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Original Article

Evaluation of Reno-Protective Effect of *Asparagus racemosus* Root Extract in Isoproterenol-Induced Rats

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Abstract: Renal dysfunction often accompanies cardiovascular damage, with oxidative stress and inflammation playing central roles. Isoproterenol (ISO)-induced renal dysfunction in rats provides a validated model for evaluating renal injury and therapeutic interventions. This study evaluates the renoprotective efficacy of ethanolic root extract of *Asparagus racemosus* (Shatamuli) against ISO-induced renal damage, with emphasis on biochemical, histopathological, and phytochemical parameters. Long Evans rats were divided into four groups: Control, ISO, Control + *A. racemosus*, and ISO + *A. racemosus*. ISO was administered subcutaneously (50 mg/kg) every third day for two weeks, while the extract was given orally (100 mg/kg daily). Renal function markers (creatinine, uric acid), oxidative stress indicators (MDA, NO, AOPP, catalase), and inflammatory mediators (MPO) were quantified. Histological assessments included H&E and Sirius red staining. HPLC-DAD analysis was performed to identify bioactive constituents in the methanolic extract. ISO significantly elevated serum creatinine, uric acid, MDA, NO, AOPP, and MPO levels, while suppressing catalase activity, indicating renal oxidative stress and inflammation. Co-treatment with *A. racemosus* extract reversed these alterations, restoring biochemical and histological integrity. HPLC-DAD profiling revealed the presence of catechin hydrate, trans-cinnamic acid, and kaempferol-polyphenolic compounds known for their antioxidant and anti-inflammatory properties. Ethanolic extract of *A. racemosus* exhibited potent renoprotective effects in ISO-induced nephrotoxicity, likely mediated by its rich polyphenolic content. These findings support its potential as a phytotherapeutic agent for renal complications associated with cardiac injury.

Keywords: Antioxidant; Oxidative stress; *Asparagus racemosus*; HPLC-DAD; Catalase.

1. Introduction

Acute kidney injury (AKI) is a major global public health concern, affecting over 13 million individuals annually and contributing to approximately 1.7 million deaths worldwide [1, 2]. Its rising incidence—particularly in low- and middle-income countries—reflects the growing burden of aging populations, chronic diseases, and limited access to renal care [3]. AKI not only increases short-term mortality and healthcare costs but also

accelerates progression to chronic kidney disease (CKD), end-stage renal disease, and cardiovascular complications [4]. CKD is a growing worldwide health concern, impacting about 800 million people roughly 9-13% of the world's population with a disproportionately high prevalence in Asia and among susceptible groups such as the elderly and those with diabetes or hypertension [5-7]. CKD is now the 12th biggest cause of death worldwide, accounting for 1.2 million fatalities in 2017 alone, with a 41.5% increase in mortality since 1990 [5]. Aside from its direct impact, CKD accounts for 35.8 million disability-adjusted life years (DALYs), significantly reducing quality of life and increasing susceptibility to cardiovascular events, infections, and early death [5, 8].

The link between cardiovascular disease and renal dysfunction is well documented [9, 10], with myocardial infarction (MI) frequently causing acute or chronic renal injury via pathways involving oxidative stress, systemic inflammation, and hemodynamic instability [9, 11-14]. Isoproterenol-induced MI in animal models mirrors these pathophysiological alterations, providing a reliable platform for testing prospective renoprotective drugs [12, 13, 15].

Systemic inflammation and oxidative stress are central to the pathogenesis of both acute and chronic kidney disease, with excessive reactive oxygen species (ROS) production and compromised antioxidant defenses driving renal injury [16]. This bidirectional interplay amplifies tissue damage, promotes fibrosis, and contributes to complications such as cardiovascular disease and anemia [17]. Isoproterenol (ISO), a synthetic β-adrenergic receptor agonist, is widely used to induce myocardial infarction in Long Evans rat models, reliably replicating oxidative stress, inflammation, and fibrotic changes in cardiac and renal tissues [18]. High doses of ISO exacerbate oxidative damage, reduce antioxidant enzyme activity, and increase lipid peroxidation, leading to infarcts and progressive organ dysfunction [19]. Antioxidant therapy has shown promise in mitigating these effects, with prior studies demonstrating its ability to restore enzymatic balance and reduce oxidative injury in ISO-administered rats—underscoring the model's relevance for evaluating reno- and cardioprotective interventions[20, 21].

Despite improvements in nephrology, current treatment techniques mostly aim to halt CKD progression rather than reverse kidney damage [22]. Furthermore, access to advanced treatments is still limited in low-resource settings, emphasizing the critical need for safe, inexpensive, and effective alternatives [23]. In this context, natural medicines have received increased interest for their antioxidant, anti-inflammatory, and antifibrotic qualities [24].

Asparagus racemosus, often known as Shatamuli, is a well-studied medicinal plant in Ayurvedic and ethnobotanical literature. It is high in saponins [25], flavonoids [25], and polyphenolic chemicals [25] and has a powerful antioxidant [25], anti-inflammatory [25], neuroprotective [26], adaptogenic [27], reproductive [28], hepatoprotective, antimicrobial, antiulcer [28], anticancer [29] and immunomodulatory properties [30]. Previous research has shown that it can reduce oxidative stress and tissue injury in a variety of organ systems, implying that it may be useful as a renoprotective drug in cardiovascular-compromised mice.

The purpose of this work is to evaluate the renoprotective effects of *Asparagus racemosus* root extract in an isoproterenol-induced myocardial infarction rat model, specifically its capacity to reduce renal dysfunction and histopathological damage. By combining traditional phytotherapy and current experimental pharmacology, this study aims to add to the expanding body of evidence supporting plant-based therapies for CKD management.

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 A. racemosus Root Extracts

The roots of *Asparagus racemosus* were sourced from the local market of Basundhara, Dhaka, Bangladesh. Specimen identification occurred at the Mirpur National Herbarium, Bangladesh, and the voucher number was documented in the Herbarium (DACB Accession Number DACB-87262). After collection, the roots were oven-dried at 50°C and then finely ground into a powder. A total of 500 g of the powdered material was macerated in methanol 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 methanolic extract. Ethanol was subsequently removed using a rotary evaporator set at 50°C and 100rpm, yielding a concentrated crude extract. An identical procedure was employed to prepare the methanolic extract. Both extracts, characterized by a sticky, dark-brown appearance, were subjected to phytochemical profiling using a high-performance liquid chromatography with diode-array detection (HPLC-DAD) method.

2.3 HPLC-DAD Analysis of A. racemosus Root Extracts

High-performance liquid chromatography coupled with diode-array detection (HPLC-DAD) was performed using a Shimadzu LC-20A system (Japan), comprising a binary pump (LC-20AT), autosampler (SIL-20A HT), column oven (CTO-20A), and photodiode array detector (SPD-M20A), all operated via LC Solution software. Chromatographic separation was carried out on a Luna C18 column (4.6 × 250 mm, 5 µm; Phenomenex), maintained at a constant temperature of 33 °C.

2.4 Chromatographic Conditions

Quantitative analysis of selected polyphenolic constituents in the ethanolic extract of *Asparagus racemosus* was performed using high-performance liquid chromatography coupled with diode-array detection (HPLC-DAD), following the protocol outlined by Ahmed et al.[31], with slight procedural adjustments. The chromatographic separation employed a binary mobile phase system comprising 1% acetic acid in acetonitrile (Solvent A) and 1% acetic acid in water (Solvent B). The gradient elution profile was programmed as follows: 5–25% A (0.01–20 min), 25–40% A (20–30 min), 40–60% A (30–35 min), 60–30% A (35–40 min), 30–5% A (40–45 min), and maintained at 5% A (45–50 min). A sample volume of 20 µL was injected per run, with a constant flow rate of 0.5 mL/min. Detection was carried out at 270 nm to ensure method validation and compound identification. Prior to use, the mobile phase was filtered through a 0.45 µm nylon membrane (India) and degassed under vacuum conditions.

2.5 Preparation of Working Standard Solutions for HPLC Analysis

Sixteen phenolic compounds were individually dissolved in methanol using 25 mL volumetric flasks to prepare standard stock solutions, with concentrations ranging from 4.0 to 50 µg/mL. Appropriate aliquots from each stock were combined and serially diluted with methanol to generate the working standard solutions. All prepared solutions were stored under refrigerated conditions to maintain stability.

For calibration curve development, a composite standard solution was formulated in methanol containing the following concentrations: gallic acid (20 µg/mL), 3,4-dihydroxybenzoic acid (15 µg/mL), catechin hydrate (50 µg/mL), catechol, (-)-epicatechin, and rosmarinic acid (30 µg/mL each), caffeic acid, vanillic acid, syringic acid, rutin hydrate, p-coumaric acid, trans-ferulic acid, and quercetin (10 µg/mL each), myricetin and kaempferol

(8 µg/mL each), and trans-cinnamic acid (4 µg/mL). Additionally, an ethanolic solution of *Asparagus racemosus* extract was prepared at a concentration of 10 mg/mL and stored under refrigeration until analysis.

2.6 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. 2024/OR-NSU/IACUC0107).

2.7 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.
2. ISO (Isoprenaline) group
3. Control + *A. racemosus* group.
4. ISO + *A. racemosus* group.

The control group was fed a diet consisting of chow food and drinking water. The ISO group was administered with 50 mg/kg of isoprenaline subcutaneously every 3 days for 2 weeks. Water and chow food were given to the ISO group along with the isoprenaline. The Control + Methanol extract of *A. racemosus* group was also given chow food and water, and additionally, the methanol extract of *A. racemosus* was also administered orally at a dose of 100 mg/kg daily for 2 weeks. The group ISO + ethanolic extract of *A. racemosus* was given both ISO and extract of *A. racemosus*. The ISO + Methanolic extract of *A. racemosus* group received isoprenaline subcutaneously at a dose of 50 mg/kg every 3 days for 2 weeks and ethanolic extract of *A. racemosus* was administered orally, using a stainless steel gavage needle, at a dose of 100 mg/kg daily for 2 weeks. The ISO + ethanolic extract of A. racemosus group was additionally fed chow food and water. 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, 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.

2.8 Induction of Myocardial Infarction

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

2.9 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.

2.10 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 being collected, the supernatants were utilized in enzymatic and protein analyses.

2.11 Malondialdehyde estimation (MDA)

The accumulation of thiobarbituric acid reactive substances (TBARS), a byproduct of polyunsaturated fatty acid peroxidation, serves as a key indicator of oxidative stress. Quantification of TBARS in kidney tissue homogenates, was performed following a previously established protocol with minor adjustments [32, 33]. The assay involved heating the reaction mixture, followed by cooling and spectrophotometric measurement of the supernatant's absorbance at 535 nm against a reagent blank. Each reaction mixture comprised 0.1 mL of sample and 2 mL of TBA-TCA-HCl reagent, containing thiobarbituric acid, trichloroacetic acid, and hydrochloric acid. TBARS concentrations were calculated using a standard curve generated from 1,1,3,3-tetramethoxypropane and expressed as nmol/g for tissue samples.

2.12 Nitric oxide estimation (NO)

Nitric oxide concentrations in tissue homogenates were quantified using a modified Griess-Illosvoy method, following previously established protocols [34-36]. Briefly, the reaction mixture—comprising phosphate-buffered saline (PBS), tissue homogenate, and the reagent—was incubated at 25 °C for 150 minutes, resulting in the formation of a pink chromophore. Absorbance was recorded at 540 nm against a reagent blank. NO levels were calculated using a standard curve and expressed as nanomoles per gram of tissue (nmol/g).

2.13 Estimation of Advanced Protein Oxidation Products (AOPP)

Advanced protein oxidation products (AOPP), a key biomarker of oxidative stress, were quantified following previously established methodologies [35, 37, 38]. The assay involved measuring the absorbance of chloramine-T in a reaction mixture composed of plasma diluted in phosphate-buffered solution, potassium iodide, and acetic acid. Absorbance was recorded immediately at 340 nm against a reagent blank. The response was linear across a concentration range of 0–100 nmol/mL, enabling accurate quantification of AOPP levels.

2.14 Estimation of Myeloperoxidase (MPO) Activity

Myeloperoxidase (MPO), a key marker of tissue inflammation, was quantified using a modified o-dianisidine–hydrogen peroxide assay, as previously described [35, 39, 40].In brief, tissue homogenates containing 10 µg of protein were added in triplicate to a reaction mixture comprising o-dianisidine dihydrochloride (0.53 mM) and hydrogen peroxide (0.15 mM) in phosphate-buffered saline (PBS). The enzymatic activity was monitored by measuring the change in absorbance at 460 nm. MPO activity was calculated and expressed as units per milligram of protein (MPO/mg protein).

2.15 Determination of Catalase (CAT) Activity

Catalase activity in serum and tissue homogenates was assessed following the method previously described by Alam et al. [41], with minor modifications. The reaction mixture consisted of 50 mmol phosphate buffer (pH 5.0), 5.9 mmol hydrogen peroxide, and 0.1 mL of enzyme extract. The decomposition of hydrogen peroxide was monitored by measuring the change in absorbance at 240 nm. A change of 0.01 absorbance units per minute was defined as one unit of catalase activity and expressed accordingly.

2.16 Histopathological Evaluation

For microscopic examination, excised kidney and heart 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 µm 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× magnification.

2.17 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical evaluations were performed using GraphPad Prism version 9. 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 Analysis of methanolic extract of A. recemosus by HPLC-DAD

An HPLC method has been developed and validated for linearity, accuracy, stability, and precision to analyze sixteen polyphenolics simultaneously. After that, a methanolic extract of *Asparagus racemosus* was used to identify and quantify these polyphenolic compounds. **Figure 1** illustrates the chromatographic separations of polyphenols in *A. racemosus* methanolic extract. A standard stock was prepared for the calibration curve, and the amount of each phenolic compound was extrapolated from its corresponding calibration curve. Catechin hydrate, Trans-cinnamic acid, and kaempferol were found in the methanolic extract of *A. racemosus* (**Table 1**).

Table 1: HPLC chromatogram of methanolic extract of *Asparagus racemosus* root

Name of Standard	***Asparagus racemosus*** (mg/100 g dry extract)
Trans-Cinnamic acid	3.54±0.13
Kaempferol	0.45±0.06
Catechin hydrate	14.44±0.09
Gallic acid	ND
(-) Epicatechin	ND
Catechol	ND
p-Coumaric acid	ND
Trans-Ferulic acid	ND
3,4-dihydroxybenzoic acid	ND
Syringic acid	ND
Rutin hydrate	ND
Caffeic acid	ND
Vanillic acid	ND
Quercetin	ND
Myricetin	ND
Rosmarinic acid	ND

**ND = Not Detected*

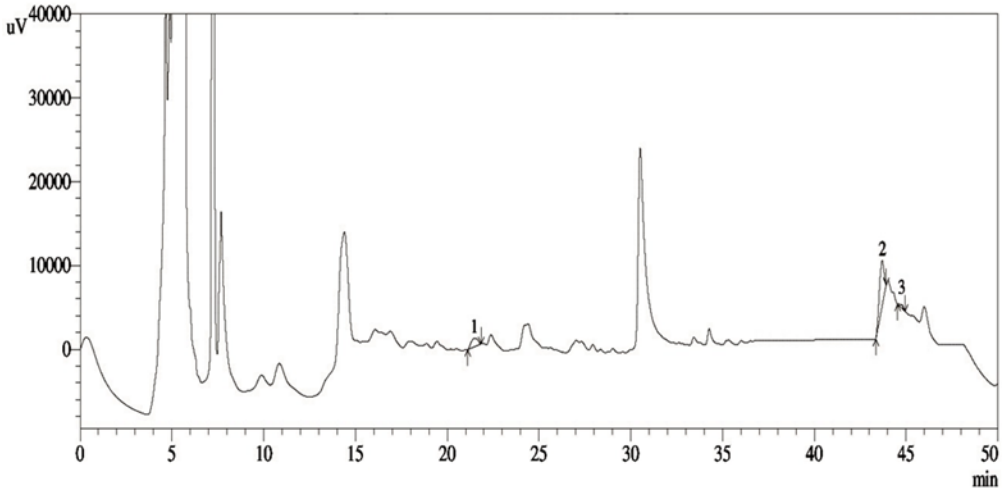

Figure 1: HPLC chromatogram of methanolic extract of *Asparagus racemosus* peaks: 1, Catechin hydrate; 2, Trans-cinnamic acid; and 3, Kaempferol

HPLC-DAD analysis identified three major polyphenolic compounds in the methanolic extract: catechin hydrate (14.44 ± 0.09 mg/100 g), trans-cinnamic acid (3.54 ± 0.13 mg/100 g), and kaempferol (0.45 ± 0.06 mg/100 g). These compounds are known for their antioxidant and anti-inflammatory properties, which may contribute to the observed reno-protective effects

3.2 Effect of A. racemosus root extract on Renal Biomarkers in ISO induced rat

To evaluate the reno-protective potential of *Asparagus racemosus* root extract, key renal biomarkers were assessed across four experimental groups: Control, Control+ *A. racemosus*, ISO, and ISO + *A. racemosus*. The results are summarized in **Figure 2** (Panels A–D).

Kidney Wet Weight has been shown in **Figure 2A**. ISO administration did not increase kidney wet weight ($p > 0.05$) significantly compared to control and control+ *A. racemosus* groups, indicating no evidence of renal hypertrophy and edema. Co-treatment with *A. racemosus* extract did not reduce kidney weight ($p > 0.05$ vs. ISO), suggesting not appreciable difference of kidney wet weight in ISO-induced rat model.

MPO Activity is demonstrated in **Figure 2B**. Myeloperoxidase (MPO) activity, a marker of neutrophil infiltration and inflammation, was significantly elevated in the ISO group ($p < 0.01$). Treatment with *A. racemosus* extract led to a significant reduction in MPO levels ($p < 0.01$ vs. ISO), indicating anti-inflammatory effects.

In **Figure 2C,** The Uric Acid Levels are represented. ISO-treated rats showed a significant rise in serum uric acid ($p < 0.01$), reflecting impaired renal clearance and oxidative stress. The ISO + *A. racemosus* group exhibited a significant decrease in uric acid levels ($p < 0.05$), approaching baseline values.

Plasma Creatinine depicted in **Figure 2D**. Creatinine levels were significantly elevated in the ISO group ($p < 0.01$), consistent with renal dysfunction. Co-administration of *A. racemosus* extract significantly lowered creatinine levels ($p < 0.01$ vs. ISO), suggesting improved renal filtration capacity.

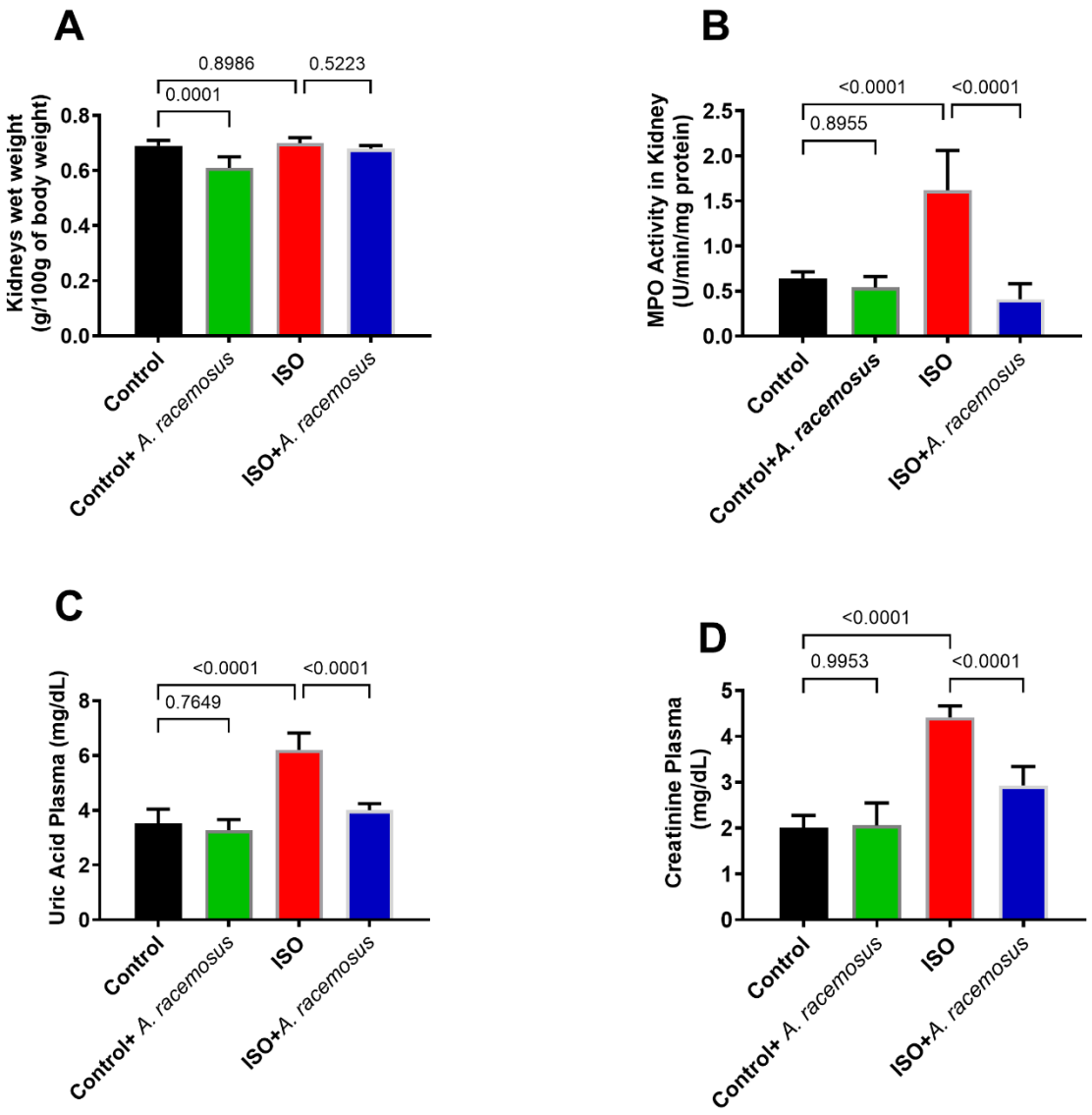

Figure 2: Effect of *A.racemosus* root extract on kidney wet weight (A); MPO assessment (B); uric acid in Plasma (C); Creatinine in Plasma (D). All data were presented as mean ± SEM. For statistical analysis, one-way ANOVA followed by Tukey's multiple comparison tests were done, where significant indicated as ns means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

3.3 Effect of Asparagus racemosus Root Extract on Oxidative Stress Markers in Kidney Homogenates: MDA, NO, AOPP, and Catalase

MDA, NO, AOPP, and Catalase provide insight about the oxidative stress in an organ. The effect of *Asparagus racemosus* on oxidative stress in kidney has been evaluated. The result is shown in **Figure 3**.

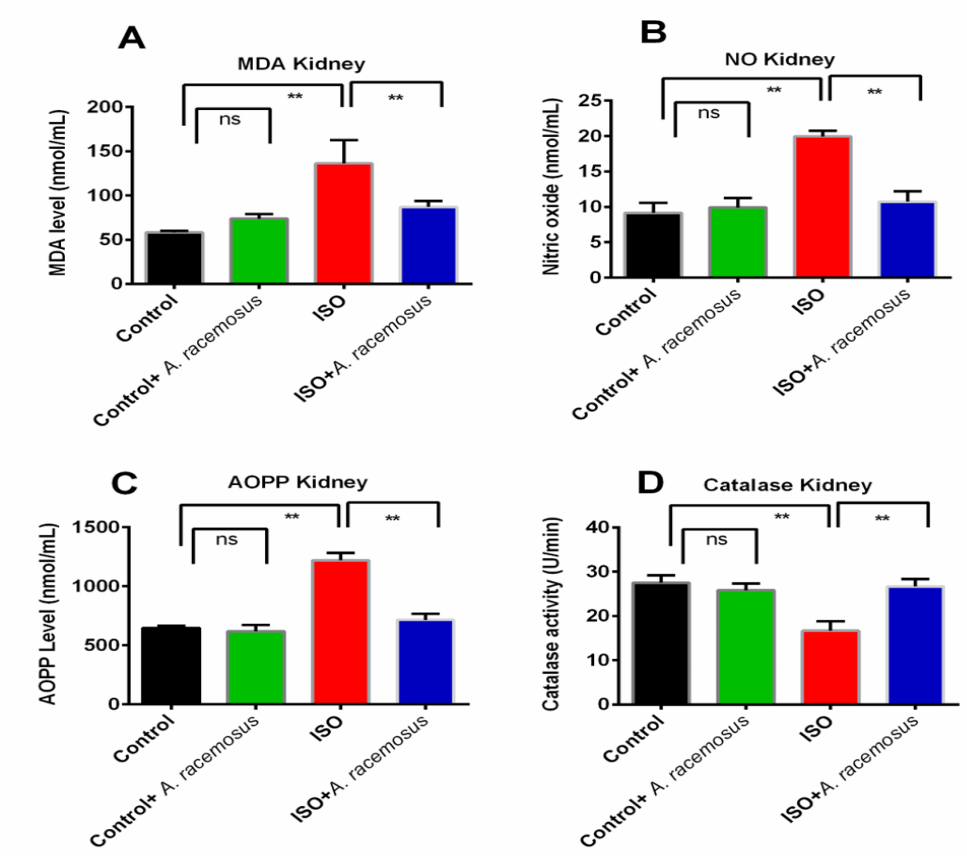

Figure 3: Effect of *A.racemosus* root extract on oxidative stress markers MDA assessment (A); NO assessment (B); AOPP assessment (C); Catalase assessment (D). All data were presented as mean ± SEM. For statistical analysis, One way ANOVA followed by Tukey's multiple comparison tests were done where significant indicated as ns means $p > 0.05$; * means $p \leq 0.05$.

Malondialdehyde (MDA), a marker of lipid peroxidation showed in **Figure 3(A)**. The ISO group exhibited a significant increase in kidney MDA levels (approximately 150 nmol/mL) compared to the control group (around 50 nmol/mL, $p < 0.01$). The group treated with ISO + *A. racemosus* had significantly lower MDA levels (around 75 nmol/mL) than the ISO group ($p < 0.01$), although they were still higher than the control group. The control + *A. racemosus* group showed no significant difference from the control group.

Figure 3(B) presents the concentration of nitric oxide (NO) in kidney tissue. Similar to the MDA results, the ISO group showed a significant elevation in NO levels (approximately 20 nmol/mL) compared to the control group (around 7.5 nmol/mL, $p < 0.01$). Treatment with *A. racemosus* (ISO + *A. racemosus* group) significantly reduced NO levels to approximately 10 nmol/mL compared to the ISO group ($p < 0.01$). The control + *A. racemosus* group was not significantly different from the control.

Advanced Oxidation Protein Products (AOPP), an indicator of protein damage displayed in **Figure 3(C)**. The ISO group had markedly higher AOPP levels (approximately 1200 nmol/mL) than the control group (around 600 nmol/mL, $p < 0.01$). The ISO + *A. racemosus* group demonstrated a significant reduction in AOPP levels to around 700 nmol/mL when compared to the ISO group ($p < 0.01$). The control + *A. racemosus* group did not show a significant change from the control.

Antioxidant enzyme activity, Catalase illustrated in **Figure 3(D)**. The ISO group showed a significant decrease in Catalase activity (approximately 15 U/min) compared to the Control group (around 30 U/min, $p < 0.01$). In contrast, the ISO + *A. racemosus* group showed a significant increase in Catalase activity (approximately 25 U/min) compared to the ISO group ($p < 0.01$). The Catalase activity in the Control + *A. racemosus* group was not significantly different from the Control.

3.4 Histopathological evaluation of A. racemosus on kidney in isoprenaline (ISO) administered rats

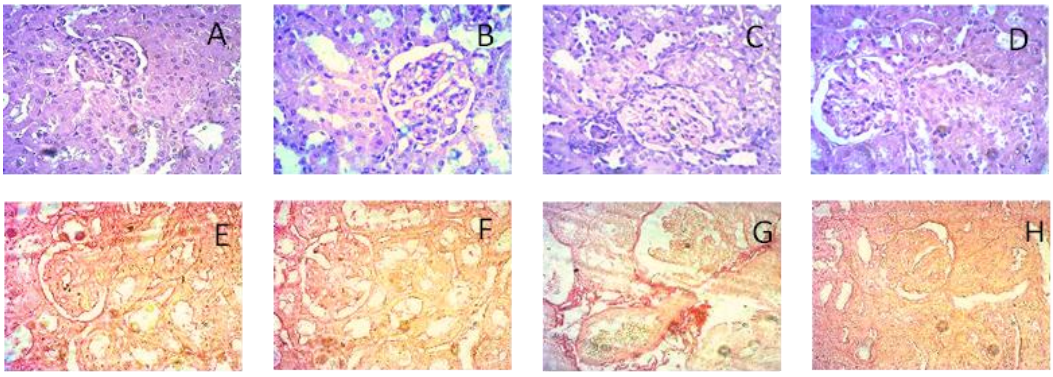

Figure 4: illustrates the histopathological changes in kidney tissue, focusing on inflammation and fibrosis, as a result of isoproterenol (ISO) administration and *A. racemosus* root extract treatment. The figure contains two panels of stained tissue images: hematoxylin and eosin (H&E) staining to show inflammatory cell infiltration (Panels A–D), and Sirius red staining to highlight collagen accumulation (Panels E–H).

3.4.1. Histological evaluation of Hematoxylin and Eosin staining of kidney section

The kidney tissue from the control group shows normal histological architecture with no signs of inflammatory cell infiltration. The ISO-treated group exhibits significant histological damage, marked by a substantial infiltration of inflammatory cells within the interstitial spaces of the kidney. The control group treated with *A. racemosus* shows a normal kidney structure, similar to the Control group, with no inflammation. The group treated with ISO and *A. racemosus* shows a notable reduction in inflammatory cell infiltration compared to the ISO-only group. The kidney structure appears much more preserved.

3.4.2. Histological evaluation of Sirius Red staining of kidney section

The lower panel (E–H) shows the Sirius red-stained kidney sections, which highlight collagen fibers in red. The Control group shows a normal distribution of collagen, with minimal red staining, indicating healthy kidney tissue. The ISO-treated group displays a pronounced increase in collagen deposition, evidenced by extensive red staining, which indicates significant fibrosis. The Control group treated with *A. racemosus* shows no change in collagen distribution compared to the Control, indicating no fibrotic effects. The group treated with ISO and *A. racemosus* shows a clear decrease in collagen accumulation compared to the ISO-only group, suggesting a reduction in fibrosis.

4. Discussion

The present study highlights the reno-protective and antioxidant effects of ethanolic extract of *Asparagus racemosus*, offering strong evidence for its therapeutic potential in reducing isoproterenol (ISO) induced renal injury. HPLC-DAD analysis confirmed the presence of three major polyphenolics catechin hydrate, trans-cinnamic acid, and kaempferol. These compounds are widely recognized for their potent antioxidant [43], anti-inflammatory [43, 44], and cytoprotective actions [45], which may underlie the beneficial outcomes observed reducing of renal dysfunction in the experimental groups [46, 47].

Biochemical analysis demonstrated that isoproterenol (ISO) induced renal dysfunction, as reflected by significant elevations in serum creatinine, uric acid, and oxidative stress markers such as increased levels of lipid peroxidation (MDA), nitric oxide (NO) and advanced oxidation protein products (AOPP). These changes are consistent with impaired renal clearance and free radical-mediated injury [45, 48]. Notably, treatment with *A. racemosus* extract markedly reduced uric acid and creatinine levels, suggesting an improvement in glomerular filtration and renal functional status. The restored catalase activity suggests that the extract strengthens the body's natural antioxidant defenses, possibly by boosting enzyme function or neutralizing free radicals directly [49, 50]. The significant reduction in myeloperoxidase (MPO) activity further supports its anti-inflammatory potential,

indicating reduced neutrophil infiltration and tissue damage [51, 52]. The reduction points to be dampened inflammatory response, which may be critical in preventing progression to chronic kidney damage [53, 54].

Markers of oxidative stress also demonstrated protective effects of the extract. ISO significantly increased lipid peroxidation (MDA), nitric oxide (NO), and advanced oxidation protein products (AOPP), while reducing antioxidant catalase activity [55-57]. Co-administration of *A. racemosus* significantly normalized these parameters, lowering oxidative damage and restoring catalase activity toward control values. This finding suggests that the extract enhances endogenous antioxidant defense while limiting radical-induced injury.

Histopathological observations corroborated the biochemical findings, providing visual confirmation of tissue-level damage and recovery. ISO exposure led to severe inflammatory cell infiltration and fibrosis [58], evident from H&E and Sirius red staining, respectively [16, 48, 59]. This fibrotic response is primarily driven by ISO-induced oxidative stress [60] and inflammatory signaling [61], which activate fibroblasts and promote excessive extracellular matrix (ECM) deposition [62] —particularly collagen types I and III. Over time, this disrupts normal kidney architecture and impairs function [62]. These pathological changes were clearly visualized using hematoxylin and eosin (H&E) and Sirius red staining. H&E staining revealed dense infiltration of inflammatory cells, tubular degeneration, and glomerular distortion [63], indicating acute tissue injury [64]. Sirius red staining, which specifically binds to collagen fibers, highlighted extensive collagen accumulation in the interstitial spaces [65], confirming the presence of fibrosis. Conversely, *A. racemosus* treatment preserved kidney architecture, reduced inflammatory infiltration, and mitigated collagen deposition. These findings indicate that the extract not only alleviates acute inflammation but may also limit fibrotic progression. Collectively, the results suggest that the bioactive compounds in *A. racemosus* contribute synergistically to renal protection by reducing oxidative stress, inflammation, and tissue remodeling.

Taken together, these findings suggest that the bioactive compounds in *A. racemosus* act synergistically to provide renal protection by reducing oxidative stress, suppressing inflammation, and preventing fibrotic remodeling. This multifaceted mechanism positions *A. racemosus* as a promising candidate for the development of phytotherapeutic interventions in renal pathologies, particularly those driven by oxidative and inflammatory insults.

5. Limitations And Future Perspectives

Although the study provides strong preclinical evidence, it has some limitations. Only three polyphenolics were quantified, while other potentially active phytochemicals remain unexplored. The study was restricted to an isoproterenol-induced rat model, which may not fully mimic human renal pathology. Future research should focus on isolating and characterizing additional bioactive compounds, exploring molecular signaling pathways, and validating these findings in clinical trials. Long-term safety profiling is also essential before therapeutic translation.

6. Conclusion

The ethanolic extract of *Asparagus racemosus*, rich in polyphenolic compounds, exhibited strong reno-protective effects against isoproterenol-induced renal injury. By attenuating oxidative stress, inflammation, and fibrosis, the extract preserved kidney function and histological integrity. These findings support its therapeutic potential as a natural adjunct in managing renal oxidative stress and related pathologies.

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