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Original Article

Comparative effects of valsartan and irbesartan combined with rosmarinic acid on serum malondialdehyde and albuminuria in diabetic nephropathy rat models

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Abstract

Diabetic nephropathy is a major microvascular complication of diabetes mellitus and remains a leading cause of chronic kidney disease. Oxidative stress is a central mechanism in its pathogenesis, and malondialdehyde (MDA) is widely used as a biomarker of lipid peroxidation. The aim of this study was to compare the effects of rosmarinic acid combined with valsartan or irbesartan on serum MDA and urinary albumin levels in a rat model of diabetic nephropathy. A laboratory-based experimental study with a randomized post-test-only controlled group design was conducted from April 2021 to April 2022. Male Wistar rats were rendered diabetic by a high-fat diet followed by streptozotocin administration and were allocated to a non-diabetic group, an untreated diabetic group, and two treatment groups receiving rosmarinic acid in combination with either valsartan or irbesartan. Serum MDA and urinary albumin levels were measured, and between-group comparisons were performed using one-way analysis of variance followed by post hoc Tukey analysis when appropriate, with statistical significance set at $p<0.05$. No significant difference in serum MDA levels was observed among groups ($p=0.512$). In contrast, urinary albumin levels differed significantly among groups ($p=0.041$). Post hoc Tukey analysis showed that urinary albumin levels differed significantly between the untreated diabetic group and rosmarinic acid–valsartan groups (mean difference (MD): 2.59; 95%CI: 0.46 to 4.73; $p=0.013$) and between the untreated diabetic and rosmarinic acid–irbesartan groups (MD: 2.59 µg/mL; 95%CI: 0.45 to 4.72; $p=0.014$). The combination of rosmarinic acid with either valsartan or irbesartan was not associated with a significant difference in serum MDA levels in this model, although lower urinary albumin levels were observed in the treatment groups. This finding suggests that serum MDA alone may not fully reflect the potential nephroprotective effects of these combinations, which should be further evaluated using a broader panel of oxidative stress biomarkers.

Keywords: Diabetic nephropathy, angiotensin receptor blocker, valsartan, irbesartan, rosmarinic acid

Introduction

Diabetic nephropathy is a major microvascular complication of diabetes mellitus and remains one of the leading causes of chronic kidney disease and end-stage renal disease worldwide [1]. A substantial clinical and public health burden is imposed by this condition, particularly in low- and middle-income countries where the prevalence of diabetes continues to rise [2]. The

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pathogenesis of diabetic nephropathy is multifactorial and involves persistent hyperglycemia, intraglomerular hypertension, activation of the renin-angiotensin-aldosterone system, inflammation, and oxidative stress [3-6]. Among these mechanisms, oxidative stress has been recognized as a central contributor to endothelial injury, mesangial expansion, podocyte dysfunction, and progressive glomerulosclerosis [3]. Malondialdehyde (MDA), a by-product of lipid peroxidation, has therefore been widely used as a biomarker of oxidative injury in diabetic kidney disease [3,7].

Current therapeutic strategies for diabetic nephropathy are primarily directed toward slowing disease progression through glycemic control, blood pressure reduction, and renin-angiotensin system blockade [1,6]. Angiotensin receptor blockers such as valsartan and irbesartan have been shown to exert renoprotective effects by reducing intraglomerular pressure, attenuating proteinuria, and improving vascular and endothelial function [11]. However, despite these benefits, residual oxidative and inflammatory injury may persist, suggesting that additional adjunctive interventions may be required. In this context, naturally derived antioxidant compounds have attracted increasing attention because of their potential to mitigate oxidative stress-related renal injury [8,12].

Rosmarinic acid is a natural polyphenolic compound with antioxidant and anti-inflammatory properties and has been reported to suppress reactive oxygen species generation and inflammatory signaling [8]. On this basis, rosmarinic acid may complement the renoprotective effects of angiotensin receptor blockade by targeting oxidative mechanisms that may not be fully addressed by conventional therapy alone [11]. Nevertheless, evidence regarding the combined effects of rosmarinic acid and angiotensin receptor blockers on oxidative stress biomarkers in diabetic nephropathy remains limited. In particular, comparative data evaluating rosmarinic acid in combination with valsartan or irbesartan are still lacking. Therefore, the aim of this study was to compare the effects of rosmarinic acid combined with either valsartan or irbesartan on serum MDA and urinary albumin levels in a rat model of diabetic nephropathy. It was expected that this study would provide further evidence regarding the potential therapeutic value of combining antioxidant and renin-angiotensin system-targeted strategies in diabetic nephropathy.

Methods

Study design and setting

A laboratory-based experimental study with a randomized post-test only controlled group design was conducted. The animal experiment was performed at the Experimental Animal Research Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. Biochemical and laboratory analyses were conducted at the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya, and the Department of Anatomical Pathology, Saiful Anwar Hospital, Malang, Indonesia. The study was designed to compare the effects of rosmarinic acid combined with valsartan or irbesartan on oxidative stress and renal injury in a rat model of diabetes, using serum MDA and urinary albumin as the main outcome measures. Diabetic rats were assigned to a positive control group or to 1 of 2 treatment groups receiving rosmarinic acid combined with either valsartan or irbesartan. The treatments were administered once daily by oral gavage for 8 weeks. At the end of the study, blood samples were obtained for serum MDA analysis and 24-hour urine samples were collected for albumin measurement.

Animal husbandry

Male Wistar rats aged 7–9 weeks and weighing 150–200 g were used in this study. Before the experiment, all animals underwent a 7-day acclimatization period. Rats were housed individually under controlled laboratory conditions and were provided free access to drinking water. Only healthy animals showing active movement and no visible hair loss were included.

Diabetes induction

To establish the diabetic model, rats in the diabetic groups were fed a high-fat diet containing 60% calories from fat for 21 days. On day 22, these animals were fasted for 6–8 hours while water

remained available ad libitum. Diabetes was then induced by a single intraperitoneal injection of streptozotocin diluted in 50 mM sodium citrate buffer (pH=4.5). Rats in the negative control group received an equivalent volume of sodium citrate buffer without streptozotocin. Blood glucose was measured 10 days after streptozotocin administration using a glucose meter, and rats with blood glucose levels >270 mg/dL were considered diabetic and eligible for the treatment phase.

Animals and group allocation

A total of 20 rats were initially enrolled, including five rats assigned to the negative control group and 15 rats subjected to diabetes induction. Rats were excluded if they failed to develop diabetes after induction or died during the study period. After successful induction of diabetes, the rats were randomly allocated into three groups: a positive control group (diabetic rats without treatment), treatment group 1 (diabetic rats receiving rosmarinic acid plus valsartan), and treatment group 2 (diabetic rats receiving rosmarinic acid plus irbesartan).

Interventions and procedure

Following confirmation of diabetes, treatment was initiated according to group allocation. In both treatment groups, rosmarinic acid was freshly prepared in 25% ethanol and administered once daily by oral gavage at a dose of 75 mg/kgBW/day for eight weeks. One hour after rosmarinic acid administration, the corresponding angiotensin receptor blocker was administered once daily by oral gavage (valsartan at a dose of 4 mg/kgBW/day or irbesartan at a dose of 15 mg/kgBW/day). Valsartan and irbesartan were dissolved in distilled water before administration. Rats in the control groups received distilled water in equal volume. At the end of the intervention period, 24-hour urine samples were collected using metabolic cages. On day 57, blood samples were collected for biochemical analysis.

Study outcomes and measurements

The primary outcome of this study was serum MDA concentration, expressed in nmol/mL, as a biomarker of oxidative stress and lipid peroxidation. Serum MDA was measured at the end of the 8-week intervention period using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Cambridge, UK), with absorbance read at 450 nm and concentrations determined from a standard curve.

The secondary outcome was urinary albumin concentration, expressed in µg/mL, as an indicator of renal injury. Twenty-four-hour urine samples were collected at the end of the study using metabolic cages and were analyzed using ELISA Kit (Abcam, Cambridge, UK) according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 27 (IBM, New York, USA). Continuous variables were summarized as mean and standard deviation (SD) or median with interquartile range, as appropriate. Data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Between-group comparisons were performed using one-way analysis of variance (ANOVA). When the overall comparison was significant, post-hoc Tukey pairwise comparisons were conducted using the least significant difference test. A two-sided $p<0.05$ was considered statistically significant.

Results

Characteristics of experimental animals

Mean post-induction blood glucose levels differed markedly among groups, with values of 77±3.65 mg/dL in negative control group, 402.0±27.86 mg/dL in positive control, 391.50±21.61 mg/dL in valsartan, and 337.50±22.78 mg/dL in irbesartan. The detailed characteristics of the experimental animals are presented in **Table 1**.

Table 1. Comparison of blood glucose level across groups after diabetes induction

Characteristics	Negative control	Positive control	Rosmarinic acid-valsartan	Rosmarinic acid-irbesartan	*p*-value
Mean post-induction blood glucose, (mg/dL±SD)	77±3.65	402.0±27.86	391.50±21.61	337.50±22.78	<0.001

Comparisons of serum MDA levels across groups

The comparison of serum MDA levels among the study groups are presented in **Table 2** and **Figure 1A**. The mean±SD serum MDA levels were 1.326±0.210 nmol/mL in the negative control group, 1.420±0.199 nmol/mL in the positive control group, 1.476±0.154 nmol/mL in the rosmarinic acid–valsartan group, and 1.368±0.219 nmol/mL in the rosmarinic acid–irbesartan group. Although the positive control and rosmarinic acid-valsartan groups showed higher mean and median MDA levels than the negative control group, and the rosmarinic acid-irbesartan group showed a lower mean MDA level than the rosmarinic acid-valsartan group, the overall between-group difference was not statistically significant (p=0.512) (**Table 2**). These findings indicate that the administration of rosmarinic acid combined with either valsartan or irbesartan was not associated with a significant difference in serum MDA levels in this diabetic nephropathy rat model.

Table 2. Comparative effects of valsartan and irbesartan combined with rosmarinic acid on serum malondialdehyde levels in rats with diabetic nephropathy

Group	Serum MDA concentration		*p*-value [a]
	Median (interquartile range) (nmol/mL)	Mean±SD (nmol/mL)	
Negative control (non-diabetic)	1.36 (1.20–1.47)	1.326±0.210	0.512
Positive control (untreated diabetic)	1.53 (1.39–1.71)	1.420±0.199	
Rosmarinic acid-valsartan	1.51 (1.44–1.61)	1.476±0.154	
Rosmarinic acid-irbesartan	1.40 (1.21–1.54)	1.368±0.219	

[a] Analyzed using one-way analysis of variance

Comparisons of urinary albumin levels across groups

The comparison of urinary albumin levels among the study groups is presented in **Table 3** and **Figure 1B**. The mean±SD urinary albumin concentrations were 1.92±0.35 µg/mL in the negative control group, 2.50±0.55 µg/mL in the positive control group, 1.55±0.26 µg/mL in the rosmarinic acid–valsartan group, and 1.94±0.39 µg/mL in the rosmarinic acid–irbesartan group. The overall between-group difference was statistically significant (p=0.041). The positive control group showed the highest urinary albumin level, whereas the rosmarinic acid–valsartan group showed the lowest level. Post hoc Tukey analysis showed no significant difference between the negative control and positive control groups (mean difference (MD): 0.55 µg/mL; 95%CI: -1.50 to 2.60; p=0.879). However, urinary albumin levels were significantly higher in the negative control group than in the rosmarinic acid–valsartan group (MD: 2.59 µg/mL; 95%CI: 0.46 to 4.73; p=0.013) and the rosmarinic acid–irbesartan group (MD: 2.59 µg/mL; 95%CI: 0.45 to 4.72; p=0.014) (**Figure 1B**).

Table 3. Comparative effects of valsartan and irbesartan combined with rosmarinic acid on urinary albumin levels in rats with diabetic nephropathy

Group	Urinary albumin		*p*-value [a]
	Median (interquartile range) (µg/mL)	Mean±SD (µg/mL)	
Negative control (non-diabetic)	1.98 (1.63–2.32)	1.92±0.35	0.041
Positive control (untreated diabetic)	2.05 (1.83–2.55)	2.50±0.55	
Rosmarinic acid-valsartan	1.55 (1.38–1.72)	1.55±0.26	
Rosmarinic acid-irbesartan	1.94 (1.68–2.21)	1.94±0.39	

[a] Analyzed using one-way analysis of variance

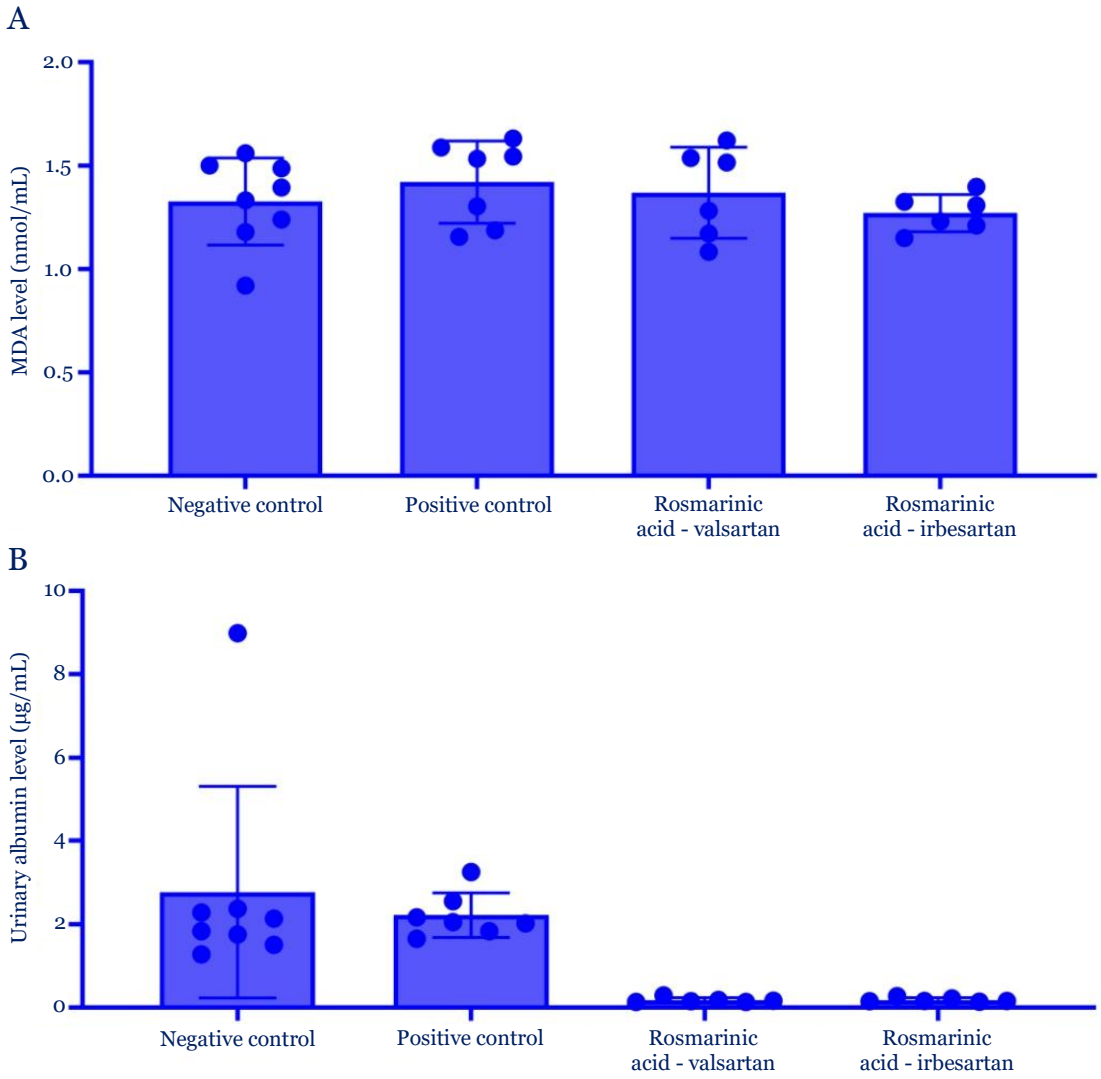

Figure 1. Comparisons of malondialdehyde (MDA) and urinary albumin levels across experimental groups. (A) Comparison of MDA. No significant between-group differences were observed. (B) Comparison of urinary albumin levels. Urinary albumin levels were significantly different only between the positive control (untreated diabetic) and rosmarinic acid–valsartan groups (mean difference (MD): 2.59; 95%CI: 0.46 to 4.73; p=0.013) and between the positive control and rosmarinic acid–irbesartan groups (MD: 2.59; 95%CI: 0.45 to 4.72; p=0.014).

Discussion

In this experimental study, the combination of rosmarinic acid with either valsartan or irbesartan was not associated with a significant difference in serum MDA levels in a rat model of diabetic nephropathy. By contrast, urinary albumin levels differed significantly among groups, with lower levels observed in the rosmarinic acid–valsartan and rosmarinic acid–irbesartan groups than in the positive control group, suggesting a potential renoprotective effect despite the absence of a measurable difference in this oxidative stress biomarker. These findings indicate that serum MDA alone may not adequately capture the biological effects of the intervention in this model.

The absence of a significant difference in serum MDA levels may be explained by several factors. First, although MDA is widely used as a marker of lipid peroxidation, it reflects only one aspect of oxidative stress and may not fully capture the complex redox disturbances involved in diabetic nephropathy [16,17]. Second, the small sample size may have limited statistical power to detect modest between-group differences, particularly for biochemical markers that are prone to biological variation. Third, the duration of the intervention may not have been sufficient for changes in systemic lipid peroxidation to become consistently detectable, even if early renal effects had already occurred. The absence of an observable difference between the valsartan- and

irbesartan-based combinations is also biologically plausible, as both agents act through angiotensin II receptor blockade and share broadly similar renoprotective mechanisms [18,19].

Potential mechanistic synergy between rosmarinic acid and angiotensin receptor blockers may nevertheless be considered. Rosmarinic acid has been reported to suppress reactive oxygen species generation and inflammatory mediator expression [8,21], suggesting that it may help reduce oxidative and inflammatory injury at the tissue level. In diabetic nephropathy, these processes are closely linked to glomerular and tubular damage, mesangial expansion, and progressive impairment of renal function. By limiting oxidative stress and inflammatory signaling, rosmarinic acid may therefore contribute to preservation of renal structural integrity. In parallel, angiotensin receptor blockers may reduce angiotensin II-mediated oxidative stress, improve endothelial function, lower intraglomerular pressure, and attenuate proteinuria [11,20,22]. These effects are particularly relevant in diabetic nephropathy, in which activation of the renin-angiotensin system plays a key role in promoting both hemodynamic stress and downstream inflammatory and fibrotic responses. The combination of rosmarinic acid with valsartan or irbesartan may offer complementary actions rather than overlapping effects alone. Rosmarinic acid may act primarily through antioxidant and anti-inflammatory pathways, whereas angiotensin receptor blockers may act mainly through modulation of intrarenal hemodynamics and inhibition of angiotensin II-related injury. Together, these pathways might provide broader protection against renal damage than either approach alone. Such combined effects may help explain why differences were more evident in urinary albumin, which more directly reflects glomerular injury and renal permeability, than in serum MDA, which represents only one systemic marker of oxidative stress.

Some implications may be drawn from this study. The absence of a significant difference in serum MDA despite differences in urinary albumin suggests that reliance on a single oxidative stress marker may provide an incomplete picture of treatment response in diabetic nephropathy. Oxidative injury in this condition is biologically complex and involves multiple pathways related to lipid peroxidation, protein oxidation, mitochondrial dysfunction, and impaired antioxidant defense. Therefore, future studies should incorporate a broader panel of biomarkers, such as 8-isoprostanes, oxidized low-density lipoprotein, superoxide dismutase, catalase, glutathione peroxidase, or total antioxidant capacity, to better characterize the redox profile associated with treatment. The lower urinary albumin levels observed in the treatment groups may indicate a potential renoprotective effect of combining rosmarinic acid with angiotensin receptor blockers, possibly through complementary actions on oxidative stress, inflammation, endothelial dysfunction, and intraglomerular hemodynamics. Although this interpretation should be made cautiously, it raises the possibility that adjunctive antioxidant therapy may enhance the renal benefits of standard renin-angiotensin system blockade. The findings highlight the need for translational studies with larger sample sizes, longer intervention periods, and more robust phenotyping of renal injury to clarify whether antioxidant supplementation provides additive benefit in diabetic nephropathy. Such studies should ideally integrate biochemical, histopathological, and functional renal outcomes to determine whether the observed changes in urinary albumin reflect true structural or clinically meaningful renal protection.

This study has some limitations that should be acknowledged. The absence of monotherapy arms precluded assessment of the independent effects of rosmarinic acid, valsartan, and irbesartan, making it difficult to determine the extent to which the observed findings were attributable to each component of the combination regimen. The small sample size may have limited statistical power and increased the risk of type II error, particularly for detecting modest differences in oxidative stress markers. In addition, baseline MDA values were not measured, which restricted evaluation of within-group changes over time and limited interpretation of treatment-related shifts in oxidative status. Therefore, these limitations should be taken into account when interpreting the findings.

Conclusion

The combination of rosmarinic acid with either valsartan or irbesartan was not associated with a significant difference in serum MDA levels in this rat model of diabetic nephropathy. However, urinary albumin levels were significantly lower in both treatment groups than in the untreated

diabetic rats, which may suggest a potential nephroprotective effect despite the absence of a detectable difference in serum MDA. These findings support further preclinical investigation with larger sample sizes, longer intervention periods, and a broader panel of oxidative stress biomarkers to better clarify the therapeutic potential of combining rosmarinic acid with angiotensin receptor blockers in diabetic kidney disease.

Ethics approval

This study has received approval from Health Research Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia, with approval number: 78.1/EC/KEPK-PSPDS/04/2022.

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None.

Competing interests

The authors declare that there are no competing interests.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

Artificial intelligence tool, ChatGPT, was used for language refinement. All AI-assisted output was critically reviewed and edited by the authors, and responsibility for the accuracy, interpretation, and final content of the manuscript was retained entirely by the authors.

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