



## *Flacourtia indica* fruit extract modulated antioxidant gene expression, prevented oxidative stress and ameliorated kidney dysfunction in isoprenaline administered rats

Saima Selim<sup>a</sup>, Noushin Akter<sup>a</sup>, Shariful Islam Nayan<sup>a</sup>, Faizul Islam Chowdhury<sup>a</sup>, Nadia Saffoon<sup>a</sup>, Ferdous Khan<sup>a</sup>, Khondoker Shahin Ahmed<sup>b</sup>, Md Iqbal Ahmed<sup>c</sup>, Mohammad Maqsud Hossain<sup>d</sup>, Md Ashraful Alam<sup>a,\*</sup>

<sup>a</sup> Department of Pharmaceutical Sciences, North South University, Bangladesh

<sup>b</sup> BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, 1205, Bangladesh

<sup>c</sup> Pharmacy Discipline, Khulna University, Khulna, Bangladesh

<sup>d</sup> NSU Genome Research Institute (NGRI), North South University, Bangladesh

### ARTICLE INFO

#### Keywords:

*Flacourtia indica*  
Isoprenaline  
Necrosis  
Antioxidant  
Inflammation

### ABSTRACT

This study evaluated the effect of *Flacourtia indica* fruit extract against isoprenaline (ISO) induced renal damage in rats. This investigation showed that ISO administration in rats increased the level oxidative stress biomarkers such as malondialdehyde (MDA), nitric oxide (NO), advanced protein oxidation product (APOP) in kidneys followed by a decrease in antioxidant enzymes functions. *Flacourtia indica* fruit extract, which is rich in strong antioxidants, also reduced the MDA, NO and APOP level in kidney of ISO administered rats. Inflammation and necrosis was also visible in kidney section of ISO administered rats which was significantly prevented by atenolol and *Flacourtia indica* fruit extract. Moreover, atenolol and *Flacourtia indica* fruit extract also modulated the genes expressions related to inflammation and oxidative stress in kidneys. The beneficial effects could be attributed to the presence of a number of phenolic antioxidants. This study suggests that *Flacourtia indica* fruit extract may prevent kidney dysfunction in ISO administered rats, probably by preventing oxidative stress and inflammation.

### 1. Introduction

Chronic kidney disease (CKD) is a public health issue, which now became the economic burden on health care sector of many countries of the world. In 2015, Global Burden of Disease Study revealed that kidney disease was responsible for 1.1 million deaths worldwide [1]. This survey also showed that CKD mortality has escalated by 31.7% during a decade long study period ranging from 2005 to 2015 [1]. At the same time, the economies of low-income and middle-income countries were heavily affected by the cost of CKD management disproportionately due to the rise of non-contagious diseases such as obesity and diabetes. The consequence of CKD is death by cardiovascular complications or progression to end-stage renal failure due to poor management of risk factors in Indian sub-continent [2,3]. CKD of unknown origin have been reported among the populations in Sri Lanka and India [4], which was

characterized by several symptoms such as tubular atrophy, interstitial fibrosis and rapid progression without proteinuria [4–6]. On the basis of risk factors and adverse side effects for kidney disease in different geographical regions and countries, proper prevention measures should be adopted.

Inflammation and oxidative stress are crucial factors in the development of CKD [7]. The enzymatic sources of free radicals in CKD are nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, inducible nitric oxide synthase (iNOS), and the mitochondrial electron transport chain [8]. Moreover, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) have been reported to be compromised in CKD [9,10]. The potential bio-markers of oxidative stress at the advanced stages of renal dysfunctions in CKD patients are the elevated plasma levels of F2-isoprostanes, malondialdehyde (MDA) and advanced oxidation protein products (AOPP) [8,11].

\* Corresponding author.

E-mail addresses: [saimaselim22@gmail.com](mailto:saimaselim22@gmail.com) (S. Selim), [noushinakter816@gmail.com](mailto:noushinakter816@gmail.com) (N. Akter), [sinayan38@gmail.com](mailto:sinayan38@gmail.com) (S.I. Nayan), [fislamrahat@gmail.com](mailto:fislamrahat@gmail.com) (F.I. Chowdhury), [nsaffoon@gmail.com](mailto:nsaffoon@gmail.com) (N. Saffoon), [khan.ferdous@northsouth.edu](mailto:khan.ferdous@northsouth.edu) (F. Khan), [shahinjnu005@gmail.com](mailto:shahinjnu005@gmail.com) (K.S. Ahmed), [iqbal-bd@hotmail.com](mailto:iqbal-bd@hotmail.com) (M.I. Ahmed), [mohammad.maqsud@northsouth.edu](mailto:mohammad.maqsud@northsouth.edu) (M.M. Hossain), [ashraful.alam@northsouth.edu](mailto:ashraful.alam@northsouth.edu), [sonaliagun@yahoo.com](mailto:sonaliagun@yahoo.com) (M.A. Alam).

<https://doi.org/10.1016/j.bbrep.2021.101012>

Received 4 November 2020; Received in revised form 13 April 2021; Accepted 30 April 2021

2405-5808/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The release of different types of inflammatory mediators such as bioactive amines, cytokines, prostaglandins, interleukins, and inflammatory lipids are increased due to tissue damage, which may cause recruitment of neutrophil in the inflamed kidney [12–14]. By the process of auto-oxidation and lipid peroxidation, reactive oxygen species are also produced by inflammatory mediators [12].

Isoprenaline causes kidney injury which acts as beta-adrenergic agonist and is also known as synthetic catecholamine. In animal model, it has been shown to act as a remarkable kidney damage initiator [15]. Free radicals have been involved in the pathophysiology of kidney damage by isoprenaline in the animal model [16]. Antioxidants showed preventive effect in the oxidative stress and inflammation in CKD [15, 17]. *Flacourtia indica* fruit extract has showed excellent antioxidant capacity due to the polyphenolic compounds [18]. *Flacourtia indica* Merr. (Family: *Flacourtiaceae*), is an indigenous medicinal plant which is found largely in tropical and temperate parts of Asia and Africa [19]. *Flacourtia indica* is also an important Ayurvedic herb and its bark, leaves and root are used as medicine to treat fever, diarrhoea and inflammations. The root and ash also have been used as an alternative therapy for kidney complaints. Recent evidences also suggest that *Flacourtia indica* is beneficial in the treatment of various pathological conditions in experimental animals. *Flacourtia indica* extract showed hepatoprotective [20], anti-microbial [21] and anti-hyperlipidemic activities [22].

Previous report also suggests that ethanol extract of *Flacourtia indica* prevented cardiac damage in doxorubicin-induced rats and re-established tissues antioxidant functions [23]. Chemical investigation revealed the isolation of pyrocatechol, homaloside D and poliothyroside compounds from *Flacourtia indica* which showed excellent antimalarial activity [21]. The antioxidant compounds detected in *Flacourtia indica* was mostly phenolic compounds such as ferulic acid, caffeic acid and vanillic acid [24,25]. However, the preventive effect of antioxidants present in *Flacourtia indica* fruits were not investigated in kidney dysfunction. From this perspective, we evaluated the capability of *Flacourtia indica* fruit extract to prevent oxidative stress in kidney of ISO administered rats. Moreover, this current investigation also evaluated *Flacourtia indica* fruits extracts in restoring the antioxidant enzyme activities in ISO administered rats.

## 2. Methods & materials

### 2.1. Plant materials

*Flacourtia indica* fruits were collected from the domestic mart of Dhaka city, Bangladesh. The specimen identification was done at the Mirpur National Herbarium and the voucher number has been filed up in the Herbarium (DACB Accession Number-47415). The ripen fruits were dried in the open air and coarse powder was made by using a grinding mill. This powdered *Flacourtia indica* fruits were soaked in ethanol for 7 days and decanted the ethanol layer. Filtration of ethanolic *Flacourtia indica* extract was performed with the help of a filter paper. The ethanol of the extraction was vaporized at room temperature (27 °C) for several days, and then the final reddish color crude extract was obtained. This extract was used for the treatment in this study.

### 2.2. Animals and treatment

All the animals (twelve to fourteen weeks old, total 24 adult Long-Evans male rats with average weight range from 200 to 220 g) were collected and housed in the animal house unit of North South University under the Department of Pharmaceutical Sciences. Isolated closet for rats, day length of 12 h, controlled temperature (25 ± 3 °C), standard diet and supply of water had been strictly maintained throughout the experiment, which comply with the in-house ethical committee standard study procedure of the department and the university. The Ethical Committee of North South University also approved of the study protocol. To obtain the desired pharmacological outcome, the rats were

segmented over four different groups, where each group gets up to six rats. Group I animals got normal diet along with water whereas group II, III and IV get subcutaneous injection of isoprenaline (ISO) for 14 days and the dose used was 50 mg per kg per day for two times a week. This dose and administration protocol was used following the survival of rats in our lab which was mentioned in previous experiments [15,16]. ISO was dissolved in water for injection and administered in the subcutaneous layer using a syringe and needle. Animals of group III was fed with *Flacourtia indica* (100 mg/kg/day) and group IV was treated with atenolol (10 mg/kg/day). All animals undergo regular body weight checking before final recording of body weight at day 14th. All animals were sacrificed on day 14th, using high dose of pentobarbital (90 mg/kg) injection in the peritoneal region of rats. Blood sample was collected from each rat. Large abdominal vein region was punctured by 18 gauge needle and collected the blood in citrate buffer (pH 4) containing tubes and placed on an ice box. After sacrifice/euthanasia of the rats, the organs (kidneys) have been collected to measure the wet organ weights. Part of the kidneys was preserved in neutral buffered formalin (pH 7.4) for histological analysis. Other part of the kidneys was preserved in freezer at -20 °C for other biochemical studies. To obtain plasma from the collected blood, centrifugation at 8000g was performed and the supernatant plasma was collected and stored at -20 °C temperature for other biochemical assays.

### 2.3. Establishment of tissue sample for the evaluation of oxidative stress markers

To evaluate the oxidative stress markers, tissue samples from the kidney were first homogenized. For homogenization of the sample tissues, phosphate buffer saline (disodium hydrogen phosphate, pH 7.4) was used and centrifuged (12,000×g and at 4 °C) up to 30 min. After centrifugation of tissue homogenates, supernatant was collected and used for biochemical analysis and analysis of antioxidant enzymes activities as narrate down below.

### 2.4. Assessment of lipid peroxidation

The presence of thiobarbituric acid reactive substances (TBARS) in renal tissues was measured as a lipid peroxidation parameter according to a method described earlier [26,27]. In short, tissue homogenate (0.1 mL) which are present in Tris-HCl buffer, pH 7.5, was mixed with 2 mL of TBA-TCA-HCl reagent. The TBA-TCA-HCl reagent was composed of equal volume of thiobarbituric acid (0.37% v/v), HCl (0.25 N), and tri-chloroacetic acid (15% v/v). The TBA-TCA-HCl reagent and tissue homogenate mixture was set in hot water bath for 15 min and then cooled down to room temperature. The absorbance of the supernatant solution was taken at 535 nm in a spectrometer together with a reference blank.

### 2.5. Evaluation of nitric oxide (NO)

For the quantification of dissolved nitric oxide (NO) in tissue homogenates, Griess-Illsvooy reagent based method was used. In this experimental analysis, naphthyl ethylene diaminedihydrochloride (0.1% w/v) was used in lieu of 1-naphthylamine (5%) to increase the solubility in aqueous system. This method is also described in details in our previous articles [26,27]. Kidney homogenate (2 mL) was incubated with Griess-Illsvooy reagent and phosphate buffered saline (0.5 mL) at 25 °C. The absorbance of the supernatant was taken at 540 nm against the blank solution. NO level of tissue (nmol/g tissue) was calculated by using a calibration curve [26,27].

### 2.6. Advanced protein oxidation products (APOP) evaluation

APOP concentration was also determined in kidney tissues followed by a previously established protocol [26,27]. At first, plasma samples (2

mL) were diluted in phosphate buffer saline at 1:5 ratios. Then, 0.1 mL of potassium iodide (1.16 M) was added to each tube containing plasma samples. Glacial acetic acid (0.2 mL) was added to stop the reaction progress. The absorbance was recorded quickly at 340 nm against a blank. The blank sample contains 2 mL of phosphate buffer saline, 0.1 mL of potassium iodide, and 0.2 mL of acetic acid. The final concentration of APOP present in the tissue homogenates was calculated as  $\text{nmol}\cdot\text{mL}^{-1}$  chloramine-T equivalents. A chloramine-T standard curve was established using the concentration range 0–100 nmol/mL which showed a linear relationship [26,27].

## 2.7. Superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO) activity assay

SOD activity in kidney tissue homogenates were determined followed by previous published literatures [26,27]. The reaction mixture (3 mL) consisted of aliquot of tissue homogenates, epinephrine (15 mM) and PBS (pH 7.4). The absorbance change at 480 nm was recorded for 1 min at 15 s interval against a reference control. A 50% enzyme inhibition is defined as one unit of enzyme activity which was generated due to the auto-oxidation of epinephrine present in the assay system.

Kidney tissue homogenates were also used to determine the catalase activities followed by previously described protocol [26,27]. In brief, tissue homogenates (0.1 mL) was combined with 2.5 mL of phosphate buffer (50 mM, pH 5.0) and 0.4 mL of  $\text{H}_2\text{O}_2$  (5.9 mM) solution. The changes in absorbance value of the reaction mixture due to the decay of  $\text{H}_2\text{O}_2$  were recorded after 1 min at 240 nm in a UV-visible spectrophotometer. The change of absorbance value 0.01 is considered as one unit catalase activity per minute [26,27].

MPO activity assay in kidney homogenates was also determined using another established protocol described earlier [26,27]. This protocol was developed based on the di-anisidine- $\text{H}_2\text{O}_2$  based mixed reagents. In brief, plasma sample (10  $\mu\text{L}$ ) was mixed with potassium phosphate buffer (50 mM, pH 6.0). Then, *O*-dianisidinedihydrochloride (0.53 mM) and  $\text{H}_2\text{O}_2$  (0.15 mM) were added to the tissue homogenates mixture. The absorbance changes for 3 min were recorded at 460 nm. MPO activity/mg protein was used as the unit expression of the protein activity.

## 2.8. Reduced glutathione assay (GSH)

The glutathione (GSH) concentration in kidney tissue was estimated followed by previous reports [26,27]. Kidney tissue homogenates (1.0 mL sample) was mixed with one mL of 4% sulfosalicylic acid. The mixture was cooled to 4 °C and kept for 1 h incubation. This mixture was then centrifuged (12000 $\times$ g) for 20 min at 4 °C temperature in a centrifuge machine. Aliquot (0.1 mL) from the supernatants were taken to a different set of tubes. The aliquot samples were then combined with 2.7 mL phosphate buffer (0.1 M, pH 7.4) and 0.2 mL 5,5-dithiobis-2-nitrobenzoic acid (100 mM) to develop color. The absorbance of the solution was measured immediately after the development of yellow color at 412 nm on a Spectrophotometer (Smart Spec™ plus) and the unit represented as ng/mg protein [26,27].

## 2.9. Creatinine and uric acid concentration assay

Creatinine and uric acid concentrations were measured by using commercial kit following the manufacturer's protocol. These kits were collected from DCI Diagnostics (Budapest, Hungary).

## 2.10. RT-PCR for oxidative stress and inflammation regulatory genes

Total RNA was extracted from renal tissues, using RNA purification Kit, obtained from Thermo-Fisher Scientific (Massachusetts, USA). After quantification of RNA by NanoDrop 2000 (Bio-Rad, California, USA), 1  $\mu\text{m}$  of the RNA from each sample was used to prepare cDNA according to

the manufacturer's instruction using RevertAid First Strand cDNA Synthesis Kit (Thermo-Fisher Scientific, USA). The primers of target genes were designed using Primer 3 online software (Table-1). The transcript levels of several factors and enzymes were analyzed by quantitative RT-PCR using Maxima SYBR Green qPCR master mix (Thermo-Scientific, USA). The PCR was performed by a CFX96 C1000 Touch Real Time PCR Detection System (Bio-Rad, California, USA) according to the protocol described previously [26]. The data analysis was done by CFX Manager™ Software (Bio-Rad, California, USA) by considering the transcript level of  $\beta$ -actin as the standard for the normalization of the mRNA level of different genes.

## 2.11. Histopathological study

Neutral buffered formalin was used to fix the kidney tissues and kept as such for several days. The fixed tissues were then processed with series of alcohol and xylene treatment and embedded in paraffin block. They were then sliced at 5  $\mu\text{m}$  thickness by a microtome and tissue sections were placed on glass slides. For the visualization of the structure of kidney sections and inflammatory cell infiltration, they were then stained with hematoxylin/eosin followed by established routine protocol. For the determination of fibrosis in kidneys, Sirius red staining was also performed. The tissue sections were undergone a series of alcohol and xylene treatment and were dipped in a mixture of Sirius red stain and aqueous picric acid solution for 45 min. Stained sections were scrutinized and pictures were taken below the light microscope (Zeiss) at 40X magnifications. At least five different snaps were taken from each section studied.

## 2.12. Statistical analysis

The data were presented as Mean  $\pm$  standard error of mean (SEM) to articulate the result outcome. The results were also checked statistically by using the One-way ANOVA accompanied by Tukey post hoc test. All analyses were conducted using Graph Pad Prism Software (Version 6). Statistical significance was considered at  $p < 0.05$  in all cases, unless otherwise stated.

**Table 1**

The forward and reverse sequence of the primer applied in this experiment.

| Name of gene                 | Type    | Sequence                       |
|------------------------------|---------|--------------------------------|
| Nrf-2                        | Forward | 5'-CCC AGCACA TCC AGACAGAC-3'  |
|                              | Reverse | 5'-TATCCAGGGCAAGCGACT C-3'     |
| Heme oxygenase-1 (HO-1)      | Forward | 5'-TGCTCGCATGAACACTCTG-3'      |
|                              | Reverse | 5'-TCCTCTGTACAGCAGTGCCCT-3'    |
| Heme oxygenase-2 (HO-2)      | Forward | 5'-CACCAGTGCACCTTTACTTCA-3'    |
|                              | Reverse | 5'-AGTGTCTGGGGAGTTTTAGTG-3'    |
| MnSOD                        | Forward | 5'-GCTCTAATCACGACCCACT-3'      |
|                              | Reverse | 5'-CATTCTCCCAGTTGATTACATTC-3'  |
| Catalase                     | Forward | 5'-ATTGCCGTCGGATTCTCC-3'       |
|                              | Reverse | 5'-CCAGTTACCATCTTCAGTGTAG-3'   |
| Glutathione peroxidase (GPx) | Forward | 5'-GGGCAAAGAAGATTCCAGGTT-3'    |
|                              | Reverse | 5'-GGACGGCTTCATCTTCAGTGA-3'    |
| IL-1                         | Forward | 5'-ATGCCTCGTGTCTGTGACC-3'      |
|                              | Reverse | 5'-CCATCTTTAGGAAGACACGGGTT-3'  |
| IL-6                         | Forward | 5'-AGCGATGATGCACTGTGAGA-3'     |
|                              | Reverse | 5'-GGTTTGCCGAGTAGACCTCA-3'     |
| TNF- $\alpha$                | Forward | 5'-ATGTGGAAGTGGCAGAGGAG-3'     |
|                              | Reverse | 5'-CCACGAGCAGGAATGAGAAGAG-3'   |
| TGF- $\beta$                 | Forward | 5'-AAGAAGTACCAGCGGTGCTA-3'     |
|                              | Reverse | 5'-TGTGTGATGTCTTTGGTTTTGTC-3'  |
| iNOS                         | Forward | 5'-TGGTCCAACCTGCAGGTCTTC-3'    |
|                              | Reverse | 5'-CAGTAATGGCCGACCTGATGTTG-3'  |
| NF- $\kappa$ B               | Forward | 5'-TGTGAAGAAGCGAGACCTGGAG-3'   |
|                              | Reverse | 5'-GGCACGGTTATCAAAAATCGGATG-3' |
| $\beta$ -Actin               | Forward | 5'-GCGAGAAGATGACCCAGATC-3'     |
|                              | Reverse | 5'-GGATAGCACAGCTGGATAG-3'      |

### 3. Results

#### 3.1. Effect of *Flacourtia indica* fruit extract on body weight and kidney wet weight in ISO administered rats

The body weights and kidney wet weights were presented in Table 2. ISO administration decreased the final body weights in rats. Moreover, *Flacourtia indica* fruit extract prevented the decline of body weights in ISO administered rats. ISO administration also increased kidney wet weight significantly ( $p < 0.05$ ) which was further prevented by *Flacourtia indica* fruit extract and atenolol treatment (Table 2).

#### 3.2. Effect of *Flacourtia indica* fruit extract on oxidative stress parameters in kidney of ISO administered rats

Lipid peroxidation is a crucial parameter for measuring oxidative stress in tissues. This section of the present study demonstrated that the biosynthesis of malondialdehyde, the byproduct of lipid peroxidation, was increased significantly ( $P < 0.05$ ) in the kidney of ISO administered rats compared to the control rats (Fig. 1A). *Flacourtia indica* fruit extract treatment was able to reduce the ISO-induced production of malondialdehyde significantly ( $P < 0.05$ ) (Fig. 1A). Atenolol treatment also decreased the malondialdehyde level in kidney of ISO administered rats (Fig. 1A).

Additionally, nitric oxide concentration was also increased significantly in the kidney of ISO administered rats ( $P < 0.05$ ) compared to the control rats (Fig. 1B). *Flacourtia indica* fruit extract and atenolol treatment significantly suppressed the elevated nitric oxide production compared to the ISO administered rats (Fig. 1B).

ISO-induced oxidative stress also caused protein oxidation and increased the level of advanced protein oxidation product markedly ( $P < 0.05$ ) compared to the control rats (Fig. 1C). *Flacourtia indica* fruit extract and atenolol treatment also reduced the APOP level in ISO administered rats (Fig. 1C).

#### 3.3. Effect of *Flacourtia indica* fruit extract on antioxidant enzyme activity parameters in kidney of ISO administered rats

Subcutaneous administration of ISO diminished the antioxidant enzyme activities in kidney tissue. In this investigation, we observed significant ( $P < 0.05$ ) reduction of catalase and SOD activities in the kidney due to subcutaneous administration of ISO. *Flacourtia indica* fruit extract treatment restored the catalase and SOD activities in the kidneys of rats received subcutaneous injection of ISO (Fig. 2A and B). Treatment with atenolol to ISO administered rats also showed increased catalase and SOD activities in the kidneys (Fig. 2A and B).

Moreover, reduced glutathione (GSH) level was also decreased in kidney of ISO administered rats significantly ( $P < 0.05$ ) which was restored by *Flacourtia indica* fruit extract treatment (Fig. 2C). Atenolol treatment also restored the glutathione (GSH) level in ISO administered

**Table 2**  
Effect of *Flacourtia indica* fruits extracts on body weight and kidney wet weight of ISO administered rats.

| Parameters                                  | Control       | ISO           | ISO +<br><i>F. indica</i> | ISO +<br>Atenolol |
|---|---------------|---------------|---------------------------|-------------------|
| Initial body weight (g)                     | 201.33 ± 3.08 | 205.5 ± 3.25  | 212.16 ± 3.04             | 205.16 ± 3.52     |
| Final body weight (g)                       | 237.66 ± 2.20 | 218.33 ± 1.28 | 242.5 ± 5.25              | 243.16 ± 2.40     |
| Kidneys wet weight (g/100 g of body weight) | 0.57 ± 0.01*  | 0.66 ± 0.03#  | 0.49 ± 0.02**             | 0.53 ± 0.01**     |

Values are presented as mean ± SEM, N = 6. One way ANOVA followed by Tukey test was performed to detect the statistical significance. Statistical significance was considered at  $P < 0.05$  in all cases. Control vs ISO, \* vs #; ISO vs ISO + FI or ISO + ATL, # vs \*\*; \*\* denoted  $P < 0.01$  and \* denotes  $p < 0.05$ .

rats significantly ( $P < 0.05$ ) (Fig. 2C).

On the contrary, significant increment of myeloperoxidase (MPO) activity was observed in the renal tissue of ISO administered rats (Fig. 2). *Flacourtia indica* fruit extract and atenolol treatment significantly diminished the ISO-induced augmentation of MPO activity (Fig. 2D).

#### 3.4. Effect of *Flacourtia indica* fruits extract on uric acid and creatinine level in plasma of ISO administered rats

ISO administration induces kidney dysfunction which can be confirmed by the increased plasma concentration of kidney dysfunction biomarkers such as uric acid and creatinine. In this study, both uric acid and creatinine concentrations were increased in ISO administered rats significantly ( $P < 0.05$ ) compared to the control rats (Fig. 3). Oral administration of *Flacourtia indica* fruit extract decreased the uric acid and creatinine level in the blood of ISO administered rats. The beneficial effect of *Flacourtia indica* fruit extract in lowering the uric acid and creatinine concentrations are comparable to the atenolol also (Fig. 3).

#### 3.5. Effect of *Flacourtia indica* fruits extract on gene expression of oxidative stress-related enzymes in the kidney of ISO administered rats

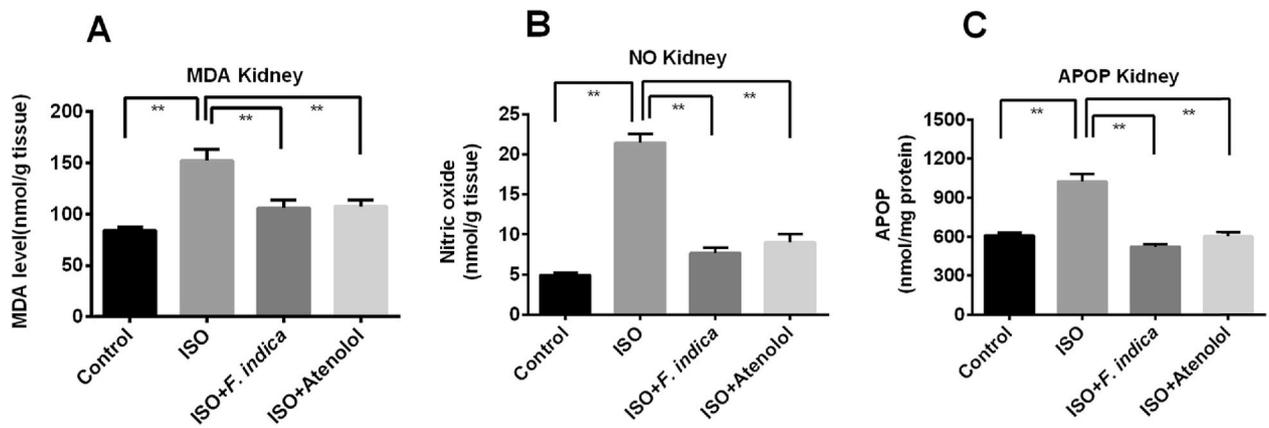
The nuclear factor erythroid 2-related factor-2 (Nrf-2), is a multi-functional transcription factor, plays an important role to regulate the expression of anti-inflammatory and anti-oxidant enzymes. In this investigation, we observed that mRNA expression of Nrf-2 in the isoproterenol (ISO) treated rats were markedly ( $P < 0.05$ ) lowered in comparison to the control rats. Two major isoforms of hemoxygenase (HO-1 and HO-2) are functionally coupled with Nrf-2. Consequently, the transcript levels of HO-1 and HO-2 were significantly ( $P < 0.05$ ) down-regulated in ISO-treated rats compared to the control rats (Fig. 4). Similarly, the expression of antioxidant enzymes like catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) were also significantly down-regulated in ISO-treated rats. The ISO-mediated suppression of Nrf-2, HO-1 and HO-2 are up-regulated significantly ( $P < 0.05$ ) due to the treatment with atenolol and *Flacourtia indica* fruit extract. Similarly, treatment with atenolol and *Flacourtia indica* extract up regulated the genes expression of catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) in ISO administered rats (Fig. 4).

#### 3.6. Effect of *Flacourtia indica* fruit extract on gene expression of inflammation and fibrosis-related proteins in kidney of ISO-induced rats

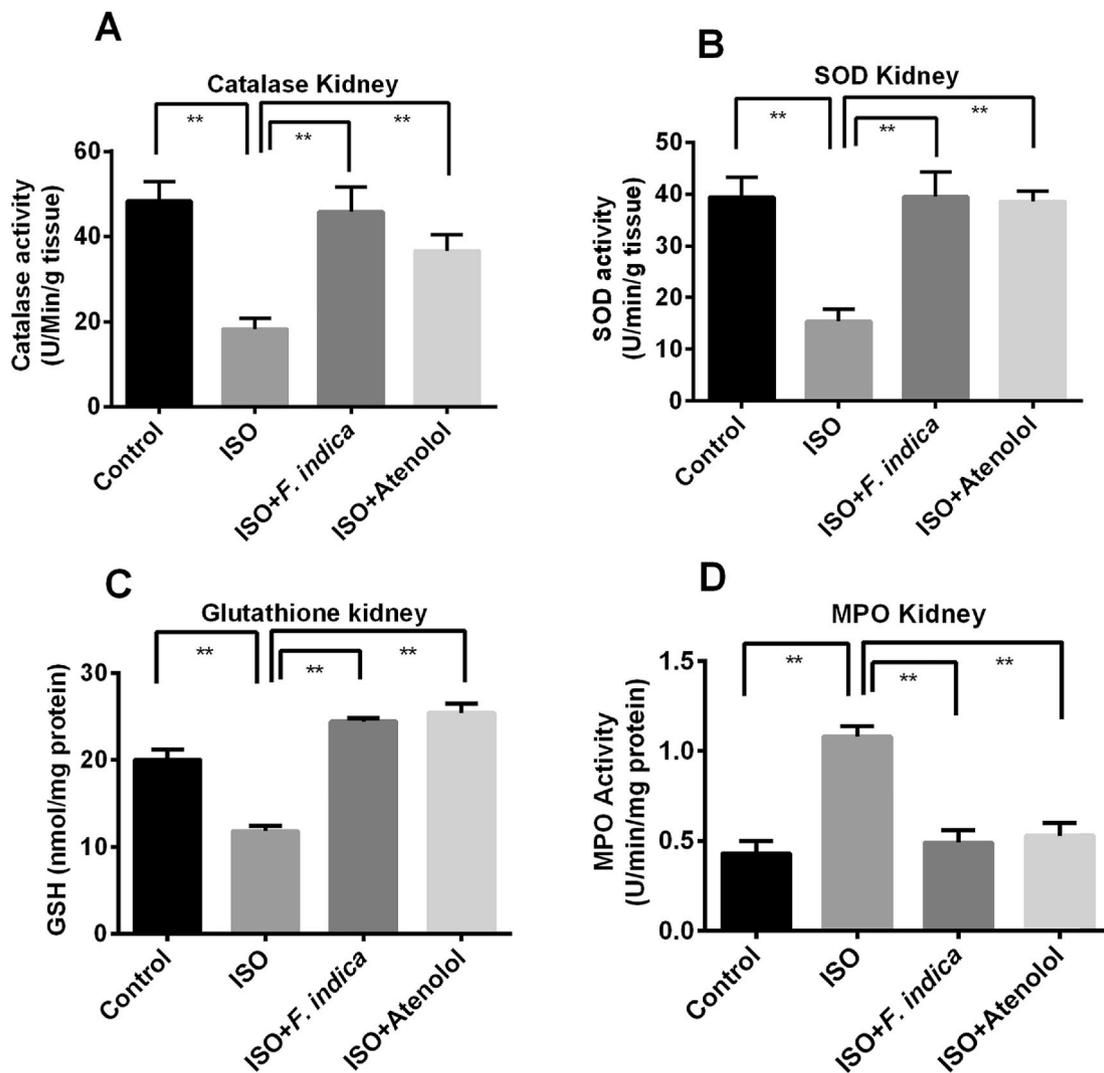
To explore the effectiveness of *Flacourtia indica* fruit extract to suppress ISO-induced inflammation and fibrosis, we also measured the gene expression of inducible nitric oxide synthase (iNOS), inflammatory cytokines including interleukin-1 (IL-1), interleukin-6, (IL-6), tumor necrosis factor-alpha (TNF- $\alpha$ ), transforming growth factor beta-1(TGF- $\beta$ 1), and NF- $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B cells). We observed that the transcript levels of iNOS, IL-1, IL-6, TNF- $\alpha$ , TGF- $\beta$ 1, and NF- $\kappa$ B were significantly ( $P < 0.05$ ) increased in the renal tissue due to ISO administration (Fig. 5). Treatment with *Flacourtia indica* fruit extract significantly ( $P < 0.05$ ) subdued the mRNA levels of these enzymes, cytokines and transcription factors. This ameliorating action of *Flacourtia indica* fruit extract is also comparable to the atenolol treatment which also lowered the inflammatory cytokines expression in kidneys of ISO administered rats (Fig. 5).

#### 3.7. Effect of *Flacourtia indica* fruit extract on histological assessments in kidney of ISO administered rats

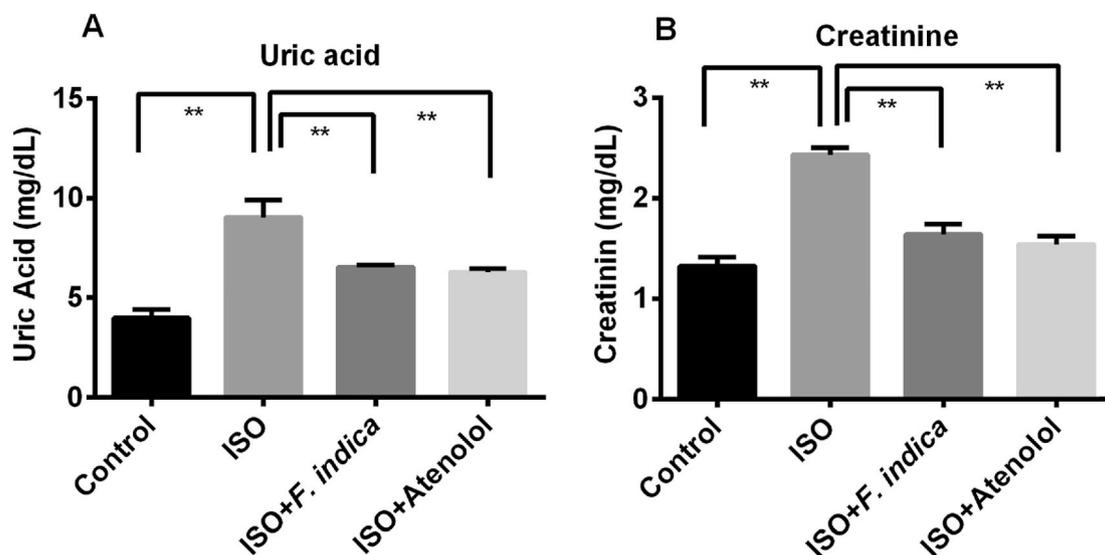
Hematoxylin and eosin staining showed normal glomerulus and tubules structure in kidney sections of control rats (Fig. 6A). ISO administration showed parenchymal cells alteration, congestion in glomerulus



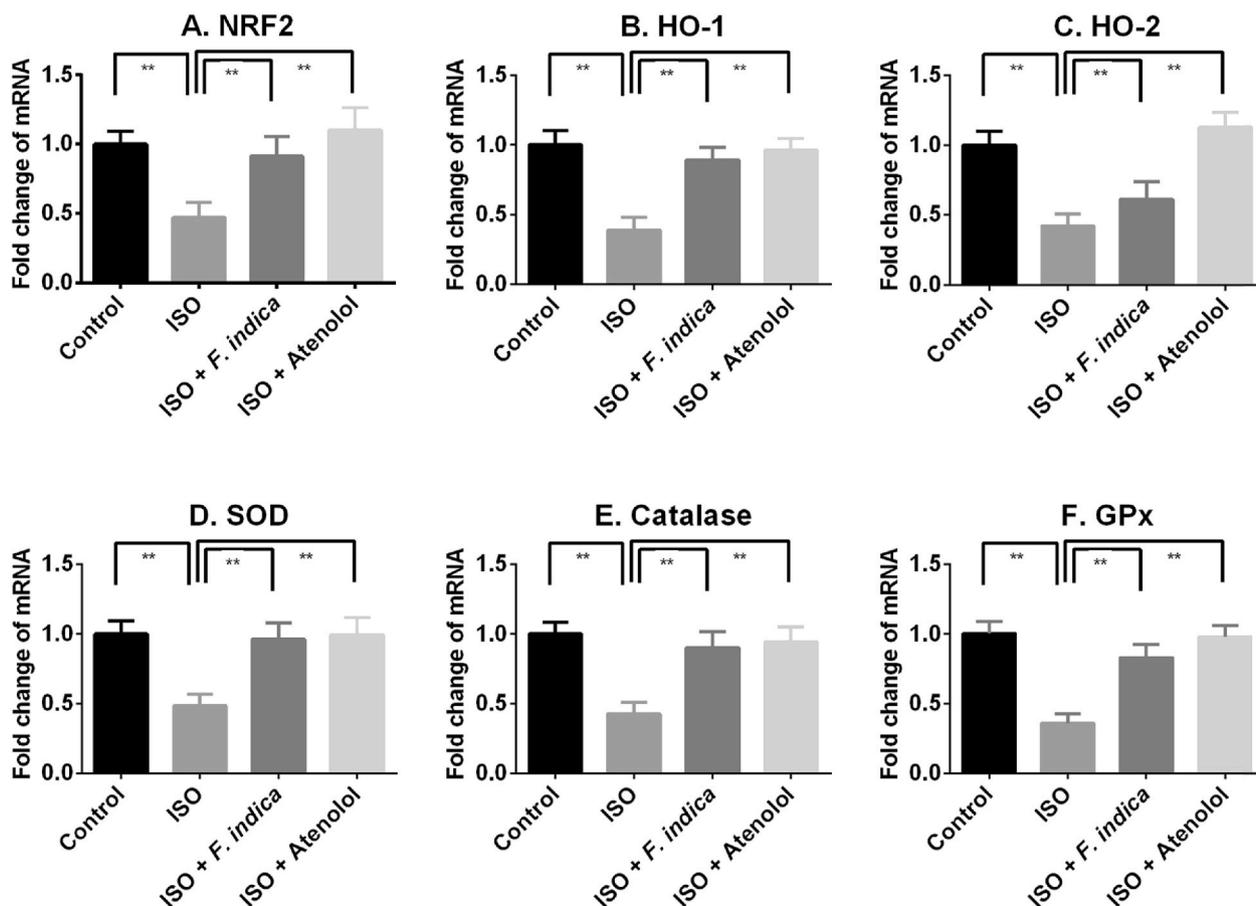
**Fig. 1.** Effect of *Flacourtia indica* fruits extracts on oxidative stress parameters in kidney of ISO administered rats. Values are presented as mean  $\pm$  SEM, N = 6. One way ANOVA followed by Tukey test was performed to detect the statistical significance. Statistical significance was considered at P < 0.05 in all cases. \*\* denoted P < 0.01 and \* denotes as p < 0.05.



**Fig. 2.** Effect of *Flacourtia indica* fruits extracts on antioxidant enzyme activities in kidney of ISO administered rats. Values are presented as mean  $\pm$  SEM, N = 6. One way ANOVA followed by Tukey test was performed to detect the statistical significance. Statistical significance was considered at P < 0.05 in all cases. \*\* denoted P < 0.01 and \* denotes as p < 0.05.



**Fig. 3.** Effect of *Flacourtia indica* fruits extracts on uric acid and creatinine in kidney of ISO administered rats. Values are presented as mean  $\pm$  SEM, N = 6. One way ANOVA followed by Tukey test was performed to detect the statistical significance. Statistical significance was considered at  $P < 0.05$  in all cases. \*\* denoted  $P < 0.01$  and \* denotes as  $p < 0.05$ .

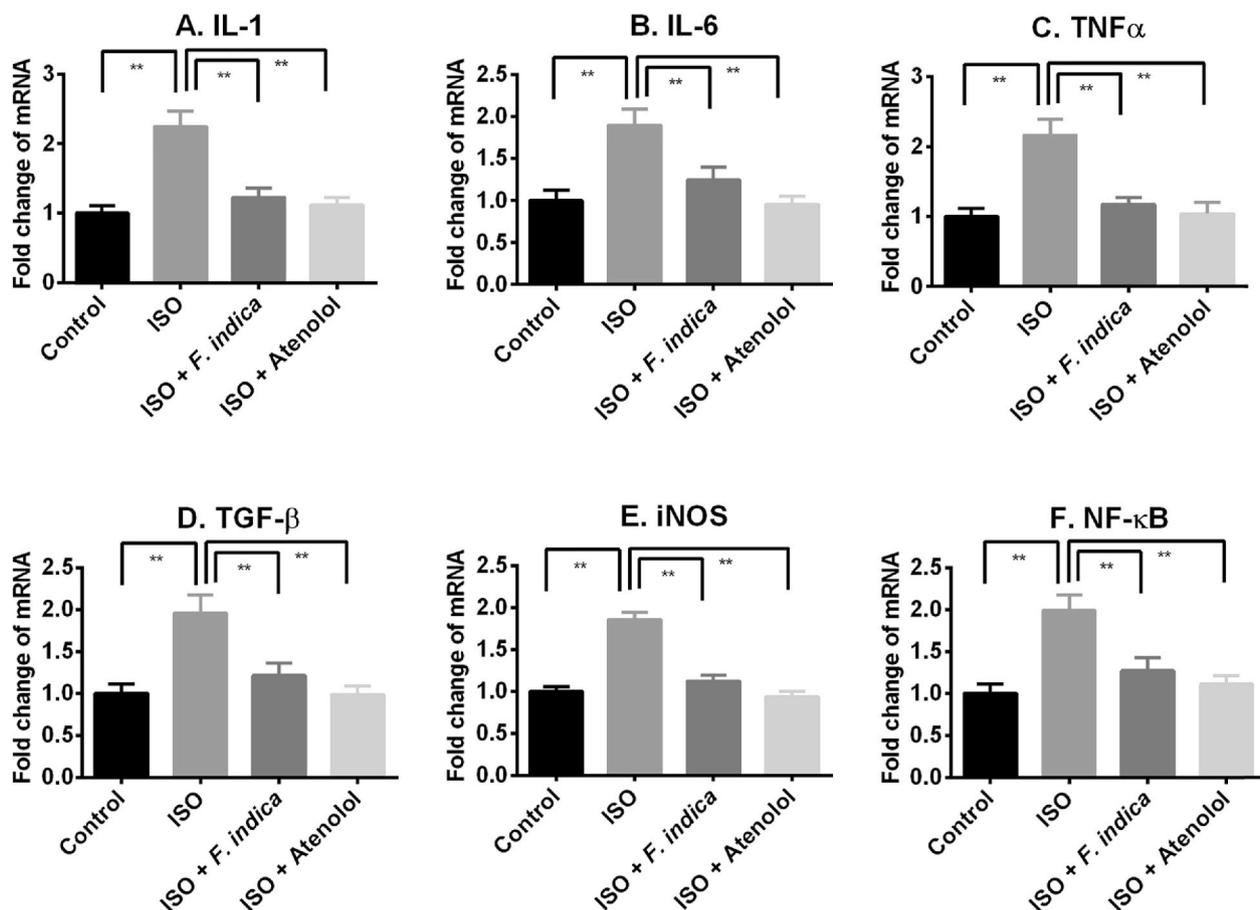


**Fig. 4.** Effect of *Flacourtia indica* fruits extracts on antioxidant gene expression in kidney of ISO administered rats. Values are presented as mean  $\pm$  SD, N = 6. One way ANOVA followed by Tukey test was performed to detect the statistical significance. Statistical significance was considered at  $P < 0.05$  in all cases. \*\*denoted  $P < 0.01$  and \* denotes as  $p < 0.05$ .

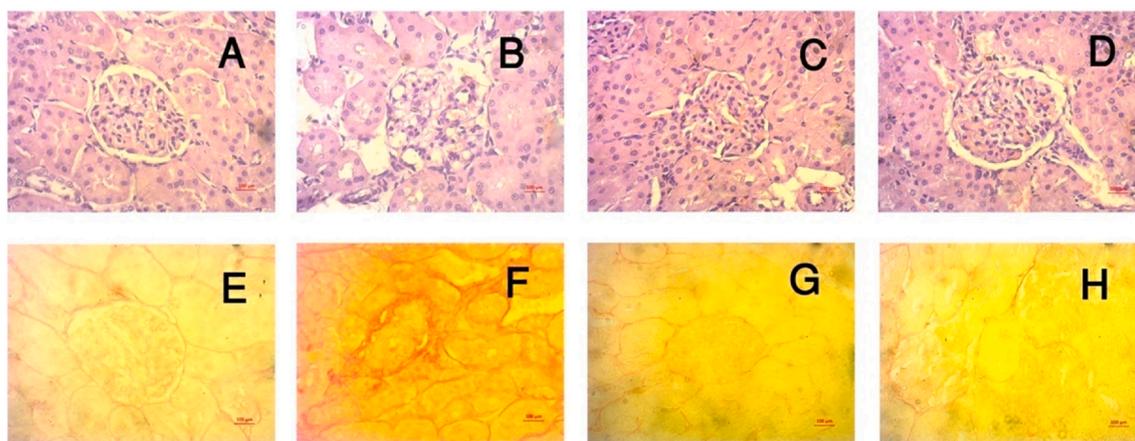
and tubular hypertrophy in kidney sections (Fig. 6 B). *Flacourtia indica* fruit extract and atenolol prevented the structural abnormalities seen in ISO administered rats (Fig. 6C and D).

Sirius red staining also showed normal baseline collagen deposition

in kidneys of control rats (Fig. 6E). Increased collagen deposition and fibrosis were observed in kidney sections of ISO administered rats (Fig. 6 F). *Flacourtia indica* fruit extract and atenolol treatment prevented the fibrosis and collagen deposition in kidney sections of ISO administered



**Fig. 5.** Effect of *Flacourtia indica* fruits extracts on inflammation marker related genes expression in kidney of ISO administered rats. Values are presented as mean  $\pm$  SD, N = 6. One way ANOVA followed by Tukey test was performed to detect the statistical significance. Statistical significance was considered at P < 0.05 in all cases. \*\*denoted P < 0.01 and \* denotes as p < 0.05.



**Fig. 6.** Effect of *Flacourtia indica* fruit extract on histological assessments in kidney of ISO administered rats. Upper panel showed Hematoxylin and eosin staining and lower panel showed Sirius red staining. A, E-control; B, F- ISO; C, G- ISO + *Flacourtia indica* and D, H- ISO + Atenolol. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

rats (Fig. 6G and H).

#### 4. Discussion

In this investigation, *Flacourtia indica* fruit extract has exhibited preventive effect against oxidative mischief and protect the kidney function by lowering the lipid peroxidation, nitric oxide formation and

restoring the antioxidants enzymes function. Moreover, *Flacourtia indica* fruit extract treatment modulated the gene expression for antioxidant enzymes and lowering the expression of inflammation related genes in kidneys of ISO administered rats.

Oxidative stress is considered as the major mediators for tissue injury in ISO administered animals [28,29]. Oxidation of catecholamine also leads to the production of superoxide anions and causes protein

oxidation, lipid oxidation, and DNA damage [28]. Previous studies also reported that stimulation of  $\beta$ 2-AR signaling in human embryonic kidney (HEK) cell is responsible for the activation of NADPH oxidase mediated biosynthesis of reactive oxygen species (ROS) [30]. Malondialdehyde is one of the obnoxious products of reactive oxygen species which is produced in exaggerated challenges due to the administration of synthetic  $\beta$ -adrenergic receptor agonist isoprenaline in higher dose. Thus, blocking of  $\beta$ -adrenergic receptor would be beneficial in preventing the oxidative stress. Previous reports suggest that beta blockers may scavenge free radicles [31] and prevents kidney dysfunction in 2K1C rats [32]. In this investigation, isoprenaline treated rodents showed increased malondialdehyde level in the tissues which was decreased to near normal level in rats treated with *Flacourtia indica* fruit extract and atenolol. *Flacourtia indica* extract showed antioxidant activities in several experimental models [18,24]. HPLC-DAD analysis of the *Flacourtia indica* fruit extract also determined the presence of quercetin and gallic acid with several other phenolic antioxidants. Previous studies suggested that quercetin scavenges free radicles in experimental animals and prevented the lipid peroxidation in kidneys [33,34]. Similar protective effect was also observed for gallic acid treatment in lead induced toxicities [35]. A recent investigation also showed that quercetin and atenolol combination therapy mitigated oxidative stress in the heart of ISO administered rats [36]. Considering this fact, we may assume that antioxidant compounds present in *Flacourtia indica* fruit extract may be responsible for the prevention of ROS mediated malondialdehyde formation and provides an additive effect [18,24].

ROS mediated kidney dysfunction is also evidenced by the rise of uric acid and creatinine level in plasma of ISO administered rats [37]. In this study, ISO administration increased the uric acid and creatinine level in plasma which was ameliorated by *Flacourtia indica* fruit extract treatment. The kidney dysfunction was also developed due to the production of free radicles in the tissues. Nitric oxide is a prominent oxidative stress marker [37]. In general, nitric oxide will be changed over time into peroxynitrite which is more reactive than superoxide and can convey irreversible cellular damage [38]. In this study, ISO administration increased nitric oxide level in kidney tissues which is co-related to the up-regulation of iNOS expression in kidney [39,40]. iNOS is the form of nitric oxide synthase, regulates the inflammatory state and lead to the dysfunction of the kidney [38,41]. In this study, *Flacourtia indica* fruit extract treatment prevented the rise of nitric oxide level and normalized the iNOS expression in kidney of ISO administered rats. This NO scavenging effect of *Flacourtia indica* fruit extract is comparable to the atenolol treatment in ISO administered rats. Earlier reports suggest that beta blockers may scavenge nitrosative stress [31] and protect kidney structure and function by modulating the NOS expression in kidneys of 2K1C rats [32]. Moreover, impelled protein oxidation products (APOP) are other inauspicious oxidative stress parameters. ISO administration also pushed protein oxidation product formation in kidney. The development of such oxidative stress was also contributed by the lowering of tissue antioxidant activities. The free radical scavenging systems such as catalase, superoxide dismutase and reduced glutathione serves to the recovery of oxidative stress. In this investigation, tissue antioxidant enzymes activities were diminished significantly in ISO administered rats; and these results are correlated with the previous reports [15,29]. Previous report also suggests the notion that, quercetin and gallic acid may augment the catalase, superoxide dismutase activities and restored reduced glutathione level in injured heart in rats [42]. Treatment with *Flacourtia indica* also showed basic augmentation and restoration of catalase and SOD activities and restored the reduced glutathione level in kidneys of ISO administered rats. Furthermore, *Flacourtia indica* fruit extract prevented the advanced protein oxidation product formation in ISO administered rats.

To look for further mechanism of protective effect of *Flacourtia indica* fruit extract in kidneys, we checked the expression of Nrf-2 mediated pathways. Nrf-2 is the key epigenetic regulator in the management of oxidative stress in tissues which will regulate several antioxidant

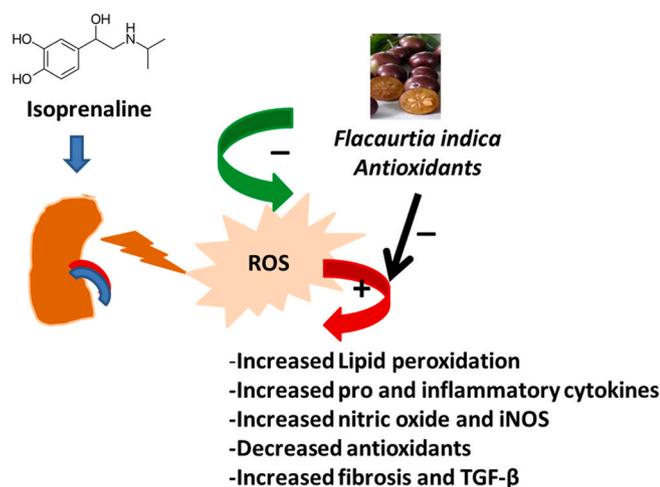
enzymes, for example SOD, GSH-Px, and catalase [43,44]. Loss of Nrf-2 showed the increased renal vascular permeability and attracts pro-inflammatory mediators in ischemia-reperfusion condition [45]. In this study, ISO administration lowered the Nrf-2 expression in kidneys. Nrf-2 translocation also regulates the induction of HO-1 [46]. Previous report also suggests that increased HO-1 expression showed cytoprotective role in cisplatin, rapamycin induced nephrotoxicity, and in ischemia and glycerol induced nephritis [47,48]. This study also showed that HO-1 and HO-2 expression were also down regulated in kidney of ISO administered rats. *Flacourtia indica* fruit extract treatment augmented Nrf-2 mediated action and up-regulated HO-1 and HO-2 expression in kidneys of ISO administered rats. These findings are also supported by previous reports suggest that natural antioxidants may restore the Nrf-2 mediated action and prevents diabetic nephropathy [49].

Oxidative stress mediated tissue damage in kidney also triggers the inflammation and fibrosis [37]. This investigation revealed that ISO administration increased the inflammatory genes expression such as IL-1, IL-6, TNF- $\alpha$  and iNOS in kidney tissues. ISO administration also showed increased MPO activity in kidney suggests the neutrophil infiltration [50,51]. *Flacourtia indica* fruit extract and atenolol treatment prevented the rise of MPO activity in ISO administered rats. Moreover, *Flacourtia indica* fruit extract treatment prevented the inflammation by down regulating NF- $\kappa$ B expression in kidney tissue. NF- $\kappa$ B transcription factor is expressed highly due to ISO administration which also leads to the generation of other inflammatory factors [52]. This current study followed this notion that inflammatory genes expression such as IL-1, IL-6, and TNF- $\alpha$  were increased in ISO administered kidneys due to NF- $\kappa$ B activation. However, currently no report was found to compare the results, that *Flacourtia indica* fruit extract treatment lowered the inflammatory genes expression in kidneys. Histological staining also suggested the increased collagen deposition in kidney of ISO administered rats. Atenolol and *Flacourtia indica* fruit extract treatment in ISO administered rats prevented the fibrosis in kidney. This result could be a direct effect of reduced inflammatory genes expression and TGF- $\beta$  down regulation in kidney by the *Flacourtia indica* fruit extract treatment. Oxidative stress and inflammatory cytokines are the key regulators for TGF- $\beta$  activation and fibrosis in kidneys [53,54]. Earlier report revealed that *Flacourtia indica* fruit extract possesses potent antioxidants such as ferulic acid and caffeic acid [24]. It has been suggested that ferulic acid and caffeic acid derivatives showed antifibrotic activity in kidney by obstructing TGF- $\beta$  mediated pathways [55,56]. However, our analysis revealed that gallic acid and quercetin are the predominant type of phenolic antioxidants present in the extract. Both these two compounds are strong antioxidants and showed anti-inflammatory activities. Quercetin treatment is also linked to the inhibition of fibrosis in kidneys probably by alleviating the TGF- $\beta$  mediated signaling pathways [57].

In conclusion, *Flacourtia indica* fruit extract showed beneficial effect in ISO administered kidney dysfunction in rats. The mechanisms of the beneficial effect of *Flacourtia indica* fruit extract are presented in the schematic diagram at Figure- 7. The prevention of lipid peroxidation and oxidative stress in kidney is augmented by restoration of antioxidants function. Further investigation is warranted to establish the benefit of antioxidants from *Flacourtia indica* fruits in a clinical setup.

#### Authors contribution

All authors have read and agreed to the submitted version of the manuscript. Conceptualization, MAA and FK; methodology, FK and MMH; Animal study/investigation, SS, SS and SI; Biochemical analysis, NA, SS, FIC, KSA; data curation, MAA, NS, HH.; writing—original draft preparation, MAA, FK, KSA and NS; writing—review and editing, MAA, MM, MMH; supervision, MAA, FK, MMH; project administration, MAA, FK.



**Fig. 7.** Schematic presentation of mechanism exerted by the *Flacourtia indica* fruit extract in kidney dysfunction of ISO administered rats. *Flacourtia indica* fruit extract are rich in polyphenols which could be responsible for the prevention of oxidative stress and inflammation in kidneys of rats. This beneficial effect could be attributed to the mechanism in which polyphenolic substance may inhibit the inflammatory cytokines expression, decreased nitric oxide production by iNOS and prevented the TGF-β mediated fibrosis signal in kidneys of ISO administered rats.

#### Funding

This project did not received any specific financial support from government, commercial, or not-for-profit organization.

#### Conflicts of interest

The authors would like to declare that there is no conflict of interests regarding the publication of this paper.

#### Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgement

The authors gratefully acknowledge the logistic supports provided by the Department of Pharmaceutical Sciences and Genome Research Institute of North South University, Bangladesh, for the conduction of different experiments.

#### References

- [1] GBD 2015 Mortality and Causes of Death Collaborators, Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the global burden of disease study 2015, *Lancet* 388 (2016) 1459–1544.
- [2] M. Tonelli, S. Agarwal, A. Cass, G.G. Garcia, V. Jha, S. Naicker, H. Wang, C.-W. Yang, D. O'Donoghue, How to advocate for the inclusion of chronic kidney disease in a national noncommunicable chronic disease program, *Kidney Int.* 85 (2014) 1269–1274.
- [3] S. Anand, D. Kondal, M. Montez-Rath, Y. Zheng, R. Shivashankar, K. Singh, P. Gupta, R. Gupta, V.S. Ajay, V. Mohan, R. Pradeepa, N. Tandon, M.K. Ali, K.M. V. Narayan, G.M. Chertow, et al., Prevalence of chronic kidney disease and risk

factors for its progression: a cross-sectional comparison of indians living in indian versus u.s. Cities, *PLoS One* 12 (2017), e0173554.

- [4] M. Kulathunga, M.A. Ayanka Wijayawardena, R. Naidu, A.W. Wijeratne, Chronic kidney disease of unknown aetiology in Sri Lanka and the exposure to environmental chemicals: a review of literature, *Environ. Geochem. Health* 41 (2019) 2329–2338.
- [5] M.W.A. de Silva, Drinking water and chronic kidney disease of unknown aetiology in anuradhapura, Sri Lanka, *Anthropol. Med.* (2018) 1–17.
- [6] C. O'Callaghan-Gordo, R. Shivashankar, S. Anand, S. Ghosh, J. Glaser, R. Gupta, K. Jakobsson, D. Kondal, A. Krishnan, S. Mohan, V. Mohan, D. Nitsch, P.A. Praveen, N. Tandon, K.M.V. Narayan, et al., Prevalence of and risk factors for chronic kidney disease of unknown aetiology in India: secondary data analysis of three population-based cross-sectional studies, *BMJ Open* 9 (2019), e023353.
- [7] A. Duni, V. Liakopoulos, K.-P. Rapsomanikis, E. Dounousi, Chronic kidney disease and disproportionately increased cardiovascular damage: does oxidative stress explain the burden? *Oxid. Med. Cell Longev.* 2017 (2017), 9036450-9036450.
- [8] D.M. Small, J.S. Coombes, N. Bennett, D.W. Johnson, G.C. Gobe, Oxidative stress, anti-oxidant therapies and chronic kidney disease, *Nephrology* 17 (2012) 311–321.
- [9] R.K. Sindhu, A. Ehdiaie, F. Farmand, K.K. Dhaliwal, T. Nguyen, C.-D. Zhan, C. K. Roberts, N.D. Vaziri, Expression of catalase and glutathione peroxidase in renal insufficiency, *Biochim. Biophys. Acta* 1743 (2005) 86–92.
- [10] F. Nuhu, S. Bhandari, Oxidative stress and cardiovascular complications in chronic kidney disease, the impact of anaemia, *Pharmaceuticals* 11 (2018).
- [11] E. Dounousi, E. Papavasiliou, A. Makedou, K. Ioannou, K.P. Katopodis, A. Tselipis, K.C. Siamopoulos, D. Tsakiris, Oxidative stress is progressively enhanced with advancing stages of ckd, *Am. J. Kidney Dis.* 48 (2006) 752–760.
- [12] B.B. Ratliff, W. Abdulmahdi, R. Pawar, M.S. Wolin, Oxidant mechanisms in renal injury and disease, *Antioxidants Redox Signal.* 25 (2016) 119–146.
- [13] D. Impellizzeri, E. Esposito, J. Attley, S. Cuzzocrea, Targeting inflammation: new therapeutic approaches in chronic kidney disease (ckd), *Pharmacol. Res.* 81 (2014) 91–102.
- [14] V. Cachofeiro, M. Goicochea, S.G. de Vinuesa, P. Oubina, V. Lahera, J. Luno, Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease, *Kidney Int.* (2008) S4–S9.
- [15] M.N. Alam, M.M. Hossain, M.M. Rahman, N. Subhan, M.A.A. Mamun, A. Ulla, H. M. Reza, M.A. Alam, Astaxanthin prevented oxidative stress in heart and kidneys of isoproterenol-administered aged rats, *J. Diet. Suppl.* 15 (2018) 42–54.
- [16] M.A.T. Sagor, N. Tabassum, M.A. Potol, M.A. Alam, Xanthine oxidase inhibitor, allopurinol, prevented oxidative stress, fibrosis, and myocardial damage in isoproterenol induced aged rats, *Oxid. Med. Cell. Longev.* 2015 (2015), 478039-478039.
- [17] S. Roumeliotis, A. Roumeliotis, E. Dounousi, T. Eleftheriadis, V. Liakopoulos, Dietary antioxidant supplements and uric acid in chronic kidney disease: a review, *Nutrients* 11 (2019) 1911.
- [18] A.R. Ndhkala, K. Chitindingu, C. Mupure, T. Murenje, F. Ndhkala, M.A. Benhura, M. Muchuweti, Antioxidant properties of methanolic extracts from *Diospyros mespiliformis* (jackal berry), *Flacourtia indica* (batoka plum), *Uapaca kirkiana* (wild loquat) and *Ziziphus mauritiana* (yellow berry) fruits, *Int. J. Food Sci. Technol.* 43 (2008) 284–288.
- [19] K.R. Kirtikar, B.D. Basu, *Indian Medicinal Plants*, Singh and MP Singh Publications, India, 1998.
- [20] M. Nazneen, M.A. Mazid, J.K. Kundu, S.C. Bachar, F. Begum, B.K. Datta, Protective effects of *Flacourtia indica* aerial parts extracts against paracetamol-induced hepatotoxicity in rats, *J. Taibah Univ. Sci.* 2 (2009) 1–6.
- [21] A.M. Kaou, V. Mahiou-Leddet, C. Canlet, L. Debrauwer, S. Hutter, M. Laget, R. Faure, N. Azas, E. Ollivier, Antimalarial compounds from the aerial parts of *Flacourtia indica* (flacourtiaceae), *J. Ethnopharmacol.* 130 (2010) 272–274.
- [22] S. Shiv Vardan, S. Atul, Jyotshna, C. Upma, S. Subhash Chandra, S. Karuna, K. S. Jitendra, B. Gitika, P. Anirban, A mechanism-based pharmacological evaluation of efficacy of flacourtia indica in management of dyslipidemia and oxidative stress in hyperlipidemic rats, *J. Basic Clin. Physiol. Pharmacol.* 27 (2016) 121–129.
- [23] S. Palani, M. Jayakumar, S. Karthi, S. Raja, Protective effects of flacourtia indica on doxorubicin-induced cardiotoxicity in rats, *Environ. Toxicol. Chem.* 94 (2012) 1014–1025.
- [24] A.R. Ndhkala, A. Kasiyamhuru, C. Mupure, K. Chitindingu, M.A. Benhura, M. Muchuweti, Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*, *Food Chem.* 103 (2007) 82–87.
- [25] A.G.A.W. Alakolanga, A.M.D.A. Siriwardane, N. Savitri Kumar, L. Jayasinghe, R. Jaiswal, N. Kuhnert, LC-MS/MS identification and characterization of the phenolic compounds from the fruits of *Flacourtia indica* (Burm. F.) Merr. and *Flacourtia inermis* Roxb, *Food Res. Int.* 62 (2014) 388–396.
- [26] N. Akter, F.I. Chowdhury, S. Selim, S.I. Nayan, F. Khan, N. Subhan, H. Hossain, M. M. Rahman, M.A. Haque, M.A. Alam, Polyphenolics in ramontchi protect cardiac tissues via suppressing isoprenaline-induced oxidative stress and inflammatory responses in Long-Evans rats, *J. Func. Foods* 75 (2020), 104250.
- [27] A. Ulla, M.A. Alam, B. Sikder, F.A. Sumi, M.M. Rahman, Z.F. Habib, M. K. Mohammed, N. Subhan, H. Hossain, H.M. Reza, Supplementation of *Syzygium cumini* seed powder prevented obesity, glucose intolerance, hyperlipidemia and oxidative stress in high carbohydrate high fat diet induced obese rats, *BMC Compl. Alternative Med.* 17 (2017), 289–289.
- [28] N.S. Dhalla, R.M. Tamsah, T. Netticadan, Role of oxidative stress in cardiovascular diseases, *J. Hypertens.* 18 (2000) 655–673.
- [29] F.A. Sumi, B. Sikder, M.M. Rahman, S.R. Lubna, A. Ulla, M.H. Hossain, I.A. Jahan, M.A. Alam, N. Subhan, Phenolic content analysis of *Aloe vera* gel and evaluation of the effect of aloe gel supplementation on oxidative stress and fibrosis in

- isoprenaline-administered cardiac damage in rats, *Prev. Nutr. Food Sci.* 24 (2019) 254–264.
- [30] N.H. Moniri, Y. Daaka, Agonist-stimulated reactive oxygen species formation regulates beta2-adrenergic receptor signal transduction, *Biochem. Pharmacol.* 74 (2007) 64–73.
- [31] A. Gomes, D. Costa, J.L. Lima, E. Fernandes, Antioxidant activity of beta-blockers: an effect mediated by scavenging reactive oxygen and nitrogen species? *Bioorg. Med. Chem.* 14 (2006) 4568–4577.
- [32] Y. Wang, M. Niu, S. Yin, F. Zhang, R. Shi, Nephroprotective effects of nebivolol in 2k1c rats through regulation of the kidney ros-adma-no pathway, *Pharmacol. Rep.* : PR. 70 (2018) 917–929.
- [33] Y. Yuksel, R. Yuksel, M. Yagmurca, H. Haltas, H. Erdamar, M. Toktas, O. Ozcan, Effects of quercetin on methotrexate-induced nephrotoxicity in rats, *Hum. Exp. Toxicol.* 36 (2016) 51–61.
- [34] J. Renugadevi, S.M. Prabu, Quercetin protects against oxidative stress-related renal dysfunction by cadmium in rats, *Exp. Toxicol. Pathol.* 62 (2010) 471–481.
- [35] P. Reckziegel, V.T. Dias, D.M. Benvenú, N. Bouffleur, R.C.S. Barcelos, H.J. Segat, C. S. Pase, C.M.M. Dos Santos, É.M.M. Flores, M.E. Bürger, Antioxidant protection of gallic acid against toxicity induced by pb in blood, liver and kidney of rats, *Toxicol. Rep.* 3 (2016) 351–356.
- [36] S. Panda, A. Kar, T. Banerjee, N. Sharma, Combined effects of quercetin and atenolol in reducing isoproterenol-induced cardiotoxicity in rats: possible mediation through scavenging free radicals, *Cardiovasc. Toxicol.* 12 (2012) 235–242.
- [37] A. Ulla, M.K. Mohamed, B. Sikder, A.F.M.T. Rahman, F.A. Sumi, M. Hossain, H. M. Reza, G.M.S. Rahman, M.A. Alam, Coenzyme Q10 prevents oxidative stress and fibrosis in isoprenaline induced cardiac remodeling in aged rats, *BMC Pharmacol. Toxicol.* 18 (2017) 29.
- [38] U. Förstermann, W.C. Sessa, Nitric oxide synthases: regulation and function, *Eur. Heart J.* 33 (2012) 829–837d.
- [39] S. Heemskerk, P. Pickkers, M.P.W.J.M. Bouw, A. Draisma, J.G. van der Hoeven, W. H.M. Peters, P. Smits, F.G.M. Russel, R. Masereeuw, Upregulation of renal inducible nitric oxide synthase during human endotoxemia and sepsis is associated with proximal tubule injury, *Clin. J. Am. Soc. Nephrol.* 1 (2006) 853.
- [40] C.J. Njoku, K.S. Patrick, P. Ruiz, J.C. Oates, Inducible nitric oxide synthase inhibitors reduce urinary markers of systemic oxidant stress in murine proliferative lupus nephritis, *J. Invest. Med.* 53 (2005) 347.
- [41] R. Zamora, Y. Vodovotz, T.R. Billiar, Inducible nitric oxide synthase and inflammatory diseases, *Mol. Med.* 6 (2000) 347–373.
- [42] V.V. Padma, P. Poornima, C. Prakash, R. Bhavani, Oral treatment with gallic acid and quercetin alleviates lindane-induced cardiotoxicity in rats, *Can. J. Physiol. Pharmacol.* 91 (2012) 134–140.
- [43] K. Yamawaki, H. Kanda, R. Shimazaki, Nrf-2 activator for the treatment of kidney diseases, *Toxicol. Appl. Pharmacol.* 360 (2018) 30–37.
- [44] C. Zoja, A. Benigni, G. Remuzzi, The Nrf-2 pathway in the progression of renal disease, *Nephrol. Dial. Transplant.* 29 (2013) i19–i24.
- [45] M. Liu, D.N. Grigoryev, M.T. Crow, M. Haas, M. Yamamoto, S.P. Reddy, H. Rabb, Transcription factor Nrf-2 is protective during ischemic and nephrotoxic acute kidney injury in mice, *Kidney Int.* 76 (2009) 277–285.
- [46] E. Balogun, M. Hoque, P. Gong, E. Killeen, C.J. Green, R. Foresti, J. Alam, R. Motterlini, Curcumin activates the haem oxygenase-1 gene via regulation of Nrf-2 and the antioxidant-responsive element, *Biochem. J.* 371 (2003) 887–895.
- [47] G.M. Goncalves, M.A. Cenedeze, C.Q. Feitoza, P.M. Wang, A.P. Bertocchi, M. J. Damiao, H.S. Pinheiro, V.P. Antunes Teixeira, M.A. dos Reis, A. Pacheco-Silva, N.O. Camara, The role of heme oxygenase 1 in rapamycin-induced renal dysfunction after ischemia and reperfusion injury, *Kidney Int.* 70 (2006) 1742–1749.
- [48] P.K. Datta, S.B. Koukouritaki, K.A. Hopp, E.A. Lianos, Heme oxygenase-1 induction attenuates inducible nitric oxide synthase expression and proteinuria in glomerulonephritis, *J. Am. Soc. Nephrol.* 10 (1999) 2540–2550.
- [49] N. Al-Waili, H. Al-Waili, T. Al-Waili, K. Salom, Natural antioxidants in the treatment and prevention of diabetic nephropathy; a potential approach that warrants clinical trials, *Redox Rep.* 22 (2017) 99–118.
- [50] D. Odobasic, A.R. Kitching, T.J. Semple, S.R. Holdsworth, Endogenous myeloperoxidase promotes neutrophil-mediated renal injury, but attenuates T-cell immunity inducing crescentic glomerulonephritis, *J. Am. Soc. Nephrol.* 18 (2007) 760.
- [51] E. Malle, T. Buch, H.-J. Grone, Myeloperoxidase in kidney disease, *Kidney Int.* 64 (2003) 1956–1967.
- [52] A.P. Davel, L.E. Fukuda, L.L. De Sa, C.D. Munhoz, C. Scavone, D. Sanz-Rosa, V. Cachofeiro, V. Lahera, L.V. Rossoni, Effects of isoproterenol treatment for 7 days on inflammatory mediators in the rat aorta, *Am. J. Physiol. Heart Circ.* 295 (2008) H211–H219.
- [53] A. Biernacka, M. Dobaczewski, N.G. Frangogiannis, TGF- $\beta$  signaling in fibrosis, *Growth Factors* 29 (2011) 196–202.
- [54] Y. Ding, M.E. Choi, Regulation of autophagy by TGF- $\beta$ : emerging role in kidney fibrosis, *Semin. Nephrol.* 34 (2014) 62–71.
- [55] M.-g. Wei, W. Sun, W.-m. He, L. Ni, Y.-y. Yang, Ferulic acid attenuates TGF- $\beta$ -induced renal cellular fibrosis in NRK-52E cells by inhibiting Smad/Ilk/Snail pathway, *Evid. Based Complement. Altern. Med.* 2015 (2015) 7.
- [56] S.-T. Chuang, Y.-H. Kuo, M.-J. Su, Antifibrotic effects of KS370G, a caffeamide derivative, in renal ischemia-reperfusion injured mice and renal tubular epithelial cells, *Sci. Rep.* 4 (2014), 5814–5814.
- [57] Y. Cao, J. Hu, J. Sui, L. Jiang, Y. Cong, G. Ren, Quercetin is able to alleviate tgfbeta-induced fibrosis in renal tubular epithelial cells by suppressing MIR-21, *Exp. Ther. Med* 16 (2018) 2442–2448.