

Ameliorative Effect of *Galium Verum* (Rubiaceae Family) Methanolic Extract on Folic Acid-induced Acute kidney Injury in Male Rats.

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Abstract

Antioxidant, sedative, anticancer, and antibacterial properties are among the numerous pharmacological characteristics of *Galium verum* plant. The primary goal of this research was to investigate the ameliorative effect of *G. verum* extract against folic acid-induced acute kidney injury. 18 male rats were randomly-categorized into three groups: Control (Group I), acute kidney injury (Group II), and *G. verum*+ folic acid-induced kidney injury (Group III). Acute kidney injury was induced by a dose of folic acid (250 mg/kg, intraperitoneally), while *G. verum* (250mg/kg) was orally administrated for 7 consecutive days by gavage tube. Phytochemical analysis was performed for *G. verum* methanol extract, in addition to kidney function tests, oxidative stress markers, and histopathological were performed for rats. *G. verum* methanol extract contains flavonoids, anthraquinones, tannins, iridoids, triterpenes, steroids, phenols, and saponins, while being free of cardiac glycosides and alkaloids. *G. verum* extract significantly decreased levels of creatinine, urea, uric acid, sodium, chloride, potassium, microalbumin, malondialdehyde and nitric oxide while it significantly increases creatinine clearance, reduced glutathione, and catalase enzyme levels in rats. Meanwhile, histological examination of kidney tissues of *G. verum* group revealed apparently normal structure. The underlying mechanism of *G. verum* action on the kidney includes antioxidant activity, which improves kidney structure and enhances renal function recovery.

Keywords: Acute kidney injury, *Galium verum*, , Natural products, Nitric oxide, Oxidative stress.

التأثير التحسيني للمستخلص الميثانولي للجويسنة الخضراء (عائلة الفوية) على الإصابة الكلوية الحادة المستحثة بحمض الفوليك في ذكور الجرذان

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الخلاصة

الخصائص المضادة للأكسدة، المسكنة، المضادة للسرطان، والمضادة للبكتيريا هي من بين الخصائص الدوائية العديدة لنبات الجويسنة الخضراء. الهدف الأساسي من هذا البحث هو فحص التأثير التحسيني لمستخلص الجويسنة الخضراء ضد الإصابة الكلوية الحادة التي يسببها حمض الفوليك. 18 من ذكور الجرذان تم تقسيمهم إلى ثلاث مجموعات: (المجموعة الأولى)، مجموعة الإصابة الكلوية (المجموعة الثانية)، ومجموعة نبات الجويسنة الخضراء (المجموعة الثالثة). تم تحفيز الفشل الكلوي بجرعة من حمض الفوليك (250 ملجم/كجم عن طريق الحقن داخل الصفاق)، بينما تم اعطاء مستخلص الجويسنة الخضراء بواسطة انبوب التجريب الفموي لمدة 7 أيام متتالية. تم إجراء تحليل كيميائي- نباتي لمستخلص الجويسنة الخضراء، بالإضافة إلى وظائف الكلى والإجهاد التأكسدي، وكذلك النسيج المرضي للجرذان. يحتوي مستخلص الجويسنة الخضراء الميثانولي على الفلافونويد، والأنثراكينون، والعفص، والإيريرويد، والتريتربين، والستيرويدات، والفينولات، والصابونين، في حين أنه خالي من جليكوسيدات القلب والقلويدات. مستخلص الجويسنة الخضراء قلل معنوياً من مستويات الكرياتينين واليوريا وحمض اليوريك والصوديوم والكلوريد والبوتاسيوم والألبومين والمالون داي الدهيد وأوكسيد النيتريك بينما زاد من تصفية الكرياتينين ومستويات الجلوتاثيون المختزل وانزيم الكاتالاز. في غضون ذلك، فإن الفحص النسيجي لأنسجة الكلى كشف عن بنية طبيعية بعد المعالجة. تضمنت الآلية لعمل المستخلص الميثانولي لنبات الجويسنة الخضراء على الكلى نشاطاً مضاداً للأكسدة، كما يحسن بنية الكلى ويعزز استعادة وظائف الكلى. الكلمات المفتاحية: إصابة الكلى الحادة، الجويسنة الخضراء، منتجات طبيعية، أكسيد النيتروجين، الإجهاد التأكسدي.

Introduction

Acute kidney injury (AKI) is a loss of renal function that occurs suddenly and may be reversible⁽¹⁾. It is marked by a fast decline in renal excretory function over hours to days, as well as a rise in the buildup of nitrogenous metabolic waste products such as creatinine, urea, and other clinically unmeasured wastes^(2,3).

Acute kidney injury (AKI) can be caused by a variety of causes, including ischemia, septicemia, and various infections⁽⁴⁾. Numerous

modern medications or medicines used in daily clinical practice, such as folic acid, gentamicin, cisplatin, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, and immunosuppressants, are responsible for more than 60% of non-hospital and hospital acute kidney injury^(5,6). Changes in intra-glomerular hemodynamics, tubular cell toxicity, decreased tubular secretion, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy are all examples of drug-induced nephrotoxicity⁽⁷⁾.

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Folic acid (FA), a bioactive molecule and an important micronutrient that cannot be produced de novo in humans, is a synthetic and fully oxidized version of the vitamin folate⁽⁸⁾. Folic acid (FA) serves as a cofactor for cellular reproduction and growth, as well as the creation of DNA and RNA strands⁽⁹⁾. Despite the significance of folic acid, excessive dose has been proven harmful to different organs, particularly the kidney⁽¹⁰⁾. The fast development of luminal crystals within renal tubules is related with folic acid-induced acute kidney damage, which is followed by a rapid loss of renal function, tubular epithelial cell necrosis, and apoptosis^(11, 12). Furthermore, new data suggests that a high folic acid dose increases the formation of reactive oxygen species (ROS) and decreases the protection against oxidative stress (OS) by depleting antioxidant enzyme activity⁽¹³⁾. Reactive oxygen species (ROS) operates directly on cells, causing lipid peroxidation, protein oxidation, nucleic acid damage, and eventually cell death and tissue destruction⁽¹⁴⁾. Acute renal injury (AKI) is characterized by severe inflammation, cellular apoptosis, and necrosis, which lead to kidney damage. Oxidative stress (OS) is a component of the pathophysiology of AKI^(15, 16). A widely used FA-induced AKI model, suggesting that FA-AKI is an excellent model that mimics human AKI.

Unfortunately, medicines used to treat AKI are insufficient and are linked to the development of significant side effects, necessitating the creation of the treatment with minimum side effects^(17, 18). Antioxidant therapy is considered to help prevent AKI by scavenging ROS and reducing lipid peroxidation in the proximal tubular cells, which is linked to excessive free radical generation⁽¹⁹⁻²¹⁾.

Galium verum, often known as Lady's Bedstraw or Yellow Bedstraw, is a *Rubiaceae* family perennial herbaceous plant native to Europe and Asia with golden yellow blooms⁽²²⁾. Due to its numerous medicinal qualities, it has been frequently used in traditional medicine as diuretics, cholera tics, to treat diarrhoea, spasmolytic, to treat malignant ulcers, and to prolong wound healing⁽²³⁾. The *G. verum* has been utilized as a sedative, an anticancer agent, and in the treatment of gout and epilepsy in previous research⁽²⁴⁾, liver disorders, and cardiovascular diseases^(23, 25). Moreover, *G. verum* extract is a more powerful antioxidant, serving as a free radical scavenger by interacting with both the hydroxyl and singlet oxygen radical⁽²³⁾. Its capacity to activate natural antioxidant enzymes is only one of its many benefits⁽²⁵⁾. In this light, the primary goal of this research was to investigate whether or not *G. verum* extract is effective in ameliorating AKI-induced by folic acid in male rats.

Materials and Methods

Chemicals and reagents

G. verum dried herb was obtained from (Shender Group LTD, UK). Folic acid was purchased from Puritan's Pride (Holbrook, NY, USA). Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Spectrum Diagnostics (Obour City, EGY) provided the sodium, chloride, potassium, and microalbumin (MAU) kits, while the Biodiagnostic Company provided the remaining kits (El Motor St, Dokki, EGY).

G. verum extraction

With slight modifications, the extraction was carried out according to Laki's⁽²³⁾ technique. One hundred (100) gm of dried *G. verum* leaves were ground (sieve 0.75) and macerated in 80 percent methanol (MeOH) (1:5 w/v) for 24 hours at room temperature. Under reduced pressure, the mixture was filtered through filter paper (Whatman, No.1) and evaporated to dryness.

Phytochemical qualitative analysis

The presence of phytochemical activity in plant extracts was determined using the following conventional techniques:

Detection of phenols

On a filter paper, the methanolic extract was added as a drop. The spots were treated with a drop of phosphomolybdic acid reagent and then exposed to ammonia fumes. The presence of phenols is indicated by the blue colour of the dots⁽²⁶⁾.

Detection of tannins

A 10% ethanolic ferric chloride solution was added to 2-3 ml of methanolic extract. The presence of tannins in the extracts is indicated by the solution's dark blue or greenish-grey hue⁽²⁷⁾.

Detection of alkaloids

In a tiny piece of pre-coated TLC plate, a drop of extract was detected, and the plate was sprayed with Dragendorff's reagent. The presence of alkaloids is indicated by the spot's orange coloration⁽²⁸⁾.

Detection of anthraquinones

A 0.05 gm of the extract was heated with 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid (35%). The extract was cooled, filtered and the filtrates were shaken with diethyl ether. Strong ammonia (33%) was added. The pink or deep red coloration of the aqueous layer indicates the presence of anthraquinones⁽²⁹⁾.

Detection of saponins

The extract (0.01 gm) was combined with hot water and shaken for 30 seconds. The presence of saponins is indicated by the development of stable foam⁽³⁰⁾.

Detection of flavonoids

A piece of magnesium ribbon and 1 ml of conc. hydrochloric acid (35%) was added to 2-3 ml of the methanolic extract. The presence of flavonoids is indicated by the pink-red or crimson colour of the solution⁽²⁶⁾.

Cardiac glycosides (Keller-Killani test)

5 mL extract was treated with 2 mL glacial acetic acid plus 1-drop ferric chloride solution. Then 1 ml of conc. sulfuric acid (90%) was added. A deoxysugar characteristic of cardiac glycosides is indicated by a brown ring at the contact⁽³⁰⁾.

Test for Steroids

1 ml of chloroform and conc. sulfuric acid (90%) was added with the 2.5 ml of extract. Red hue emerged in the bottom chloroform layer, indicating the presence of steroids⁽²⁹⁾.

Test for Iridoids

The *G. verum* extract (1ml) was boiled for a few minutes after adding 1 ml of Trim-Hill reagent. Iridoids were identified by their blue-green or red hue⁽³⁰⁾.

Experimental animals

Male Wistar rats (*Rattus norvegicus*) weighing 150 ±10 g was used in this study. Rats were purchased from the National Research Center (NRC, Dokki, Giza). In the well-ventilated animal house of the Zoology Department, Faculty of Science, Cairo University, male rats were housed in polyacrylic cages and randomly distributed into equal groups (six animals per cage). Rats were kept in a comfortable setting with 12 hours of daily-automated light at ambient temperature (22°C). The animals adapted to the laboratory conditions for 14 days before the experiment. Water and pellets were provided *ad libitum*. Rats were maintained under a 12:12-h hours light: dark cycle at 22 to 25 °C in a well-ventilated animal facility (humidity 35%) before to the start of the experiment.

Ethical Consideration

The Cairo University Institutional Animal Care and Use Committee (CUFS/F/69/19) authorized the experimental techniques and protocols utilized in this work. All operations involving groups of animals and their care were carried out in compliance with international standards.

Induction of acute kidney injury (AKI)

Acute kidney injury (AKI) was induced by intraperitoneal injection of a single dose of folic acid (250 mg/kg) dissolved in 0.3M NaHCO₃⁽³¹⁾.

Experimental design

Eighteen male Wistar rats were randomly-distributed into three groups after one week of acclimation as follows:

Control (Group I): After a single dose of NaHCO₃ (0.3M, i.p), rats received [10% dimethylsulfoxide (DMSO)] orally daily for one week.

Acute kidney injury (AKI) (Group II): After a single dose of folic acid (250 mg/kg, i.p) (31) that is dissolved in NaHCO₃, rats received (10% DMSO, orally) daily for one week.

***G.verum* + folic acid-induced kidney damage (Group III):** After a single dose of folic acid (250 mg/kg, i.p), rats received *G.verum* extract (500 mg/kg body weight in 10% DMSO, orally) daily for one week according to Bradic, *et al.*⁽³²⁾.

Animal handling and specimen collection

Male rats were placed in metabolic cages 24 hours before the experiment ended to collect the urine, they had passed in the previous 24 hours. Rats had unrestricted access to water during this period. The rats were completely anaesthetized with sodium pentobarbital (50 mg/kg) and were euthanized by carotid exsanguination shortly after losing consciousness. The chest was opened, and the blood was collected by the cardiac puncture. Blood samples were taken in centrifuge tubes. The kidney of each rat was removed and wiped with filter paper to eliminate any blood or weight traces. Half of each rat's kidney was put in 10% formal saline for histological inspection, while the other half was kept at -80°C for biochemical analysis⁽³³⁾.

Samples preparation

The volume of collected urine of each rat was quantified using the measuring cylinder, and the urine samples were centrifuged for 10 minutes at 3000rpm to separate the supernatant, which was kept at -20°C until analyzation of urea, uric acid, sodium (Na⁺), potassium (K⁺), chloride (Cl⁻), and microalbumin^(34, 35).

Blood sample of each male rat was spun at 3000 rpm for 20 minutes after being collected in centrifuge tubes. The obtained serum was kept at -20 °C until it was utilized in biochemical tests of kidney functions tests (urea, uric acid, Na⁺, K⁺, and Cl⁻).

In ice-cold 0.1 M Tris-HCl buffers, kidney tissue was homogenized (10% w/v) (pH7.4). The homogenate was centrifuged at 3000 rpm for 15 minutes at 4°C to separate the supernatant. Homogenate supernatants were stored at -80 °C until biochemical analysis (MDA, GSH, GST, and NO).

Biochemical parameters

The creatinine and creatinine clearance were assessed by the technique described by Schirmeister⁽³⁶⁾. Moreover, urea⁽³⁷⁾, uric acid⁽³⁸⁾, sodium⁽³⁹⁾, chloride⁽⁴⁰⁾, potassium⁽⁴¹⁾, and microalbumin (MAU)⁽⁴²⁾ according to the manufacturer's instructions using Bio-diagnostic kits (Giza, Egypt).

Furthermore, the level of MDA, which is an index of lipid peroxidation was estimated by the method described by Ohkawa *et al*⁽⁴³⁾. Additionally, reduced glutathione (GSH)⁽⁴⁴⁾, nitric oxide (NO·)⁽⁴⁵⁾, glutathione-S-transferase (GST)⁽⁴⁶⁾, and catalase (CAT)⁽⁴⁷⁾ were determined in the kidney homogenate supernatant according to the manufactures instructions using Biodiagnostic kits (Giza, Egypt).

Histological examination

The kidney of each rat was dissected out and fixed in neutral buffered formalin (10%). After fixation, kidney tissue samples were routinely processed, cut and stained by hematoxylin and eosin (H&E). The specimens were examined under Olympus BX43 light microscope and captured using CellSens dimensions software (Olympus) linked to Olympus DP27 camera⁽⁴⁸⁾.

The digital photos were used to determine the Bowman's capsule and glomeruli area (μm^2) of the kidneys tissue in different experimental groups using Image J 1.45 software (National Institute of Health, USA). The difference between Bowman's capsule and glomeruli areas was considered the Bowman's space area for each renal corpuscle. The mean of each parameter was detected for statistical evaluation⁽⁴⁹⁾.

Statistical analysis

Results were expressed as mean \pm SEM. The comparisons within groups were tested using one-way analysis of variance (ANOVA) with Duncan post hoc test used to compare the group means. $P < 0.05$ was considered as statistically significant. SPSS, for Windows (version 15.0) was used for the statistical analysis.

Results and Discussion

Yield value of *G. verum* extraction

Each 100 gm of dried *G. verum* leaves yield 5g methanolic extract, which kept in a dark glass container at -30°C.

Phytochemical qualitative analysis

The determination of components from *G. verum* extract was indicated by the data in Table 1. Flavonoids, anthraquinones, tannins, iridoids, triterpenes, steroids, phenols, and saponins are all present in the extract, but it is devoid of cardiac glycosides and alkaloids.

Table 1. Phytochemical analysis of *G. verum* methanolic extract

Test	<i>G. verum</i> methanolic extract
Flavonoids	++
Anthraquinones	++
Tannins	++
Iridoids	++
Triterpenes	++
Steroids	++
Phenols	+
Saponins	+
Cardiac Glycosides	-
Alkaloids	-

(-) negative reaction, + positive reaction, ++ strong positive reaction.

Biochemical markers

Biochemical analysis of urine and serum samples showing a significant increase ($P < 0.05$) in creatinine, urea, uric acid, sodium, chloride, potassium, and urine microalbumin in the AKI (**Group II**) as compared to the control (Group I); while, creatinine clearance was significantly decreased ($P < 0.05$) (Table 2). Treatment with *G. verum* extract plus folic acid-induced kidney (**Group III**) decreased significantly ($P < 0.05$) the levels of creatinine, urea, uric acid, sodium, chloride, potassium, and urine microalbumin as compared to AKI (**Group II**) while, it increased creatinine clearance.

Oxidative stress markers

The MDA and NO levels in the kidneys of AKI (**Group II**) was significantly increased ($P < 0.05$); while, GSH, GST, and CAT levels were significantly- decreased ($P < 0.05$) in **Group II** compared to corresponding levels in control (**Group I**) rats. Furthermore, in rats of **Group III** (*G. verum* extract+folic acid-induced kidney injury), there were significant decreases ($P < 0.05$) in kidney MDA and NO levels compared to corresponding levels in the AKI (**Group II**) rats; but there was significant increase ($P < 0.05$) in kidney GSH, GST, and CAT levels compared to such levels in the AKI (**Group II**) rats.

Table 2. Ameliorative effect of *G. verum* methanolic extract on kidney function markers of acute kidney injury (AKI) in male rats.

Parameters	Sample	Groups		
		Group I	Group II	Group II
Creatinine (mg/dl)	Serum	0.35±0.03	0.48±0.02 ^a	0.41±0.04 ^b
	Urine	15.84±1.28	31.94±3.15 ^a	24.32±2.13 ^b
Urea (g/dl)	Serum	14.99±1.74	28.51±1.75 ^a	16.34±1.68 ^b
	Urine	44.49±3.41	64.16±4.93 ^a	47.20±4.23 ^b
Uric acid (mg/dl)	Serum	6.43±0.27	7.77±0.32 ^a	6.52±0.13 ^b
	Urine	6.16±0.29	8.07±0.34 ^a	6.68±0.20 ^b
Sodium (mEq/l)	Serum	110.5±6.9	131.13±10.2 ^a	115.70±4.12 ^b
	Urine	89.85±7.99	121.41±3.62 ^a	103.16±3.55 ^b
Chloride (mmol/l)	Serum	59.30±3.23	108.14±7.25 ^a	72.25±7.03 ^b
	Urine	104.07±3.49	140.76±2.04 ^a	119.96±3.81 ^b
Potassium (mmol/l)	Serum	3.77±0.46	5.82±0.44 ^a	3.86±0.14 ^b
	Urine	5.56±0.50	7.58±0.91 ^a	5.82±0.40 ^b
Microalbumin (mg/L)	Urine	360.00±30.98	476.67±30.29 ^a	384.00±21.54 ^b
Creatinine clearance(ml/min)	Urine	0.68±0.03	0.36±0.06 ^a	0.57±0.04 ^b

*Group I (Control), Group II (AKI) and Group III (*G. verum*+folic acid-induced kidney injury).

*n=18 (6 male in each group).

*(a): Significantly different (P<0.05) from Group I.

*(b): Significantly different (P<0.05) from Group II.

*Values are expressed as mean ± SEM. *The comparisons within groups were tested using one-way analysis.

Table 3. Ameliorative effect of *G. verum* methanolic extract on oxidative stress markers of acute kidney injury (AKI) in male rats

Parameters	Groups		
	Group I	Group II	Group II
MDA (nmol/g.tissue)	1.71±0.12	2.34±0.10 ^a	1.83±0.11 ^b
NO (µmol/g.tissue)	45.83±4.50	72.79±3.09 ^a	51.79±4.92 ^b
GSH (mg/ g.tissue)	5.46±0.36	4.58±0.26 ^a	5.20±0.22 ^b
GST (µmol/g.tissue)	0.83±0.03	0.55±0.08 ^a	0.73±0.06 ^b
CAT (U/g.tissue)	19.78±2.84	5.22±0.83 ^a	16.21±1.54 ^b

*Group I (Control), Group II (AKI) and Group III (*G. verum*+folic acid-induced kidney injury).

*n=18 (6 male in each group).

*(a): Significantly different (P<0.05) from Group I.

*(b): Significantly different (P<0.05) from Group II.

*Values are expressed as mean ± SEM.

*The comparisons within groups were tested using one-way analysis.

Histological examination

Microscopic examination of the kidney of control rats (**Group I**) showed the normal histological structure of the renal tissue, that is composed of cortex and medulla; in which the cortex appeared containing numerous glomeruli and both types of renal tubules. The glomeruli showed normal size and structure (Figures 1A, 2, 3, 4). In **Group II** of male rats that administered folic acid, there were various histopathological alterations in the kidney; where the cortex showed multifocal areas of hemorrhages associated with severe congestion of the cortical blood vessels. Furthermore, there were necrobiotic changes in renal tubules with an accumulation of eosinophilic proteinaceous renal cast in the lumen of renal

tubules. The glomeruli showed a marked increase in the size of the glomerulus capsule, mesangial cell proliferation and Bowman's space. In addition, the glomeruli showed evidence of capillary tuft atrophy in some instances (Figures 1B, 2, 3, 4). Only a small number of glomeruli in **Group III** were enlarged in size. Meanwhile, tissues of kidneys were evaluated and found to have a normal structure in the majority of the sections tested. (Figures 1C, 2, 3, 4).

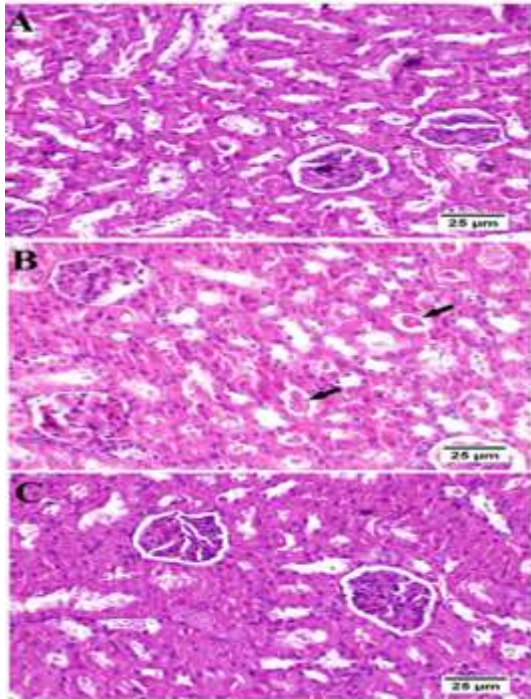


Figure 1. Histopathological examination of the kidney (H&E).

(A) Control group, higher magnification showing normal size and structure of renal corpuscles. (B) Folic acid group, showing accumulation of casts in the renal tubular lumen (arrows). (C) Kidneys of rat, *G. verum* group, showing apparently normal renal cortex (H&E).

Bowman's capsule area, Glomeruli area, and Bowman's space area decreased significantly in AKI (Group II) compared with the control (Group I). While a significant decrease was noticed in Bowman's capsule area, Glomeruli area, and Bowman's space area after *G. verum* administration (Group III) compared with AKI (Group II) (Figures 2,3,4).

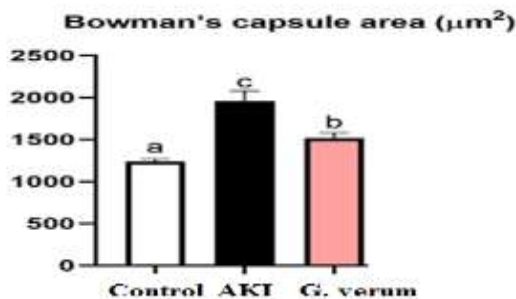


Figure 2. Bowman's capsule area in different experimental groups. Different letters (a-c) above the error bar indicate statistically significant differences at $P<0.05$.

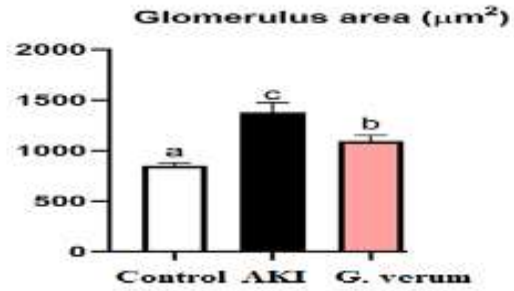


Figure 3. Glomeruli area in different experimental groups. Different letters (a-c) above the error bar indicate statistically significant differences at $P<0.05$.

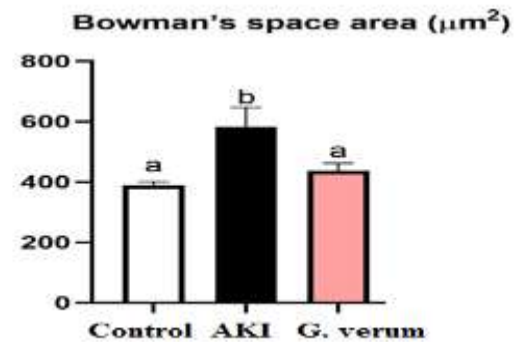


Figure 4. Bowman's space area in different experimental groups. Different letters (a and b) above the error bar indicate statistically significant differences at $P<0.05$.

Creatinine and urea are metabolic waste products that are removed from the bloodstream by glomerular filtration and proximal tubular secretion of the kidney. Serum elevations of these parameters are consequently viewed as sensitive markers of kidney disease, implying that the kidney's excretory function has been impaired⁽⁵⁰⁻⁵²⁾. Increased glomerular capillary pressure, leaky glomerular capillaries, and impaired tubule-glomeruli all affect creatinine clearance, which is an indication of glomerular filtration rate. The rate of diffusion by the thicker capillary basement membrane decreases as glomerular injury progresses, finally culminating in a reduction in GFR^(53, 54). Renal failure was verified in FA-induced AKI in rats, with a substantial rise in creatinine, urea, and a significant reduction in creatinine clearance. Table 2. The kidney is important in maintaining homeostasis because it keeps the concentrations of numerous plasma electrolytes within tight ranges⁽⁵⁵⁾. Folic acid-induced substantial changes in serum sodium, potassium, and chloride concentrations, according to our findings. Furthermore, a decrease of the glomerular function with severe tubular oncosis, blockage of Na⁺ K⁺ ATPase enzyme, and a reduction in renal function might be the mechanism by which folic acid causes these harmful consequences.

Renal function tests revealed that a single dose of 250 mg/kg folic acid-induced AKI (Group

II) rats caused significant increases in serum creatinine, urea, uric acid, Na⁺, K⁺, and Cl⁻, with a significant reduction ($P < 0.05$) in creatinine clearance (table 3). Previous studies support these findings⁽⁵⁶⁻⁵⁸⁾. Furthermore, folic acid is present in the blood in the form of 5-methyl tetrahydrofolate⁽⁵⁹⁾; and folate can accumulate in large quantities in the kidney due to the strong affinity to the folate receptor⁽⁶⁰⁾. At both neutral and acidic pH, folic acid has a poor solubility. As a result, at large doses, decreasing the tubular fluid pH along the tubule causes folic acid deposition as a folate crystal, resulting in tubular blockage⁽⁶¹⁾. Tubular blockage is the most common cause of epithelial hypoxia, renal tubular necrosis, renal failure, and potentially mortality within hours or days^(62, 63). Folate crystals form mostly in the thick ascending limbs of Henle's loop and less in the distal section of the proximal convoluted tubules, causing Na/K ATPase inhibition⁽⁶⁴⁾. Micro-albuminuria is a wide marker for AKI. In the current study, folic acid caused significant increase in microalbumin level ($P < 0.05$) (Group II) compared to control (Group I) (table 2). Poor proximal tubular performance can cause an increase in urinary albumin levels⁽⁶⁵⁾ as well as changes in glomerular shape and function⁽⁶⁶⁾.

Furthermore, histological examination revealed that folic acid induced kidney damage (figure 1B). Moreover, an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense capability is referred to as oxidative stress (OS)^(67, 68). It is a key factor in the development of a number of illnesses, including AKI⁽⁶⁹⁾. Folic acid, according to the results of this study in (Group II) rats caused OS, which is characterized by a rise in MDA and NO levels and a reduction in GSH, GST, and CAT levels. Table 3. Lipid peroxides are formed when ROS oxidize membrane lipids, and these lipid peroxides can create a variety of aldehydes, including MDA^(70, 71). Folic acid stimulates the formation of mitochondrial ROS and nitric oxide species (NOX), which increases OS and inflammation in the kidney, according to two studies^(72, 73). Furthermore, NADPH and L-arginine-linked ROS generation can be increased when a high quantity of folic acid is stored in mitochondria⁽⁷³⁾. Moreover, excessive NO generation is linked to renal tubular injury, which can impair mitochondrial respiration as well as damage membranes and DNA^(74, 75). NO may also combine with certain metabolites to form the more hazardous peroxynitrite, worsening oxidative damage even further⁽⁷⁶⁾. Reduced glutathione (GSH) is an essential intracellular anti-oxidant molecule that protects cells from a variety of unpleasant stimuli, including OS⁽⁷⁷⁾. Increased engagement of GSH as an antioxidant for trapping free radicals generated by folic acid treatment might explain the lower amounts of GSH in the folic acid given⁽¹³⁾. Table 3. Furthermore, antioxidant

enzymes such as CAT and glutathione-S-transferase have been proposed as indicators of OS (GST)^(78, 79). These enzymes work by deactivating free radicals before they cause harm to cells, as well as donating free electrons to make them stable⁽⁸⁰⁾. The buildup of hydrogen peroxide owing to mitochondrial malfunction and an increase in reactive oxygen species (ROS) causes a decrease in CAT and GST activity⁽⁸¹⁾.

After treatment with *G. verum*, data of this work showed that kidney function markers returned near to normal levels (tables 2 and 3), as well as there were improvements in the kidney's histological structure (figure 1C). Additionally, researchers mentioned that active components in *G. verum* extract, including as flavonoids, tannins, and saponins may contribute to its diuretic effect⁽⁸²⁾, which can increase the amount of fluid passing through the kidneys; and the increase in urine volume can lower the salt saturation and consequently can avoid the formation of crystals⁽⁸³⁾. In the current study, the treatment with *G. verum* extract+folic acid-induced kidney injury (Group III) enhanced CAT, GST, and GSH activity while decreasing MDA and NO levels (table 3). The *G. verum* has been utilized as an anti-inflammatory and antioxidant for a long time⁽⁸⁴⁾. The presence of flavonoids, and phenolics in *G. verum* extracts, possessed a stronger antioxidant activity for neutralization of OH radical, H₂O₂, and inhibition of lipid peroxidation⁽²³⁾. Moreover, researchers mentioned that, the phenolic component in *G. verum* is not only scavenge free radicals; but it also inhibits endothelial NO oxidase (NOX), leading to reduction in ROS release and formation of peroxynitrite. A reduction of NO concentration may indicate an anti-inflammatory activity of *G. verum*⁽⁸⁵⁾.

Conclusion

The underlying renal mechanism of *G. verum* action includes antioxidant activity, which improves kidney structure and enhances renal function recovery.

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Conflict of Interest

The authors declare no conflict of interest.

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Ethics Statements

This study was performed in accordance with the ethical standards and protocols approved by the Committee of The Cairo University Institutional Animal Care and Use Committee (CUFS/F/69/19) authorized the experimental techniques and protocols utilized in this work. All operations involving groups of animals and their care were carried out in compliance with international standards.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Amel M. Soliman, Ayman Saber Mohamed; data collection: Aya Ramadan Rashed; analysis and interpretation of results: Aya Ramadan Rashed, Ayman Saber Mohamed. Author; draft manuscript preparation: Amel M. Soliman, Aya Ramadan Rashed. All authors reviewed the results and approved the final version of the manuscript.

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