LABORATORY STUDY

Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: role of FXR and KIM-1

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ABSTRACT

Context: Acetaminophen (APAP) is an analgesic and antipyretic agent commonly known to cause hepatic and renal toxicity at a higher dose. Naringin, a bioflavonoid possesses multiple pharmacological properties such as antioxidant, anti-inflammatory, analgesic and anti-hyperlipidemic activity.

Objective: To evaluate the effect of naringin against the APAP-induced hepatic and renal toxicity.

Materials and methods: Male Wistar albino rats (180–220 g) were divided into various groups, and toxicity was induced by APAP (700 mg/kg, p.o., 14 days). Naringin (20, 40 and 80 mg/kg, p.o.) or Silymarin (25 mg/kg) was administered to rats 2 h before APAP oral administration. Various biochemical, molecular and histopathological parameter were accessed in hepatic and renal tissue.

Results: Naringin pretreatment significantly decreased ($p < 0.05$) serum creatinine, blood urea nitrogen, bilirubin, aspartate transaminase, alanine transaminase, lactate dehydrogenase, low-density lipoprotein, very low-density lipoprotein, cholesterol and triglycerides as compared with APAP control rats. Decreased level of serum albumin, uric acid, and high-density lipoprotein were also significantly restored ($p < 0.05$) by naringin pretreatment. It also significantly restores ($p < 0.05$) the altered level of superoxide dismutase, reduced glutathione, malondialdehyde and nitric oxide in hepatic and renal tissue. Moreover, altered mRNA expression of hepatic farnesoid X receptor and renal injury molecule-1 (KIM-1) were significantly restored ($p < 0.05$) by naringin treatment. Naringin treatment also reduced histological alteration induced by APAP in the liver and kidney. Conclusion: Naringin exerts its hepato- and nephroprotective effect via modulation of oxi-do-nitrosative stress, FXR and KIM-1 mRNA expression.

Introduction

Acetaminophen (N-acetyl-p-aminophenol also known as APAP and Paracetamol), is a widely prescribed as well as over the counter analgesic and antipyretic drugs either as a single compound or combination with other drugs.1–3 APAP is safe at a therapeutic dose, but an acute overdose or extreme chronic administration can cause serious adverse events including hepatic and renal toxicity.4,5 When ingested at a therapeutic dose, APAP is metabolized by a cytochrome P450 and detoxified by glucuronidation as well as sulfation whereas N-acetyl-p-benzoquinone imine (NAPQI) with conjugated glutathione (GSH).6 On the other hand, high dose saturates the detoxication pathways of APAP due to glucuronidation and sulfation insufficiency. Thus, excessive drugs accumulations become accessible for metabolism by cytochrome P450, which leads to GSH depletion. Exhausted GSH levels allow NAPQI free to bind with other targeted cellular proteins which aggravate cellular oxidative stress and indulge in the cellular necrosis process.7,8

Reactive oxygen species (ROS) are strongly associated with oxidative stress.9,10 The equilibrium between ROS generation and the non-enzymatic antioxidant (GSH), superoxide dismutase (SOD), catalase (CAT) notion of being important for the APAP-induced toxicity.5,11

Farnesoid X receptor (FXR) or NR1H4 is a nuclear receptor transcript or factor play a vital role in the regulation of bile acids, cholesterol, glucose metabolism, and lipoproteins. It has been reported that FXR activation could be beneficial in the APAP-induced hepatic toxicity.12 Moreover, several other antioxidants such as liver fatty acid binding protein (L-FABP), with its methionine and cysteine amino acids moiety defense the cells against the oxidative stress by quenching free radicals.13,14 It has been demonstrated that recombinant rat L-FABP can be effective cellular antioxidant against hydrophilic or lipophilic free radical generator.15

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Naval renal prognostic biomarker, i.e., Kidney Injury Molecule (KIM-1) belongs to trans-membrane globular protein plays a central role in the phagocytic clearance of apoptotic and necrotic cells in renal injury.\textsuperscript{16,17}

Pharmacotherapy for the management of APAP-induced hepatic and renal toxicity is multivariate as many interactive pathways are involved in their pathophysiology. It has been shown that administration of gadolinium chloride or dextran sulfate played a vital role in the macrophages inactivation whereas amino-guanidine, a nitric oxide (NO) synthase inhibitor also utilized for the amelioration of APAP-induced toxicity.\textsuperscript{18,19} Treatments with zinc sulfate and lobenzarit are other treatment options for APAP-induced toxicity that improved hepatic GSH levels.\textsuperscript{20} Intravenous administration of N-acetylcysteine is also an early treatment option for APAP poisoning.\textsuperscript{21}

In Ayurveda, plenty of medicinal plants have been recommended as a treatment option for the renal and hepatic diseases.\textsuperscript{22} Moreover, >600 commercial preparations with hepatoprotective potential are available all over the world which contains ~100 medicinal plants from Ayurveda.\textsuperscript{23} But, due to lack of scientific validation of efficacy and safety, these medicinal plants are often ignored by many and considered to be an alternative to conventional medicine. Therefore, their systematic scientific validation in terms of biological assays, animal models, clinical trials, chemical standardization as well as documentation is today’s need of hours.\textsuperscript{24,25} The medicinal plant based drugs have the added advantage of being simple, effective and offering a broad spectrum of activity with an emphasis on the preventive actions of drugs. Because of these factors, the demand for plant-based medicines (photomedicine and phytopharmaceutical) is increasing worldwide.\textsuperscript{26}

Bioflavonoids considered as a potent bioactive moiety against free radical and oxidative stress.\textsuperscript{27–30} Naringin is a polyphenolic bioflavonoid compound derived from citrus fruits and related grapefruits.\textsuperscript{31} Oral naringin intake gets converted into naringenin, an absorbable form through \(\alpha\)-rhamnosidase and \(\beta\)-glucosidase enzymes.\textsuperscript{32} Naringin possesses multiple biological and pharmacological properties such as lipid alleviating, anti-apoptotic, anti-diabetic, neuroprotective, anti-inflammatory, wound healing, metal chelating, anti-microbial, anti-mutagenic, anti-cancer, free radical trapping, and antioxidant properties.\textsuperscript{27,30,33–36} Moreover, our laboratory has recently demonstrated that naringin may exert its free radical scavenging and antioxidative effect in sodium arsenite-induced renal and hepatic toxicity.\textsuperscript{37} To best of our knowledge, no study has been carried out on the effect of naringin on APAP-induced hepatic and renal toxicity. We, therefore, evaluated the protective effect of naringin on APAP-induced renal and hepatotoxicity via examination of FXR, L-FABP, KIM-1, and other antioxidants as well as serum prognostic markers in Wistar albino rats.

**Materials and methods**

**Animals**

Adult male Wistar rats (180–220 g) were obtained from the National Institute of Biosciences, Pune, India. They were housed in cages in a facility maintained at 24 ± 1°C, with a relative humidity of 45–55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pranav Agro Industries Ltd., Sangli, India) and filtered water throughout the experimental period. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved (CPCSEA/67/2012) by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India, and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. Animals were transferred to testing laboratory 1 h before the experiment for adaptation.

**Chemicals**

Acetaminophen and silymarin were obtained as gift samples from Symed Pharmaceutical Pvt. Ltd, Hyderabad, India. Naringin (\(\geq 95\%\) by HPLC) was purchased from Sigma Chemical Co. (St Louis, MO). 1,1,3,3-Tetraethoxypropane, crystalline beef liver CAT, reduced GSH, and 5,5’-dithiobis (2-nitrobenzoic acid) were purchased from SD Fine Chemicals, Mumbai, India. Sulfanilamides, naphthylamine diamine HCl, and phosphoric acid were obtained from Loba Chemi Pvt. Ltd., Mumbai, India.

**Induction of APAP-induced toxicity and drug treatment schedule**

The selection of dose for APAP was based on the studies carried out previously.\textsuperscript{38} Silymarin was administered to rats orally at a dose of 25 mg/kg for 14 days.\textsuperscript{39} APAP was administered to rats orally at a dose of 700 mg/kg.\textsuperscript{38} Fasted rats were randomly divided into seven groups of six rats as follows:

**Group I:** Normal control: rats were administered a single daily dose of distilled water (10 unit), p.o. for 14 days.
**Group II:** APAP: rats that served as the model control was administered a single daily dose of distilled water (10 mg/kg, p.o.) 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

**Group III:** Silymarin (25): rats were administered a single daily dose of silymarin (25 mg/kg, p.o.) in distilled water, 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

**Group IV:** Naringin (20 mg/kg) [N (20)]: rats were administered single daily dose of Naringin (20 mg/kg, p.o.) 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

**Group V:** Naringin (40 mg/kg) [N (40)]: rats were administered single daily dose of Naringin (40 mg/kg, p.o.) 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

**Group VI:** Naringin (80 mg/kg) [N (80)]: rats were administered single daily dose of Naringin (80 mg/kg, p.o.) 2 h before oral administration APAP suspension (700 mg/kg) for 14 days.

**Group VII:** Per se: rats were administered a single daily dose of Naringin (80 mg/kg, p.o.) for 14 days. However, it did not receive any oral administration APAP suspension.

After 14-day treatment, rats fasted overnight and, after 24 h, they were sequentially anesthetized with anesthetic ether for \( \sim 30-40 \) s. The blood was withdrawn by the retro-orbital puncture. Each blood sample was collected into separate vials for the determination of serum parameters. After blood collection, the animals were sacrificed by cervical dislocation and then liver as well as kidney were removed. The specimens were divided into two portions: one portion was used for biochemical estimation, and another portion was processed for histopathological examination.

**Biochemical estimations**

**Preparation of tissue homogenate**

For liver and kidney homogenization, tissue segments were mixed with 0.1 M phosphate buffer and homogenized on ice for \( \sim 60 \) s at 10,000 rpm in a homogenizer (Remi Equipment Pvt. Ltd., Remi Motors Ltd., Mumbai, India). Supernatant of tissue homogenates was employed to estimate SOD, reduced GSH, lipid peroxidation [malondialdehyde (MDA) content], and NO content.

**Determination of total protein, SOD, GSH, MDA, and NO**

The level of total protein, SOD, GSH, MDA, and NO in liver and kidney homogenate was determined according to earlier reported methods.

**Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis**

Total RNA was extracted from liver and kidney tissues according to earlier reported methods. The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Eppendorf India Ltd, Chennai) by using gene specific primers. The primer sequences for FXR, KIM-1, and \( \beta \)-actin were selected according to the previously reported method (Supplementary file). PCR products were run on 1% agarose gels, stained with ethidium bromide. The expression of all the genes was assessed by generating densitometry data for band intensities in different sets of experiments and was generated by analyzing the gel images in the Image J program (Version 1.33, Wayne Rasband, National Institutes of Health (NIH), Bethesda, MD). The band intensities were compared with constitutively expressed \( \beta \)-actin which served as a control for sample loading and integrity. The intensity of mRNAs was standardized against that of the \( \beta \)-actin mRNA from each sample and the results are expressed as PCR-product/\( \beta \)-actin mRNA ratio.

**Histopathological examination**

Liver and kidney tissues were stored in 10% formalin for 24 h. The specimen was dehydrated and placed in xylene for 1 h (3 times) and later in ethyl alcohol (70%, 90% and 100%) for 2 h. The infiltration and impregnation were carried out by treating with paraffin wax twice, each time for 1 h. Tissue specimens were cut into sections of 3–5 mm thickness and were stained with hematoxylin and eosin (H&E). The specimen was mounted on the slide by use of distrain phthalate xylene as a mounting medium. Sections were examined...
under a light microscope for the inspection of the histopathology features of specimen and infiltration of cells. The various changes in histological features were graded as Grade 0 (not present or very slight), Grade 1 (mild), Grade 2 (moderate), and Grade 3 (severe) as described earlier.37,48

**Statistical analysis**

Data were expressed as mean ± SEM. Data analysis was performed using software (v 5.0, Graph Pad, San Diego, CA). Data of biochemical parameters were analyzed using one-way analysis of variance (ANOVA) and followed by Tukey’s multiple range tests for each parameter separately. A value of $p < 0.05$ was considered to be statistically significant.

**Results**

**Effect of naringin treatment on APAP-induced alteration in body weight and relative organ weight in rats**

There was significant ($p < 0.05$) decrease in body weight of APAP rats as compared to normal rats. Whereas relative organ weights of liver and spleen were significantly ($p < 0.05$) increased in APAP rats as compared with normal rats. Treatment with naringin (40 and 80 mg/kg, p.o.) showed a significant increase in body weight as compared to APAP rats whereas, relative liver weight, as well as relative spleen weight, were significantly ($p < 0.05$) decreased as compared to APAP rats. Administration of silymarin (25 mg/kg, p.o.) for 14 days significantly ($p < 0.05$) increased body weight as compared to APAP rats whereas relative liver weight and spleen weight were significantly ($p < 0.05$) decreased as compared to APAP rats. There was no significant difference in the body weight and relative organ weight in normal as well as per se treated group (Table 1).

**Effect of naringin treatment on APAP-induced alteration in serum albumin, blood urea nitrogen, serum creatinine and serum uric acid in rats**

The level of serum albumin and serum uric acid were significantly ($p < 0.05$) decreased in APAP rats as compared to normal rats whereas BUN and serum creatinine level were significantly ($p < 0.05$) increased in the APAP rats as compared to normal rats. Chronic administration of naringin (40 and 80 mg/kg, p.o.) significantly increased serum albumin ($p < 0.05$) and serum uric acid ($p < 0.05$) level whereas the level of BUN and serum creatinine were significantly ($p < 0.05$) decreased by naringin treatment (40 and 80 mg/kg, p.o.) as compared to APAP control rats. When compared with APAP rats, the serum albumin and serum uric acid levels were significantly ($p < 0.05$) increased in silymarin (25 mg/kg, p.o.) treated rats whereas the levels of BUN, and serum creatinine were significantly ($p < 0.05$) decreased. However, serum albumin, BUN, serum creatinine and serum uric did not differ significantly in normal as well as per se treated group (Table 1).

**Effect of naringin treatment on APAP-induced alteration in alkaline phosphatase, total bilirubin, direct bilirubin, AST and ALT in rats**

Chronic administration of APAP caused significant ($p < 0.05$) increase in the level of alkaline phosphatase, total bilirubin, direct bilirubin, AST and ALT in APAP rats as compared to normal rats. When compared with APAP rats, there was significant ($p < 0.05$) decrease in the level of alkaline phosphatase, total bilirubin, direct bilirubin, AST and ALT after chronic naringin treatment (40 and 80 mg/kg, p.o.). Silymarin (25 mg/kg, p.o.) administration significantly ($p < 0.05$) reduced these elevated levels of alkaline phosphatase, total bilirubin, direct bilirubin, AST and ALT when compared with APAP rats (Table 2).

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### Table 1. Effect of naringin treatment on body weight, relative liver weight, relative spleen weight, albumin, blood urea nitrogen, serum creatinine and uric acid in APAP-induced toxicity in rats.

| Treatment          | Body weight (BW) (gm) | Relative liver weight | Relative spleen weight | Albumin (mg %) | BUN (mg/dl) | Serum creatinine (mg/dl) | Uric acid (mg %) |
|--------------------|-----------------------|-----------------------|------------------------|----------------|-------------|--------------------------|-----------------|
| Normal             | 232.50 ± 1.33         | 0.017 ± 0.001         | 0.36 ± 0.07            | 3.21 ± 0.34    | 7.25 ± 1.50 | 1.41 ± 0.15              | 2.24 ± 0.16     |
| APAP control       | 189.20 ± 0.65         | 0.028 ± 0.002         | 0.89 ± 0.04            | 0.61 ± 0.20    | 24.31 ± 0.73 | 7.27 ± 0.66              | 0.55 ± 0.04     |
| Silymarin (25)     | 226.83 ± 0.37±        | 0.024 ± 0.001         | 0.42 ± 0.03            | 2.07 ± 0.32    | 10.63 ± 2.31 | 2.98 ± 0.64              | 1.84 ± 0.26     |
| Naringin (20)      | 204.16 ± 1.90         | 0.027 ± 0.002         | 0.85 ± 0.06            | 0.93 ± 0.24    | 20.43 ± 1.45 | 4.79 ± 0.75              | 0.71 ± 0.07     |
| Naringin (40)      | 220.83 ± 0.10±        | 0.024 ± 0.001         | 0.51 ± 0.05            | 1.20 ± 0.26    | 14.30 ± 1.46 | 3.83 ± 0.45              | 1.70 ± 0.31     |
| Naringin (80)      | 227.33 ± 4.25±        | 0.022 ± 0.002         | 0.41 ± 0.03            | 2.39 ± 0.27    | 6.95 ± 1.22  | 3.27 ± 0.60              | 1.74 ± 0.16     |
| Per se             | 228.00 ± 1.34         | 0.019 ± 0.001         | 0.35 ± 0.04            | 2.90 ± 0.26    | 6.95 ± 1.22  | 1.47 ± 0.38              | 1.85 ± 0.17     |

Notes: Data are expressed as mean ± SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey’s multiple range test for each parameter separately. $^a p < 0.05$ as compared to APAP group, $^b p < 0.05$ as compared to normal group and $^c p < 0.05$ as compared to one another group.

APAP: acetaminophen; BUN: blood urea nitrogen.
Table 2. Effect of naringin treatment on alkaline phosphatase, total bilirubin, direct bilirubin, AST and ALT in APAP-induced toxicity in rats.

| Treatment        | Alkaline phosphatase (IU/l) | Total bilirubin (mg %) | Direct bilirubin (mg %) | AST (IU/l) | ALT (IU/l) |
|------------------|-----------------------------|------------------------|------------------------|------------|------------|
| Normal           | 54.62 ± 8.51                | 0.10 ± 0.01            | 0.19 ± 0.03            | 130.5 ± 16.33 | 38.52 ± 6.65 |
| APAP control     | 284.0 ± 38.44^a             | 0.39 ± 0.04^a          | 0.67 ± 0.06^a          | 360.1 ± 16.73^a | 145.6 ± 10.48^a |
| Silymarin (25)   | 131.3 ± 8.98^b              | 0.12 ± 0.01e^c         | 0.26 ± 0.03^e          | 174.5 ± 20.43^e | 48.32 ± 10.38^e |
| Naringin (20)    | 210.5 ± 26.65               | 0.33 ± 0.05            | 0.51 ± 0.11            | 290 ± 22.11   | 142.6 ± 12.36 |
| Naringin (40)    | 198.4 ± 28.51               | 0.23 ± 0.02^c          | 0.33 ± 0.08^c          | 246.3 ± 31.16^c | 96.4 ± 16.68^c |
| Naringin (80)    | 96.92 ± 9.68^d              | 0.17 ± 0.04^e          | 0.26 ± 0.04^e          | 177.1 ± 32.26^e | 57.93 ± 4.68^e  |
| Per se           | 49.72 ± 3.57                | 0.13 ± 0.02            | 0.22 ± 0.04            | 155.6 ± 21.63 | 46.64 ± 10.60 |

Notes: Data are expressed as mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey’s multiple range test for each parameter separately.

^p < 0.05 as compared to APAP group,
^p < 0.05 as compared to normal group and
*p < 0.05 as compared to one another group.

APAP: acetaminophen; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LDH: lactate dehydrogenase; VLDL: very low-density lipoprotein.

Effect of naringin treatment on APAP-induced alteration in serum cholesterol, HDL, LDL, LDH and triglyceride in rats

There were significant (p < 0.05) increase in the level of serum cholesterol, LDL, LDH and triglyceride in APAP rats as compared to normal rats. Chronic administration of APAP significantly (p < 0.05) decreased the level of HDL in APAP rats as compared to normal rats. The level of serum HDL was significantly (p < 0.05) increased by the chronic treatment with naringin (40 and 80 mg/kg, p.o.) when compared with APAP rats. Moreover, administration of naringin (40 and 80 mg/kg, p.o.) significantly (p < 0.05) prevented APAP caused an increase in the level of serum cholesterol, LDL and triglyceride as compared to APAP rats. Silymarin (25 mg/kg, p.o.) significantly (p < 0.05) decreased the elevated levels of serum cholesterol, LDL, and triglyceride as compared to APAP rats. However, administration of silymarin (25 mg/kg, p.o.) did not produce any significant change in the level of HDL as compared to APAP rats. The levels of serum cholesterol, HDL, LDL, LDH and triglyceride after APAP administration did not differ significantly in per se group as compared to normal group (Table 3).

Effect of naringin treatment on APAP-induced alteration in hepatic as well as renal SOD and GSH in rats

Chronic administration of APAP for 14 days resulted in significant (p < 0.05) decrease in the level of SOD and GSH in hepatic as well as renal tissue in APAP rats as compared to normal rats. Treatment with naringin (40 and 80 mg/kg, p.o.) showed significant elevation (p < 0.05) in the level of hepatic and renal SOD and GSH as compared to APAP rats. Silymarin (25 mg/kg, p.o.) showed significant (p < 0.05) increase in level of hepatic SOD, renal SOD and rennal GSH as compared to APAP rats but, it failed to produce any significant increase in the level of hepatic GSH as compared to APAP rats. The level of SOD and GSH in hepatic as well as renal tissue did not significantly differ in per se group as compared to normal group (Figure 1A and B).

Effect of naringin treatment on APAP-induced alteration in hepatic as well as renal MDA and NO in rats

There was significant increase (p < 0.05) in the levels of hepatic MDA and NO as well as renal MDA and NO in APAP rats as compared to normal rats. Treatment with naringin (40 and 80 mg/kg, p.o.) showed a significant decrease (p < 0.05) in hepatic and renal MDA as well as hepatic and renal NO levels as compared to APAP rats. When compared with APAP rats, silymarin (25 mg/kg, p.o.) treated rats showed a significant decrease (p < 0.05) in the level of hepatic as well as renal MDA and NO. However, per se treated group did not show any significant alterations in hepatic as well as renal...
Figure 1. Effect of naringin treatment on hepatic and renal SOD (A), GSH (B), MDA (C) and NO (D) in APAP-induced toxicity in rats. Data are expressed as mean ± SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey’s multiple range test for each parameter separately. *p < 0.05 as compared to APAP group, †p < 0.05 as compared to normal group and ‡p < 0.05 as compared to one another group. APAP: acetaminophen; N (20): naringin (20 mg/kg) treated; N (40): naringin (40 mg/kg) treated; N (80): naringin (80 mg/kg) treated; SOD: superoxide dismutase; GSH: glutathione; MDA: malondialdehyde; NO: nitric oxide.
Effect of naringin treatment on APAP-induced alteration in hepatic FXR and renal KIM-1 mRNA expression in rats

There was significant decrease \( (p < 0.05) \) hepatic FXR mRNA expression whereas renal KIM-1 mRNA expression was increased \( (p < 0.05) \) in APAP rats as compared to normal rats. Treatment with naringin (40 and 80 mg/kg, p.o.) showed significant amelioration \( (p < 0.05) \) in the alterations induced in hepatic FXR and renal KIM-1 mRNA expression as compared to APAP rats. When compared with APAP rats, silymarin (25 mg/kg, p.o.) treated rats showed a significant increase \( (p < 0.05) \) in hepatic FXR mRNA expression whereas there was significant decrease \( (p < 0.05) \) in renal KIM-1 mRNA expression.
expression. However, per se treated group did not show any significant alterations in hepatic FXR and renal KIM-1 mRNA expression as compared to normal rats (Figure 2).

Effect of naringin treatment on APAP-induced pathological alteration in rat liver and kidney

In the histopathological studies, normal control, as well as per se animals, showed normal central vein in liver parenchymal cells and without any signs of inflammation as well as necrosis in cells (Figure 3A and F, respectively). However, there was evidence of congestion (grade 1). The histopathological examination of liver from APAP administered rats was showed inflammatory cells (grade 4) and shrunken hepatocytes with chromatin condensation. Administration of APAP caused hepatocellular injury reflected by the presence of diffuse cytoplasmic vacuolation (grade 3), centrilobular necrosis (grade 4), vascular congestion (grade 3) and nuclear pyknosis (grade 3) of the hepatocytes. Macrovesicular fatty changes (grade 2) were also evident in liver histological observation of APAP rats (Figure 3B). However, silymarin (25 mg/kg) showed moderate histopathological changes in liver marked by sinusoidal congestion (grade 2), cytoplasmic vacuolation (grade 1) and the presence of only a few inflammatory cells (grade 1) along with few fatty globules (grade 1) (Figure 3C). In the naringin (40 mg/kg, p.o.) treated rats, the histology of liver showed a mild degree of vacuolation (grade 1), necrosis (grade 1) and congestion (grade 1) around central vein (Figure 3D). Moderate inflammatory cells with few plasma cells (grade 2) were present in naringin (80 mg/kg, p.o.) treated rats (Figure 3E) (Table 4).

Figure 4(A) and (F) showed the normal architecture of kidney from normal as well per se group. Chronic administration of APAP caused renal damage evident by glomerular structural disruption (grade 4) and partial endothelial rupture in the capsule. APAP animals showed the presence of intraluminal cell debris, karyorrhexis and pyknosis (grade 2) indicating cell death (Figure 4B). Treatment with silymarin (25 mg/kg) as well as naringin (80 mg/kg, p.o.) showed a moderate number of inflammatory (grade 1) and apoptotic cells (Figure 4C and E). Naringin (40 mg/kg, p.o.) treated rats showed mild architectural damage with few intraluminal cell debris (grade 2) and infiltration of inflammatory cells (grade 2) as compared to APAP (Figure 4E) (Table 4).

Discussion

Clinically, it has been found that high dose of APAP tightly associated with hepatic and renal calamity. Naringin has been reported to possess potent antioxidant, anti-hypercholesterolemic, free radical scavenging, metal chelating and anticancer properties. The aim of present study was to investigate the role of naringin against APAP-induced hepatic toxicity and renal toxicity. We observed that the APAP administration caused hepatic and renal toxicity by disturbing its antioxidant status, lipid profiles and histomorphological architecture.
Antioxidant such as SOD enzyme and GSH, a non-enzymatic antioxidant are the first line defense system that confines the toxicity allied with the free radicals.53–56 Antioxidants and ROS balance might be important for deletion of oxidative stress in intracellular organelles.57–59 Nonetheless, in the APAP-induced toxicity, ROS generation enormously deter this balance with an enhanced demand on the antioxidant protection system. Moreover, Lipid peroxidation associated with the increased free radicals production and/or decreased antioxidant defense system activities.60–62 Several lines of studies suggested that free radical scavenging enzymes, i.e., SOD and GSH decreased and MDA, a lipid peroxidation end product increased when administered with APAP.11 In line with previous studies53,64 the present study also found significantly decreased SOD and GSH levels as well as increased MDA level during APAP treatment in the hepatic and renal tissue. Naringin treatment significantly enhanced tissue SOD and GSH as well as decreased MDA levels, demonstrating that naringin could maintain the abnormal antioxidant and ROS generation, which in turn modulate the membrane integrity against the APAP-induced cellular injury.

Figure 4. Effect of naringin treatment on APAP-induced pathological alteration in rat kidney. Photomicrograph of sections of kidney of normal (A), APAP controls (B), silymarin (25 mg/kg) treated (C), naringin (40 mg/kg) treated (D), naringin (80 mg/kg) treated (E) and per se treated (F) rats. Glomerular hypertrophy (blue arrow), inflammatory infiltration (black arrow), edema (yellow arrow), congestion (green arrow) and necrosis (red arrow). H&E staining at 40× and 100× (inset).

Table 4. Effect of naringin treatment on APAP-induced pathological alteration in rat hepatic and renal histology.

| Treatment       | Normal | APAP control | Silymarin (25) | Naringin (20) | Naringin (40) | Naringin (80) | Per se |
|-----------------|--------|--------------|----------------|---------------|---------------|---------------|-------|
| **Hepatic changes** |
| Vesicular fat   | 0      | ++           | +              | ++            | ++            | ++            | +     |
| Inflammatory infiltration | 0      | +++          | +              | +++           | +++           | +++           | +     |
| Plasma cells    | 0      | ++          | +              | ++            | ++            | ++            | +     |
| Congestion      | +      | ++          | ++             | ++            | ++            | ++            | +     |
| Edema           | 0      | +++         | +              | ++            | ++            | ++            | +     |
| Vacuolization   | 0      | +++         | +              | ++            | ++            | ++            | +     |
| Pyknosis        | 0      | ++          | +              | ++            | ++            | ++            | +     |
| Necrosis        | 0      | +++         | 0              | +++           | +++           | +++           | +     |
| **Renal changes** |
| Glomerular hypertrophy | 0      | ++++       | ++             | +++           | +++           | +++           | +     |
| Inflammatory infiltration | 0      | +++         | +              | ++            | ++            | ++            | +     |
| Congestion      | 0      | +++         | ++             | ++            | ++            | ++            | +     |
| Edema           | +      | +++         | ++             | ++            | ++            | ++            | +     |
| Pyknosis        | 0      | ++          | +              | ++            | ++            | ++            | +     |
| Necrosis        | 0      | +++         | 0              | +++           | +++           | +++           | +     |

Notes: APAP: acetaminophen; 0: no abnormality detected; +: damage/active changes up to <25%; ++: damage/active changes up to <50%; +++: damage/active changes up to <75%; ++++: damage/active changes up to >75%.
higher dose of APAP resulted in significantly increased hepatic and renal NO levels as compared to normal rats. It in agreement with the number of previous studies which showed that generation of peroxynitrite free radical due to increase in NO levels might be responsible for the oxidative stress in APAP-induced hepatic and renal toxicity.69–71 In addition, earlier reports suggested that enhanced NO levels also attributed with GSH deple- tion.72–74 APAP-induced oxidative stress significantly decreased by naringin treatment via attenuation of increased hepatic and renal NO levels.

ALT and AST are important endogenous diagnostic biomarkers for hepatic toxicity. These two important enzymes found in high concentration in the cytoplasm and during hepatic insult leaks into the blood stream in conformity with the extent of hepatotoxicity. An increased concentration of AST and ALT in serum is an indicator of cellular leakage and loss of functional integrity of cell membrane in liver.75 In the current study, elevated ALT and AST levels in serum are the results of APAP-induced liver impairment. In our histopathological examination, we found fat accumulation and centrilobular necrosis of liver tissue of APAP rats. It has been also documented that APAP-induced hepatotoxicity caused ALT and AST overproduction due to hepatic parenchymal injury and increased alkaline phosphatase (ALP) synthesis.23,38 An earlier report also suggested that bile canaliculi cell lining caused ALP discharge in response to cholestasis and increased biliary pressure. Furthermore, serum bilirubin including total as well as direct bilirubin considered as a prognostic marker for the investigation of hepatic abnormalities. Liver parenchymal cells consumed bilirubin from the blood and in the presence of glucuronol transferase enzymes conjugates with glucuronic acid and then excreted into bile. Hyperbilirubinemia is an important consequence of excessive heme destruction and biliary tract obstruction. Previous studies revealed that hepatic parenchymal cells mishap are the main reason for the increased total and direct serum bilirubin.

LDH enzymes generally occur in the cytoplasmic cells and ooze out into the blood during apoptotic and necrotic cell death.30,76 Serum LDH considered as a prognostic marker for the investigation of hepatic injury.77 On the other hand, serum creatinine, uric acid, BUN also play very important role in the diagnosis of renal impair- ment.78 In the present study, APAP administration resulted in significantly increased LDH, creatinine, and BUN while, serum uric acid level was significantly decreased. These findings were significantly corroborated with the previous finding.38 In the current study, APAP administration significantly increases the serum total cholesterol, LDL, and triglycerides as well as decreases the HDL level. This result comes in agreement with the result of the previous investigator.54 In the past, it has been mentioned that APAP causes impairment in lipoprotein and cholesterol metabolism.79 Naringin treatment showed remarkable attenuation of altered serum lipid profile level which is in line with the previous investigation.80

Farnesoid X receptor also known as NR1H4, a ligand-activated transcription factor belonging to a category of nuclear receptor super-family. FXR greatly expressed in the liver, kidney, intestine and adrenal glands81 and involved in the expression of various proteins and bio- synthetic enzymes which are important for the physiological maintenance of cholesterol and bile acid homeostasis.82 It has been reported that FXR activation drop off the levels of liver bile acids, turn on Foxm1b and other genes responsible for the progression of cell cycle and stimulate liver regeneration.83 Previously; it has been suggested that FXR agonist treatment signifi- cantly protects the liver through FXR activation from APAP-induced toxicity in mice model.12 In addition, hep- atic steatosis and hypertriglyceridemia developed in FXR-deficient mice reflect the key role of FXR in the regulation of hepatic lipid metabolism.84 Similarly, in the present study, we also found that APAP-induced hepatic toxicity demur the expression of FXR, which was in accordance with the previous finding.12 Naringin treatment notably increases the FXR expression in APAP-induced hepatic toxicity as compared to APAP control group.

Liver fatty acid binding protein (L-FABP), an endo- genous antioxidant occurs inside the cytoplasm and nucleus of hepatocytes as well as also expressed in various tissues, such as kidney, intestine and heart.85 L-FABP bind with polyunsaturated fatty acids and long chain fatty acid peroxidation products, control the accessibility of fatty acids to oxidative pathways and thus monitor the ROS release.86,87 It has been demonstrated that L-FABP could act as an antioxidant through its free radical scav- enging property.88 In addition, the previous report also explained that L-FABP considered as a cytoprotective agent against oxidative stress in APAP-induced toxicity model. It has been well reported that APAP-induced toxicity may be associated with ROS generation.18 In our animal study, results suggest that APAP ingestion causes antioxidant depletion might be responsible for the decreased L-FABP due to excess generation of ROS. Till now, best of our knowledge this is the first animal study to explore the role of L-FABP in APAP-induced toxicity. Further studies on the role of L-FABP in APAP-induced toxicity are required. Treatment with naringin signifi- cantly increased the L-FABP, suggesting that this effect
might contribute to its protective effect against APAP-induced oxidative stress.

Ischemic or toxic insult, a series of events including loss of cytoskeleton integrity, apoptotic and necrotic cell casualty, cells proliferation and dedifferentiation undergoes in renal proximal tubule cells. KIM-1 with its unique feature, such as dedifferentiation of regenerating cells is one of the striking pathological manifestations for the kidney injury.90–91 Recently, in a mice model it has been shown that APAP-induced renal toxicity increases KIM-1 expression.91 Similarly, in our study, we also observed significantly increased KIM-1 expression in APAP-induced renal toxicity. The exact mechanism behind the enhanced KIM-1 expression in APAP-induced renal toxicity is still not elucidated, but from our histopathological investigation, we can conclude that elevated KIM-1 expression in the renal tissue could be due to oxidative stress followed by necrotic cell death. Whereas, administration of naringin appreciably ameliorated the APAP-induced increases in renal KIM-1 expression.

Conclusion
In conclusion, chronic APAP ingestion caused oxidative impairment in the hepatic and renal tissue of the experimental animals, and that could be averted by the treatment of naringin. Protective role of naringin could be due to its antioxidant, membrane stabilizing as well as anti-hyperlipidemic actions exerted through modulation of FXR and KIM-1 mRNA expression. In addition, results also suggest that naringin might be beneficial for the finding of therapeutic potential against APAP-induced hepatic and renal toxicity.

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Disclosure statement
There is no conflict of interest between any of the authors.

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