PROTECTIVE IMPACT OF UMBELLIFERONE AGAINST FOLIC ACID-INDUCED HEMATOLOGICAL TOXICITY IN MICE

Comment [AN1]: Gender & strain of animals to be mentioned

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.

Methodology: Adult male C57BL/6 mice were administered folic acid at a dose of 250 mg/kg body weight on the 1st day to elicit toxicity, while UMB was concurrently given at a dosage of 60 mg/kg body weight. Haematological 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 methodically appraised.

Results: Hematology results showed that in folic acid treated group a significantreduction in TEC, Hb, PCV, MCV, and lymphocytes, along with an increase in total leukocyte count (TLC) and neutrophils, was observed.UMB treated group showed improvement in all these parameters.

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

Comment [AN2]: Study design, number of animals, number of groups to be mentioned

Keywords: Folic acid, haematotoxicity, umbelliferone, therapeutic

Comment [AN3]: Keywords repeated from title. Kindly refer MESH terms

1. INTRODUCTION

Folates, water-soluble derivatives of vitamin B9, serve as Methyl donors in the Cellular synthesis of thymidylate and purines (1). 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 (2). In spite folic acid supplementation is worthwhile for health, Elevated doses can cause harmful effects on various organs, particularly the kidneys and liver (3).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 (4). Umbelliferone, a coumarin variant present in edible fruits such as golden apple, bitter orange, and banana, has been universally analyzed for its antioxidant traits (5). The therapeutic profile of umbelliferone is notable for its antidiabetic (6), anti-inflammatory, anti-nociceptive (7), anticancer (8), as well as hepato (9) and nephro(10) protective attributes, highlighting its impact in clinical studies and drug discovery.

Comment [AN4]: References can be put at the end of sentence.

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 housed in polypropylene cages, kept on a 12-hour light/dark cycle in the animal facility, maintained under clean conditions with an ambient

Comment [AN5]: Number of animals per cage needs to be mentioned

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temperature ranging from 22 to 24°C. 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	Sham (Normal saline P/O for 14 days).
G-Ⅱ	Folic acid (@ 250 mg/kg) I/P single dose on the 1st 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 1st day + Umbelliferone (@60 mg/kg) P/O daily for 14 days.

Comment [AN7]: Is any procedure done on animals? If not then it is a placebo control

2.2 Sample collection and analysis:

Before blood collection, 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.

Comment [AN8]: When was blood collection was done?

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 $p<0.05$(11).

3. Results

Analysis of haemotological parameters revealed a significant decline in TEC, Hb, PCV, MCV and lymphocytes, 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.

Table 1: Effect of umbelliferone on haematological parameters

Comment [AN9]: Same data no need to represent in table as well as figures (Graphical representation). It is considered as Repetition of data

Parameter	Group-I	Group-Ⅱ	Group-Ⅲ	Group-Ⅳ
TEC (Millions/µL)	8.58±0.29	4.93±0.18	7.93±0.30	6.25±0.19
Hb (g percent)	15.70±0.37	7.65±0.37	14.45±0.34	11.97±0.42
PCV/Hct (percent)	38.50±0.96	27.83±0.87	36.90±0.99	32.90±1.04

MCV (fL)	61.72±1.85	42.50±1.45	59.45±1.71	51.20±1.71
TLC (Thousands/µL)	10.67±0.66	17.08±0.57	9.80±0.61	13.57±0.46
Neutrophils (%)	16.67±1.40	39.67±1.33	16.00±1.48	30.17±1.20
Lymphocytes (%)	62.00±1.61	41.83±1.50	60.17±1.50	54.00±1.63

3.1 Graphical representation of effect of UMB on various haematological parameters

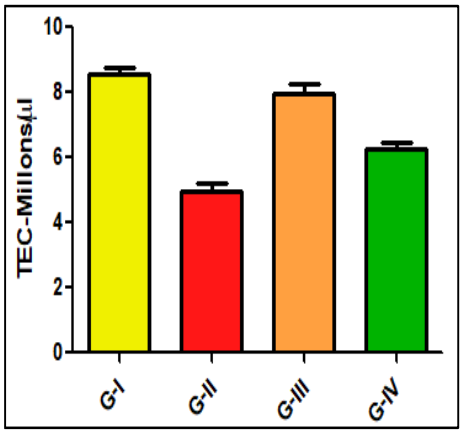

Fig 1: Ameliorative effect of Umbelliferone on Total Erythrocyte count (TEC)

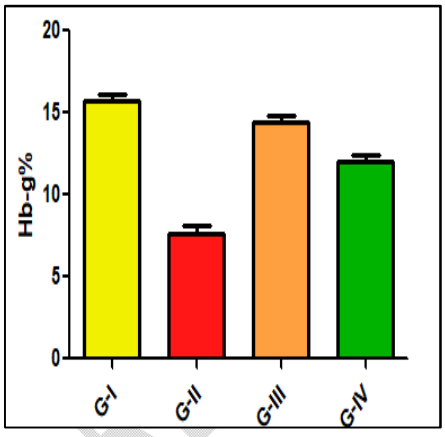

Fig 2: Ameliorative effect of Umbelliferone on Haemoglobin concentration

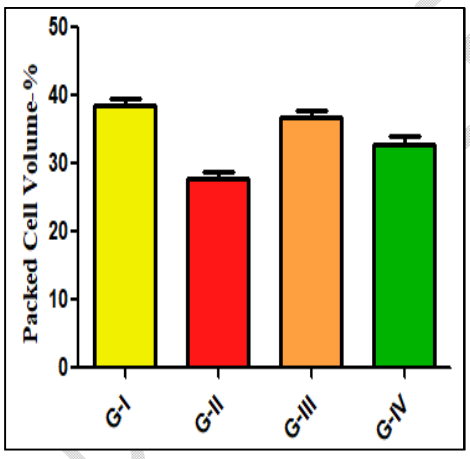

Fig 3: Ameliorative effect of Umbelliferone on packed cell volume (PCV)

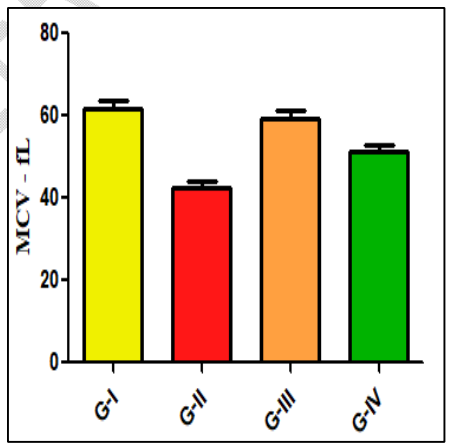

Fig 4: Ameliorative effect of Umbelliferone on mean cell volume (MCV)

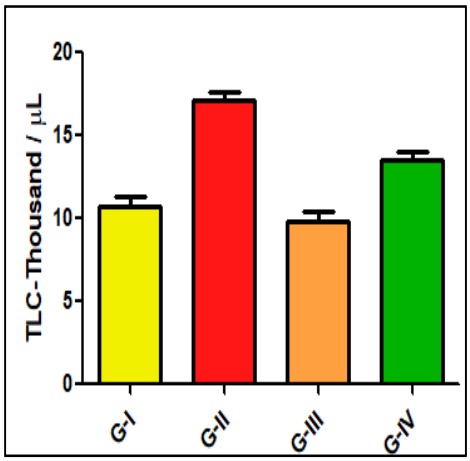

Fig 5: Ameliorative effect of Umbelliferone on total leukocyte count (TLC)

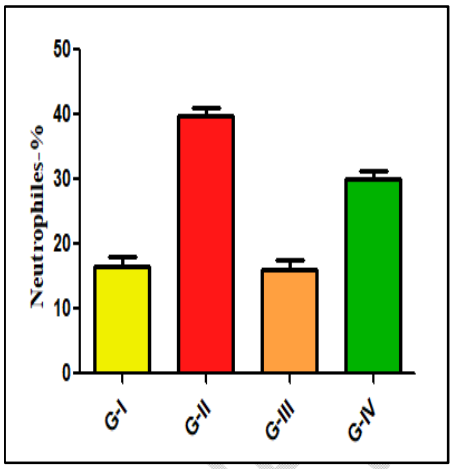

Fig 6: Ameliorative effect of Umbelliferone on neutrophiles (%)

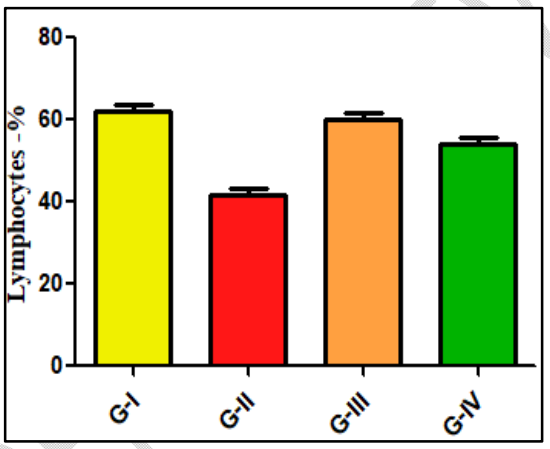

Fig 7: Ameliorative effect of Umbelliferone on lymphocytes (%)

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 (12). Haematological assessments play a crucial role in tracking disease progression and evaluating treatment efficacy. 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 neutrophiles were observed in group Ⅱ when compared with group Ⅰ. These results align with and are supported by the findings of earlier studies Vagdevi *et al*., 2024 and Henry *et al*., 1985 (13 and 14). 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. 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 (13). In comparison, Group IV mice exhibited a

Comment [AN10]: Add about why only male *C57BL/6* mice were used?

Comment on mechanism of Umbelliferone in discussion.

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Add about strengths & limitations of the study.

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 (15).

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-κB signalling pathway while enhancing the regulation of the Nrf-2 pathway.

REFERENCES

Comment [AN11]: Need 80-90 % recent (Last 5 Years) references.

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