Eksakta : Berkala Ilmiah Bidang MIPA
VOLUME 27 NO 06 2026, pp 1099-1110
ISSN : Print 1411-3724 — Online 2549-7464
DOI : https://doi.org/10.24036/eksakta/vol27-iss06/715

Eksakta
Berkala Ilmiah Bidang MIPA
http://www.eksakta.ppj.unp.ac.id/index.php/eksakta

Article

The Effect of Apigenin as an Antioxidant in the Kidney Rats Induced by Excess Iron-Dextran

Article Info

Article history *:*
Received June 28, 2026
Revised October 25, 2026
Accepted November 12, 2026
Published December 30, 2026
In Press

Keywords *:*
Apigenin,
iron overload,
kidney, GSH,
antioxidant

Salsabila Raniah[1], Ari Estunigtyas[2*], Rani Wardani Hakim[3]

[1]Master's Programme in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Department of Pharmacology and Therapeutic, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[3]Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Abstract. Excess renal iron may generate reactive oxygen species, which may deplete antioxidant systems. Apigenin may limit renal iron accumulation and support antioxidant defenses. This study evaluated apigenin in rats given excess iron. Sprague-Dawley rats were randomized into normal (N), iron overload (IO), deferiprone, and apigenin (25, 50, and 100 mg/kg BW) groups (n = 4/group). Iron dextran (15 mg/250 g BW) was administered for four weeks and continued four more weeks with treatment. Renal iron and GSH were measured. Renal iron in the IO group increased 3.18-fold compared with the N group (504.8 vs. 158.8 mg/kg; $p < 0.05$). Deferiprone numerically lowered renal iron by 21.69% (395.3 mg/kg) compared with IO ($p > 0.05$). None of the apigenin doses significantly reduced renal iron (644.5, 579.8, and 699.5 mg/kg) or increased GSH (39.3, 43.3, and 36.0 nmol/g) compared with IO (504.8 mg/kg and 43.5 nmol/g; $p > 0.05$), whereas GSH was numerically higher in deferiprone than in IO (49.5 nmol/g; $p > 0.05$). Renal GSH in the apigenin 100 mg/kg BW group was significantly lower than deferiprone ($p < 0.05$). Overall, apigenin was not shown to significantly reduce renal iron or increase GSH.

This is an open access article under the CC-BY license.

This is an open access article distributed under the Creative Commons 4.0 Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. ©2026 by author.

Corresponding Author:
Ari Estuningtyas
Department of Pharmacology and Therapeutic, Faculty of Medicine,
Universitas Indonesia, Jakarta, Indonesia
Email : aestuningtyas04@gmail.com

1. Introduction

Iron is an essential nutrient for cellular function, supporting various biological processes and playing a vital role in redox reactions [1]. Iron can act as either an electron donor or acceptor by switching between its ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms, thereby supporting various biochemical reactions in the body [2]. This redox activity is potentially toxic, as iron can catalyze the Fenton and Haber-Weiss reactions, producing harmful hydroxyl radicals [3]. Given its redox activity, iron is kept in a relatively inert form by transferrin in the circulation and ferritin in cells, under tight systemic and cellular regulation [4]. Maintaining adequate iron availability is essential for balancing physiological function and preventing iron toxicity, as iron levels are primarily regulated through absorption with no active excretory mechanism to eliminate excess iron [5-6].

Iron overload is one example of this imbalance, commonly observed in transfusion-dependent thalassemia patients, with each unit of packed red blood cells contributing approximately 200–250 mg of iron [7]. This excess iron can exceed transferrin-binding capacity, leading to iron accumulation within organ cells. As transferrin becomes saturated, non-transferrin-bound iron (NTBI) forms and contributes to the labile iron pool, promoting reactive oxygen species (ROS) production and oxidative damage [2],[8]. Under normal conditions, cells neutralize ROS through antioxidant defenses such as glutathione (GSH), a tripeptide that serves as substrate for GPX4 [9]. However, when iron-induced ROS production becomes excessive, it may disrupt the glutathione system and deplete GSH, further compromising antioxidant defense [10]. As a result, oxidative stress may lead to iron-dependent cell death (ferroptosis), which may ultimately affect the function of vital organ [11].

The kidneys are a vital organ that receives approximately 20-25% of cardiac output [12]. Recent studies have highlighted the involvement of the kidneys in iron handling through glomerular filtration and tubular reabsorption, making them highly susceptible to systemic iron overload [13]. Under conditions of excessive iron exposure, these processes may disrupt renal iron homeostasis, contributing to renal iron accumulation and oxidative injury that may lead to kidney injury [11]. This is particularly relevant given the increasing recognition of renal complications in patients with thalassemia [14-15]. Since the body lacks an active mechanism for iron excretion, pharmacological intervention is required to facilitate excess iron elimination.

Iron chelators such as deferiprone, deferasirox, and deferoxamine are used as standard therapies to reduce iron overload [16]. Nevertheless, long-term chelation requires careful monitoring for adverse effects [17]. Therefore, the potential of natural compounds is increasingly being explored. Apigenin (4',5,7-trihydroxyflavone), a natural flavonoid widely distributed in plants such as celery and chamomile, is a potential candidate for limiting iron-related oxidative damage [18]. Its hydroxyl and carbonyl functional groups have been reported to confer iron-binding properties [18-19]. These structural features may also contribute to its antioxidant activity by limiting Fenton reaction-mediated ROS generation [20].

A previous in-silico study [21] demonstrated that apigenin exhibits higher binding affinity for Fe (III) compared with Cu (II). Apigenin has also been reported to exert various pharmacological effects including the modulation of endogenous antioxidant defenses [22]. In experimental animal models, apigenin increased GSH levels while reducing ROS in rats with doxorubicin-induced renal injury [23] and hypercholesterolemic-induced kidney injury [24]. Furthermore, apigenin has demonstrated low intrinsic toxicity [25], supporting its further pharmacological investigation via the oral route.

Based on previous findings, apigenin may be a promising agent to limit renal iron accumulation and support antioxidant defenses, owing to its iron-binding and antioxidant properties. However, these properties have been demonstrated mainly through in silico studies and renal injury models not induced by iron, so whether they translate into benefits on renal iron burden and GSH level under iron excess remains an unresolved issue. To date, the in vivo effect of apigenin under iron overload has been largely unexplored. This issue is growing importance, as the kidney is increasingly recognized as vulnerable to excess iron exposure, which promotes oxidative stress in renal tissue that may progress to kidney injury.

To address this issue, this study aimed to evaluate the effects of apigenin on total renal iron content and GSH, an endogenous antioxidant that may provide an initial indication of redox status, in rats administered iron dextran. Iron dextran was given repeatedly to represent frequent iron exposure. Apigenin dose was based on Liu et al. (2024) [26], who reported that 50 mg/kg BW attenuated ferroptosis in a mouse model, and an equivalent rat dose of 25 mg/kg BW was derived using body surface area conversion. Two additional doses, 50 and 100 mg/kg BW, were established using geometric progression. Together, these three doses were used to assess the dose-response relationship of apigenin on renal iron and GSH levels.

2. Experimental Section

2.1. Materials

Apigenin was obtained from Plamed Green Science Group, China, with a certified purity of 98.5% as determined by high-performance liquid chromatography (HPLC). Deferiprone (Oferlod) was obtained from PT Novel Pharmaceutical Laboratories. Iron dextran (Hemadex) was obtained from Sanbe Farma. Male Sprague-Dawley rats were purchased from Indonesian Food and Drug Authority. All animal experiments were approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Ethical Approval No. KET-932/UN2.F1/ETIK/PPM.00.02/2025).

2.2. Animals

This study was conducted in the Animal Research Facilities (ARF) of Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia. A total of twenty-four Sprague-Dawley rats were used, selected based on the following inclusion criteria: healthy condition, male sex, six weeks of age, and body weight of 250-450 g. Exclusion criteria were female sex, age under six weeks, body weight below 250 g, and signs of illness during acclimatization. The rats were acclimatized for seven days prior to treatment under standardized conditions (25°C, 40-60% humidity, a 12:12 h light/dark cycle, and standard laboratory feed with water provided ad libitum). Following acclimatization, the rats were randomly assigned to six experimental groups using simple randomization via a random number generator, as follows: N (normal control) received no treatment; IO (iron overload) received iron dextran 15 mg/250 g BW and CMC Na 0.5%; D received iron dextran 15 mg/250 g BW and deferiprone 462.5 mg/kg BW; A1, A2, and A3 received iron dextran 15 mg/250 g BW and apigenin at 25, 50, and 100 mg/kg BW, respectively.

All treatment groups except normal control received intraperitoneal iron dextran injections twice weekly for four weeks. Beginning in the fifth week, the respective therapies were additionally administered by oral gavage, while iron dextran induction continued. At the end of the eight weeks, all rats were weighed and euthanized using ketamine/xylazine. The kidneys were harvested and weighed for subsequent analysis. Renal iron measurements were performed by an analyst blinded to the treatment group assignments using coded samples. The complete experimental workflow is illustrated in Figure 1.

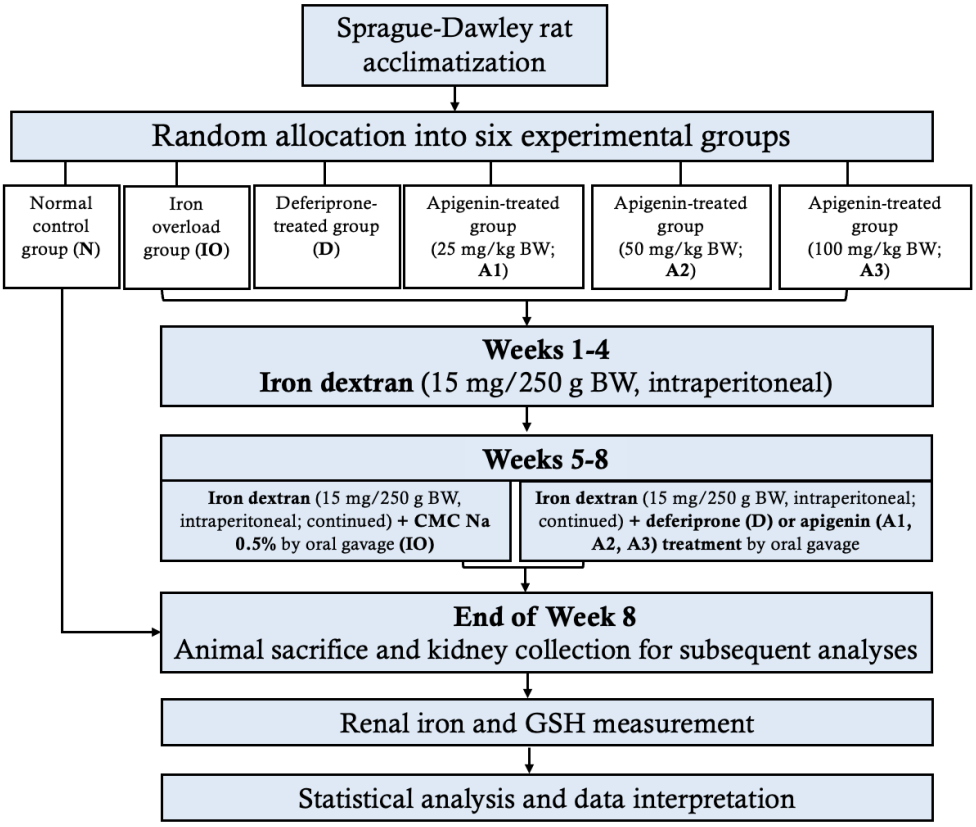


Figure 1. Schematic Overview of the Experimental Design.

2.3 Renal Iron Content

Renal iron content was measured according to the atomic absorption spectrophotometry AAS method. Briefly, a 200 mg kidney sample was placed in an Erlenmeyer flask, immersed in 2 mL of nitric acid (HNO_3), and treated with one to two drops of picric acid. The sample was then heated on a hotplate until the brown vapor disappeared, after which it was cooled to room temperature. Distilled water was added to the total volume to 50 mL. The iron concentration was subsequently measured using AAS at a wavelength of 248.3 nm.

2.4 Renal GSH

Renal total GSH levels were measured using a commercial colorimetric kit (Elabscience, Catalog No. E-BC-K097-M), based on the reaction of GSH with DTNB to form a yellow product. Briefly, 100 mg of kidney tissue was washed with cold PBS (0.01 M, pH 7.4), homogenized in 900 µL of protein precipitator, and centrifuged at 10,000 × *g* for 10 minutes at 4°C. A 10 µL aliquot of the supernatant was loaded to sample wells. A total of 150 µL of reaction working solution was then added to each well and incubated for 5 minutes at room temperature. Subsequently, 50 µL of substrate working solution was added to each well, mixed for 5 seconds, and incubated for a further 25 minutes. The optical density (OD) was then measured at 412 nm. Total GSH content was calculated using the following formula: GSH content = (ΔA1-b1) ÷ a1 x 2 ÷ m/v1 x f. Where ΔA1 is the difference between the sample and blank OD values; b1 and a1 are the intercept and slope of the GSH standard curve, respectively; m is the fresh weight of the sample; v1 is the volume of protein precipitator used during tissue sample preparation; and f is the dilution factor of the sample prior to testing.

2.5 Statistical Analysis and Software

Statistical analyses were performed using SPSS software (version 29). The data were subjected to normality and homogeneity tests. If the data were normally distributed and displayed homogeneous variance, statistical significance was analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests to determine differences between groups. If the data were normally distributed but lacked homogeneity of variance, Welch's ANOVA was performed, followed by Games-Howell post hoc analysis. Graphical representations were generated using GraphPad Prism version 10.

3. Results and Discussion

As presented in Figure 2, the excess iron dextran administration significantly increased renal iron content in the IO group (504.8 mg/kg) compared with the N group (158.8 mg/kg), as demonstrated by the Welch's ANOVA test ($p < 0.001$) followed by Games-Howell post hoc test ($p < 0.05$). This elevation represents a 3.18-fold increase, indicating that the iron dextran regimen increased renal accumulation. Liang et al. (2023) [27] reported that iron dextran was distributed not only to the serum and liver but also to the lymphatic system. The present study is consistent with this finding, as the distribution extends beyond the primary organs of iron metabolism and reaches the kidneys as well. This result is aligned with the findings of study by Heriatmo et al., (2024) [28], who similarly reported significant renal iron deposition following iron dextran administration in a rat model.

Under physiological conditions, the kidney filters transferrin-bound iron (TBI) which is reabsorbed by the tubules. However, under iron overload conditions, non-transferrin-bound iron (NTBI) is formed, subsequently filtered and enters tubular epithelial cells through several transport mechanisms [29,30]. The excess filtered iron increases the deposition within the kidney that accumulates in the renal cortex, particularly in the glomerular and proximal tubular region [31]. This deposition may turn trigger cellular damage to renal tissue [32]. Consequently, if not counteracted by endogenous defense systems, this process can lead to an increase in renal function biomarkers, as typically observed in iron overload conditions [33]. Taken together, the eight-week intraperitoneal iron dextran protocol used in this study was sufficient to establish a model of renal iron accumulation.

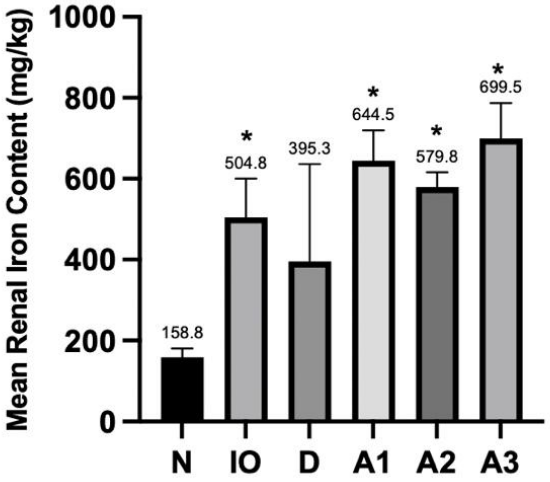


Figure 2. Mean Renal Iron Content in Rat After Treatment. Bars represent the mean and error bars represent the standard deviation (SD); n = 4 per group. *) $p < 0.05$ vs. N group (Welch's ANOVA, $p < 0.001$; Games-Howell post hoc, $p < 0.05$).

To mitigate iron accumulation, iron chelation therapy has been established as the standard intervention for reducing body iron burden in iron overload. Deferiprone, one of iron chelating agent, is a bidentate chelator with a low molecular weight which enables to penetrate cells and bind directly to damaging labile free iron, forming a stable 3:1 (deferiprone: iron) complex. It mobilizes iron from various body iron pools and organs, and forms orange-red complex that will be excreted via urine [34]. According to Figure 2, renal iron content in the deferiprone group was 395.3 mg/kg, 21.69% lower than in the IO group, although the difference was not statistically significant ($p > 0.05$).

Deferiprone has been reported to be effective in reducing cardiac iron, whereas deferoxamine achieves better outcomes in the liver [35]. This suggests that the iron-lowering effects of chelators may vary across tissues. However, in another study on renal iron accumulation that was not driven by systemic iron loading from Sharawy et al., (2024) [36], deferiprone effectively lowered renal iron levels and conferred protection against oxidative damage under conditions of kidney injury that disrupt renal iron homeostasis. In the present study, deferiprone had numerically lower renal iron than IO group, but the large standard deviation observed in the deferiprone group (Figure 2) suggests considerable inter-individual variability, which may have reduced the statistical power to detect a significant difference.

While deferiprone produced at least a numerical lowered in renal iron, none of the apigenin-treated groups showed a reduce in renal iron (644.5 mg/kg, 579.8 mg/kg, and 699.5 mg/kg for the 25, 50, and 100 mg/kg doses, respectively) compared to IO (504.8 mg/kg; $p > 0.05$). Renal iron content in the apigenin group remained significantly higher than in the N group ($p < 0.05$) and no consistent dose-response relationship was observed. In present study, apigenin did not show to reduce renal iron as predicted by the in-silico study [21]. In contrast to this finding, this may be related to its chemical properties, as an in vitro study by Kurpios et al., (2021) [37] demonstrated that apigenin was a slightly weaker Fe(II) chelator than catechol-containing flavonoids and the weakest Fe(III) reductant among those tested, suggesting that the absence of a catechol group may limit its iron-chelating activity.

Besides its chelating capacity, the stability of apigenin under physiological conditions may also play a role. Previous studies have suggested that the stability of apigenin is influenced by environmental factors, including physiological temperature and pH changes, which may lower the availability of the active compound. Accordingly, this may reduce its antioxidant and iron-chelating activities [20],[38]. In addition, AAS method measures total iron renal and does not distinguish its Fe(II) or Fe(III) form. Therefore, any effect of apigenin on specific iron forms may not be detected, which may limit the interpretation of its chelating to reduce renal iron accumulation.

To assess the redox consequences of renal iron accumulation, GSH was measured as a sensitive marker of cellular antioxidant capacity against iron-induced oxidative stress [39]. Under iron overload, increased ferritin storage may enhance NCOA4-mediated ferritinophagy, expanding the labile iron pool and promoting Fenton-driven ROS generation [40]. This labile iron accelerates Fenton-driven ROS generation and triggers lipid peroxidation. To counteract this oxidative insult, glutathione peroxidase 4 (GPX4) utilizes GSH as an essential cofactor to neutralize phospholipid hydroperoxides and prevent iron-dependent cell death [41]. Given that, GSH levels provide further insight into renal iron accumulation as presented in Figure 3.

According to Figure 3, renal GSH levels in the IO group (43.5 nmol/g) were not lower than the N group (41 nmol/g), instead showed a slight upward trend although not significant ($p > 0.05$). Excess iron administration in this model did not appear to be accompanied by a reduction in renal GSH. This is consistent with Rahma et al., (2024) [42], who showed that iron dextran induction elevated GSH levels as part of an initial compensatory response to elevated ROS. This may suggest that GSH synthesis was increased in the IO group as a response to higher ROS production. This phenomenon also aligns with a recent clinical study by Srichairatanakool et al., (2026) [43], which reported increased GSH levels in patients with thalassemia and marked iron accumulation, interpreted as a compensatory mechanism to maintain cellular redox balance.

In contrast, according to Piloni et al., (2021) [44] an acute iron dextran exposure in rat brain was reported to deplete GSH while inducing Nrf2-controlled GPx and GST expression as an adaptive response. Study by Odeh et al., (2022) [45] has also reported GSH depletion after iron dextran administration in different rat model. In addition, Dar et al. (2026) [46] also reported reported lower GSH levels following chronic iron exposure, whereas acute exposure did not. These discrepancies may be related to variations in iron exposure duration and dose, which can influence oxidative stress and GSH levels [46-47]. This non-significant trend may suggest that IO groups were still within a compensato ry phase, before GSH depletion.

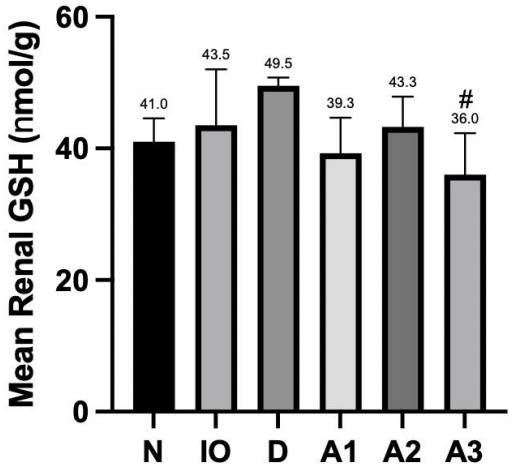


Figure 3. Mean Renal GSH in Rat after Treatment. Bars represent the mean and error bars represent the standard deviation; n = 4 per group. $^{\#)}p < 0.05$ vs. D group (One Way ANOVA, $p = 0.049$; post hoc Tukey, $p < 0.05$).

On the other hand, The D group had the highest GSH (49.5 nmol/g) accompanied by numerically lower renal iron content, though neither differed significantly from IO group ($p > 0.05$; Figures 2 and 3). Unlike in the IO group, this tendency may interpret as preserved antioxidant capacity under a lower iron burden than a compensatory response. Consistent with Morales et al., (2022) [48], deferiprone protected cells against iron-induced oxidative damage indirectly by reducing redox-active iron and thereby limiting Fenton-driven hydroxyl radical formation. In another study of iron-overloaded patients from Merkel et al., (2024) [49], deferiprone reduced the labile iron pool by 35-46% in blood cells and increased reduced glutathione. In addition, another study by Li et al., (2024) [50] showed deferiprone to restore the iron-induced decrease in GSH in iron-loaded hepatocytes. Taken together, the observed pattern is consistent with this interpretation, although the nonsignificant findings provide limited statistical support.

In contrast, apigenin did not show the expected increase in GSH levels. All three doses showed numerically lower levels than the IO group ($p > 0.05$), while the 100 mg/kg BW group showed significantly lower GSH levels than the D group ($p < 0.05$). An in vitro study by Han et al., 2022 [51] showed that apigenin increased the GSH/GSSG ratio and suppressed intracellular iron accumulation in DEHP-induced liver injury cells [49]. In contrast, the numerically lower GSH levels and higher renal iron content in the apigenin groups may be consistent with reports linking prolonged or greater iron exposure to GSH depletion [46],[52]. Interestingly, among the apigenin-treated groups, the mean GSH levels A2 (43.3 nmol/g), A1 (39.3 nmol/g) and A3 (36.0 nmol/g) showed an inverse pattern to renal iron content (Figures 2 and 3). However, this pattern warrants further investigation, as the relationship was not statistically tested.

Shi et al., (2023) [53] also reported that higher iron levels were accompanied by reduced GSH and GPX4 in other rat models of kidney injury. Similarly, the significant difference in GSH between the D and A3 groups may be associated with their contrasting renal iron levels, with D showing the lowest and A3 the highest renal iron content. This difference may help explain the significantly higher GSH levels in D than in A3, providing further support for a possible link between renal iron levels and GSH. However, the lack of a statistically significant difference in renal iron levels between D and A3 makes it difficult to establish a clear association with the observed GSH difference. In addition, the absence of oxidative stress markers and ROS measurements also limits this interpretation.

Considering the overall findings of this study, these results suggest that the effects of apigenin were limited under the conditions of the present study. Hrelia and Angeloni (2021) [54] highlighted that the direct scavenging of ROS by natural antioxidants may be insufficient to provide effective antioxidant in vivo because of kinetic limitations. These limitations are particularly relevant to polyphenols, whose low bioavailability and extensive biotransformation may reduce their antioxidant efficacy in vivo. This provides a potential explanation for the findings observed with apigenin in the present model. DeRango-Adem and Blay (2021) [55] reported that only approximately 30% of orally administered apigenin reaches the systemic circulation, indicating low proportion of a compound that is absorbed and becomes available in the systemic circulation.

Hsu et al., (2025) [56] demonstrated that apigenin undergoes extensive phase I and II metabolism in enterocytes and hepatocytes. The formation of glucuronide conjugates may increase the molecular weight of apigenin, consequently reducing its intestinal absorption [38]. In addition, the poor aqueous solubility of apigenin may also contribute to its absorption, so higher doses may not proportionally increase the amount available in dissolved form [57]. The lack of a clear dose-dependent pattern across the three doses may therefore be partly related to the variable pharmacokinetics of apigenin.

These in vivo findings may reflect challenges in demonstrating the efficacy of apigenin. In silico and in vitro studies assess molecular interactions under static conditions, whereas in vivo studies involve dynamic pharmacokinetic processes within a living organism. We speculate that the limited efficacy of apigenin may be related to its insufficient availability at the site of action and its chelation properties, which may depend on its functional groups, including the absence of a catechol moiety, although these factors were not assessed in this study. The lower GSH level in the highest dose apigenin group than in the deferiprone group may be related to differences in renal iron levels, although neither group differed significantly from the IO group. Given the limitations of this study, these should be considered when interpreting the findings, which do not rule out potential effects under different experimental conditions. Future studies could explore modifications to the delivery system and include the measurement of oxidative damage markers, labile iron, and tissue apigenin concentrations.

4. Conclusion

This study demonstrated that iron dextran administration successfully established renal iron accumulation in rats, increasing renal iron content 3.18-fold compared with normal controls (504.8 vs. 158.8 mg/kg). However, renal GSH levels in the iron overload group did not differ significantly from normal controls and displayed a slight upward trend rather than depletion. Deferiprone resulted in numerically lower renal iron content (395.3 mg/kg; 21.69% lower than IO) and slightly higher renal GSH levels, although neither difference was statistically significant. Oral administration of apigenin at 25, 50, and 100 mg/kg BW did not reduce renal iron (644.5, 579.8, and 699.5 mg/kg) or increase GSH (39.3, 43.3, and 36.0 nmol/g) significantly compared with the IO group (504.8 mg/kg and 43.5 nmol/g), and no consistent dose-response relationship was observed. Overall, under the specific experimental conditions and dosing regimens evaluated in this study, oral apigenin was not shown to significantly reduce renal iron accumulation or increase renal GSH levels in rat administered iron dextran.

5. Acknowledgement
This study was supported by Funding from Direktorat Jenderal Riset Dan Pengembangan Kementerian Pendidikan Tinggi, Sains, dan Teknologi 2026.

References

[1] Obeagu, E. I. (2025). Iron homeostasis and health: understanding its role beyond blood health – a narrative review. *Annals of Medicine & Surgery*, *87*(6), 3362–3371.
[2] Duca, L., Di Pierro, E., Scaramellini, N., Granata, F., & Graziadei, G. (2025). The Relationship Between Non-Transferrin-Bound Iron (NTBI), Labile Plasma Iron (LPI), and Iron Toxicity. *International Journal of Molecular Sciences*, *26*(13).
[3] Silva, A. M. N., & Rangel, M. (2022). The (Bio)Chemistry of Non-Transferrin-Bound Iron. *Molecules*, *27*(6).
[4] Testa, U., Pelosi, E., & Castelli, G. (2025). Outline of Iron Metabolism, with Emphasis on Erythroid Cells. *Mediterranean Journal of Hematology and Infectious Diseases*, *17*(1).
[5] Garbowski, M. W., Cabantchik, I., Hershko, C., Hider, R., & Porter, J. B. (2023). The clinical relevance of detectable plasma iron species in iron overload states and subsequent to intravenous iron-carbohydrate administration. *American Journal of Hematology*, *98*(3), 533–540.
[6] Qiu, L., Frazer, D. M., Hu, M., Song, R., Liu, X., Qin, X., Ma, J., Zhou, J., Tan, Z., Ren, F., Collins, J. F., & Wang, X. (2025). Mechanism and regulation of iron absorption throughout the life cycle. *Journal of Advanced Research*, *77*, 107–118.
[7] Woei-A-Jin, F. J. S. H., Zheng, S. Z., Kiliçsoy, I., Hudig, F., Luelmo, S. A. C., Kroep, J. R., Lamb, H. J., & Osanto, S. (2020). Lifetime Transfusion Burden and Transfusion-Related Iron Overload in Adult Survivors of Solid Malignancies. *The Oncologist*, *25*(2), e341–e350.
[8] Pilo, F., Cilloni, D., Della Porta, M. G., Forni, G. L., Piperno, A., Santini, V., & Angelucci, E. (2022). Iron-mediated tissue damage in acquired ineffective erythropoiesis disease: It's more a matter of burden or more of exposure to toxic iron form? *Leukemia Research*, *114*.
[9] Chen, X., Li, J., Kang, R., Klionsky, D. J., & Tang, D. (2021). Ferroptosis: machinery and regulation. *Autophagy*, *17*(9), 2054–2081.
[10] Afzal, S., Abdul Manap, A. S., Attiq, A., Albokhadaim, I., Kandeel, M., & Alhojaily, S. M. (2023). From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. *Frontiers in Pharmacology*, *14*.
[11] Huang, X., Liu, R., Zhan, C., Wu, H., Fan, J., Li, Z., & Yang, X. (2024). Aristolochic acid induces acute kidney injury through ferroptosis. *Frontiers in Pharmacology*, *15*.
[12] Wareing, M., & Smith, C. P. (2021). Iron Is Filtered by the Kidney and Is Reabsorbed by the Proximal Tubule. *Frontiers in Physiology*, *12*.
[13] Packer, M. (2024). Iron homeostasis, recycling and vulnerability in the stressed kidney: A neglected dimension of iron-deficient heart failure. *European Journal of Heart Failure*, *26*(7), 1631–1641.
[14] Kabbara, F., Charbel, N., El Khoury, S., Klim, J., Ismail, G., Ismail, F., Fawaz, H., Koussa, S., & Taher, A. (2026). Liver iron concentration and renal dysfunction in β-thalassemia: a case series and exploratory clinical observations. *Annals of Hematology*, *105*(9).
[15] Yılmaz, İ., Şimşek, A., Yılmaz, H., Cevizli, D., & Leventoğlu, E. (2025). Renal and cardiac involvements in children with transfusion-dependent thalassemia: A cross-sectional analysis. *European Journal of Pediatrics*, *184*(11).
[16] Bruzzese, A., Martino, E. A., Mendicino, F., Lucia, E., Olivito, V., Bova, C., Filippelli, G., Capodanno, I., Neri, A., Morabito, F., Gentile, M., & Vigna, E. (2023). Iron chelation therapy. *European Journal of Haematology*, *110*(5), 490–497.

[17] Siregar, O. R., Siregar, R. S., & Lubis, B. (2020). Renal Function in Children with β-Thalassemia Major Treated with Iron Chelating Agent. *Indonesian Biomedical Journal*, *12*(3), 214–219.
[18] Kashyap, P., Shikha, D., Thakur, M., & Aneja, A. (2022). Functionality of apigenin as a potent antioxidant with emphasis on bioavailability, metabolism, action mechanism and in vitro and in vivo studies: A review. *Journal of Food Biochemistry*, *46*(4).
[19] Liu, S., Zheng, X., Luo, Z., Tang, C., Hu, Y., Peng, Q., Mi, P., Chen, H., & Yao, X. (2024). The synthesis and bioactivity of apigenin derivatives. *Fitoterapia*, *179*.
[20] Kejík, Z., Kaplánek, R., Masařík, M., Babula, P., Matkowski, A., Filipenský, P., Veselá, K., Gburek, J., Sýkora, D., Martásek, P., & Jakubek, M. (2021). Iron complexes of flavonoids-antioxidant capacity and beyond. *International Journal of Molecular Sciences*, *22*(2), 1–20.
[21] Spiegel, M., & Sroka, Z. (2023). Quantum-mechanical characteristics of apigenin: Antiradical, metal chelation and inhibitory properties in physiologically relevant media. *Fitoterapia*, *164*.
[22] Allemailem, K. S., Almatroudi, A., Alharbi, H. O. A., AlSuhaymi, N., Alsugoor, M. H., Aldakheel, F. M., Khan, A. A., & Rahmani, A. H. (2024). Apigenin: A Bioflavonoid with a Promising Role in Disease Prevention and Treatment. *Biomedicines*, *12*(6).
[23] Wu, Q., Li, W., Zhao, J., Sun, W., Yang, Q., Chen, C., Xia, P., Zhu, J., Zhou, Y., Huang, G., Yong, C., Zheng, M., Zhou, E., & Gao, K. (2021). Apigenin ameliorates doxorubicin-induced renal injury via inhibition of oxidative stress and inflammation. *Biomedicine and Pharmacotherapy*, *137*.
[24] Almaghrabi, S. Y. (2023). Apigenin ameliorates hypercholesterolemic-induced kidney injury via modulating renal KIM-1, Fn1, and Nrf2 signaling pathways. *European Review for Medical and Pharmacological Sciences*, *27*, 1155–1169.
[25] Sato, V. H., Sato, H., Sangfuang, M., Nontakham, J., Junyaprasert, V. B., Teeranachaideekul, V., & Morakul, B. (2024). Enhancement of in vitro transcellular absorption and in vivo oral bioavailability of apigenin by self-nanoemulsifying drug delivery systems. *Scientific Reports*, *14*(1).
[26] Liu, Q., Mao, T., Liu, F., Chen, B., Liu, Z., Pathak, J. L., & Li, J. (2024). Apigenin alleviates Sjögren's syndrome-induced salivary gland epithelial cell ferroptosis via ERα signaling-mediated regulation of the ATF3/SLC7A11 axis. *International Immunopharmacology*, *143*.
[27] Liang, M., Chen, L., He, Q., Mi, X., Qu, L., Xie, J., & Song, N. (2023). Intraperitoneal injection of iron dextran induces peripheral iron overload and mild neurodegeneration in the nigrostriatal system in C57BL/6 mice. *Life Sciences*, *320*.
[28] Larasinta Heriatmo, N., Estuningtyas, A., Soetikno, V., & Poerwaningsih, E. H. (2024). Attenuation of TNF-α and Iron Levels in Renal Hemosiderosis by Phaleria macrocarpa (Scheff.) Boerl Extract in a Rat Iron Overload Model. *RESEARCH ARTICLE Indonesian Journal of Pharmacy Indonesian J Pharm*, *35*(3), 481–490.
[29] van Swelm, R. P. L., Wetzels, J. F. M., & Swinkels, D. W. (2020). The multifaceted role of iron in renal health and disease. *Nature Reviews Nephrology*, *16*(2), 77–98.
[30] Fisher, A. L., & Babitt, J. L. (2021). Pumping iron in the kidney. *Kidney International*, *100*(3), 505–508.
[31] Chultemsuren, M., Song, S.-J., Han, K.-H., & Shin, J.-A. (2025). Iron Overload Accelerates Aging-Associated Kidney Injury in Mice: Implications for Iron Supplementation in the Elderly. *Nutrients*, *17*(16), 2580.
[32] Punchai, S., Chaiyagot, N., Artkaew, N., Jusakul, A., Cha'on, U., Thanan, R., Vaeteewoottacharn, K., & Lert-Itthiporn, W. (2024). Iron-induced kidney cell damage: insights into molecular mechanisms and potential diagnostic significance of urinary FTL. *Frontiers in Molecular Biosciences*, *11*.

[33] Song, Y., Yang, N., Si, H., Liu, T., Wang, H., Geng, H., Qin, Q., & Guo, Z. (2022). Iron overload impairs renal function and is associated with vascular calcification in rat aorta. *BioMetals*, *35*(6), 1325–1339.

[34] Kontoghiorghes, G. J. (2023). The Vital Role Played by Deferiprone in the Transition of Thalassaemia from a Fatal to a Chronic Disease and Challenges in Its Repurposing for Use in Non-Iron-Loaded Diseases. *Pharmaceuticals*, *16*(7).

[35] Entezari, S., Haghi, S. M., Norouzkhani, N., Sahebnazar, B., Vosoughian, F., Akbarzadeh, D., Islampanah, M., Naghsh, N., Abbasalizadeh, M., & Deravi, N. (2022). Iron Chelators in Treatment of Iron Overload. *Journal of Toxicology*, *2022*.

[36] Sharawy, N., Aboulhoda, B. E., Khalifa, M. M., Morcos, G. N., Morsy, S. A. A. G., Alghamdi, M. A., Khalifa, I. M., & Algaleel, W. A. A. (2024). Amelioration of nephrotoxicity by targeting ferroptosis: role of NCOA4, IREB2, and SLC7a11 signaling. *Brazilian Journal of Medical and Biological Research*, *57*.

[37] Kurpios-Piec, D., Majewska-Wierzbicka, M., Czeczot, H., & Podsiad, M. (2021). Flavonoids as Hydroxyl Radical Scavengers, Iron Reductants and Chelators: In Vitro Antioxidant Action. *Acta Poloniae Pharmaceutica - Drug Research*, *78*(5), 635–648.

[38] Chen, P., Chen, F., Guo, Z. L., Lei, J., & Zhou, B. (2023). Recent advancement in bioeffect, metabolism, stability, and delivery systems of apigenin, a natural flavonoid compound: challenges and perspectives. *Frontiers in Nutrition*, *10*.

[39] Sönmez Aydın, F., Hukkamlı, B., & Budak, H. (2021). Coaction of hepatic thioredoxin and glutathione systems in iron overload-induced oxidative stress. *Journal of Biochemical and Molecular Toxicology*, *35*(4).

[40] Li, X., Meng, F., Wang, H., Sun, L., Chang, S., Li, G., & Chen, F. (2023). Iron accumulation and lipid peroxidation: implication of ferroptosis in hepatocellular carcinoma. *Frontiers in Endocrinology*, *14*.

[41] Kuang, F., Liu, J., Tang, D., & Kang, R. (2020). Oxidative Damage and Antioxidant Defense in Ferroptosis. *Frontiers in Cell and Developmental Biology*, *8*.

[42] Rahma, Estuningtyas, A., Kusmardi, & Poerwaningsih, E. H. (2024). Effect of the combination of deferiprone with ethanolic extract of Phaleria macrocarpa L. fruit in overcoming iron excess in the liver of hemosiderosis model rats. *Pharmacia*, *71*, 1–14.

[43] Srichairatanakool, S., Paradee, N., Pokharel, B., Zhong, Y., Kerdto, O., Kiratipaisarl, W., Tantiworawit, A., Chai-Adisaksopha, C., & Srichairatanakool, S. (2026). Iron Overload-Associated Oxidative Stress and Immune Cell Dysfunction in Thalassemia: Integrative Analysis of Hematological, Biochemical, and Flow Cytometric Biomarkers. *Antioxidants*, *15*(4).

[44] Piloni, N. E., Vargas, R., Fernández, V., Videla, L. A., & Puntarulo, S. (2021). Effects of acute iron overload on Nrf2-related glutathione metabolism in rat brain. *BioMetals*, *34*(5), 1017–1027.

[45] Odeh, D., Oršolić, N., Adrović, E., Gaćina, L., Perić, P., Odeh, S., Balta, V., Lesar, N., & Kukolj, M. (2022). Effects of Volatile Anaesthetics and Iron Dextran on Chronic Inflammation and Antioxidant Defense System in Rats. *Antioxidants*, *11*(4).

[46] Dar, N. J., Soriano-Castell, D., & Maher, P. (2026). Sustained dysregulation of iron and glutathione homeostasis induces chronoferroptosis, a persistent ferroptotic adaptation in neuronal cells. *Cell Death Discovery*, *12*(1).

[47] Balas, M., Iconaru, S. L., Dinischiotu, A., Buton, N., & Predoi, D. (2023). Response of the Endogenous Antioxidant Defense System Induced in RAW 264.7 Macrophages upon Exposure to Dextran-Coated Iron Oxide Nanoparticles. *Pharmaceutics*, *15*(2).

[48] Morales, N. P., Rodrat, S., Piromkraipak, P., Yamanont, P., Paiboonsukwong, K., & Fucharoen, S. (2022). Iron chelation therapy with deferiprone improves oxidative status and red blood cell quality and reduces redox-active iron in β-thalassemia/hemoglobin E patients. *Biomedicine and Pharmacotherapy*, *145*.

[49] Merkel, D., Soffer, S., Filanovsky, K., Braester, A., Fibach, E., Dana, M., Ofran, Y., Greenbaum, U., Nagler, A., Amitai, I., & Mittelman, M. (2024). The Effect of Oral Iron Chelator Deferiprone on Iron Overload and Oxidative Stress in Patients with Myelodysplastic Syndromes: A Study by the Israeli MDS Working Group. *Acta Haematologica*, *147*(4), 427–434.

[50] Li, J., Koonyosying, P., Korsieporn, W., Paradee, N., Hutachok, N., Xu, H., Ma, Y., Chuljerm, H., & Srichairatanakool, S. (2024). Deferiprone–resveratrol hybrid attenuates iron accumulation, oxidative stress, and antioxidant defenses in iron-loaded human Huh7 hepatic cells. *Frontiers in Molecular Biosciences*, *11*.

[51] Han, D., Yao, Y., Chen, L., Miao, Z., & Xu, S. (2022). Apigenin ameliorates di(2-ethylhexyl) phthalate-induced ferroptosis: The activation of glutathione peroxidase 4 and suppression of iron intake. *Food and Chemical Toxicology*, *164*.

[52] Wang, X., Li, W., Yuan, W., Wei, Z., Yang, Z., Zhang, Z., Sun, Z., Ji, G., & Hu, H. (2026). Quercetin Attenuates Iron Overload-Induced Renal Injury via Activating Nrf2/xCT/GPX4 Signaling to Inhibit Ferroptosis. *Life*, *16*(3).

[53] Shi, X., Zhang, Q., Chang, M., Zhang, Y., Zhao, M. M., Yang, B., Li, P., & Zhang, Y. (2023). Ferroptosis is involved in passive Heymann nephritis in rats. *Heliyon*, *9*(10).

[54] Hrelia, S., & Angeloni, C. (2021). New mechanisms of action of natural antioxidants in health and disease II. *Antioxidants*, *10*(8).

[55] DeRango-Adem, E. F., & Blay, J. (2021). Does Oral Apigenin Have Real Potential for a Therapeutic Effect in the Context of Human Gastrointestinal and Other Cancers? *Frontiers in Pharmacology*, *12*.

[56] Hsu, S. J., Chung, H. C., Chang, C. H., Liao, Y. C., Liu, T. W., Lin, S. M., & Lee, C. K. (2025). Rapid evaluation of apigenin bioavailability and hypouricemic bioactivity by targeted metabolomics study in enterohepatic microenvironment mimetic cell culture model. *Food Research International*, *209*.

[57] Berga, M., Logviss, K., Lauberte, L., Paulausks, A., & Mohylyuk, V. (2023). Flavonoids in the Spotlight: Bridging the Gap between Physicochemical Properties and Formulation Strategies. *Pharmaceuticals*, *16*(10).