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Original Research Article

Effects of Saffron *(Crocus sativus L.)* vs. Folic Acid on Spleen Structure and Function in Intrauterine Growth Restriction

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ABSTRACT

Intrauterine growth restriction (IUGR) is a serious perinatal complication that weakens neonatal immune function, rendering affected infants highly susceptible to infections and consequently increasing their risk of morbidity and mortality. This vulnerability results from impaired development of vital immune organs, particularly the spleen. This study investigated the anti-inflammatory and immunoprotective effects of saffron *vs.* folic acid supplementation on the spleen of IUGR rat models induced through dietary restriction. Researchers divided 25 rat pups into four groups derived from adult *Rattus norvegicus*. The third group received saffron (15.68 mg/kg of body weight/day), while the fourth group received folic acid (1.5 mg/kg of body weight/day) from pregnancy through postnatal day 21. The study investigated birth weight, spleen index, histopathology, and tumor necrosis factor-alpha (TNF-α) expression. Birth weight significantly differed between the IUGR control group and those treated with saffron and folic acid. The spleen index showed a significant difference between the IUGR control group and the saffron-treated group. Spleen histopathology analysis revealed significant differences in white pulp components: periarteriolar lymphoid sheath (PALS), follicles, marginal zone, germinal center (GC) and red pulp between the IUGR control group and the group treated with folic acid. There were notable variations in TNF-α expression between the IUGR control group and the groups who received saffron and folic acid. The findings suggest that saffron can potentially increase birth weight and prevent spleen tissue damage due to IUGR. A decrease in inflammatory cytokines in the spleen indicates the organ-protective mechanism, possibly through anti-inflammatory effects.

Keywords: Diet restriction, Fetal growth restriction, Folic acid, Saffron, Spleen histopathology, TNF- α.

Introduction

Intrauterine growth restriction (IUGR) is a condition where fetal development is impaired, resulting in fetal weight falling below the 10th percentile for gestational age, typically diagnosed via ultrasonography. Globally, IUGR affects approximately 24% of newborns, with an estimated 30 million cases occurring each year. It remains a major global contributor to perinatal mortality and morbidity, posing extensive challenges to maternal and neonatal health worldwide.[1] In developing countries, the incidence of IUGR is approximately 40% higher than in developed regions.[2] In Indonesia, the prevalence of IUGR is often commonly reflected in low birth weight (LBW) statistics, with a 30-40% increase reported by the World Health Organization (WHO) in 2013.[3] One of the primary contributors to IUGR is inadequate maternal intake of essential macro- and micronutrients during pregnancy. Dietary restrictions or maternal malnutrition can negatively affect fetal growth and immune system.[4] Deficiencies in energy, protein, iron, calcium, vitamin A, and folate hinder both placental and fetal development. These nutritional insufficiencies inadequacies also disrupt fetal immune homeostasis by promoting an excessive inflammatory response, reducing splenic B lymphocyte populations, and impairing immunoglobulin production.[1,5] Nutritional deprivation during pregnancy triggers oxidative stress and systemic inflammation, compromising endothelial function and placental perfusion. These effects contribute to placental hypoxia, a hallmark of IUGR.[6] Nutritional stress also stimulates overproduction of pro-inflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α),[7] which modulate immune responses and affects tissue remodelling in lymphoid organs like the spleen.[8] Chronic elevation of TNF-α leads to dysfunction in lymphoid organs, resulting in atrophy and immune dysregulation that worsening fetal developmental outcomes.[9] Consequently, prenatal malnutrition not only increases the risk of IUGR but also weakens neonatal immune defenses, making infants more vulnerable to infections due to an underdeveloped immune system. In many developing countries, limited access to nutritious food increases the prevalence of maternal malnutrition, thus elevating the risk of IUGR. Socioeconomic factors such as poverty, poor sanitation, and limited healthcare access further exacerbate maternal undernutrition.[10] Additionally, the increased nutritional demands of pregnancy often remain unmet, particularly in terms of folate. Studies have linked folate deficiency to increased homocysteine levels, which contribute to placental endothelial dysfunction and abnormal inflammatory responses. Nutritional insufficiency also impairs hormonal and metabolic regulation, further disrupting fetal development. Folic acid supplementation has proven effective in mitigating IUGR risk by reducing homocysteine levels, improving endothelial function, and modulating inflammatory pathways. However, concerns over potential side effects of synthetic folic acid, such as allergic reactions and gastrointestinal discomfort have prompted

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interest in natural alternatives. In this context, Saffron (*Crocus sativus L.*) has emerged as a promising prospect due to its rich content of bioactive compounds: *crocin*, *crocetin*, *picrocrocin* and *safranal*. Saffron exhibits potent anti-inflammatory and immunomodulatory properties, suppressing TNF-α expression and enhancing immune responses.[11] Previous studies have shown that saffron improves immune response by reducing pro-inflammatory cytokines without causing significant haematological or histological side effects.[12] This study aims to compare the immunoregulatory, anti-inflammatory, and spleen-protective effects of saffron extract and folic acid in an *in vivo* IUGR model using Wistar rats. The findings are expected to provide new insights into complementary therapeutic approaches for managing IUGR and improving neonatal health through nutritional modulation and inflammation control.

Materials and Methods

Ethical approval

The study utilized a post-test-only control group design. The Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia approved the research protocol (Ethics #290/EC/KEPK/FKUA/2023). Researches randomly divided 25 Wistar pups into four groups: control (n = 8), diet restriction (n = 8), diet restriction plus saffron (n = 5), and diet restriction plus folic acid (n = 4).

Drugs and chemicals

This study used saffron (*Crocus sativus L.*) extract, containing the active compounds such as *safranal, crocin*, and *picocrocin* (88.5 mg), manufactured in New York, USA by Piping Rock Health Products, LLC 2120 (Latitude: 40°49'19.9488" N, Longitude: 73°7'47.8236" W). Folic acid (1 mg) was obtained from Novapharin Pharmaceutical, Gresik, Indonesia (Registration No. GKL 1834011910A1; Batch No. 2402-03-510; Latitude: 7°24'6.1025" S and Longitude: 112°59'56.5607" E).

Animal experimental design

The researchers acclimatized 8 male and 40 female Wistar rats aged 8 to 10 weeks with body weights ranging from 150 to 250 grams for 7 days under 12:12-hour light-dark cycles at a stable temperature of 24°C.[13,14] Food (BR-1 511, PT Charoen Pokphand, Indonesia) and tap water were provided *ad libitum*. After acclimatization the rats were housed in the cages for the 5:1 female-to-male ratio for overnight mating. The presence of a vaginal plaque was considered day 0 of pregnancy.[15,16] The pregnant rats were randomly divided into four groups of ten female per group: (1) Served as the control group receiving a standard diet (SD) (BR-1 511, PT Charoen Pokphand, Indonesia) with the composition: water 11-12%, protein 21-23%, fat 58%, fiber 3-5%, ash 4-7%, 2800-3100 kcal, (2) Subjected to a 50% diet restriction (DR) using the 50% total restriction method of the SD, thus limiting the total volume of food consumed for the purpose of creating an intrauterine growth restriction (IUGR) animal model,[17,18] (3) DR were given saffron supplementation, and (4) DR were given folic acid supplementation from pregnancy (19-21 days) to lactation period (21 days).[18,19] Food and water remained available *ad libitum*. Following the gestation period, the researchers regularly monitored the body weight of the 25 pups and allowed them to nurse until postnatal day 21.[15] At the end of the experiment, the rat pups were euthanized via cervical dislocation, as described in previous studies.[20,21] Tissue samples were subsequently collected for further analysis.

Induction of Saffron and Folic Acid

The researchers prepared both saffron (*Crocus sativus L.*) extract and folic acid as suspensions. The saffron suspension was administered orally to group 3 at a dose of 15.68 mg/kg of body weight/day.[22] The folic acid suspension was administered orally to group 4 at a dose of 1.5 mg/kg of body weight/day.[23] Both suspensions were given daily during pregnancy until the lactation period or postnatal day 21.[22,23]

Measurement of body weight

To reduce stress, the researchers handled animals using cloth-covered hands to avoid direct skin contact. Skin secretions can induce stress responses in Wistar rats; therefore, gloves helped prevent the animals from coming into direct contact with the researcher's skin.[20] The researchers measured the body weight of the dams using a large digital scale (SF-400).

Measurement of spleen index

The spleen index, a standard indicator of immune activity, was calculated by determining the ratio of spleen weight to total body weight, expressed the result as a percentage (%).[24] This index provides valuable insights into the size and potential functional status of the spleen. Previous studies have confirmed that the spleen index can serve as a sensitive indicator of immune system activation in response to various stressors,[25] including IUGR. In this study, the spleen index was used to assess the effects of saffron vs. folic acid on immune response in IUGR rat pups.

Spleen TNF-α expression and histopathological evaluation

To assess TNF-α expression, spleen sections were stained with a TNF-α antibody (Bioss, BSM-0387M, USA) and counterstained with Mayer's Hematoxylin. Spleen tissue sections were also stained with Haematoxylin-Eosin (H&E) and examined under a light microscope (Olympus CX23, Tokyo, Japan) at 100x, 200x, and 400x magnification. Histopathological changes, including alterations in white pulp (periarteriolar lymphoid sheath/PALS, follicles, marginal zones, and germinal centers) and red pulp, were assessed using a modified scoring system adapted from Elmore.[26] Lymphoid nodules were measured by calculating taking the mean (± SD) of 10 fields of view and counting the number of lymphoid nodules per field of view.[27] PALS measurements were obtained by averaging of the three longest sizes of PALS per field of view (µm) and counting the number of PALS per field of view.[26,28] Germinal center measurements were made by averaging the three widest germinal center sizes per field of view (µm) and counting the number of germinal centers. Marginal zone measurements were made by averaging the three widest marginal zone sizes per field of view (µm), as well as counting the number of marginal zones of germinal centers per field of view, taken from all follicular nodules/germinal centers per field of view. Measurement of red pulp area was performed using the ImageJ application (µm^2).[26,29,30]

Statistical analysis

Data were analysed using SPSS version 26.0. The Shapiro-Wilk test assessed data normality, and Levene's test evaluated homogeneity of variances. One-way ANOVA followed by the Least Significant Difference (LSD) post-hoc test was used to compare birth weight, spleen index, and TNF-α expression. The Kruskal-Wallis test, with subsequent Mann-Whitney U post-hoc comparisons, was employed to assess differences in histopathological abnormalities between groups. A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

Birth weight

Intrauterine growth restriction (IUGR) significantly reduced birth weight in the diet restriction (DR) group compared to the control group (2.42 ± 0.39g vs. 5.88 ± 0.53g, $p < 0.01$) as shown in Figure1. This finding aligns with previous studies, which attribute IUGR to placental insufficiency and reduced nutrient transfer.[31] Both saffron and folic acid supplementation significantly reversed the reduction in birth weight in the DR groups (4.30 ± 0.65g of saffron and 4.33 ± 0.46g of folic acid, $p < 0.05$). The beneficial effects of saffron may be attributed to its antioxidant properties,[32] which help mitigate oxidative stress and improve placental function. Similarly, folic acid, an essential nutrient for DNA synthesis and cellular proliferation, likely enhances fetal growth by supporting cellular replication and reducing oxidative damage.[33]

Organ Index of the Spleen

As shown in Figure 2, the spleen index was significantly reduced in the diet restriction (DR) group (0.54 ± 0.12%) compared to controls (0.79 ± 0.14%, $p < 0.05$), indicating impaired splenic development due to chronic hypoxia and placental insufficiency. Both saffron and folic acid

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supplementation significantly ameliorated this reduction and increased spleen weights (0.61 ± 0.14% for saffron and 0.55 ± 0.04% for folic acid, $p < 0.05$). Saffron's potent antioxidant properties likely contributed to its greater efficacy in protecting splenic tissue from oxidative damage and promoting recovery. Folic acid, essential for cellular proliferation and immune function, may have supported splenic growth and development.[34]

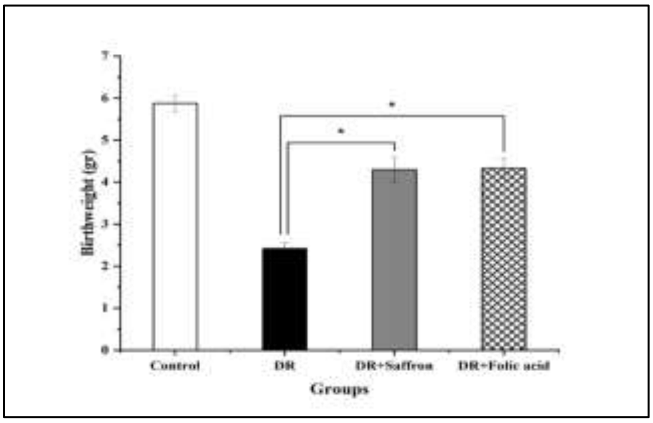

Figure 1: Effect of saffron (*Crocus sativus L.*) vs. folic acid supplementation on birth weight of the Wistar rat pups. The diet restriction (DR) group shows the 50% total restriction method of the standard diet. The* indicates significant differences between the DR group.

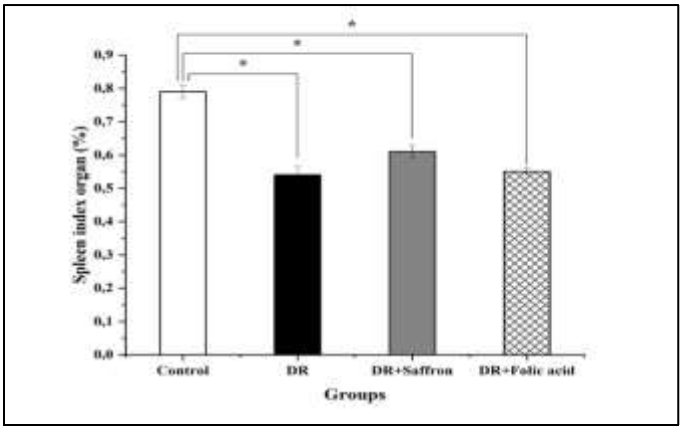

Figure 2: Effect of saffron (*Crocus sativus L.*) *vs.* folic acid on spleen index organ of Wistar rat pups. The diet restriction (DR) group shows a 50% total restriction method of the standard diet. The* indicates significant differences compared to the control group (Wistar rat pups receiving a standard diet).

TNF-α expression

Analysis of spleen tissue revealed distinct TNF-α expression patterns among the treatment groups. TNF-α expression was significantly elevated in the diet restriction (DR) group (47.54 ± 10.01, $p < 0.01$) compared to the control group (13.03 ± 3.69, $p < 0.01$), indicating a strong pro-inflammatory response. This observation aligns with established literature identifying TNF- α as a key mediator of inflammation in IUGR.[35,36] The increased expression of TNF-α, a key pro-inflammatory cytokine, is consistent with the systemic inflammation and oxidative stress commonly observed in IUGR.[37] These changes reflect an underlying state of chronic inflammation, impairing fetal organogenesis and contributing to the development of critical conditions such as placental insufficiency and fetal hypoxia.[38]

As shown in Figure 3, both saffron and folic acid offer promising therapeutic strategies to counteract the inflammatory consequences of IUGR, with folic acid demonstrating a more pronounced effect (19.29 ± 8.02 of saffron vs. 11.29 ± 7.08 of folic acid, $p < 0.01$). These results suggest that dietary interventions targeting oxidative stress and inflammation may improve immune function and overall health outcomes in neonates affected by IUGR. Further studies investigating the molecular mechanisms involved may provide deeper insights into the therapeutic potential of these compounds for managing inflammation in IUGR cases.

Our findings indicate a clear association between elevated TNF-α levels in the spleens of IUGR rats and the possible activation of NF-κB signalling, an essential element in inflammatory processes. NF-κB, a transcription factor residing within cells, regulates the production of pro-inflammatory cytokines like TNF-α upon activation.[39] In IUGR, factors such as placental dysfunction, hypoxia, and oxidative stress can trigger this activation cascade.[35]

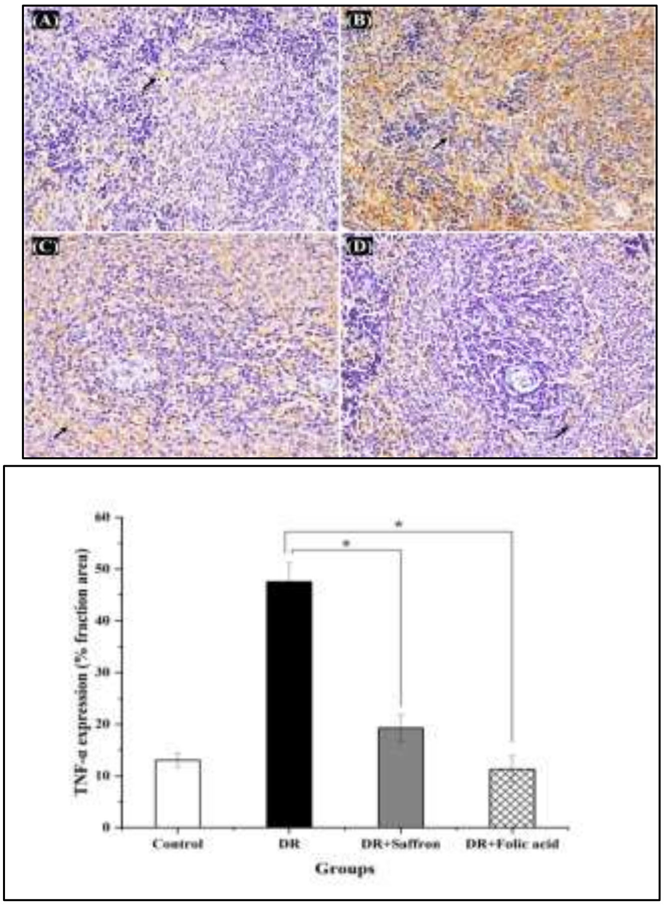

Figure 3: Effect of saffron (*Crocus sativus L.*) vs. folic acid on tumor necrosis factor-alpha (TNF-α) expression in the spleen of 22-day-old rat pups. **(a).** Representative immunohistochemical images of TNF-α staining in spleen tissue: (A) control group, (B) diet restriction (DR) group, (C) DR plus saffron group, and (D) DR plus folic acid group. Note the reduced TNF-α expression in the DR plus saffron and DR plus folic acid groups compared to the DR group. **(b)** Quantitative analysis of TNF-α expression. *p<0.05.

The resulting increase in TNF-α contributes to a systemic inflammatory environment, potentially causing the detrimental effects in the developing fetal organs, including the spleen. The observed reduction in TNF-α following treatment with saffron and folic acid suggests a possible influence on NF-κB activity, although further research is needed to comprehensively reveal the specific molecular pathways involved. This modulation may explain the improvements observed in splenic tissue structure and other measured parameters.

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Histopathological analysis of Spleen tissue

Histopathological analysis was conducted to evaluate morphological changes in the white pulp, which includes the periarteriolar lymphoid sheath (PALS), follicles, marginal zone and germinal centers, as well as the red pulp (Figure 4). By comparing the control group, the diet restriction (DR) group, and the DR plus intervention groups (saffron vs. folic acid), it is expected to identify specific changes associated with IUGR and the protective effects of both interventions.

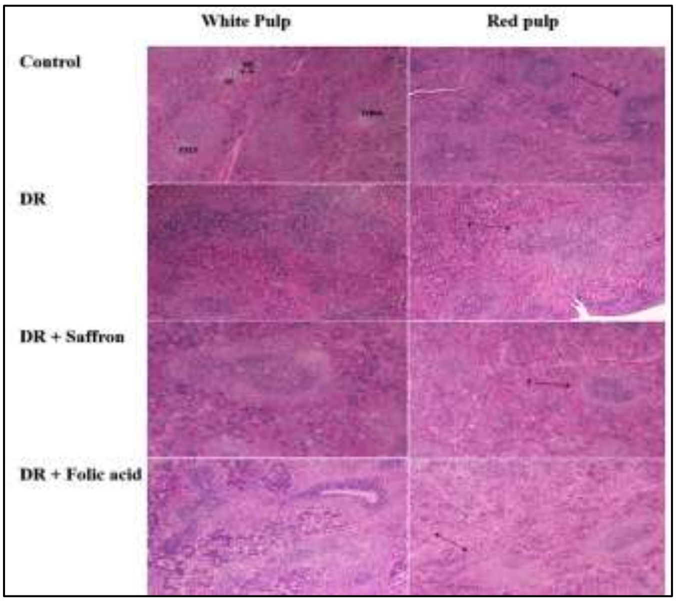

Figure 4: Representative picture of spleen tissue sections from the four treatment groups, stained with haematoxylin and eosin (H&E) and imaged at 100x magnification. White pulp components: periarteriolar lymphoid sheath (PALS), follicles, germinal centers (GC) and marginal zone (MZ) and red pulp are shown.

Periarteriolar Lymphoid Sheath (PALS)

Histopathological analysis demonstrated a significant increase in the linear measurement of the periarteriolar lymphoid sheath (PALS) in the DR group compared to the control group as shown in Figure 5 (7.34 ± 0.09µm vs. 6.03 ± 0.17µm, $p < 0.001$). This finding suggests elevated immune activity in the IUGR group, possibly due to an increased number of antigen-presenting cells. The enlargement of PALS, which houses T lymphocytes, may indicate an enhance T cell-mediated immune response in IUGR pups, likely as a response to chronic inflammation or other stressors. Both saffron and folic acid interventions significantly decreased PALS size compared to the DR group (6.81 ± 0.18 µm of saffron and 6.05 ± 0.11µm of folic acid; $p < 0.001$). These findings strongly suggest the potential of both compounds in modulating the excessive immune response in IUGR conditions. Saffron, with its antioxidant properties, may help attenuate inflammation and prevent tissue damage,[40] whereas folic acid, which is essential for cell proliferation and immune function, may contribute to restoring immune homeostasis[41].

Follicles

Histopathological analysis revealed a significant increase in the diameter of lymphoid follicles in the diet restriction (DR) group compared to the control group (9.24 ± 0.20µm vs. 7.44 ± 0.25µm, $p < 0.001$) as shown in Figure 5. This finding suggests enhanced B cell proliferation within germinal centers of lymphoid follicles in the IUGR group, likely as a response to subclinical infection and/or inflammation in pregnancy, although it might also kill the fetal cells and produces fetal growth restriction.[42] Saffron and folic acid interventions significantly decreased follicle diameter compared to the IUGR group (7.62 ± 0.21µm of saffron vs. 7.90 ± 0.29µm of folic acid, $p < 0.001$). These findings highlight the potential of both compounds in modulating the excessive immune response under IUGR conditions.

Germinal center

Histopathological analysis revealed a significant decrease in the diameter of germinal centers in the diet restriction (DR) group compared to the control group, as shown in Figure 5 (1.74 ± 0.28µm vs. 3.85 ± 0.88µm, $p < 0.001$). This finding suggests impaired B cell proliferation within germinal centers in the DR group, indicating a compromised adaptive immune response. Interestingly, both saffron and folic acid interventions showed a trend towards increased germinal center diameter compared to the DR group (2.30 ± 0.41µm of saffron vs. 3.27 ± 0.16µm of folic acid, $p < 0.001$). These results underscore the potential of both compounds in improving the immune response under IUGR conditions.

Marginal zone

Histopathological analysis revealed a significant enlargement of the splenic marginal zone in the diet restriction (DR) group compared to the control group in Figure 5 (3.94 ± 0.19µm vs. 1.93 ± 0.16µm, $p < 0.001$). This finding suggests increased activity of B cell proliferation within the splenic marginal zone of the IUGR group, potentially driven by subclinical maternal infection and/or inflammation during pregnancy, although elevated kill cell might contribute to fetal growth restriction.[42,43] Saffron vs. folic acid interventions significantly reduced marginal zone size compared to the DR group (3.16 ± 0.58µm of saffron vs. 2.48 ± 0.25µm of folic acid, $p < 0.001$). These findings strongly suggest the potential of both compounds in modulating the activity of the marginal zone.

Red pulp

Red pulp is a major component of the spleen, consisting of sinusoids that play a crucial role in blood filtration and immune function. Red pulp facilitates cell passage from the circulation into splenic tissue. Splenic red pulp contains the Cords of Billroth, which are responsible for removing senescent or damaged erythrocytes.[44,45] Histopathological analysis revealed a significant decrease in splenic red pulp size in the IUGR group compared to the control group ($p < 0.05$), as depicted in Figure 5. This decrease suggests impaired splenic filtration, potential anemia, and broader alterations in the red pulp structure and function, likely stem from an underlying inflammatory process and/or cellular damage. Although further research is needed to ascertain whether the inflammation is subacute or chronic, the observed morphological changes may be associated with factors: vascular congestion, cellular atrophy, or fibrosis. It is worth noting that both saffron and folic acid interventions showed a trend towards increased red pulp size, indicating their potential in modulating these IUGR-induced effects. The periarteriolar lymphoid sheath (PALS), reveals significant immune alterations in the IUGR group. The observed increase in PALS size ($p < 0.05$) suggests heightened immune activation, possibly due to an increased presence of antigen-presenting cells (APCs) such as dendritic cells and macrophages. This response may represent a compensatory mechanism to systemic inflammation commonly associated with IUGR. However, excessive APC activity can disrupt immune regulation and impair spleen function. Folic acid intervention demonstrated greater efficacy in reducing PALS hypertrophy, suggesting its role in modulating immune hyperactivity. By restoring the normal structure of the PALS, folic acid may help reestablish immune homeostasis, reducing the overactive immune responses seen in IUGR. Saffron, while also effective, demonstrated less impact in comparison. The follicles, primarily composed of B cells, have a crucial role in humoral immunity.[28] In IUGR conditions, a noticeable enlargement of the follicular diameter ($p < 0.05$) was observed, likely reflecting increased proliferation of B cells. This may represent a compensatory mechanism due to chronic inflammation or immune dysregulation.

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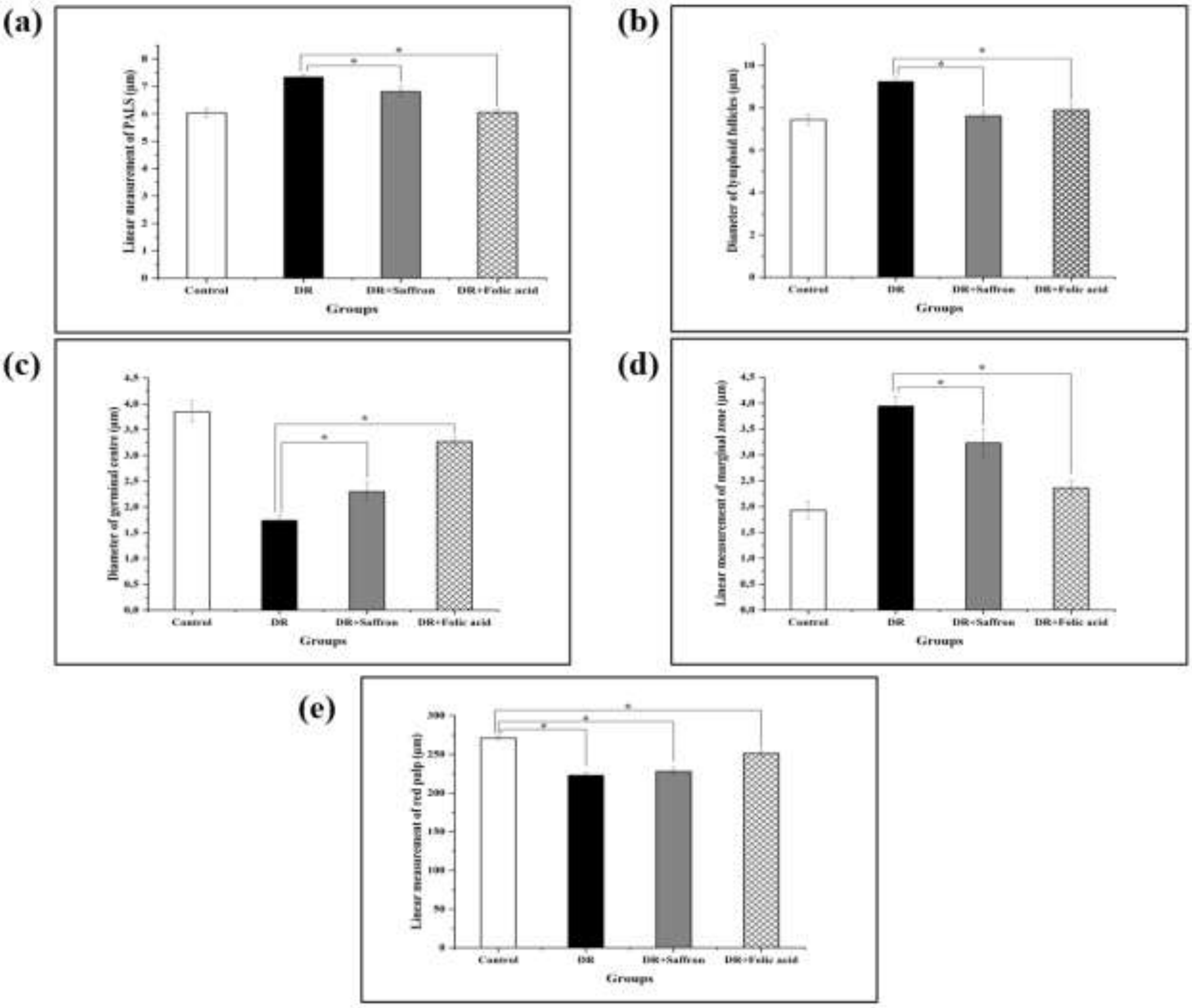

Figure 5: Effect of saffron (*Crocus sativus L.*) and folic acid on splenic histopathology of the Wistar rat pups. (a) periarteriolar lymphoid sheath (PALS), (b) lymphoid follicle, (c) germinal center, (d) marginal zone and (d) red pulp. The * indicate significant differences between the diet restriction (DR) group.

The enlarged follicles in the IUGR group suggest heightened B-cell activation and expansion, potentially driven by an increased antigen load or persistent immune stimulation. Biochemically, this hyperactivation of B cells could be associated with upregulated signalling through receptors like CD40, along with increased production of cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10).[46] These cytokines promote B-cell proliferation, differentiation into plasma cells, and antibody production.[47] This hyperactivity, however, may lead to immune exhaustion or an ineffective immune response, contributing to impaired pathogen defence or autoimmunity in IUGR-affected offspring.

The marginal zone, which serves as a transitional area between the red pulp and white pulp, contains macrophages and specialized B cells that respond rapidly to blood-borne pathogens.[28] The significant thickening of the marginal zone in IUGR ($p < 0.05$) suggests increased macrophage activity and heightened antigens captures from the bloodstream. Pathophysiologically, this may result from systemic inflammation associated with IUGR, where elevated levels of circulating inflammatory cytokines (e.g., TNF-α, IL-6) enhance macrophage recruitment and activation.[48] Additionally, increased lipid peroxidation and oxidative stress, commonly found in IUGR, further stimulate macrophage activation.[49] Although this might initially appear as a beneficial response to counteract infection or inflammation, chronic activation of the marginal zone may disrupt immune surveillance and result in inefficient antigen presentation or improper immune response, further compromising the immune function.

Germinal centers within the follicles are key sites of intense B-cell proliferation, somatic hypermutation, and affinity maturation.[28] In IUGR, the observed decrease in germinal center size ($p < 0.05$) indicated an impairment in the process of generating high-affinity antibodies. This shrinkage suggested a failure in the development of effective B-cell responses, possibly due to dysregulated T-cell help, which is essential for germinal center function. Biochemically, germinal centers depend on a delicate interplay of signals from follicular helper T cells (Tfh) and cytokines like IL-21, to promote B-cell survival and differentiation. The diminished germinal center response in IUGR might have resulted from reduced Tfh cell support or altered expression of co-stimulatory molecules, such as CD40L and ICOS.[44] This impairment could lead to the production of lower-affinity antibodies, thereby weakening the humoral immune defense in IUGR-affected individuals.[50,51]

Pathologically, this reduction may have resulted from decreased erythropoiesis or increased red blood cells destruction due to oxidative stress. IUGR is frequently associated with hypoxia, leading to increased reactive oxygen species (ROS) production and damage to red blood cells. The diminished red pulp area observed in IUGR animals suggests a compromised ability to clear damaged red blood cells, resulting in splenic congestion or ineffective erythropoiesis. Additionally, the red pulp plays a critical role in the immune response by harbouring monocytes and dendritic cells. Reduced red pulp size may indicate a weakened immune surveillance, further compromising the body's ability to mount an effective response to infection or inflammation.[52,53]

Both saffron and folic acid treatments improved the histological features of the spleen, although their effects were compartment-specific. Statistically, saffron showed significant improvement in follicle structure ($p < 0.05$), while folic acid was more effective in restoring PALS, marginal zone, germinal center, and red pulp ($p < 0.05$). These differences underscore the distinct mechanisms by which each intervention exerts its therapeutic effects.

The known antioxidant and anti-inflammatory properties of saffron likely contributed to the restoration of normal follicle structure by modulating B-cell activation and proliferation, improving follicular size and function. In contrast, folic acid demonstrated greater efficacy in modulating the PALS, marginal zone, and germinal center, suggesting its role in supporting T-cell responses, macrophage activity, and B-cell maturation, thus addressing the immune dysregulation seen in IUGR.

In the red pulp, folic acid outperformed saffron in restoring tissue area, improving the spleen's filtration capacity and mitigating erythrocyte damage. These findings suggest that folic acid may have broader impact on both immune and hematological functions.

Inflammation appears to be a significant factor in the pathogenesis of IUGR, evidenced by elevated TNF-α as a pro-inflammatory cytokine and characteristic histopathological changes. This inflammation stemming from placental insufficiency and cellular stress acts as a key driver of various downstream pathologies, including oxidative stress, tissue damage, altered cellular function, and vascular dysfunction[54]. Overall, the underlying pathology in IUGR is chronic hypoxia ischemia due to alteration in fetoplacental circulation.

In conclusion, IUGR induces widespread pathological changes in the spleen, affecting both immune function and erythropoiesis, while also significantly impacting birth weight. Our study reveals that both saffron and folic acid interventions improve fetal birth weight, with folic acid demonstrating superior efficacy. Regarding organ health, saffron more effectively improves the spleen index compared to folic acid. Furthermore, while both compounds modulate immune responses, folic acid significantly outperforms saffron in reducing splenic TNF-α expression as determined by immunohistochemistry. Histopathological analyses confirmed distinct effects, with saffron effectively improving follicular health, while folic acid provides broader restoration across multiple splenic compartments, including PALS, marginal zone, germinal center, and red pulp. These findings highlight the distinct yet complementary benefits of each individual intervention in mitigating IUGR-induced adverse outcomes on both birth weight and splenic function.

Conclusion

Based on the findings of the study, the administration of saffron and folic acid effectively prevented birth weight reduction and spleen organ impairment in offspring rats with intrauterine growth restriction (IUGR) due to dietary restriction. Both interventions demonstrated the ability to reduce spleen tissue damage and TNF-α expression, with folic acid showing superior efficacy compared to saffron. Although folic acid was more effective, these findings highlight the potential of saffron as a novel nutritional intervention in mitigating the adverse effects of IUGR.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and assume full accountability for any issues or claims arising from its publication.

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