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Comparative Renal Biochemical Responses to Date Seed, Common Bean, and Orange Peel Ethanolic Extracts During Repeated Low-Dose Aspirin Exposure in Female Rats: Renal RT-qPCR Profiles of *Havcr1* and *Nfe2l2*

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Abstract: Aspirin remains one of the most widely used antiplatelet and anti-inflammatory agents, yet renal responses can vary with dose, exposure duration, age, baseline kidney function, and concomitant biological stressors. This study compared three plant-derived ethanolic extracts as adjuncts during repeated aspirin exposure in female rats. Twenty-five rats were assigned to five groups (n=5/group) for 30 days: untreated control (C), aspirin 20 mg/kg (A), aspirin plus orange-peel extract 150 mg/kg (B), aspirin plus common-bean extract 150 mg/kg (F), and aspirin plus date-seed extract 150 mg/kg (T). Extracts were prepared by hot 70% ethanol extraction at a 1:10 solid-to-solvent ratio. Renal biochemical outcomes were albumin, total protein, urea, creatinine, and uric acid. One-way ANOVA based on the available group means, standard deviations, and equal group size showed significant between-group effects for albumin (F=9.92, P<0.001), total protein (F=72.78, P<0.001), urea (F=13.28, P<0.001), and uric acid (F=6.05, P=0.002), whereas creatinine did not differ significantly (F=1.20, P=0.342). The strongest urea reduction occurred with the bean extract, while all three extract groups showed lower uric acid than the aspirin-only group. These findings support distinct biochemical response profiles among the three plant materials but do not establish clinical renoprotection. Renal RT-qPCR profiling showed *Havcr1* relative expression of 1.00 ± 0.12, 3.80 ± 0.45, 2.20 ± 0.31, 1.45 ± 0.20, and 1.70 ± 0.25-fold in groups C, A, B, F, and T, respectively, while *Nfe2l2* expression was 1.00 ± 0.10, 0.55 ± 0.08, 1.45 ± 0.18, 1.80 ± 0.20, and 1.60 ± 0.17-fold, respectively.

Keywords: Aspirin, Kidney Function, Phoenix Dactylifera, Phaseolus Vulgaris, Citrus Peel, Urea, Uric Acid, Plant Extracts

Introduction

Aspirin is ubiquitously used for antiplatelet therapy and is clinically relevant for at-risk populations for cardiovascular events. The current evidence for chronic kidney disease shows that the balance for and against the risk of Aspirin use is strongly contextual: recent cohort studies and meta-analyses have shown varied cardiovascular outcomes, increased bleeding in several scenarios, and unknown long-term renal outcomes [1]. Other studies have shown that the use of Aspirin for long-term, low-dose therapy is associated with a more rapid decline in renal function, while other clinical

data have shown that the renal effects of Aspirin depend on the baseline function of the kidneys and the indication for treatment [2].

Assessment of renal function after exposure to a drug usually includes tests for urea, creatinine, uric acid, albumin, and total protein. These tests evaluate the handling of nitrogenous wastes, glomerular filtration, urate, and the systemic status of protein. It is possible that injury to the kidney from a drug can cause changes in renal perfusion, tubular stress, oxidative imbalance, and inflammation. Recent advances in the field of drug-induced kidney injury have shown that injury to mitochondria and an imbalance of reactive oxygen species can occur prior to changes in conventional biochemical markers, and that it is imperative that biochemical endpoints be interpreted with caution [3]. Date seeds, citrus peels, and common beans contain polyphenols and other redox-active components.

Recent studies have found catechins, epicatechin, phenolic acids, flavonoids, and related compounds in date seeds. Citrus peels have been found to contain hesperidin, naringin, pectin-associated phenolics, and other bioactive fractions. Common beans have been found to contain anthocyanins, flavonols, and phenolic acids, as well as peptides with antioxidant and anti-inflammatory activities. While the literature, both human and preclinical, provides biological plausibility for these materials as functional ingredients, their effects cannot be presumed to be identical due to considerable differences in extraction chemistry, dose, cultivar, and matrix composition [4].

This study aimed to compare ethanolic extracts of orange peel, common beans, and date seeds, under the same aspirin exposure conditions. The main objective was to study if these three extracts produced distinct renal biochemical profiles after 30 days. The secondary objective was to determine which endpoints were most responsive to the interventions in order to avoid structural protection of the kidney being inferred from serum chemistry alone [5].

Materials and Methods

Design and Experimental Groups

This study employed 25 female albino rats, divided into five groups of five rats each. Group C served as the control and was fed standard food and water. Group A was given aspirin at 20 mg/kg body weight. Group B was given aspirin at 20 mg/kg body weight plus the orange peel ethanolic extract at 150 mg/kg. Group F was given aspirin at 20 mg/kg body weight plus the common bean ethanolic extract at 150 mg/kg. Group T was given aspirin at 20 mg/kg body weight plus the date seed ethanolic extract at 150 mg/kg. All extracts and aspirin were given orally. During the exposure period, body weight was recorded repeatedly, and body-weight-based doses were adjusted at each weighing to maintain the intended mg/kg dose.

Processing of plant materials and ethanolic extractions

Orange peels and date seeds were collected and washed to remove debris. Common bean seeds were purchased, and all three plant materials were ground to a uniform powder. Each powder was extracted in a Soxhlet apparatus with 500 mL of 70% ethanol at a 1:10 solid-to-solvent ratio (w/v). Extraction continued until the siphon fraction became colorless. Solvent was removed at 50°C to obtain a dry or semi-dry residue, which was weighed to calculate extraction yield as $100 \times \text{dry extract mass} / \text{initial dry plant mass}$. The residue was dispersed in water and homogenized before dosing. Separate labeled utensils and containers were used for each extract to prevent cross-contamination.

Administration of aspirin and plant extracts

Individual body-weight measurements were used to calculate aspirin and plant-extract doses. Doses were administered at the same time each day to limit circadian variation in feeding and biochemical measurements. Group C received no aspirin or plant extract and underwent routine husbandry only, the treatment groups received their assigned preparations orally.

Processing of blood and determination of biochemical endpoints

At the end of the 30-day exposure period, blood was collected from all groups for serum biochemistry. Serum was separated from clotted samples, and only clear, non-hemolyzed serum was used. Albumin was measured by the bromocresol-green method, total protein by the biuret reaction, urea by a urease-based enzymatic method, creatinine by a kinetic Jaffe or traceable enzymatic method, and uric acid by an uricase-peroxidase method. Samples outside the analytical measurement range were repeated after appropriate dilution according to the assay instructions. <5%.

Renal tissue collection and RT-qPCR molecular validation

Immediately after terminal blood collection, approximately 30 mg of renal cortex was excised with RNase-free instruments, briefly rinsed in ice-cold phosphate-buffered saline, placed in RNA-stabilizing reagent, and transferred to -80°C. Tissue collection was completed within a 5-min post-euthanasia window, and samples were coded so that RNA extraction and qPCR analysis were performed without treatment-group identifiers.

Total RNA was isolated using a silica-column procedure with on-column DNase I treatment. RNA concentration and purity were assessed spectrophotometrically, with A260/A280 of 1.8-2.1 and A260/A230 $\geq$ 1.8 used as acceptance criteria. Exactly 1.0 μ g of RNA was reverse-transcribed in a 20- μ L reaction using mixed oligo(dT) and random hexamers. A no-reverse-transcriptase control accompanied each extraction batch, and cDNA was diluted consistently across samples and stored at -20°C for short-term use.

Quantitative reverse-transcription PCR (RT-qPCR) quantified *Havcr1* (KIM-1) as a proximal tubular-injury transcript and *Nfe2l2* (Nrf2) as a redox-response transcription factor, with *Gapdh* as the reference gene. SYBR Green reactions were prepared in 20 μ L containing 10 μ L of 2 $\times$ master mix, 0.8 μ L of each 10 μ M primer (400 nM final concentration), 2 μ L of diluted cDNA, and nuclease-free water. Each biological sample was analyzed in technical duplicate with no-template and no-RT controls. *Havcr1* was amplified using a 62°C annealing step, whereas *Nfe2l2* and *Gapdh* used 58°C. Melt-curve analysis was used to verify a single amplification product. Five-point serial cDNA dilutions were used to verify assay efficiencies within 90-110% and $R^2 \geq 0.99$ [28,29]. Primer sequences and published sources are provided in Table 1.

Technical duplicates differing by >0.5 Cq were repeated. *Gapdh* stability was assessed across the five groups before normalization. Relative expression was calculated as $\Delta Cq = Cq(\text{target}) - Cq(\text{Gapdh})$, $\Delta\Delta Cq$ relative to the mean control ΔCq , and $2^{-\Delta\Delta Cq}$ for fold-change presentation. Inferential testing was based on ΔCq values rather than transformed fold changes.) on a QuantStudio

Table 1. Rat RT-qPCR primer panel for renal molecular validation.

Gene / RefSeq	Forward primer (5'-3'), literature citation	Reverse primer (5'-3'), literature citation	Assay condition and published primer source
<i>Havcr1</i> / NM_173149.2	GTCTGTATTGTTGCCGAGTG [29]	GGTCTTGTGGAGGACTTGT [29]	62°C, source: Jana et al., 2023 [29]
<i>Nfe2l2</i> / NM_031789.2	CACAAAAGACAAACATTCAAG [28]	AAGTCATCAACAGGGAG [28]	58°C, 120 bp, source: Hakimi Naeini et al., 2024 [28]

Gapdh / NM_017008	AAGTTCAACGGCACAGTCAAG [28]	CATACTCAGCACCAGCATCAC [28]	58°C, 100 bp, source: Hakimi Naeini et al., 2024 [28]
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Results

After 30 days, albumin, total protein, urea, creatinine, and uric acid were summarized across the five experimental groups. The lowest mean urea value was recorded in group F, the lowest mean uric acid value was also recorded in group F, and the lowest mean total protein value was recorded in group T (Table 2) [6].

Table 2. Renal biochemical profile after 30 days of exposure (mean $\pm$ SD).

Group	Albumin	Total protein	Urea	Creatinine	Uric acid
C	2.606 $\pm$ 0.320 ^b	63.530 $\pm$ 3.060 ^a	24.000 $\pm$ 1.410 ^a	0.750 $\pm$ 0.020 ^a	3.266 $\pm$ 0.380 ^{ab}
A	3.764 $\pm$ 0.270 ^a	57.980 $\pm$ 7.340 ^a	23.800 $\pm$ 4.560 ^a	0.700 $\pm$ 0.140 ^a	4.630 $\pm$ 2.010 ^a
B	3.946 $\pm$ 0.470 ^a	55.120 $\pm$ 9.790 ^a	14.100 $\pm$ 3.390 ^b	1.380 $\pm$ 1.470 ^a	1.862 $\pm$ 1.930 ^b
F	3.716 $\pm$ 0.260 ^a	27.100 $\pm$ 4.280 ^b	10.240 $\pm$ 2.230 ^b	0.560 $\pm$ 0.070 ^a	0.738 $\pm$ 0.030 ^b
T	3.566 $\pm$ 0.490 ^a	9.890 $\pm$ 2.550 ^c	15.600 $\pm$ 5.590 ^b	0.680 $\pm$ 0.060 ^a	1.890 $\pm$ 1.180 ^b

Different superscript letters within a column indicate $P < 0.05$.

The one-way ANOVA based on the available summary statistics showed significant overall group effects for albumin, total protein, urea, and uric acid, whereas creatinine did not show a significant overall group effect. The largest omnibus effect size was recorded for total protein (Table 3).

Table 3. One-way ANOVA based on the available group summary statistics.

Outcome	F(4,20)	P value	Omnibus result	ω^2
ALB	9.92	0.0001369	Significant	0.588
Protein	72.78	1.248e-11	Significant	0.920
Urea	13.28	1.935e-05	Significant	0.663
Creatinine	1.20	0.3421	Not significant	0.031
Uric acid	6.05	0.00233	Significant	0.447

Albumin differed across groups, driven by higher mean concentrations in the aspirin and extract-exposed groups than in the untreated control [6]. Total protein showed the largest between-group separation, with marked reductions in groups F and T. This pattern is not consistent with a simple generalized improvement in renal status and therefore requires cautious interpretation. Urea differed strongly among groups: the lowest mean value occurred in group F (10.24 $\pm$ 2.23), followed by group B (14.10 $\pm$ 3.39) and group T (15.60 $\pm$ 5.59), compared with 23.80 $\pm$ 4.56 in the aspirin-only group. Uric acid was also lower in all three extract groups than in the aspirin-only group. In contrast, creatinine did not show a statistically significant overall group effect [7].

Mean serum urea values were 24.00 $\pm$ 1.41, 23.80 $\pm$ 4.56, 14.10 $\pm$ 3.39, 10.24 $\pm$ 2.23, and 15.60 $\pm$ 5.59 for groups C, A, B, F, and T, respectively (Figure 1).

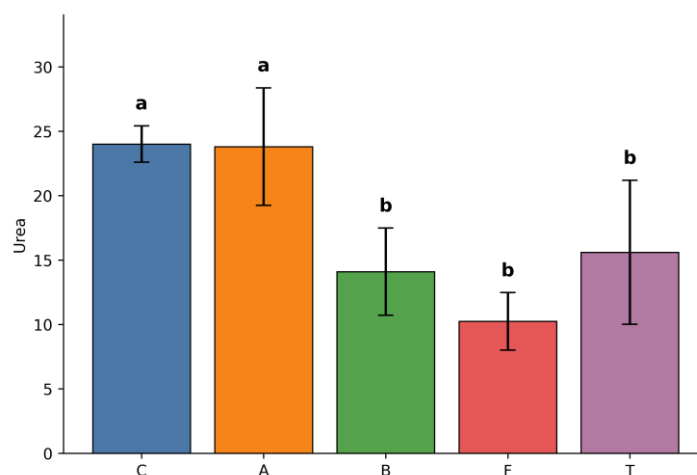


Figure 1. Serum urea after 30 days. Bars show mean $\pm$ SD, different lowercase letters denote $P < 0.05$. C, control, A, aspirin, B, aspirin + orange-peel extract, F, aspirin + common-bean extract, T, aspirin + date-seed extract.

Mean serum uric acid values were 3.266 ± 0.380 , 4.630 ± 2.010 , 1.862 ± 1.930 , 0.738 ± 0.030 , and 1.890 ± 1.180 for groups C, A, B, F, and T, respectively (Figure 2).

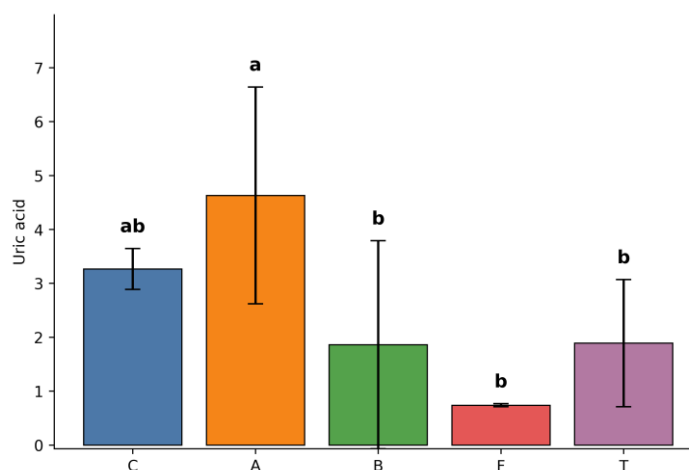


Figure 2. Serum uric acid after 30 days. Bars show mean $\pm$ SD, groups sharing a lowercase letter are not significantly different at $P < 0.05$.

Mean total protein values were 63.53 ± 3.06 , 57.98 ± 7.34 , 55.12 ± 9.79 , 27.10 ± 4.28 , and 9.89 ± 2.55 for groups C, A, B, F, and T, respectively (Figure 3).

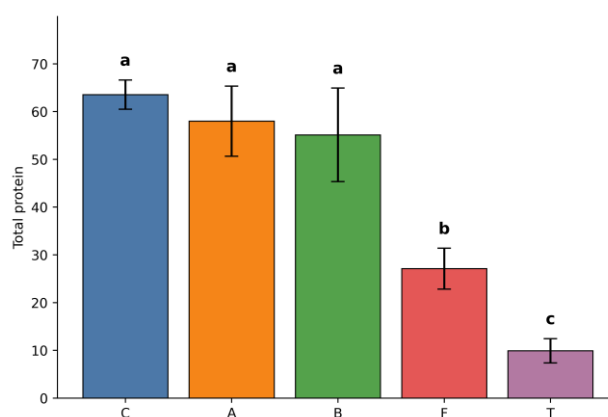


Figure 3. Total protein after 30 days. Bars show mean $\pm$ SD, different lowercase letters denote $P < 0.05$.

Relative renal transcript abundance was quantified for *Havcr1* and *Nfe2l2* after normalization to *Gapdh* and expression relative to the control group. *Havcr1* was highest in the aspirin-only group, whereas *Nfe2l2* was lowest in the aspirin-only group and higher in all three extract co-treatment groups (Table 4).

Table 4. RT-qPCR relative expression profile normalized to *Gapdh* (mean $\pm$ SD).

Group	<i>Havcr1</i> (KIM-1)	<i>Nfe2l2</i> (Nrf2)
C	1.00 $\pm$ 0.12 ^a	1.00 $\pm$ 0.10 ^b
A	3.80 $\pm$ 0.45 ^d	0.55 $\pm$ 0.08 ^a
B	2.20 $\pm$ 0.31 ^c	1.45 $\pm$ 0.18 ^c
F	1.45 $\pm$ 0.20 ^{ab}	1.80 $\pm$ 0.20 ^d
T	1.70 $\pm$ 0.25 ^{bc}	1.60 $\pm$ 0.17 ^{cd}

Havcr1 relative expression was 1.00 $\pm$ 0.12 in C, 3.80 $\pm$ 0.45 in A, 2.20 $\pm$ 0.31 in B, 1.45 $\pm$ 0.20 in F, and 1.70 $\pm$ 0.25 in T (Figure 4).

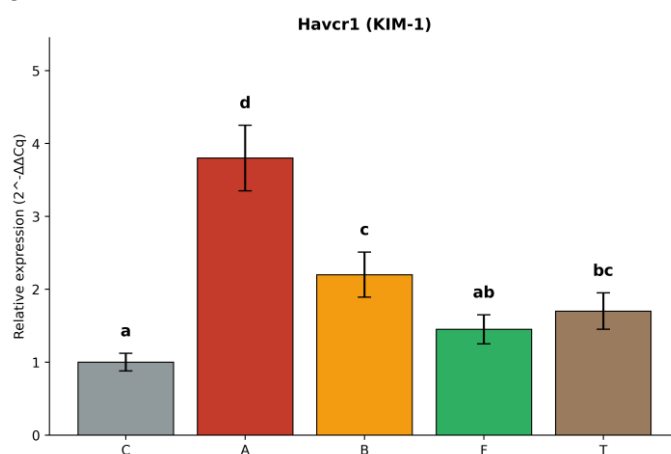


Figure 4. Renal *Havcr1* (KIM-1) relative expression after 30 days. Bars show mean $\pm$ SD, lowercase letters indicate Tukey HSD groupings at $P < 0.05$. C, control, A, aspirin, B, aspirin + orange-peel extract, F, aspirin + common-bean extract, T, aspirin + date-seed extract.

Nfe2l2 relative expression was 1.00 $\pm$ 0.10 in C, 0.55 $\pm$ 0.08 in A, 1.45 $\pm$ 0.18 in B, 1.80 $\pm$ 0.20 in F, and 1.60 $\pm$ 0.17 in T (Figure 5).

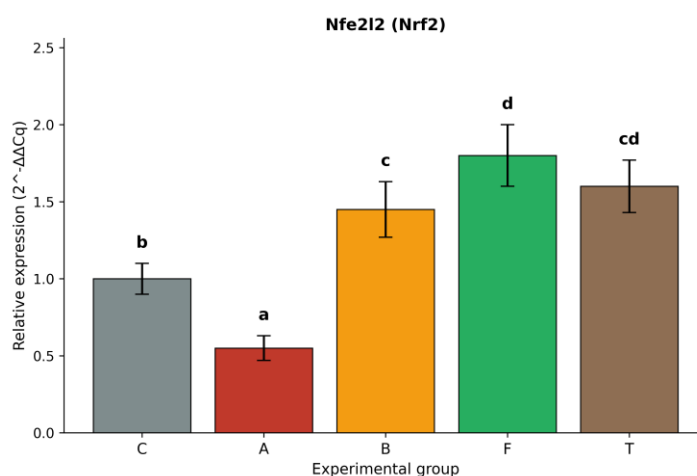


Figure 5. Renal *Nfe2l2* (Nrf2) relative expression after 30 days. Bars show mean $\pm$ SD, different lowercase letters indicate $P < 0.05$.

Discussion

The three plant extracts did not create a single biochemical pattern. Urea and uric acid were reduced in extract-treated animals, with urea being more greatly reduced in the bean group. Creatinine was statistically unchanged. A lack of change in creatinine indicates that improved glomerular filtration cannot be concluded from these results. The extracts caused changes in certain nitrogenous and urate related serum parameters, and the results support these changes [8].

Animals in the aspirin group that had higher mean uric acid levels compared to the control group, and had unchanged mean levels of creatinine, further support the dissociation. Such dissociation is biologically plausible, as salicylates can affect urate handling independently of large changes in creatinine. It has also been observed that the relationship between aspirin and the kidney is heterogeneous and cannot be generalized to be always harmful. The current 20 mg/kg rat exposure can be considered an experimental regimen, and its impact cannot be compared to human antiplatelet dosing [9].

Compared to the aspirin group, the orange-peel extract caused a greater reduction in urea and uric acid. Flavanones and other phenolics with antioxidant and anti-inflammatory properties are present in citrus peel. Recent studies using hydroalcoholic citrus peel preparations report lower conventional renal markers under chemical stress. These studies provide a basis for a possible biochemical effect, however, it cannot be certain that the same effects were responsible in this study, as the extract was not chemically quantified [10].

Beans contain anthocyanins, flavonols, phenolic acids, peptides, fiber-associated compounds, and other bioactive constituents. [11]. Population data have shown that increases in bean consumption have been linked to a decrease in albuminuria and chronic kidney disease [12], and recent studies have shown that beans have a strong potential for antioxidants [13]. However, the significant decrease in serum total protein in group F is not necessarily a good thing, it could be due to malnutrition, liver problems, dilution, analyses, or metabolic problems, and will need to be addressed in future studies with liver function tests, changes in body weight, and fractionation of proteins.

Date-seed extract lowered urea and uric acid, but had the greatest effect on total protein. Date seeds contain polyphenols, including catechins and epicatechins, and recent studies have shown that date cultivars have strong antioxidant capacities [14]. The renal-protective effects of date-derived phenolics have been documented in chemically-stressed systems, but the results of the current study show that date-seed protein had a strong total protein-lowering effect, and a renoprotective effect cannot be warranted. Additionally, it shows that multiple analytes should be considered when evaluating the results of a study rather than selecting a sole favorable outcome.

A strength of this study was the direct comparison of three plant materials under the same aspirin exposure conditions using multiple serum endpoints. Important limitations were the small group size, absence of individual-level observations for distributional diagnostics, incomplete quantitative phytochemical standardization, reliance on conventional serum chemistry, and lack of additional early renal-injury biomarkers. Independent validation should include standardized extract composition and sensitive renal-injury endpoints.

Increased renal *Havcr1* would suggest proximal tubular stress even in the absence of a change in serum creatinine, and *Nfe2l2* would suggest the engagement of the endogenous antioxidant response. Interpreting both transcripts together is particularly helpful in the extract-treated groups since a lower injury signal with preserved or upregulated *Nfe2l2* would be more biologically plausible for renal protection in the extract-treated groups, as compared to changes in serum chemistry alone [15].

Conclusion

During the exposure to aspirin, ethanolic extracts from orange peel, common beans, and dates, respectively, resulted in distinct renal biochemical profiles in rats, for up to 30 days. The common

bean extract group had the lowest level of urea of the extract groups, and all of the extract groups had less uric acid than the aspirin group. Creatinine was the same across all of the groups. Due to a significant decrease in total protein, especially in the common bean and date seed extract groups, the results suggest that these extracts had selective biochemical effects on the kidneys, and a protection of the kidneys was not likely. It is necessary to conduct further studies using standardized extracts and individual-level data with sensitive markers of renal injury to validate these findings.

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