Comparative Efficacy of Folic Acid and Adenine in Inducing Renal Anemia in Rat Models

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Article Info:

Received 16 July 2024
Revised 20 Oct 2024
Accepted 27 Nov 2024
Published 30 Oct 2025
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DOI: https://doi.org/10.32947/ajps.v25i4.1239

Abstract:

Abstract: Chronic kidney disease occurs when a disease or condition impairs kidney function, causing kidney damage to worsen over several months or years**.** Anemia is an important complication of chronic kidney disease (CKD), with increasing prevalence in the more advanced stages of the disease. The etiology of anemia in CKD is multifactorial, and the key mechanisms involve relative deficiency of erythropoietin (EPO), iron deficiency and maldistribution, and shortened erythrocyte life span.

Objective: To determine the most effective method for inducing chronic kidney disease (CKD) with anemia in male rats by administering adenine and folic acid and comparing the outcomes of four different models.

First, folic acid was intraperitoneally injected into rats at a dose of 250mg/kg per week. Second, folic acid was also intraperitoneally injected into rats at a dose of 250mg/kg every 2 weeks. Third, adenine was intraperitoneally injected into rats at a dose of 250mg/kg/ per week. Finally, adenine was intraperitoneally injected into rats at a dose of 250mg/kg every 2 weeks.

Methods: Thirty male Wistar albino rats were divided into five groups, with n=6 in each group. Group I, the healthy control group, received a weekly intraperitoneal injection of normal saline for four weeks. Group II, the Adenine model group, received a weekly intraperitoneal injection of adenine at 250 mg/kg. Group III, also part of the adenine model group, received an intraperitoneal injection of adenine at a dose of 250 mg/kg every two weeks for four weeks. Group IV, the Folic acid model group, received a weekly intraperitoneal injection of folic acid at 250 mg/kg for four weeks. Lastly, Group V, also part of the folic acid model group, received an intraperitoneal injection of folic acid at 250 mg/kg every two weeks for four weeks. After a 28-day induction period, the rats were sedated and euthanized. Blood samples were obtained, and the serum was collected for further biomarker testing using ELISA kits.

Results: Upon comparing the rats' body weight at the start and end of the experiment, no group showed a statistically significant change. The dosage of folic acid per week was the most important factor in the increase of relative kidney weight, while there was a significant variation in relative

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kidney weight across the analyzed groups. After measuring kidney function tests (urea, creatinine), hematological parameters (hematocrit, ferritin), it was found that the folic acid model (250 mg/kg/wk, IP for 4 weeks) was significantly the best to induce renal anemia in male rats, it caused a critical increase in the levels of urea and creatinine, which indicates the occurrence of chronic kidney failure. The aforementioned model also caused a decrease in the hematocrit rate and a reduction in the level of ferritin in the blood, which gives the impression of renal anemia. The adenine model, in the same dose and duration, came second in ranking of the optimal induction model.

Conclusion: Compared to the other rat models, the folic acid model group (250 mg/Kg/wk) is more effective in producing anemia associated with chronic renal disease. Due to the observed alterations in many parameters including kidney function, oxidative stress markers, and inflammatory markers, the weekly dosage of folic acid demonstrated its superiority in the context of renal anemia. This will be useful in the future for discovering innovative drugs and understanding the renal anaemia process.

Keywords: Chronic kidney disease (CKD), Erythropoietin (EPO), Folic acid (FA), Adenine (AD), Renal anemia

اختيار أفضل نموذج للجرذان لحث فقر الدم الكلوي: حامض الفوليك مقارنة بالأدينين

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الخلاصة

يحدث مرض الكلى المزمن عندما يؤدي مرض أو حالة إلى إعاقة عمل الكلى، مما يؤدي إلى تفاقم تلف الكلى على مدى عدة أشهر أو سنوات. ويعتبر فقر الدم من مضاعفات مرض الكلى المزمن(CKD) ، مع زيادة انتشاره في المراحل الأكثر تقدماً من المرض. وينطوي علم فقر الدم في CKD على عوامل متعددة، وتنطوي الآليات الرئيسية على نقص نسبي في الإيراثروبوتيتين(EPO) ، ونقص الحديد وسوء توزيعه، وفترة حياة كريات الإيريثروسيل المقصرة .

الهدف: تحديد أنجع طريقة لتحفز أمراض الكلى المزمنة مع وجود فقر الدم في ذكور الجرذان عن طريق حقن حامض الفوليك والأدينين او مقارنة نتائج أربعة نماذج مختلفة (وحامض الفوليك 250 ملغ/اسبوع، وحامض الفوليك 250 ملغ/كغ/2اسبوع، وأدينين 250 ملغ/كغ / اسبوع، وأدينين 250 ملغ/كغ/2 اسبوع).

طريقة العمل: 30 ذكر جرذان واستر ألبينو، مقسمة إلى خمس مجموعات (مع 6n= لكل مجموعة): وقد تلقت المجموعة الأولى، وهي مجموعة التحكم الصحي، حقنة أسبوعية من الملح الطبيعي داخل الصفاق وتلقت المجموعة الثانية، وهي المجموعة النموذجية للأدينين، حقنة أسبوعية داخل الصفاق من الادينين عند 250 مغ/كغ. وتلقى الفريق الثالث، الذي يشكل أيضاً جزءاً من المجموعة النموذجية للأدينين، حقنة داخل الصفاق للأدينين بجرعة قدرها 250 ملغم/كغم كل أسبوعين. وتلقت المجموعة الرابعة، وهي المجموعة النموذجية لحامض الفوليك، حقنة أسبوعية من حامض الفوليك داخل الصفاق عند 250 مغ/كغ. وأخيراً، تلقت المجموعة الخامسة، وهي أيضاً جزء من المجموعة النموذجية لحامض الفوليك، حقنة حامض الفوليك داخل الصفاق عند 250 مغ/كغ كل أسبوعين. وبعد فترة تعريف مدتها 28 يوما، تم تخدير الجرذان. وتم الحصول على عينات من الدم، وأُبقي على المصل لإجراء مزيد من الاختبارات على العلامات البيولوجية باستخدام مجموعات من مواد ELISA. النتائج: بعد مقارنة وزن جسم الجرذان في بداية ونهاية التجربة، لم يكن هناك فرق كبير بين جميع المجموعات. وفي الوقت نفسه، كان هناك فرق كبير في الوزن النسبي للكلية بين المجموعات التي شملتها الدراسة، وكانت الجرعة النموذجية لحامض الفوليك في الأسبوع هي الأكثر أهمية، فيما يتعلق بارتفاع الوزن النسبي للكلية. وبعد قياس اختبارات وظائف الكلى (اليوريا، الكرياتينين)، والبارامترات الدموية (فرتين، هيماتوكرت)، تبين

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أن نموذج حمض الفوليك 250 ملغم/كغم/أسبوع، داخل الصفاق لمدة 4 أسابيع (هو أفضل نموذج لإحداث فقر الدم الكلوي في ذكور الجرذان، وتسبب في زيادة حادة في مستويات اليوريا والكرياتينين، مما يشير إلى حدوث فشل كلي مزمن. كما أدى النموذج المذكور أعلاه إلى انخفاض في معدل الهيماتوكرت وانخفاض في مستوى الفريتين في الدم، مما يعطي انطباعاً لحدوث فقر الدم الكلوي. وجاء نموذج الأدينين، بنفس الجرعة والمدة، في المرتبة الثانية في ترتيب نموذج الحث الأمثل .

الاستنتاج: مقارنة بنماذج الجرذان الأخرى، فإن مجموعة نماذج حامض الفوليك (250 مغ/كغ/اسبوع) أكثر فعالية في إنتاج فقر الدم المرتبط بالأمراض الكلوية المزمنة. وسيكون ذلك مفيدا في المستقبل في تحديد الأدوية الجديدة وفهم آلية فقر الدم الكلوي.

الكلمات المفتاحية: مرض الكلى المزمن(CKD) ، إريثروبويتين(EPO) ، حمض الفوليك(FA) ، الأدينين(AD) ، فقر الدم الكلوي

Introduction

Rats with chronic kidney disease (CKD) are used as models to study the causes and mechanisms of CKD and to develop treatment strategies. Anemia is a common complication in individuals with CKD, characterized by reduced red blood cell production, decreased erythropoietin (EPO) levels, and abnormal iron metabolism (1–4). The traditional definition of anemia is based on serum ferritin, a measure of stored iron, and the hematocrit ratio, which is the proportion of red blood cells by volume. One in five patients with intermediate chronic kidney disease (CKD; estimated glomerular filtration rate [eGFR] 30 to 59 mL/min/1.73 m2) suffer from anemia, a prevalence that increases noticeably with declining kidney function(5). Studies illuminate the interactions of variables, including EPO insufficiency, iron dysregulation, chronic inflammation, bone marrow dysfunction, a shortened red cell life span, or vitamin B12 or folic acid deficits, so our knowledge of anemia in chronic kidney disease (CKD) has changed dramatically(2,6–8). In those with chronic kidney disease (CKD), anemia is a sign of the severity of the illness as well as a predictor of unfavorable outcomes such as cardiovascular events and death. Additionally, compared to those without this illness, those with anemia have a worse quality of life and a higher chance of hospitalization. This implies that treating CKD patients with anemia comorbidity requires additional healthcare resources, which raises the expense of healthcare(9,10). Qiao Yang *et al. (*2024) found that adenine at a dose of 200mg/kg daily for five weeks causes kidney failure leading to anemia(11), folic acid has nephrotoxic activity at various levels of the nephron because it induces a pro-oxidant state by increasing lipid binding and reducing protective-anti-oxidant enzymes (Gupta et al., 2012)(12). The purpose of this study is to compare two models and determine the most effective method for inducing chronic kidney disease (CKD) with anemia in male rats using adenine and folic acid.

Methods:

Animals

Thirty male albino Wistar rats were supplied by the Iraqi Center for Cancer Research and Medical Inheritance's animal house at Mustansiriya University. They were approved by the ethics committee for animal experimentation at the College of Pharmacy at Mustansiriya University, where the study was conducted. Their ages ranged from 10 to 12 weeks, and their weight varied between 200 and 300 grams.

Animals were housed in well-ventilated cages and provided unlimited access to water and food *ad Libitum (*standard chow pellets), a temperature of 25°C± 5° under natural light/dark cycles and a humidity level of 30%-40%. They were allowed a 14-day acclimatization period before starting the experiment. This study was done in the animal house and the postgraduate laboratory

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after obtaining approvals from the scientific and ethical committee at the College of Pharmacy / Mustansiriyah University between 8 November and 8 December 2023.

Preparation of drug and doses

The dose administration was performed between 9:00 and 10:00 am to minimize the effects of circadian changes. Adenine was dissolved in NaOH solution, the latter was prepared by method. A 1M NaOH solution is created by dissolving 40 grams of sodium hydroxide pellets in 250 milliliters of distilled water and then adding more water to reach a liter. 500 milliliters (500 ml) of distilled water dissolves 19.95g of NaOH pellets(13,14). Adenine injections of 250 mg/kg were administered by 2 models once or twice a week(15). Adenine dose was prepared by taking mean of body weight for each group so every rat received 60 milligrams of adenine, which was dissolved in ten milliliters of 1M NaOH and mixed with 7 drops of strong HCl to bring the pH within the rat's biological range. Folic acid was dissolved in $NaHCO_3$ solution, the latter was prepared by method. A 0.3 M NaHCO3, dissolve 1 grams of sodium bicarbonate in 10 milliliters of distilled water and add 1 grams of folic acid to 10 milliliters of a mixture of $NaHCO_3$ and use stirring at a temperature of 40℃ for 5 minutes(16,17); after that, according to body weight take 0.57 milliliters and injected folic acid for group (IV) once weekly between 9:00 and 10:00 am. Group (V) of folic acid was given 0.6milliliters/ 2 weeks according to mean of body weight.

Experimental design

Thirty albino Wister male rats were used in this investigation, were conquest and division into the following five groups (six in each group). According to previous studies, the use of Normal Line in the control group was implemented to ensure that no changes occurred at the level of kidney tubules. This group will serve as a baseline for comparison with the other groups, the folic acid and adenine groups were dissolved sequentially in 0.3M $NaHCO_3$ and 1M NaOH, this study lasted for 4 weeks:

Group-I (healthy control): - 6 male rats received 0.5ml normal saline once weekly intraperitoneally for 4 weeks.

Group-II (Adenine model group): - 6 male rats intraperitoneally injected with 0.55 ml adenine in 250mg/kg once weekly for 4 weeks.

Group III (Adenine model group): - 6 male rats intraperitoneally injected with 0.6 ml adenine in 250mg/kg / 2 weeks for 4 weeks.

Group IV (Folic acid model group): - 6 male rats intraperitoneally injected with 0.58 ml a single dose of folic acid 250 mg/kg once weekly for 4 weeks.

Group V (Folic acid model group): - 6 male rats intraperitoneally injected with 0.58 ml folic acid 250 mg/kg / 2 weeks for 4 weeks.

Materials:

Materials include Adenine (powder Bide pharm) China, Folic acid (powder Bide pharm) China, Formaldehyde (37%-40%) (solvochem) UK, Ketamine 10% vial (Alfasan) Holland, Normal saline (0.9%) bottle (Pioneer) Iraq, Sterilized water for injections (Pioneer) Iraq, Xylazine 20mg/ml vial (Kepro) Holland.

Rat's body weight

An electronic balance was used to evaluate the mean body weight for all animal groups at the beginning and ending of experiment.

Relative kidney weight

After rat's sacrifisation, kidney was taken and weighed to evaluate its index according to the following equation(18).

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$$\text{Kidney index} = \frac{Weight\ of\ kidney}{Total\ body\ weight} \times 100$$

Enzyme-linked immunosorbent assays (ELISA) method: for measuring ferritin

The ferritin levels among groups were measured 28 days after the experiment started. According to the manufacturer's instructions, the serum levels were quantified using the enzyme-linked immunosorbent assay (ELISA) method (Catalog No. SEA518Ra, USCN – USA) (19,20).

Determination of creatinine and urea levels by colorimetric method

This method was used to determine the levels of creatinine and urea in the experimental groups following the guidelines provided by the manufacturer(21).

Determination of package cell volume (PCV)

2 mls of blood samples were collected from rats into bottles containing Ethylene di amine tetra acetic acid (EDTA) anticoagulant and was properly mixed using a blood mixer. PCV was determined by the microhematocrit by placing the centrifuge heparinized blood in a capillary tube (also known as microhematocrit tube), the capillary tube was filled to 75% of its length, sealed with plasticine and centrifuged in a micro haematocrit centrifuge at 10,000 RPM for five minutes, this separates the blood into layers. It is then placed on the HC 702 hematocrit and read(22).

Statistical analysis

The data in the current study was represented using the mean ± standard error of the mean (M±SEM). The statistical investigation used version 16.0 of Statistical Packages for the Social Sciences (SPSS). The significance of difference means was determined using analysis of variance (ANOVA) and a post hoc Tukey test. Differences were considered significant, highly significant, and highly significant if their p-values were below 0.05, 0.01, and 0.001, respectively.

Results

Effect of adenine and folic acid on body weight.

Regarding the percentage of change in the weight of the rats, as shown in Figure (1), there was a slight decrease in the weight of the 250 mg/kg/wk FA group and the 250 mg/kg/wk AD group. In the other two groups, the 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups, there was no change in weight compared to the control group. In other words, there are no significant differences in weight between the four groups and the control group. However, there is a slight, almost non-significant, decrease in the weight of the folic acid group dosed weekly and the adenine group dosed weekly, compared to the control group.

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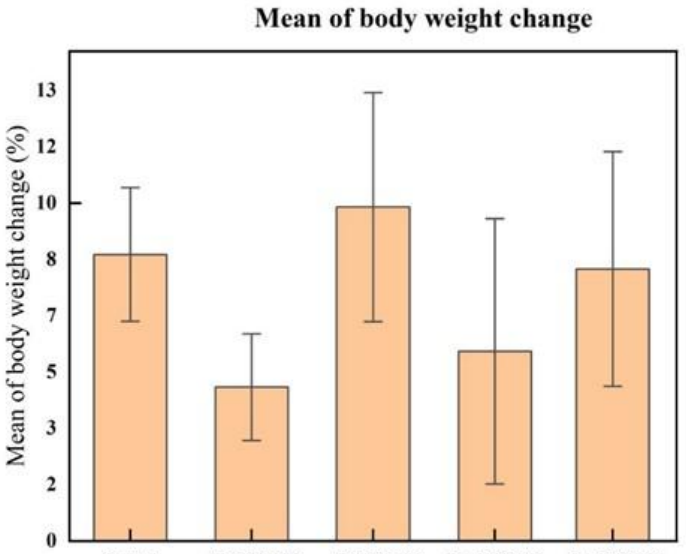

	Control	AD 250/1WK	AD 250/2WK	F.A 250/1WK	F.A 250/2WK
Mean of body weight change (%)	8.48	4.555	9.885	5.615	8.055
SEM	1.98	1.58	3.39	3.93	3.47

Figure (1): **Adenine and folic acid's effects on body weight**

- AD (Adenine), FA (Folic acid).

Effect of adenine and folic acid on relative kidney weight

As clarified in figure (2), the mean relative kidney weight of the 250 mg/kg/wk AD, 250 mg/kg/wk FA, 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups were elevated in a very highly significant extent (P-value<0.001) when compared to that of control group. Meanwhile, mean relative kidney weight of the 250 mg/kg/2wk AD, 250 mg/kg/wk FA and 250 mg/kg/2wk FA showed a non-significant difference compared to that of 250 mg/kg/wk AD group (p- value>0.1). Finally, mean relative kidney weight for the 250 mg/kg/wk FA group demonstrated a non-significant difference (p-value >0. 1) in comparison to that of 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups. Meanwhile, mean relative kidney weight of the 250 mg/kg/2wk AD showed a non- significant difference compared to that of 250 mg/kg/2wk FA group (p-value >0.1).

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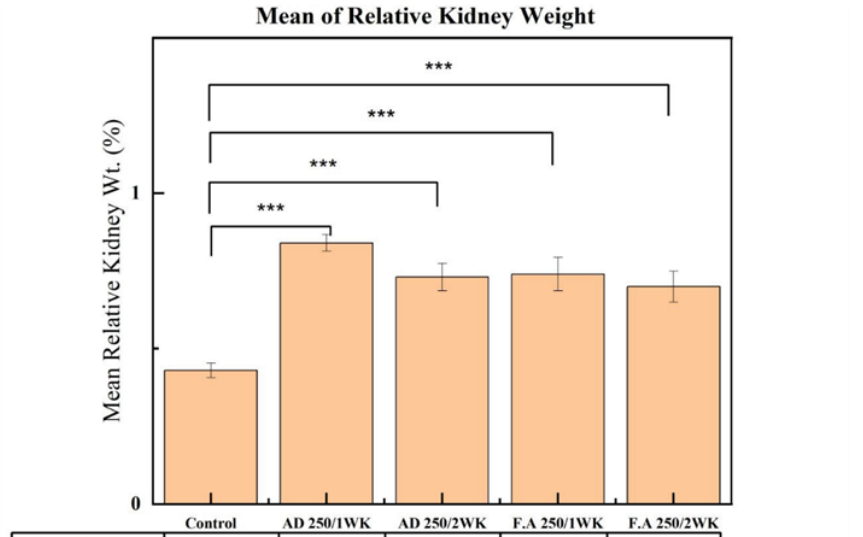

	Control	AD 250/1WK	AD 250/2WK	F.A 250/1WK	F.A 250/2WK
Mean R. Kidney Wt. (%)	0.43	0.84	0.73	0.74	0.7
SEM	0.024	0.027	0.044	0.054	0.05

Figure (2): **Adenine and folic acid's effects on relative kidney weight**

- ***→ p-value <0.001 (very highly significant difference).
- AD (Adenine), FA (Folic acid).

Effect of adenine and folic acid on kidney function biomarkers

Effect on urea levels

As seen in figure (3), the mean serum urea levels of the 250 mg/kg/wk AD and 250 mg/kg/wk FA groups were elevated in a very highly significant extent (41.657±2.007 and 42.071±2.218 mmol/L, p-value < 0.001, respectively) when compared to that of control group (22.936±0.146 mmol/L). Meanwhile, the mean serum levels of this marker for the 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups showed no statistically significant difference (24.996±0.604 and 26.823±0.790 mmol/L, p-value = 0.838 and 0.320, respectively) compared to that of control group and approached to its value (22.936±0.146 mmol/L).

On the other hand, mean serum urea levels of the 250 mg/kg/wk AD group were increased in a very highly significant extent (p-value < 0.001) when compared to the 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups. In contrast, mean serum urea levels of the same group showed no significant difference (p-value > 0.05) compared to that of 250 mg/kg/wk FA group.

Finally, mean serum levels of this marker for the 250 mg/kg/wk FA group were raised in a very highly significant extent (p-value <0.001) in comparison to that of 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups. Meanwhile, mean serum urea levels of the 250 mg/kg/2wk AD showed a non- significant difference compared to that of 250 mg/kg/2wk FA group (p-value = 0.888).

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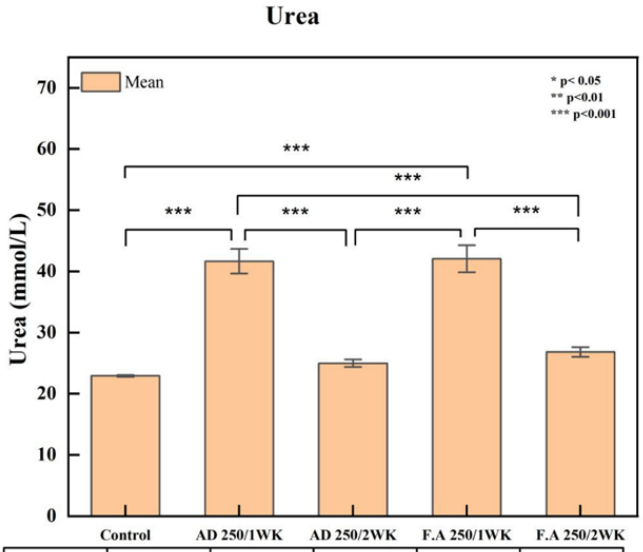

	Control	AD 250/1WK	AD 250/2WK	F.A 250/1WK	F.A 250/2WK
Mean	22.9365	41.6578	24.9962	42.0717	26.8238
SEM	0.14696	2.00752	0.60405	2.21862	0.79099

Figure (3): Adenine and folic acid's effects on serum urea levels within different animal groups.

- Data were represented by mean ± SEM (standard error of mean).
- ***→ p-value <0.001 (very highly significant difference).
- AD (Adenine), FA (Folic acid).

Effect on creatinine levels

As clarified in figure (4), the mean serum creatinine levels of the 250 mg/kg/wk AD and 250 mg/kg/wk FA groups were elevated in a very highly significant extent (1.486±0.142&1.520±0.161 mg /dL) (P-value<0.001) when compared to that of control group (0.355±0.007 mg /dL). Meanwhile, the mean serum levels of this marker for the 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups showed no statistically significant difference (0.516±0 .027&0.572±0 .035 mg/dL respectively) compared to that of control group and approached to its value)P-value =0.775,0.534 respectively).

On the other hand, mean serum creatinine levels of the 250 mg/kg/wk AD group were significantly elevated in a very high manner (p-value < 0.001) when compared to the 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups. In contrast, mean serum creatinine levels of the same group showed no significant difference (p-value =0.999) compared to that of 250 mg/kg/wk FA group.

Finally, mean serum levels of this marker for the 250 mg/kg/wk FA group were raised in a very highly significant extent (p-value <0.001) in comparison to that of 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups. Meanwhile, mean serum creatinine levels of the 250 mg/kg/2wk AD showed a non- significant difference compared to that of 250 mg/kg/2wk FA group (p-value = 0.994).

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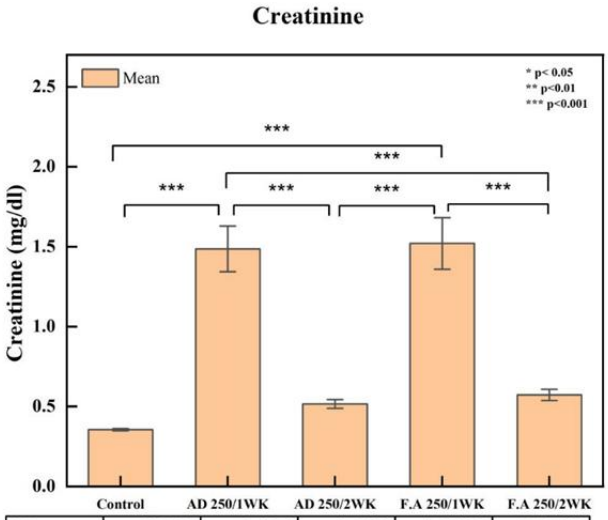

	Control	AD 250/1WK	AD 250/2WK	F.A 250/1WK	F.A 250/2WK
Mean	0.355	1.486	0.516	1.5205	0.5722
SEM	0.00705	0.14246	0.02775	0.16109	0.03578

Figure (4): Adenine and folic acid's effects on serum creatinine levels in different animal groups.

- Data were represented by mean ± SEM (standard error of mean).
- ***→ p-value <0.001 (very highly significant difference).
- AD (Adenine), FA (Folic acid).

Effect of adenine and folic acid on hematological biomarkers

Effect on hematocrit levels

As demonstrated in figure (5), the mean hematocrit percentage of the 250 mg/kg/wk AD , 250 mg/kg/wk FA, 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups were decreased in a very highly significant extent (39%±0.632 ,36.666%±1.406 ,42.166%±0.909 and 39.666%±0.5 ,P-value <0.001 ,respectively) when compared to that of control group.(1.085±%48.666)

On the other hand, mean hematocrit percentage of the 250 mg/kg/wk AD group showed no-significant difference when compared to the 250 mg/kg/2wk AD, 250 mg/kg/wk FA group and 250 mg/kg/2wk FA groups (p-value=0.175, 0.450 and 0.988, respectively).

Finally, mean percentage of this marker for the 250 mg/kg/wk FA group showed a non-significant difference in comparison to that of 250 mg/kg/2wk groups (P-value=0.216), also mean hemoglobin percentage of the 250 mg/kg/2wk AD showed no significant difference compared to that of 250 mg/kg/2wk FA group (p-value = 0.383).In contrast, mean hematocrit percentage 250 mg/ wk FA decreased in a highly significant extent (P-value =0.004) when compared to that of 250 mg/kg/2wk AD.

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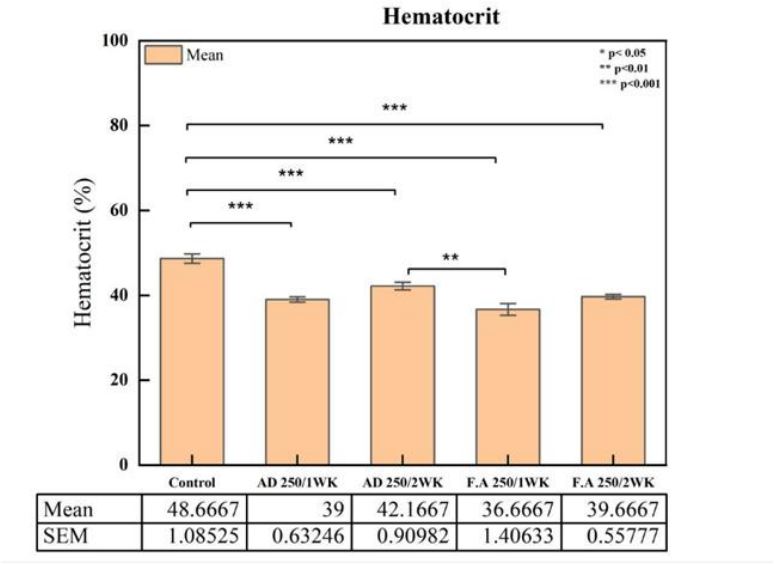

	Control	AD 250/1WK	AD 250/2WK	F.A 250/1WK	F.A 250/2WK
Mean	48.6667	39	42.1667	36.6667	39.6667
SEM	1.08525	0.63246	0.90982	1.40633	0.55777

Figure (5): Adenine and folic acid's effects on hematocrit percentage in different animal groups.

- Data were represented by mean ± SEM (standard error of mean).
- **→ p-value <0.01 (highly significant difference),
- ***→ p-value <0.001 (very highly significant difference).
- AD (Adenine), FA (Folic acid).

Effect on ferritin levels

As clarified in figure (6), the mean serum ferritin levels of the 250 mg/kg/wk AD , 250 mg/kg/wk FA, 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups were reduced in a very highly significant extent (24.713±2.877, 23.374±1.949, 60.378±2.465 and 60.396±1.988 ng/mL, P-value <0.001 ,respectively) when compared to that of control group (79.484±1.151 ng/mL).

On the other hand, mean serum ferritin levels of the 250 mg/kg/wk AD group reduced in a very highly significant extent when compared to the 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups (p-value<0.001). In contrast, mean serum of the same group showed a no significant difference (p-value>0.001) compared to that of 250 mg/kg/wk FA group.

Finally, mean serum levels of this marker for the 250 mg/kg/wk FA group were diminished in a very highly significant extent (p-value <0.001) in comparison to that of 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups. Meanwhile, mean serum ferritin levels of the 250 mg/kg/2wk AD showed a non-significant difference compared to that of 250 mg/kg/2wk FA group (p-value = 0.680).

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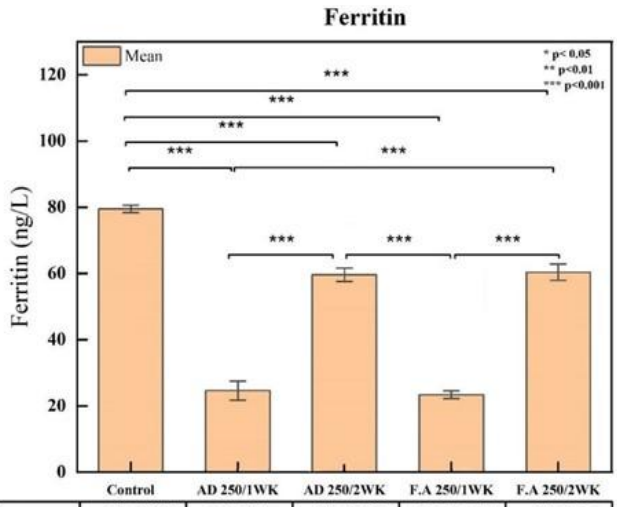

	Control	AD 250/1WK	AD 250/2WK	F.A 250/1WK	F.A 250/2WK
Mean	79.4842	24.713	60.3783	23.3747	60.396
SEM	1.15177	2.87765	2.46513	1.19495	1.988

Figure (6) Adenine and folic acid's effects on serum ferritin levels in different animal groups.

- Data were represented by mean ± SEM (standard error of mean).
- ***→ p-value <0.001 (very highly significant difference).
- AD (Adenine), FA (Folic acid).

Discussion

As kidney disease progresses, anemia prevalence increases, reducing quality of life and increasing hospitalization and mortality. Understanding the molecular mechanism of anemia is crucial for developing therapeutic tools and new pharmacological approaches. As shown in Figure (1), there was slight change in rats body weight among groups but was statistically nonsignificant, a slight decrease in the weight of 250 mg/kg/wk FA group and 250 mg/kg/wk AD group was observed. Gabriel *et al.* (2021) and Liang-Jun Yan *et al.* (2021) found a reduction in rats body weight after using FA injection. Also, Yasuhiro *et al*. (2008) and Asadur *et al.* (2018) noticed that there was a decrease in body weight when rats gave adenine, these results came in agreement with that of the current study. As shown in Figure (2), the mean relative kidney weight of the 250 mg/kg/wk AD, 250 mg/kg/wk FA, 250 mg/kg/2wk AD and 250 mg/kg/2wk FA groups were elevated in a very highly significant extent (P-value<0.001) when compared to that of control. Folic acid injection causes increased relative kidney weight in rats due to chronic kidney damage, leading to tubular injury, and fibrosis. This damage results in enlargement of distal tubules and extracellular matrix accumulation, contributing to kidney hypertrophy and increased relative kidney weight(23). Panthip *et al.* (2016) and Omer et al. (2020) observed a raising in relative kidney weight after using folic acid injection in rats, Mohammed Al Za'abia *et al.* (2018) and B. H. ALI *et al.* (2014) also found that increasing in relative kidney weight occur after gave adenine to rats, the findings of these previous studies were compatible with results of the present study.

In the current study, and as seen in Figure (3), the mean serum urea levels of the 250 mg/kg/wk FA group was elevated in a very highly significant extent than other groups. The adenine group with 250 mg/kg/wk was ranked second in terms of increasing urea level. Folic acid model with 250 mg/kg/wk may be consider more optimal to induce CKD, ending with anemia, than other models in this

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experiment. As seen in figure (4), the mean serum creatinine levels of the 250 mg/kg/wk AD and 250 mg/kg/wk FA groups were elevated in a very highly significant extent (P-value <0.001) when compared to that of other groups. Previous study done by panther *et al.* (2016) and Xue Li *et al.* (2020) revealed that folic acid injection cause increase in creatinine level, these results were in parallel with the results of the present study(24). Rahman *et al.* (2018) gave mice 200 mg/kg of adenine by mouth every day for 28 days, and Estefanía Vázquez-Méndez *et al.* (2020) gave mice 100 mg/kg of adenine by mouth every day for 28 days, it was clear that after kidney dysfunction start, the levels of urea and creatine begin to rise, which is exactly what the current study report. This may because the adenine-treated group exhibiting tubular distention with a loss of the brush border of the proximal tubules, where adenine ending with CKD(25).

As shown in Figure (5) the mean serum level of hematocrit in the 250 mg/kg/wk FA group decreased to 36.66% compared to the other models, the 250 mg/kg/wk AD group had a mean serum level of this marker that decreased to 39%, while FA 250 mg/kg/2wk group and AD 250 mg/kg/2wk group decreased the hematocrit by a lesser percentage. The findings of the present study agree with the of previous studies done by BH ALI *et al*. (2014) and Lei Li *et al*. (2020) used mice which administered adenine orally daily for four weeks to induce renal impairment and after measuring hematocrit established that there is a reduction in serum levels of this hematological parameter. The CKD occur when red blood cells are not produced due to the suppression of renal erythropoietin (EPO) production. Adenine and folic acid injections in rats can lead to renal anemia by suppressing renal EPO mRNA expression, accumulating uremic toxins, and disrupting iron metabolism, leading to decreased hemoglobin and hematocrit levels(26–28). In this study, the mean serum ferritin levels of the 250 mg/kg/wk FA group were significantly lower (p-value <0.001) than those of the other groups, as shown in Figure (6). This finding was in line with that reported by Xue Li et al. (2020), Gabriel et al. (2021), and Liang-Jun Yan et al. (2021) who found a reduction in rats ferritin levels after four weeks of injection of folic acid. Since low ferritin levels are linked to iron deficiency anemia, they are a prevalent characteristic of chronic kidney disease, where low levels of ferritin, a protein that the body uses to store iron, indicate insufficient iron for erythropoiesis(29).

Conclusion

Based on the decrease in hematocrit levels in the folic acid group at a dose of 250 mg/kg/weekly, as well as an increase in ferritin levels in the same group compared to the others, along with elevated urea and creatinine levels and an enlarged relative kidney size in the folic acid weekly group, particularly when compared to the adenine group at a dose of 250 mg/kg per week, it is believed that a folic acid model administered weekly is most effective in inducing renal anemia. The knowledge presented here will be of great importance in the future for comprehending the mechanism and development of renal anemia, as well as evaluating and uncovering treatments for this condition.

CONFLICTS OF INTEREST

There are no conflicts to declare.

A CKNOWLEDGMENTS

The authors would like to express their gratitude to college of pharmacy/Mustansiriyah University (www.uomustansiriyiah.edu.iq), Baghdad, Iraq, university of Baghdad / college of veterinary medicine (https://covm.uobaghdad.edu.iq) for its support with this work.

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