

Mangrove Fruit Keora Peel Extract Prevents Oxidative Stress and Fibrosis in the Kidney of ISO Administered Rats

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Abstract: Cardiorenal complications often arise from oxidative stress which ultimately disrupts the renal structure and function. For the exploration of the therapeutic potential of natural antioxidants, this study examined the effects of *Sonneratia apetala* (Keora) fruit peel extract in an Isoproterenol (ISO) – induced model of renal injury in rats. The Long Evans rats were grouped as Control, ISO, Control + Keora, and ISO + Keora. ISO (50 mg/kg) was administered subcutaneously every third day for two weeks, which produced significant oxidative stress to renal tissues, evidenced by an elevated MDA, NO, AOPP and MPO levels, along with a reduced SOD, CAT and GSH activities, increased plasma creatinine and uric acid concentrations. Oral pretreatment with Keora peel extract (100 mg/kg/day) provided an improved antioxidant enzyme activity, while lowering the oxidative and nitrosative stress markers thus normalizing the renal function indicators. Histopathological changes also backed up these biochemical results, showing a more preserved glomerular morphology and reduced collagen accumulation in the renal tissues of the Keora-treated rats. Overall, Keora fruit peel extract showed a strong renoprotective potential against ISO-induced nephrotoxicity, which suggests that its bioactive constituents may counteract oxidative damage and fibrosis through the enhancement of antioxidant defenses and limiting oxidative injury.

Keywords: Mangrove; Keora; Oxidative stress; Antioxidant; Renoprotective.

1. Introduction

Individuals who have chronic kidney disease (CKD) are prone to develop cardiovascular disorders, which can appear as heart failure, arrhythmias, coronary artery disease, and cardiac arrest [1]. Furthermore, if both comorbidities are present, the prognosis of patients with cardiovascular disease is significantly impacted by the presence of CKD, which raises morbidity and mortality [2]. The intricate relationship with CKD pathophysiological features, such as oxidative stress, fluid retention, or renin-angiotensin system activation, results in cardiac abnormalities such as ventricular hypertrophy or fibrosis [3]. By contrast, heart failure (HF) promotes kidney failure by contributing to congestion, hypoperfusion, and deteriorating kidney function [4]. High triglycerides, LDL, and low HDL cholesterol are lipid abnormalities that are typically observed in patients with CKD and are also linked to atherosclerosis development

and thus might further progress to a heart attack [5, 6]. There is a direct connection between cardiovascular disease and kidney disease [7]. When one organ is affected, the other becomes dysfunctional and eventually fails [8]. Oxidative stress, activation of the renin-angiotensin system, and fluid retention due to kidney disease can lead to the progression of heart disease due to excess stress [3]. Likewise, heart disease can contribute to damaging kidney function due to congestion, hypoperfusion, etc. [4]. Kidney patients are more likely to develop atherosclerosis due to high cholesterol levels and triglycerides, which can also lead to the possible development of myocardial infarction. [5, 6].

Among the planet's most prolific and varied ecosystems, mangrove forests are essential for preserving biodiversity, protecting coastlines, and slowing down the effects of climate change. *Sonneratia apetala* (Keora), one of the many mangrove species found across the world, is notable for its distinct traits and ecological roles [9]. Along the coasts of Bangladesh, India, Australia, Malaysia, and other nations, the mangrove fruit *S. apetala* is a quickly growing and pioneering tree species in ecological succession in the Sundarbans. Local coastal populations consume a lot of *S. apetala* fruits [10]. Although they are unaware of the fruit's impact on their physiological health, the people who live close to the Sundarbans frequently eat the fruit boiled and occasionally in other preparations. Half-ripe fruit is used to treat coughs, and ripe fruits are utilized to get rid of intestinal parasites [11]. This fruit's fermented juice can be used to stop bleeding. Asthma, fever, ulcers, swellings, sprains, bleeding, hemorrhages, and piles can all be treated using the fruits and bark of plants in the genus *Sonneratia* [12]. Polyphenols, flavonoids, tannins, and carbohydrates are Keora's primary bioactive components. These compounds enhance its antioxidant activity and make it a possible natural antioxidant source. Furthermore, the biological characteristics of Keora are also influenced by triterpenes, steroids, carboxylic acids, and lactones [13]. The aim of this research to evaluate the renoprotective effects of Keora fruit peel extract in isoprenaline-induced rats by preventing oxidative stress, fibrosis, and restoring antioxidant enzymes in kidney tissues.

2. Materials and Methods

2.1 Chemicals

Isoprenaline hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO, USA). Reagents and standards required for myeloperoxidase (MPO), malondialdehyde (MDA), nitric oxide (NO), and advanced oxidation protein products (AOPP) assays, Catalase (CAT), along with Picrosirius red staining materials, were sourced from Merck (Darmstadt, Germany) and Sigma-Aldrich. standards and associated assay components were procured from SR Group (Delhi, India). Commercial kits for uric acid and creatinine analyses were purchased from DCI Diagnostics (Budapest, Hungary).

2.2 Preparation of *Sonneratia apetala* Root Extracts

The fruits of Keora were sourced from the Mangrove forest Sundarbans, located in Khulna, Bangladesh. After collection, the roots were oven-dried at 50 °C and finely ground into powder. A total of 500 g of the powdered material was macerated in ethanol within a sealed glass container and stored in a dark environment at ambient temperature for five days, with intermittent shaking. The resulting mixture was filtered to obtain the ethanolic extract. Ethanol was subsequently removed using a rotary evaporator set at 50 °C and 100 rpm, yielding a concentrated crude extract. An identical procedure was employed to prepare the methanolic extract.

2.3 Experimental animals

Twenty-four male Long Evans rats, aged 8–10 weeks and weighing between 180–190 g, were procured from the animal facility of the Department of Pharmaceutical Sciences at North South University, Dhaka, Bangladesh, for this study. The animals were housed under standardized laboratory conditions, including a 12-

hour light/dark cycle, relative humidity of approximately 55%, and a controlled temperature of 25 ± 2 °C. All procedures adhered to international ethical guidelines outlined by the Council for International Organization of Medical Sciences (CIOMS) and the International Council for Laboratory Animal Science (ICLAS). Ethical approval for the experimental protocols was granted by the Institutional Animal Care and Use Committee (IACUC) of North South University (Approval No- 2025/OR-NSU/IACUC/0209).

2.4 Experimental design and treatment protocol

The rats were divided into four groups, each group consisting of 6 rats. The groups were as such.

1. Control group: For two weeks, the rats in this group were fed chow meals and regular water daily.
2. ISO (Isoprenaline) group: For two weeks, the ISO group received subcutaneous injections of isoprenaline at a rate of 50 mg/kg every three days. The ISO group was given isoprenaline along with water and chow food.
3. Control + Keora: For two weeks, the rats in this group were given regular water and chow meals treated with Keora fruit peel extract (100 mg/kg of body weight).
4. ISO + Keora: For two weeks, the rats in this group were given 50 mg/kg of isoprenaline subcutaneously every three days, along with chow food and water. This group also received a 100 mg/kg of body weight dose of Keora fruit peel extract.

After completion of 14 days of the procedure, the rats were weighed and then sacrificed on the 15th day. Blood was collected and centrifuged at 4000 rpm for 10 min, with a temperature of 4 °C in order to collect the plasma. Following that, the required organs left and right kidneys, were collected and weighed. The harvested organs, including kidney tissues, were stored for bioassay, and one part of the kidney was stored in neutral buffer formalin for the use of histology staining. The plasma and tissues collected for bioassay were stored at -18 °C preserved for bioassay analysis. A portion of the kidney tissue was fixed in neutral buffered formalin for histological examination. All plasma and tissue samples designated for bioassay were stored at -18 °C until further use.

2.5 Induction of Myocardial Infarction

Rats were given subcutaneous injections of 50 mg/kg of isoproterenol (ISO) hydrochloride, dissolved in physiological solution, to induce an experimental myocardial infarction.

2.6 Determination of Kidney Function via Creatinine and Uric Acid Measurement

Kidney specific marker tests such as creatinine, and uric acid were also performed using plasma following the manufacturer's protocol.

2.7 Preparing a Tissue Sample for Oxidative Stress Marker Evaluation

To separate the supernatant, the heart and kidney tissues were homogenized in 10% phosphate buffer saline (pH 7.4) and centrifuged at 8000 rpm for 15 minutes at 4°C. After collecting, the supernatants were utilized in enzymatic and protein analyses.

2.8 Malondialdehyde estimation (MDA)

To detect plasma and heart lipid peroxidation, reactive thiobarbituric acid substances (TBARS) were measured colorimetrically using a previously published technique [14]. Malondialdehyde (MDA), a consequence of lipid peroxidation, is measured using the TBARS test. Samples of plasma and heart tissue were diluted with PBS, 100 µL of glacial acetic acid was added, and the mixture was left for 10 minutes to measure the MDA concentration. After adding 200 µL of TBA (0.37%), the mixture was put in a sealed Eppendorf tube and heated for about fifteen minutes in a hot water bath. After cooling to room temperature, 200 µL of the mixture was put into a 96-well plate, and the absorbance at 535 nm was measured. Using 1,1,3,3-tetramethoxypropane as a reference, an MDA standard curve was created to determine the MDA levels in tissue and plasma samples. The measuring units of plasma and tissue were mmol/mL and mmol/g, respectively.

2.9 Nitric oxide estimation (NO)

Using the earlier technique (Griess reaction), the NO concentration was determined [14]. This procedure involved taking 20 μ L samples on a 96-well plate with 80 μ L PBS and waiting 10 minutes. The sample mixture was then mixed with 50 μ L of 0.1% w/v naphthyl ethylene diamine dihydrochloride (NED) and 50 μ L of sulfanilamide solution. The liquid was then made acidic by adding 50 μ L of 15% glacial acetic acid. After ten minutes of waiting, the absorbance at 540 nm was measured. The units were expressed as mmol/mL or mmol/g of tissue, and the standard curve was created using NaNO₂ as a standard.

2.10 Estimation of Advanced Protein Oxidation Products (APOP)

The aforementioned technique was used to measure the amount of AOPP in tissues and plasma [15]. In order to create an acidic medium, 50 μ L of glacial acetic acid (15%) was added to 10 μ L samples that had been collected with 90 μ L PBS on a 96-well plate. To create a colored complex, 50 μ L of potassium iodide (1.16 mM) was then added. The absorbance at 405 nm was measured after two minutes of waiting. The units were expressed as mmol/mL or mmol/g of tissue, with chloramine T serving as the reference.

2.11 Estimation of Myeloperoxidase (MPO) Activity

MPO activity was assessed using the previously developed di-anisidine-H₂O₂-based test technique [16]. This method was modified to function with 96-well plates. 10 μ L sample were mixed with 250 μ L PBS and then 3 μ L of H₂O₂ (0.15 mM) were added. After that, 3 μ L of o-dianisidine solution was added, and the absorbance was taken at 460 nm and reported as MPO activity/mg protein.

2.12 Assessment of the activity of antioxidant enzymes

SOD, catalase, and GSH activities were also measured in the heart and plasma using modified methods that have been previously reported in the literature [17, 18]. To put it briefly, 90 μ L of PBS was combined with 10 μ L of the sample, and then 100 μ L of adrenaline was added. Absorbance was measured at 15-second intervals for 1 minute at 480 nm during 50% adrenaline auto-oxidation. One minute after starting, changes in absorbance at 240 nm were monitored. To measure catalase activity, 10 μ L of the sample was combined with 90 μ L of PBS, and then 50 μ L of H₂O₂ (5.9 mmol) was added. After mixing 10 μ L of the sample with 90 μ L of PBS, 100 μ L of DTNB reagent (100 mM 5,5-dithiobis-2-nitrobenzoic acid) was added to measure the GSH activity.

2.13 Histopathological Evaluation

For microscopic examination, excised kidney tissues from experimental rats were initially fixed in 10% neutral buffered formalin (NBF). The fixed specimens were then dehydrated through a graded ethanol series, cleared with xylene, and embedded in paraffin to prevent air entrapment. Thin sections approximately 5 μ m in thickness were prepared using a rotary microtome and mounted onto clean glass slides. These sections were stained with hematoxylin and eosin (H&E) to assess inflammatory cell infiltration. Additionally, Picrosirius Red staining was performed to visualize and evaluate fibrotic alterations. All stained slides were imaged and analyzed under a light microscope (Zeiss Axioscope) at 40 $\times$ magnification.

2.14 Statistical Analysis

All experimental data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical evaluations were performed using GraphPad Prism version 10. Group comparisons were conducted via one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify significant differences among treatment groups. A *p*-value less than 0.05 was considered indicative of statistical significance.

3. Results

3.1 Effect of Keora fruit peel extract on the histology of the kidney in isoprenaline (ISO) administered rats

In **Figure 1**, the effect of ISO administration and Keora peel extract supplementation on Kidney Wet weight has been shown and it was seen that ISO group showed negligible changes in kidney wet weight compared to the

Control group ($p = 0.3372$). However, the supplementation of Keora peel extract in ISO administered rats showed a significant reduction in kidney weight compared to the ISO only group ($p = 0.0041$). There was no significant difference between the Control and Control + Keora group in the wet weights of the kidney ($p = 0.8800$).

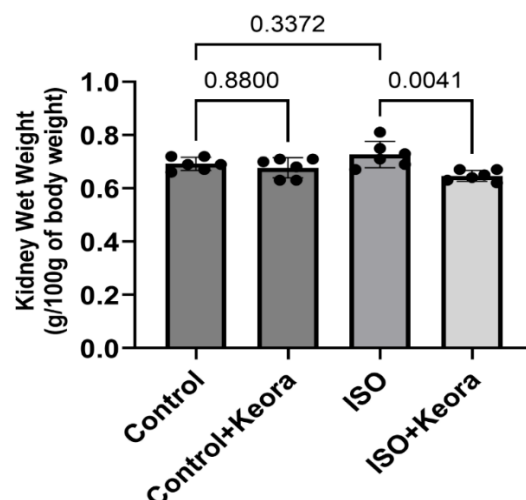


Figure 1: Effect of Keora peel extract supplementation on Kidney Wet weight in ISO administered Rats. Data are expressed as mean $\pm$ Standard Error of Mean (SEM) ($n=6$). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. For significance, $P < 0.05$ was selected for all measurements.

3.2. Effect of Keora Peel Extract Supplementation on Oxidative Stress Markers in Kidney Tissue

Figure 2 represents the levels of oxidative stress markers in the kidney tissues. From **Figure 2A**, it can be seen that the concentration of Malondialdehyde (MDA) was significantly increased in the ISO treated group when compared to the Control group ($p < 0.0001$). Treatment with Keora peel extract in the ISO + Keora group resulted in a significant decrease in MDA concentration when compared to the group treated with ISO only ($p < 0.0001$). Similarly, in **Figure 2B**, which represents the Nitric Oxide concentration in the kidney tissues, it was seen that the ISO treated group had a significantly higher concentration of NO compared to the Control group ($p < 0.0001$). Treatment with Keora peel extract in the ISO + Keora group showed a marked decrease in NO concentration when compared to the ISO administered group ($p < 0.0001$). Furthermore, in **Figure 2C**, the level of Advanced Oxidation Protein Products (AOPP) was found to be significantly increased in the ISO administered group when compared to the Control group ($p < 0.0001$). Keora supplementation to ISO administered rats in the ISO + Keora groups seems to have significantly decreased the AOPP concentration when compared to the ISO group ($p < 0.0001$). Finally, no significant difference was found between the Control group and the Control + Keora group among all Oxidative Stress markers.

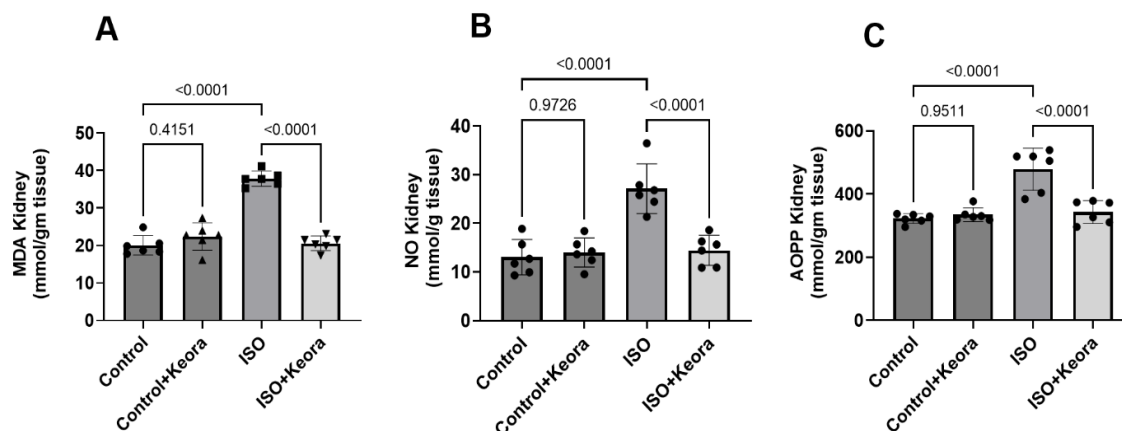


Figure 2: Effect of Keora peel extract on Kidney Oxidative Stress Markers: (A) MDA, (B) NO, and (C) AOPP in ISO-administered Rats. Data are expressed as mean $\pm$ Standard Error of Mean (SEM) (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. For significance, $P < 0.05$ was selected for all measurements.

3.3. Effect of Keora Peel Extract Supplementation on Antioxidant Activity in Kidney Tissue

The antioxidant systems in the kidney tissues have been shown in **Figure 3**. From **Figure 3A**, it can be seen that the Superoxide dismutase (SOD) activity was considerably reduced in the ISO group when compared to the Control group ($P = 0.0008$). Conversely, the ISO + Keora group showed a significant increase in SOD activity when compared to the ISO only group ($P = 0.0171$). In **Figure 3B**, the Catalase activity of the ISO group was also significantly reduced when compared to the Control group ($P = 0.0014$). However, Keora peel extract treatment in the ISO administered group, ISO + Keora, showed a considerable increase in the Catalase activity levels when compared to the ISO only group ($P = 0.0068$). For reduced Glutathione (GSH) represented in **Figure 3C**, the ISO group had significantly diminished levels when compared with the Control group ($P < 0.0001$). ISO + Keora group exhibited a significant increase in the GSH levels when compared to the ISO only group ($P = 0.0002$). No statistically significant difference was found between the Control group and the Control + Keora group for any of the tested Kidney Antioxidant Activities.

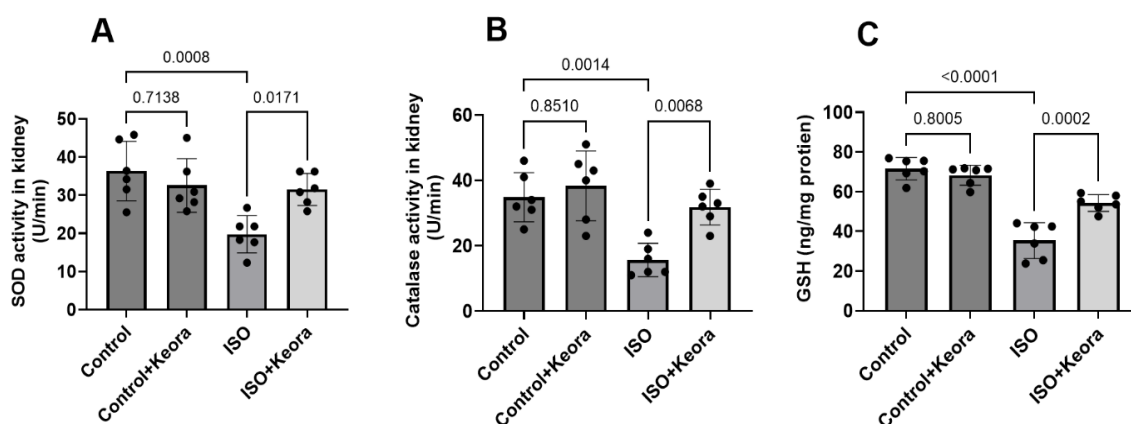


Figure 3: Effect of Keora peel extract on Kidney Antioxidant Activity: (A) SOD, (B) Catalase, and (C) GSH in ISO-administered Rats. Data are expressed as mean $\pm$ Standard Error of Mean (SEM) (n=6). Statistical analysis

was performed using one-way ANOVA followed by Tukey's post hoc test. For significance, $P < 0.05$ was selected for all measurements.

3.4. Effect of Keora Peel Extract Supplementation on MPO Activity, Uric Acid, and Creatinine Levels

The results for Myeloperoxidase (MPO) activity in the kidney have been represented in **Figure 4A**, which shows that ISO had significantly increased the MPO activity in the kidney tissues when compared to the Control group ($P < 0.0001$). Treatment with Keora in the ISO + Keora group drastically reduced the elevated levels when compared to the ISO only treated group ($P < 0.0001$). Figure 4B representing the Uric Acid concentration in plasma also showed a significantly increased concentration of Uric Acid in the ISO group when compared with the Control group ($P < 0.0001$). ISO + Keora-treated group showed a significant decrease in the Uric Acid concentration when compared with the group only administered with ISO ($P < 0.0001$). The Creatinine Concentration in plasma, represented in **Figure 4C**, was highly elevated in the ISO group when compared with the Control group ($P < 0.0001$). The addition of Keora peel extract to ISO administered treatment group, ISO + Keora, exhibited a significantly reduced Creatinine concentration ($P < 0.0001$) when compared with the ISO only group. No significant difference was observed between the Control and Control + Keora groups across MPO activity, Uric Acid, and Creatinine Levels.

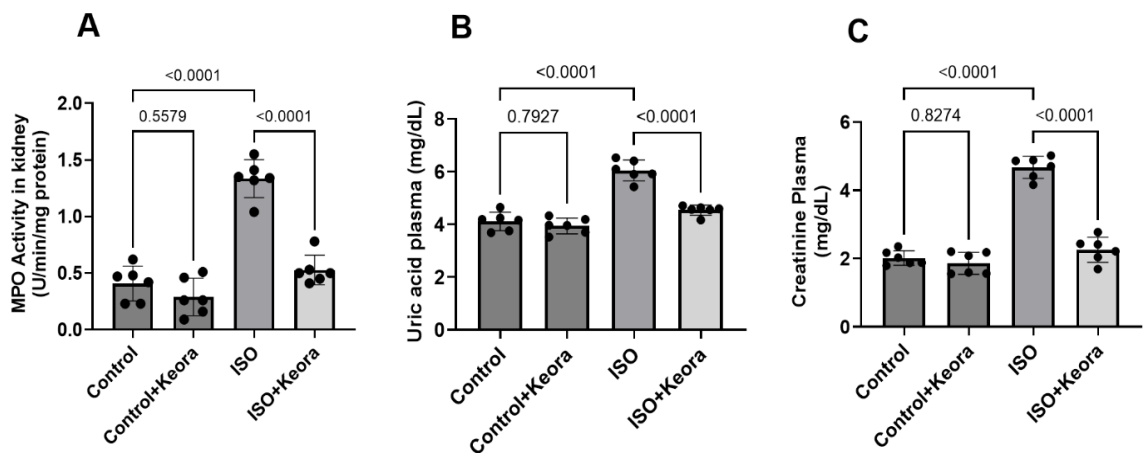


Figure 4: Effect of Keora peel extract on (A) MPO, (B) Uric Acid, and (C) Creatinine in ISO-administered Rats. Data are expressed as mean $\pm$ Standard Error of Mean (SEM) ($n=6$). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. For significance, $P < 0.05$ was selected for all measurements.

3.4 Effect of *S. apetala* fruit peel extract on histology of the kidney in isoprenaline (ISO) administered rats

Figure 5 represents the microscopic observations from H&E and Picrosirius Red staining of kidney tissues. Hematoxylin & Eosin (H&E) staining showed that the Control (A) and Control + Keora (C) groups showed similar normal renal morphology, with the glomeruli and tubules being intact. The ISO only group (B) revealed pronounced pathological changes, including infiltration of inflammatory cells. The ISO + Keora group (D) showed preservation of the renal tissue architecture, having noticeable reduction in infiltration of inflammatory cells when compared with the ISO only group (B).

Picrosirius Red staining for collagen deposition showed minimal basal staining in the Control (E) and Control + Keora (G) groups. The ISO group (F) displayed an intense and widespread collagen deposition, mostly concentrated around the glomerulus. The ISO + Keora group (H) showed a remarkable reduction of collagen deposition when compared with the ISO only group (F).

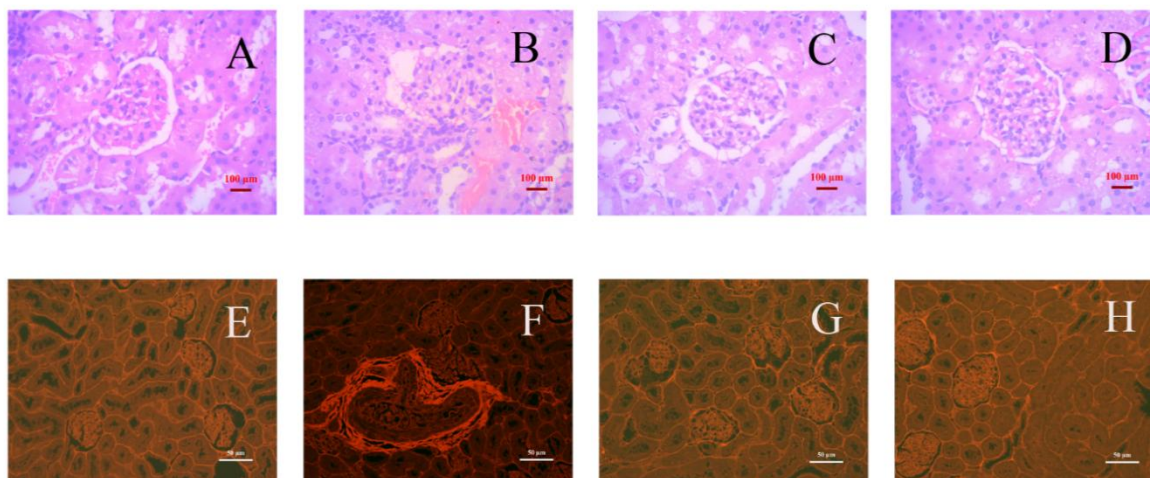


Figure 5: Histopathological and Anti-fibrotic effect of Keora peel extract on Kidney Tissue. (A-D) H&E staining showing morphological changes (40×). (E-H) Picrosirius Red staining showing collagen deposition (20×). Groups are Control (A, E), ISO B, F), Control + Keora (C, G), and ISO + Keora (D, H)

4. Discussion

The study's key discovery is that Keora extracts reduced oxidative stress and lipid peroxidation while increasing antioxidant enzymes, therefore attenuating ISO-induced fibrosis in renal tissue. Additionally, ISO-induced histological alterations were reduced by the extract. The β -adrenergic receptors are expressed by juxtaglomerular granular cells, vascular smooth muscle cells from arteries, and kidney tubules (proximal and distal tubular cells). Norepinephrine sends adrenergic signals to these tissues, which control fluid balance and renal hemodynamics [19]. Cellular macromolecules and membrane phospholipids are oxidized in turn by the excess ROS. These free radicals, which are generated in tissues, can damage and necrotize cells by attacking the lipid layers of the cell membrane [14]. Increased MDA levels and oxidative damage to lipid membranes result from ISO's imbalance between ROS generation and antioxidant defenses. Increased MDA levels signify oxidative stress-induced renal cell membrane damage, which leads to cellular malfunction and apoptosis [20]. The amounts of lipid hydroperoxides and TBARS in the kidney tissue were considerably decreased by pretreatment with Keora fruit peel. This result demonstrated Keora's ability to prevent lipid peroxidation. The development of renal dysfunction was also triggered by the generation of free radicals in the tissues. One common indicator of oxidative stress is Nitric oxide, which is converted to nitrite via oxidation. Normal endothelium and vascular activity trigger the generation of NO, which is known to rise under inflammatory conditions and promote cell death [21]. Inducible nitric oxide synthase, or iNOS, is involved in the creation of NO in vivo. When oxidative stress is present, iNOS expression rises, producing too much NO, which can easily cause lipid peroxidation [22]. By generating excessive quantities of NO, which combine with superoxide to generate peroxynitrite, iNOS plays a crucial role in mediating nitro-oxidative stress [23]. Peroxynitrite, which is more reactive than superoxide and can cause irreversible cellular damage, is often produced over time from nitric oxide [24]. One significant indicator of oxidative stress in tissues is advanced oxidation protein product (AOPP). Plasma proteins may react with chlorine-free radicals

to produce AOPP as a result of elevated oxidative stress [25]. The current study found that ISO rats had higher levels of APOP in their kidney tissues than the control group, which Keora helped to reduce.

Some free radicals may still be present in tissues because of the antioxidants' defense mechanism. SOD, CAT, and GSH are examples of free radical scavenging enzymes that can help mitigate oxidative and nitrosative stress. Furthermore, ISO-induced myocardial injury also results in a decrease in endogenous antioxidants [26]. H_2O_2 is primarily converted to water by catalase, while superoxide radicals are scavenged by SOD to produce hydrogen peroxide (H_2O_2) [27]. Cellular defense against reactive free radicals and other oxidant species is largely dependent on the glutathione antioxidant system. GSH, a cellular tripeptide, neutralizes free radicals to prevent peroxidative damage [28]. Reduced GSH levels in rats given isoproterenol may result from its increased use to shield proteins with SH groups from free radical damage [29]. Glutathione (GSH) and antiperoxidative enzyme (CAT & SOD) activities were significantly reduced in the kidney tissue of the rats given ISO; however, these antioxidant activities were enhanced after pretreatment with Keora fruit peel extract.

MPO is an indicator of oxidative damage and inflammation. It is the most prevalent pro-inflammatory biomarker found in neutrophilic granulocytes [30]. Prior studies found that ISO-induced kidney dysfunction in animals was associated with noticeably elevated MPO levels and activity [31]. MPO makes H_2O_2 more reactive by generating RNS, free radicals, and hypochlorous acid. In acute MI, MPO and all of these products encourage lipid peroxidation, protein nitration, and further oxidative alterations [30]. Our study found a significantly elevated level of MPO in the kidney tissue of ISO-administered rats, which was significantly reduced by the Keora fruit extract treatment. This might be due to the Reno protective effect of Keora.

We also verified in this investigation that elevated kidney injury indicators, such as elevated plasma levels of urea and creatinine, which indicate lower glomerular filtration rates, were linked to isoprenaline-induced kidney damage. Uric acid is the final byproduct of purine metabolism and a waste product that comes from food and diet [32]. Uric acid is primarily controlled by xanthine oxidoreductase, which transforms hypoxanthine into xanthine and xanthine into uric acid [33]. Elevated blood uric acid levels can raise the risk of heart and kidney disease. If the kidneys are unable to eliminate uric acid from our bodies adequately, it can accumulate in the blood and may be a sign of renal dysfunction [34, 35]. In our study, we found that the ISO-administered disease group showed a high level of plasma uric acid compared to the normal control group of rats. The treatment with Keora fruit significantly reduced the elevated uric acid present in the plasma in the ISO-administered rats. This may suggest that the treatment of *S. apetala* fruit enhanced the renal function.

Another commonly used biomarker for kidney function and estimation of glomerular filtration rate is creatinine [36]. When muscles contract, creatinine is generated from the muscle tissue. It is a waste product from metabolism that is easily removed by the kidneys. Therefore, renal insufficiency, which is manifested as a decreased estimated glomerular filtration rate, is linked to extremely high levels of plasma creatinine [37]. Our result displayed a significantly high plasma creatinine level in the ISO-administered group, which indicates renal insufficiency. The high plasma creatinine level was reduced significantly in the treatment group of ISO-administered rats. This might

also indicate the ameliorating effect of the kidney function of the Keora fruit. In addition, the Control+Keora treatment group showed similar creatinine levels, which may prove that there was no kidney toxicity in the given dose of Keora fruit extract.

Lastly, kidney samples were examined histopathologically. Hematoxylin and Eosin were used to stain the kidney sections. Rats given ISO developed aberrant glomerular structure and renal necrosis, which are avoided by treating them with Keora fruit peel. The biochemical investigations that are connected to renal inflammation and oxidative stress corroborate these histological alterations. Collagen deposition in kidney slices is determined by another staining method, such as Sirius red staining. Collagen deposition was observed to be much elevated in the rats given ISO, but it was decreased when Keora fruit was given.

5. Conclusion

The current investigation indicated that pretreatment with Keora fruit peel lowered oxidative stress and lipid peroxidation following ISO-induced renal damage. It enhanced antioxidant enzymes, which lowered the plasma levels of creatinine and uric acid. The reno-protective effects of Keora fruit peel may be related to the presence of antioxidant ingredients and the mitigation of oxidative stress. However, the identification of precise mechanisms of action needs more exploration.

Author Contributions: Conceptualization, MAA and NS; methodology, SS, MA, TJ, MTH, ABJ, and HCG; software, MA and SS; validation, MAA, NS, and HCG; formal analysis, MA, ABJ, AA, TA, JA and NS; investigation, SS, ATH, ABJ, AK, TA and JA; resources, TA and JA; data curation, MAA and MA; writing—original draft preparation, SS, HCG, TJ; writing—review and editing, MA, TA and JA; visualization, MAA, NS; supervision, MAA and NS project administration, MAA; funding acquisition, MAA. All authors have read and agreed to the published version of the manuscript.

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