Original Research Paper

Effect of Combined Fenofibrate and Nicotinamide on Oxidative Stress and Inflammatory Cytokines Involved in Cisplatin-Induced Nephrotoxicity in Rats

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Abstract: Cisplatin (Cis) is an anticancer drug, which is accompanied with major side effects including nephrotoxicity. The current study was performed to assess the possible prophylactic effects of fenofibrate (FEN), Nicotinamide (NAM) and their combination on oxidative stress and inflammatory cytokines associated with cisplatin-induced renal damage. Rats were randomly divided into seven groups (8 each) as follows; control group; FEN group (100 mg/kg/day p.o.); NAM group (200 mg/kg/day p.o.); FEN and NAM were administered for eight days. Cis group (7 mg/kg i.p. as a single dose on day five); FEN + Cis group; NAM + Cis group and FEN + NAM + Cis group. Urine, blood and kidneys were taken out for biochemical and histopathological analysis and scoring. Oxidative stress induced by Cis was evidenced by significant elevation in renal Malondialdehyde (MDA) level acompanied by significant decrease in Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in kidney tissues. Moreover, Cis produced significant increase in kidney Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6), the proinflammatory cytokines and significant decrease in Interleukin-10 (IL-10), the anti-inflammatory cytokine. However, administration of either FEN or NAM attenuated cisplatin-induced increased oxidative stress and inflammation in the kidney of rats, associated with improvement of the impaired renal function and histopathological changes, but their combination was found to be more effective in protection against cisplatin-induced renal damage than each drug alone. In conclusion, FEN and NAM combination protected the kidney tissue against cisplatin-induced nephrotoxicity through their antioxidant and anti-inflammatory activities.

Keywords: Cisplatin, Fenofibrate, Nicotinamide, Nephrotoxicity

Introduction

Nephrotoxicity is associate adverse effect of the anticancer drugs for solid and hematologic malignancies (Kintzel, 2001). Cisplatin, cisplatinum or cis-diamminedichloroplatinum (II), a platinum-based drug, is one of the most frequently used anti-neoplastic agents. It has a potent anti-tumor action against wide range of malignancies, including testicular, ovarian, cervical, bladder and lung cancers as well as solid tumors resistant to other treatment regimens (Hanigan and Devarajan, 2003). The kidney is not only responsible for the excretion of cisplatin, but is also the primary site of its accumulation (Miller *et al.*, 2010).

Although several strategies have been suggested to prevent this adverse effect, no specific treatments are currently recommended, except for vigorous hydration with normal saline (Launay-Vacher *et al.*, 2008). Therefore, new and effective therapeutic strategies are needed for prevention of cisplatin-induced nephrotoxicity. The precise mechanisms underlying this toxicity are not fully elucidated. However, the production of nephrotoxic metabolites, vascular injury, inflammation, generation of free radicals and apoptotic pathways seem to play a crucial role (Pabla *et al.*, 2009; Miller *et al.*, 2010).

Peroxisome Proliferator-Activated Receptor-alpha (PPAR-α) is one of the members of nuclear receptor

PPAR family and is a modulator of inflammation (Daynes and Jones, 2002). Fenofibrate, a selective PPAR-α activator, has been primarily developed to treat human dyslipidemia (Willson *et al.*, 2000). It also improves insulin sensitivity and protects against microvascular events in patients with diabetes (Hahm *et al.*, 2010). Furthermore, fenofibrate exerts an antioxidant, anti-inflammatory and anti-ischemic protective effects on the heart (Yue *et al.*, 2003), intestine (Cuzzocrea *et al.*, 2004), brain (Chen *et al.*, 2007) and kidney (Bhalodia *et al.*, 2010).

Nicotinamide is the amide form of vitamin B3 (niacin), which is synthesized from nicotinic acid and is obtained through synthesis in the body or as a dietary source and supplement (DiPalma and Thayer, 1991). It is a precursor for the coenzyme ß-Nicotinamide Adenine Dinucleotide (NAD^+), which serves as an essential nutrient for cellular growth and participates in the regulation of multiple cellular functions including apoptosis (Klaidman *et al.*, 1996; Mukherjee *et al.*, 1997). Nicotinamide has been reported to exert a number of physiological and pharmacological effects including prevention of ATP depletion, inhibition of poly ADP- Ribose Polymerase (PARP), prevention of apoptosis and suppression of lipid peroxidation (Ogata *et al.*, 2002; Klaidman *et al.*, 2003). Also, it has been shown that nicotinamide exerts the anti-inflammatory properties both in-vitro and in-vivo (Fernandes *et al.*, 2011).

In light of the foregoing data, the current study was designed to evaluate the possible prophylactic effects of fenofibrate, nicotinamide and their combination on oxidative stress and inflammatory cytokines associated with cisplatin-induced nephrotoxicity.

Materials and Methods

Animals

Adult male Sprague- Dawley rats weighing 180- 200 g were purchased from Helwan farm (VACSERA), Egypt. The animals were housed (4 per cage), in the animal facility of Faculty of Medicine, Benha University, Egypt, for one week before the onset of the experiment for acclimatization. Rats were kept under the standard laboratory conditions (12 h light/dark cycles at 25±2°C) with free access to standard balanced diet and fresh-water supply. All animal experiments were approved by the ethical committee (Faculty of Medicine, Benha University, Egypt).

Drugs

Fenofibrate (FEN) and Nicotinamide (NAM) were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). FEN was dissolved in water containing 0.5% carboxymethyl cellulose while NAM was dissolved in water, so that 1 mL of the vehicle contained the desired dose. Drugs were given by the oral route through an orogastric catheter. FEN and NAM solutions were freshly prepared for each use. Cisplatin (Cis) (10 mg/10 mL), product of Aventis Pharma, in the form of solution diluted with saline. All other chemicals and reagents used in the present study were of analytical grade.

Experimental Design

The rats were arbitrarily divided into seven groups, each group containing eight rats as follows: Control group, rats received 1 mL of water containing 0.5% carboxymethyl cellulose daily for eight days (the study period) with single i.p. injection of 1 mL saline on day five; FEN group, received FEN 100 mg/kg/day orally (Camara-Lemarroy *et al.*, 2009) for eight days; NAM group, received NAM 200 mg/kg/day orally (John *et al.*, 2012) for eight days. Cis group, received single i.p. dose of Cis (7 mg/kg b.w.), the dose of Cis was selected according to previous work that demonstrated significant damage in renal parameters of rats (Atessahin *et al.*, 2006); FEN + Cis group, received FEN 100 mg/kg/day orally for eight days with single i.p. dose of Cis (7 mg/kg b.w.) on day five of the study period; NAM + Cis group, received NAM 200 mg/kg/day orally for eight days with single i.p. dose of Cis (7 mg/kg b.w.) on day five; FEN + NAM + Cis group received FEN 100 mg/kg/day p.o. and NAM 200 mg/kg/day p.o. for eight days with single i.p. dose of Cis (7 mg/kg b.w.) on day five of the study period.

Samples Preparation

At the end of the study period (72 h after Cis injection), rats were individually housed in metabolic cages, animals were fasted and allowed free access to water only, for 24 h urine collection. The volume of the urine samples were measured and recorded. Creatinine concentration (Cr), protein and urinary N-Acetyl-ß-Glucosaminidase (NAG) were estimated. The following day, rats were sacrificed under ether anesthesia, a midline abdominal incision was performed and blood was collected from the abdominal aorta. Samples were collected in clean and dry centrifuge tubes, which were left for 15 min to clot and then centrifuged at 3000 rpm for 15 min to separate the serum. They were used for biochemical analysis. At the same time, kidneys were removed, washed with physiological saline. Part of kidney was homogenized in ice cold 100 mM phosphate buffer (pH 7.4). Homogenates were centrifuged and the resulting supernatant was used for biochemical analysis. The other part was preserved in 10% formalin for histopathological examination.

Biochemical Analysis

Serum urea and Cr were determined colorimetrically as described by (Fawcett and Scott, 1960; Fabiny and Ertingshausen, 1971) respectively, additionally total protein contents in 24 h urine samples were determined

according to the method of Nishi and Elin (1985) using commercially available diagnostic kits from Biodiagnostic Company, Giza, Egypt. NAG activity was assayed in urine using a colorimetric assay according to the method of Wellwood *et al.* (1976) using commercially available diagnostic kits from the Egyptian American Company for Laboratory Services, Egypt. Urine Cr was also determined colorimetrically as described by Fabiny and Ertingshausen (1971) for calculation of creatinine clearance (Crcl) using the standard Equation:

$$Crcl = \frac{mg\,creatinine/100\,ml\,urine\,x\,ml\,urine/24\,hrs}{mg\,creatinine/100\,ml\,serum\,x\,1440}\ ml/min$$

Renal lipid peroxidation product, Malondialdehyde (MDA) was measured in renal tissue homogenates by the method described by Ellman (1959), Superoxide Dismutase (SOD) activity was estimated according to Beauchamp and Fridovich (1971) and Glutathione Peroxidase (GPx) activity was measured according to Flohé and Gunzler (1984) using commercially available diagnostic kits from Biodiagnostic Company, Giza, Egypt.

Levels of interleukin (IL)-6, IL-10 and Tumor Necrosis Factor-alpha (TNF-α) in supernatants from kidney tissue homogenates were quantitated by Enzyme-Linked Immunosorbent Assay (ELISA) using Quantikine rat specific kits; R and D Systems Inc., Minneapolis, Minnesota, USA, which were obtained from Clinilab Company, Cairo, Egypt, according to manufacturer's directions.

Histopathological Evaluation of the Kidney

Kidneys were fixed in paraformaldehyde (4%) and embedded in paraffin. Tissue sections (4 µm) were placed on slides, stained with Hematoxylin and Eosin (H and E) and the morphology was analyzed under light microscopy. Tubular injury was assessed using semi-quantitative scale (Ramesh and Reeves, 2004). Scores was assigned according to the percentage of cortical tubules having epithelial necrosis as follows: 0, 0%; 1, <10%; 2, 10%-25; 3, 26%-75; or 4, >75%. Ten fields per kidney under high-power magnification were assessed and then assigned as necrosis severity scores.

Statistical Analysis

Data are expressed as mean ± S.D. Statistical comparison between different groups was conducted using one-way analysis of variance (ANOVA) followed by a student's *t*-test to judge the difference among values of individual parameters using the Statistical Program of Social Sciences (SPSS) for windows (version 16; SPSS, Chicago, IL, USA). Significance was accepted at p<0.05.

Results

Biochemical Results

At the end of experiment, there were no statistically significant changes in all measured biochemical parameters between FEN-or NAM-treated groups compared with the control group (p>0.05).

Effect on Urine Volume

As illustrated in Fig. 1, single dose of cisplatin elicited a significant (p<0.001) increase in urine volume as compared to control rats. Treatment with FEN or NAM plus Cis was able to reduce this increase (p<0.05) compared to Cis group. Also, combination of FEN and NAM plus Cis significantly (p<0.001) decreased the volume of the urine collected 24 h when compared to Cis group. Administration of FEN and NAM in combination plus Cis resulted in significant (p<0.05) decrease in urine volume when compared to FEN plus Cis group and NAM plus Cis group.

Effect on Urinary Protein and NAG

The data presented in Table 1, revealed that the urinary protein level and NAG were significantly increased (p<0.0001) in the Cis-treated group compared to the control group. Compared to Cis group, protein and NAG levels in FEN plus Cis group were significantly (p<0.0001, 0.001) reduced. Similarly, NAM plus Cis group showed significant (p<0.0001, 0.05) decrease in their levels. At the same time, combined administration of FEN and NAM plus Cis showed significant (p<0.0001) decrease in urinary protein and NAG levels. The rats pretreated with FEN and NAM plus Cis showed a significant decrease in urinary protein (p<0.001) and urinary NAG (p<0.05) when compared with rats pretreated with FEN plus Cis. Also, FEN and NAM plus Cis group showed a significant decrease in urine protein (p<0.0001) and urinary NAG (p<0.001) when compared with NAM plus Cis group.

Effect on Urea, Cr and Crcl

Cis-induced nephrotoxicity was reflected by the significant increase in serum urea level (p<0.0001) and serum Cr (p<0.01) compared to control rats. The concentrations of serum urea and Cr in the FEN plus Cis-treated group were significantly (p<0.01, 0.05) reduced with respect to Cis-treated group. The same results were observed in NAM plus Cis-treated group with significant decrease in concentrations of serum urea (p<0.05) and Cr (p<0.05) in comparison with Cis-treated group. Administration of FEN and NAM plus Cis produced significant reduction in urea (p<0.0001) and Cr (p<0.01) when compared to Cis-treated rats. Combination of FEN and NAM plus Cis resulted in significant decrease in urea (p<0.01) and Cr (p<0.05) when compared to FEN plus Cis group. At the same time, combined administration of FEN and NAM resulted in significant decrease in urea (p<0.001) and Cr (p<0.01) when compared to NAM plus Cis group (Table 1).
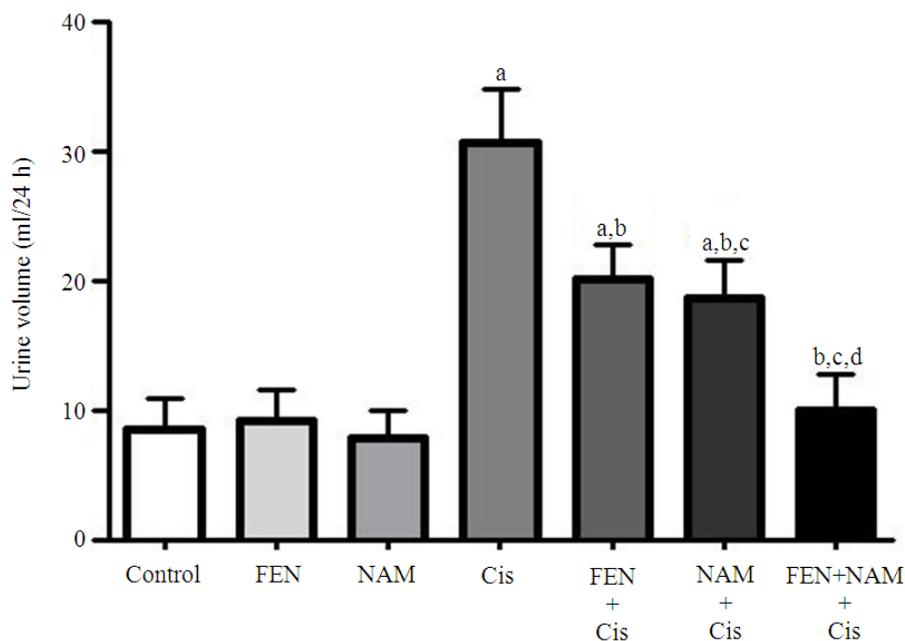
Fig. 1. Effect of administration of Fenofibrate (FEN), Nicotinamide (NAM) alone or in combination on urine volume in Cisplatin (Cis)-induced nephrotoxicity in adult albino rats; Values represent (Mean ± SE), n = 8 rats/group; a: Significantly different as compared to the control group; b: Significantly different as compared to Cis group; c: Significantly different as compared to FEN + Cis group; d: Significantly different as compared to NAM + Cis group

Table 1. Effect of fenofibrate (FEN) and nicotinamide (NAM) alone or in combination on serum urea, creatinine (Cr), urinary N-Acetyl-β-Glucosaminidase (NAG) and total protein in cisplatin (Cis)-induced nephrotoxicity in adult albino rats

Groups	Urine		Serum	
	NAG (U/mL)	Protein (mg/24 h)	Urea (mg/dL)	Cr (mg/dL)
Control	8.57±0.96	25.3±1.24	28.8±1.6	0.86±0.21
FEN	9.26±0.87	23.9±0.74	29.4±1.8	0.85±0.32
NAM	9.04±1.10	25.6±0.99	26.9±1.5	0.92±0.18
Cis	43.6±2.8[a]	75.2±1.70[a]	70.9±4.1[a]	3.94±0.85[a]
FEN+ Cis	31.3±1.6[a,b]	54.1±1.30[a,b]	54.2±3.2[a,b]	1.87±0.34[a,b]
NAM+ Cis	36.2±1.9[a,b]	58.8±1.27[a,b,c]	59.1±2.9[a,b]	2.01±0.25[a,b]
FEN+NAM+ Cis	24.4±2.2[a,b,c,d]	46.9±1.20[a,b,c,d]	42.6±2.3[a,b,c,d]	1.05±0.17[b,c,d]

Values represent (Mean ± SE), n = 8 rats/group; a: Significantly different as compared to the control group; b: Significantly different as compared to Cis group; c: Significantly different as compared to FEN + Cis group; d: Significantly different as compared to NAM + Cis group

Crcl is used to estimate the glomerular filtration rate as it compares the level of Cr in urine with the Cr level in the blood. Cis-induced nephrotoxicity was manifested by marked reduction in Crcl ($p < 0.0001$) compared to control rats. Significant ($p < 0.001$) elevation of Crcl in FEN plus Cis-treated group was noticed with respect to Cis-treated group. The same results were recorded in NAM plus Cis-treated group with significant ($p < 0.05$) increase in Crcl in comparison with Cis-treated group. Combination of FEN and NAM plus Cis resulted in significant increase in Crcl ($p < 0.0001$) when compared to Cis group, ($p < 0.05$) when compared to FEN plus Cis group ($p < 0.05$) and significant increase in Crcl ($p < 0.05$) when compared to NAM plus Cis group (Fig. 2).

Effect on Lipid Peroxidation and Antioxidant Enzymes

MDA level, as a marker of free radical mediated lipid peroxidation injury, was significantly ($p < 0.0001$) elevated in the kidney homogenate of Cis-treated group compared with that of the normal control group. The administrations of FEN and NAM alone or in combination before Cis administration resulted in significant ($p < 0.0001$) decrease in MDA level as compared to Cis group. Combination of FEN and NAM plus Cis resulted in significant decrease in MDA level when compared to FEN plus Cis group ($p < 0.01$) and NAM plus Cis group ($p < 0.05$) (Table 2).
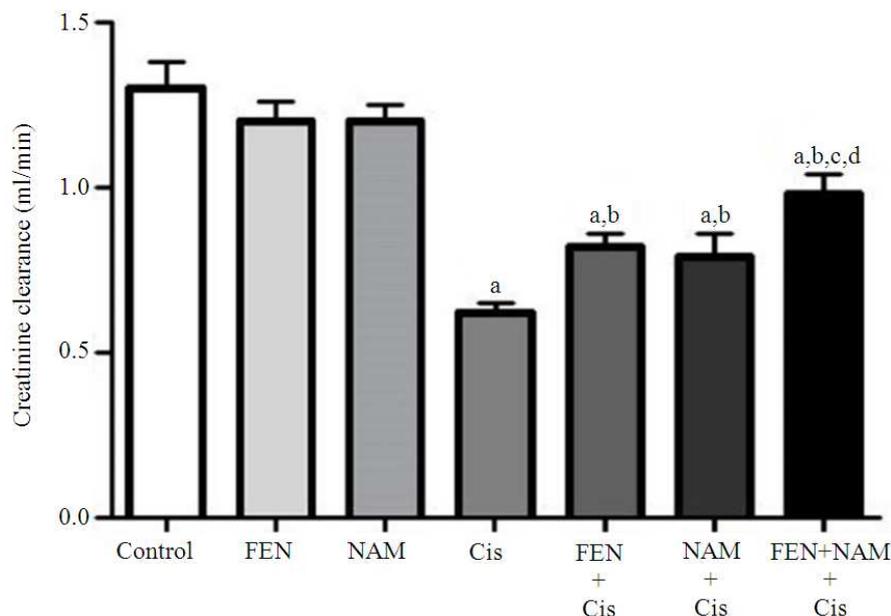
Fig. 2. Effect of administration of Fenofibrate (FEN), Nicotinamide (NAM) alone or in combination on creatinine clearance in Cisplatin (Cis)-induced nephrotoxicity in adult albino rats; Values represent (Mean ± SE), n = 8 rats/group; a: Significantly different as compared to the control group; b: Significantly different as compared to Cis group; c: Significantly different as compared to FEN + Cis group; d: Significantly different as compared to NAM + Cis group

Table 2. Effect of fenofibrate (FEN) and nicotinamide (NAM) alone or in combination on malondialdehyde (MDA), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in cisplatin (Cis)-induced nephrotoxicity in adult albino rats

Groups	MDA (nmol/mg protein)	SOD (U/mg)	GPx (IU/G)
Control	0.48 ± 0.03	22.6 ± 0.97	29.7 ± 0.87
FEN	0.46 ± 0.01	22.3 ± 1.2	29.4 ± 1.1
NAM	0.49 ± 0.01	24.8 ± 1.1	28.9 ± 0.96
Cis	2.03 ± 0.07^{a}	10.7 ± 0.63^{a}	16.7 ± 0.66^{a}
FEN+ Cis	$0.86 \pm 0.05^{a,b}$	$13.9 \pm 0.86^{a,b}$	$20.5 \pm 0.85^{a,b}$
NAM+ Cis	$0.73 \pm 0.03^{a,b,c}$	$17.2 \pm 0.84^{a,b,c}$	$23.1 \pm 0.74^{a,b,c}$
FEN+NAM+ Cis	$0.62 \pm 0.05^{a,b,c,d}$	$20.6 \pm 1.3^{b,c,d}$	$26.3 \pm 1.2^{a,b,c,d}$

Values represent (Mean ± SE), n = 8 rats/group; a: Significantly different as compared to the control group; b: Significantly different as compared to Cis group; c: Significantly different as compared to FEN + Cis group; d: Significantly different as compared to NAM + Cis group

Compared to the control group, there were statistically significant ($p<0.0001$) decrease in the SOD activity in the Cis-treated rats. However, SOD activity was significantly increased in FEN plus Cis ($p<0.01$), NAM plus Cis- ($p<0.0001$) and FEN and NAM and Cis in combination ($p<0.0001$) compared with the Cis-treated rats. Renal SOD activity was increased significantly in rats administered combination of FEN and NAM plus Cis as compared to rats administered FEN plus Cis ($p<0.001$) or NAM plus Cis ($p<0.05$) (Table 2).

Table 2 shows the changes of GPx in all groups. Cis produced a significant ($p<0.0001$) reduction in renal GPx activity compared with that in the control group while treatment with FEN or NAM plus Cis significantly increased ($p<0.01$, 0.0001) its level when compared to Cis-treated group. Combination of FEN and NAM plus Cis resulted in significant increase ($p<0.0001$) compared to Cis-treated group. At the same time, combination of FEN and NAM plus Cis showed a significant increase ($p<0.01$) in GPx activity when compared to FEN plus Cis group and a significant increase ($p<0.05$) when compared to NAM plus Cis group.

Effect on TNF-α, IL-6 and IL-10

Cis induced significant ($p<0.0001$) increase in renal TNF-α compared to control rats. Administration of either FEN or NAM to rats treated with Cis resulted in significant ($p<0.001$, 0.01) decrease in tissue TNF-α compared to Cis treated group. Combined administration of FEN and NAM plus Cis resulted in significant decrease in TNF-α compared to Cis-treated rats ($p<0.0001$), FEN plus Cis-treated rats ($p<0.001$) and NAM-treated rats ($p<0.0001$) (Fig. 3).

Cis significantly (p<0.0001) increased renal IL-6 compared to control group. A significant decrease in IL-6 level was observed in the rats treated with FEN plus Cis (p<0.001) and NAM plus Cis (p<0.05) and combined FEN and NAM plus Cis (p<0.0001) compared with the Cis group. Combined administration of FEN and NAM plus Cis resulted in significant decrease in IL-6 when compared to FEN plus Cis (p<0.0001) or NAM plus Cis-treated group (p<0.0001) (Fig. 4).

Conversely, Cis significantly (p<0.0001) decreased renal IL-10 compared to control group. FEN or NAM alone or in combination plus Cis significantly (p<0.001, 0.05) increased its level compared to Cis group. Combined administration of FEN and NAM plus Cis resulted in significant increase in IL-10 when compared to FEN plus Cis (p<0.0001) or NAM plus Cis- treated group (p<0.001) (Fig. 5).

Histopathological Results

Kidneys of control rats showed normal architecture of renal tissue, being composed of a number of glomeruli embedded among a great number of proximal and distal convoluted tubules. Proximal convoluted tubules are lined by pyramidal cells with narrow lumen and distal convoluted tubules are lined with cubical cells with narrow lumen (Fig. 6). No lesions were observed in the sections of FEN- and NAM-treated rats. The histological appearance of these groups was similar to that of the control group (are not shown). By contrast, Cis-treated rats showed a pattern of extensive acute tubular necrosis, which included widespread degeneration of the tubular architecture, tubular dilatation, vacuolar degeneration, detachment and sloughing of epithelial cells from the basement membrane, intratubular cast formation and luminal congestion with extensive loss of the brush border. Moreover, interstitial hemorrhage, inflammatory cellular infiltration and pyknotic nuclei were detected (Fig. 7 to 10). A high microscopic damage score of Cis-treated rats (3.81±0.11) was determined in the histological sections when compared to control group (0.11±0.03) with a significant difference (p<0.0001).

Kidney specimens from rats pretreated with FEN plus Cis revealed significant improvement of some renal tubules in the form of mild tubular dilatation with more or less normal cells and presence of interstitial hemorrhage compared with the Cis-treated group (Fig. 11). In sections of NAM plus Cis-treated rats, there was mild proximal tubular necrosis with luminal dilatation and mononuclear cellular infiltration were seen (Fig. 12). The microscopic score of FEN plus Cis and NAM plus Cis groups were found to be significantly reduced (2.13±0.08 and 2.43±0.09, respectively) when compared to the Cis group (3.81±0.11) (p<0.0001).

Kidney sections of the rats pretreated with combination of FEN and NAM plus Cis revealed preservation of tubular histology. The histopathological renal damage induced by Cis was minimal in animals received FEN or NAM treatment alone or with combination (Fig. 13).

In FEN and NAM plus Cis group: The histopathological score (0.23±0.05) was significantly (p<0.0001) reduced when compared to Cis group (3.81±0.11). This reduction was significant when compared to FEN plus Cis group (p<0.0001) and NAM+ Cis group (p<0.0001) (Fig. 14).
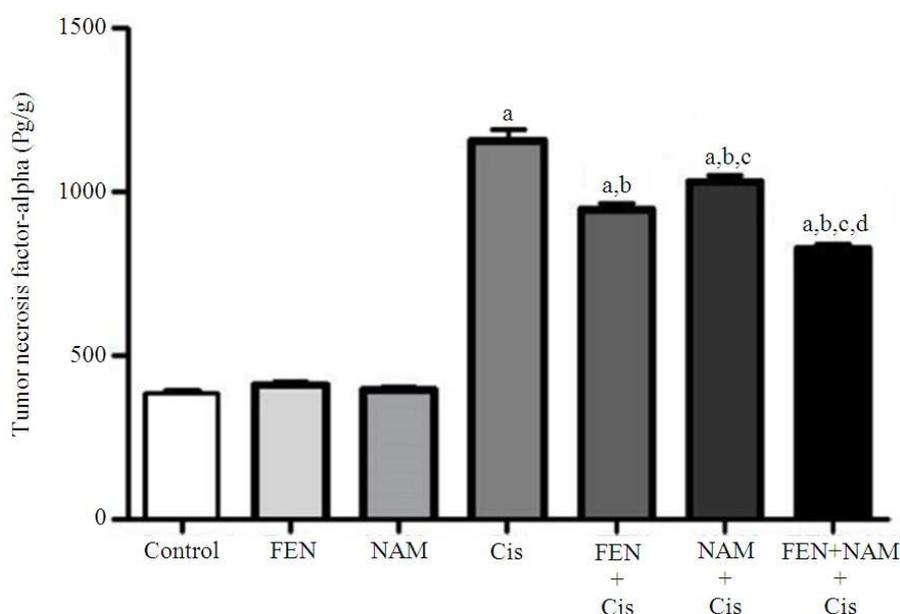

Fig. 3. Effect of administration of Fenofibrate (FEN), Nicotinamide (NAM) alone or in combination on renal Tumor Necrosis Factor-α (TNF-α) in Cisplatin (Cis)-induced nephrotoxicity in adult albino rats; Values represent (Mean ± SE), n = 8 rats/group; a: Significantly different as compared to the control group; b: Significantly different as compared to Cis group; c: Significantly different as compared to FEN + Cis group; d: Significantly different as compared to NAM + Cis group
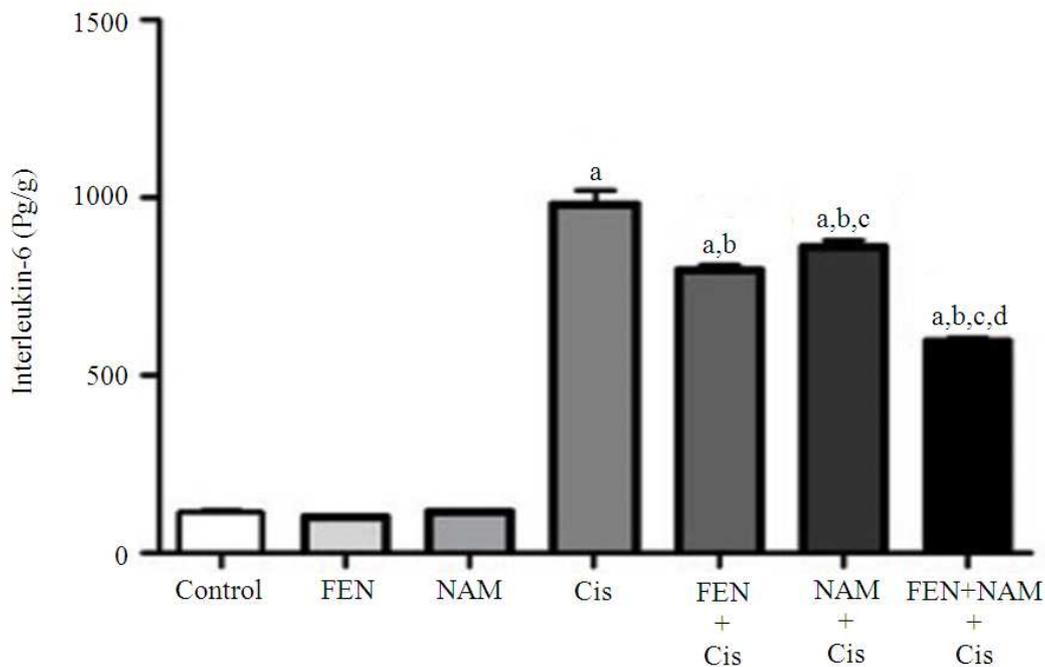
Fig. 4. Effect of administration of Fenofibrate (FEN), Nicotinamide (NAM) alone or in combination on renal Interleukin-6 (IL-6) in Cisplatin (Cis) -induced nephrotoxicity in adult albino rats; Values represent (Mean ± SE), n = 8 rats/group; a: Significantly different as compared to the control group; b: Significantly different as compared to Cis group; c: Significantly different as compared to FEN + Cis group; d: Significantly different as compared to NAM + Cis group
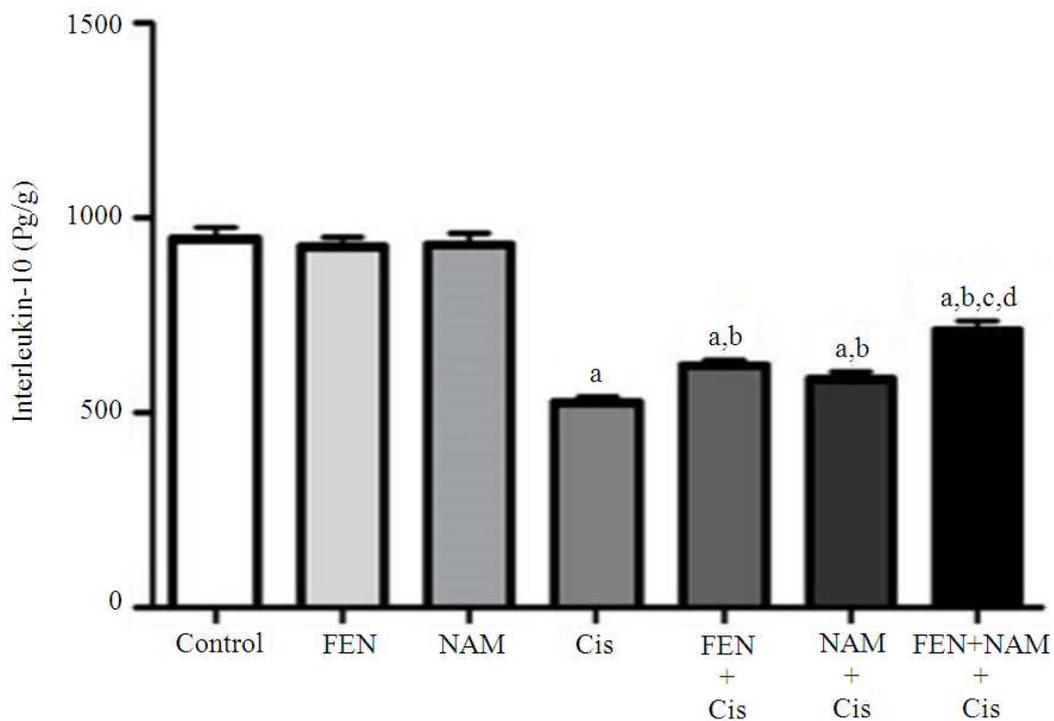

Fig. 5. Effect of administration of Fenofibrate (FEN), Nicotinamide (NAM) alone or in combination on renal Interleukin-10 (IL-10) Cisplatin (Cis)- induced nephrotoxicity in adult albino rats; Values represent (Mean ± SE), n = 8 rats/group; a: Significantly different as compared to the control group; b: Significantly different as compared to Cis group; c: Significantly different as compared to FEN + Cis group; d: Significantly different as compared to NAM + Cis group.
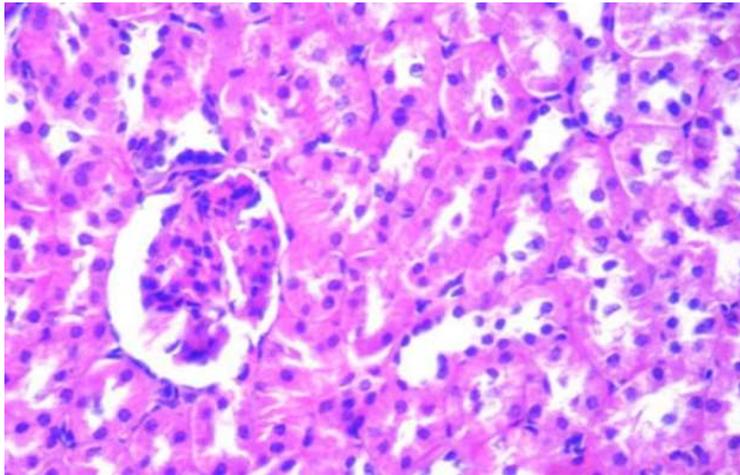
Fig. 6. A photomicrograph of kidney of control rats showing normal glomeruli, distal and proximal convoluted tubules (H and E ×400)
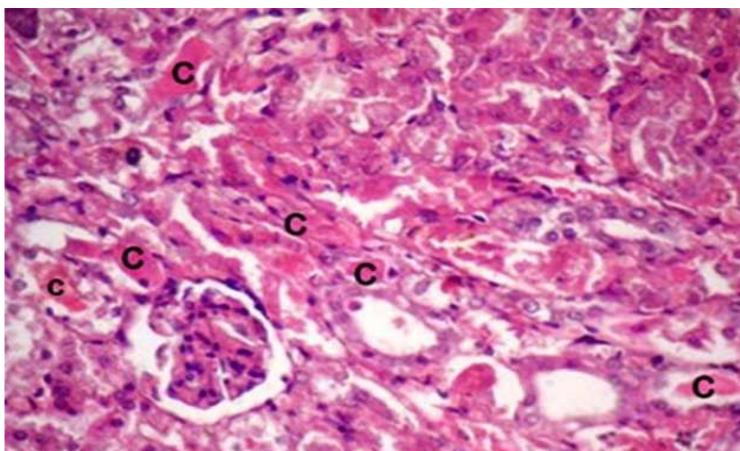

Fig. 7. A photomicrograph of kidney of cisplatin-treated rats showing acute tubular necrosis with intratubular Cast formation (C) (H and E ×400)
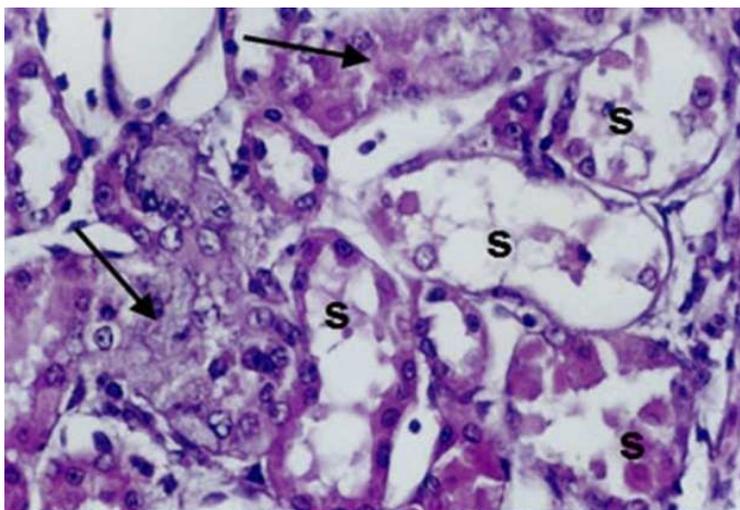

Fig. 8. A photomicrograph of kidney of cisplatin-treated rats showing acute tubular necrosis characterized by dilatation of tubules, epithelial flattening, brush border loss and Sloughing of cells in some of them (S) and complete denudation of others with complete obstruction of the lumen (arrow) (H and E ×400)
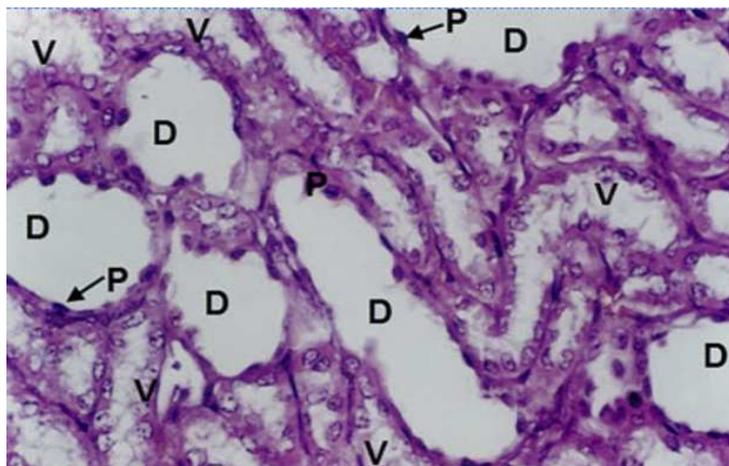
Fig. 9. A photomicrograph of kidney of cisplatin-treated rats showing acute tubular necrosis characterized by severe tubular Dilatation (D) with flattening of epithelial cells, Vacuolar degeneration (V) and Pyknotic nuclei (P). (H and E ×400)
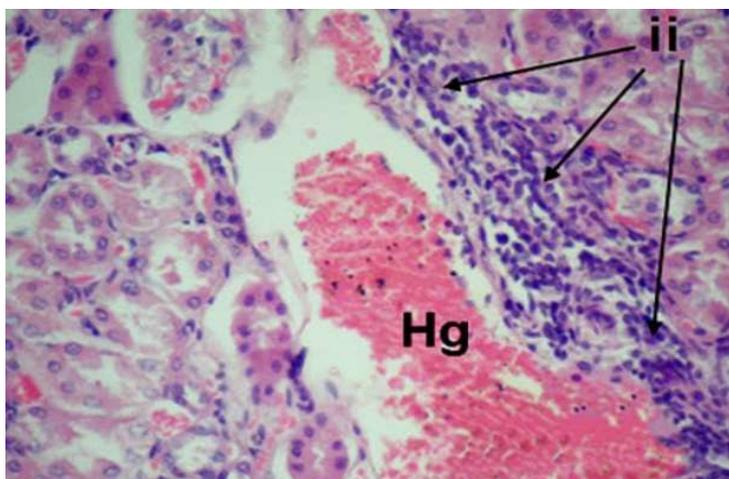

Fig. 10. A photomicrograph of kidney of cisplatin-treated rats showing acute tubular necrosis, interstitial Hemorrhage (Hg) and inflammatory cellular infiltration (ii) (H and E ×400)
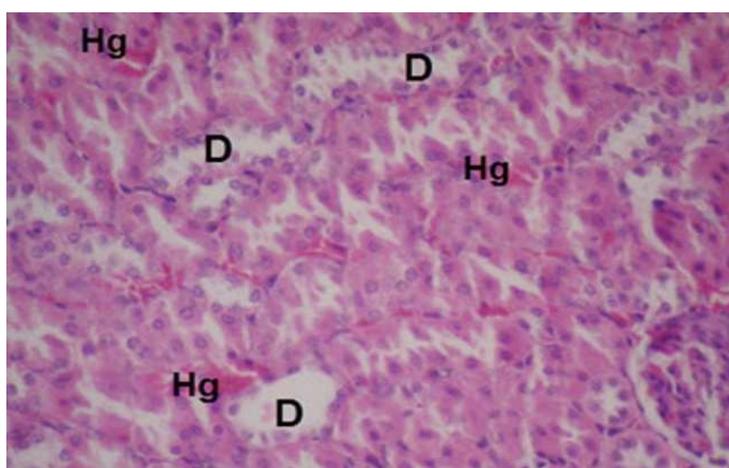

Fig. 11. A photomicrograph of kidney of fenofibrate plus cisplatin-treated rats showing mild Dilatation of some tubules (D) and slight interstitial Hemorrhage (Hg) (H and E ×400)
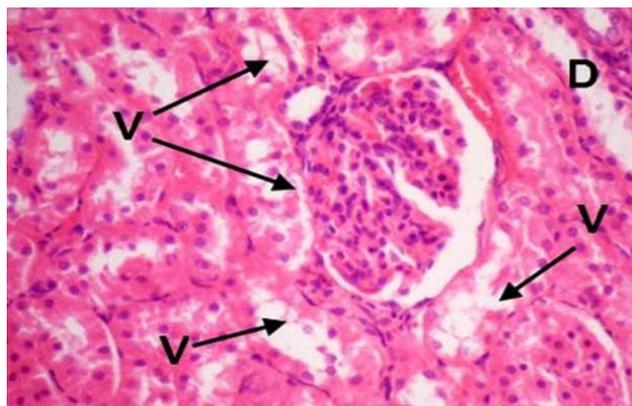
Fig. 12. A photomicrograph of kidney of nicotinamide plus cisplatin-treated rats showing moderate tubular Dilatation (D). Lining epithelium of some tubules were Vacuolated (V) and others are normal (H and E ×400)

Fig. 13. A photomicrograph of kidney of fenofibrate and nicotinamide plus cisplatin-treated rats showing marked improvement of histological appearance with almost normal histological structure (H and E ×400)

Fig. 14. Renal tubular damage score in control, Fenofibrate (FEN), Nicotinamide (NAM), Cisplatin (Cis), FEN plus Cis, NAM plus Cis-, combined FEN and NAM plus Cis-treated rats; Values represent (Mean ± SE), n = 8 rats/group; a: Significant when compared to the control group; b: Significant when compared to Cis group; c: Significant when compared to FEN + Cis group; d: Significant when compared to NAM + Cis group

Discussion

In the present study, we have evaluated the prophylactic effects of FEN, NAM and their combination on oxidative stress and inflammatory cytokines associated with Cis-induced renal damage and consequent kidney dysfunction in rats. Induction of nephrotoxicity by Cis is a rapid process involving reaction with proteins in the renal tubules (Howe-G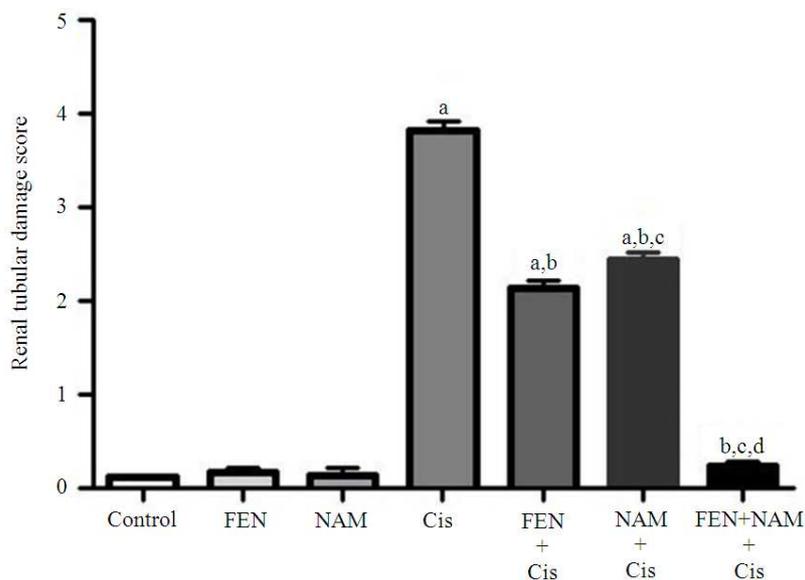rant and Lippard, 1980). Because this renal damage occurs in the first hour after administration, it is important that the protective agent is present at sufficient concentrations in renal tissue before the damage occurs (Elferink *et al.*, 1986). This is the rationale behind the prophylactic treatment.

The results of the current study revealed that treatment of rats either with FEN or NAM before Cis administration attenuated Cis-induced increased oxidative stress and inflammation in the kidney of rats, associated with improvement of compromised renal function and histopathological changes. However, the combination of FEN and NAM was found to be more effective in protection against Cis-induced nephrotoxicity than each drug alone.

This study showed that single injection of Cis in rats resulted in deterioration of renal function as evidenced by significant elevation in serum urea, Cr, urinary NAG, urinary protein and significant decrease in Crcl with increase in urine output. These results are consistent with the previous studies on Cis-induced nephrotoxicity in experimental animals (Sinha *et al.*, 2013; Kimoto *et al.*, 2013). It has been reported that Cis administration causes a decrease in renal blood flow by renal vasoconstriction as well as ischemic damage in the proximal tubules and distorts its ability to reabsorb water and cause polyuria, which can also lead to polydipsia (Wong *et al.*, 1993). Recent studies reported that Cis administration leads to increase urine output (Ali *et al.*, 2008; Abdelrahman *et al.*, 2010). The pathological findings i.e., the tubular necrosis in kidney of Cis treated animals are giving further evidence to the renal damage caused by Cis.

Several studies have demonstrated the important role of oxidative stress (Davis *et al.*, 2001), inflammation (Faubel *et al.*, 2007) and cytokine activity (Ramesh and Reeves, 2002; Zhang *et al.*, 2007) in the pathogenesis of Cis-induced nephrotoxicity. It has been suggested that Cis-induced oxidative stress via its binding to the renal base transport system resulted in peroxidation of membrane lipids and subsequently generation of Reactive Oxygen Species (ROS) (Baek *et al.*, 2003). Mukhopadhyay *et al.* (2011) reported that Cis triggers oxidative stress in the mitochondria of kidney proximal tubular and endothelial cells, which is followed by ROS generation, deterioration of mitochondrial structure and function, an intense inflammatory response, cell death,

kidney dysfunction and nephropathy. Inflammation may further amplify oxidative/nitrative stress and these interrelated processes eventually culminate in more concerted renal tubular and endothelial cell death.

Concerning oxidative stress, our results showed that administration of Cis resulted in a marked decrease in activity of antioxidative enzymes: SOD and GPx with concomitant significant increase in MDA level in renal tissue homogenate. This finding is in agreement with previous reports demonstrating that Cis induces oxidative stress in renal tissue as evidenced by increasing MDA level and decreasing SOD and GPx activities (Lee *et al.*, 2013a; Pandir and Kara, 2013). Both FEN and NAM reduced oxidative stress caused by Cis in renal tissue. However, their combination was found to be more effective than each drug alone.

FEN, a PPAR-α activator, is a drug of the fibrate class, used to treat dyslipidemia. PPAR-α plays an important role in the physiological and pathological responses involving oxidative stress and antioxidant enzymes (Schrader and Fahimi, 2006). PPAR- activation, using pharmacological ligands has been shown to reduce Ischemia/Reperfusion (I/R) injury in the liver and kidney in experimental models (Sivarajah *et al.*, 2002; Xu *et al.*, 2008). Studies suggest that similar to ischemia-reperfusion, Cis injury can cause endothelial cell dysfunction and neutrophil infiltration, which then leads to the release of cytokines/chemokines (Bonventre, 2004; Friedewald and Rabb, 2004).

In this study, FEN treatment was associated with a preservation of serum antioxidant capacity and reduction in MDA level. This suggests that FEN acts also through anti-oxidant pathways. This result is supported by the finding of Hou *et al.* (2010) who concluded that FEN treatment markedly reduced oxidative stress accompanied by reduced activity of renal NAD (P) H oxidase and increased activity of SOD in the kidney of spontaneously hypertensive rats, consequently, it can protect against hypertensive renal injury by inhibiting inflammation and fibrosis via suppression of oxidative stress. In addition, Kadian *et al.* (2013) suggested a protective effect of low-dose FEN pretreatment against biochemical (raised serum Cr level, blood urea nitrogen and microalbuminuria) and histological changes (glomerular capsular wall distortion, mesangial expansion and tubular damage) of diabetic nephropathy in streptozocin-induced diabetic rats. Several studies demonstrated that oxidative stress in diabetic kidneys can be decreased by FEN (Chen and Quilley, 2008; Hiukka *et al.*, 2010; Bishnoi *et al.*, 2012).

NAM has been shown to have antioxidant activity. Besides inhibiting protein oxidation and lipid peroxidation, it also inhibits ROS-induced apoptosis (Cuzzocrea *et al.*, 2001). The findings of many researches confirmed the antioxidant effect of NAM. A study by Ayla *et al.* (2011) revealed that pretreatment

with NAM protected renal tissue against doxorubicin-induced nephrotoxicity via its antioxidant action as it increased the activities of glutathione, catalase, GPx and protein oxidation levels in renal tissue. Also, NAM prevented the histopathological changes occurred due to doxorubicin toxicity in rat kidney. Piro *et al.* (2002) and recently Lee *et al.* (2013b) concluded that NAM was able to diminish beta cell apoptosis and suggested that protective effect of NAM on beta cell glucolipotoxicity is attributed to its antioxidant activity.

Previous studies have shown increased tissue content of inflammatory mediators together with inflammatory cell infiltration, suggesting that inflammation plays an important role in Cis-induced renal injury (Faubel *et al.*, 2007; Lu *et al.*, 2008). Cis induced marked up-regulation of TNF-α and IL-1β mRNA in the kidney (Ramesh and Reeves, 2002; Zhang *et al.*, 2007). Zhang *et al.* (2007) demonstrated that local production of TNF-α by renal parenchymal cells likely promotes the elaboration of chemokines and an influx of inflammatory cells resulting in functional and structural damage.

Although the precise inflammatory mechanisms are unknown, marked attenuation of Cis-induced renal damage by inhibition of TNF-α indicates that it has a central role in mediating Cis induced inflammatory renal injury (Ramesh and Reeves, 2003). TNF-α reduces renal blood flow and glomerular filtration rate by acting as a vasoconstrictor and can cause natriuresis by inhibiting renal epithelial sodium channel activity (Shahid *et al.*, 2010; Majid, 2011).

The results of the present study also showed that Cis enhance tissue inflammation with increase in various inflammatory mediators. We observed that the levels of TNF-α and IL-6 are markedly increased in Cis-treated kidneys. In contrast to proinflammatory mediators, the IL-10 level decreased markedly in renal tissues of Cis-treated rats. IL-10 is a well-known anti-inflammatory cytokine that has been known to suppress proinflammatory cytokines and chemokines (Rossato *et al.*, 2012), so these findings suggested that decreased endogenous IL-10 release might contribute to enhanced inflammation and subsequent tissue injury by Cis. Pretreatment of rats with either FEN or NAM significantly improved endogenous IL-10 release with simultaneous suppression of TNF-α and IL-6 in renal tissues. However, their combination was found to be more effective than each drug alone in restoring the balance between proinflammatory and anti-inflammatory cytokines.

FEN has potent anti-inflammatory effect via activation of PPAR-α in animal experiments and clinical studies (Duez *et al.*, 2002; Okopien *et al.*, 2006). The findings of this study are in line with those of previous experimental studies, Li *et al.* (2005) demonstrated that pretreatment with PPAR-α ligand WY, a fibrate class of PPAR-α ligand, afforded protection from Cis

nephrotoxicity and significantly inhibited Cis-induced increased expression of many cytokines including TNF-α and IL-6. Furthermore, Nagothu *et al.* (2005) reported that bezafibrate treatment inhibits Cis mediated tubular injury by preventing the activation of various cellular mechanisms that lead to proximal tubule cell death. These findings support previous observations by Li *et al.* (2004) who suggested that the use of fibrates represents a novel strategy to ameliorate Cis-induced acute renal failure by preventing proximal tubule cell apoptosis and necrosis.

In addition, observations of decreased leucocytes adherence to mesangial cells imply anti-inflammatory effects of FEN on kidney (Li *et al.*, 2010). In this context, increased expression of inflammatory mediators in diabetic kidneys was inhibited by FEN *in vivo* (Chen *et al.*, 2008). Gelosa *et al.* (2010) found that FEN delayed the onset of proteinuria and prevented morphological renal alterations in salt-loaded spontaneously hypertensive stroke-prone rats, an animal model that develops a complex pathology characterized by systemic inflammation, hypertension and proteinuria and leads to end-organ injury, FEN treatment decreased the renal expression of IL-1β, Transforming Growth Factor-beta (TGF-ß) and Monocyte Chemoattractant Protein-1(MCP-1). It also prevented the plasma and urine accumulation of acute-phase and oxidized proteins, suggesting that the protection induced by FEN was at least partially caused by its anti-inflammatory and antioxidative properties.

Similar findings have been described in clinical trials in which FEN-treated patients with atherosclerosis and cardiovascular diseases had reduced plasma levels of cytokines, C-reactive protein and fibrinogen (Krysiak *et al.*, 2011; Min *et al.*, 2012). FEN has also been proven to reduce plasma levels of TNF-α, among other inflammatory markers in human metabolic-syndrome studies (Rosenson *et al.*, 2009). On the other hand, NAM can regulate cellular inflammation. NAM blocks pro-inflammatory cytokines, such as IL-1ß, IL-6, IL-8 and TNF-α (Reddy *et al.*, 2001; Ungerstedt *et al.*, 2003) as well as TGF β2, IL-1 β, TNF-α and macrophage chemotactic protein-1 in hepatic cells (Traister *et al.*, 2005). Moreover, Shi *et al.* (2012) found that NAM induced a significant reduction in the plasma levels of pro-inflammatory cytokines TNF-α, interferon-γ and IL-6 and suggested that NAM possesses protective effects on acetaminophen-induced liver injury, which may involve the anti-inflammatory action.

Furthermore, NAM modulates TNF-α in vascular cells (Fukuzawa *et al.*, 1997). NAM also may control inflammatory mechanisms that lead to arthritis, such as the inhibition of collagen II expression (Kroger *et al.*, 1999) as well as contact hypersensitivity reactions (Bryniarski *et al.*, 2008). A recent study by Monfrecola *et al.* (2013) reported that NAM

significantly downregulated IL-6, MCP-1 and TNF-α mRNA expression in Ultraviolet (UV)-irradiated keratinocytes, so NAM can be used as a possible therapy to improve or prevent conditions induced by (UV) radiation which has profound effects on human skin, causing inflammation, cellular-tissue injury and cell death. However, the role of NAM during inflammation is not entirely clear, since some investigations that examined the ability of oral NAM administration to reduce cytokine production following endotoxin challenge in healthy volunteers did not demonstrate a significant effect upon serum cytokine levels (Soop *et al.*, 2004).

Conclusion

The beneficial prophylactic effects of FEN and NAM against Cis-induced nephrotoxicity observed in this study were attributable to the anti-oxidant and anti-inflammatory action of each of them. Also, the extracted data justified that combined administration of FEN and NAM has more potential ameliorative effects on Cis-induced nephrotoxicity compared to their individual effect; hence the combined therapy could be more effective.

Acknowledgment

Declaration of Interest

The authors report no conflicts of interest.

Author's Contributions

Omaima M. Abd Allah: *Defined the* research *theme/Designed methods and experiments/*Wrote the first draft of the manuscript/Critical revision of the article/Read and approved the final manuscript.

Abeer A.I. Sharaf El-Din: Wrote the protocol of the study/*Designed methods and experiments*/performed the statistical analysis/Critical revision of the article/Read and approved the final manuscript.

Fouad El Debakey: Acquisition of biochemical samples and carried out the biochemical analysis/Critical revision of the article/Read and approved the final manuscript.

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