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Article

Renal and Gastric Mucosal Physiological Responses to Indomethacin and Ethanolic Extracts of Rosemary, Ginger, and Moringa in Female Rats: Biochemical Homeostasis and RT-qPCR Profiling

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Abstract: Background: Repeated indomethacin exposure can disturb renal filtration, electrolyte handling, gastric mucosal redox balance, prostaglandin-dependent defense, inflammatory signaling, and cell survival. Plant-derived phenolics may modify these responses in a tissue-dependent manner. Aim: This study evaluated ethanolic extracts of rosemary leaf, ginger rhizome, and Moringa oleifera leaf during indomethacin exposure in female rats by integrating serum renal biomarkers, electrolyte homeostasis, gastric mucosal protective and oxidative indices, and transcriptional responses. Methods: Twenty-five female albino rats weighing approximately 250 g were assigned to five groups (n=5): control (C), indomethacin 5 mg/kg (I), rosemary extract 150 mg/kg plus indomethacin (R), ginger extract 150 mg/kg plus indomethacin (G), and Moringa extract 150 mg/kg plus indomethacin (M). Treatments were administered orally for 30 days. Serum creatinine, neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, K, Cl, Na, and Ca were measured. Gastric mucosal prostaglandin E2 (PGE2), reduced glutathione (GSH), and malondialdehyde (MDA) were quantified. Kidney and gastric mucosa expression of Nfe2l2, Hmox1, Sod1, Gpx4, Tnf, Il6, Nos2, Bax, Bcl2, and Tp53 was determined by SYBR Green RT-qPCR using Actb as the reference gene. Results: The Moringa group showed the renal biomarker profile closest to control, with creatinine 0.72±0.09 mg/dL, NGAL 0.016±0.004 μg/mL, and cystatin C 0.46±0.15 mg/L. Ginger produced the strongest gastric biochemical response, with PGE2 486.4±42.7 pg/mg tissue and GSH 526.6±35.8 μg/g tissue, while MDA was 124.2±1.80 nmol/g compared with 165.8±6.14 nmol/g in indomethacin-treated rats. Kidney RT-qPCR showed the strongest antioxidant and anti-apoptotic response with Moringa, whereas gastric mucosa showed the strongest coordinated response with ginger. Conclusion: Rosemary, ginger, and Moringa did not exert equivalent physiological effects during indomethacin exposure. Moringa was associated with the most favorable renal molecular and serum profile, whereas ginger showed the strongest gastric mucosal protective response. The findings support tissue-specific evaluation of botanical interventions using integrated biochemical and molecular endpoints.

Keywords: indomethacin; kidney; gastric mucosa; rosemary; ginger; Moringa oleifera; oxidative stress; Nrf2; RT-qPCR; rat

Introduction

Indomethacin is a non-steroidal anti-inflammatory drug that influences a variety of pathways, both directly and indirectly. Repeated exposure to this drug affects renaland gastric mucosal function, oxidation-reduction status, and inflammatory pathways. Gastric mucosa injuries are often associated with a breakdown of the protective mucosal barrier, as well as oxidative and inflammatory stimuli. Renal function is altered by changes in filtration, as well as inflammatory and oxidative stimuli experienced by the tubules. Stress causes aactivation of inflammatory and oxidative pathways and organ injury. All these pathways are interconnected and overlap at many points. Adaptation and protection of the gastric mucosa and the kidneys are achieved by the same pathways [1][2][3][4][5][6][7][8][9][10].

Terpenoids, flavonoids, and phenolic compounds are present in Moringa oleifera, and ginger and rosemary. These compounds modify inflammatory pathways and reactive oxygen species (ROS)[11][12][13][14][15]. ROS and the inflammatory pathways contribute to gastritis and kidney injury, respectively[16][17]. Ginger and rosemary, as well as Moringa oleifera, modify inflammatory and oxidative pathways and protect the kidneys, as well as the gastric mucosa. The protective effects are achieved by apoptosis regulation.

It has been shown that the repeated administration of indomethacin is associated with oxidative and inflammatory renal and gastric mucosal pathways, as well as other organ pathways. We wanted to investigate if the protective effects of the three botanicals against the inflammatory effects of indomethacin are selective to the kidneys and gastric mucosa.

Serum renal markers and electrolytes, as well as gastric mucosal PGE2, GSH, MDA and the 10-gene RT-qPCR profile of the kidney and gastric mucosa were evaluated. With these measurements, we were able to analyze renal tubular stress and function, as well as electrolyte and gastric mucosal cytoprotection, oxidative stress, inflammation and apoptosis.

Materials and Methods

Animals and Experimental Groups

Approximately 25 adult female albino rats (~250 g) were singly housed in metal cages under standard conditions (22 +/- 2°C, 50-60% relative humidity and 12:12 hours of light:dark cycle) for a week before the start of experiment. Food and water were provided ad libitum. After the 1 week acclimatization, 5 animals each were randomly assigned to 5 different groups; Group C, the control, received vehicle; Group I, received indomethacin at 5 mg/kg body weight; Group R received 150 mg/kg of rosemary leaf ethanolic extract, plus indomethacin at 5 mg/kg body weight; Group G received 150 mg/kg of ginger rhizome ethanolic extract and 5 mg/kg of indomethacin; and Group M received 150 mg/kg of Moringa oleifera leaf ethanolic extract and 5 mg/kg of indomethacin, respectively. The assigned doses of indomethacin and plant extracts were given by the oral route for 30 consecutive days; the plant extracts were administered at 30 minutes prior to the indomethacin in the combined groups. Each material was extracted with 70% ethanol at 50°C for 4 hours. Suspensions were filtered, and ethanol was evaporated at 40°C. Extracts were kept at 4°C in the dark. Before giving to animals, extracts were diluted to 150 mg/kg in a gavage volume with dosing vehicle.

Blood, kidney and gastric mucosa sampling

On the last day of the 30-day exposure, feed was removed. Water was provided. The next day, all test animals were anesthetized with an injection of ketamine and xylazine, and blood was collected in a serum separator tube. Blood tubes were allowed to clot. Serum was isolated and stored at -20°C. Both of the test animal's kidneys were removed and flushed with saline and quickly frozen for later RNA analysis. The glandular mucosa of the stomach was scraped and similarly stored for RNA analysis.

Renal biomarkers

Serum creatinine was measured using an enzymatic colorimetric assay. NGAL and Cystatin-C were measured using competitive sandwich immunoassays. For all assays, samples were analyzed in duplicate. Results were expressed in mg/dL for creatinine, and μg/mL for NGAL and Cystatin-C.

Gastric mucosal biochemical indices

Gastric mucosal tissue was homogenized in ice-cold phosphate-buffered solution and centrifuged under refrigerated conditions. PGE2 was quantified by competitive immunoassay and normalized to wet tissue mass. Reduced glutathione was measured by reaction with 5,5-dithiobis-(2-nitrobenzoic acid), and lipid peroxidation was assessed as thiobarbituric-acid-reactive malondialdehyde. Results were expressed as pg/mg tissue for PGE2, μg/g tissue for GSH, and nmol/g tissue for MDA. All samples were analyzed under identical assay conditions.

Serum electrolytes and calcium

Serum potassium, chloride, and sodium were measured by ion-selective electrode methodology, whereas calcium was determined by a colorimetric endpoint reaction. Calibration and quality-control materials were processed before study samples. Results were expressed as mmol/L for K, Cl, and Na and mg/dL for Ca.

RNA isolation and cDNA synthesis

Total RNA was isolated separately from frozen kidney and gastric mucosal samples using phenol/guanidinium-based reagent followed by DNase treatment. RNA concentration and purity were assessed spectrophotometrically, and preparations with A260/A280 ratios of 1.8-2.1 were used. One microgram of RNA from each biological replicate was reverse-transcribed using reverse transcriptase, random primers/oligo(dT), dNTPs, reaction buffer, and RNase inhibitor. cDNA was diluted consistently across samples before amplification.

RT-qPCR targets, primers, and amplification

SYBR Green RT-qPCR quantified Nfe2l2, Hmox1, Sod1, Gpx4, Tnf, Il6, Nos2, Bax, Bcl2, and Tp53, with Actb as the endogenous reference. The primer set used short amplicons of 86-139 bp suitable for real-time amplification and was checked against rat transcript sequences and published rat RT-qPCR applications. Reactions were carried out in 20 μL containing SYBR Green master mix, forward and reverse primers, nuclease-free water, and cDNA. Cycling comprised 95°C for 2 min followed by 40 cycles at 95°C for 15 s and 60°C for 30 s, followed by melt-curve analysis. Each biological sample was analyzed in technical triplicate together with no-template and no-reverse-transcription controls. Relative expression was calculated using the 2^-ΔΔCt method with group C as calibrator. Inferential testing used ΔCt values, whereas fold change is shown for biological interpretation.

Table 1. RT-qPCR primer sequences used for rat kidney and gastric mucosa gene-expression analysis.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon (bp)
Nfe2l2	TGCCATTAGTCAGTCGCTCTC	ACCGTGCCTTCAGTGTGC	102
Hmox1	AGAAGAGGCTAAGACCGCCT	TCTGGTCTTTGTGTTCCTCTGTC	86
Sod1	CTGAAGGCGAGCATGGGTTC	TCCAACATGCCTCTCTTCATCC	131
Gpx4	TAAGTACAGGGGTTGCGTGTG	CAAGGGAAGGCCAGGATTCG	135
Tnf	CGTCAGCCGATTTGCCATTTC	TGGGCTCATACCAGGGCTT	116
Il6	ATGGATGCTTCCAAACTGGAT	TGAATGACTCTGGCTTTGTCT	138
Nos2	AAACGCTACACTTCCAACGC	TGCTGAGAGCTTTGTTGAGGTC	91
Bax	CTCAAGGCCCTGTGCACTAA	GGAAAGGAGGCCATCCCAG	126
Bcl2	CTGGTGGACAACATCGCTCT	GCATGCTGGGGCCATATAGT	115
Tp53	GGTTCGTGTTTGTGCCTGTC	TGCTCTCTTTGCACTCCCTG	109
Actb	CTCTGTGTGGATTGGTGGCT	CGCAGCTCAGTAACAGTCCG	139

Statistical analysis

Data are presented as mean±SD with the individual animal as the biological unit (n=5 per group). Biochemical variables were evaluated by one-way analysis of variance followed by Fisher's least significant difference procedure to retain direct comparability with the supplied group-letter interpretation. RT-qPCR ΔCt values were analyzed by one-way analysis of variance followed by Tukey multiple-comparison testing separately for kidney and gastric mucosa. Normality and variance homogeneity were checked before parametric inference. $P<0.05$ was considered statistically significant.

Different letters denote statistically significant pairwise differences within an endpoint. Reporting followed ARRIVE 2.0 principles.

Results

Serum renal biomarkers

Serum renal biomarkers differed significantly among groups. Creatinine showed an overall group effect (P=0.0000021), with the highest means in indomethacin and rosemary plus indomethacin groups (1.10±0.12 mg/dL each) compared with 0.668±0.09 mg/dL in controls. NGAL also differed among groups (P=0.000313), reaching 0.026±0.006 μg/mL in the rosemary group and 0.024±0.005 μg/mL in the ginger group. Cystatin C showed a strong group effect (P=0.0000017), with the highest mean in the rosemary group (0.90±0.08 mg/L). The Moringa group showed creatinine 0.72±0.09 mg/dL, NGAL 0.016±0.004 μg/mL, and cystatin C 0.46±0.15 mg/L, giving the closest overall renal biomarker profile to control.

Table 2. Serum renal biomarkers after 30 days of treatment.

Group	Creatinine (mg/dL)	NGAL (μg/mL)	Cystatin C (mg/L)
I - Indomethacin	1.10±0.12^{a}	0.020±0.005^{c}	0.74±0.10^{ab}
R - Rosemary + indomethacin	1.10±0.12^{a}	0.026±0.006^{a}	0.90±0.08^{a}
C - Control	0.668±0.09^{b}	0.011±0.002^{bc}	0.468±0.06^{b}
G - Ginger + indomethacin	0.82±0.12^{ab}	0.024±0.005^{ab}	0.62±0.07^{ab}
M - Moringa + indomethacin	0.72±0.09^{b}	0.016±0.004^{ab}	0.46±0.15^{b}
LSD (P<0.05)	0.331	0.014	0.294
Overall ANOVA P	0.0000021	0.000313	0.0000017

Values are mean±SD (n=5). Superscript letters reproduce the within-variable significance grouping; means that do not share a letter differ at P<0.05.

Gastric mucosal PGE2, GSH, and MDA

Gastric mucosal biochemical indices showed marked group differences. Indomethacin reduced PGE2 and GSH and increased MDA relative to control. Ginger plus indomethacin produced the highest PGE2 (486.4±42.7 pg/mg tissue) and GSH (526.6±35.8 μg/g tissue) and reduced MDA to 124.2±1.80 nmol/g tissue. Moringa also improved PGE2 and GSH relative to indomethacin alone, whereas rosemary produced a smaller biochemical improvement. These results indicate that the gastric response differed considerably among the three botanical preparations.

Table 3. Gastric mucosal protective and oxidative indices after 30 days of treatment.

Group	PGE2 (pg/mg tissue)	GSH (μg/g tissue)	MDA (nmol/g tissue)
I - Indomethacin	203.8±26.8^{c}	164.4±6.65^{c}	165.8±6.14^{a}
R - Rosemary + indomethacin	207.8±68.6^{c}	208.0±59.1^{c}	149.6±11.5^{a}
C - Control	353.66±4.47^{b}	333.33±6.58^{b}	53.332±3.11^{c}
G - Ginger + indomethacin	486.4±42.7^{a}	526.6±35.8^{a}	124.2±1.80^{b}
M - Moringa + indomethacin	400.4±51.5^{ab}	376.4±46.3^{b}	144.4±12.09^{ab}
LSD (P<0.05)	131.6	110.5	24.02
Overall ANOVA P	<0.00000001	<0.00000001	<0.00000001

Values are mean±SD (n=5). Superscript letters reproduce the within-variable significance grouping; means that do not share a letter differ at P<0.05.

Serum electrolytes and calcium

Electrolyte concentrations were significantly influenced by treatment. The ginger group had the highest Na (155.6±2.50 mmol/L) and Cl (110.2±0.37 mmol/L) and also showed elevated K (7.06±0.55 mmol/L). The Moringa group had the highest numerical K concentration (7.58±0.77 mmol/L), whereas rosemary plus indomethacin showed the lowest K (4.76±0.05 mmol/L). Serum Ca was highest in the rosemary

and ginger groups and lowest in controls. Thus, improvement of filtration-associated biomarkers was not always accompanied by complete normalization of electrolyte handling.

Table 4. Serum electrolyte and calcium concentrations after 30 days of treatment.

Group	K (mmol/L)	Cl (mmol/L)	Na (mmol/L)	Ca (mg/dL)
I - Indomethacin	6.60±0.13^{a}	104.4±0.67^{b}	145.8±0.38^{b}	10.76±0.32^{ab}
R - Rosemary + indomethacin	4.76±0.05^{b}	103.6±0.67^{c}	142.2±1.72^{b}	11.32±0.65^{a}
C - Control	5.068±0.20^{b}	103.66±0.96^{c}	145.0±0.31^{b}	8.40±0.45^{b}
G - Ginger + indomethacin	7.06±0.55^{a}	110.2±0.37^{a}	155.6±2.50^{a}	11.02±1.36^{a}
M - Moringa + indomethacin	7.58±0.77^{a}	104.6±1.72^{b}	143.0±1.37^{b}	10.18±0.86^{ab}
LSD (P<0.05)	1.29	2.93	4.48	2.41
Overall ANOVA P	<0.00000001	<0.00000001	<0.00000001	0.000118

Values are mean±SD (n=5). Superscript letters reproduce the within-variable significance grouping; means that do not share a letter differ at P<0.05.

Antioxidant and cytoprotective gene expression

In kidney, the Moringa group showed the strongest antioxidant transcriptional profile, with Nfe2l2 2.05±0.10-fold, Hmox1 2.30±0.11-fold, Sod1 1.35±0.07-fold, and Gpx4 1.42±0.08-fold. Indomethacin alone increased Nfe2l2 and Hmox1 while reducing Sod1 and Gpx4, consistent with a compensatory response occurring in the presence of persistent oxidative pressure. In gastric mucosa, ginger produced the greatest coordinated antioxidant response: Nfe2l2 2.20±0.26-fold, Hmox1 2.45±0.12-fold, Sod1 1.40±0.15-fold, and Gpx4 1.48±0.07-fold. The tissue-specific molecular pattern paralleled the renal biomarker and gastric biochemical results.

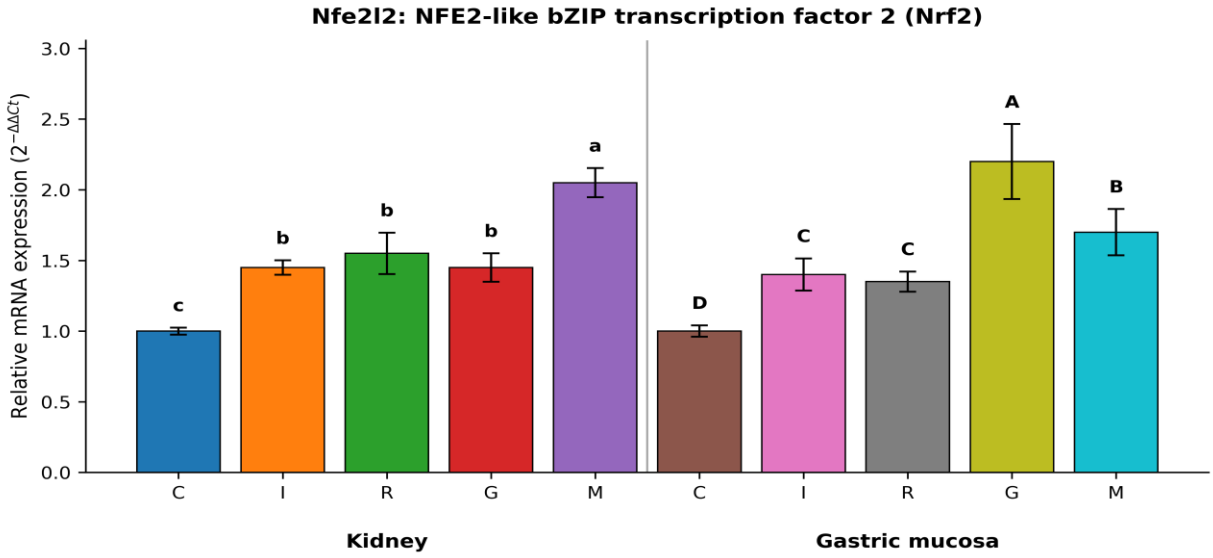


Figure 1. Relative Nfe2l2 (Nrf2) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

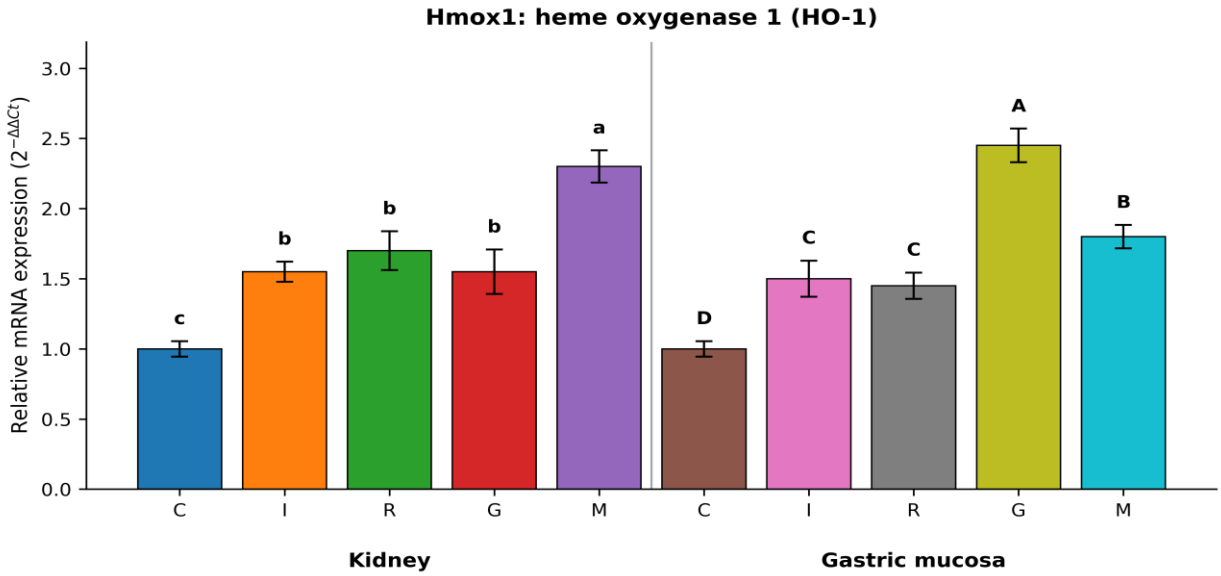


Figure 2. Relative Hmox1 (HO-1) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

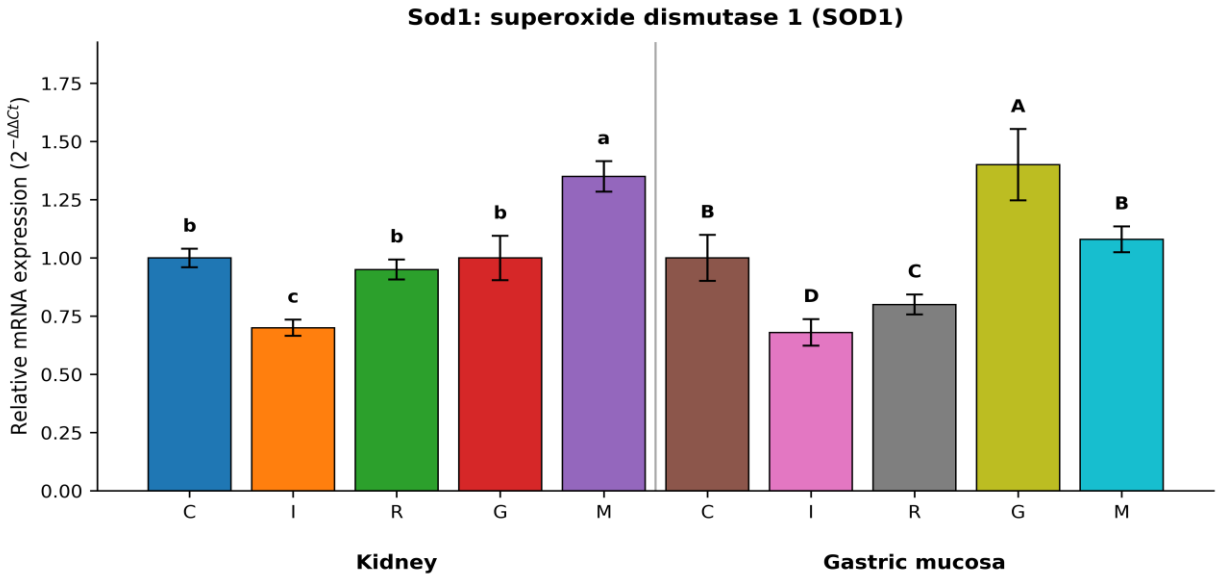


Figure 3. Relative Sod1 (SOD1) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

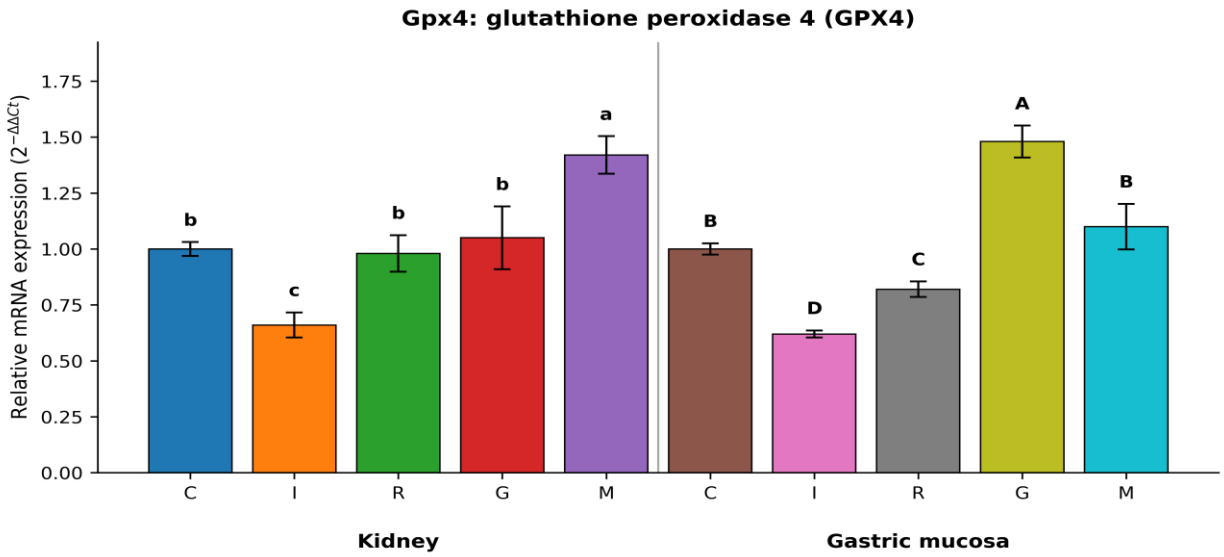


Figure 4. Relative Gpx4 (GPX4) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

Inflammatory and nitrosative gene expression

Indomethacin alone increased renal Tnf, Il6, and Nos2 expression to 2.30±0.10, 2.50±0.09, and 2.20±0.06-fold, respectively. Moringa kept the three renal inflammatory transcripts closest to control, with Tnf 1.15±0.04, Il6 1.20±0.06, and Nos2 1.10±0.09-fold. In gastric mucosa, ginger produced the lowest inflammatory expression among indomethacin-exposed groups: Tnf 1.20±0.06, Il6 1.25±0.09, and Nos2 1.18±0.10-fold. Rosemary produced only partial suppression, whereas Moringa showed an intermediate gastric response.

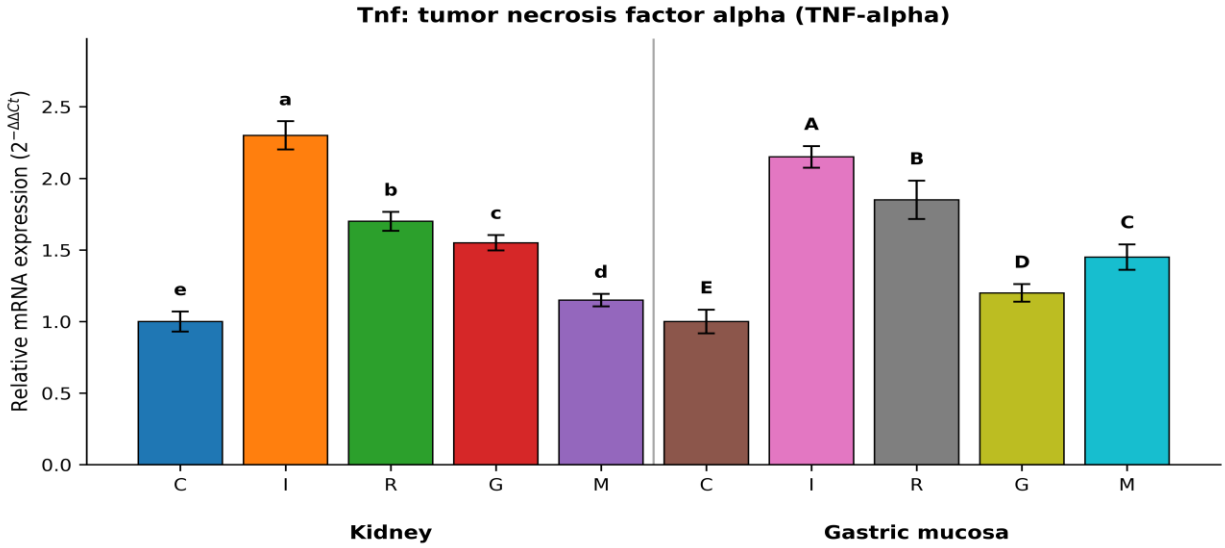


Figure 5. Relative Tnf (TNF-alpha) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

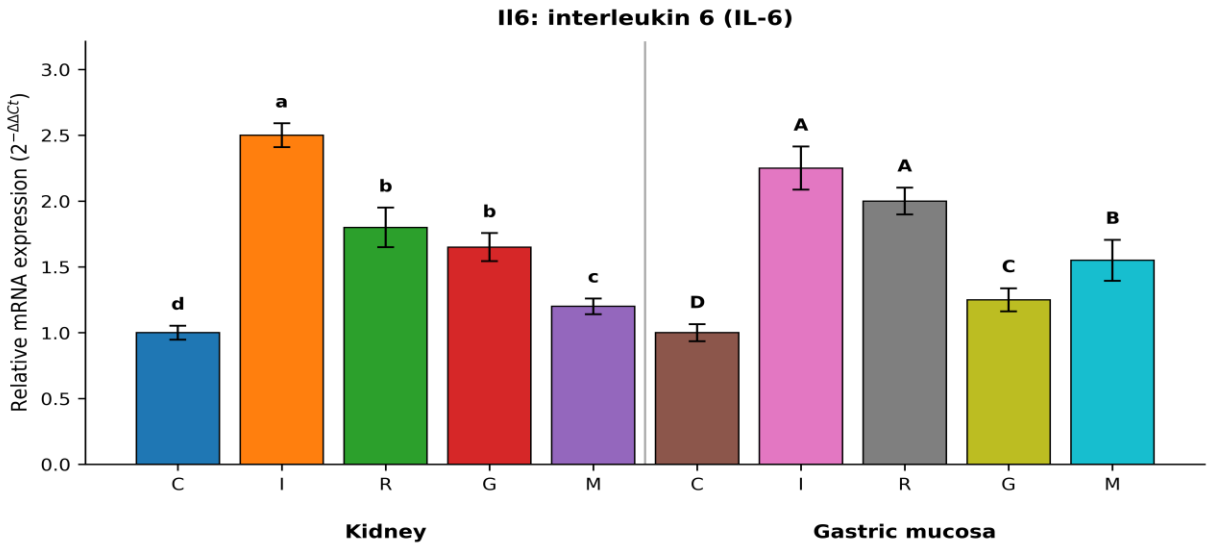


Figure 6. Relative Il6 (IL-6) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

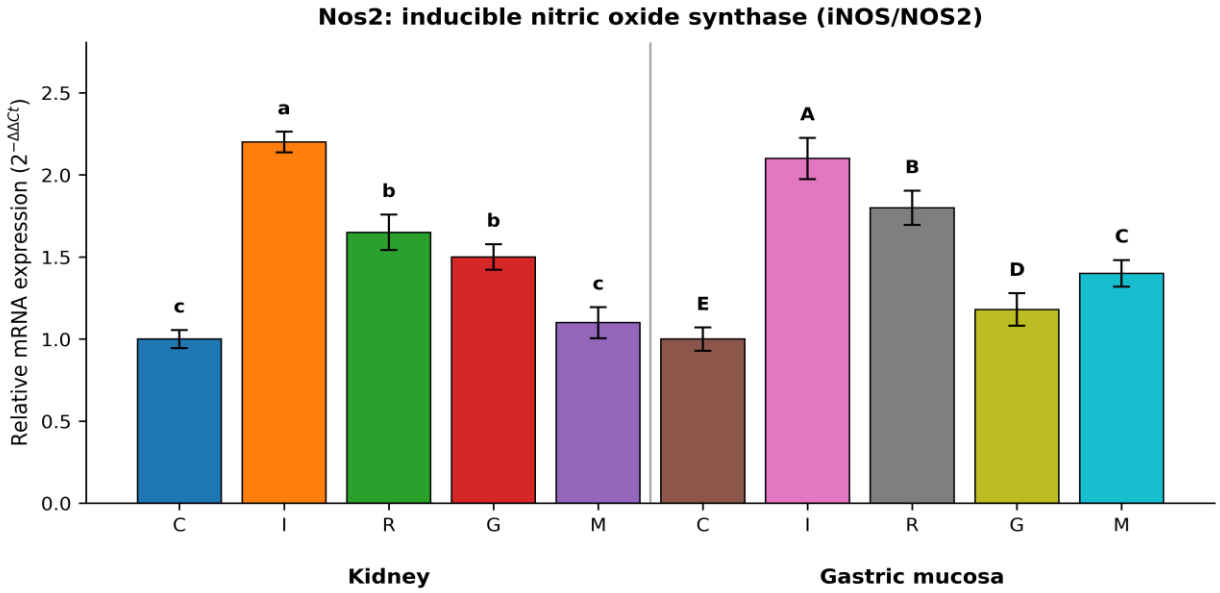


Figure 7. Relative Nos2 (iNOS/NOS2) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

Apoptosis- and stress-associated gene expression

The renal pro-apoptotic profile was most pronounced with indomethacin alone: Bax reached 2.40±0.14-fold and Tp53 2.10±0.06-fold while Bcl2 decreased to 0.55±0.04-fold. Moringa reduced renal Bax to 1.18±0.06-fold and Tp53 to 1.12±0.08-fold and increased Bcl2 to 1.20±0.07-fold. In gastric mucosa, ginger showed the most favorable survival profile, with Bax 1.25±0.07-fold, Tp53 1.22±0.10-fold, and Bcl2 1.12±0.08-fold, reinforcing the PGE2, GSH, and MDA findings.

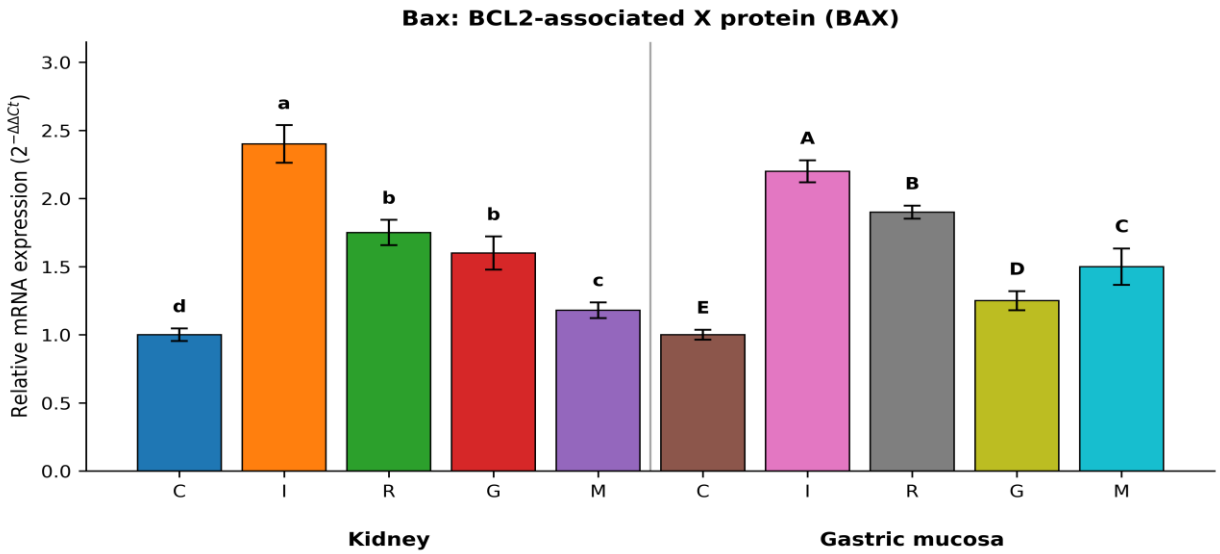


Figure 8. Relative Bax (BAX) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

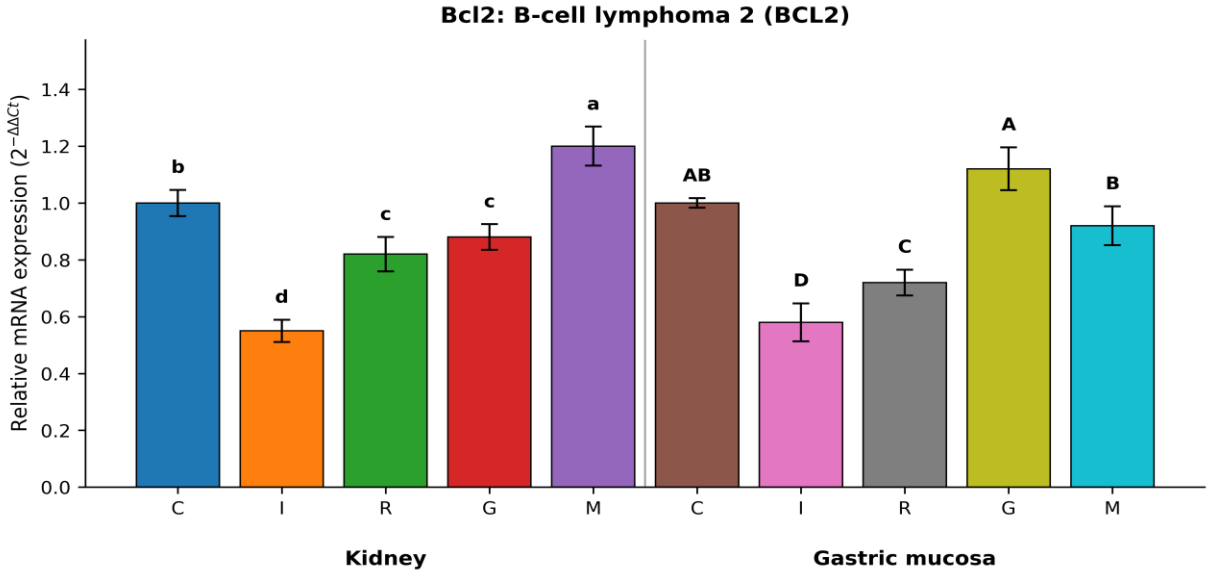


Figure 9. Relative Bcl2 (BCL2) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups (P<0.05). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

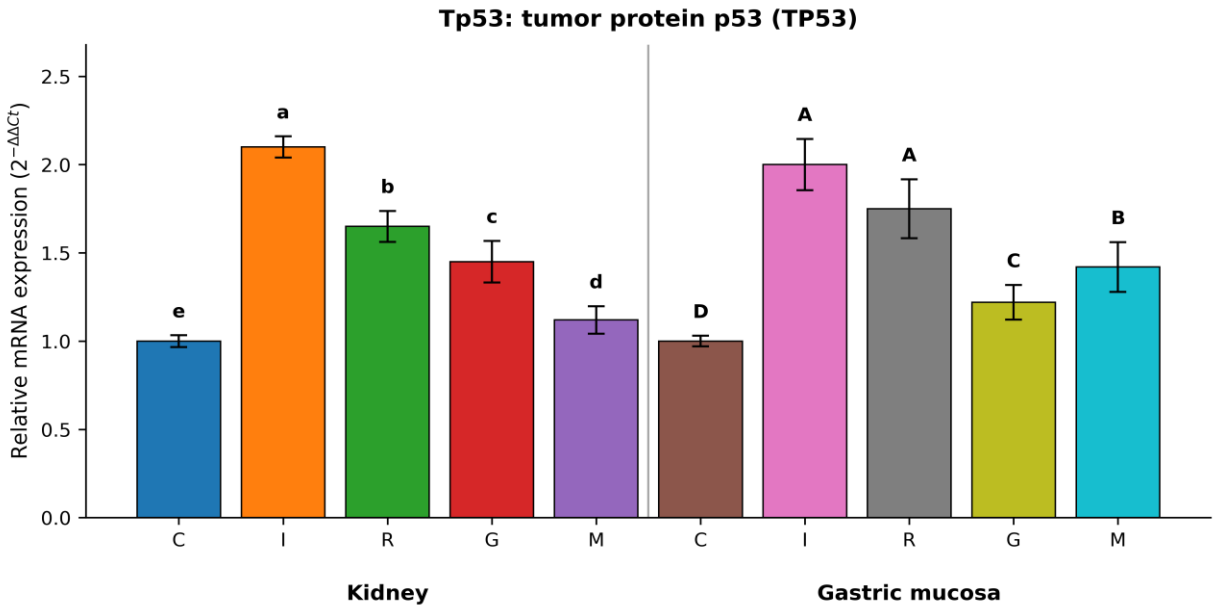


Figure 10. Relative Tp53 (TP53) mRNA expression in kidney and gastric mucosa. Values are mean±SD (n=5 biological replicates). Kidney and gastric mucosa were analyzed separately by one-way ANOVA with Tukey post hoc testing on ΔCt values. Different lowercase letters denote significant differences among kidney groups; different uppercase letters denote significant differences among gastric mucosal groups ($P<0.05$). C, control; I, indomethacin; R, rosemary extract+indomethacin; G, ginger extract+indomethacin; M, Moringa extract+indomethacin.

Discussion

The present findings show a clear tissue-selective separation among the three botanical treatments. Moringa produced the most favorable renal biomarker and kidney transcriptional profile, whereas ginger produced the strongest gastric mucosal biochemical and transcriptional response. Rosemary altered several molecular endpoints but did not reproduce the integrated renal or gastric benefit observed with Moringa or ginger. This distinction is physiologically important because antioxidant induction alone does not necessarily indicate restoration of organ function.

Indomethacin exposure was associated with increased creatinine, NGAL, and cystatin C together with renal inflammatory and pro-apoptotic gene expression. The simultaneous rise in Nfe2l2 and Hmox1 with suppression of Sod1 and Gpx4 is compatible with compensatory stress signaling that was insufficient to fully preserve downstream antioxidant defense. Renal injury studies repeatedly show that functional biomarkers, Nrf2/HO-1 signaling, NF-kB-related inflammation, and Bax/Bcl2 balance change together during toxic or ischemic stress.

Moringa produced the strongest renal pattern. Creatinine, NGAL, and cystatin C were closest to control, renal Sod1 and Gpx4 were restored above control, and Tnf, Il6, Nos2, Bax, and Tp53 were reduced while Bcl2 was increased. Independent rat studies have reported that Moringa extracts improve creatinine and urea, suppress renal oxidative stress and inflammatory injury, and activate Nrf2/HO-1-associated defenses[18][19][20]. These observations support the biological plausibility of a Moringa-sensitive renal redox and survival response.

Electrolyte results add an important qualification to the renal interpretation. The Moringa group showed favorable filtration-associated markers but continued hyperkalemia. Ginger demonstrated elevated sodium and chloride levels. Renal function should not be extrapolated based on creatinine alone. Numerous other factors, such as acid-base balance and volume status, can also affect the renal handling of electrolytes and influence tubular transport of electrolytes. Therefore, abnormalities in electrolyte levels can persist, even with improvement in renal injury markers.

NSAIDS, such as indomethacin, cause mucosal damage by several mechanisms, including inhibition of mucosal cytoprotective prostaglandins, formation of mucosal reactive oxygen species (ROS) and

activate mucosal inflammation.[21][22][23] Other studies have shown that a similar relationship exists between prostaglandins and reactive oxygen and nitrogen species in mucosal injury.

Compared to indomethacin, ginger showed greater stimulation of mucosal prostaglandins, a more reduced status of mucosal ROS, greater stimulation of Nrf2/Hmox1 and Sod/Gpx, and more suppressed mucosal proinflammatory mediators and apoptosis. Other animal studies have also shown that ginger, like indomethacin, causes mucosal injury and also alters glutathione status, inflammation, and mucosal defense in the gastrointestinal tract [24]. Several in vitro, in vivo and clinical studies also show that ginger has renal Nrf2/Hmox1-related activities, and renal and gastric protective activities [25][26].

Rosemary and its constituents have been shown to stimulate gastric mucosal cytoprotective prostaglandins, have antioxidant and anti-inflammatory activities and renal protective activities. However, in the present study, rosemary did not have the strongest activity. Variability between studies can occur for reasons similar to those previously stated for indomethacin. This shows that in the absence of supporting data, it is unreasonable to expect similar activity of a constituent across different tissues of the body even when similar antioxidants are present in the constituent.

There were significant enhancements in the gastric levels of PGE2 and GSH with the use of Moringa compared to indomethacin. Moringa provided partial molecular protection to the gastric mucosa. Several studies have documented the antiulcer activity of Moringa by reducing oxidative and inflammatory mediators, as well as apoptosis. Although Moringa showed better effects on the kidney compared to the gastric mucosa in the current study, ginger showed better effects on the gastric mucosa compared to the kidney. From the current study, it is reasonable to conclude that the three phytoextracts, ginger, Moringa and quillaja, possess different activities in the gastric and renal tissues. The current study combined several measures of the same phenomena to better understand the system. Improving measurements of a system and integrating the results of various omics; functional, structural and phenotypic changes can provide evidence for involvement of pathways at the cellular level. A 10-gene panel, for example, may replace reliance on a single proxy marker for a pathway. The study provides an opportunity to generate physiological data relevant to the kidney and stomach and relate it to variability in bioactive compounds, while controlling other variables.

Conclusion

Indomethacin induced reversible renal and gastric mucosal lesions in female rats. Moringa oleifera leaves had the best impact on the kidney. Improvement in biomarkers was associated with enhanced Nfe2l2/Hmox1/Sod1/Gpx4, reduced proinflammatory and pro-apoptotic gene expression and increased Bcl2. Increased expression of PGE2 and GSH and reduced MDA along with down-regulation of inflammatory and apoptotic genes in ginger accounted for the gastric mucosal protection. Partial molecular effects and less complete biochemical protection was achieved by rosemary. The study shows that the modulation of the indomethacin-induced lesion by the test samples has greater impact when assessed through integration of functional and molecular parameters.

Alterations in renal function and gastric mucosal integrity through interconnected oxidative stress, inflammatory, and apoptotic mechanisms. The findings indicate that the protective effects of plant-derived extracts are tissue-dependent and vary according to their influence on biochemical, physiological, and molecular pathways. Among the three botanical preparations investigated, Moringa oleifera leaves demonstrated the most pronounced renal protective potential, while ginger exhibited the strongest gastroprotective effects.

The renal protective activity of Moringa oleifera was reflected in the improvement of serum creatinine, NGAL, and cystatin C levels, together with favorable regulation of antioxidant and inflammatory gene expression. Increased expression of Nfe2l2, Hmox1, Sod1, Gpx4, and Bcl2, accompanied by reduced expression of Tnf, Il6, Nos2, Bax, and Tp53, suggests that Moringa may support renal cellular defense by improving antioxidant capacity, suppressing inflammatory responses, and regulating apoptosis. These findings highlight the potential involvement of the Nrf2/HO-1 signaling pathway in the observed renal protective response.

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