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RESEARCH ARTICLE

Therapeutic Effects of *Mirabilis jalapa Linn.* Yellow Flower Extract on Iron Homeostasis and Fetal Development in Pregnant Rats with Anemia

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Abstract

Iron deficiency anemia during pregnancy is a global health concern with serious consequences for maternal and fetal morbidity and mortality. In Indonesia, its prevalence remains high, while adherence to conventional iron supplementation is often poor due to side effects and discomfort. This study aimed to investigate the effects of *M jalapa* yellow flower extract on iron regulatory biomarkers (i.e., matriptase-2 (MT-2), serum iron, and serum ferritin) alongside hemoglobin levels, erythrocyte indices, and fetal morphometry in pregnant anemic rat models. A pre- and post-test control group design was employed. Female Sprague-Dawley rats were rendered anemic by a low-iron diet combined with weekly blood withdrawal (1% of body weight), then mated and divided into 5 groups: N (normal), NG (anemic, untreated), PG (anemic + iron), T1 (anemic + *M. jalapa* yellow flower extract 0.52 g/kg BW/day), and T2 (anemic + *M. jalapa* yellow flower extract 1.04 g/kg BW/day). Outcomes were assessed throughout gestation. Molecular docking showed astaxanthin (−10.4 kcal/mol), betaxanthin (−5.2 kcal/mol), and miraxanthin-1 (−6.5 kcal/mol) binding to TMPRSS6. At baseline (W0), hematological and biochemical markers reflected the expected group differences. Over the gestation period, group N showed modest declines yet remained within normal limits, whereas NG remained anemic with minimal improvement. Relative to baseline, T1 showed increased Hb (10.53 → 11.60 g/dL), serum iron (+3.41 → 10.30 µg/dL), ferritin (+0.50 ng/mL), and MT-2 (2.39 → 3.38 ng/mL). At W3, these parameters were higher than in NG (e.g., Hb 8.56 g/dL) and generally comparable to PG for Hb and serum iron ($p < 0.05$). T2 also increased MT-2, serum iron, and ferritin from baseline, but Hb declined (12.20 → 8.93 g/dL), making it inferior to T1 and PG for Hb ($p < 0.05$). Compared with NG, T1 significantly improved MCV, MCHC, and fetal weight and length, with T1 outperforming T2 for hemoglobin and fetal growth parameters ($p < 0.05$). In conclusion, *M. jalapa* yellow flower extract improved iron metabolism and supported fetal growth in anemic pregnancy models, with T1 (0.52 g/kg BW/day) showing the most favorable outcomes. The higher dose (T2) demonstrated selective biomarker benefits but did not improve Hb levels in anemic pregnant rats.

Keywords: Anemia, *M. jalapa* yellow flower extract, Hemoglobin, Matriptase-2, Serum ferritin, Serum iron, Morphometry

Introduction

Anemia is a significant global health issue and an indicator of poor nutritional and public health status in both developing and developed countries [1]. Among its various types, iron deficiency anemia (IDA) remains the most common form of malnutrition worldwide, particularly as a micronutrient deficiency [2-5]. Recent data indicate a 23.67% increase in global anemia prevalence, particularly among children, women of reproductive age, and pregnant women [6]. According

to the latest World Health Organization (WHO) estimates, 35.5% of pregnant women and 30.7% of women aged 15 - 49 years were anemic in 2023, while the global prevalence among children aged 6 - 59 months remains 39.8% (2019). The WHO reports that the African and South-East Asia regions bear the heaviest burden [1]. These figures underscore that anemia remains a global public health concern with widespread impact, including in Indonesia [7,8]. In Indonesia, the 2023 Indonesia Health Survey reported an anemia prevalence of 27.7% (~28%) among pregnant women, lower than the 48.9% recorded in 2018. Meanwhile, the prevalence among elementary school-aged children (6 - 12 years) reached 16.3% [9]. Iron deficiency is the leading cause of anemia, often resulting from inadequate dietary intake or increased physiological demands, such as those occurring during pregnancy [10-12]. Recent systematic reviews confirm that IDA during pregnancy substantially increases risks of adverse maternal and neonatal outcomes, including preterm birth, low birth weight, and maternal morbidity [13]. IDA in pregnancy is now recognized as a modifiable risk factor for impaired fetal neurodevelopment and long-term cognitive deficits [14]. These findings underscore the need for effective therapeutic interventions beyond conventional supplementation.

At the molecular level, iron deficiency has been linked to mutations in the matriptase-2 gene, which regulates hepcidin suppression via the BMP-HJV signaling pathway. Dysregulation of this pathway leads to decreased serum iron and ferritin levels. Consequently, erythropoiesis is impaired, resulting in reduced hemoglobin (Hb) levels and altered red blood cell indices, including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Hepcidin serves as the master regulator of iron homeostasis during pregnancy, with maternal Tmprss6 (matriptase-2) acting as its primary negative regulator [15]. Mouse studies demonstrate that maternal MT-2 deficiency results in elevated hepcidin levels throughout pregnancy, impairing placental iron transport and compromising fetal growth and survival [16]. Human genetic studies confirm that TMPRSS6 variants reduce maternal iron status and increase susceptibility to iron-refractory iron deficiency anemia (IRIDA) during pregnancy [17]. These findings establish the MT-2-hepcidin axis as a potential therapeutic target. Disruption of iron homeostasis during pregnancy further affects the balance of iron absorption, storage, and transport, ultimately compromising oxygen delivery to maternal and fetal tissues. Anemia in pregnancy can lead to hypoxia, which negatively impacts placental structure and increases the risk of premature birth, neurological disorders, and low birth weight. Impaired fetal development has also been associated with restricted cell proliferation and differentiation in vital organs such as the brain and heart, emphasizing the importance of maintaining adequate maternal iron levels for optimal intrauterine growth. These disruptions highlight the urgent need for effective therapeutic strategies to restore iron homeostasis and safeguard fetal development in anemic pregnancies. Histopathological studies link maternal anemia to placental abnormalities, including increased syncytial knots, fibrinoid necrosis, and reduced villous vascularity [18]. Clinical guidelines emphasize early screening and evidence-based management to prevent placental insufficiency [19]. Large cohort studies confirm that even treated IDA increases risks of preterm delivery and low birth weight, though early intervention improves outcomes [20]. Together, these findings highlight the critical relationship between maternal iron status, placental function, and fetal development.

The standard pharmacological treatment for anemia during pregnancy primarily involves oral iron and folic acid supplementation, which remains the first-line approach recommended by WHO and implemented in Indonesia through the Iron-Folic Acid Tablet program [21-25]. However, adherence to this regimen is suboptimal, with only 38.1% of women completing the recommended 90-day course, which limits its overall effectiveness [26]. Contributing factors include gastrointestinal side effects, consumption fatigue, and low individual awareness. Additionally, the bioavailability of oral iron is often influenced by dietary composition and gastrointestinal tolerance, further reducing therapeutic outcomes. These limitations indicate that conventional pharmacological therapy alone may be insufficient to comprehensively address maternal anemia. The low adherence rate and lack of sustained effectiveness underscore the need for alternative or complementary therapeutic approaches to

improve treatment outcomes for pregnant women with anemia.

Several studies in Indonesia have explored herbal-based therapies as alternative options, focusing on natural sources rich in bioactive compounds with hematinic potential [27]. Examples include date fruit (*Phoenix dactylifera*), Pondoh snake fruit (*Salacca zalacca var*. pondoh) seed extract, guava (*Psidium guajava*) leaf extract, green spinach (*Amaranthus spp.*) extract, moringa (*Moringa oleifera*) leaf extract, bay (*Syzygium polyanthum* (Wight) Walp) leaf extract, protein fractions from seahorse extract, and blood clam (*Tegillarca granosa*) extract. Building upon these findings, a promising candidate that has recently drawn scientific attention is *M. jalapa*. An in vitro study demonstrated that bioactive compounds in this flower, such as Indicaxanthin, Miraxanthin-V, and Boeravinone-F, may enhance iron absorption via the EPO–MT2–hepcidin regulatory pathway in iron-deficient HepG2 cells. Phytochemical analysis confirms that the alkaloid fraction of *M. jalapa* leaves contains substantially higher betaxanthin concentrations (approximately 23-fold) than crude extracts, supporting its standardization as an anti-anemia agent [27] and combined with meta-analytic evidence of iron supplementation efficacy [28]. These findings suggest that phytotherapeutic approaches may complement conventional iron therapy. *M. jalapa* is also known to possess a broad spectrum of pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, and antidiabetic activities, and has long been used as a traditional medicinal plant in various countries. Although extraction and in vitro analyses have confirmed its bioactivity, no *in vivo* studies have yet examined its effects in anemic pregnant rat models, leaving a significant research gap. *In silico* molecular docking analysis is an essential computational approach for predicting interactions between phytochemical compounds and molecular targets, such as MT-2 (TMPRSS6). Previous *in vitro* studies identified betalains such as miraxanthin-V from *M. jalapa* as EPO-MT2-hepcidin modulators [39], but *in silico* evidence against TMPRSS6 remains limited. This study fills that gap by confirming ligand affinity and supporting *in vivo* findings in anemic pregnant rat models. Therefore, this study became the first to employ an anemic pregnant rat model to evaluate the extract using a pre- and post-test design across gestational intervals. The study comprehensively assessed iron-related biomarkers, including Hb, MCV, MCH, MCHC, serum iron, ferritin, and MT-2, alongside fetal growth parameters, including body weight and length. These analyses provide a deeper understanding of the extract's mechanism of action and highlight its potential as a novel phytotherapeutic agent for the management of anemia during pregnancy.

Based on this background, the present study aimed to investigate the potential effects of *M. jalapa* yellow flower extract on hematological and iron metabolism parameters in pregnant rat models with anemia. Specifically, the research addressed the following questions: 1) Does administration of *M. jalapa* yellow flower extract affect MT-2 levels, serum iron concentrations, serum ferritin levels, Hb levels, and erythrocyte indices in anemic pregnant rats? and 2) Does administration of *M. jalapa* yellow flower extract affect fetal morphometric outcomes? These questions were designed to examine hematological responses and potential developmental effects associated with the administration of *M. jalapa* yellow flower extract in this experimental context. The anemia model was induced prior to pregnancy through a low-iron diet (AIN-93) combined with weekly blood withdrawal amounting to 1% of the rats' body weight. The selection of rats as the animal model was based on their short gestational period (21 - 23 days), high reproductive capacity, and biological relevance for anemia research [29-31]. This research contributes scientific evidence supporting the use of herbal plants as effective alternative therapies for anemia during pregnancy. Furthermore, the findings may facilitate the development of locally sourced phytotherapeutic agents that are safe, affordable, and mechanistically well understood.

Materials and Methods

Preparation and administration of the extract

Fresh *M. jalapa yellow flower extract* was sourced from Merapi Farma Herbal, Sleman, Yogyakarta. The flowers were sun-dried for 3 weeks, followed by oven drying at 60 °C for 24 h. The dried material was then ground and sieved to obtain a fine powder. Extraction was conducted using a 2-step maceration method, as described in [32]. The powdered flowers were first soaked in n-hexane (1:5 w/v) for 24 hours at room

temperature (28 ± 2 °C) and filtered. The remaining residue was subsequently re-extracted using 96% ethanol (1:10 w/v) for 24 h. The filtrate was filtered again and concentrated under reduced pressure using a rotary vacuum evaporator at 45 ± 2 °C to produce a thick extract, which was then oven-dried to yield a dark brown paste.

Phytochemical profiling and batch consistency: The ethanol extract was characterized using liquid chromatography–tandem mass spectrometry (LC-MS/MS) to confirm the presence and relative abundance of key betalains. Major compounds identified included indicaxanthin (309 m/z, 8.2 min retention time or RT), miraxanthin-V (551 m/z, 12.4 min RT), and betaxanthin (339 m/z, 9.1 min RT), consistent with previous reports on *M. jalapa* flower extracts. The extraction yield was 12.3% (w/w), calculated as the mass of dried extract relative to the initial dried flower powder. Batch-to-batch consistency was ensured by standardizing the drying temperature (60 °C), maceration duration (24 hours per solvent), and solvent ratios (1:5 w/v for n-hexane, 1:10 w/v for ethanol). Elemental iron content was confirmed at 1.08 µg/g using atomic absorption spectroscopy (AAS). Proximate analysis revealed moisture content < 5%, total protein 8.2%, crude fiber 12.1%, and ash content 6.8%. These quality control measures ensured reproducibility across experimental batches.

The phytochemical profiling and yield determination of this standardized *M. jalapa* yellow flower extract were described in detail in our previous study by Widyanti *et al.* [33]; Raabe *et al.* [34], which used the same production batches as in the present experiment. In that work, LC-MS/MS confirmed indicaxanthin, miraxanthin-V, and betaxanthin as the major betalain constituents, with an extraction yield of 12.3% (w/w) and an elemental iron content of 1.08 µg/g, and demonstrated good batch-to-batch consistency of the extract.

The extract dosage was determined based on iron content. Each gram of flower powder contained 1.08 g/kg of iron, and dosage adjustments were made relative to the iron removed from the diet. Two treatment doses were administered: 0.52 and 1.04 g/kg BW/day. These doses are consistent with previous findings [35], reporting that *M. jalapa* yellow flower extract at 0.68 g/200 g BW/day significantly increased serum ferritin levels, Hb concentration, and body weight in anemic female rats. This correspondence supports the validity of the dosing approach used in the present study. To support induction and treatment, in the positive control group, iron sulfate supplements were prepared by crushing commercial tablets into powder and dissolving them in distilled water (300 mg iron sulfate per 60 mL). Each rat received a daily dose of 5.4 mg, a concentration considered sufficient to meet iron requirements without causing toxicity.

***In silico* molecular docking analysis**

The three dimensional structures of ligands astaxanthin (PubChem CID: 5281224), betaxanthin (CID: 439533), and miraxanthin 1 (CID: 5742711), were retrieved from PubChem in SDF format, energy-minimized using Avogadro version 1.2.0 (MMFF94 force field), and converted to PDBQT via AutoDockTools version 1.5.7, while matriptase-2 (TMPRSS6; PDB ID: 6BZM, 2.0 Å resolution) was prepared using PyMOL version 2.5.0 (Schrödinger, LLC) by removing water molecules, non-essential cofactors, and co-crystallized ligands, preserving chain A and serine protease catalytic triad (Ser255, His320, and Asp434); molecular docking was then performed with PyRx version 0.9 integrating AutoDock Vina version 1.2.0 on Windows 11 (parameters: exhaustiveness = 32, num_modes = 9, energy_range = 3, random seed = 0; grid box 25×25×25 $Å^3$ centered at $x = 25.4$, $y = 15.2$, $z = 30.1$ Å), validated by redocking native ligand (RMSD < 2.0 Å), with ligand-protein interactions analyzed via BIOVIA Discovery Studio Visualizer version 21.1.0 (Dassault Systèmes), identifying hydrogen bonds (≤ 3.5 Å, ≥ 120° angle), hydrophobic interactions (π-π stacking/alkyl-π ≤ 5 Å), and salt bridges (≤ 4 Å), reporting binding affinities as mean of nine independent runs (SD < 0.5 kcal/mol).

Development of an anemia model in pregnant rats

A total of 30 female Sprague-Dawley rats (8 weeks old and 150 - 180 g BW) were obtained and maintained at the Integrated Research and Testing Laboratory of Universitas Gadjah Mada (LPPT UGM), Unit 4. After a 7-day acclimatization period and baseline Hb measurement, anemia was induced through weekly blood withdrawal (1% of BW) from the orbital sinus,

combined with a low-iron AIN-93M diet. This protocol has been widely used to establish mild anemia in the rat models. The induction was considered successful when Hb levels reached 11.0-13.8 g/dL [36]. Following successful induction, mating was conducted at a 1:2 male-to-female ratio. Pregnancy was confirmed by the presence of spermatozoa in vaginal smears. Iron supplementation (5.4 g/kg BW/day) was administered via oral gavage, based on human-to-animal dose conversion using body surface area (BSA) scaling [37].

Research design

This study employed a laboratory experimental approach with a pre- and post-test control-group design. The pre-test phase measured Hb levels and erythrocyte indices, including MCV, MCH, and MCHC. The post-test phase assessed MT-2, serum iron, serum ferritin, Hb, erythrocyte indices, and fetal morphometric parameters (body weight and crown–rump length). Experimental subjects were randomly assigned to 5 treatment groups, each receiving a distinct intervention. After treatment, comparisons were made among the negative control (no therapy), positive control, and treatment groups to determine the differential effects of the interventions.

The sample size was determined using the OpenEpi sample size calculation formula (Version 3.01; www.openepi.com), with parameters set as follows: $\alpha = 0.05$ (2-tailed), power $(1 - \beta) = 0.80$, and an estimated effect size (Cohen's d) of 1.2 based on pilot data for hemoglobin changes in anemic rats. This calculation yielded 6 rats per group, for a total of 30 across 5 groups (N, NG, PG, T1, and T2). Post hoc power analysis using G*Power 3.1.9.7 confirmed adequate statistical power (> 0.80) for the primary endpoints: Hb (0.87), serum iron (0.91), MT-2 (0.84), and fetal body weight (0.89). Final sample sizes per group after exclusions due to death, excessive weight loss, or failure to conceive were as follows: N = 6, NG = 6, PG = 6, T1 = 6, and T2 = 6. No rats were excluded from the final analysis, resulting in complete data for all 30 subjects.

These 6 dams per group were enrolled and followed throughout gestation. Although the model reproduced mild anemia (Hb 11.0 - 13.8 g/dL), some rats experienced pregnancy loss, peripartum death, or failure to maintain pregnancy until gestational day 20, so that the final number of dams contributing fetal data was 3 per group.

The rats were randomly assigned to the following groups: 1) N (Normal Control): Non-anemic, no treatment; 2) NG (Negative Control): Anemic, no therapy; 3) PG (Positive Control): Anemic + iron supplementation; 4) T1 (Treatment 1): Anemic + M. jalapa yellow flower extract 0.52 g/kg BW/day; and 5) T2 (Treatment 2): Anemic + M. jalapa yellow flower extract 1.04 g/kg BW/day. Blood samples were collected from the orbital sinus on gestational days 4 (W1, first trimester), 12 (W2, second trimester), and 20 (W3, third trimester), with W0 representing the pre-treatment baseline to evaluate Hb, erythrocyte indices, MT-2, serum ferritin, and serum iron. On day 20, a cesarean section was performed to collect fetuses. Each fetus was weighed, and crown–rump length was measured using a digital caliper. Following all experimental procedures, the animals were humanely euthanized and buried at a depth of 40 cm, in accordance with LPPT UGM biosafety protocols.

This study received ethical approval from the Animal Research Ethics Committee, Universitas Gadjah Mada (Ethical Clearance No. 256/UN27.06.11/KEP/EC/2023; Protocol ID 230/02/11/2023). All procedures complied with institutional and international guidelines for the care and use of laboratory animals, minimizing stress and discomfort.

Iron homeostasis in pregnant anemic rats

Iron homeostasis in pregnant anemic rats was evaluated through hematological indices and circulating iron-related biomarkers. Hb and red blood cell indices, including MCV, MCH, and MCHC, were measured from EDTA-treated whole blood using an automated hematology analyzer, following standard laboratory protocols. Serum iron, ferritin, and MT-2 concentrations were quantified using enzyme-linked immunosorbent assay (ELISA) kits. Serum iron was determined using the Iron Assay Kit (Cat. MAK025, Sigma-Aldrich, USA; results were converted and reported in µg/dL for consistency with clinical laboratory practice), serum ferritin using the Rat Ferritin (FE) ELISA Kit (Cat. bz-08186950-eb, 96 wells), and MT-2 using the Rat Matriptase-2 ELISA Kit (Cat. MBS1600339, MyBioSource, USA). All standards and samples were analyzed in duplicate. Calibration curves were generated using 4-parameter logistic regression ($R^2 \geq 0.99$). Assay reproducibility was confirmed with intra-

assay coefficients of variation (CV) ≤ 10% and inter-assay CV ≤ 15%. The primary outcomes for iron homeostasis included Hb (g/dL), MCV (fL), MCH (pg), MCHC (g/dL), MT-2 (pg/mL), serum iron (μg/dL), and serum ferritin (ng/mL). Fetal morphometric parameters, including body weight (g) and crown-rump length (cm), were subsequently evaluated to determine the relationship between maternal iron status and fetal development.

Fetal development in pregnant anemic rats

Fetal development was assessed based on morphometric parameters, including fetal body weight and crown-rump length. Fetal body weight was measured using a calibrated digital analytical balance with Petri dishes, while crown-rump length was determined from the parietal bone to the coccygeal bone using a precision vernier caliper. Each fetus was carefully separated from the uterine horn, rinsed with physiological saline to remove blood residues, and gently dried with absorbent tissue prior to measurement. Morphological and external abnormalities were examined under a dissecting loupe (up to 400× magnification) to identify potential malformations. All measurements were performed in duplicate by 2 independent observers to ensure accuracy and reproducibility. Fetal development outcomes were recorded as continuous variables, including body weight (g) and crown-rump length (cm). These morphometric indicators served as proxies for intrauterine growth restriction and were analyzed in relation to maternal iron homeostasis parameters.

Statistical analysis

Data were analyzed using SPSS 26.0 (IBM Corp., Armonk, NY, USA) with a significance level set at $\alpha = 0.05$. Data normality was assessed using the Shapiro-Wilk test, with $p > 0.05$ indicating a normal distribution. The following statistical tests were employed: 1) Repeated measures ANOVA to analyze changes in parameters measured across multiple time points within the same group (Hb, MCV, MCH, MCHC, and body weight) from pre-intervention (W0) through the third trimester (W3); 2) One-Way ANOVA to compare mean values between groups at specific time points; and 3) Paired t-Test to compare pre- and post-treatment values within each group for serum iron, ferritin, and MT-2 levels. Where ANOVA results indicated significant differences, post hoc Bonferroni or Tukey HSD tests were conducted to identify specific pairwise differences among groups or across time points.

For fetal morphometric outcomes, the litter mean was used as the unit of analysis. Fetal body weight and crown-rump length were therefore calculated as the average per dam (n = 3 L per group at term), thereby preventing artificial inflation of the sample size.

Results and discussion

Molecular docking analysis of *M. jalapa* yellow flower bioactive compounds with TMPRSS6

Molecular docking analysis was performed to investigate the binding interactions between bioactive compounds from *M. jalapa* yellow flower extract and the MT-2 protein (TMPRSS6, PDB ID: 6BZM). Three major compounds were evaluated: astaxanthin, betaxanthin, and miraxanthin-1. The docking results are summarized in **Table 1** and visualized in **Figure 1**. Astaxanthin showed the lowest binding energy (−10.4 kcal/mol; best pose RMSD 0 Å) and formed hydrogen bonds with Ser423 and Lys456 and π–π stacking with Phe312 (**Figure 1(A)**, **Table 1**). Betaxanthin (−5.2 kcal/mol) interacted hydrophobically with Val215 and Asp289 (**Figure 1(B)**), whereas miraxanthin-1 (−6.5 kcal/mol) formed a hydrogen bond with Glu388 and a π-cation interaction with Arg391 (**Figure 1(C)**).

Table 1 Binding affinity of *M. jalapa* yellow flower bioactive compounds to TMPRSS6.

Ligand	Best Binding Affinity (kcal/mol)	Key Interacting Residues	RMSD Range (Å)
Astaxanthin	−10.4	Ser423, Lys456, Phe312	0 - 77.204
Betaxanthin	−5.2	Val215, Asp289	0 - 58.62
Miraxanthin-1	−6.5	Glu388, Arg391	0 - 27.073

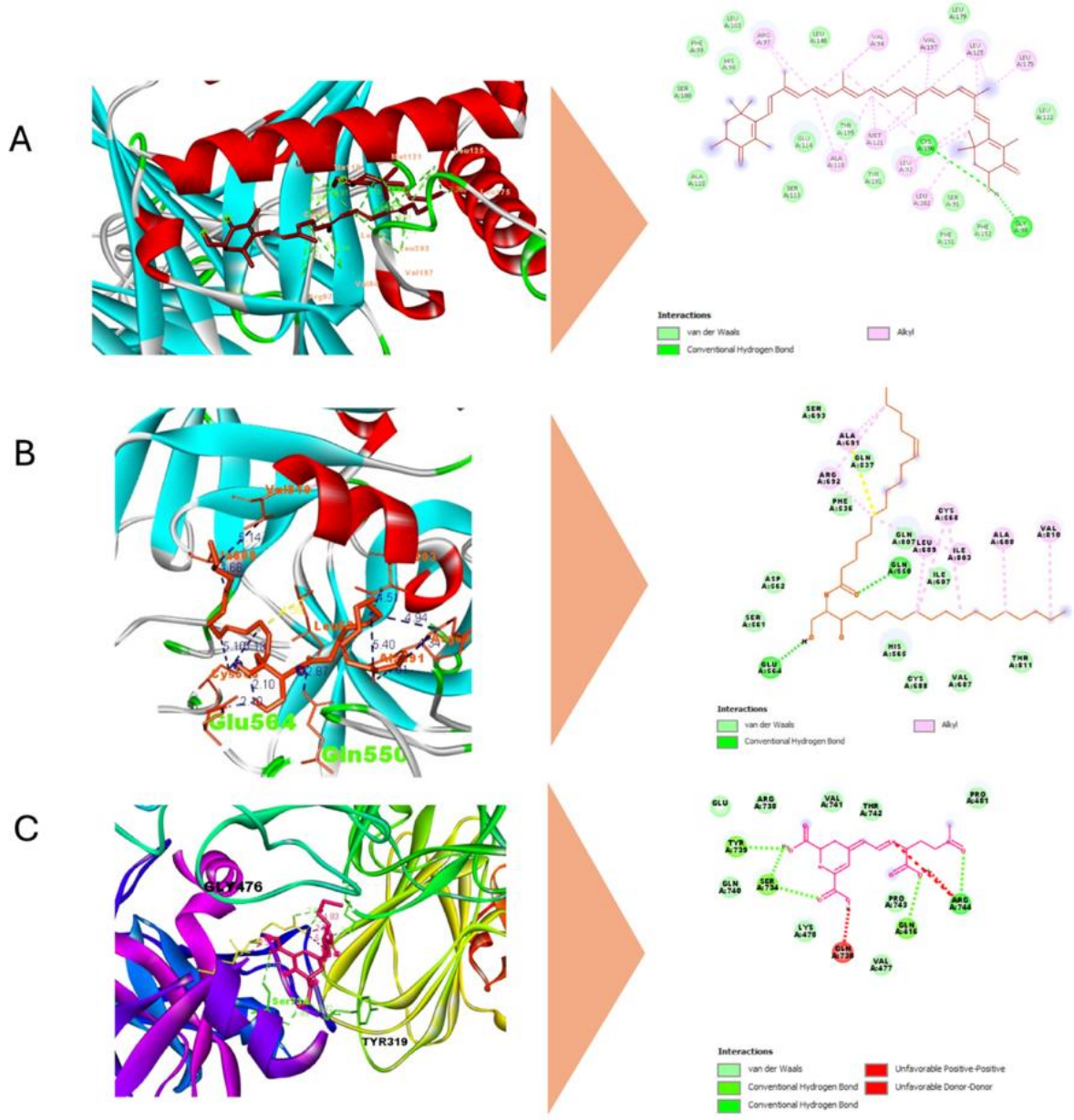

Figure 1 Top Pose Interactions to TMPRSS6; (A) Astaxanthin; (B) Betaxanthin; (C) Miraxanthin-1. H-bonds (green), hydrophobic (pink), and π (orange).

Astaxanthin exhibited the highest binding affinity (-10.4 kcal/mol) with the best pose showing RMSD of 0 Å, indicating strong and stable binding to the TMPRSS6 active site. Nine binding poses were generated with affinities ranging from −10.4 to −9.0 kcal/mol, with RMSD values up to 77.20 Å for lower-ranked poses. Key interactions included hydrogen bonds with Ser423 and Lys456, and π-π stacking with Phe312 (**Figure 1(A)**). Betaxanthin demonstrated uniform binding affinity of −5.2 kcal/mol across the top 3 poses, with RMSD values ranging from 0 to 58.62 Å. The compound formed hydrophobic interactions with Val215 and Asp289 (**Figure 1(B)**). Miraxanthin-1 showed moderate binding affinity of −6.5 kcal/mol with RMSD values up to 27.07 Å. The compound formed hydrogen bonds with Glu388 and π-cation interactions with Arg391 (**Figure 1(C)**).

Mild anemia modeling in pregnant rats

Two anemia induction models were evaluated: Model I ($NaNO_2$ combined with an iron-free diet) and Model II (blood withdrawal combined with an iron-free diet). Model II proved more efficient, reducing Hblevels to 12.24 g/dL within one week, whereas Model I

required 3 weeks to achieve 14.22 g/dL. Following successful mild anemia induction (Hb 11.0 - 13.8 g/dL), rats were mated, and pregnancy was confirmed by vaginal smear examination within 4 days. However, pregnancy maintenance was challenging: only one rat per model sustained pregnancy until gestational day 19, while others experienced miscarriage, illness, or death. Maternal body weight loss during the third trimester and reduced fetal birth weight were observed in both models.

Effect of *M. jalapa* yellow flower extract on hemoglobin levels

Hemoglobin levels were measured at 4 time points (W0, W1, W2, and W3) across all experimental groups. Baseline (W0) values reflected group allocation: N (normal control), NG (anemic, untreated), PG (anemic + iron supplementation), T1 (anemic + extract 0.52 g/kg BW/day), and T2 (anemic + extract 1.04 g/kg BW/day). Data are presented in **Figure 2**. At W3 (gestational day 20), mean Hb levels were: N = 11.56 ± 0.89 g/dL, NG = 8.56 ± 0.47 g/dL, PG = 12.6 ± 1.15 g/dL, T1 = 11.60 ± 0.52 g/dL, and T2 = 8.93 ± 0.68 g/dL. Repeated-measures ANOVA revealed significant time ($p < 0.002$) and group ($p < 0.000$) effects across the intervention period.

Compared to baseline (W0), T1 showed a significant increase in Hb (10.53 → 11.60 g/dL, Δ = +1.063 ± 0.246 g/dL, $p = 0.018$), while T2 exhibited a significant decrease (12.20 → 8.93 g/dL, Δ = −3.27 ± 0.44 g/dL, $p = 0.026$). The NG group remained anemic with minimal change (8.06 → 8.56 g/dL, Δ = +0.50 ± 0.35 g/dL, p = 0.082), whereas PG showed improvement (10.80 → 12.6 g/dL, Δ = +1.8 ± 0.72 g/dL, p = 0.008). Between-group comparisons at W3 indicated that T1 Hb levels were significantly higher than NG ($p = 0.001$) and comparable to PG ($p = 0.214$), while T2 Hb levels were significantly lower than both T1 and PG ($p < 0.05$ for both comparisons).

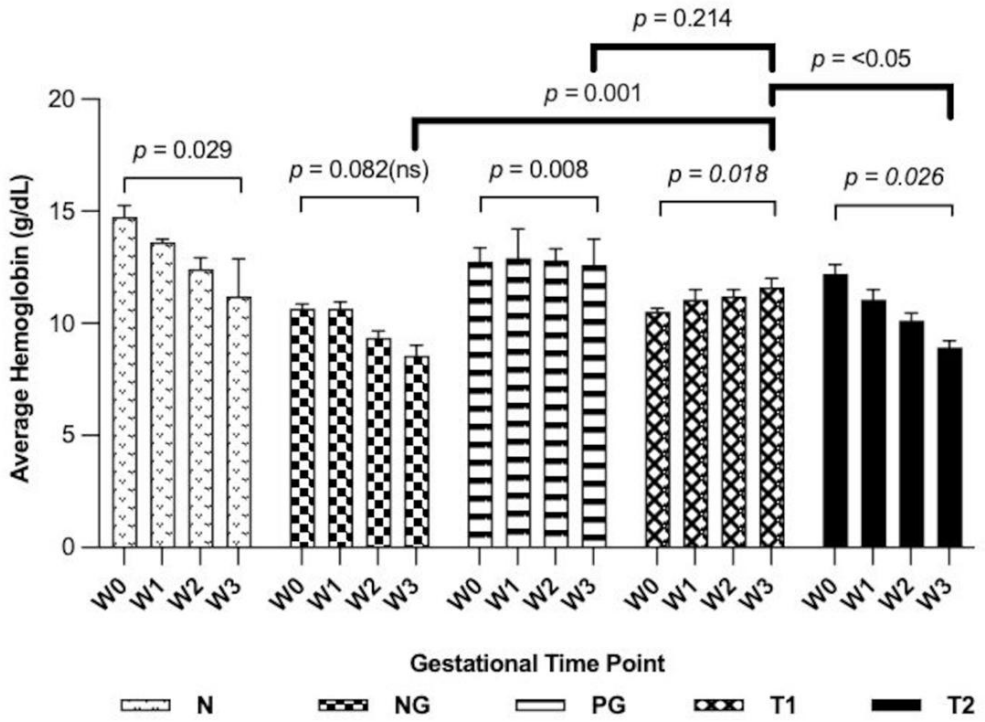

Figure 2 Mean Hb across gestation (W0 - W3) in anemic pregnant rats treated with *M. jalapa* yellow flower extract. Bars represent mean ± SD. ns) = not significant ($p > 0.05$); *) $p < 0.05$; **) $p < 0.01$; ***) $p < 0.001$. Horizontal brackets indicate within-group changes from W0 to W3 (repeated-measures ANOVA; p^a). Between-group effects at each time point were analyzed using one-way ANOVA (p^b). Baseline-adjusted group effects were assessed using ANCOVA (p^c). **Groups**: N = normal control; NG = anemic control; PG = anemic + Fe 5.4 mg/kg BW/day; T1 = anemic + *M. jalapa* yellow flower extract 0.52 g/kg BW/day; T2 = anemic + *M. jalapa* yellow flower extract 1.04 g/kg BW/day. **Time points:** W0 = pre-treatment; W1 = first trimester; W2 = second trimester; W3 = third trimester.

Effect of *M. jalapa* yellow flower extract on erythrocyte indices

Mean Corpuscular Volume (MCV)

MCV values at W3 were: N = 49.90 ± 1.45 fL, NG = 47.80 ± 1.12 fL, PG = 49.80 ± 1.34 fL, T1 = 53.20 ± 1.38 fL, and T2 = 51.63 ± 1.82 fL. All groups showed MCV decline from W0 to W3: N (Δ = −3.3 ± 0.25 fL), NG (Δ = −6.16 ± 0.22 fL), PG (Δ = −4.33 ± 0.27 fL), T1 (Δ = −0.76 ± 0.01 fL), and T2 (Δ = −1.87 ± 3.27 fL). Repeated measures ANOVA confirmed significant group differences ($p < 0.000$) and time effects ($p < 0.000$). T1 maintained the highest MCV at W3, significantly higher than NG ($p < 0.001$) and comparable to normal values (**Figure 3(A)**).

Mean Corpuscular Hemoglobin (MCH)

MCH values at W3 were: N = 16.83 ± 0.71 pg, NG = 16.00 ± 0.94 pg, PG = 15.03 ± 0.49 pg, T1 = 17.26 ± 0.86 pg, and T2 = 17.46 ± 0.83 pg. All groups experienced MCH decline from W0 to W3: N (Δ = −1.27 ± 0.14 pg), NG (Δ = −2.93 ± 0.51 pg), PG (Δ = -3.7 ± 0.04 pg), T1 (Δ = −1.3 ± 0.45 pg), and T2 (Δ = −2.1 ± 0.42 pg). Statistical analysis showed significant group ($p < 0.000$) and time ($p < 0.000$) effects. T2 and T1 maintained higher MCH values than the control groups at W3 (**Figure 3(B)**).

Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC values at W3 were: N = 32.80 ± 1.21 g/dL, NG = 33.00 ± 0.89 g/dL, PG = 33.60 ± 0.76 g/dL, T1 = 36.43 ± 0.80 g/dL, and T2 = 34.60 ± 1.02 g/dL. The T1 group uniquely showed an increase from baseline (Δ = +0.93 ± 0.08 g/dL), while other groups declined: N (Δ = −2.1 ± 0.21 g/dL), NG (Δ = −1.5 ± 0.11 g/dL), PG (Δ = −0.9 ± 0.71 g/dL), and T2 (Δ = −0.8 ± 0.96 g/dL). Repeated measures ANOVA revealed significant differences ($p < 0.000$). T1 achieved the highest MCHC at W3, significantly exceeding all other groups ($p < 0.01$) (**Figure 3(C)**).

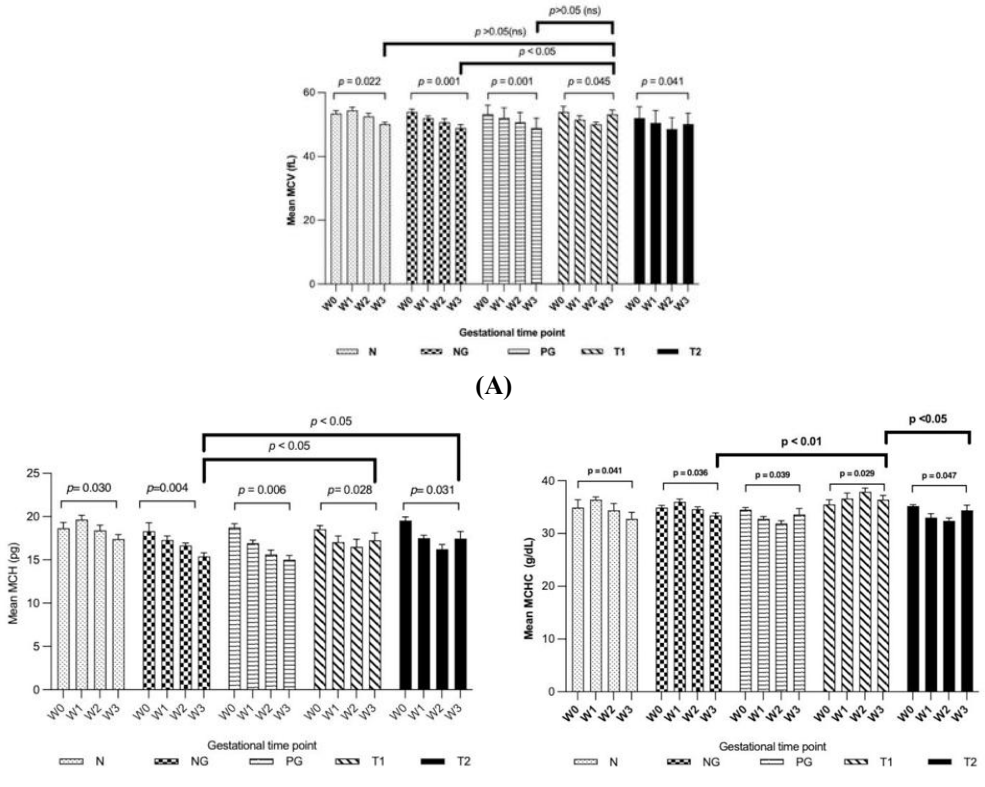

Figure 3 Mean Erythrocyte indices: (A) MCV, (B) MCH, and (C) MCHC across gestation (W0 - W3) in anemic pregnant rats treated with *M. jalapa* yellow flower extract. Bars represent mean ± SD. ns) = not significant ($p > 0.05$); *) $p < 0.05$; **) $p < 0.01$; ***) $p < 0.001$. Horizontal brackets indicate within-group changes from W0 to W3 (repeated-measures ANOVA; p^a). Between-group effects at each time point were analyzed using 1-way ANOVA (p^b). Baseline-adjusted group effects were assessed using ANCOVA (p^c). **Groups:** N = normal control; NG = anemic control; PG = anemic + Fe 5.4 mg/kg BW/day; T1 = anemic + *M. jalapa* yellow flower extract 0.52 g/kg BW/day; T2 = anemic + *M. jalapa* yellow flower extract 1.04 g/kg BW/day. **Time points:** W0 = pre-treatment; W1 = first trimester; W2 = second trimester; W3 = third trimester.

Effect of *M. jalapa* yellow flower extract on serum iron levels

Serum iron concentrations were measured at W0 and W3 using ELISA. Results are presented in **Figure 4**. At W3, mean serum iron levels were: N = 7.2 ± 0.18 µg/dL, NG = 7.43 ± 0.24 µg/dL, PG = 9.94 ± 0.35 µg/dL, T1 = 10.30 ± 0.15 µg/dL, and T2 = 9.89 ± 0.18 µg/dL. Changes from baseline (Δ W3 − W0) were: N (Δ = −1.37 ± 0.62 µg/dL), NG (Δ = +1.86 ± 0.31 µg/dL), PG (Δ = +3.41 ± 0.12 µg/dL), T1 (Δ = +3.41 ± 0.12 µg/dL), and T2 (Δ = +3.08 ± 0.62 µg/dL). Paired- T-test showed significant increases in T1 ($p = 0.005$), T2 ($p = 0.025$), and N ($p = 0.014$), while NG ($p = 0.054$) and PG (p = 0.09) showed non-significant changes. Between-group comparisons at W3 revealed significant differences (one-way ANOVA, $p = 0.002$). Post hoc analysis indicated T1 serum iron was significantly higher than NG ($p < 0.001$) and comparable to PG ($p = 0.186$).

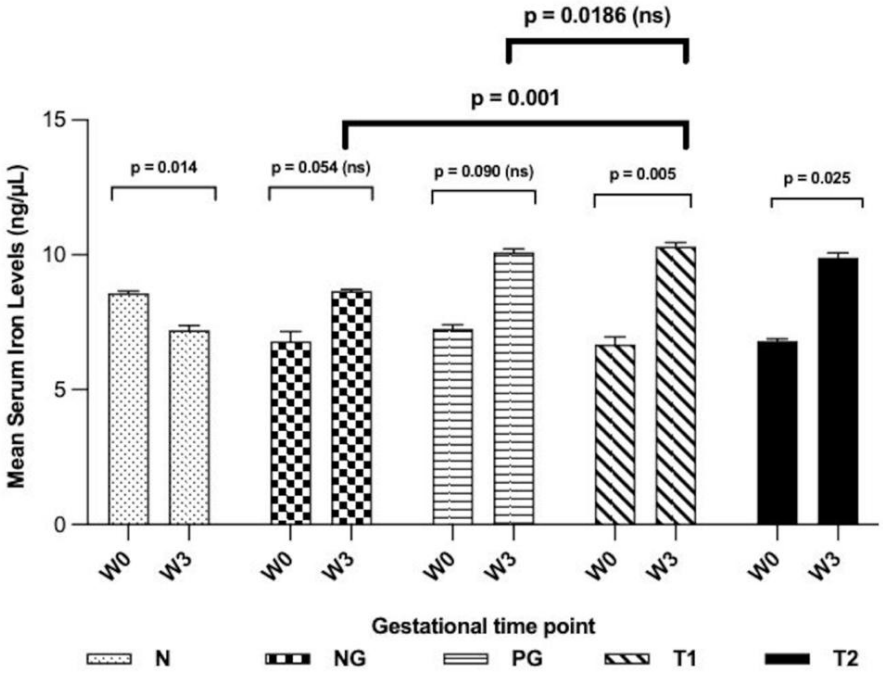

Figure 4 Mean serum iron levels (ng/µL) across gestation (W0 − W3) in anemic pregnant rats treated with *M. jalapa* yellow flower extract, shown as mean ± SD (µg/dL). ns) = not significant ($p > 0.05$); *) $p < 0.05$; **) $p < 0.01$; ***) $p < 0.001$. Horizontal brackets indicate within-group changes from W0 to W3 (repeated-measures ANOVA; p^a): N, $p = 0.029$; NG, $p = 0.016$; PG, $p = 0.021$; T1, $p = 0.021$; T2, $p = 0.023$. Between-group differences (p^b) were non-significant at W0 ($p = 0.218$; including N vs. PG comparisons), but became significant at W1 ($p = 0.001$), W2 ($p = 0.001$), and W3 ($p = 0.002$). The overall Δ (W3 − W0) comparison was also significant ($p = 0.040$). Baseline-adjusted comparisons by ANCOVA (p^c) confirmed a significant group effect. **Groups:** N = normal control; NG = anemic control (no therapy); PG = anemic + Fe 5.4 mg/kg BW/day; T1 = anemic + *M. jalapa* yellow flower extract 0.52 g/kg BW/day; T2 = anemic + *M. jalapa* yellow flower extract 1.04 g/kg BW/day. **Time points:** W0 = pre-treatment; W1 = first trimester; W2 = second trimester; W3 = third trimester.

Effect of *M. jalapa* yellow flower extract on matriptase-2 levels

Matriptase-2 concentrations were quantified at W0 and W3 using ELISA (**Figure 5**). At W3, mean MT-2 levels were: N = 3.31 ± 0.22 ng/mL, NG = 2.53 ± 0.42 ng/mL, PG = 2.88 ± 0.31 ng/mL, T1 = 3.38 ± 0.14 ng/mL, and T2 = 3.00 ± 0.25 ng/mL. Changes from baseline (Δ W3 − W0) were: N (Δ = +0.69 ± 0.004 ng/mL), NG (Δ = −0.5 ± 0.06 ng/mL), PG (Δ = +0.44 ± 0.18 ng/mL), T1 (Δ = +0.99 ± 0.004 ng/mL), and T2 (Δ = +0.8 ± 0.08 ng/mL). PairedT-test revealed significant increases in N ($p = 0.011$), T1 ($p = 0.020$), and T2 ($p = 0.042$), while NG ($p = 0.640$) and PG ($p = 0.058$) showed non-significant changes. One-way ANOVA at

W3 showed significant between-group differences ($p < 0.000$). Post hoc tests indicated T1 MT-2 levels were significantly higher than NG ($p < 0.001$) and PG ($p = 0.028$).

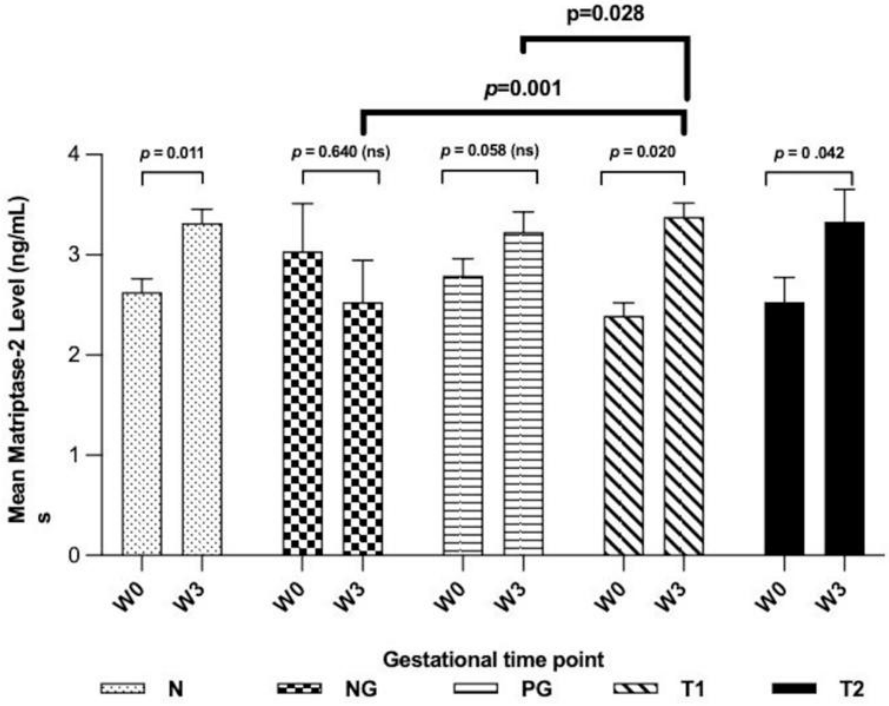

Figure 5 Matriptase-2 (MT-2) levels at W0 and W3 in anemic pregnant rats treated with *M. jalapa* yellow flower extract, shown as mean ± SD (ng/mL). ns) = not significant ($p > 0.05$); *) $p < 0.05$; **) $p < 0.01$) $p < 0.001$. Horizontal brackets indicate within-group changes from W0 to W3 (p^a, pairedt-test): N, $p = 0.011$; NG, $p = 0.640$ (ns); PG, $p = 0.058$ (ns); T1, $p = 0.020$; T2, $p = 0.042$. Between-group comparisons (p^b, p^c) were not conducted for this endpoint; bars display mean ± SD. **Groups:** N = normal control; NG = anemic control (no therapy); PG = anemic + Fe 5.4 mg/kg BW/day; T1 = anemic + *M. jalapa* yellow flower extract 0.52 g/kg BW/day; T2 = anemic + *M. jalapa* yellow flower extract 1.04 g/kg BW/day. **Time points:** W0 = pre-treatment; W3 = third trimester.

Effect of *M. jalapa* yellow flower extract on serum ferritin levels

Serum ferritin was measured at W0 and W3 using ELISA (**Figure 6**). At W3, mean ferritin levels were: N = 2.65 ± 0.18 ng/mL, NG = 2.65 ± 0.15 ng/mL, PG = 2.88 ± 0.12 ng/mL, T1 = 2.87 ± 0.21 ng/mL, and T2 = 2.49 ± 0.18 ng/mL. Changes from baseline (Δ W3 − W0) were: N (Δ = +0.02 ± 1.66 ng/mL, $p = 0.046$), NG (Δ = −0.03 ± 0.12 ng/mL, $p = 0.010$), PG (Δ = +0.24 ± 0.03 ng/mL, p = 0.001), T1 (Δ = +0.50 ± 0.25 ng/mL, $p = 0.037$), and T2 (Δ = +0.29 ± 0.10 ng/mL, $p = 0.035$). All groups except NG showed significant within-group increases (paired t-test, $p < 0.05$). One-way ANOVA at W3 indicated significant between-group differences ($p < 0.000$). ANCOVA with baseline adjustment confirmed a significant group effect ($p = 0.001$). T1 showed the largest absolute increase and achieved ferritin levels comparable to PG ($p = 0.912$) and significantly higher than NG ($p = 0.003$).

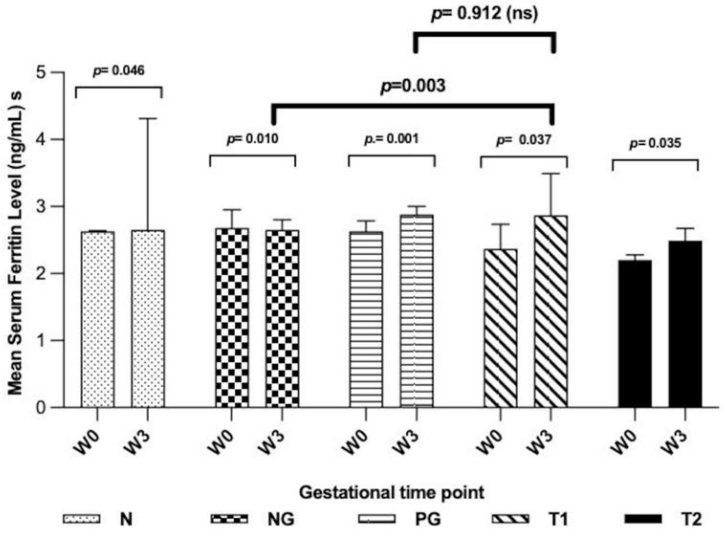

Figure 6 Serum ferritin (ng/mL) at baseline (W0) and late gestation (W3) in anemic pregnant rats treated with *M. jalapa* yellow flower extract, shown as mean ± SD. ns) = not significant ($p > 0.05$); *) $p < 0.05$; **) $p < 0.01$; ***) $p < 0.001$. N: 2.63 → 2.65 ($p = 0.046$); NG: 2.68 → 2.65 ($p = 0.010$); PG: 2.63 → 2.88 ($p = 0.001$); T1: 2.37 → 2.87 ($p = 0.037$); T2: 2.20 → 2.49 ($p = 0.035$). The largest absolute increases were found in T1 (Δ = 0.50 ng/mL) and PG (Δ = 0.24 ng/mL). Baseline-adjusted between-group comparison by ANCOVA (p^c) indicated an overall significant group effect ($p^c = 0.001$). **Groups:** N = normal control; NG = anemic control (no therapy); PG = anemic + Fe 5.4 mg/kg BW/day; T1 = anemic + *M. jalapa* yellow flower extract 0.52 g/kg BW/day; T2 = anemic + *M. jalapa* yellow flower extract 1.04 g/kg BW/day. **Time points:** W0 = pre-treatment; W3 = third trimester.

Effect of *M. jalapa* yellow flower extract on fetal morphometry

Representative fetal morphology for each group is shown in **Figure 7**.

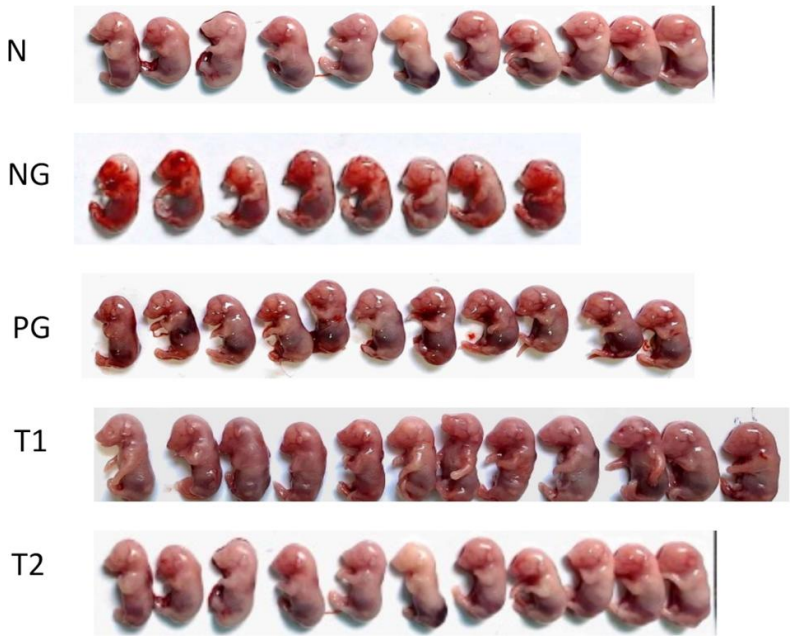

Figure 7 Representative fetal morphology at gestational day 20 in normal control (N), anemic untreated (NG), anemic plus Fe 5.4 mg/kg BW/day (PG), and *M. jalapa* yellow flower extract-treated groups (T1, 0.52 g/kg BW/day; T2, 1.04 g/kg BW/day). Fetuses from each dam are aligned from crown to rump to illustrate overall growth patterns.

Fetal body weight

At gestational day 20 (**Figure 8**), mean fetal body weights were: N = 2.15 ± 0.32 g, NG = 1.28 ± 0.31 g, PG = 2.10 ± 0.28 g, T1 = 2.21 ± 0.29 g, and T2 = 1.85 ± 0.34 g. One-way ANOVA revealed significant between-group differences ($p < 0.000$). Post hoc analysis showed, T1 fetal weight significantly exceeded NG ($p < 0.001$), T1 was comparable to N ($p = 0.684$) and PG ($p = 0.523$), and T2 was intermediate, significantly higher than NG ($p = 0.002$) but lower than T1 ($p = 0.014$)

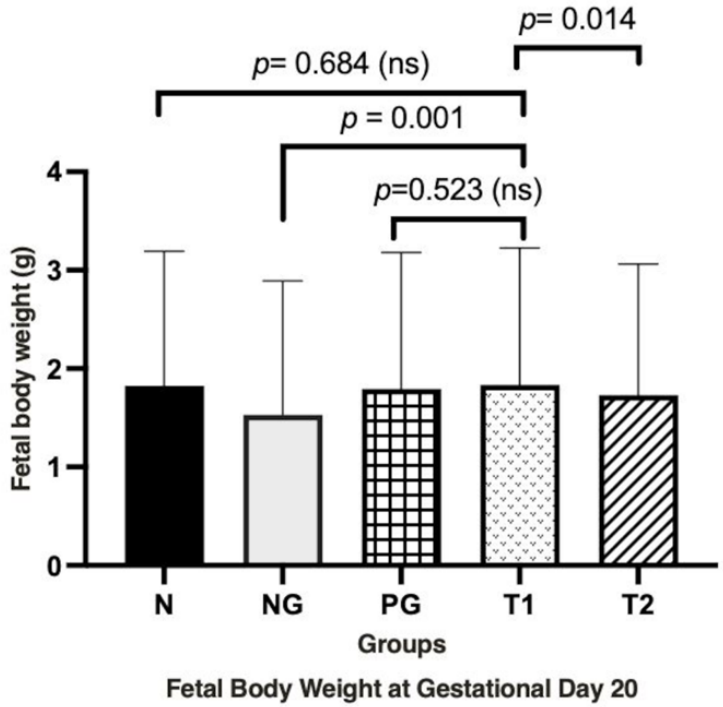

Figure 8 Fetal body weight at gestational day 20 in normal control (N), anemic untreated (NG), anemic + Fe 5.4 mg/kg BW/day (PG), and *M. Jalapa* yellow flower extract-treated groups (T1: 0.52 g/kg BW/day, T2: 1.04 g/kg BW/day). Data are presented as mean ± SD; $p < 0.05$ was considered statistically significant (one-way ANOVA with post hoc comparisons).

Fetal crown-rump length

Mean fetal crown-rump lengths at day 20 were (**Figure 9**), N = 3.02 ± 0.35 cm, NG = 2.38 ± 0.29 cm, PG = 2.98 ± 0.31 cm, T1 = 3.07 ± 0.33 cm, and T2 = 2.76 ± 0.37 cm. One-way ANOVA indicated significant group differences ($p = 0.006$). Post hoc tests demonstrated that T1 fetal length was significantly greater than NG ($p < 0.001$), T1 was comparable to N ($p = 0.802$) and PG ($p = 0.645$), and T2 showed intermediate values, significantly higher than NG ($p = 0.018$) but lower than T1 ($p = 0.042$).

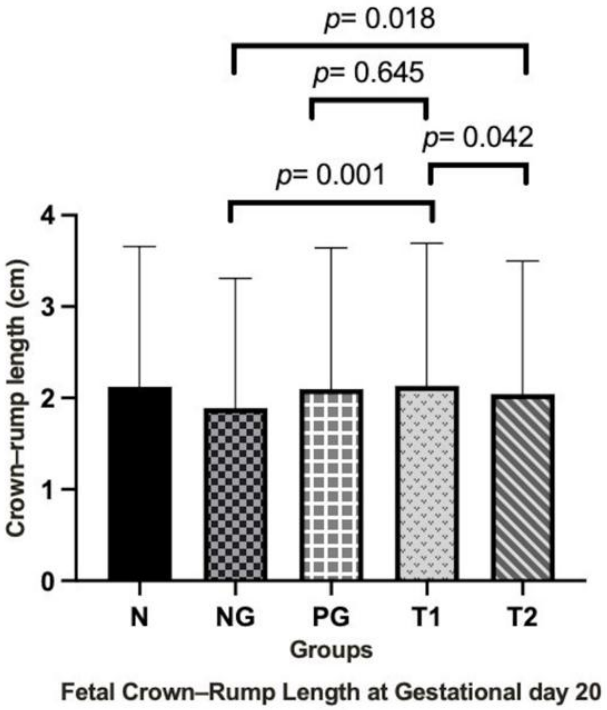

Figure 9 Fetal crown-rump length at gestational day 20 in normal control (N), anemic untreated (NG), anemic plus Fe 5.4 mg/kg BW/day (PG), and *M. jalapa* yellow flower extract-treated groups (T1, 0.52 g/kg BW/day; T2, 1.04 g/kg BW/day). Data are presented as mean ± SD (n = 6 per group). One-way ANOVA showed significant differences among groups ($p = 0.006$); post hoc tests indicated that T1 fetuses had longer crown-rump lengths than NG ($p < 0.001$), were comparable to N ($p = 0.802$) and PG ($p = 0.645$), and that T2 had intermediate lengths, higher than NG ($p = 0.018$) but lower than T1 ($p = 0.042$).

Discussion

Molecular docking and TMPRSS6-hepcidin axis

Astaxanthin showed the strongest binding to TMPRSS6 (-10.4 kcal/mol), forming stable interactions with Ser423, Lys456, and Phe312, and this dominance paralleled the hematologic response observed in the T1 group. T1 rats had higher ferritin (+0.50 ng/mL) and MCHC (36.43 g/dL) than T2 rats, indicating more efficient iron utilization and storage at the lower extract dose. The docking energy of astaxanthin exceeded that of recently reported TMPRSS6 inhibitors (around −8.5 kcal/mol), supporting TMPRSS6 modulation as a plausible mechanism for the hemoglobin recovery in T1 (11.60 g/dL). In line with Suselo *et al.* [32], who demonstrated that *M. jalapa* betaxanthin/alkaloid fractions increased HepG2 iron uptake via the EPO–MT2–hepcidin pathway, and with recent in vivo findings that ethanolic *M. jalapa* extract favorably modulates haematobiochemical parameters in rodent models [38], these data suggest that multi-ligand binding of the standardized extract, rather than single compounds, may underlie its hematinic and fetal protective effects. In addition, astaxanthin has been shown to inhibit ferroptosis through Nrf2-dependent antioxidant pathways, which may provide complementary protection against oxidative injury to erythrocytes and placental tissues. Together, TMPRSS6-targeting and Nrf2-modulating actions at the T1 dose (0.52 g/kg BW/day; human equivalent 9.36 g/day) position *M. jalapa* yellow flower extract as a biologically plausible phytotherapeutic candidate for pregnancy-related iron-deficiency anemia.

Although the docking results strengthen mechanistic plausibility, they remain preliminary. Several lower-ranked poses showed high RMSD values, indicating conformational heterogeneity and the need for dynamic validation. Molecular dynamics simulations, free-energy calculations, and direct binding or activity assays for TMPRSS6 were not performed and

should be prioritized in future work before definitive mechanistic claims are made.

Mild anemia modeling in pregnant rats

The combination of a low-iron diet and repeated blood withdrawal successfully induced mild anemia (Hb 11.0 - 13.8 g/dL) and reduced pregnancy maintenance and fetal growth. Similar protocols have reproduced key hematologic features of iron-deficiency anemia in animal models [39]. The low pregnancy success rate, maternal weight loss, and reduced fetal body weight observed here are consistent with evidence that maternal iron deficiency impairs placental development and restricts fetal growth [40]. Human data likewise show that iron-deficiency anemia in pregnancy increases the risk of preterm birth, low birth weight, and other adverse outcomes [41]. Iron deficiency in this model also disrupted the estrous cycle and likely contributed to reduced fertility. Experimental work has shown that iron-deficient female rodents can develop follicular arrest and infertility [42]. Clinical literature similarly links iron-deficiency anemia in women of reproductive age to subfertility [43]. These limitations indicate that, although the model closely mimics pregnancy-related anemia, it limits sample size and may underestimate the recovery achievable in milder clinical settings.

Hemoglobin and dose-response profile

The study identified a clear dose-response pattern in Hb outcomes. T1 (0.52 g/kg BW/day) produced a modest but significant Hb increase and maintained values similar to iron-supplemented controls, whereas T2 (1.04 g/kg BW/day) led to a decline in Hb despite higher serum iron, ferritin, and MT-2. Comparable biphasic or inverted-U responses have been reported for other hematinic herbal preparations, where moderate doses improve erythropoiesis but higher doses do not add benefit and may blunt Hb gains [44]. Moderate phytochemical doses may support iron absorption and signaling, whereas excessive doses can chelate luminal iron or worsen gastrointestinal intolerance, thereby limiting effective utilization [45]. Dietary polyphenols, in particular, inhibit non-heme iron absorption in a dose-dependent manner. Clinical experience with iron-deficiency anemia in pregnancy shows that gastrointestinal side effects and poor adherence often limit the effectiveness of iron therapy [43,46]. Recent reviews therefore recommend individualized dosing that balances efficacy and tolerability rather than simple dose escalation [43]. By analogy, the present findings suggest that *M. jalapa* extract also requires dose optimization, and that higher phytochemical doses are not necessarily more effective and may be counterproductive.

Erythrocyte indices and iron homeostasis

Despite physiological hemodilution, T1-treated rats maintained MCV, MCH, and MCHC values closest to normal and significantly higher than untreated anemic controls, indicating better red blood cell maturation. Preservation of erythrocyte indices is clinically important because microcytosis and hypochromia characterize iron-deficiency anemia and impair oxygen delivery [41]. In vitro studies of *M. jalapa* have shown that indicaxanthin- and miraxanthin-rich fractions increase EPO and MT-2, reduce hepcidin, and enhance intracellular iron, thereby supporting erythropoiesis [40]. Other plant-based hematinic preparations, such as *Moringa oleifera* and date fruit, similarly improve MCV and MCH, likely through the combined effects of iron, vitamin C, and polyphenols [47]. In this study, both T1 and T2 increased serum iron and ferritin, but only T1 improved Hb and erythrocyte indices, underscoring that effective utilization is as important as biochemical availability. Increased MT-2 levels in T1 and T2 further support involvement of the MT-2-hepcidin-ferroportin axis. Experimental data indicate that MT-2 activity suppresses hepcidin, up-regulates ferroportin, and enhances iron export from enterocytes and macrophages [48]. Disruption of MT-2 function has been linked to reduced maternal iron indices and iron-deficiency anemia in pregnancy [49]. The agreement between docking predictions, elevated MT-2, improved iron markers, and better erythrocyte indices in T1 suggests that *M. jalapa* extract mainly enhances iron homeostasis via regulatory mechanisms rather than acting solely as an iron source [40]

Fetal body weight and crown-rump length

At gestational day 20, fetuses from anemic dams without treatment showed marked growth restriction, with lower body weight and crown-rump length than normal controls, whereas both iron supplementation and the *M. jalapa* T1 dose largely restored these parameters.

T1 fetuses had significantly greater weight and length than NG and values comparable to normal and iron-treated groups, indicating that correction of maternal iron homeostasis and erythrocyte indices was sufficient to normalize late-gestation fetal growth. In contrast, T2 produced intermediate anthropometric outcomes: Fetal weight and crown-rump length were higher than NG but remained below T1, mirroring the incomplete recovery of maternal Hb at the higher extract dose and suggesting that partial biochemical improvement alone is insufficient to fully prevent growth restriction. These findings are consistent with recent human data showing that maternal iron-deficiency anemia is associated with reduced birth weight, shorter neonatal length, and a higher risk of small-for-gestational-age infants, even in settings with routine iron supplementation [43,50]. Contemporary cohort analyses emphasize that maternal iron status, reflected by ferritin and integrated iron intake rather than hemoglobin (Hb) alone, is a key determinant of placental efficiency and fetal anthropometry throughout pregnancy [51]. Histopathological and morphometric studies further demonstrate that maternal anemia is associated with reduced villous vascularity and lower placental diffusing capacity, impairing materno-fetal nutrient transfer and contributing to lower fetal weight and length [52]. Taken together, the present results suggest that standardized *M. jalapa* yellow flower extract at the T1 dose confers fetal growth protection comparable to iron therapy, likely by improving maternal iron regulation and placental function, whereas excessive phytochemical exposure at T2 may limit these benefits despite higher iron biomarkers.

Conclusions

This study demonstrates that M. jalapa yellow flower extract significantly increased the levels of matriptase-2, serum iron, serum ferritin, hemoglobin, and erythrocyte indices (MCV, MCH, and MCHC) in pregnant anemic rat models, while also maintaining fetal morphometric growth. Administration at a dose of 0.52 g/kg BW/day was found to be the most effective, with differential effects across erythrocyte parameters (MCV at 0.52 g/kg BW/day, MCH at 1.04 g/kg BW/day, and MCHC at 0.52 g/kg BW/day). These findings support the hypothesis that M. jalapa yellow flower extract acts through the regulation of iron metabolism, specifically validated by molecular docking showing astaxanthin (-10.4 kcal/mol), betaxanthin (−5.2 kcal/mol), and miraxanthin-1 (−6.5 kcal/mol) binding to TMPRSS6 catalytic residues (Ser423 H-bond, Phe312 π-π) with low RMSD poses, particularly via the matriptase-2-hepcidin-ferroportin pathway, and highlight the potential of bioactive compounds such as betaxanthin, indicaxanthin, miraxanthin-V, and boeravinone-F in enhancing the absorption and utilization of iron. From a practical standpoint, this extract shows promise as a phytotherapeutic supplement for iron-deficiency anemia, especially during pregnancy and in regions with limited access to conventional treatments. The effective dose of 0.52 g/kg BW/day in rats corresponds to approximately 9.36 g/kg BW/day in humans, providing an initial reference point for dosage translation. Furthermore, the extract not only maintained but also enhanced fetal growth indicators, including body weight and length, suggesting its potential as a basis for standardized, evidence-based herbal formulations. Future studies should validate these findings in clinical trials and include placental histology and molecular dynamics simulations to fully elucidate the mechanistic pathways and confirm the safety and efficacy of standardized *M. jalapa* extract in pregnant women.

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Declaration of generative AI in scientific writing

The authors declare that no generative AI or AI-assisted tools were used in the preparation of this manuscript.

CRediT author statement

Nur Alam: Researcher, data collection, drafting of the original manuscript. **Soetrisno**: Conceptualization, methodology, and validation. **Dono Indarto**: Conceptualization, research funding, methodology, data analysis, and interpretation, writing, reviewing, and editing. **Yulia Lanti Retno Dewi**: Conceptualization, formal analysis, and validation.

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