ORIGINAL ARTICLE

Nefopam induced dose-related changes in renal and hepatic functions

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ABSTRACT

Nefopam is a non-opioid, centrally acting, non-steroidal analgesic drug. It is used to treat mild to moderate painful conditions. Although developed about five decades ago, its use has gained resurgence in recent years. This study was designed to investigate the effects of different analgesic doses of nefopam on the liver and kidneys of mice. Forty albino mice were divided into four groups of 10 mice for each group. Group 1, 2 &3 received a daily intraperitoneal injection of Nefopam at 10, 20, and 30 mg/kg doses, respectively. The fourth group (control group) received injections of normal saline. After two weeks of treatment, the animals were weighed and sacrificed, and then blood was collected for liver enzymes analysis and renal function test as well as histological assessment. The results revealed that the bodyweight increase ratio was significantly lower in group 3 (*P*-value <0.01). All tested liver enzymes i.e., ALT, AST, and ALP levels showed a highly significant (*P*-value <0.01) change among the tested groups. Although ALT remained within normal limits in the first two groups and normal group, in group 3 (30 mg/kg), its level exceeded the normal value. Liver enzyme changes reflected and supported the histopathological findings in the liver tissue. Group 2 & 3 showed varying degrees of hepatotoxicity, ranging from granulomatous lymphocytic infiltration to micro-vesicular steatosis and apoptotic pictures. Both kidney function test and histopathological examination, on the other hand, illustrated insignificant effect (*P*-value >0.05) of Nefopam on the kidneys. Nefopam is well tolerable by the liver at low analgesic doses but may have detrimental effects at higher analgesic doses and prolonged duration of intake.

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INTRODUCTION

The liver is considered to be one of the most vital organs that functions as a center of metabolism for nutrients and excretion of waste metabolites, but mainly providing protection against foreign substances by detoxification and elimination (Roy *et al.*, 2012). The liver can metabolize xenobiotics, which can lead to drug-induced liver injury (DILI) and is a potential complication of many drugs. DILI broadly classified into two types: intrinsic and idiosyncratic types; intrinsic DILI generally is dose-dependent and predictable (e.g., acetaminophen toxicity), whereas idiosyncratic DILI is unpredictable and does not depend directly on dose (Chalasani and Björnsson, 2010). Drug-induced liver injury is one of the main reasons for the withdrawal of many drugs from the market. The mechanism of hepatotoxicity can be investigated through mitochondrial dysfunction and DNA damage — this imperial function caused by the drug itself or its cytochrome P450 mediated metabolite. Reports on hepatotoxicity suggest that oxidative

stress, microvascular steatosis, imbalance energy storage are a major outcome of mitochondrial dysfunction (Au *et al.*, 2011).

The kidney is an essential organ required by the body to perform several important functions, including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification and excretion of toxic metabolites and drugs. Therefore, the kidney can be considered as a major target organ for exogenous toxicants. Therefore, approximately 20% of renal injury or damage is caused by drugs (Kim and Moon, 2012). Renal injury or damage can be diagnosed through a simple blood test. Evaluation of nephrotoxicity through blood tests includes the measurements of blood urea nitrogen (BUN), the concentration of serum creatinine, glomerular filtration rate, and creatinine clearance (Rached *et al.*, 2008). Drug-induced renal injury or damage can be caused by mechanisms, including changes in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic micro-angiopathy (Ferguson *et al.*, 2008).

Nefopam is a benzoxazine that was developed in the 1960s as a muscle relaxant, but clinical use revealed that it has non-opioid, centrally acting, non-steroidal analgesic abilities, and it was used for relief of moderate pain (Seetohul *et al.*, 2015). Its unique mode of action involves inhibition of the reuptake of serotonin, noradrenaline, and dopamine and may interact with the glutamatergic system (Saghaei *et al.*, 2012). The drug undergoes significant first-pass metabolism to inactive metabolites, and only 4-6% is excreted unchanged in urine (Djerada *et al.*, 2014). Due to the resurgence of its use over the past years for the treatment of chronic painful conditions e.g., neuropathic pain, it gained more attraction from researchers to examine its potential effects on different body organs (Kim and Abdi, 2014). The aim of the study was to investigate the effect of different doses of nefopam on renal and hepatic function in mice.

MATERIALS AND METHODS

Animal Grouping

The study was conducted at the pharmacology/toxicology lab in Al-Rasheed University College/ Pharmacy Department after obtaining an animal ethical approval. Forty adult male albino mice were obtained for this study. They had a median age of $8 \pm (1.55)$ weeks. The animals were divided into four groups, 10 mice in each group. All animals in each group were weighed pre, and post-intervention, and the body weight increasing

ratio was calculated as well. The intervention included receiving a single daily intraperitoneal (i.p.) injection. The i.p. doses were as following: Group 1 received 10 mg/kg Nefopam i.p.; group 2 received 20 mg/kg Nefopam i.p.; group 3 received 30 mg/kg Nefopam i.p. for 2 weeks; while group 4 (control group) received normal saline i.p. for 2 weeks. Nefopam (Acupan®) was used as a 20mg/2 mL injectable ampoule, and the dose was calculated for each animal according to its body weight, and smaller doses were diluted with distilled water.

Serum Biochemical Analysis

At the end of the experiment, the animals were put under anesthesia with diethyl ether, and blood was collected by heart puncture for biochemical analysis. Blood was centrifuged at 4000 rpm for 4 min, and plasma was isolated for the assessment of liver enzymes and renal function by means of spectrophotometry. The assessed liver enzymes included; alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), whilst the kidney function tests were tested through blood urea and serum creatinine.

Histological Examination

The liver and right kidney were excised, weighed, washed, and fixed in 10% formaldehyde for tissue processing and histological examination. Hepatic and renal tissues were prepared for routine H&E staining, according to Bancroft and Stevens (Bancroft and Stevens, 1982). Five sections (5μm thick) from each liver were examined. Digital images were acquired using Micros microscope (Austria) with built-in Micros Camera using the provided NMS Software (v 1.5.3.291) running on Microsoft Windows CE (v 5.00, build 1400).

Statistical Analysis

Data were analyzed using SPSS 23 software (SPSS Inc., Chicago, IL, USA), and the results were presented as mean + SD. Mann-Whitney test was used to compare the differences in body weight, increasing ration, organs weight, and organ/ body ratios of each group with the control group as they show non-normal distribution. While One-way ANOVA was used to compare the differences in the levels of the biomarkers among the groups. *P*-values less than 0.05 were regarded as statistically significant, while *P*-values less than 0.01 were regarded as high statistical significance.

RESULTS AND DISCUSSION

The results revealed that the average initial body weight of all animals in all groups was $17.98 \pm$

(2.36) gm, while the final body weight was 21.44 ± (2.38) gm. The bodyweight increase ratio was significantly lower in group 3 (*P*-value <0.01), which was (14%) compared to the control group (21%). The bodyweight increase ratio between group1 and group2 did not show the significant change (*P*-value >0.05) in comparison to the control group, as shown in Table 1.
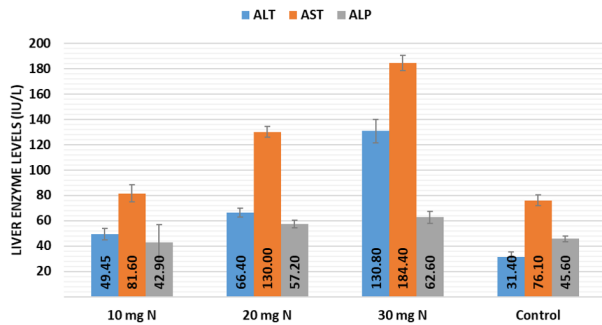

Figure 1: Liver enzyme levels in all groups receiving different doses of Nefopam. (results are presented as mean± SD, n = 10, significance level at P < 0.01) (ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphate)
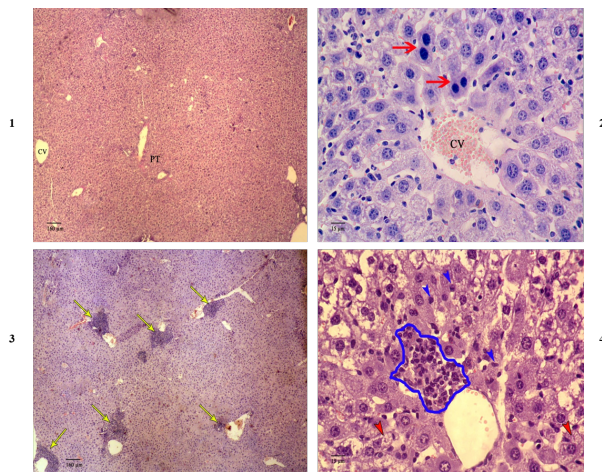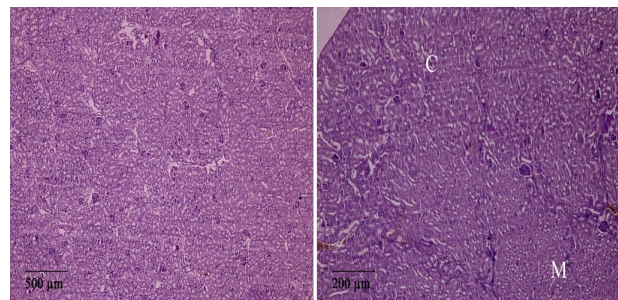

Figure 2: Histological examination of liver tissue. Image (1) represents the control group, Image (2) represents (10 mg N) group, Image (3) represents (20 mg N)group and Image (4) represents (30 mg N) group. H&E at X400 magnification (CV: CentralVein, PT: Portal Triad)

Liver weight in the control group was significantly (*P*-value <0.01) lower than the other groups. However, the liver/body weight ration did not show a statistical significance (*P*-value >0.05) difference between each group and the control group Table 2.

Liver enzyme levels revealed a highly significant difference in their levels among all groups (*P*-value
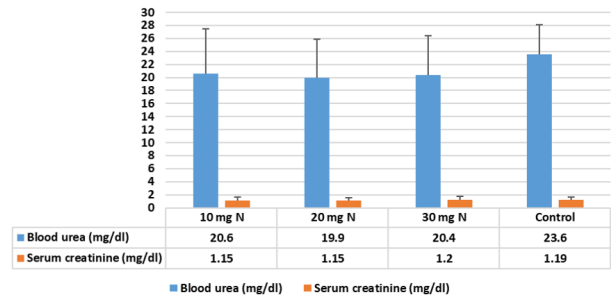
Figure 3: Renal function tests in animal groups receiving different doses of Nefopam. The Control group received normal saline till the end of the experiment. (results are presented as mean ±SD, n = 10)

Figure 4: Histological examination of kidney tissues. Image (1) represents the renal tissue of the control group. H&E, X40; Image (2) represents renal tissue of (30mg N) group. H&E,X100 (C = Cortical Area, M = MedullaryArea)

<0.01) Figure 1. However, ALT remained within normal value in all groups except in group 3 (30 mg N), which was significantly higher when compared to the other groups, and showed abnormally elevated value (>120 IU/l) (Otto *et al.*, 2016). AST remained within the normal limit (191 IU/l) in all groups. ALP remained within normal limits (43- 64 IU/l) in all groups, although it showed a high statistically significant difference (*P*-value <0.01) among the groups.

Hepatic tissue examination revealed normal hepatic lobular structure and morphology in control and (10 mg N) groups. Branching and anastomosing cords of hepatocytes were seen radiating from central veins towards portal triads. The cords were separated by narrow sinusoidal spaces. Hepatocytes had vesicular nuclei, and some of them were binucleated (indicated by red arrows). In the (20 mg N) group, hepatic architecture was preserved, and hepatocyte appeared normal, but there were scattered granulomatous lymphocyte infiltration with particular location affinity around the central veins

Table 1: Initial and final body weight of mice at the beginning and end of the study and the pre/post bodyweight increasing ratio in each group

Group	Initial body weight (g)	Final body weight (g)	The pre/post bodyweight increasing ratio %
Group 1 (10mg/kg)	16.62±2.05	20±2.9	0.20
Group 2 (20mg/kg)	18.52±1.87	22.43± 1.98	0.21
Group 3 (30mg/kg)	19.58±2.77	22.44± 2.94	0.14**
Group 4 Control	17.22±1.72	20.90± 1.69	0.21

(Data presented as mean ± SD, n = 10, **high statistical significance at P-value <0.01)

Table 2: The liver weight and liver weight / body weight ratio

Group	Liver weight (g)	Liver weight/Bodyweight ratio
Group 1 (10mg/kg)	1.76±0.25**	10.19±2.18
Group 2 (20mg/kg)	1.74±0.16**	9±1.43
Group 3 (30mg/kg)	1.81±0.13**	8.44±1.33
Group 4 (control)	1.56±0.19	8.27±0.7

(Data presented as means ± SD, n = 10, ** high statistical significance at P-value <0.01)

Table 3: The kidney weight and kidney weight/body weight ratio

Group	Kidney weight (mg)	Kidney weight: Bodyweight ratio
Group 1 (10mg/kg)	256.3±4.52	1.3±0.12
Group 2 (20mg/kg)	242.4±13.6	1.08±0.11
Group 3 (30mg/kg)	253±14	1.14±0.16
Group 4 (control)	250.4±18.99	1.21±0.12

(Data represent means ±SD)

(yellow arrows and blue surrounded area). Hepatic tissue section of (30 mg N) group revealed that, the tissue showed evidence of hepatic cell injury as shown by several apoptotic pictures (blue arrowheads), peri-venular lymphocyte infiltration, and mid-lobular hepatocytic micro-vesicular steatosis (numerous small lipid droplets are present in hepatocyte cytoplasm) (red arrowheads) as shown in Figure 2.

The analysis of kidney weights revealed that weights (absolute & relative) showed insignificant (*P*-value >0.05) change among all the groups, as shown in Table 3.

Renal function tests remained within normal reference values, and there was no statistically significant difference in their levels among the treated nor control groups (*P*-value >0.05) Figure 3. This was complemented by normal histological features of renal tissue Figure 4. The cortical area showed normal glomerular arrangements with normal urinary spaces and The normal analgesic dose of Nefopam in rodents ranges from low analgesia at 10mg/kg to high analgesia at 30mg/kg (Girard *et al.*, 2008).

However, the incidence of adverse effects increases with the dose (Sanga *et al.*, 2016). Among the common side effects are nausea, dizziness, blurred vision, and confusion (Gregori-Puigjane *et al.*, 2012). In this study, only the (30 mg N) group failed to gain weight as the other groups. This may be most likely related to the aforementioned side effects encountered with high analgesic doses. Since Nefopam is non-sedative (Bilotta *et al.*, 2005), its central actions are unlikely to be related to the reward and satisfaction centers in the brain and thus does not affect appetite and food intake directly (Buritova and Besson, 2002). Although liver weights were different in the control group, the liver weight/body weight ratios were comparable in all groups. This is best explained by the difference in growth rates among the animals causing the control group animals that had the median initial weight to catch up with the other groups, i.e., the gain in body weight exceeded the gain organ weight.

Enzymatic changes in different groups are reflective of the dose régime given. Low analgesic doses (10 mg N) and control (placebo) groups had nor-

mal comparable results reflecting the ability of the liver to handle the 10mg/kg dose without enzymatic activation. However, at 20mg/kg dose, the enzyme levels increased significantly but still within normal levels, and at 30mg/kg, the ALT exceeded the normal levels while AST was at its high normal. ALT is a more specific marker than AST in detecting hepatocytic damage since AST is also present in cardiac and skeletal muscles and red blood cells and may be elevated in diseases related to these sites (Ramaiah, 2007). Elevated levels of ALT with low AST/ALT ratio are indicative of liver cell necrosis (Nyblom et al., 2004). Elevated ALP levels are associated with cholestatic liver damage. It appears that hepatocytic necrosis precedes cholestasis or maybe the major form of liver injury caused by the metabolic pathway of Nefopam. Nefopam is mainly metabolized by N-demethylation but may involve other routes, since the amount excreted unchanged is minimal (Sanga et al., 2016).

The enzymatic changes are complemented by the histopathological findings. While the control and (10 m N) groups showed normal liver histology, the other two groups showed advancing levels of cell damage ranging from granulomatous legions in the (20 m N) group to clear cell apoptosis and micro-vesicular steatosis in the (30 mg N) group. Drug-induced liver damage depends on the type of the drug, dose, and duration of intake and encompasses a range of lesions including granulomas, fatty liver change (steatosis), zonal necrosis, cholestasis, and cholestatic-hepatitis (Lee, 2003). While any drug may cause any lesion, certain drugs are associated with more lesions than others are. Analgesics are more commonly associated with granulomatous hepatitis (Andrade et al., 2005), while valproate and cytotoxic drugs are more associated with steatosis (Amacher and Chalasani, 2014). Both lesions are associated with elevated ALT and AST. The absence of cholestasis is supportive of the normal ALT levels and may indicate that the metabolic pathway of Nefopam does not interfere with cholestatic pathology, at least for the duration and dose of the study.

Regarding the effect of different doses of nefopam on kidney function and histological features, there are insufficient studies about its effect on kidney function. The study revealed that the kidney function for all animal groups was not affected, and the levels of BUN and creatinine remained within the reference normal values; even the histological examination did not show any feature deviation from the normal. One study can prove this, where Oliver and co-workers in 2010 studied the effect of using nefopam in end-stage kidney disease patients as it was less likely to decline kidney function further, but it should be used with caution due to possibility of elevated serum level (Mimoz et al., 2010).

CONCLUSION

Nefopam is well tolerable by the liver at low analgesic doses but may have detrimental effects at higher analgesic doses with prolonged duration of intake and with no significant changes in kidney function.

Conflict of interest

All authors declare that there is no conflict of interest among authors.

REFERENCES

Amacher, D., Chalasani, N. 2014. Drug-Induced Hepatic Steatosis. *Seminars in Liver Disease*, 34(02):205–214.

Andrade, R., Lucena, M., Fernandez, M., Pelaez, G., Pachkoria, K., Garciaruiz, E., Duran, J. 2005. Drug-Induced Liver Injury: An Analysis of 461 Incidences Submitted to the Spanish Registry Over a 10-Year Period. *Gastroenterology*, 129(2):512–521.

Au, J. S., Navarro, V. J., Rossi, S. 2011. Review article: drug-induced liver injury - its pathophysiology and evolving diagnostic tools. *Alimentary Pharmacology & Therapeutics*, 34(1):11–20.

Bancroft, J. D., Stevens, A. 1982. Theory and practice of histological techniques. pages 482–502. 2nd ed.

Bilotta, F., Ferri, F., Giovannini, F., Pinto, G., Rosa, G. 2005. Nefopam or clonidine in the pharmacologic prevention of shivering in patients undergoing conscious sedation for interventional neuroradiology. *Anaesthesia*, 60(2):124–128.

Buritova, J., Besson, J. M. 2002. Effects of nefopam on the spinal nociceptive processes: a c-Fos protein study in the rat. *European Journal of Pharmacology*, 441(1-2):67–74.

Chalasani, N., Björnsson, E. 2010. Risk Factors for Idiosyncratic Drug-Induced Liver Injury. *Gastroenterology*, 138(7):2246–2259.

Djerada, Z., Fournet-Fayard, A., Gozalo, C., Lelarge, C., Lamiable, D., Millart, H., Malinovsky, J. M. 2014. Population pharmacokinetics of nefopam in elderly, with or without renal impairment, and its link to treatment response. *British Journal of Clinical Pharmacology*, 77(6):1027–1038.

Ferguson, M. A., Vaidya, V. S., Bonventre, J. V. 2008. Biomarkers of nephrotoxic acute kidney injury. *Toxicology*, 245(3):182–193.

Girard, P., Verniers, D., Coppé, M. C., Pansart, Y.,

Gillardin, J. M. 2008. Nefopam and ketoprofen synergy in rodent models of antinociception. *European Journal of Pharmacology*, 584(2-3):263–271.

Gregori-Puigjane, E., Setola, V., Hert, J., Crews, B. A., Irwin, J. J., Lounkine, E., Shoichet, B. K. 2012. Identifying mechanism-of-action targets for drugs and probes. *Proceedings of the National Academy of Sciences*, 109(28):11178–11183.

Kim, K. H., Abdi, S. 2014. Rediscovery of Nefopam for the Treatment of Neuropathic Pain. *The Korean Journal of Pain*, 27(2):103–103.

Kim, S. Y., Moon, A. 2012. Drug-Induced Nephrotoxicity and Its Biomarkers. *Biomolecules and Therapeutics*, 20(3):268–272.

Lee, W. M. 2003. Drug-Induced Hepatotoxicity. *New England Journal of Medicine*, 349(5):474–485.

Mimoz, O., Chauvet, S., Grégoire, N., Marchand, S., Guern, M. E. L., Saleh, A., Levy, R. H. 2010.

Nyblom, H., Berggren, J., Olsson, R. 2004. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. *Alcohol and Alcoholism*, 39(4):336–339.

Otto, G. P., Rathkolb, B., Oestereicher, M. A., Lengger, C. J., Moerth, C., Micklich, K., Angelis, M. H. D. 2016. Clinical Chemistry Reference Intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ Mice (Mus musculus). *Journal of the American Association for Laboratory Animal Science*, 55(4):375–386. JAALAS.

Rached, E., Hoffmann, D., Blumbach, K., Weber, K., Dekant, W., Mally, A. 2008. Evaluation of Putative Biomarkers of Nephrotoxicity after Exposure to Ochratoxin A In Vivo and In Vitro. *Toxicological Sciences*, 103(2):371–381.

Ramaiah, S. K. 2007. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food and Chemical Toxicology*, 45(9):1551–1557.

Roy, S. D., Das, S., Shil, D., Dutta, K. N. 2012. Herbal hepatoprotective agents: a review. *World J. Pharma. Res*, 1(2):87–99.

Saghaei, E., Zanjani, T. M., Sabetkasaei, M., Naseri, K. 2012. Enhancement of Antinociception by Co-administrations of Nefopam, Morphine, and Nimesulide in a Rat Model of Neuropathic Pain. *The Korean Journal of Pain*, 25(1).

Sanga, M., Banach, J., Ledvina, A., Modi, N. B., Mittur, A. 2016. Pharmacokinetics, metabolism, and excretion of nefopam, a dual reuptake inhibitor in healthy male volunteers. *Xenobiotica*, 46(11):1001–1016.

Seetohul, L. N., Paoli, G. D., Drummond, G., Maskell, P. D. 2015. Nefopam Hydrochloride: A Fatal Overdose. *Journal of Analytical Toxicology*, 39(6):486–489.