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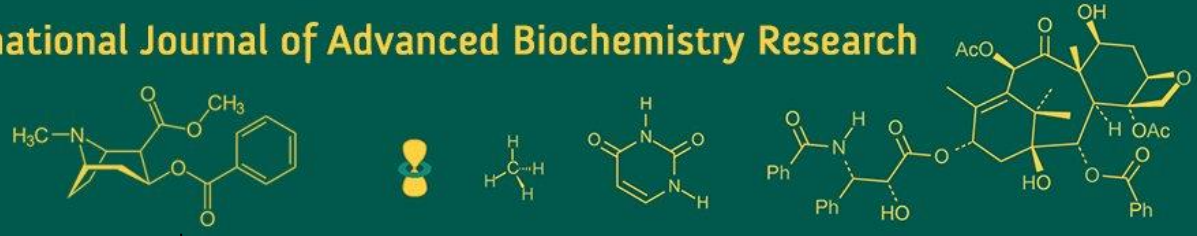

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Folic acid induced Haematotoxicity in mice and its amelioration with Ferulic acid

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Abstract
Folic acid or Folate, an essential B-vitamin, plays a pivotal role in various metabolic processes, including DNA synthesis and gene expression regulation. But excessive intake of folic acid has been attributed to several detrimental health effects. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is a phenolic compound having wide range of potency. The protective effect of ferulic acid against haematotoxicity induced by folic acid was assessed in the current study. Utilizing *in vivo* experimental designs, the study aimed to elucidate the ameliorative impact of ferulic acid on haematotoxicity induced by folic acid, focusing on the analysis of key haematological parameters. Adult male C57BL/6 mice were administered folic acid at a dose of 250 mg/kg body weight for a period of 14 days to induce toxicity, while ferulic acid was concurrently administered at a dosage of 100 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 meticulously assessed. Significant (p<0.05) decrease in TEC, Hb, PCV, MCV, lymphocytes and increase in Total leukocyte count (TLC) and neutrophils were observed. The findings of this investigation strongly suggest that ferulic acid exerts substantial mitigating effects against folic acid-induced haematotoxicity.

Keywords: Folic acid, DNA, Ferulic acid, haematotoxicity, ameliorative

Introduction
Folic acid (B_9) serves as a water-soluble vitamin crucial for multiple metabolic pathways, encompassing DNA synthesis and the modulation of gene expression (Patel and Sobczynska-Malefora, 2017) [1]. Despite being nutritionally beneficial, high dose of folic acid supplementation can elicit detrimental effects on different body organs, especially kidney and liver (Al Shawoush *et al*., 2022) [2]. Folic acid-induced acute kidney injury (AKI) is a classic model characterized by tubular cell death and tubulointerstitial inflammatory cell infiltration. Oxidative stress, apoptosis, inflammation, ferroptosis which leads to necrosis (Hu *et al*.,2019) [3] and increased expression of fibroblast growth factor 23 (FGF 23) are the central mediators in Folic acid (FA) induced acute kidney injury and chronic kidney disease which results due to renal inflammation and renal fibrosis (Yan *et al*., 2021) [4]. Folic acid is bio transformed to 5-methyl-tetrahydrofolic acid in hepatic portal vein which is responsible for hepatotoxicity (Elrouby *et al*., 2021) [5].
Phytoconstituents, also referred to as phytochemicals, are widely embraced in traditional medicinal systems and are derived from botanical sources (Basist *et al*., 2022) [6]. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is a phenolic compound formed during tyrosine and phenylalanine metabolism and is mostly found in wheat, barely, banana, tomato, citrus fruits, coffee and berries (Roghani *et al*., 2022) [7]. Ferulic acid has a wide range of potency, including anti- inflammatory (Mir *et al*., 2018) [8], antioxidant, antidiabetic, anticarcinogenic, antiapoptotic, antiaging, hepatoprotective, pulmonary protective, antiatherogenic, hypotensive and vasodilation effects (Erseckin *et al*., 2022) [9]. Notably, ferulic acid demonstrates significant antifibrotic effects on liver, renal, and pulmonary fibrosis (Li *et al*., 2021) [10].

Materials and Methods

Chemicals: Folic acid was procured from Sigma-Aldrich Limited (CAS: 59-30-3) and Ferulic acid was procured from Sisco Research Laboratories Pvt. limited., Maharashtra (CAS:1135-24-6).

Experimental Animals

A total of 24 male C57BL/6 mice weighing between 25-30 g were procured from Vyas Labs, Hyderabad. The experimental protocol was approved by Institutional Animal Ethics Committee, C.V.Sc., Rajendranagar, Hyderabad (08 /26/CVSc, Hyd. IAEC /2023). The mice were housed in solid bottom polypropylene cages at a laboratory animal house facility. All the animals were maintained in a controlled environment (20-22 ℃), with provision of sterile corn cob as a standard bedding material, a standard pellet diet and deionized water *ad libitum* throughout the experimental period. Prior to experiment, all animals were allowed to acclimatize for about 7 days.

Experimental design

Table 1: Experimental design with group wise treatment protocol

Groups	Treatment	No. of animals
G1	Sham (Normal saline for 14 days)	6
G2	Ferulic acid *per se* (@ 100 mg/kg) P/O daily for 14 days	6
G3	Folic acid (@ 250 mg/kg) I/P single dose on the 1st day of experiment.	6
G4	Folic acid (@ 250 mg/kg) I/P single dose on the 1st day + Ferulic acid (@100 mg/kg) P/O daily for 14 days	6

Sample collection and analysis

Six (6) mice from each group were sacrificed on 15th day of experiment. At the time of sacrifice, blood samples (0.25 to 0.5 mL) were aseptically collected from the retro-orbital plexus using a capillary tube and deposited into anticoagulant-coated vacutainers (K3-EDTA tube, 13 mm x 75 mm, 4 mL, Rapid Diagnostics Pvt. Ltd., Delhi). This facilitated the preservation of blood in an anticoagulated state for subsequent hematological analyses. Prior to blood collection, the experimental rodents underwent a 12-hour fasting period. All the blood samples were used for estimation of Total Erythrocyte Count (TEC-Millions/µL), Total Leukocyte Count (TLC-Thousands/µL), Hemoglobin (Hb-g %) concentration, Packed Cell Volume/Haematocrit (PCV/Hct-percent) and Mean Corpuscular volume (MCV-fL), lymphocytes and neutrophils by using automatic whole blood analyzer (Huma count, Med Source Ozone Biomedical Pvt. Ltd., Faridabad, Haryana) and results were systematically tabulated for subsequent statistical analysis.

Statistical analysis

Data obtained were subjected to statistical analysis by applying one-way analysis of variance (ANOVA) using 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 $p<0.05$ (Snedecor and Cochran, 1994) [11].

Results

Analysis of hematological parameters revealed a significant ($p<0.05$) reduction in mean values of TEC (Fig 1), Hb (Fig 2), PCV (Fig 4), MCV (Fig 5) and lymphocytes (Fig 6) accompanied by a substantial increase increase in TLC (Fig 3) and neutrophils (Fig 7) in group 3 mice when compared to group 1 mice. Statistically, no significant ($p<0.05$) difference was observed between the groups 1 and 2. A mild to moderate improvement in all the parameters was observed in group 4 in comparison to group 3 mice.

Table 2: Effect of Ferulic acid on haematological parameters

Parameter	Group 1	Group 2	Group 3	Group 4
TEC (Millions/µL)	8.13±0.48	8.06±0.45	5.16±0.23	6.38±0.21
Hb (g percent)	15.42±0.70	14.62±0.71	7.73±0.96	10.57±0.83
TLC (Thousands/µL)	7.4 ±0.89	8.05 ±0.65	17.43 ±1.60	12.07 ±0.52
PCV/Hct (percent)	38.67±0.88	38.50±1.25	26.72±1.42	33.33±0.88
MCV (fL)	65±2.82	63.83±2.72	41.27±3.07	52.93±2.02
Lymphocytes (%)	78.83±1.64	78±1.52	46.0±0.88	64.67±1.35
Neutrophils (%)	15.83±1.42	17.50±1.25	33.0±1.52	25.67±0.88

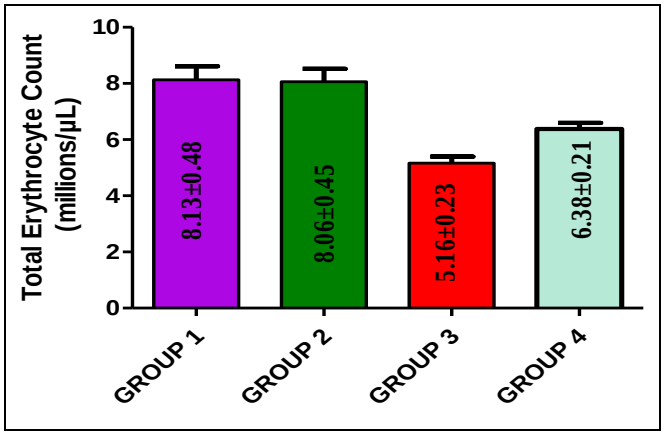

Fig 1: Ameliorative effect of Ferulic acid on Total Erythrocyte count (TEC)

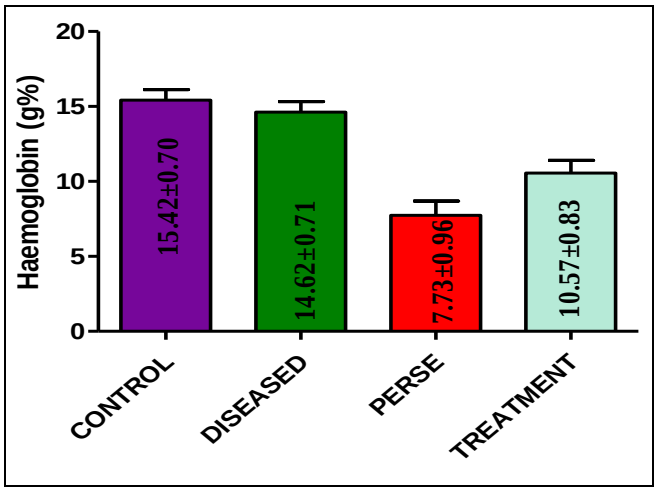

Fig 2: Ameliorative effect of Ferulic acid on Haemoglobin concentration

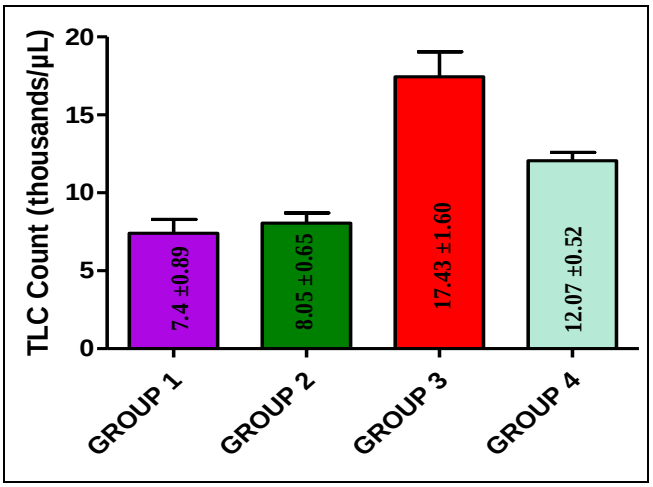

Fig 3: Ameliorative effect of Ferulic acid on Total leukocyte count (TLC)

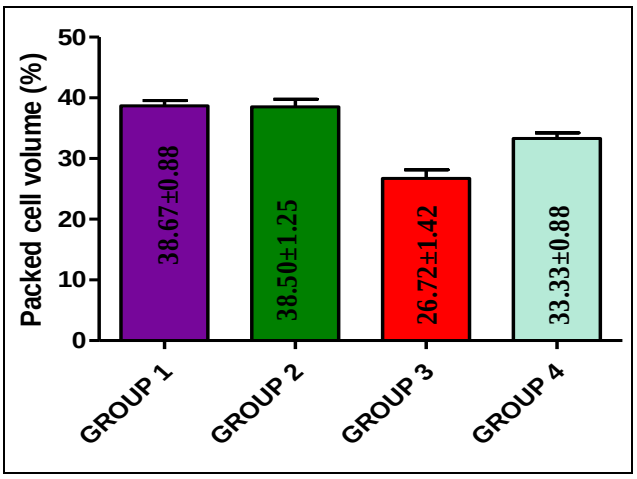

Fig 4: Ameliorative effect of Ferulic acid on Packed cell volume (PCV)

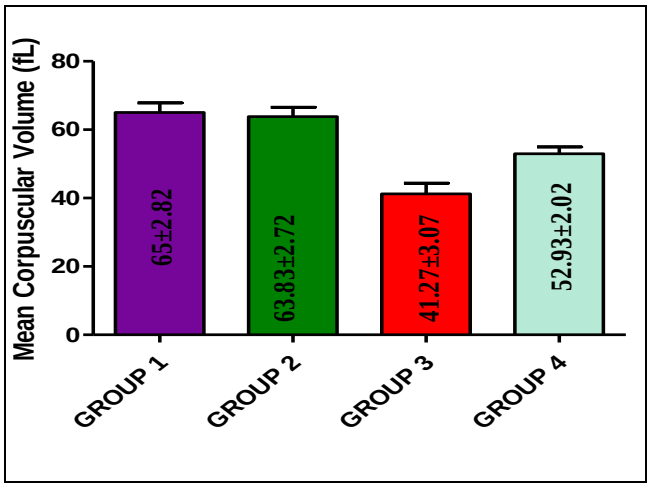

Fig 5: Ameliorative effect of Ferulic acid on Mean Corpuscular Volume (MCV)

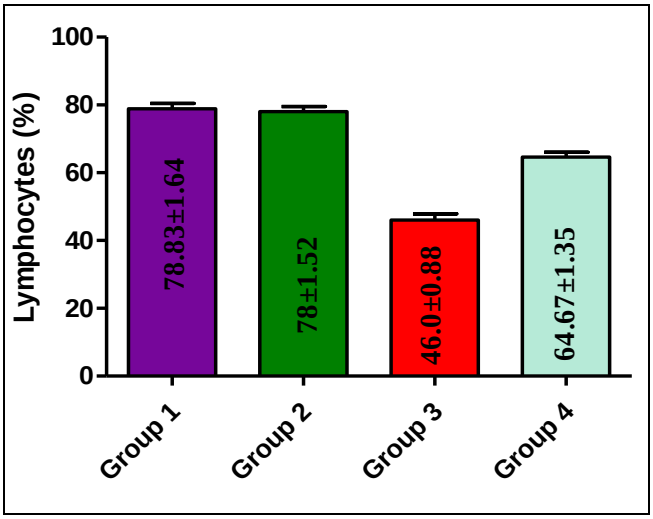

Fig 6: Ameliorative effect of Ferulic acid on Lymphocytes (%)

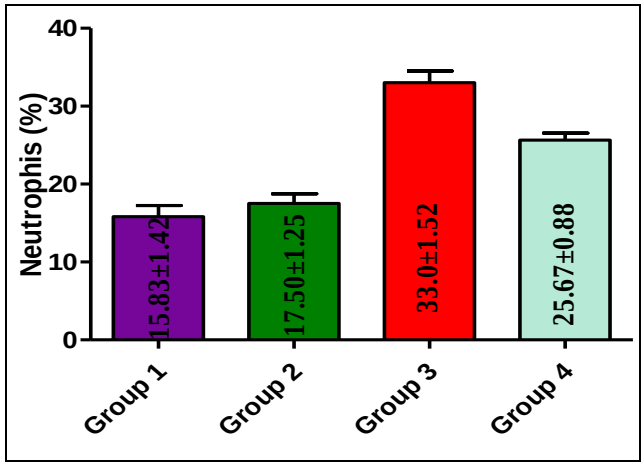

Fig 7: Ameliorative effect of Ferulic acid on Neutrophils (%)

Discussion

Haematological examination serves as a valuable tool for assessing the deleterious effects of chemotherapeutic drugs on living organisms, offering insight into the presence of metabolites and other constituents in animal bodies. It plays a pivotal role in determining the physiological, nutritional, and pathological status of an animal (Liju *et al*., 2013) [12]. Deficiency of folate leads to macrocytic anaemia, which results from an impaired red blood cells synthesis, peripheral neuropathy and increases the risk of formation of neural tube defects (NTD) in foetuses. But high dose of folic acid can also cause serious effects on liver, kidney and haemopoietic system. Normal serum folate concentrations typically range from 0.5 to 0.015 µg/mL. Generally, serum folate concentrations below 0.005 µg/mL indicate folate deficiency, and concentrations below 0.002 µg/mL usually lead to megaloblastic anaemia. In terms of toxicity, folates exhibit low toxicity, with high LD50 values. The mean LD50 values for intraperitoneal (i.p.) administered folic acid in the range of 85-330 mg/kg body weight (b.wt.) for different mouse strains tested (Van Amsterdam *et al*. 2004) [13].

In the present study, significantly (p<0.05) decreased mean values of various haematological parameters *viz*., TEC, Hb concentration, PCV, MCV, lymphocytes and numerically elevated mean values of TLC and neutrophils were observed among group 3 mice when compared group 1 mice. These findings are corroborated with findings of previous studies (Henry *et al*., 1985) [14]. A significant change in haematological parameters might be attributed to altered RBCs membrane permeability, increased LPO and MDA levels of erythrocytes (Sravathi *et al*., 2022) [15] and impaired DNA synthesis that could be due to enzyme inhibition in purine and pyrimidine synthetic pathways (Henry *et al*., 1985) [14]. Decrease in the PCV percent might be due to the increased rate of breakdown of RBCs. The significant (p<0.05) increase in mean values of TLC and neutrophils could be associated with the generation of reactive oxygen species (ROS) production, indicative of an intense inflammatory process leading to the influx of inflammatory cells through the activation of the animal's defense mechanism (Sravathi *et al*., 2022) [15].

In contrast, group 4 mice exhibited a significant increase in TEC, Hb concentration, PCV, MCV, lymphocytes, and a decline in TLC and neutrophils compared to group 3 mice. This improvement is attributed to the antioxidant, anti-inflammatory, and antiapoptotic properties of ferulic acid.

Conclusion

In conclusion, Folic acid cause significant reduction in TEC, Hb concentration, PCV, MCV, lymphocytes and increase in TLC and neutrophils due to impaired DNA synthesis and oxidative stress. However ferulic acid supplementation to mice attenuated the toxic effect of folic acid, indicating its anti-inflammatory and anti-oxidant properties. Thus, present investigation confirmed the ameliorative role of ferulic acid against folic acid induced toxicity. To advance our understanding and combat haematotoxicity as well as to improve therapeutic outcomes in the future, more thorough and in-depth study on mechanism of folic acid induced haematotoxicity is required.

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