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In Vivo Identification of the Iron-Chelating Potential of Kwini Mango (Mangifera odorata Griff) Leaf Extract in Iron Overload Cases

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Abstract: Iron overload (IO) is a condition characterized by an abnormal accumulation of iron in the body, which affects vital organs such as the liver, heart, pancreas, and endocrine tissues. Kwini mango (Mangifera odorata Griff) leaves, which contain mangiferin (a phenolic compound), have the ability to chelate Fe3+ by promoting the oxidation of Fe2+, potentially lowering blood iron levels. This study aimed to explore the potential of kwini mango leaf extract (KMLE) as an in vivo iron chelator for treating IO, using blood samples from an IO patient. The in vivo study assessed the effects of KMLE on ferritin, SGPT, SGOT, BUN, creatinine, and serum hematology levels in blood samples. Additionally, liver and kidney histopathology were examined as markers of iron chelation. The extract's standardization was performed to determine the mangiferin content in KMLE. The in vivo results showed a decrease in ferritin, SGPT, SGOT, BUN, creatinine, and hematological parameters. Comparisons between the KMLE group, deferoxamine group, and mangiferin group indicated a significant reduction in ferritin levels in both the deferoxamine and mangiferin groups when compared to the KMLE group at doses of 50 mg/200 g BW and 100 mg/200 g BW (Asym. Sig. (2-tailed) < 0.05). A similar pattern was observed for reductions in SGPT, SGOT, BUN, and creatinine levels at the same doses (Asym. Sig. (2-tailed) < 0.05). No significant difference was observed in the KMLE group at a dose of 200 mg/200 g BW (Asym. Sig. (2-tailed) > 0.05). KMLE demonstrates the potential to reduce serum ferritin, SGOT, SGPT, BUN, creatinine, and improve liver histopathology, suggesting its effectiveness as an iron chelator for treating IO.

Keywords: Histopatology; In vivo; Kwini Manggo; Liver

Introduction

Iron overload (IO) refers to a condition where the body accumulates more iron than necessary due to iron imbalance (Mutaz et al., 2019). This condition encompasses a variety of disorders that arise from both genetic and acquired factors (Özbolat & Yegani, 2019). Excess iron can have detrimental effects on health, as it has been associated with an increased risk of heart failure, liver disease, diabetes, and metabolic syndrome (Rachmasari & Sugiarso, 2017). It has also been linked to neurodegenerative conditions, such as Alzheimer's and epilepsy (Kontoghiorghe et al., 2015). In 2021, the global prevalence of IO was estimated at 5-10%, affecting around 176 million people. In Indonesia, 7,029 cases of IO were reported in 2020 (Permatasari et al., 2020), a

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significant rise from 4,431 cases in 2015 (Brissot et al., 2020).

Current treatments for IO involve the use of synthetic drugs like deferoxamine, which works by forming iron complexes that can be excreted through urine (Widarti & Nurqaidah, 2019). However, deferoxamine has several side effects, including visual disturbances, hearing impairments, digestive, hematological, nervous, and musculoskeletal disorders, liver diseases, and elevated liver enzymes (Fitria et al., 2019; Oktiyani, 2017). Additionally, patients may experience growth retardation, pallor, muscle hypotrophy, hepatosplenomegaly, genu valgum, foot ulcers, and bone deformities due to expanding bone marrow (Fatkuriyah & Hidayati, 2022). These drugs can also be costly and uncomfortable as they require subcutaneous infusion for at least 8-12 hours a day, 5-7 days per week (Cloonan et al., 2017). With the increasing number of IO cases every year, it is crucial to explore and develop alternative treatments or iron-chelating agents, ideally sourced from accessible natural ingredients (Chaudhary et al., 2021).

Kwini mango (Mangifera odorata Griff) leaves contain mangiferin, an iron (Fe^{3+}) chelator that can oxidize Fe2+ and prevent the reduction of Fe^{3+}, thereby limiting the iron ions available for lipid peroxidation (Permatasari et al., 2020). Mangiferin is a phenolic compound with various pharmacological effects (Bhargava et al., 2015). As a natural bioactive compound, mangiferin possesses iron-chelating and antioxidant properties (Verna et al., 2022). It is a metal chelator composed of pure phenolics (Salsabila et al., 2019) and contains a condensed aromatic ring that binds to glucose by forming a C-C bond (Al-Shimaysawee, 2018).

Additionally, kwini mango leaves are rich in flavonoids and polyphenols, both of which exhibit iron-chelating and antioxidant properties. The specific structure of flavonoids enables them to chelate iron and form soluble, stable complexes with the metal (Arivukkarasu et al., 2018). Flavonoids have multiple metal-chelating sites, such as the 3-hydroxy-4-ketone on the C-ring, the 5-hydroxy on the A-ring, the 4-carbonyl on the C-ring, and the 3,4-dihydroxy on the B-ring (Jahurul et al., 2015). Polyphenols, on the other hand, neutralize free radicals by donating hydrogen atoms or electrons, which helps reduce iron levels in the blood and lower serum ferritin levels (Pohan et al., 2013). Previous studies have shown that mangiferin effectively reduces excess iron in the blood of mice with iron overload (Arivukkarasu & Rajasekaran, 2015). The main goal of this research was to create alternative IO medications by harnessing the iron-chelating properties of kwini mango leaf extract (KMLE).

Method

Preparation of Leaf Sample and Extract

Kweni mango leaves were sourced from Kebumen, Central Java, Indonesia, and authenticated at the Laboratory of Biology, Ahmad Dahlan University, Indonesia, on June 21, 2023 (No. 296/Lab.Bio/B/VI/2023). The leaves were sorted, washed, sun-dried, and re-sorted to eliminate impurities. The dried leaves were ground into a powder and macerated with 96% ethanol using a 1:10 powder-to-solvent ratio. After a 3x24-hour maceration period, the resulting filtrate was concentrated using a rotary evaporator to produce a thick extract (Prasetyorini et al., 2020).

This study followed ethical guidelines, utilizing male Wistar strain white rats as experimental subjects. Ethical approval was granted under number 012306095 on July 4, 2023. Phytochemical screening involved tests for mangiferin, steroids, flavonoids, alkaloids, saponins, and tannins using test-tube reactions and thin-layer chromatography (TLC) (Estuningtyas et al., 2018).

Extract Standardization:

KMLE was standardized using both non-specific and specific parameters. Non-specific parameters included organoleptic properties, water content (Karl Fischer titration), loss on drying (thermogravimetry), total ash content (gravimetry), acid-insoluble ash content (gravimetry), water-soluble and ethanol-soluble essence (moisture analyzer), and specific gravity (aerometer). Specific parameters included mangiferin, saponin, tannin, flavonoid, and alkaloid content, as determined from the phytochemical screening (Thomas et al., 2016).

In Vivo Testing

Thirty-five male Wistar strain rats (180-200g, 8-10 weeks old) were divided into seven groups. Group 1 received 0.5% CMC (normal group), Group 2 received ferrous sulfate at 40 mg/kg BW (disease control), Group 3 received ferrous sulfate and deferoxamine (1 mg/200 g BW) as a positive control, Group 4 received ferrous sulfate and mangiferin (0.5 g/kg BW) as a comparator, Group 5 received ferrous sulfate and extract at 50 mg/200 g BW, Group 6 received ferrous sulfate and extract at 100 mg/kg BW, and Group 7 received ferrous sulfate and extract at 200 mg/kg BW (Barakat et al., 2022).

Ferrous sulfate was administered for 14 days. Extracts, comparator, and deferoxamine treatments were applied for 28 days after inducing iron overload (IO). Blood samples were collected on the day IO occurred and on day 29 post-treatment to measure biochemical parameters. Rats were sacrificed, and their

kidneys and livers were collected for microscopic analysis.

Ferritin Activity Measurement

To measure ferritin activity, 25 µL of blood serum was added to each well, followed by 100 µL of biotin reagent and incubation at 37°C for 30 minutes. The serum was then washed three times with 300 µL of washing solution, followed by the addition of 300 µL of enzyme, incubated for another 30 minutes at 37°C. After further washing, 100 µL of TMB substrate was added and incubated at room temperature for 15 minutes. A stop solution was added, and the absorbance was measured at 450 nm using an Elisa Vidas device (Ariawan et al., 2022).

SGPT and SGOT Activity Measurement

SGPT and SGOT activities were assessed using the substrate start method, where serum was mixed with reagent and incubated at 37°C. The mixture was analyzed using a spectrophotometer to quantify enzyme levels (Sukmayanti et al., 2020; Widarti & Nurqaidah, 2019).

BUN and Creatinine Level Measurement

BUN and creatinine levels were measured using a semi-automated clinical chemistry analyzer (Microlab 300) and Diasys® kits at LPPT-UGM Unit I (Fitria et al., 2019).

Hematology Test

Hematology analysis, including red and white blood cell counts, MCV, and MCHC, was conducted using the Mindray BC-3200 Auto Hematology Analyzer.

Histopathological Analysis of Liver and Kidney Tissues

Liver and kidney tissue preparations followed standard protocols at the Faculty of Veterinary Medicine, Gadjah Mada University. Tissue samples were analyzed under a light microscope, and necrotic cell percentages were determined from five fields of view per slide.

Iron Level Measurement in Liver Tissues Using AAS

One gram of liver tissue was digested in aqua regia, heated, filtered, and stored. Calibration curves were created using concentrations of 0-10 ppm, and iron content was measured at 248.3 nm using Atomic Absorption Spectrophotometry (AAS) (Estuningtyas et al., 2019).

Data Analysis

Data from the in vivo tests were analyzed for ferritin, SGPT, SGOT, BUN, creatinine, and hematology parameters. Normality was tested using the Shapiro-Wilk test, and homogeneity was checked with Levene's test. ANOVA followed by Post hoc LSD or Games-Howell tests was performed on normally distributed data. Kruskal-Wallis or Mann-Whitney tests were used for non-normal data. A significance level of $p < 0.05$ was used, with analyses conducted using IBM SPSS software.

Result and Discussion

The identification process was carried out to ensure the correct species of plants used in this research. The process occurred at the Biology Laboratory, Ahmad Dahlan University, confirming the sample as Mangifera odorata Griff. A certificate of determination, numbered 296/Lab.Bio/B/VI/2023, was issued on June 21, 2023. Ethical approval for animal subject research was granted under reference number 012306097 on July 18, 2023.

The yield refers to the amount of compound extracted, where a higher yield percentage indicates a more concentrated presence of secondary metabolites in the extract (Nahor et al., 2020). The yield of Kweni mango leaf extract in this study was 8.3%, with 83 grams of thick extract produced from 1000 grams of simplicia powder, exceeding the minimum yield requirement of 7.2%.

Standardized Kwini Mango Leaf Extract (KMLE)

KMLE was standardized based on both specific and non-specific parameters. As presented in Table 1, all non-specific parameters of the extract, such as yield, water content, water-soluble essence, ethanol-soluble essence, specific gravity, total ash content, acid-insoluble ash content, and organoleptic properties, met the established criteria. Table 2 summarizes the results of the phytochemical screening, which confirmed the presence of saponins, tannins, flavonoids, and alkaloids, fulfilling the specific requirements. The TLC plates used for detecting these compounds, including mangiferin, are displayed in Figures 1.

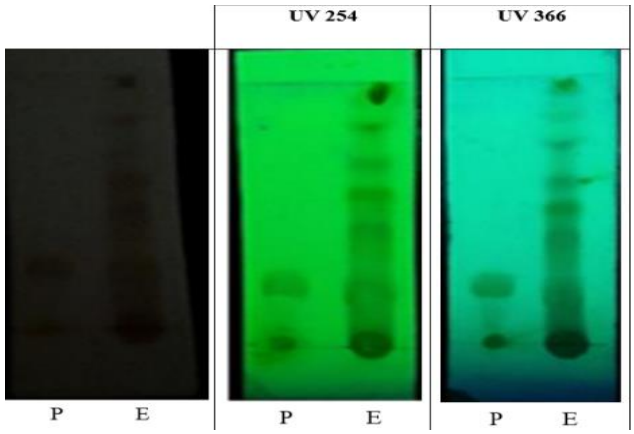


Figure 1. TLC for mangiferin detection with the mobile phase acetate:formic acid:acetone:water (7:2:1:1).

To ensure the extract's quality, safety, and consistency, the Kweni mango leaf extract underwent standardization. This study examined both non-specific and specific standardization parameters. Non-specific parameters included moisture content, total ash content, and acid-insoluble ash content. Specific parameters included organoleptic characteristics (odor, taste, color, and physical form), water-soluble extractive values, and ethanol-soluble extractive values, as shown in Table 1.

Table 1. Standardization of the kwini mango leaf extract (KMLE) based on non-specific parameters.

Non-specific parameter	Value	Standard	Compliance
Extract yield	8.3%	>30	Yes
Water content	8.80±0.58%	>10%	Yes
Water-soluble essence	69.78%	>30	Yes
Ethanol-soluble essence	75.51%	>10%	Yes
Specific gravity	1.0230 g/ml	1.055	Yes
Total ash content	3.35%	3–5%	Yes
Acid-insoluble ash content	1.84%	>0.9%	Yes
Organoleptic properties:			Yes
- Odor	Specific	Specific	Yes
- Taste	Bitter	Bitter	Yes
- Color	Brownish-black	Brownish-black	Yes
- Viscosity	Thick	Thick	Yes

Table 2. Standardization of the kwini mango leaf extract (KMLE) based on specific parameters (phytochemical screening).

Specific parameter	Phytochemical screening result	Compliance
Saponin	Stable foam (positive)	Yes
Tannin	Blackish-green color (positive)	Yes
Flavonoid	Amyl alcohol layer with orange color (positive)	Yes
Alkaloid	Orange color (positive)	Yes

Notes: Standard: Mangiferin (P, Rf=0.63); Sample: KMLE (E, Rf=0.53).

Preparation of Leaf Sample and Extract

Kwini mango leaves were harvested from Kebumen, Central Java, Indonesia, and authenticated at the Laboratory of Biology, Ahmad Dahlan University, Indonesia, on June 21, 2023 (No. 296/Lab.Bio/B/VI/2023). The leaves were sorted, washed with running water, sun-dried, and then re-sorted to eliminate any impurities. The dried leaves (referred to as the crude drug) were ground into a powder and macerated with 96% ethanol using a 1:10 powder-to-solvent ratio.

In Vivo Activity of Kweni Mango Leaf Extract on Ferritin Levels

Statistical analysis showed that ferritin level data were non-normally distributed and heterogeneous (p-value < 0.05). Follow-up tests, including Kruskal-Wallis and Mann-Whitney tests, were conducted to assess the significant reduction in ferritin levels after administration of Kweni mango leaf extract (EDMK) compared to $FeSO_4$, deferoxamine, and mangiferin groups. The Mann-Whitney test revealed significant differences between $FeSO_4$ and EDMK groups at doses of 100 mg/200 g BW and 200 mg/200 g BW (Asym. Sig. (2-tailed) < 0.05), while no significant difference was observed at a dose of 50 mg/200 g BW (Asym. Sig. (2-tailed) > 0.05). Similar trends were noted in comparisons with deferoxamine and mangiferin, with significant reductions at doses of 50 mg/200 g BW and 100 mg/200 g BW, but not at 200 mg/200 g BW.

Table 3. Activity of ferritin level

Group	Average ferritin level (ng/mL)
Normal	140.45 ± 8.06 [a]
FeSO4	163.17 ± 3.33
Deferoxamine	125.58 ± 5.93 [a]
Mangiferin	127.60 ± 4.30
KMLE Dose 50mg/200kgBW	158.25 ± 4.48 [bc]
KMLE Dose100mg/200kgBW	136.73 ± 4.94 [abc]
KMLE Dose 200mg/200kgBW	103.05 ± 4.82 [a]

In Vivo Activity of Kweni Mango Leaf Extract on SGPT Levels

Table 4. Assessment of SGPT Activity Level

Group	Average SGPT level (U/L)
Normal	24.38±6.98[a]
FeSO4	138.76±16.64
Deferoxamine	26.86±5.34[a]
Mangiferin	28.38±2.31
KMLE Dose 50mg/200kgBW	102.44±13.27 [ab]
KMLE Dose100mg/200kgBW	61.16±8.20 [ab]
KMLE Dose 200mg/200kgBW	32.8±4.89

Kruskal-Wallis and Mann-Whitney tests analyzed significant reductions in SGPT levels. The administration of $FeSO_4$ caused liver damage, including hemosiderosis and hemochromatosis, resulting from iron accumulation. Reactive iron species facilitated the generation of harmful free radicals, leading to cellular damage, fibrosis, and organ dysfunction. Post Hoc LSD tests indicated that EDMK doses of 50 mg/200 g BW, 100 mg/200 g BW, and 200 mg/200 g BW significantly reduced SGPT levels compared to $FeSO_4$. However,

comparisons with deferoxamine and mangiferin revealed no significant differences at a dose of 200 mg/200 g BW.

In Vivo Activity of Kweni Mango Leaf Extract on SGOT Levels

Analysis showed SGOT data were non-normally distributed but homogeneous (p-value > 0.05). Mann-Whitney tests revealed significant reductions in SGOT levels for EDMK compared to $FeSO_4$ at all doses (Asym. Sig. (2-tailed) < 0.05). Comparisons with deferoxamine and mangiferin also showed significant reductions at lower doses (50 mg/200 g BW and 100 mg/200 g BW), with no significant differences at 200 mg/200 g BW.

Table 5. Assessment of SGOT Activity Level

Group	Average ferritin level (mg/mL)
Normal	28.4±4.09 a
FeSO4	140.16±16.13
Deferoxamine	34.6±3.48 a
Mangiferin	34.25±4.36
KMLE Dose 50mg/200kgBW	102.44±13.27^{bc}
KMLE Dose100mg/200kgBW	42.18±4.63^{bc}

In Vivo Activity of Kweni Mango Leaf Extract on BUN Levels

Table 6. Assessment of BUN Activity Level

Group	Average BUN level (mg/dL)
Normal	22.3±1.75 a
FeSO4	125.22±7.32
Deferoxamine	27.3±0.28 a
Mangiferin	29.16±4.00
KMLE Dose 50mg/200kgBW	94.16±5.95^{bc}
KMLE Dose100mg/200kgBW	50.24±3.84^{bc}

BUN level data were non-normally distributed and heterogeneous (p-value < 0.05). Mann-Whitney tests showed significant reductions in BUN levels for EDMK doses of 50 mg/200 g BW, 100 mg/200 g BW, and 200 mg/200 g BW compared to $FeSO_4$. Significant reductions were also observed in comparisons with deferoxamine and mangiferin, except for the 200 mg/200 g BW dose.

In Vivo Activity of Kweni Mango Leaf Extract on Creatinine Levels

Statistical analysis indicated non-normal and heterogeneous distribution for creatinine data (p-value < 0.05). Mann-Whitney tests revealed significant reductions in creatinine levels for EDMK doses of 50 mg/200 g BW, 100 mg/200 g BW, and 200 mg/200 g BW compared to $FeSO_4$.

Table 7. Assessment of SGPT Activity Level

Group	Average Creatinin level (mg/dL)
Normal	0.61±0.01 a
FeSO4	0.912±0.01
Deferoxamine	0.716±0.02 a
Mangiferin	0.618±0.01
KMLE Dose 50mg/200kgBW	0.684±0.01$^{b\ c}$
KMLE Dose100mg/200kgBW	0.624±0.01$^{b\ c}$

In Vivo Activity of Kweni Mango Leaf Extract on Hematological Parameters:

One-way ANOVA identified significant differences in hematological parameters, including WBC, RBC, HCT, MCH, LYM%, NEUT%, LYM#, and RDW (p-value < 0.05). No significant differences were observed in parameters such as HGB, MCV, MCHC, PLT, NEUT#, PDW, MPV, and P-LRC (p-value > 0.05).

Table 8. Blood Hematology Activity

Group	Average													
	Level HGB (g/dL)	Level HCT (%)	Level MCV (juta/µ)	Level MCH (ribu/µL)	Level MCHC (g/dL)	Level PLT (10^3/µL)	Level LYM% (%)	Level NEUT% (%)	Level LYM# (10^3/µL	Level NEUT# (10^3/µL	Level RDW (%)	Level PDW (%)	Level MPV (fL	Level P-LRC (%)
K1	13.45±0.23	41.975±1.25	57.7±0.28	18.5±0.27	32.05±0.47	831500±105180.79	78.725±4.60	21.325±4.65	9175±613.05	2550±732.57	29.675±0.41	8.225±0.28	6.95±0.12	6.7±0.45
K2	13.1±0.80	40.5±2.01	62.8±1.57	20.3±0.24	32.325±0.73	801250±421474.69	81.375±6.75	18.625±6.75	7750±443.47	7175±11165.53	34.175±0.80	7.85±0.58	6.7±0.25	5.8±1.44
K3	13.65±0.42	41.275±1.18	62.35±0.54	20.625±0.26	33.075±0.17	903000±160775.20	88.15±1.21	11.85±11.21	9700±1998.33	2450±2217.35	32.425±0.97	7.95±0.57	6.775±0.29	5.925±1.58
K4	13.825±0.25	42.1±1.00	63.65±0.31	20.9±0.2	32.85±0.17	675000±303585.24	78.1±2.77	19.975±6.40	7450±1330.41	6850±9602.95	33.975±1.78	8.175±0.94	6.85±0.45	6.375±2.43
K5	13.325±1.67	41.125±3.01	60.25±1.07	19.575±0.59	32.525±1.45	732250±372833.63	76.325±8.93	23.675±8.93	6400±836.66	2150±1292.28	32.6±0.87	8.125±0.48	6.85±0.19	6.275±1.26
K6	13.8±2.82	46.5±1.62	50.2±1.9.83	20.25±0.37	29.725±6.10	1320500±148280.14	77.825±10.44	22.175±1.0.44	7225±895.82	2325±1873.27	32.55±1.13	7.9±0.33	6.7±0.18	5.6±0.84
K7	13.675±0.67	43.6±1.70	62.2±1.88	19.55±1.45	33.175±0.17	864000±535994.40	97.175±1.19	2.85±1.16	15675±4809.27	575±287.22	34.8±1.53	7.875±1.42	6.625±0.84	6.375±3.55
nova	0.984	<0.002	>0.212	<0.001	>0.466	>0.183	<0.007	<0.013	<0.000	>0.589	<0.000	>0.982	>0.927	>0.984

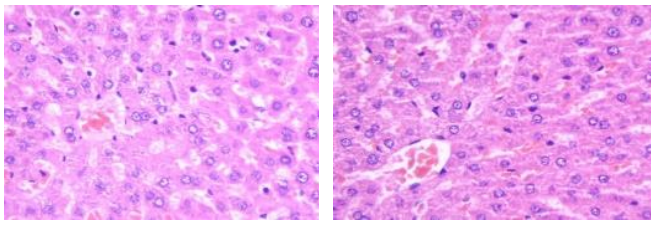
Description: <0.05 shows a significant difference; and >0.05 indicates the absence of a significant difference.

Table 9. Result Histopathological Analysis of Liver Tissues in Wistar Strain White Rats Used as Iron Chelators.

Group	Degenerative						Nekrosis						Kongesti					
	Total score of rats					Average	Total score of rats					Average	Total score of rats					Average
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
1	1	1	1	1	1	1±0.000	1	1	1	1	1	1±0.000	1	1	1	1	1	1±0.000
2	3	2	3	2	2	2.4±0.548	2	2	2	3	3	2.4±0.548	3	3	3	3	2	2.8±0.447
3	1	1	1	1	1	1±0.000	1	1	1	1	1	1±0.000	1	1	1	1	1	1±0.000
4	1	1	1	2	1	1.2±0.447	2	1	1	1	1	1.2±0.447	1	1	2	1	1	1.2±0.447
5	1	1	1	2	2	1.4±0.548[a]	2	2	1	1	1	1.4±0.548[a]	1	2	2	0	1	1.2±0.837[a]
6	2	1	1	1	1	1.2±0.447[a]	2	1	1	1	1	1.2±0.447[a]	1	1	1	1	2	1.2±0.447[a]
7	1	1	1	1	1	1±0.000[a]	1	1	1	1	1	1±0.000[a]	1	1	1	1	1	1±0.000[a]

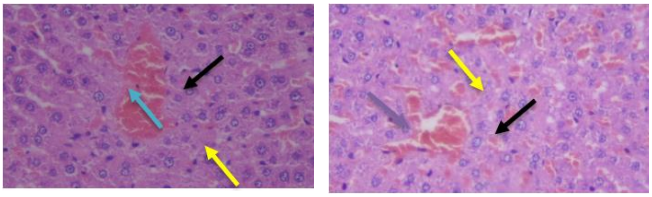
Histopathological Analysis of Liver and Kidney Organs

Histopathological examinations revealed both reversible (fatty degeneration congestion) and irreversible (necrosis) damage mostly mild to moderate, Siswanto & Astriani (2019), which state that reversible cellular damage can be resolved, allowing cells to return to normal function.



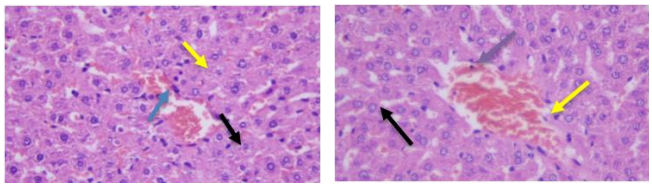
(a) (b)

Figure 2. Liver histopathology of Wistar strain rats treated with 0.5% CMC as the normal control group: (a) No notable indicators of necrosis were detected, including pyknotic nuclei (shrunken and dense); (b) Degenerative changes such as cell swelling or vacuolization. and clear signs of congestion (HE. 400x).



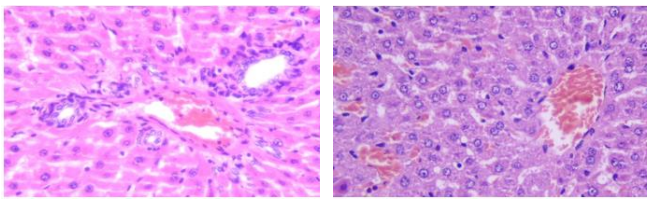
(a) (b)

Figure 3. Liver histopathology of Wistar strain rats treated with FeSO4 at a dose of 40 mg/kg BW as the negative control group; (a) Observations revealed degeneration (indicated by the black arrow). necrosis (yellow arrow); (b) congestion (blue arrow) (HE. 400x).



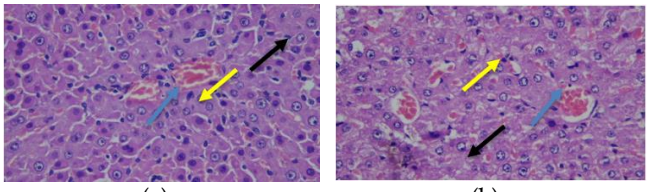
(a) (b)

Figure 4. Histopathology of the liver in Wistar strain rats treated with Deferoxamine: (a) Degeneration (black arrow) necrosis (yellow arrow); (b) Congestion (blue arrow) were observed (HE. 400x).



(a) (b)

Figure 5. Histopathology of the liver in Wistar strain rats treated with Mangiferin extract: (a) Degeneration (black arrow). necrosis (yellow arrow); (b) congestion (blue arrow) were observed (HE. 400x).

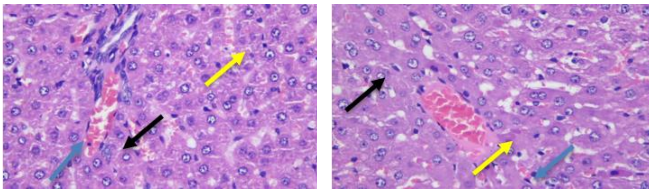


(a) (b)

Figure 6. Histopathology of the liver in Wistar strain rats treated with EDMK at a dose of 50 mg/kg BW: (a) Degeneration (black arrow), necrosis (yellow arrow); (b) congestion (blue arrow) were observed (HE. 400x).

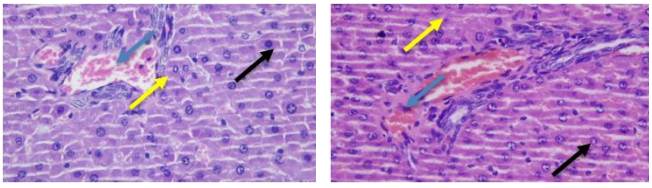
Table 10. Measurement Results in Liver Tissues Using the AAS (Atomic Absorption Spectrophotometer) Method

Group	Sample Weight	Animal test			Average	Iron Content
		1	2	3		(% w/w)
1	0.5	160.93	159.98	158.89	159.933±1.020	0.01599
2	0.5	339.76	338.66	339.88	339.433±0.672	0.03394
3	0.5	197.66	198.44	198.76	198.286±0.565	0.01987
4	0.5	167.88	168.02	166.77	167.556±0.684	0.01675
5	0.5	310.99	311.12	311.077	311.062±0.066[abc]	0.03111
6	0.5	271.99	273.01	272.99	272.663±0.583[abc]	0.02729
7	0.5	197.88	198.22	197.66	197.920±0.282[ac]	0.01976



(a) (b)

Figure 7. Histopathology of the liver in Wistar strain rats treated with EDMK at a dose of 100 mg/kg BW: (a) Degeneration (black arrow), necrosis (yellow arrow); (b) and congestion (blue arrow) were observed (HE. 400x).



(a) (b)

Figure 8. Histopathology of the liver in Wistar strain rats treated with EDMK at a dose of 200 mg/kg BW: (a) Degeneration (black arrow). necrosis (yellow arrow); (b) and congestion (blue arrow) were observed (HE. 400x).

Assessment of Iron Content in Liver Organs

Atomic Absorption Spectroscopy (AAS), an analytical chemistry tool based on the principle of atoms absorbing energy, was used to determine the concentration of analytes in samples. AAS measures the spectrum associated with atomic absorption and emission. The system includes a radiation source. atomization system, monochromator, photodetector. and read-out system (Sugito, 2022), highlighted that iron accumulation in the liver generates reactive oxygen species. leading to hepatocyte damage and increased inflammation. It can also harm nucleic acids. proteins. and trigger lipid peroxidation. depleting liver antioxidants and posing risks to hepatocytes. contributing to liver cirrhosis and hepatocellular carcinoma. The extract's effect on reducing excessive iron concentration in the liver was demonstrated. helping to prevent liver cirrhosis and hepatocellular carcinoma.

Conclusion

The ethanolic extract of mango kweni leaves demonstrates potential as an iron chelating agent in vivo in test animals. as indicated by decreased ferritin levels. SGPT. SGOT. BUN. and creatinine. along with improved hematological parameters and histopathological conditions of the liver organs.

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Conflicts of Interest

The authors declare no conflict of interest.

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