

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2026): 5.29

IJABR 2026; 10(5): 09-13

www.biochemjournal.com

Received: 07-02-2026

Accepted: 11-03-2026

Shireesha Koolla

MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Rajendar Bobbili

Associate Professor, Department of Veterinary Pharmacology and Toxicology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

B Anil Kumar

Associate Professor and Head, Department of Veterinary Pharmacology and Toxicology, PVNRTGVU, College of Veterinary Science, Mamoor, Warangal, Telangana, India

S Soujanya

Associate Professor and Head, Department of Veterinary Pathology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

S Amani

MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Ch. Priya

MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Y Poojitha

MVSc Scholar, Department of Veterinary Pathology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Hanuman D

Ph.D. Scholar, Department of Veterinary Pharmacology and Toxicology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Gopala Reddy A

Professor and University Head, Department of Veterinary Pharmacology and Toxicology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:**Shireesha Koolla**

MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, PVNRTGVU, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Protective effect of Apigenin against folic acid-induced renal toxicity: Evaluation of body weight, kidney weight and haematological alterations

Shireesha Koolla, Rajendar Bobbili, B Anil Kumar, S Soujanya, S Amani, Ch. Priya, Y Poojitha, Hanuman D and Gopala Reddy A

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5a.8301>

Abstract

An experimental investigation was carried out to assess the nephroprotective potential of apigenin against folic acid-induced toxicity in mice. Thirty Swiss albino mice (8-12 weeks old) were acclimatized and randomly assigned into five groups (n = 6) for a 14-day study. Group I served as the normal control and received normal saline, while Group II was administered folic acid (250 mg/kg, IP) on 1st day of experiment. Group III received apigenin alone (20 mg/kg, IP) for 14 days. Groups IV and V were administered with folic acid (@ 250 mg/kg, IP) along with apigenin (10 mg/kg and 20 mg/kg), IP, respectively. On day 15, blood samples were collected prior to sacrifice for haematological evaluation, and kidneys were subsequently excised for recording organ weights. In present study, folic acid administration (Group II) exhibited significant alterations in body weight, kidney weight, and haematological indices compared to Group I. Treatment with apigenin in Groups IV and V effectively ameliorated these changes, whereas Groups I and III remained statistically comparable. In conclusion, findings from this study indicates that apigenin effectively mitigates folic acid-induced renal injury, with the higher dose (20 mg/kg b.wt.) producing the most pronounced improvement by normalizing the altered parameters.

Keywords: Folic acid, Apigenin, swiss albino, haematology

Introduction

Renal disorders have become a significant global health concern, contributing to both acute kidney injury (AKI) and chronic kidney disease (CKD), which are closely interconnected (Jiang *et al.*, 2020) [1]. AKI is defined as a rapid and potentially reversible loss of kidney function and is commonly observed in critically ill patients (Stallons *et al.*, 2014) [2]. Clinically, AKI is a critical condition with high rates of morbidity and mortality, affecting around 13% of hospitalized patients and contributing to nearly 1.7 million deaths annually. Additionally, it predisposes patients to CKD and end-stage renal disease (ESRD), affecting nearly 10% of the global population (Yang *et al.*, 2025) [3]. Management of AKI remains challenging due to limited treatment options and their associated adverse effects, emphasizing the need for the development of safer therapeutic strategies (Rizwan *et al.*, 2019 and Sadek *et al.*, 2022) [4,5].

Folic acid (FA) is an essential, water-soluble micronutrient and an oxidized form of folate, which cannot be synthesized by humans. It serves as an important cofactor in DNA and RNA synthesis and plays a crucial role in cell growth and proliferation (Silva *et al.*, 2017) [6]. Excessive folic acid intake has been associated with the formation of intratubular crystals, leading to impaired renal function, tubular epithelial damage, and cell death. Additionally, it promotes oxidative stress (OS) by increasing ROS generation and suppressing antioxidant enzyme activity. ROS-induced damage to lipids, proteins, and nucleic acids that triggers inflammation and contributes to the progression of AKI (Nikolic *et al.*, 2020) [7].

Plant-based traditional medicines have been used for centuries in the treatment of various diseases. Natural products continue to serve as an important source for identifying and developing novel drugs (Fang *et al.*, 2021) [8]. Apigenin (API), (4',5,7-trihydroxyflavone) is a naturally occurring flavone belonging to the flavonoid family, commonly found in fruits and vegetables (Fossatelli *et al.*, 2023) [9].

Apigenin exhibits diverse biological activities, including antioxidant, anti-inflammatory, antiapoptotic, antimutagenic, and antitumor effects (Malik *et al.*, 2017) [10]. Therefore, the present study was designed to evaluate the nephroprotective effect of apigenin against folic acid-induced AKI in mice.

Materials and Methods

Chemicals

Folic acid was procured from Sigma Aldrich, USA-(Cat. No. F7876) and Apigenin was procured from Tokyo Chemical Industry, Japan-(Cat. No. A1514).

Experimental Animals

A total of 30 adult Swiss albino mice (8-12 weeks old, 20-

25 g) were obtained from Jeeva Life Sciences, Hyderabad. The animals were housed in polypropylene cages under controlled environmental conditions (temperature 22-24 °C, relative humidity 30-70%, and 12 h light/dark cycle). The mice were acclimatized for 7 days before the start of the experiment and the animals were provided with a standard pellet diet and water ad-libitum, with corncob as a bedding material. Health status was monitored daily throughout the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), with approval no. (07/30/C.V.Sc, Hyd. IAEC). The animals were randomly allocated into 5 groups of six mice each (n = 6), and maintained for 14 days as per the treatment protocol given below.

Table 1: Experimental Design

Groups	Treatment	No. of animals
I.	Normal Control (Normal saline), P/O for 14 days	6
II.	Folic acid control @ 250 mg/kg, single dose IP on the first day	6
III.	Apigenin control @ 20 mg/kg, IP, daily for 14 days	6
IV.	Folic acid @ 250 mg/kg, single dose IP on first day + Apigenin LD @ 10 mg/kg, IP daily for 14 days	6
V.	Folic acid @ 250 mg/kg, single dose IP on first day + Apigenin HD @ 20 mg/kg, IP daily for 14 days	6

Body weights

Body weight of each mouse was recorded using an electronic weighing balance (Shimadzu Pvt. Ltd.) day 0 and subsequently on days 7 and 14 of the experimental period.

Absolute kidney weights

Following sacrifice on 15th day of experiment, the kidneys were promptly excised and gently cleared of adhering tissues. Each kidney was weighed individually using an electronic weighing balance (Shimadzu Pvt. Ltd.), and the measurements were documented.

Blood collection

At the end of the experiment (day 15), mice were fasted for 12 hours before sacrifice. Prior to euthanasia, animals were anesthetized, and blood samples were aseptically collected from the retro-orbital plexus using capillary tubes into anticoagulant-coated vacutainers (K3-EDTA tube, 13 mm x 75 mm, 6 mL; Rapid Diagnostics Pvt. Ltd., Delhi), which ensures that blood is preserved in an anticoagulated state until further analysis. The collected blood was used for the estimation of haematological parameters, including Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC), Haemoglobin (Hb), and Packed Cell Volume (PCV), were analyzed using an automatic whole blood analyzer (Huma count, Med source ozone Bio-chemicals Pvt. Ltd., Faridabad, Haryana) and the results were tabulated.

Statistical analysis

Data were expressed as mean±SE (n = 6). Statistical analysis was performed using SPSS software (version 21) by one-way ANOVA, followed by Duncan's multiple range test for post hoc comparisons. A value of $p < 0.05$ was

considered statistically significant.

Results

Body weights

A significant decline ($p < 0.05$) in body weight was observed in Group II on days 7 and 14 when compared to Group I. In contrast, apigenin-treated groups (IV and V) demonstrated a significant ($p < 0.05$) improvement in body weight relative to Group II, indicating recovery. The body weights of Group III remained comparable to those of the control group (Group I). The findings are summarized in Table 2 and Fig. 1.

Absolute kidney weights

The absolute kidney weight in Group II was significantly ($p < 0.05$) higher compared to the control group (Group I), indicating renal enlargement. However, treatment with apigenin in Groups IV and V resulted in a reduction in kidney weight in a dose-dependent manner, though the decrease was not statistically significant ($p < 0.05$). Group III maintained kidney weights similar to those of Group I. Detailed results are shown in Table 3 and Fig. 2.

Haematology

Haematological analysis showed a significant ($p < 0.05$) reduction in TEC (Fig. 3), Hb (Fig. 4), PCV (Fig. 5), lymphocytes (Fig. 6), and eosinophils (Fig. 7), along with an increase in TLC (Fig. 8), neutrophils (Fig. 9), and monocytes (Fig. 10) in group II compared to group I. Apigenin treated groups IV and V effectively reversed these changes. Whereas, no significant ($p < 0.05$) difference was observed between groups I and III. The data are summarized in Table 4.

Table 2: Weekly body weights (g) in different groups of mice

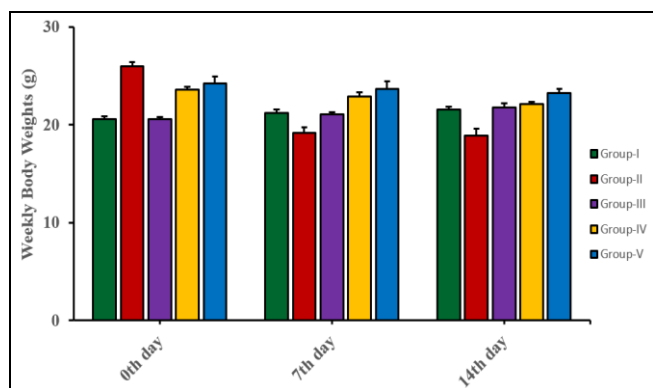
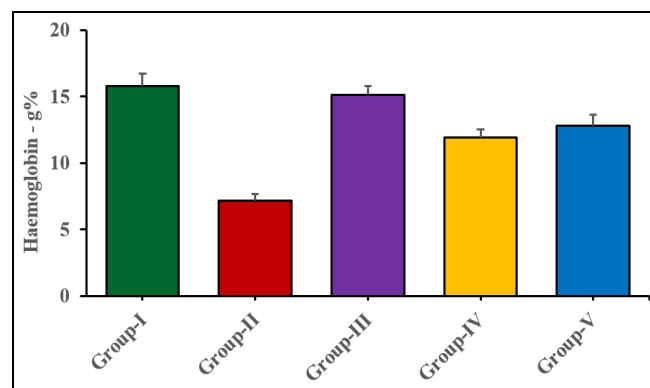
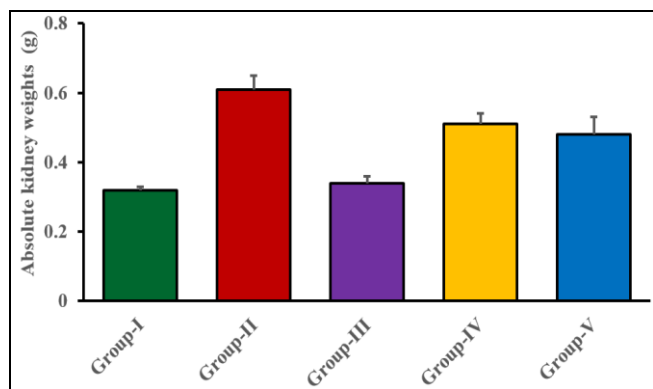
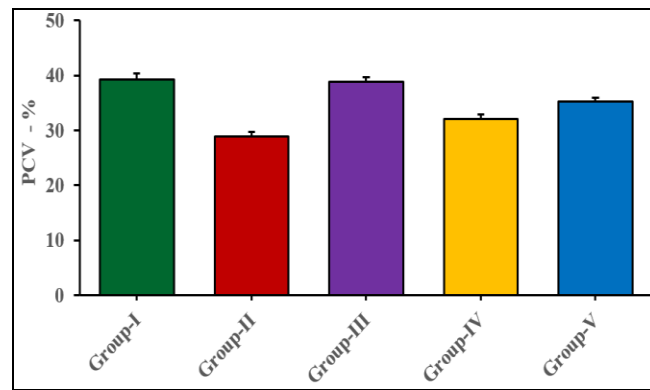
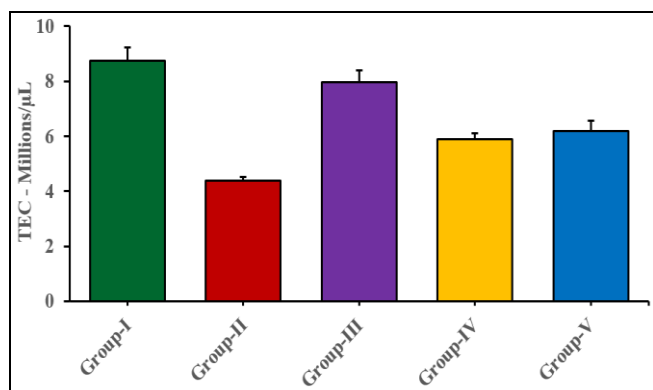
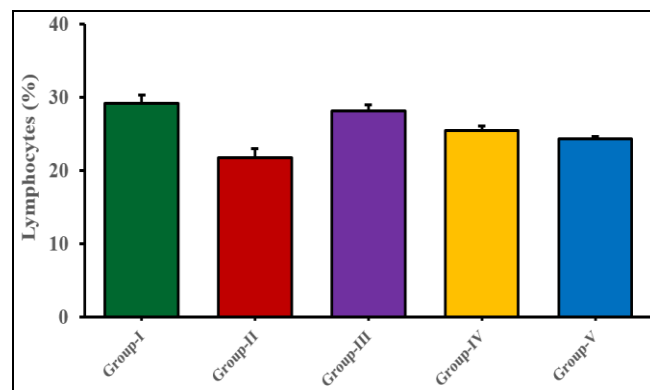
Group	0 th day	7 th day	14 th day
I	20.56±0.32 ^a	21.23±0.36 ^b	21.57±0.31 ^b
II	25.98±0.44 ^c	19.21±0.54 ^a	18.87±0.71 ^a
III	20.61±0.21 ^a	21.07±0.25 ^b	21.77±0.44 ^b
IV	23.58±0.33 ^b	22.92±0.37 ^c	22.11±0.25 ^{bc}
V	24.23±0.69 ^b	23.67±0.79 ^c	23.24±0.45 ^c

Table 3: Absolute kidney weights (g) in different groups of mice

Group	Absolute kidney weights (g)
I	0.32±0.01 ^a
II	0.61±0.04 ^c
III	0.34±0.02 ^a
IV	0.51±0.03 ^{bc}
V	0.48±0.05 ^b

Table 4: Haematological parameters in different groups of mice

Parameter	Group I	Group II	Group III	Group IV	Group V
TEC (Millions/ μ L)	8.74±0.49 ^c	4.39±0.13 ^a	7.97±0.42 ^c	5.89±0.23 ^b	6.18±0.39 ^b
TLC (Thousands/ μ L)	7.87±0.49 ^a	18.24±0.98 ^d	8.14±0.57 ^a	14.93±0.75 ^c	12.63±0.68 ^b
Haemoglobin (g%)	15.78±0.94 ^c	7.16±0.52 ^a	15.13±0.67 ^c	11.91±0.64 ^b	12.83±0.83 ^b
PCV (%)	39.24±1.09 ^d	28.96±0.77 ^a	38.79±0.94 ^d	32.07±0.83 ^b	35.22±0.69 ^c
Neutrophils (%)	56.34±1.07 ^a	75.07±2.03 ^c	53.50±1.19 ^a	66.12±1.76 ^b	63.67±1.20 ^b
Lymphocytes (%)	29.17±1.12 ^c	21.75±1.24 ^a	28.17±0.79 ^c	25.51±0.56 ^b	24.32±0.32 ^b
Monocytes (%)	2.02±0.07 ^a	3.78±0.15 ^d	2.32±0.14 ^a	3.15±0.08 ^c	2.82±0.06 ^b
Eosinophils (%)	2.35±0.09 ^d	1.42±0.05 ^a	2.28±0.07 ^d	1.73±0.05 ^b	1.95±0.06 ^c

**Fig 1:** Weekly body weights (g) in different groups of mice**Fig 4:** Haemoglobin (g%) in different groups of mice**Fig 2:** Absolute kidney weights (g) in different groups of mice**Fig 5:** PCV (%) in different groups of mice**Fig 3:** TEC (Millions/ μ L) in different groups of mice**Fig 6:** Lymphocytes (%) in different groups of mice

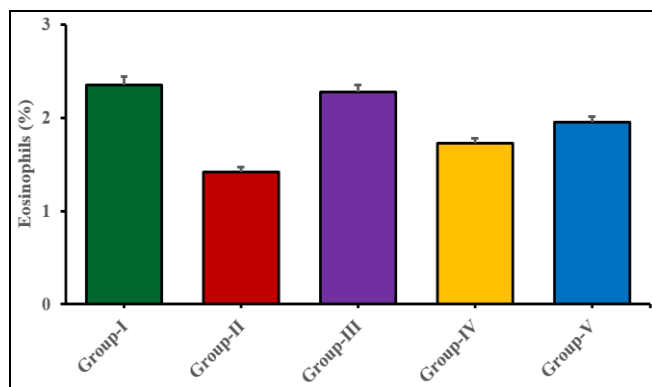


Fig 7: Eosinophils (%) in different groups of mice

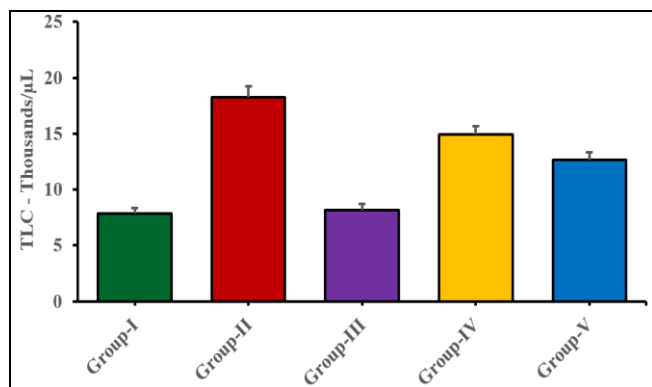


Fig 8: TLC (Thousands/μL) in different groups of mice

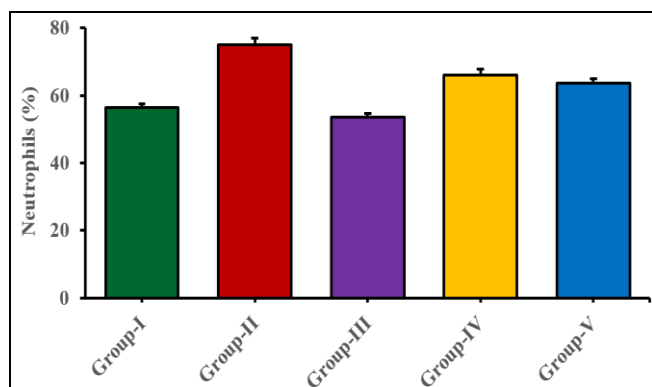


Fig 9: Neutrophils (%) in different groups of mice

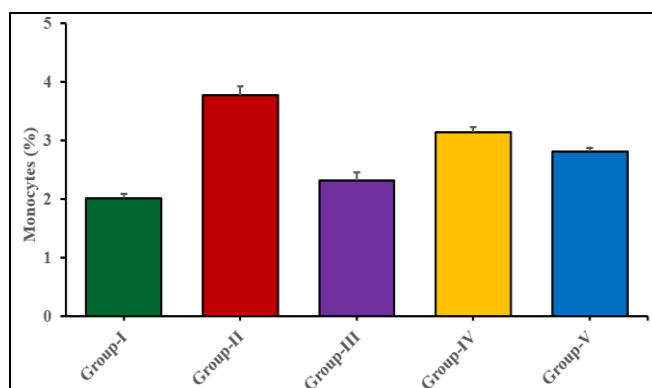


Fig 10: Monocytes (%) in different groups of mice

Discussion

Exposure to high doses of folic acid impaired the animal's physiological status, as evidenced by reduced feed intake, muscle weakness, decreased alertness, and roughening of the coat (Gupta *et al.*, 2012) ^[11]. These clinical signs are

indicative of folic acid-induced acute renal injury, which contributed to the observed decline in body weight. In this study, Group II animals exhibited a significant decline in body weight when compared to other groups. This reduction may be due to decreased feed intake and dehydration caused by renal damage and impaired tubular function. These findings are consistent with earlier reports by Nabae *et al.* (2006) ^[12], Wan *et al.* (2006) ^[13], and Gupta *et al.* (2012) ^[11]. In contrast, treatment with apigenin in Groups IV and V resulted in a marked improvement in body weight when compared to Group II. The beneficial effect may be attributed to the restoration of antioxidant balance and the ability of apigenin to mitigate oxidative stress and inflammation, thereby improving overall physiological status.

The kidney index, expressed as the ratio of kidney weight to body weight, serves as a useful indicator of renal impairment. In the present study, folic acid-treated mice (group II) showed a marked increase in kidney weight compared to other groups, suggesting renal damage. This increase may be due to compensatory hypertrophy and regenerative responses following tissue damage. Earlier findings have also linked increased kidney weight associated with elevated nucleic acid synthesis (Gupta *et al.*, 2012) ^[11]. These observations align with findings reported by Gupta *et al.* (2012) ^[11] and Abdelrahman *et al.* (2022) ^[14]. However, apigenin treatment in Groups IV and V resulted in a decrease in kidney weight, suggesting its protective role, possibly through anti-inflammatory mechanisms.

Folic acid is essential for the synthesis of nucleic acids, amino acids, and proteins (Ratajczak *et al.*, 2021) ^[15]. It also acts as a key cofactor in one-carbon metabolism required for cell growth and proliferation. Deficiency of folate impairs erythropoiesis, causing macrocytic anaemia, neurological complications such as peripheral neuropathy and increased risk of neural tube defects (NTD) in the foetus (Yan, 2021) ^[16]. However, high doses of folic acid can be nephrotoxic, leading to acute renal failure characterized by crystal formation in renal tubules, tubular epithelial damage, and cortical scarring (Gupta *et al.*, 2012) ^[11]. In the current investigation, FA administration caused a marked ($p < 0.05$) decline in TEC, Hb, PCV, lymphocytes and eosinophils, along with a rise in TLC, neutrophils and monocytes in group II when compared to group I. This reduction in group II haematological parameters may be attributed to impaired renal tissue producing insufficient erythropoietin (EPO), a key hormone for stimulating erythropoiesis in the bone marrow. These results are in alignment with the findings of Estrela *et al.* (2021) ^[17] and Divya *et al.* (2025) ^[18]. Haematological alterations could be attributed to enhanced oxidative stress, leading to increased RBC membrane permeability and lipid peroxidation. The significant rise in TLC, neutrophils and monocytes reflect ROS-mediated inflammation, that suggests activation of the host defense system and recruitment of inflammatory cells (Sravathi *et al.*, 2022) ^[19]. In contrast, apigenin-treated groups (Group IV and V) have shown significant ($p < 0.05$) improvement in haematological values, suggesting apigenin has beneficial role on homeostasis of hematopoietic system.

Conclusion

In conclusion, the present study revealed that folic acid (@ 250 mg/kg, IP) administration resulted in a significant alteration in body weights, kidney weights and

haematological parameters. However, apigenin-treated groups (10 and 20 mg/kg, IP) along with folic acid (@ 250 mg/kg, IP) significantly improved these parameters, indicating its protective effects. The improvement was more pronounced at the higher dose (20 mg/kg, IP), suggesting a dose-dependent effect.

Acknowledgements

The authors are thankful to the College of Veterinary Science, P. V. Narsimha Rao Telangana Veterinary University (PVNRTVU), Rajendranagar, Hyderabad for providing the necessary facilities to carry out the research work.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Jiang M, Bai M, Lei J, Xie Y, Xu S, Jia Z, *et al.* Mitochondrial dysfunction and the AKI-to-CKD transition. *Am J Physiol Renal Physiol.* 2020;319(6):F1105-F1116.
- Stallons LJ, Whitaker RM, Schnellmann RG. Suppressed mitochondrial biogenesis in folic acid-induced acute kidney injury and early fibrosis. *Toxicol Lett.* 2014;224(3):326-332.
- Yang Y, Wang P, Zhou K, Zhang W, Liu S, Ouyang J, *et al.* HUWE1-mediated degradation of MUTYH facilitates DNA damage and mitochondrial dysfunction to promote acute kidney injury. *Adv Sci.* 2025;12(13):2412250-2412250.
- Rizwan F, Yesmine S, Banu SG, Chowdhury IA, Hasan R, Chatterjee TK. Renoprotective effects of stevia (*Stevia rebaudiana* Bertoni), amlodipine, valsartan, and losartan in gentamycin-induced nephrotoxicity in the rat model: biochemical, hematological and histological approaches. *Toxicol Rep.* 2019;6:683-691.
- Sadek SA, Hassanein SS, Mohamed AS, Soliman AM, Fahmy SR. Echinochrome pigment extracted from sea urchin suppresses bacterial activity, inflammation, nociception, and oxidative stress resulting in inhibition of renal injury in septic rats. *J Food Biochem.* 2022;46(3):e13729.
- Silva C, Keating E, Pinto E. The impact of folic acid supplementation on gestational and long-term health: critical temporal windows, benefits and risks. *Porto Biomed J.* 2017;2(6):315-332.
- Nikolic T, Petrovic D, Matic S, Turnic TN, Jeremic J, Radonjic K, *et al.* The influence of folic acid-induced acute kidney injury on cardiac function and redox status in rats. *Naunyn Schmiedeberg Arch Pharmacol.* 2020;393(1):99-109.
- Fang Y, Yang C, Yu Z, Li X, Mu Q, Liao G, *et al.* Natural products as LSD1 inhibitors for cancer therapy. *Acta Pharm Sin B.* 2021;11(3):621-631.
- Fossatelli L, Maroccia Z, Fiorentini C, Bonucci M. Resources for human health from the plant kingdom: the potential role of the flavonoid apigenin in cancer counteraction. *Int J Mol Sci.* 2023;25(1):251-251.
- Malik S, Suchal K, Khan SI, Bhatia J, Kishore K, Dinda AK, *et al.* Apigenin ameliorates streptozotocin-induced diabetic nephropathy in rats via MAPK-NF- κ B-TNF- α and TGF- β 1-MAPK-fibronectin pathways. *Am J Physiol Renal Physiol.* 2017;313(2):F414-F422.
- Gupta A, Puri V, Sharma R, Puri S. Folic acid induces acute renal failure (ARF) by enhancing renal prooxidant state. *Exp Toxicol Pathol.* 2012;64(3):225-232.
- Nabae K, Doi Y, Takahashi S, Ichihara T, Toda C, Ueda K, *et al.* Toxicity of di(2-ethylhexyl) phthalate (DEHP) and di(2-ethylhexyl) adipate (DEHA) under conditions of renal dysfunction induced with folic acid in rats: enhancement of male reproductive toxicity of DEHP is associated with an increase of the mono-derivative. *Reprod Toxicol.* 2006;22(3):411-417.
- Wan B, Hao L, Qiu Y, Sun Z, Cao Q, Zhang Y, *et al.* Blocking tumor necrosis factor- α inhibits folic acid-induced acute renal failure. *Exp Mol Pathol.* 2006;81(3):211-216.
- Abdelrahman RS, Abdelsalam RA, Zaghloul MS. Beneficial effect of trimetazidine on folic acid-induced acute kidney injury in mice: role of HIF-1 α /HO-1. *J Biochem Mol Toxicol.* 2022;36(5):e23011.
- Ratajczak AE, Szymczak-Tomczak A, Rychter AM, Zawada A, Dobrowolska A, Krela-Kazmierczak I. Does folic acid protect patients with inflammatory bowel disease from complications? *Nutrients.* 2021;13(11):4036-4036.
- Yan LJ. Folic acid-induced animal model of kidney disease. *Anim Models Exp Med.* 2021;4(4):329-342.
- Estrela GR, Freitas-Lima LC, Budu A, Arruda AC, Perilhão MS, Fock RA, *et al.* Chronic kidney disease induced by cisplatin, folic acid and renal ischemia reperfusion induces anemia and promotes GATA-2 activation in mice. *Biomedicines.* 2021;9(7):769-769.
- Divya K, Kumar Y, Swathi B, Kumar B, Chandrika P, Ashwini K, *et al.* Protective effect of umbelliferone in folic acid-induced hematological toxicity in male C57BL/6. *J Adv Biol Biotechnol.* 2025;28(1):469-475.
- Sravathi V, Jeevanalatha M, Ravikumar Y, Gopalareddy AT, Chandravathi BS, Lakshman M. 5-fluorouracil-induced hematological alterations in Wistar rats and its amelioration with naringenin. 2022;4:12-12.