

PROTECTIVE EFFECT OF UMBELLIFERONE INFOLIC ACID-INDUCED HEMATOLOGICAL TOXICITY IN MALE C57BL/6

ABSTRACT

Aims: Folic acid, also referred to as vitamin B9, functions as a catalyst in mono-carbon metabolism, playing a pivotal position in supporting cellular growth and proliferation. Although beneficial, excessive amounts of folic acid can negatively impact hematological health. Herbal remedies are acquiring accelerated popularity in both developed and developing countries, primarily because they are affordable and have fewer complications. Umbelliferone (UMB), known by the name of 7-hydroxycoumarin, is a pharmacologically active compound derived from coumarin. This yellowish-white, crystalline substance found in various plants, exhibiting a wide array of potency. Using in vivo experimental models, the study sought to explore the beneficial effects of UMB on haematotoxicity caused by folic acid, with a particular emphasis on examining important haematological parameters.

Frame work of the study: Twenty-four adult C57BL/6 mice were randomly assigned to four groups. Group 1 served as the placebo control. Group 2 was administered folic acid at a dose of 250mg/kg through intraperitoneum on first day to induce toxicity. Group 3 received only UMB at a dose of 60mg/kg body weight through oral route for 14 days. Group 4 was given folic acid on first day and concurrently treated with UMB for 14 days. After 14-day period, hemotological parameters including Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Haemoglobin (Hb) concentration, Packed Cell Volume/Haematocrit (PCV/Hct), and Mean Corpuscular Volume (MCV), were meticulously evaluated.

Results: Analysis of haematological parameters revealed a significant ($p < 0.05$) decrease in the mean values of TEC, Hb, PCV, MCV, and lymphocytes, along with a marked increase in TLC and neutrophils in group 2 mice compared to other groups. No significant ($p < 0.05$) differences were found between groups 1 and 3. Group 4 showed a mild to moderate improvement in all parameters compared to group 3.

Conclusion: This study demonstrates that unambiguously revealed that UMB provides safeguarding counter to haematological toxicity induced by folic acids suggesting its potential as a therapeutic agent.

Keywords: phytochemicals, one carbon metabolism, oxidative stress, neutrophiles

1. INTRODUCTION

Folates, water-soluble derivatives of vitamin B9, serve as Methyl donors in the Cellular synthesis of thymidylate and purines (1). Yeast, green vegetables, animal liver and egg yolks are sources of folic acid (2). Folic acid is essential for rapidly growing tissues, including foetal development, blood-forming organs and epithelial cells (3). Due to its key contribution in the synthesis of RNA and DNA components, as well as in DNA methylation, blocking the folate pathway is considered one of the most potent strategies for anti-cancer therapy (4). In spite folic acid supplementation is worthwhile for health, Elevated doses can cause harmful effects on various organs, particularly the kidneys and liver (5). Folic acid is essential for many functions, but excessive intake can mask vitamin B12 deficiency, disrupt methylation, and affect red blood cell production. It may also deplete serum holotranscobalamin, further reducing vitamin B12 availability for critical metabolic processes (6). Serum folate concentrations below 0.005 $\mu\text{g/mL}$ are considered indicative of folate deficiency, while

levels below 0.002 µg/mL typically lead to megaloblastic anaemia. Folates generally exhibit low toxicity, with high LD50 values. The mean LD50 for intraperitoneally (i.p.) administered folic acid ranges from 85 to 330 mg/kg body weight (b.wt.) across different mouse strains (7). Phytoconstituents, or phytochemicals, are commonly utilized in traditional medicinal practices and are obtained from plant sources (8). On a global scale, numerous phytochemicals and folk remedies, along with their origins, are being studied to verify their effectiveness in alleviating a wide range of afflictions (9). Coumarins are natural phenolic substances widely present in numerous medicinal plants (10). Umbelliferone (UMB) is a coumarin compound recognized for its antioxidant properties and potent anti-inflammatory properties (11). Umbelliferone is naturally derived from various plant sources, including coriander (*Coriandrum sativum*), carrot (*Daucus carota*), and garden angelica (*Angelica archangelica*) (12). The therapeutic profile of umbelliferone is notable for its antidiabetic, anti-inflammatory, anti-nociceptive, anticancer, as well as hepato and nephroprotective attributes (13), highlighting its impact in clinical studies and drug discovery. Haematological examination is an essential tool for evaluating the harmful effects of drugs on living organisms. It provides crucial information about metabolites and other components within the body, playing a key role in assessing the animal's physiological, nutritional, and pathological condition (14).

2. MATERIAL AND METHODS

2.1 Animals:

A total of 24 C57BL/6 mice, weighing around 20-25 g, aged 8-12 weeks, were procured from National Institute of Nutrition, Hyderabad. The animals were maintained with an temperature ranging from 22 to 24°C and relative humidity of 50-55% with 12-hour light/dark cycle in propylene cages with each cage containing 3 animals. The mice were allowed a one-week habituation period before the trial commenced. During the study, the animals were given a regular commercial sterilized pellet diet and had accessory to water at leisure. The Institutional Animal Ethics Committee certified the experimental method with approval no (02/28/C.V.Sc., Hyd. IAEC). The mice were equally allocated into four groups, each containing six mice, and the experiment lasted for 14 days. Each group received the assigned treatment according to the schedule outlined below.

2.2 Experimental Design:

Group	Treatments
G-I	Placebo control (Normal saline P/O for 14 days).
G-II	Folic acid (@ 250 mg/kg) I/P single dose on the 1 st day of experiment.
G-III	Umbelliferone per se (@60 mg/kg) P/O daily for 14 days.
G-IV	Folic acid (@ 250 mg/kg) I/P single dose on the 1 st day + Umbelliferone (@60 mg/kg) P/O daily for 14 days.

2.2 Sample collection and analysis:

On 15th day the experimental rodents were fasted for 12 hours. Blood was aseptically collected from the retro-orbital plexus of each animal using a capillary tube and transferred into K3-EDTA vacutainers (13 mm x 75 mm, 4 mL, Rapid Diagnostics Pvt. Ltd., Delhi). This ensures the blood is preserved in an anticoagulated state until further analysis. The collected blood samples were utilized for Total Erythrocyte Count (TEC-Millions/µL), Haemoglobin (Hb-g %) concentration, Packed Cell Volume/Haematocrit (PCV/Hct-percent) and Mean Corpuscular volume (MCV-fL), Total Leukocyte Count (TLC-Thousands/µL), lymphocytes and neutrophils by using automatic whole blood analyzer (Huma count, Med Source Ozone Biomedical Pvt. Ltd., Faridabad, Haryana) and the results were organized in a systematic table for subsequent statistical analysis.

2.3 Statistical analysis

The data collected were analyzed quantitatively using one-way analysis of variance (ANOVA) with GraphPad Prism 5, version 5.01. (GraphPad Software, California, USA). To discern differences between means, Tukey's test, a multiple comparison procedure, was employed, with the significance level set at $p < 0.05$ (15).

3. Results

Analysis of haematological parameters revealed a significant decline in TEC, Hb, PCV, MCV and lymphocytes in group II when compared to other groups, however there is considerable increase in TLC and neutrophil count in group II. Statistically, no significant ($P < 0.05$) difference observed between group I and III. A moderate improvement in all these parameters observed in group IV. Any substance that is new to the body is recognized as an antigen, prompting the body to respond and adapt. While umbelliferone is generally considered a safe product, its introduction to the animal results in slight changes in parameters when compared to group 1, even though these changes are statistically non-significant.

3.1 Graphical representation of effect of UMB on various haematological parameters

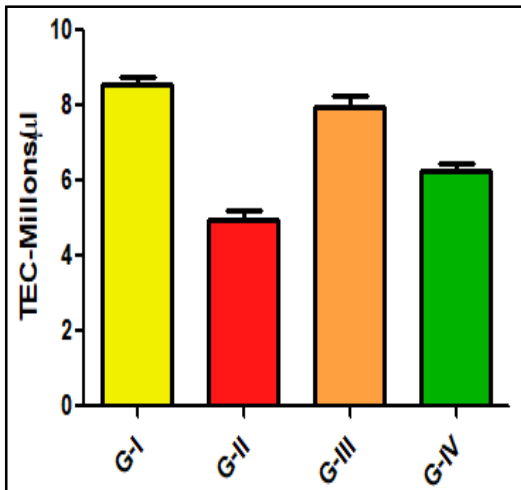


Fig 1: Ameliorative effect of Umbelliferone on Total Erythrocyte count (TEC)

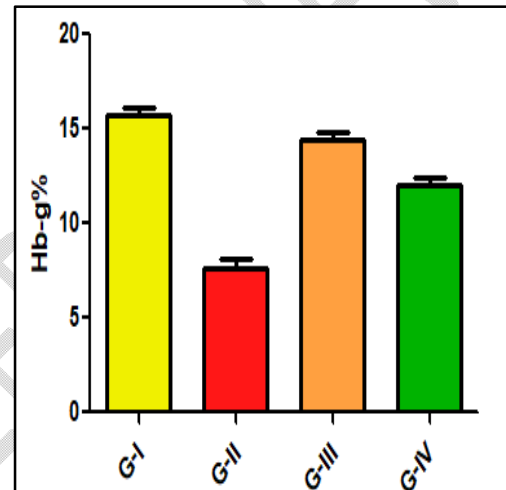


Fig 2: Ameliorative effect of Umbelliferone on Haemoglobin concentration

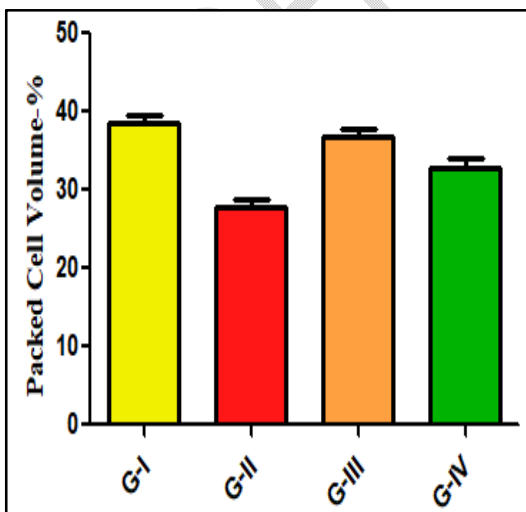


Fig 3: Ameliorative effect of Umbelliferone on packed cell volume (PCV)

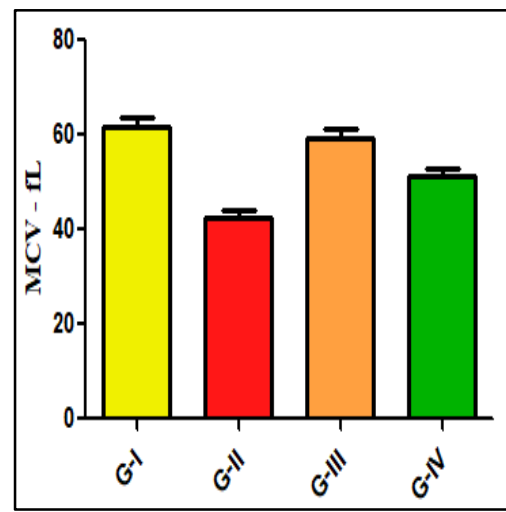


Fig 4: Ameliorative effect of Umbelliferone on mean cell volume (MCV)

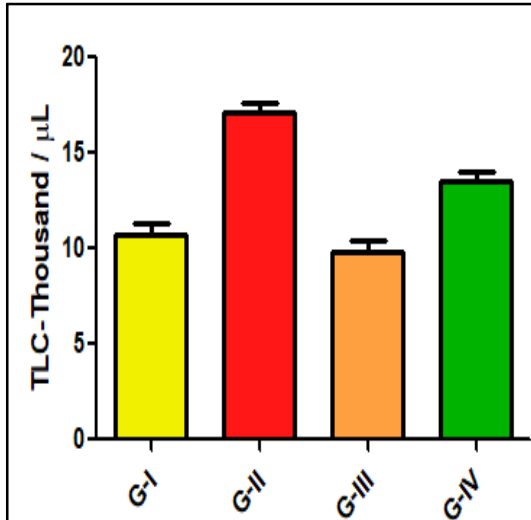


Fig 5: Ameliorative effect of Umbelliferone on total leukocyte count (TLC)

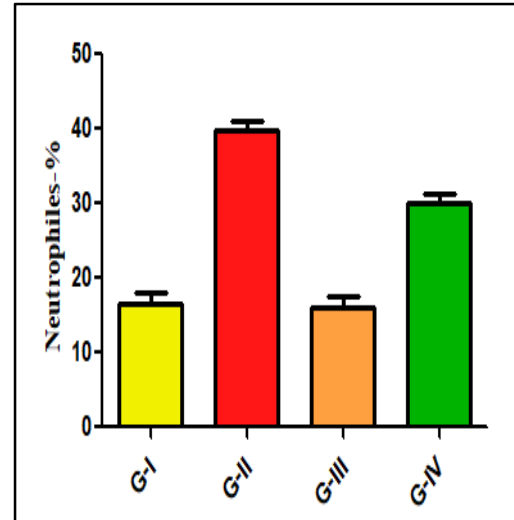


Fig 6: Ameliorative effect of Umbelliferone on neutrophiles (%)

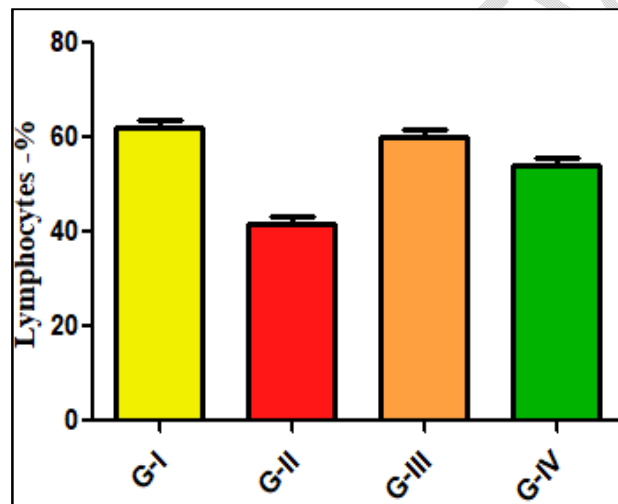


Fig 7: Ameliorative effect of Umbelliferone on lymphocytes (%)

4. DISCUSSION

Blood is a vital and adaptive component of body that serves as a reflection of the body's overall health, providing insights into both normal physiological processes and the presence of disease or dysfunction (16). Haematological assessments play a crucial role in tracking disease progression and evaluating treatment efficacy (17). Changes in blood parameters frequently manifest earlier than clinical symptoms, offering an early indication of underlying health conditions. In the present study, a notable ($P < 0.05$) decreased mean values of TEC, Hb, PCV, MCV, Lymphocytes and increased mean values of TLC and neutrophils were observed in group II when compared with group I. These results align with and are supported by the findings of earlier studies(18). Excessive folic acid disrupts the one-carbon metabolism pathway, causing imbalance in purine and pyrimidine synthesis, which subsequently interferes with normal haematopoiesis. Elevated folic acid levels can interfere with Regular metabolic activities, possibly leading to mild inflammation or an increase in oxidative stress (19). This disruption can impact various biological mechanisms, leading to the activation of the immune system which may trigger activation of inflammatory pathways. This inflammatory condition can trigger the release of cytokines, which enhance the production and mobilization of neutrophils from the bone marrow (20). The significant ($p < 0.05$) increase in the mean values of TLC and

neutrophils may be attributed to the production of reactive oxygen species (ROS), reflecting a heightened inflammatory response that activates the animal's defense mechanism, resulting in the influx of inflammatory cells (21). It is indicative of leukocytosis, which is attributed to enhanced immune function to protect the animals from folic acid-induced toxicity(22). In comparison, Group IV mice exhibited a significant rise in TEC, Hb, PCV, MCV, and lymphocyte levels, along with a decrease in the mean values of TLC and neutrophils compared to Group II. This improvement is likely attributed to the compound's antioxidant and anti-inflammatory properties (23). The antioxidant property of UMB is through upregulation of nrf2 pathway(24). The anti-inflammatory effects of umbelliferone are primarily mediated through the inhibition of the NF- κ B pathway(10).

5. CONCLUSION

In conclusion, folic acid leads to a marked decrease in TEC, Hb, PCV, MCV, and lymphocyte levels, along with reductions in TLC and neutrophil counts. In Group IV mice, supplementation with umbelliferone led to a significant ($P < 0.05$) increase in TEC, Hb, PCV, and MCV, along with a significant ($P < 0.05$) decrease in TLC levels when compared to Group II mice. The current study demonstrated the protective effect of umbelliferone in mitigating toxicity caused by folic acid. It can be concluded that umbelliferone alleviates cytokine storms and oxidative stress by suppressing the activation of the NF- κ B signalling pathway while enhancing the regulation of the Nrf-2 pathway. To deepen our understanding and mitigate haematotoxicity, as well as improve therapeutic outcomes, further in-depth studies on the mechanisms of folic acid-induced haematotoxicity are crucial. Understanding the mechanism of folic acid toxicity is an important area of research, especially in relation to its metabolism and potential effects at high doses.

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2.

3.

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