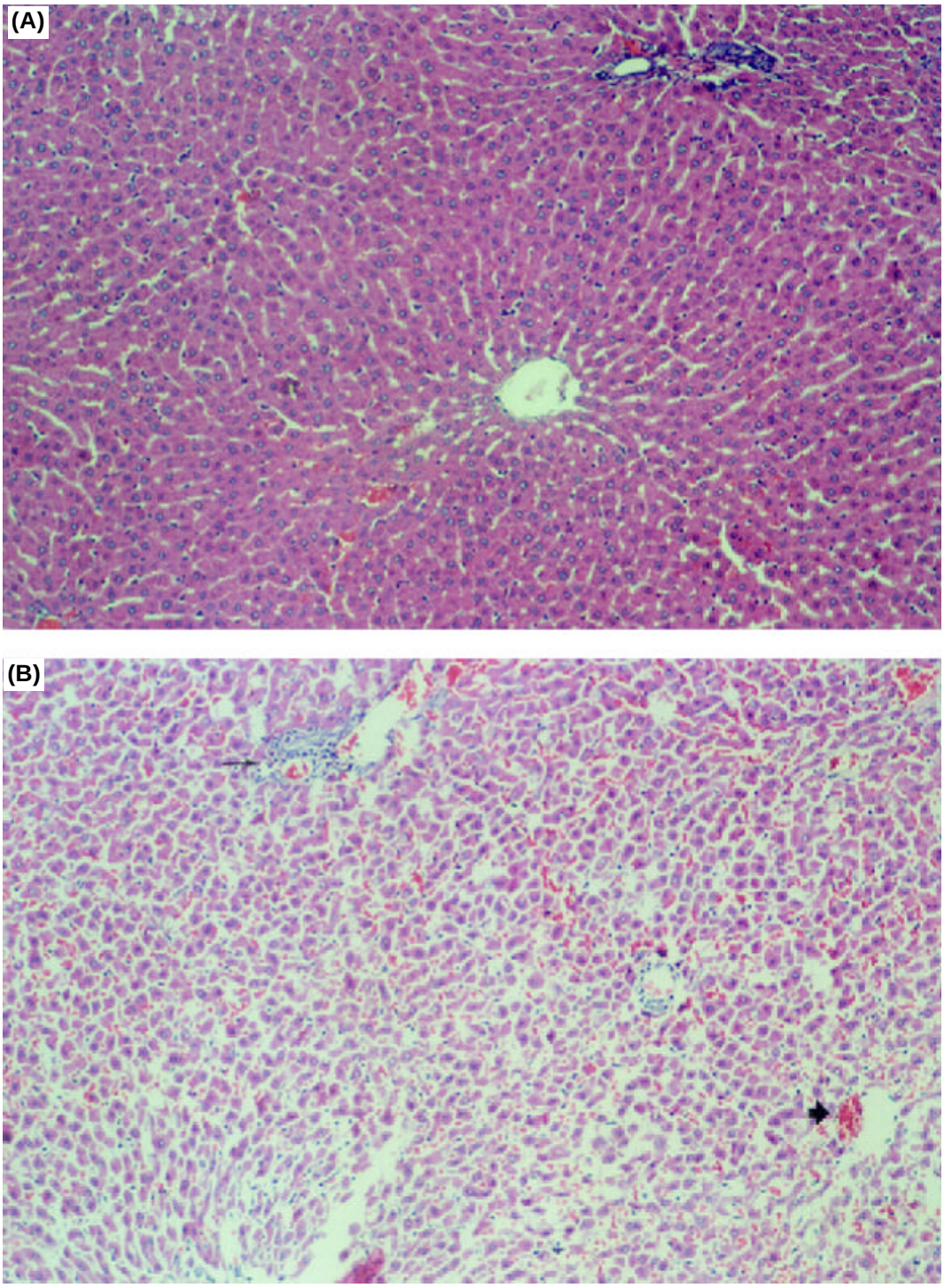


Figure 1. Serum MDA levels were significantly higher in morphine group compared to tramadol and control groups (*$P < 0{\cdot}01$). No difference was found between control and tramadol groups ($P > 0{\cdot}05$).

The liver and kidneys are responsible for the metabolism and excretion of morphine (Coughtrie *et al* 1989; Milne *et al* 1997). Morphine may cause hepatotoxicity and nephrotoxicity during its metabolism (Van der Laan *et al* 1995). Incubation of adult human hepatocytes with opioids, in therapeutic doses, for 24 h, is unlikely to produce irreversible damage to these cells in chemically defined culture conditions (Gomez–Lechon *et al* 1988). On the other hand, a significant increase in the levels of ALT, LDH and lipid peroxides was reported among chro-



Figures 2. (**A**) and (**B**). For caption see page No. 249.

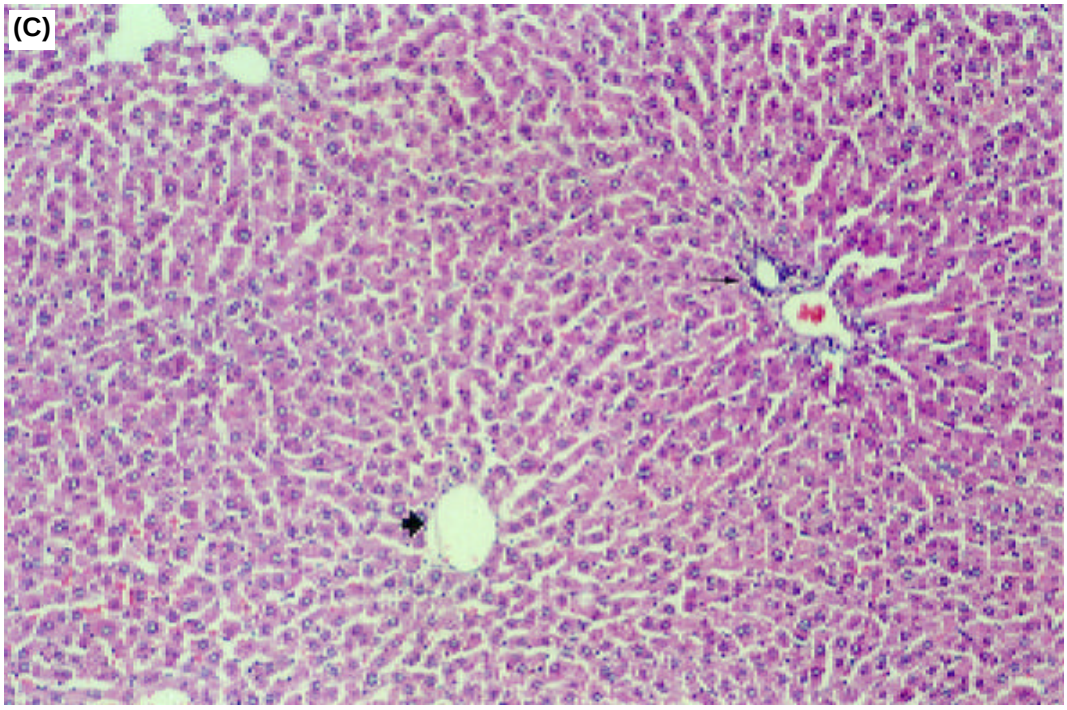


Figure 2. (**A**) High-power photomicrograph of normal appearing liver in control rats (hematoxylin-eosin × 100 original magnification). (**B**) Necrosis and haemorrhage in midzonal (zone 2) and perivenular regions (zone 3), sinusoidal dilatation, apoptotic body, congestion and ballooning degeneration in rats receiving morphine (hematoxylin-eosin × 100 original magnification). (**C**) Sinusoidal dilatation, perivenular hydropic degeneration and cytolysis in rats receiving tramadol (hematoxylin-eosin × 100 original magnification). Thin arrow indicates portal area; thick arrow indicates central vein.

Table 2. Histopathologic findings in liver and kidney.

	Morphine (n = 10)	Tramadol (n = 10)
Liver		
Hydropic degeneration	8	10
Haemorrhage	3	–
Congestion	10	–
Sinusoidal dilatation	10 (severe)	2 (mild)
Cytolysis	7	4
Granuloma formation	2	2
Apoptotic body	7	–
Perivenular necrosis	3	–
Focal microvesicular steatosis	4	–
Kidney		
Tubular epithelial vacuolization	10 (severe)	6 (mild)
Interstitial mononuclear cell infiltration	5	2
Focal necrosis and haemorrhage	3	1

nic heroin users (Panchenko *et al* 1999). Experimental studies have also supported toxic effects of chronic use of opioids on liver and kidneys. Borzelleca *et al* (1994) reported increased levels of ALT, AST and LDH in rats after long-term usage of morphine like agent levo-alpha–acetylmethadol (LAAM) HCL. Centrolobular hypertrophy in the liver was also reported in the same study. Nogamatsu *et al* (1986) demonstrated that addition of morphine to the isolated rat hepatocytes induced a marked decrease in the cells and resulted in cell death. We

similarly found significant increase in the levels of ALT, AST and LDH in the group receiving morphine for long-time compared to controls. ALT was also found significantly increased among rats receiving tramadol. These findings suggest the possible hepatotoxic effects of both morphine and tramadol in long term. But when histopathologic findings together with biochemical findings are taken into consideration, the toxic effects of tramadol seem to be less severe compared to morphine.

Renal damage like focal cortico-medullary mineralization, focal regeneration in tubular epithelium, and mineral/crystal deposition in intertubular region in kidneys

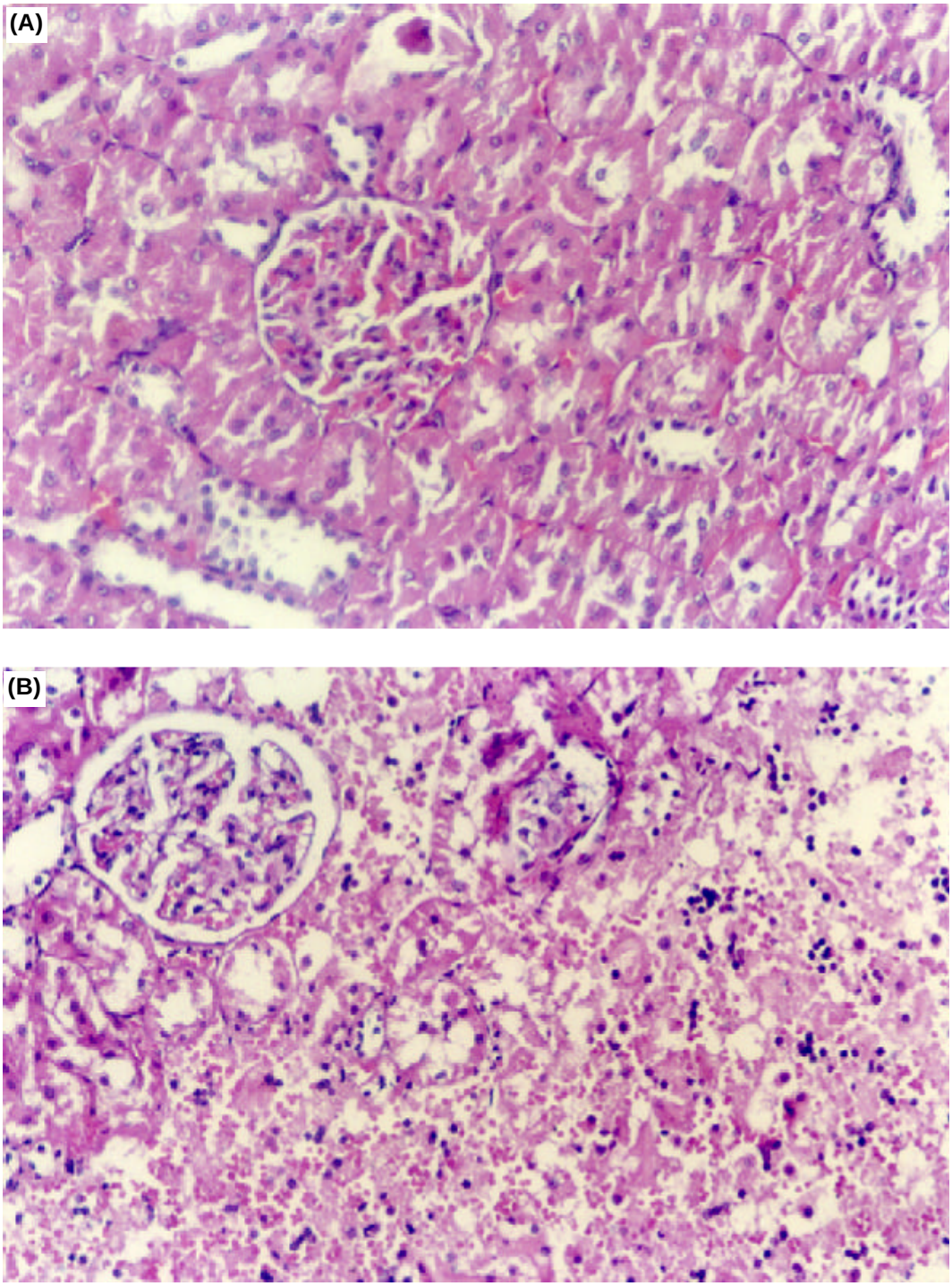


Figure 3. (**A**) and (**B**). For caption see page No. 251.

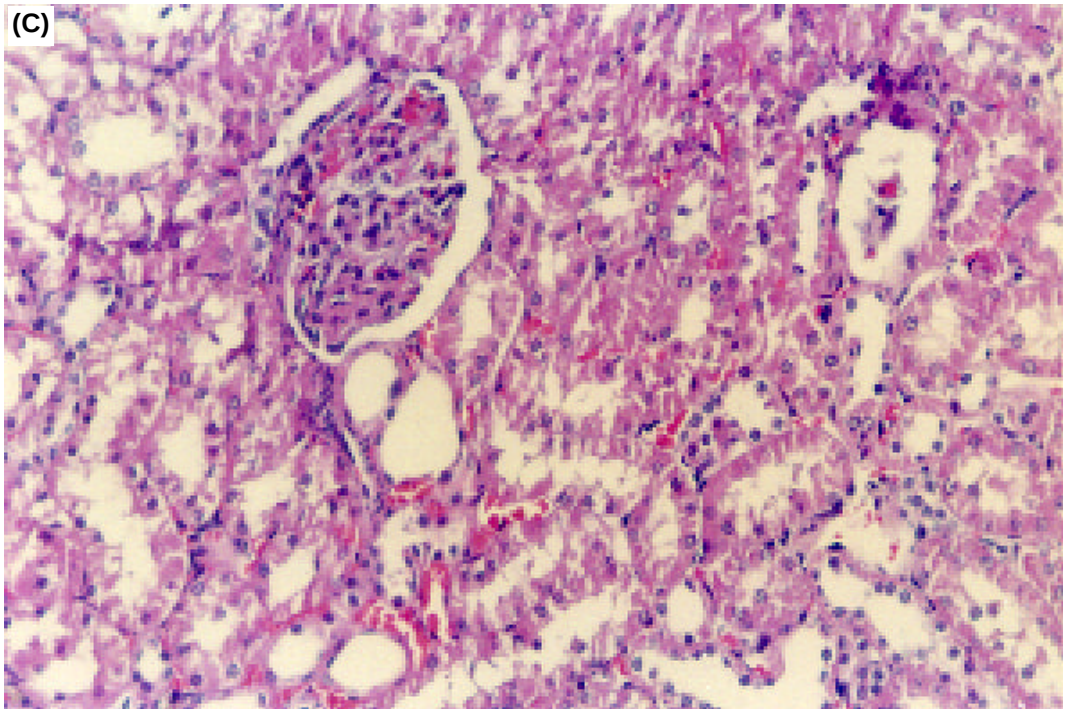


Figure 3. (**A**) High-power photomicrograph of normal appearing kidney in control rats (hematoxylin-eosin × 200 original magnification). (**B**) Extensive necrosis (bottom right) in rats receiving morphine (hematoxylin-eosin × 200 original magnification). (**C**) Tubular epithelial vacuolization and congestion in rats receiving tramadol (hematoxylin-eosin × 200 original magnification).

has been shown after long-term use of LAAM (Borzelleca *et al* 1994). In our study renal tubular vacuolization, mononuclear cell infiltration, focal necrosis and haemorrhage as well as increase in BUN and creatinin levels in rats receiving morphine can be considered as evidence of renal damage. On the other hand rats receiving tramadol showed minimal histopathologic changes in kidneys limited only to tubular cells. BUN and creatinin levels remained unchanged in the tramadol group, indicating that tramadol may be a safer drug in terms of side effects compared to morphine.

Toxic effects of opioids at cellular level may be explained by lipid peroxidation (Lurie *et al* 1995). Biological membranes contain a large amount of polyunsaturated fatty acids, which are particularly susceptible to peroxidative attacks by oxidants resulting in lipid peroxidation. Therefore, lipid peroxidation has been used as an indirect marker of oxidant-induced cell injury. A significant increase in lipid peroxidation was reported in rats receiving an acute dose of cocaine (Masini *et al* 1997). Similarly lipid peroxides were found significantly increased among chronic heroin users (Panchenko *et al* 1999). In another experimental study, isolated rat hepatocytes exhibited a marked decrease in glutathione level when incubated with various concentrations of morphine and resulted in cell death (William *et al* 1991). Our study showed a significant increase in serum MDA levels in the morphine group compared to control and tramadol groups, indicating an increase in lipid peroxidation.

Published patient surveys and case-reports support the efficacy and safety of long-term use of opioid analgesics in chronic non-malignant nociceptive and neuropathic pain (Portenoy and Foley 1986; Turk *et al* 1994). But our findings pointed out the risk of increased lipid peroxidation, hepatic and renal damage due to long term use of opioids, especially morphine. In conclusion, although opioids are reported to be effective in pain management, their toxic effects should be kept in mind.

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MS received 24 April 2004; accepted 12 January 2005

*e*Publication: 2 February 2005

Corresponding editor: SHASHI WADHWA