Functional and Structural Study on the Effect of Curcumin on Folic Acid-Induced Acute Kidney Injury in Albino Rats

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Abstract

Objective. Systemic administration of folic acid (FA) in rats was used for studying the pathogenesis associated with acute renal damage. However, the mechanism by which FA induces renal damage remains poorly understood. Up to our knowledge, no effective preventive or therapeutic drugs have been developed to protect against acute kidney injury. Curcumin (CUR) is commonly used worldwide as a spice and has been demonstrated to possess various biological activities. The present study was planned to investigate the effect of folic acid administration on renal function, inflammatory cytokines and associated histological changes in renal tissue. In addition, we examined the possible protective effect of curcumin on a rat model of folic acid (FA)-induced acute kidney injury (AKI). Methods. Rats were divided into 3 groups; (FA) folic acid treated group rats were exposed to FA (250 mg/kg) i.p. injection as a single dose. (FA+CUR) folic acid plus curcumin treatment group rats were given curcumin (200 mg/kg) administered by gavage daily for 11 days prior to folic acid (250 mg/kg) i.p injection and the last dose of curcumin was given one day after folic acid injection. Control group are given distilled water by gavage daily for 12 days and saline i.p. as a single dose on the 11th day. Animals were scarified one day following i.p. injection in all groups. Deterioration of kidney function was detected by blood urea and creatinine levels. Inflammatory response was monitored with blood levels of interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor (TNF-α). Results. We found that FA treatment significantly raised blood urea, creatinine, IL-6, IL-10, TNF-α levels and caused marked structural changes of the kidney. CUR treatment for 12 days significantly reduced blood urea, IL-6, IL-10, TNF- α, and protected partially against renal structural damage. Conclusion. These findings suggest that curcumin is a promising protective agent against AKI induced by FA.

Keywords
- Acute kidney injury (AKI)
- Curcumin (CUR)
- Folic acid (FA)
- Interleukin-6 (IL-6)
- Interleukin-10 (IL-10)
- Tumor necrosis factor-alpha (TNF- α)

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INTRODUCTION

Acute renal damage is considered as a major clinical issue since it affects many critically diseased patients. Toxic, ischemic, as well as inflammatory factors were found to be combined causes underlying acute renal damage. Folic acid is a commonly used compound to study the pathophysiology associated with acute renal failure. Curcumin, a bright yellow aromatic powder commonly used as spice, was found to have a beneficial effect on acute kidney injury (AKI) markers. Acute kidney damage is defined as a rapid decrease in the glomerular filtration rate leading to retention and elevation of blood urea and creatinine levels. Previous studies showed a correlation between proinflammatory cytokines and renal injury.

Numerous previous studies, including in vitro and animal experiments suggest a major impact of tumor necrosis factor-superfamily (TNFSF), interleukin-6 (IL-6) and interleukin-10 (IL-10) in inflammatory, malignant, and autoimmune diseases. Members of the TNFSF of cytokines are the key mediators of renal injury. Necrosis and apoptosis were found to be the major histological changes in nephrotoxic AKI. The remaining slightly injured tissues are considered as a source of immunological signals that activate the immune system resulting in further damage of renal tissues. Folic acid nephropathy is a model of AKI characterized by acute renal failure and tubule-interstitial inflammation. Up to our knowledge no previous study investigated the role of IL-6 and IL-10, or CUR in folic acid-induced acute renal damage. This study was designed to investigate the mechanisms underlying folic acid induced renal damage by measuring the inflammatory markers IL-6, IL-10, and TNF-α as well as the histological and structural changes in the kidney. In addition, we assessed the role of curcumin as a potential intervention to ameliorate the folic acid-induced changes in the proinflammatory markers and renal structure.

MATERIALS AND METHODS

Drugs and chemicals
Folic acid (FA) Oxford (Laboratory Reagent) C19H19N7O6 M.W: 441.40 CAS NO: 7791-13-1 (Assay by complexometry): 97-102% PKD by Oxford laboratory Mumbai-400 002, India; Batch NO: 208092 was dissolved in 1ml distilled water and given as a single dose of 250 mg/kg i.p. injection. Curcumin Crystalline (CUR) 99% extra pure, Batch NO. C0632 (Alpha Chemika, Savgan Heights, 102, B wing, RTO Lane, Andheri (W), Mumbai-400 053, Maharashtra, India) was dissolved in 1 ml distilled water and administered orally by gavage immediately after preparation as single daily dose of 200 mg/kg for 12 consecutive days. Rat TNF-alpha ELIZA Kit (K0331196) Koma Biotech INC. AVITEX CRP (Ref OD073/OD023/OD023/E) Omega Diagnostics. Rat Interleukin-6 (IL-6) ELIZA Kit (Koma Biotech INC, Cat NO. K0331229). Rat Interleukin-10 (IL-10) ELISA Kit (Koma Biotech INC, Cat NO. K0332134). Both IL-6 and IL-10 company address the same (19F, IS BIZ Tower, Sunyudo Station 1cha, Yangpyeong-dong 5 ga 1-1, Yeongdeungpo-gu,Seoul 150-105, South Korea). Creatinine Jaffe. Colorimetric – kinetic (Diamond Diagnostics, 333 Fiske St, Holliston, MA 01746, United States, REF 2 x 100 ml). Urea-Kit S 180
Animal preparation and experimental approach
Experiments were performed on a total number of 18 adult male albino rats weighing 115-150 grams. Animals were obtained from the animal’s house facility, Faculty of Medicine, Assiut University, Assiut, Egypt. Two days before experimentation, the animals were kept in the laboratory for accommodation in the condition of 12 hours light/dark cycle with free access to standard rat chow and tap water. The experiments were carried out according to the protocol approved by the Local Ethical Committee of the Faculty of Medicine, Assiut University in accordance with the ethical guidelines for scientific research in conscious animals. Animals were randomized into three groups, six animals each: FA-treated group rats were given distilled water orally by gavage for 12 days and exposed to FA (250 mg/kg) i.p. injection as single dose on the eleventh day. FA+CUR treatment group rats were given curcumin (200 mg/kg) orally administered by gavage daily for 12 days: 11 days prior to folic acid i.p. injection and 1 day thereafter. Control (C) group rats were given distilled water orally for 12 days and saline i.p. as single dose on the eleventh day. Animals were scarified one day following the i.p. injection in all groups.

Experimental protocol and assays
Blood samples were withdrawn from the retro-orbital veins into heparinized tubes, centrifuged at 3000 rpm for 10 minutes and plasma were separated and kept at -20°C until analysis. Plasma urea level was detected using Urea- Kit S using urease- modified Berthelot reaction (end point enzymatic determination) at 578 nm, plasma creatinine level using Jaffe Colorimetric-kinetic at 492nm, IL-6, IL-10, and TNF-α detected using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions. The samples were tested in duplicate.

Histology
Kidney specimens from all groups were taken for histological evaluation. Kidneys were fixed in 10% neutral buffered formalin for 24 h. After dehydration through a graded alcohol series, the tissues were embedded in paraffin, sectioned at 5 micron thickness stained with hematoxylin and eosin. Semithin sections stained with toluidine blue, before being evaluated using a Leitz light research microscope (Leitz Wetzlar, Germany). Blind analysis of morphological changes was performed. We looked at the integrity of glomeruli, focal glomerular fragmentation, space in corpuscle, swelling and glomerular capillaries; tubular epithelium for dilatation, luminal digestion, loss of the brush border, and nucleus changes; possible apoptotic changes as cell shrinkage or chromatin condensation.

Statistical analysis
GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA) was used for data analysis. Data were presented as mean +/-SEM. Data were compared among groups using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. A (P) value of less than 0.05 was considered to represent a statistically significant difference.
RESULTS

**Effect of folic acid (FA) versus FA and curcumin (CUR) on blood urea and creatinine levels:**

Treatment with FA as a single dose 250 mg i.p. significantly elevated blood urea level about 3 fold (***P< 0.005) and creatinine level (***P< 0.05). Treatment with CUR 200mg/kg orally daily for 11 days prior to FA administration and 1 day thereafter significantly decreased blood urea level compared to folic acid group (**P< 0.005). There was insignificant difference in blood urea level between control group (C) and CUR+FA group (Fig.1). Treatment with CUR insignificantly decreased blood creatinine level compared to FA group (P> 0.05). There was insignificant difference in blood creatinine level between control group (C) and CUR+FA group (Fig. 2).

**Effect of folic acid (FA) versus FA and curcumin (CUR) on blood Cytokines: interleukin-6 (IL-6), interleukin-10 (IL-10), and Tumor necrosis factor-alpha (TNF-α):**

Treatment with FA as a single dose 250 mg i.p. significantly elevated blood IL-6 level (***P< 0.005), IL-10 level (***P< 0.005) and TNF-α level (***P< 0.005). Treatment with CUR 200mg/kg orally daily for 11 days prior and 1 day after FA injection significantly decreased blood IL-6 level compared to FA group (+++P< 0.005; Fig. 3), IL-10 level compared to FA group (+++P< 0.005; Fig. 4), and TNF-α level compared to FA group (++P< 0.01; Fig. 5). There was insignificant difference in blood IL-6 or IL-10 levels between C group and CUR+FA group (Fig. 3 & 4). There was significant difference in blood TNF-α level
between C group and CUR+FA group (🏘P< 0.05; Fig. 5).

**Figure (3):** Effect of folic acid (FA) versus FA and curcumin (CUR) to control (C) on blood interleukin-6 (IL-6): C: control; FA: folic acid treated group; CUR+FA: curcumin and folic acid group. One Way ANOVA with Bonferroni’s Multiple Comparison Test. (FA vs FA+CUR to C; ***P< 0.005 C compared to FA; ++P< 0.01 FA compared to CUR+FA; n=6) (P< 0.05).

**Figure (4):** Effect of folic acid (FA) versus FA and curcumin (CUR) to control (C) on blood interleukin-10 (IL-10): C: control; FA: folic acid treated group; CUR+FA: curcumin and folic acid group. One Way ANOVA with Bonferroni’s Multiple Comparison Test. (FA vs FA+CUR to C; ***P< 0.005 C compared to FA; ++P< 0.01 FA compared to CUR+FA; n=6). (P< 0.05).

**Effect of folic acid (FA) versus FA and curcumin (CUR) on renal structure**

Curcumine (CUR) treatment 11 days prior FA administration and 1 day thereafter protected renal tubular cells from damage. Histological examination showed disrupted brush borders flattening of epithelia, condensed nucleus, and plasma membrane blebbings in the kidneys of rats receiving FA (FA) but not of control rats (C), indicating the occurrence of acute tubular necrosis (Fig. 6). CUR ameliorated proximal and distal tubular epithelial cell flattening in the kidney (Fig. 6, FA+CUR).
Renal structure

Light microscopy: In the control group animals of the present study the renal cortex was formed of renal corpuscles, proximal and distal convoluted tubules (Figs. 6a and 6b). The renal corpuscle was formed of a tuft of capillaries surrounded by Bowman’s capsule consisting of two layers with Bowman’s urinary space in between (Fig. 6a). The outer parietal layer was formed of a simple squamous epithelium, whereas the inner visceral layer had modified epithelial cells called podocytes. The proximal convoluted tubules were lined by a cuboidal epithelium with basally located and spherical nuclei and prominent brush border at the apical surface (Figs. 6a and 6b). The distal convoluted tubules were lined by cuboidal epithelial cells that lacked a brush border. The nuclei were spherical and apically located with pale cytoplasm compared with proximal convoluted tubules (Figs. 6a and 6b).

In the FA treated group rat renal cortex showed marked changes in the renal corpuscles and proximal and distal convoluted tubules. The renal corpuscles showed atrophic shrinkage and retraction of the glomerular tuft of capillaries with dilated Bowman’s urinary space (Fig. 6c). There was dilatation of both of the proximal and distal convoluted tubules with flattening of their epithelia, and destruction of the brush border of the proximal convoluted tubules (Figs. 6c and 6d). Cellular necrosis was seen in the form of condensed shrunken nuclei (pyknotic) and nuclear lysis with loss of affinity of its chromatin for basic dyes (karyolytic nuclei), and desquamated cells observed inside the lumen of the tubules (Fig. 6c).

Marked congestion of capillaries was shown as dilated vascular spaces with blood cells (Fig. 6d).

In the renoprotection group (FA + CUR) there was milder cellular destruction as compared to FA treated group, retraction of the glomerular tuft of capillaries with dilation of Bowman’s urinary space. The brush border of proximal convoluted tubules is preserved with apparent thickening of its basement membrane however, some dilatation of both of proximal convoluted tubules and distal convoluted tubules were observed (Fig. 6e & 6f).

DISCUSSION

This study demonstrated that FA treatment significantly raised blood urea and creatinine levels. This result is supported by several previous studies that proved the role of FA as a model drug for induction of kidney injury manifested by elevation of waste products mainly urea and creatinine². Gupta and his group used two doses of FA 100mg/kg for seven days and 250 mg/kg as single i.p. injection and both doses caused serological changes as well as histopathological renal damage. The efficiency of giving a single dose of FA at 250 mg/kg i.p. injection for induction of acute renal damage is supported by many previous studies¹,²,¹³.

Histological examination of kidney tissues showed that a high single dose of folic acid injection had wide range of toxic effects on the kidney. Widening of the Bowman’s urinary space and dilatation of the proximal and distal convoluted tubules were observed indicating the occurrence of acute tubular necrosis. The widening of the Bowman’s urinary space may be secondary to
Figure (6): Photomicrographs of sections of rat renal cortex (Left panel) and semithin sections (right panel), (a & b) control, (c & d) FA treated, (e & f) FA + CUR treated groups, showing glomerular tuft of capillaries (G), Bowman’s urinary space (asterisk), parietal layer of Bowman’s capsule (thick arrow), proximal convoluted tubules (P), distal convoluted tubules (D), brush border (thin arrow), pyknotic nuclei (thick arrow), karyolytic nuclei (black arrowheads), vascular spaces (V), and cavitations (C), and thickened basement membrane (white arrowhead). Fig. a, c, & e stained with H&E, magnification × 400 and Fig. b, d, & f stained with Toluidine blue, magnification × 1000.
glomerular atrophy, whereas widening of the proximal tubules may be secondary to destruction of the brush border or due to decreasing height of the lining cells as a result of degeneration.

Previous studies have shown that tubular epithelial cells of FA-induced AKI undergo apoptosis that is characterized with disruption of brush borders and flattening of epithelia, condensed nuclei, plasma membrane blebbings, DNA fragmentation, and formation of apoptotic bodies. Due to its poor solubility, folic acid at acid pH precipitates intratubularly as a consequence of fluid reabsorption and increasing acidity along the nephron. This intratubular obstruction due to folic acid crystals could be the primary cause of ARF induced by acute administration of folic acid. Apoptosis was observed in the renal tubules epithelium that appeared with highly acidophilic cytoplasm with deeply stained pyknotic nuclei of dying cells. Apoptosis, in general, was triggered by exogenous and endogenous stimuli such as ultraviolet radiation, oxidative stress, and genotoxic chemicals.

The results of this study proved that CUR 200 mg/kg, given orally for 11 days prior to FA treatment, and one day thereafter, significantly reduced blood urea level and ameliorated the structural damage in rat kidney. Few proximal and distal convoluted tubules were widened, and mild thickening in the basement membrane was observed in CUR+FA group compared to FA group. The apparent thickening of the basement membrane may be secondary to increased collagen fiber formation. Moreover, thickening of the basement membrane might be a part of a healing process to impede the tubular distension. We found insignificant difference in blood creatinine level between FA- and CUR+FA-treated groups. Previous studies showed a reno-protective effect of CUR through lowering blood urea and creatinine level in diabetic nephropathy, in cisplatin-induced nephrotoxicity, and in renal ischemia reperfusion injury. In contrast to previous studies, our study showed that CUR lowered the FA-induced elevation of blood urea but failed to lower the creatinine level. This result could be explained on the basis of the difference in the experimental design and the model of AKI.

We found that FA treatment 250 mg/kg as single dose significantly elevated a known apoptosis-induced inflammatory cytokine TNF-α. Our finding is supported by the study of Wan and his colleagues who reported that FA-mediated acute renal failure at 250mg/kg is mainly caused by TNF-alpha and the administration of anti-TNF-alpha antagonist may be effective for prevention and treatment of acute renal failure. Another in vitro study demonstrated that a member of the TNF superfamily named (TNFSF12) or tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) act as weak inducer of apoptosis and inflammation processes that play a significant role in renal tubular cell injury. In addition, the work of Nakayama proved that TWEAK-induced cell death in human rhabdomyosarcoma cell line (Kym-1 cells) was indirectly mediated by TNF-alpha and was inhibited by cycloheximide. In our study, folic acid caused marked elevation in the level of tumor necrosis factor.
factor alpha which inhibits cell growth and proliferation thus leading to apoptosis.

This study also revealed that IL-6 and IL-10 are elevated significantly in FA treated group compared to C group. This result is supported by previous studies done in several body tissues activated by other pathological factors. One study showed that IL-6 stimulates the inflammatory and autoimmune processes in many diseases such as diabetes and atherosclerosis. Previous studies suggest that IL-10 has stimulatory effect on the immune response that helps to scavenge foreign particles whether infectious or noninfectious to reduce inflammation. Other studies revealed that IL-6 has an anti-inflammatory effect that is mediated by inhibition of TNF-alpha and IL-1, and activation of interleukin-1 receptor antagonist (IL-1RA) and IL-10. Furthermore, IL-6 receptor (IL-6R) cleavage is activated by IL-1, TNF-α and apoptosis. Moreover, soluble IL-6/IL-6R signaling can be further enhanced, e.g., by IL-10. Activated skin macrophages and Langerhans cells release IL-1, IL-6, and TNF-α causing proliferation and activation of keratinocytes resulting in early desquamation and late hyperkeratosis as well as fibrosis. Although it is not easy to link increased cytokine levels to pathogenesis, our results suggest that the elevation of not only TNF-α, but also IL-6 and IL-10 may play a major role in FA-induced acute kidney injury.

Moreover, we demonstrated that CUR treatment significantly decreased IL-6, IL-10 and TNF-α. These results are supported by a previous study reporting that CUR reduced the levels of IL-6 and TNF-α induced by intestinal ischemia-reperfusion on hepatic tissue. Another study proved that curcumin treatment in a dose of (50 mg/kg i.p.) beginning 5 days prior to viral infection and daily thereafter for 14 days caused significant reduction of IL-6 and IL-10 in whole lung tissue in a mouse model of acute respiratory distress syndrome. They also reported that CUR treatment caused significant reduction of NFκB and the key markers of myofibroblast activation; α-smooth muscle actin and Tenascin-C in a mouse model of viral-induced acute respiratory distress syndrome. These findings suggest that CUR is a promising partially protective agent against AKI induced by FA.

Conclusion:
This study revealed that FA treatment significantly raised blood urea, creatinine, IL-6, IL-10, and TNF-α level and caused marked histological changes in renal epithelial cells. CUR treatment for 12 days significantly reduced blood urea, IL-6, IL-10 and TNF-α, and produced marked improvement of kidney structure. This study offers increased understanding of role of folic acid in the induction of AKI and indicates that CUR presents a promising prophylactic treatment for a yet intractable disease.

Conflict of Interest
There is no conflict of interest.

REFERENCES


دراسة فسيولوجية و تركيبية عن تأثير الكركمين الوقائي ضد إصابات الكلى الحادة الناجمة عن حامض الفوليك في الجرذان البضاء

المستعرض العربي

الملخص: مع استخدام حامض الفوليك في المحاولات لعلاج فشل الكلى الحاد، فإن الآليات التي تحدث بها الضرر الكلوي لا تزال غير مفهومة تمامًا. على ذلك، لا تزال任務 الفوائدة لعلاج الفوليك منطقة مختلطة. الهدف من الدراسة الحالي هو اكتشاف وتدشين الدراسات الأولى لدراسة التأثيرات المحتملة لهذا العامل على إصابات الكلية الحادة، بالإضافة إلى ذلك، يتم البحث عن علاج أو وقائي محتمل لحمض الفوليك من خلال استخدام الكركم، وهو عامل بيولوجي مختلف.

ليستحتوي جميع المجموعات على مجموعتين: (1) مجموعة الجرذان المعالجة بجرعة واحدة من حامض الفوليك (0.5 مللي كجم/كغم) في اليوم، (2) مجموعة الجرذان التي أُعطت حامض الفوليك بالاضافة إلى الكركمين (0.2 مللي كجم/كغم) والجرعة الأخيرة من الكركمين أُعطيت بعد يوم من حق حمض الفوليك. (3) مجموعة الجرذان الضابطة تم إعطاؤها الماء المكثف من خلال أنبوب تغذية عن طريق الفم يوميا لمدة 12 يومًا و محلل الدم في الغشاء البريتوني كجرعة واحدة في اليوم الحادي عشر. تم قياس مستويات اليوبريا في الدم، و يدقيكي الكرياتين، و معدل نخر الورم في الدم. وجدنا أن العلاج بحمض الفوليك أدى إلى زيادة نسبة الأيضافات الكرياتين في الدم، و زيادة مستويات الكركمين في الدم، و نخر الورم. و تسببت في تغييرات سيجية وهيكلية ملحوظة في الكرياتين. كما أظهرت هذه الدراسة أن العلاج بالكركمين لمدة 12 يومًا خفضت بشكل ملحوظ نسبة اليوبريا في الدم، و نخر الورم. كما أدت إلى حماية جزئية ضد الأضرار التي أحدثها حامض الفوليك في النسيج الكلوي.

استنتاج: وتشير هذه النتائج إلى أن الكركمين هو عامل وقائي ضد إصابات الكلى الحادة الناجمة عن استخدام حامض الفوليك بجرعة عالية.