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Comparative analysis of inflammatory and oxidative stress indicators in rat models of CKD induced by adenine versus folic acid; an experimental study

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ABSTRACT

Introduction: Chronic kidney disease (CKD) is a progressive condition characterized by inflammation, oxidative stress, and significant renal dysfunction. Animal models are indispensable for studying CKD pathophysiology and testing therapeutic strategies. Among these, adenine and folic acid-induced CKD models are widely used due to their simplicity and reproducibility. **Objectives:** This study aims to compare inflammatory and oxidative stress indicators in rat models of CKD induced by adenine and folic acid.

Materials and Methods: This experimental study was conducted on 30 male Wistar rats at Mustansiriyah University, Iraq, in 2023, to investigate the effects of CKD induced by adenine and folic acid. The rats were divided into five groups, each consisting of six animals, and treated over four weeks with varying dosages of adenine or folic acid weekly and bi-weekly, while a control group received normal saline. Data collection involved anesthetizing the rats and extracting blood for serum analysis. The serum was subsequently used to measure inflammatory markers such as tumor necrosis factor α (TNF- α) and interleukin-6 (IL-6), along with oxidative stress biomarkers like glutathione (GSH) and malondialdehyde (MDA), using enzyme-linked immunosorbent assays (ELISA). Data were compared between groups using statistical tests.

Results: This study compared inflammatory and oxidative stress markers in CKD rat models induced by adenine and folic acid, revealing notable differences based on treatment type and frequency. Folic acid, particularly at a weekly dosage, elicited stronger pro-inflammatory effects, significantly increasing TNF- α and IL-6 levels compared to adenine. Weekly folic acid administration also demonstrated a dose-dependent response within its group, with greater effects than bi-weekly dosing. Regarding oxidative stress markers, both folic acid and adenine reduced GSH levels and increased MDA levels compared to controls, but weekly folic acid was the most potent in reducing GSH and increasing MDA. Bi-weekly adenine had the least impact on these markers.

Conclusion: This study highlights that both folic acid and adenine induce inflammatory and oxidative stress in CKD rat models, with folic acid, particularly at a weekly dosage, showing a stronger pro-inflammatory and oxidative impact. The findings emphasize the dose-dependent effects of folic acid and its greater potency compared to adenine, offering insights into the differential mechanisms of CKD progression and the importance of treatment frequency and type in experimental models.

Introduction

Chronic kidney disease (CKD) is a progressive condition defined by persistent kidney damage or a glomerular filtration rate (GFR) below 60 mL/min/1.73 m² for at least three months, irrespective of the underlying cause, and it often leads to end-stage renal disease requiring dialysis or transplantation (1). Globally, CKD affects

approximately 11%-13% of the population, with diabetes and hypertension being the most common causes, though other factors such as glomerulonephritis and genetic predispositions also contribute (2,3). The progression of CKD involves mechanisms such as oxidative stress, inflammation, and fibrosis, which lead to structural and functional deterioration of the kidneys (4,5).

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Implication for health policy/practice/research/medical education:

The findings of this study indicated that both folic acid and adenine trigger significant inflammatory and oxidative stress responses in rat models of chronic kidney disease (CKD), with distinct variations depending on dosage and treatment regimen. The pronounced pro-inflammatory and oxidative effects of weekly folic acid administration, compared to adenine, suggest that treatment regimens involving folic acid should be carefully tailored to minimize potential adverse effects. For research, these results highlight the necessity of understanding the differential mechanisms of CKD progression induced by various compounds to develop more targeted therapeutic strategies. In medical education, these insights can enhance training on the complexities of CKD pathophysiology and the nuanced role of treatment regimens in influencing disease outcomes. These findings also call for further investigation into optimizing experimental models to better reflect clinical scenarios and inform evidence-based health policies..

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Chronic kidney disease can be induced in animal models through the administration of adenine or folic acid, both leading to kidney function abnormalities and structural changes (6). The adenine-induced model involves feeding rodents an adenine-supplemented diet, resulting in tubulointerstitial damage resembling human CKD (6,7). This process upregulates integrin-linked kinase (ILK), and its depletion can prevent disease progression (7). Conversely, the folic acid-induced model utilizes intraperitoneal injections of folic acid to induce kidney fibrosis and inflammation (6). The folic acid model can be used to study acute kidney injury (AKI), chronic renal failure, and the AKI-to-CKD transition (8). While both models result in kidney fibrosis, the adenine diet-fed model exhibits a higher inflammatory response (6).

In rat models of chronic renal failure, both inflammation and oxidative stress play significant roles in the progression of renal damage (9,10). Studies have confirmed that chronic renal failure leads to increased systemic oxidative stress, with elevated levels of oxidative markers such as advanced oxidation protein products (10,11). Additionally, markers of inflammation, including interleukins (ILs) and tumor necrosis factor- α (TNF- α), are significantly altered in rat models of CKD (12). Specifically, oxidative stress and inflammation disrupt renal regulatory mechanisms, preventing the maintenance of intra-systemic homeostasis and leading to the accumulation of metabolic products (10). Treatment with oxidative stress modulators has shown promise in attenuating oxidative stress, inflammation, and renal injury in rat models of chronic renal failure (13). The imbalance between endogenous oxidants and antioxidants causes oxidative stress, contributing to vascular dysfunction and further increasing inflammation (10). This study was conducted to provide insights into selecting the most appropriate model for specific research objectives related to CKD.

Objectives

The objective of this experimental study is to conduct a comparative analysis of inflammatory and oxidative stress

indicators in rat models of chronic renal failure induced by adenine versus folic acid. This will involve assessing and comparing the levels of key inflammatory markers, such as IL-6 and TNF- α . Additionally, the study will evaluate oxidative stress markers, including glutathione (GSH) and malondialdehyde (MDA), to determine the extent of oxidative damage in both CKD models. By comparing these indicators, the study aims to provide insights into the distinct inflammatory and oxidative stress pathways associated with adenine-induced and folic acid-induced CKD in rats. The results may help in understanding the pathogenesis of chronic renal failure and identifying potential therapeutic targets

Materials and Methods*Study design*

This study was designed as an experimental study as mentioned in our previous study (14), and conducted on 30 male Wistar rats at Mustansiriyah University, Iraq, in 2023.

Rats and drug preparation

- The subjects of this study included 30 male Wistar rats. Their age ranged from 2.5 to 3 months, and their weight varied between 200 and 300 g. To house the animals, a facility with proper ventilation, access to food and water, and a natural light/dark cycle was provided (room temperature at 25 °C, humidity ranging from 30% to 40%). To acclimate the rats to the new conditions, they were kept in the new environment for two weeks before the start of the intervention.
- Adenine solution was prepared by dissolving adenine in 10 milliliters of a 1M sodium hydroxide (NaOH) solution, which was made by dissolving 40 grams of NaOH in one liter of distilled water. 7 drops of concentrated hydrochloric acid (HCl) were added to adjust the solution to the physiological pH suitable for rats. The prepared solution was then administered to the rats at a dosage of 250 mg/kg,

with one group receiving injections every 7 days and another receiving injections every 14 days (15-18).

- To prepare the folic acid solution, one gram of the drug was dissolved in a sodium bicarbonate (NaHCO₃) solution (1 g NaHCO₃ in 10 milliliters of distilled water). To ensure complete solubility, the mixture was stirred for 5 minutes at 40 °C (19).

Experimental design for animal study

This investigation utilized thirty male albino Wistar rats, which were divided into five groups of six animals each. The study was conducted over a four-week period with the following experimental design:

- Group I (healthy control) received intraperitoneal injections of 0.5 mL of normal saline once weekly for four weeks.
- Group II (weekly adenine model) received intraperitoneal injections of 0.55 mL of adenine at a dosage of 250 mg/kg once weekly for four weeks.
- Group III (bi-weekly adenine model) received intraperitoneal injections of 0.6 mL of adenine at a dosage of 250 mg/kg every two weeks for four weeks.
- Group IV (weekly folic acid model) received intraperitoneal injections of 0.58 mL of folic acid at a single dose of 250 mg/kg once weekly for four weeks. Finally,
- Group V (bi-weekly folic acid model) received intraperitoneal injections of 0.58 mL of folic acid at 250 mg/kg every two weeks for four weeks.
- For adjusting to hormonal fluctuation in rats, the solution was administered during a consistent morning time window (8:30 to 9:30 AM).

Data collection and serum sampling

First, the rats were anesthetized via intraperitoneal injection of ketamine and xylazine (100 and 10 mg/kg, respectively) (20). Then, blood was drawn directly from

the right ventricle and centrifuged to separate the serum from the cellular components of the blood. The serum was then stored at -20 °C. The collected serum was used to measure and quantify inflammatory markers, specifically TNF- α and IL-6, as well as oxidative stress biomarkers, including GSH and MDA. These measurements were conducted using enzyme-linked immunosorbent assays (ELISA) (21).

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, IBM Corp, USA). To compare the mean levels of TNF- α , IL-6, GSH, and MDA among the five groups of rats, an analysis of variance (ANOVA) was performed, followed by post hoc Least Significant Difference (LSD) tests. Statistical significance was defined as *P* values < 0.05.

Results

The study involved 30 rats divided into five groups, with six rats in each group: healthy controls, rats administered adenine at 250 mg/kg weekly and bi-weekly, and rats administered folic acid at 250 mg/kg weekly and bi-weekly. Data analysis revealed statistically significant differences in the frequency distribution of inflammatory markers, including TNF- α and IL-6, as well as oxidative stress biomarkers, such as GSH and MDA, across the groups (Table 1).

Analysis of TNF- α levels across the study groups revealed distinct effects based on treatment. Both weekly and bi-weekly administration of folic acid significantly increased TNF- α compared to the control group. In contrast, only the weekly dosage of adenine resulted in a significant increase, while the bi-weekly adenine dosage did not. When comparing the treatments, the weekly adenine dosage showed similar effects to both the weekly and bi-weekly folic acid doses. However, the increase in TNF- α induced by both weekly and bi-weekly folic acid was

Table 1. Frequency distribution of inflammatory and oxidative stress indicators among studied rat groups

Studied group	Indicators							
	Inflammatory				Oxidative stress			
	TNF- α (ng/L)		IL-6 (ng/L)		GSH (ng/L)		MDA (μ mol/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy rats	118.78	12.70	141.28	59.27	31.51	0.47	113.38	10.46
Weekly adenine	353.42	8.34	463.47	16.36	7.32	2.39	399.92	136.76
Bi-weekly adenine	164.26	84.67	306.01	16.67	15.63	1.35	162.29	11.04
Weekly FA	383.06	4.94	476.20	20.63	5.29	0.83	1124.44	77.53
Bi-weekly FA	320.61	35.12	407.09	26.62	10.51	0.56	331.19	42.48
<i>P</i> value*	<0.001		<0.001		<0.001		<0.001	

TNF- α , Tumor necrosis factor α ; IL-6, Interleukin-6; GSH, Glutathione; MDA, Malondialdehyde; SD, Standard deviation; FA, Folic acid.

*ANOVA test.

significantly greater than that induced by the bi-weekly adenine dose. Additionally, the weekly folic acid dosage significantly increased TNF- α compared to the bi-weekly folic acid dosage, highlighting a dose-dependent response within the folic acid treatment group. Regarding IL-6, the comparative analysis across the study groups revealed that both folic acid and adenine administration, at weekly and bi-weekly intervals, led to a significant increase in IL-6 compared to the control group. When directly comparing folic acid and adenine, the weekly dosages of both substances yielded similar results, and both were more effective than their bi-weekly counterparts. Furthermore,

the bi-weekly dose of folic acid resulted in higher IL-6 levels than the bi-weekly dose of adenine, indicating a differential impact on this inflammatory marker based on the substance and frequency of administration (Table 2 and Figure 1).

The comparative analysis of GSH levels among the studied groups indicated that both weekly and bi-weekly administrations of folic acid and adenine significantly decreased GSH compared to the healthy control group. When comparing folic acid and adenine treatments, the weekly dose of folic acid proved most effective at reducing GSH levels, surpassing both its bi-weekly counterpart and

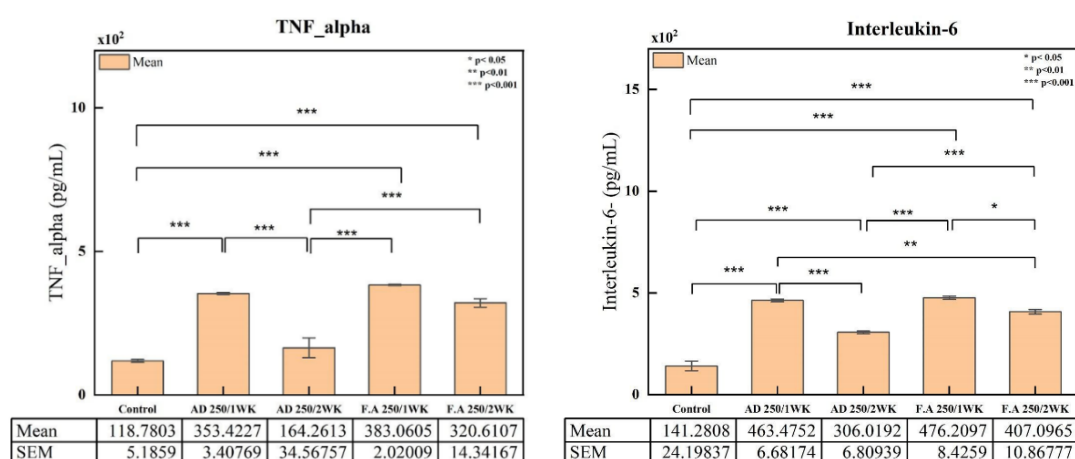


Figure 1. Comparison of inflammatory indicators among experimental groups. ** *P* value <0.01 (highly significant difference), *** *P* value <0.001 (very highly significant difference). AD, Adenine; F.A, Folic acid; TNF- α , Tumor necrosis factor α .

Table 2. Comparative analysis of inflammatory indicators across experimental rat groups

First group	Second group	Mean difference	<i>P</i> value*
Inflammatory indicators	Weekly AD	234.64	<0.001
	Bi-weekly AD	45.48	0.070
	Weekly FA	264.28	<0.001
	Bi-weekly FA	201.83	<0.001
	Bi-weekly AD	189.16	<0.001
	Weekly FA	29.63	0.229
	Bi-weekly FA	32.81	0.184
	Weekly FA	218.79	<0.001
	Bi-weekly FA	156.34	<0.001
	Weekly FA	62.44	0.015
	Weekly AD	322.19	<0.001
	Bi-weekly AD	164.73	<0.001
	Weekly FA	334.92	<0.001
	Bi-weekly FA	265.81	<0.001
	Bi-weekly AD	157.45	<0.001
	Weekly FA	12.73	0.500
	Bi-weekly FA	56.37	0.006
	Weekly FA	170.19	<0.001
	Bi-weekly FA	101.07	<0.001
	Weekly FA	69.11	0.001

TNF- α , Tumor necrosis factor α ; IL-6, Interleukin-6; AD, adenine; FA, Folic acid.

*Post hoc LSD.

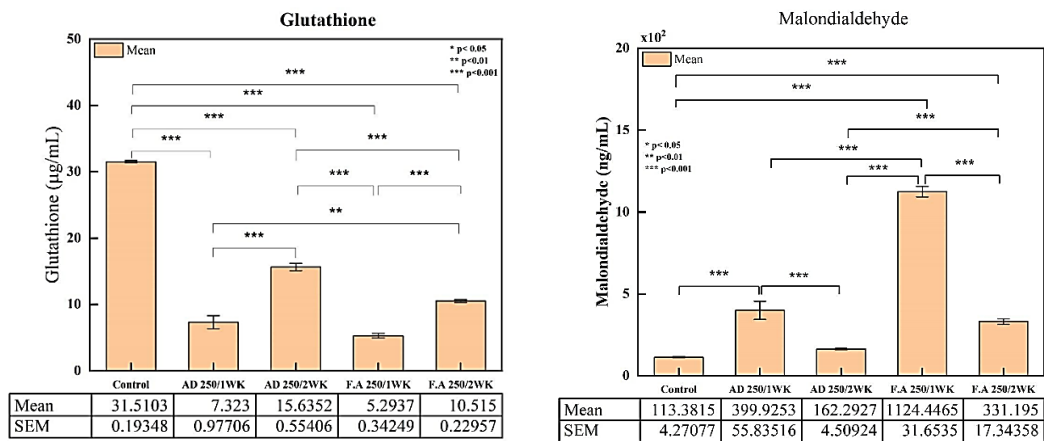


Figure 2. Comparison of oxidative stress biomarkers among experimental groups. ** *P* value <0.01 (highly significant difference), *** *P* value <0.001 (very highly significant difference). AD, Adenine; F.A, Folic acid.

both dosages of adenine. Additionally, the weekly dose of adenine outperformed the bi-weekly dose. Comparing the bi-weekly dosages of folic acid and adenine, folic acid resulted in a greater reduction of GSH. The comparative analysis of MDA levels across the experimental groups revealed that both dosages of folic acid and the weekly dose of adenine significantly increased MDA compared to the control group. Conversely, the bi-weekly adenine dose did not produce a statistically significant difference in MDA levels compared to the control group. When comparing the effects of folic acid and adenine, the weekly dose of folic acid resulted in a greater increase in MDA than its bi-weekly counterpart and both dosages of adenine. While

the bi-weekly dose of folic acid also led to a higher MDA level than the bi-weekly dose of adenine, it did not differ significantly from the weekly dose of adenine (Table 3 and Figure 2).

Discussion

The present study assessed CKD within the inflammatory and oxidative stress markers framework. This approach aims to deepen the understanding of the pathophysiological mechanisms underlying CKD progression, particularly how inflammation and oxidative stress contribute to renal dysfunction. By examining these indicators, the study seeks to identify potential therapeutic targets and

Table 3. Comparative analysis of oxidative stress across experimental rat groups

First group		Second group	Mean difference	P value*
Oxidative Stress	GSH (ng/L)	Weekly AD	24.18	<0.001
		Bi-weekly AD	15.87	<0.001
		Weekly FA	26.21	<0.001
		Bi-weekly FA	20.99	<0.001
		Bi-weekly AD	8.31	<0.001
		Weekly FA	2.02	0.014
		Bi-weekly FA	3.19	<0.001
		Weekly FA	10.34	<0.001
	MDA (µmol/L)	Bi-weekly FA	5.12	<0.001
		Bi-weekly FA	5.22	<0.001
		Weekly AD	286.54	<0.001
		Bi-weekly AD	48.91	0.258
		Weekly FA	1011.06	<0.001
		Bi-weekly FA	217.81	<0.001
	Weekly AD	Bi-weekly AD	237.63	<0.001
		Weekly FA	724.52	<0.001
		Bi-weekly FA	68.73	0.116
		Weekly FA	962.15	<0.001
	Bi-weekly AD	Bi-weekly FA	168.90	<0.001
		Weekly FA	793.25	<0.001

GSH, Glutathione; MDA, Malondialdehyde; AD, adenine; FA, Folic acid.
*Post hoc LSD.

biomarkers that could improve the management of CKD beyond its hematological manifestations. Our previous study on this subject focused on the evaluating CKD induced by adenine and folic acid, specifically in the context of anemia-associated CKD (14). At this study, we focused on the other features of inflammatory and oxidative stress indicators in rat models of CKD induced by adenine and folic acid, which is separate with previous one (14). This investigation conducted a comparative analysis of inflammatory and oxidative stress biomarkers in CKD rat models induced by adenine and folic acid, identifying significant variations contingent upon the type and frequency of treatment. Folic acid, particularly when administered weekly, provoked more pronounced pro-inflammatory responses, markedly elevating TNF- α , and IL-6 concentrations compared to adenine. The administration of folic acid on a weekly schedule also exhibited a dose-dependent relationship within its cohort, producing more substantial effects than bi-weekly administration. In terms of oxidative stress markers, both folic acid and adenine were observed to diminish GSH levels and augment MDA levels relative to control groups, with weekly folic acid demonstrating the highest efficacy in reducing GSH and enhancing MDA. The bi-weekly administration of adenine exerted minimal influence on these biomarkers. The observed increase in inflammatory markers, such as TNF- α and IL-9, in adenine-treated rats aligns with previous studies that highlight the role of adenine in CKD and systemic inflammation. Elevated TNF- α levels have been consistently reported in adenine-induced CKD models, where they contribute to renal injury, fibrosis, and systemic inflammation. For instance, Etanercept, a TNF- α inhibitor, was shown to significantly reduce TNF- α levels and ameliorate kidney damage in adenine-treated rats, underscoring the cytokine's pathological role in CKD (22). Similarly, other studies have demonstrated that adenine-induced CKD leads to increased pro-inflammatory cytokines, including TNF- α and IL-1 β , which exacerbate oxidative stress and tissue damage (23,24). The findings of elevated IL-9 in this context are novel but consistent with the broader inflammatory milieu induced by adenine. Overall, these results reinforce the understanding that adenine exacerbates inflammation through the up-regulation of key cytokines like TNF- α , contributing to systemic and organ-specific damage. Further research could explore therapeutic interventions targeting these inflammatory pathways to mitigate the adverse effects of adenine-induced inflammation.

The observed result that adenine increased oxidative stress, as indicated by elevated MDA levels and decreased GSH in rats, aligns with findings from previous studies. For instance, adenine-induced chronic renal failure in

rats has been shown to significantly increase oxidative stress markers like MDA while reducing antioxidant defenses such as GSH, a critical molecule for neutralizing free radicals and maintaining redox balance (24,25). Similarly, oxidative stress has been implicated in various pathological conditions, including metabolic syndrome and acute stress models, where increased MDA and decreased GSH levels were consistently reported (26,27). These findings collectively highlight that adenine disrupts the oxidative balance by promoting lipid peroxidation and depleting antioxidant reserves, thereby contributing to tissue damage and disease progression. In conclusion, the current study corroborates the established role of adenine in inducing oxidative stress through mechanisms involving increased MDA and decreased GSH, emphasizing the need for therapeutic strategies targeting oxidative damage in adenine-related pathologies.

The finding that folic acid increased inflammatory markers such as TNF- α and IL-9 in rats, contrasts with several prior studies that generally highlight its anti-inflammatory properties. For instance, folic acid has been shown to reduce TNF- α levels in sepsis-induced lung damage in rats, suggesting its protective role against inflammation in specific contexts (28). Similarly, in vitro studies demonstrated that folic acid inhibits pro-inflammatory cytokines like TNF- α and IL-1 β while promoting anti-inflammatory cytokines such as IL-10 through mechanisms involving NF- κ B inhibition and suppressor of cytokine signaling (SOCS) protein up-regulation (29). Furthermore, evidence also exists that excess folic acid may exacerbate inflammation under certain conditions, such as high-fat diets, by promoting lipid accumulation and weight gain (30). The observed pro-inflammatory effects in the current study could be context-dependent, potentially influenced by dosage, experimental conditions, or the specific inflammatory pathway involved. Overall, while folic acid generally exhibits anti-inflammatory effects, certain conditions may reverse this trend, underscoring the need for further research to clarify its role in inflammation regulation.

The observation that folic acid increased MDA levels and decreased GSH levels, indicative of increased oxidative stress, contrasts with several studies demonstrating folic acid's protective role against oxidative damage. For example, folic acid has been shown to alleviate kidney oxidative stress induced by lead exposure by decreasing MDA levels and increasing GSH levels in rats (31). Similarly, folic acid treatment in diabetic rats reduced activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) (32), suggesting an alleviation of oxidative stress. Furthermore, folic acid supplementation mitigated oxidative stress caused by maternal ethanol consumption, decreasing thiobarbituric

acid reactive substances (TBARS) (33). In contrast, folate deficiency has been associated with increased oxidative stress and reduced antioxidant enzyme activity (34). However, studies also indicate that the combination of vitamin E and folic acid can restore GSH content and reduce MDA levels in diabetic rats (35). Additionally, folic acid has demonstrated the ability to alleviate oxidative stress-induced telomere attrition (36) and increase GSH levels in the brain of rats with induced oxidative stress (37), as well as reduce oxidative stress in rats treated with ethanol (38). The increase in MDA and decrease in GSH observed in the current study may be context-dependent, influenced by factors such as dosage, duration, or specific experimental conditions, highlighting the complex and sometimes contradictory role of folic acid in modulating oxidative stress.

Overall, this study provides a comparative analysis of the effects of adenine and folic acid on inflammatory and oxidative stress biomarkers in CKD rat models, revealing significant variations based on treatment type and frequency. Adenine was confirmed to exacerbate oxidative stress and inflammation, as evidenced by increased MDA levels, decreased GSH levels, and elevated pro-inflammatory cytokines such as TNF- α , consistent with prior studies on adenine-induced CKD. Folic acid, particularly when administered weekly, demonstrated a more pronounced pro-inflammatory response and oxidative stress compared to adenine. While folic acid is generally associated with anti-inflammatory and antioxidant properties in other contexts, its pro-inflammatory and oxidative effects in this study may be context-dependent, influenced by factors such as dosage and administration frequency. These findings underscore the complex roles of adenine and folic acid in CKD pathophysiology and highlight the need for further research to elucidate the mechanisms underlying these effects, as well as to explore therapeutic strategies targeting inflammation and oxidative stress in CKD.

Conclusion

This study demonstrates that both folic acid and adenine induce significant inflammatory and oxidative stress responses in rat models of CKD, with notable differences based on dosage and treatment type. Folic acid, particularly at a weekly dosage, elicited a stronger pro-inflammatory effect, as evidenced by higher TNF- α and IL-6 levels, compared to adenine. Oxidative stress markers showed that both treatments significantly reduced GSH levels and increased MDA, with weekly folic acid administration having the most pronounced effects. The findings highlight the dose-dependent nature of folic acid's impact and its greater potency relative to adenine in driving inflammation and oxidative stress. These results provide valuable insights into the differential mechanisms

of CKD progression induced by these compounds, emphasizing the importance of treatment frequency and type in experimental models.

Authors' contribution

Conceptualization: Asaad Abass Fadhel Khalif, Bahir Abdul Razzaq Mshimesh, and Deyaa Abdul Hussein Abood.

Data curation: Safaa Abdulsattar Oudah Al-Qaysi.

Formal analysis: Safaa Abdulsattar Oudah Al-Qaysi and Asaad Abass Fadhel Khalif.

Investigation: Asaad Abass Fadhel Khalif.

Methodology: Asaad Abass Fadhel Khalif.

Project administration: Bahir Abdul Razzaq Mshimesh.

Resources: All authors.

Software: Safaa Abdulsattar Oudah Al-Qaysi.

Supervision: Bahir Abdul Razzaq Mshimesh and Deyaa Abdul Hussein Abood.

Validation: Bahir Abdul Razzaq Mshimesh and Deyaa Abdul Hussein Abood.

Visualization: Bahir Abdul Razzaq Mshimesh.

Writing—original draft: All authors.

Writing—review & editing: All authors.

Conflicts of interest

The authors declare no conflict of interest.

Ethical issues

This study resulted from pharmacology thesis of Asaad Abass Fadhel Khalif (Thesis No. #99), at the College of Pharmacy, Mustansiriyah University in Iraq. A part of this study was published previously (14), since this study presents separate findings of this thesis. The research and the protocol of this study followed the guidelines of animal studies. We tried to conduct the guidelines related to animal experiments, approved by the United States National Institutes of Health (NIH, 1978). Besides, the authors have ultimately observed ethical issues (including plagiarism, data fabrication, and double publication).

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