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Investigating the Effects of Methanol Leaf Extract of *Chasmanthera dependens* on Liver and Blood in a Simulated Iron Overload Model

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ABSTRACT

Natural iron-chelating agents from plants offer a promising alternative to synthetic chelators with potentially fewer side effects. Phytochemical analysis of the methanol leaf extract of *Chasmanthera dependens* (MLECD) confirms the presence of antioxidants known for their metal-chelating properties. This study investigated the effects of *Chasmanthera dependens* on the liver and blood in a simulated iron overload model. Twenty-five adult Wistar rats of both sexes with access to feed and water *ad libitum* were randomly assigned into five groups (A, B, C, D, & E) of five rats each. Group A was administered 1 ml of distilled water; B received 2 mg/kg of iron chloride ($FeCl_2$); C received co-administration of 2 mg/kg $FeCl_2$ and 200 mg/kg of MLECD; D received co-administration of 2 mg/kg $FeCl_2$ and 400 mg/kg of MLECD and E received co-administration of 2 mg/kg $FeCl_2$ and 800 mg/kg of MLECD daily for twenty-eight days. Initial and final weights of rats were taken before euthanization under chloroform anesthesia and subsequent tissue processing. One-way ANOVA statistical analysis was carried out, with significance set at $P<0.05$ relative to the control. Results showed elevated serum ferritin levels, decreased liver weight in iron overloaded group. Histological results showed perivascular infiltrates of inflammatory cells, portal vascular congestion and ulceration, and Kupffer cell activation in liver tissue. MLECD-administered groups showed dose-dependent effects of MLECD on liver enzymes, hematocrit levels, hemoglobin concentration, red blood cell count, and organ weight as well as improved hepatic histoarchitecture. MLECD prevented excessive iron deposits in hepatic and hematological tissues.

Keywords: iron overload, *Chasmanthera dependens*, natural chelators, liver, blood

INTRODUCTION

Excessive accumulation of iron in the body termed Iron overload, also known as hemochromatosis results in organ damage[1]. According to a study on the prevalence of iron overload in Nigerian women by Fasola[2], the frequency of iron overload was 8.2% in women aged 26 to 71 years. Skilled workers made up 44.9% of the population, while semiskilled and housewives made up 50% and 5.1%, respectively. Eighty-four percent of the women had taken iron vitamin supplements at some point.

Hepatocytes and macrophages store excess iron as ferritin[3].

Plant-derived substances that bind to excess iron present a hopeful substitute for artificial iron-binding compounds, with the potential for reduced adverse effects. *Chasmanthera dependens* commonly called Chasmanthera, is a genus of flowering plants belonging to the family Menispermacea. Its native range is in Tropical Africa[4]. The plant is a woody climber with a rough stem. It has a heart-shaped base, a shortly drawn apex and produces many flowers in the axils of the leaves[5]. *Chasmanthera dependens* is valued in Nigerian traditional medicine for its multifaceted healing properties, targeting abdominal pain, red-eye infections, sprained joints, and bruises[6]. In West Africa, its leaf and stem sap serve as local remedies for sprains, bruises, and fractures, often combined with shea butter for pain relief[7].

The liver is a vital organ responsible for nutrient metabolism, detoxification, bile production, and blood clotting regulation[8]. It is located in the right hypochondrium and epigastric areas, with two main surfaces: the smooth diaphragmatic surface and the irregular visceral surface, shaped by surrounding organs. The liver is divided into the larger right lobe and the smaller left lobe, separated by the falciform ligament. Microscopically, it consists of lobules containing hepatocytes, sinusoids, and central veins. Blood supply is dual, via the hepatic artery and portal vein. The liver also plays a key role in bile production, with its development beginning early in embryogenesis[8].

Iron is an essential micronutrient required for critical physiological functions, including oxygen transport, DNA synthesis, and enzymatic reactions[3]. However, excessive iron accumulation, as seen in iron overload conditions, poses significant risks, particularly to the liver and hematological system. The liver, as the primary site of iron storage and metabolism, is highly susceptible to iron-induced oxidative stress, leading to hepatocellular damage, inflammation, and fibrosis[8]. In the blood, excess iron disrupts erythropoiesis, alters red blood cell integrity, and contributes to anemia through oxidative stress-mediated hemolysis. Given these pathological consequences, the search for natural, less toxic iron-chelating agents has gained attention. *Chasmanthera dependens*, widely utilized in traditional medicine, contain phytochemicals such as flavonoids, polyphenols, and saponins, known for their antioxidant and metal-chelating properties[4]. The methanol leaf extract of *Chasmanthera dependens* (MLECD) was therefore selected for this study due to its potential to mitigate iron-induced hepatotoxicity and hematological alterations. By assessing the effects of MLECD in a simulated iron overload model using Wistar rats, this research aims to provide scientific validation for its use as a natural therapeutic intervention for iron-related toxicity.

MATERIALS AND METHODS

Plant procurement and extraction

Chasmanthera dependens leaves, collected from Ekosodin, Edo State, were authenticated at the University of Benin (voucher number UBHC387). The leaves were air-dried and milled into powder, and 110 g was soaked in 50% methanol for 48 hours. The mixture was filtered, and the filtrate was concentrated at 45°C, yielding 33.6 g of extract (30.5% yield). The extract was stored at 4°C in the Department of Anatomy, University of Benin[9].

$$Yield(\%) = \frac{Final\ weight\ of\ extract\ (g)}{Weight\ of\ powdered\ C.\ dependens\ leaves\ (g)}$$

Phytochemical screening of the extract

The phytochemical composition of the methanol leaf extract of *Chasmanthera dependens* was analyzed using established standard procedures[10-12].

Ethical approval

In the course of this research work, ethical clearance was applied for and approved by the ethics committee of the College of Medical Sciences, University of Benin, Benin City, Nigeria (CMS/REC/2023/340)

Dose determination of MLECD and iron chloride

Acute toxicity was assessed using Lorke's method[13]. Observations over 48 hours revealed no fatalities, indicating an LD50 greater than 5,000 mg/kg. Consequently, 200, 400, and 800 mg/kg were designated as low, medium, and high doses for subsequent treatments. 10 g of Iron II chloride ($FeCl_2$) crystals (Xilong Science Co., Ltd,) with batch number 209923-2 was dissolved in 100 ml of distilled water to form an Iron stock solution. 2 mg/kg body weight of $FeCl_2$ was administered orally to induce iron overload[9].

Animal care and management

Twenty-five (25) adult Wistar rats of either sex weighing 185-225 g were used for this study. The rats were bred in the animal holding of the Anatomy Department, University of Benin, Benin City[9]. Animal management and care followed the National Research Council's Guide for the Care and Use of Laboratory Animals[14], ensuring ethical treatment and welfare.

Tissue processing

Fixed tissues were processed using hematoxylin and eosin staining technique according to Drury and Wallington[15]. Processed tissue slides were examined under a Leica DM750 research microscope with a digital camera (LeicaICC50) attached. Digital photomicrographs of the tissue sections were taken at 100 and 400x magnifications[9] respectively.

Liver enzymes tests

Serum was isolated from blood samples by centrifugation (3,000 × g, 15 min). The levels of liver enzymes and proteins were analyzed using an automated biochemical analyzer (Hitachi7100, Japan).

Serum ferritin test

Serum ferritin concentration was determined using a ferritin ELISA Kit (BioVendor GmbH, Heidelberg, Germany). The absorbance of the reaction was read at 450 nm using a microplate reader (Bio-Rad Laboratories Inc., Hercules, CA).

Full blood count test

Full blood count was analyzed using an automated hematology analyzer (BC-6000, Shenzhen Mindray Bio-Medical Electronics Co., Ltd).

Table 1: Experimental design

GROUPS	DOSES
A (CONTROL)	Feed and water only
B	2 mg/kg b.w of $FeCl_2$ solution only
C	2 mg/kg b.w of $FeCl_2$ + 200 mg/kg b.w of MLECD (Low dose)
D	2 mg/kg b.w of $FeCl_2$ + 400 mg/kg b.w of MLECD (Medium dose)
E	2 mg/kg b.w of $FeCl_2$ + 800 mg/kg b.w of MLECD[9] (High dose)

Statistical analysis

Data were subjected to statistical analysis using GraphPad Prism version 8.1 statistical package and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean ± standard error of the mean (SEM). The least significant difference (LSD) post-hoc test was used. Values of $p<0.05$ were considered statistically significant. The statistical values obtained were converted into graphical representations in the form of bar charts and tables.

RESULTS

Change in body weight

There were significant increases in final weight compared with initial weight for control, $FeCl_2$-induced administered with 200 mg/kg, 400 mg/kg and 800 mg/kg of MLECD respectively, though there were no significant differences in final weight compared with initial weight for $FeCl_2$ only as shown in Table 2.

Table 2: Comparing the mean values of body weight and organ weight in $FeCl_2$-induced Wistar rats administered with MLECD of different doses.

Parameters	Control	$FeCl_2$ only	$FeCl_2$ + 200mg/kg MLECD	$FeCl_2$ + 400mg/kg MLECD	$FeCl_2$ + 800mg/kg MLECD
Initial weight (g)	224.0 ± 0.578	206.3 ± 11.46	187.0 ± 8.82	188.7 ± 11.02	198.3 ± 4.19
Final weight (g)	304.7 ± 1.45	237.3 ± 15.76×	222.0 ± 4.88×	224.3 ± 8.57×	239.5 ± 8.13×
Liver weight (g)	9.500 ± 0.265	7.300 ± 0.569×	8.733 ± 0.884	9.733 ± 0.371	9.733 ± 0.267

×P < 0.05 indicates significant difference

Change in organ weight

There was a significant decrease in $FeCl_2$-induced only compared with control, though there were no significant differences in $FeCl_2$-induced administered with 200 mg/kg, 400 mg/kg and 800 mg/kg of MLECD compared with control respectively as shown in Table 2.

Hepato-somatic index

There were significant increases in the hepatosomatic index of $FeCl_2$-administered rats treated with 400 mg/kg and 800 mg/kg MLECD compared with the control group. However, there were no significant differences in the hepatosomatic index of $FeCl_2$-induced iron overload rats treated with 200 mg/kg of MLECD and untreated $FeCl_2$-induced iron overload rats compared with

the control group, as shown in Fig. 1.

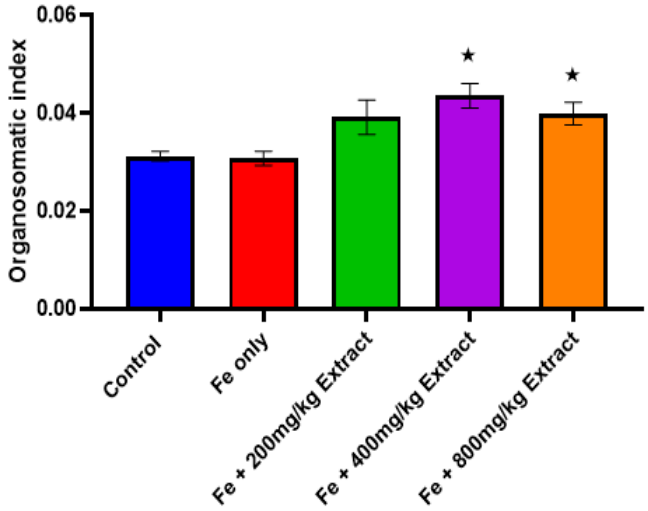

Fig 1: The organosomatic index (liver weight/body weight) in $FeCl_2$ administered Wistar rats treated with MLECD at different doses.

Liver enzyme test results

The results showed that $FeCl_2$ significantly affected liver enzymes, particularly alkaline phosphatase and AST, with MLECD showing a dose-dependent protective effect, especially at 800mg/kg for AST. Other parameters like ALT, total bilirubin, total protein, albumin, and globulin were not significantly altered, except for globulin, which decreased with $FeCl_2$ alone and at 400mg/kg MLECD as shown in Table: 3. The findings suggest a complex interaction between $FeCl_2$ and MLECD, with the latter showing some protective effects at specific doses.

Table 3: Comparing the mean values of hematological parameters in $FeCl_2$-induced iron overload in Wistar rats administered with MLECD of different doses.

Parameters	Control	$FeCl_2$ only	$FeCl_2$ + 200mg/kg MLECD	$FeCl_2$ + 400mg/kg MLECD	$FeCl_2$ + 800mg/kg MLECD
WBC count	6.733 ± 0.751	8.700 ± 1.00	8.825 ± 2.05	8.833 ± 0.821	11.95 ± 0.97
Lymphocytes (%)	92.70 ± 0.95	93.47 ± 0.38	90.65 ± 0.98	92.03 ± 0.50	90.88 ± 1.15
MID	5.267 ± 0.62	5.033 ± 0.32	6.475 ± 1.13	6.133 ± 0.48	7.325 ± 0.95
Granulocytes	2.033 ± 0.33	1.500 ± 0.06	2.925 ± 0.52	1.833 ± 0.20	1.800 ± 0.22
RBC count	8.280 ± 0.31	7.953 ± 0.16	6.400 ± 0.69	6.710 ± 0.25	7.405 ± 0.28
Hemoglobin	15.30 ± 0.45	15.03 ± 0.19	11.83 ± 1.32	13.00 ± 0.42	14.10 ± 0.60
Hematocrit	44.20 ± 1.14	43.13 ± 0.90	36.18 ± 3.20	37.17 ± 1.17	40.55 ± 0.87
MCV	53.50 ± 1.46	54.33 ± 1.07	57.05 ± 1.84	55.47 ± 1.09	54.93 ± 1.03
MCH	18.43 ± 0.19	18.83 ± 0.19	18.40 ± 0.17	19.33 ± 0.34	18.98 ± 0.20
MCHC	34.57 ± 0.58	34.83 ± 0.48	32.45 ± 1.22	34.93 ± 0.03	34.68 ± 0.75
ALP	434.3 ± 90.04	192.0 ± 19.73	533.0 ± 66.48	430.7 ± 91.85	384.5 ± 24.91
AST	231.0 ± 13.65	163.7 ± 6.89	183.5 ± 24.40	199.7 ± 12.60	142.0 ± 9.01
ALT	113.7 ± 1.67	100.3 ± 19.94	99.75 ± 13.12	115.3 ± 3.28	106.0 ± 14.65
Total bilirubin	0.267 ± 0.03	0.200 ± 0.00	0.275 ± 0.05	0.267 ± 0.03	0.275 ± 0.05
Total protein	6.600 ± 0.058	6.067 ± 0.176ˣ	6.100 ± 0.505	6.300 ± 0.00	6.150 ± 0.312
Albumin	3.433 ± 0.09	3.267 ± 0.145	3.325 ± 0.287	3.400 ± 0.058	3.425 ± 0.266
Globulin	3.167 ± 0.033	2.800 ± 0.058	2.775 ± 0.239	2.900 ± 0.058	2.650 ± 0.150
Ferritin concentration	197.0 ± 9.54	369.0 ± 17.10ˣ	195.0 ± 53.00	252.7 ± 53.73	382.8 ± 55.16

ˣ$P < 0.05$ indicates significant difference

Full blood count test results

The results indicated that MLECD at different doses had varied effects on hematological parameters in $FeCl_2$-administered Wistar rats. Significant changes were observed in WBC count at 800 mg/kg (Table 3), with a notable increase, and in RBC count, hemoglobin concentration, and hematocrit levels at 400 mg/kg and 800 mg/kg, where decreases were noted. These findings suggest dose-dependent effects of MLECD on certain blood parameters, particularly at higher doses, with potential implications for its safety and efficacy in modulating blood-related outcomes.

Serum ferritin test results

The results indicated that $FeCl_2$ administration led to a significant increase in serum ferritin levels, reflecting increased iron storage. However, MLECD treatment had dose-dependent effect, with the highest dose (800 mg/kg) failing to prevent the $FeCl_2$-induced ferritin elevation, while the lower doses (200 mg/kg and 400 mg/kg) did not significantly alter ferritin levels as shown in fig 2. This suggests a complex interaction between MLECD and iron metabolism, where lower doses might offer some protection, but higher doses could potentially enhance the effects of $FeCl_2$-induced iron overload.

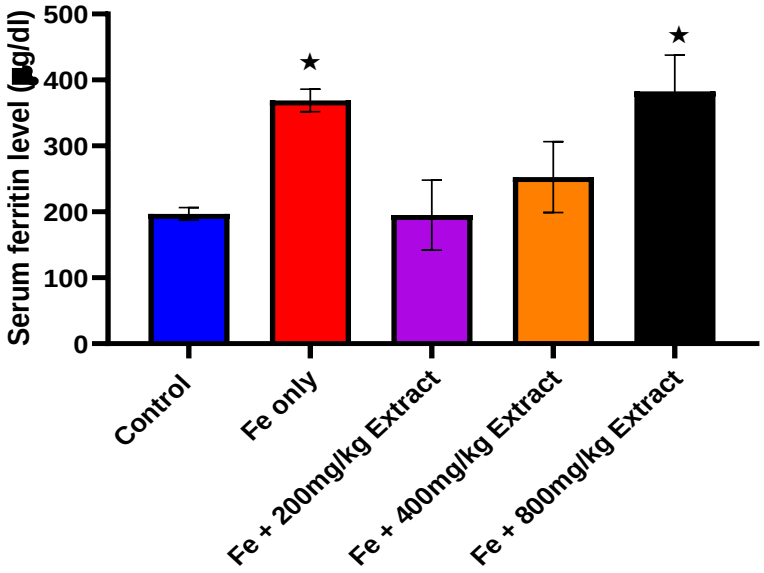

Fig 2: The serum ferritin level in $FeCl_2$ administered Wistar rats treated with MLECD at different doses.

There were significant increases in untreated $FeCl_2$-administered rats, and $FeCl_2$-administered treated with 800 mg/kg MLECD compared with control, though there were no significant differences in $FeCl_2$-induced administered with 200 mg/kg and 400 mg/kg of MLECD compared with control.

Histological assessment

The histological results show that $FeCl_2$ alone caused considerable liver damage, while MLECD treatment offered varying levels of protection depending on the dose. The highest dose of MLECD (800 mg/kg) showed the most substantial protective effect, with liver tissue appearing completely normal. In contrast, lower doses still provided protection but with some mild pathological changes as shown in Fig 3.

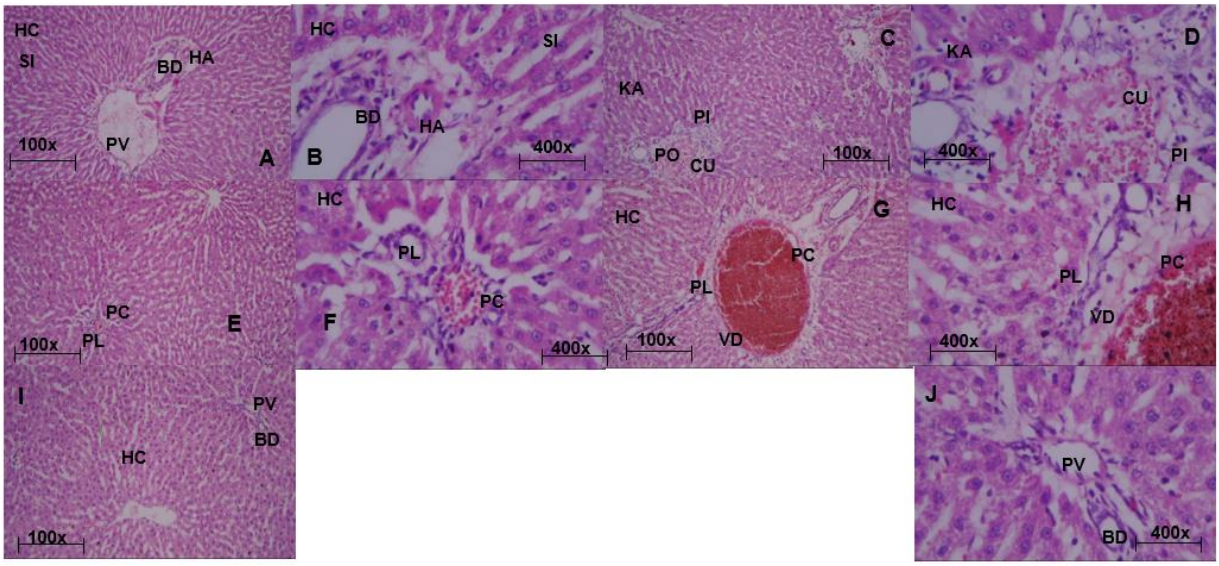

Fig. 3: Histological plates: A and B: Control group rat liver showing normal architecture: H&E 100x, 400x. C and D: 2 mg/kg $FeCl_2$ group showing PO, PI, CU, KA: H&E 100x, 400x. E and F: 200 mg/kg MLECD + 2 mg/kg $FeCl_2$ group showing normal HC, mild PC, mild PL, H&E 100x, 400x. G and H: 400 mg/kg MLECD + 2mg/kg $FeCl_2$ group showing normal HC, mild PL, PC, VD: H&E 100x, 400x. I and J: 800mg/kg MLECD + 2 mg/kg $FeCl_2$ group showing normal HC, PV, BD: H&E 100x, 400x.

Key: HC: Hepatocytes, SI: Sinusoids, BD: Bile duct, HA: Hepatic artery, PV: Portal vein, KA: Kupffer cell activation, PO: Periportal oedema, PI: Perivascular infiltrates of inflammatory cells, CU: Portal vascular congestion and ulceration, PL: Periportal mobilization of lymphocytes, PC: mild portal congestion, VD: Vasodilation

Phytochemical results

The qualitative and quantitative analysis of *Chasmanthera dependens* methanol leaf extract revealed the presence of saponins, polyphenols, flavonoids, coumarins, alkaloids, and terpenoids, while tannins, steroids, anthraquinones, glycosides, and amino acids were absent. Among the detected phytochemicals, terpenoids (31.34 mg/g) and alkaloids (29.54 mg/g) were the most abundant, followed by flavonoids (27.20 mg/g) and polyphenols (22.21 mg/g), while saponins had the lowest concentration (0.69 mg/g) (Table 4 and 5). Studies have demonstrated the metal-chelating properties of polyphenols, flavonoids, and alkaloids[34].

Table 4: Qualitative analysis of methanol leaf extract of *Chasmanthera dependens*

Phytochemicals	**Indication**
Saponins	+
Tannins	-
Polyphenols	+
Steroids	-
Anthraquinones	-
Coumarins	+
Flavonoids	+
Alkaloids	+
Amino acids	-
Glycosides	-
Terpenoids	+
Amino acids	-

Key*:* + present; - absent

Table 5: Quantitative analysis of methanol leaf extract of *Chasmanthera dependens*

Phytochemicals	**Quantity (mg/g)**
Saponins	0.69
Polyphenols	22.21
Flavonoids	27.20
Coumarins	10.00
Terpenoids	31.34
Alkaloids	29.54

DISCUSSION

Iron metabolism is necessary for many biological functions, including oxygen delivery, energy synthesis, and enzymatic reactions[16]. Dietary iron comes from both heme and nonheme sources, with heme iron being more easily absorbed[17]. Iron absorption in the duodenum and proximal jejunum is mediated by transport proteins such as DMT1 and FPN[18]. Recent studies emphasize the increased susceptibility of both the blood[19] and heart[20] to excessive iron exposure. Iron is absorbed in the duodenal enterocytes, where heme is broken down by Heme Oxygenase 1 (HO1)[21]. Iron is stored as ferritin or enters circulation through ferroportin. Nonheme iron is reduced by duodenal cytochrome b (DCYTB) and transported via divalent metal transporter 1 (DMT1). In circulation, iron bound to transferrin is oxidized by CP, and is transported to tissues. In hepatocytes, iron is stored in ferritin[22]. When systemic iron is high, hepcidin inhibits ferroportin.

Chasmanthera dependens, widely utilized in traditional medicine, contains phytochemicals such as flavonoids, polyphenols, and saponins, known for their antioxidant and metal-chelating properties[23]. Previous studies have demonstrated the metal chelating properties of polyphenols, flavonoids, and alkaloids, which can form stable complexes with iron (Fe^{2+}/Fe^{3+}), copper (Cu^{2+}), and lead (Pb^{2+})[24], thereby reducing oxidative stress and metal-induced toxicity. Terpenoids and coumarins have also been reported to chelate iron (Fe^{3+}) and cadmium (Cd^{2+}), contributing to their potential therapeutic roles in managing metal overload conditions. Phytochemical studies of qualitative and quantitative analysis of *Chasmanthera dependens* methanol leaf extract from this study revealed the presence of saponins, polyphenols, flavonoids, coumarins, alkaloids, and terpenoids, while tannins, steroids, anthraquinones, glycosides, and amino

acids were absent. Among the detected phytochemicals, terpenoids and alkaloids were the most abundant, followed by flavonoids and polyphenols, while saponins had the lowest concentration[25,26].

Results from this study showed significant changes in body and organ weights among the experimental groups. Liver weight demonstrated an interesting trend, with a decrease observed in rats treated with iron chloride alone. This reduction could be attributed to iron-induced liver damage. However, administration of MLECD stabilized liver weight, indicating a protective effect against iron-induced liver damage. MLECD at low doses proved to be effective in protecting the liver from the adverse effects of excessive iron exposure.

The hepatosomatic index (HSI) measures the relative weight of the liver to the body weight of the Wistar rats. Elevated hepatosomatic index in iron-overloaded rats indicates liver enlargement due to iron accumulation, inflammation, and potential fibrosis. Reduced indices suggest advanced organ damage, atrophy, and severe functional impairment[27]. From our findings, the organo-somatic index of the liver reflected the impact of iron overload and extract treatment on liver health. The extract demonstrated a positive influence on this index, suggesting a potential for preserving organ function and structure.

Serum ferritin levels, a key indicator of iron overload, were significantly increased in iron overloaded group, and increased serum ferritin levels (hyperferritinemia) in iron-overloaded Wistar rats indicated significant iron accumulation, liver damage, and potential multi-organ dysfunction. These elevated levels reflect severe iron toxicity and the attempt by the body to manage iron overload[28]. Serum ferritin levels were reduced in rats treated with MLECD in this study. This reduction suggests a chelating role of the extract in binding excess iron and preventing its accumulation in tissues.

Liver function enzymes, including alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase, showed improvements with extract administration in this study. Lower enzyme levels indicate reduced liver damage and improved hepatic function, further supporting the protective effects of the extract against iron-induced hepatotoxicity[29]. Elevated levels are expected in iron overload but then, chronic iron overload could alter the synthesis of enzymes in the liver, potentially downregulating their production. Also, significant fibrosis or cirrhosis caused by iron overload might disrupt the liver architecture so that enzyme release into the bloodstream is impaired, however, normal levels can occur in the early stages of iron overload. Compromised liver function due to iron overload leads to a struggle to synthesize essential proteins like albumin and clotting factors, leading to hypoalbuminemia and coagulation abnormalities. Additionally, the ability of the liver to metabolize drugs and toxins may be impaired, impacting overall detoxification processes. Findings from the study revealed that iron overload significantly elevated liver enzyme parameters, indicating hepatic damage, while administration of *Chasmanthera dependens* methanol leaf extract (MLECD) exhibited varying protective effects. Specifically, the iron-overloaded group showed a marked increase in ALP compared to the control, while groups receiving MLECD exhibited dose-dependent reductions at low-dose high doses of MLECD. AST and ALT levels also followed a similar trend, with the iron-only group showing elevated AST and ALT, whereas MLECD administration reduced these levels, particularly at high doses. Total bilirubin levels remained relatively stable across groups, while total protein, albumin, and globulin levels indicated mild variations, with the iron-overloaded group showing a significant reduction in total protein compared to control. These results suggest that MLECD exerted hepatoprotective effects by mitigating iron-induced hepatic injury and improving biochemical markers of liver function.

Findings from the study showed that while certain hematological parameters like WBC count and RBC-related indices (RBC count, hemoglobin concentration, hematocrit level) were influenced by $FeCl_2$ and varying doses of MLECD, other parameters such as lymphocyte count, mid-cell count, and granulocyte count remained relatively stable. No significant differences were noted in lymphocyte count among groups, including $FeCl_2$ alone and $FeCl_2$ with different MLECD doses, compared to the control group. In iron overload, the

normal range for lymphocyte count remains largely unchanged unless there's a concurrent condition affecting lymphocytes, such as an infection or inflammatory response[30]. $FeCl_2$ alone or with low and medium doses of MLECD did not significantly alter the WBC count. However, there was a notable increase in WBC count when $FeCl_2$ was administered with a high dose of MLECD compared to the control. Similar to lymphocyte count, mid-cell count remained stable across all groups, indicating no significant changes with $FeCl_2$ or MLECD administration. An increase in MID cell count may indicate an active bone marrow response, possibly due to an infection or inflammatory process, conversely, a decrease in MID cell count might suggest bone marrow suppression or a shift in white blood cell production[31]. $FeCl_2$ administration, regardless of MLECD doses, did not result in significant changes in granulocyte count compared to the control group. The addition of medium dose MLECD to $FeCl_2$ led to a significant decrease in RBC count, hemoglobin concentration, and hematocrit level compared to the control. Other MLECD doses did not show significant differences in these parameters. RBC and hemoglobin production increases in iron overload as a compensatory mechanism due to the body's attempt to utilize excess iron for hemoglobin synthesis[32]. This leads to an elevated RBC and hemoglobin count. Decreased red blood cell and hemoglobin count during iron overload treatment indicates successful iron reduction and hematological response to therapy[33]. Mean Corpuscular Volume (MCV) indicates red blood cell size and helps classify anemia types. Elevated MCV suggests macrocytic anemia, indicating early iron overload. No significant changes were observed in MCV, MCH, and MCHC levels across all groups, indicating stability in these hematological indices irrespective of $FeCl_2$ or MLECD administration.

Iron chloride administration resulted in histopathological changes in liver tissue, including portal congestion and inflammatory infiltrates. In contrast, rats treated with MLECD showed mitigated histopathological alterations, indicating a protective effect on liver tissues. Iron overload has significant effects on the liver, posing a threat to its health and function[34]. The accumulation of excess iron within liver cells can trigger a cascade of detrimental processes. Chronic iron overload can also instigate liver fibrosis, characterized by the excessive deposition of collagen and other extracellular matrix components[34]. This fibrotic response disrupts the liver's normal architecture and can progress to cirrhosis if left unchecked. Cirrhosis is a severe condition where liver tissue becomes nodular and dysfunctional, potentially culminating in liver failure[35].

The highest dose of MLECD was the most effective and was sufficient to ensure protection, this implies that there is a dose-dependent relationship, where the lower doses of MLECD treated groups proved to be less histo-protective suggesting that at lower doses, MLECD itself is inefficient in preventing iron accumulation in hepatic tissues. The mechanism of action to this effect is yet to be understood.

In addition to exploring the protective effects of *Chasmanthera dependens* on liver and blood tissues, future studies should focus on the molecular pathways involved in its iron-chelating activity. Investigating the modulation of iron transporters such as divalent metal transporter 1 (DMT1) and ferroportin, as well as the role of hepcidin in iron homeostasis, will provide deeper insights. Pathway analyses involving oxidative stress markers, the Nrf2-antioxidant response pathway, and inflammatory pathways like NF-κB could reveal the precise molecular mechanisms by which *Chasmanthera dependens* protects tissues from iron overload-induced damage.

CONCLUSION

This study demonstrated the potential of *Chasmanthera dependens* methanol leaf extract as an effective natural iron chelator. When administered in varying doses to Wistar rats with iron overload, MLECD exhibited dose-dependent effects on hepatic and hematological parameters, with the high dose emerging as the optimal level for preventing excessive iron deposition in hepatic tissue in the histological examination.

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Contributions: SON: concept, design, data analysis, critical revision of manuscript; DC: laboratory work, histological analysis and interpretation of results, preparation of manuscript.

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