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Effectiveness Of Combination Of Ethanol Extract Of Turmeric Rhizome And Green Tea Leaves On Serum Iron Levels In Wistar Rats Induced With Hemosiderosis

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ABSTRACT

Hemosiderosis is a condition of iron overload characterized by excessive iron deposition in body tissues and increased circulating iron levels, including serum Fe, which commonly occurs as a result of repeated blood transfusions and may lead to oxidative stress. This condition requires alternative adjuvant therapies based on natural products. This study aimed to evaluate the effectiveness of ethanol extracts of turmeric rhizome, green tea leaves, and their combination on serum Fe levels in Wistar rats with induced hemosiderosis. This laboratory experimental study used a post-test only control group design with 24 male Wistar rats divided into six groups: negative control, positive control (iron dextran induction), turmeric extract 100 mg/kgBW, green tea extract 100 mg/kgBW, combination 100 mg/kgBW, and combination 200 mg/kgBW. Serum Fe levels were measured using the Ferrozine colorimetric spectrophotometric method and analyzed with One Way ANOVA followed by a Post-Hoc Tukey test. The results showed that all treatment groups experienced a significant reduction in serum Fe levels compared to the positive control ($p < 0.05$). The combination groups demonstrated a greater reduction than single treatments; however, no significant difference was observed between the combination doses of 100 mg/kgBW and 200 mg/kgBW ($p > 0.05$). This finding suggests a synergistic effect between curcumin and catechins, and indicates that the 100 mg/kgBW dose may have reached the minimum effective dose. In conclusion, the combination of turmeric and green tea extracts effectively reduced serum Fe levels in Wistar rats with hemosiderosis and has potential as an adjuvant therapy for iron overload conditions.

Keywords: *Green Tea, Hemosiderosis, Serum Iron, Turmeric, Wistar Rats*

ABSTRAK

Hemosiderosis merupakan kondisi kelebihan zat besi yang ditandai oleh penumpukan besi di jaringan tubuh dan peningkatan kadar besi dalam sirkulasi, termasuk Fe serum, yang umumnya terjadi akibat transfusi darah berulang serta berpotensi menimbulkan stres oksidatif. Kondisi ini memerlukan alternatif terapi adjuvan berbasis bahan alam. Penelitian ini bertujuan menilai efektivitas ekstrak etanol rimpang kunyit, ekstrak etanol daun teh hijau, dan kombinasi keduanya terhadap kadar Fe serum pada tikus Wistar yang diinduksi hemosiderosis. Penelitian eksperimental laboratorik dengan desain post-test only control group ini menggunakan 24 ekor tikus Wistar yang dibagi menjadi enam kelompok: kontrol negatif, kontrol positif (induksi iron dextran), kunyit 100 mg/kgBB, teh hijau 100 mg/kgBB, kombinasi 100 mg/kgBB, dan kombinasi 200 mg/kgBB. Kadar Fe serum diukur dengan metode spektrofotometri kolorimetri Ferrozine dan dianalisis menggunakan One Way ANOVA dilanjutkan uji Post-Hoc Tukey. Hasil penelitian menunjukkan seluruh kelompok perlakuan mengalami penurunan kadar Fe serum yang bermakna dibandingkan kontrol positif ($p < 0,05$). Kombinasi ekstrak memberikan penurunan lebih besar dibandingkan perlakuan tunggal, namun tidak terdapat perbedaan bermakna antara dosis kombinasi 100 mg/kgBB dan 200 mg/kgBB ($p > 0,05$). Hal ini menunjukkan adanya efek sinergis kurkumin dan katekin, serta bahwa dosis 100 mg/kgBB telah mencapai minimum effective dose. Disimpulkan bahwa kombinasi ekstrak kunyit dan teh hijau efektif menurunkan kadar Fe serum dan berpotensi sebagai terapi adjuvan pada kondisi kelebihan zat besi.

Kata kunci: Hemosiderosis, Kadar Besi Serum, Kunyit, Teh Hijau, Tikus Wistar

INTRODUCTION

Hemosiderosis is a condition characterized by excessive iron accumulation in body tissues. This condition commonly occurs in patients who receive repeated blood transfusions over a long period, such as those with thalassemia major (Faranoush et al., 2023). Each unit of transfused blood contains approximately 200–250 mg of iron, while the human body lacks an effective mechanism for iron excretion (Yadav & Singh, 2022). As a result, excess iron accumulates in vital organs, including the liver, heart, and pancreas, triggering the formation of reactive oxygen species through the Fenton reaction. This process leads to oxidative stress, which may cause tissue damage and organ dysfunction (Rachmawati Triwardhani et al., 2022).

Thalassemia major is one of the most common clinical conditions associated with transfusion-related hemosiderosis. Patients with thalassemia require regular blood transfusions to maintain adequate hemoglobin levels. In addition to exogenous iron exposure from transfusions, increased intestinal iron absorption due to dysregulation of hepcidin further aggravates iron overload (Taher et al., 2017). Uncontrolled iron accumulation can lead to serious complications, such as liver fibrosis, cardiomyopathy, endocrine disorders, and increased morbidity and mortality.

Globally, transfusion-related hemosiderosis remains a major health problem, particularly in countries with a high prevalence of thalassemia. It is estimated that 5–7% of the world's population are carriers of hemoglobin disorders, and thousands of patients with thalassemia major require lifelong regular transfusions. In Indonesia, the prevalence of thalassemia carriers is estimated to be around 3–7%, with the number of patients increasing each year. West Java is reported to have one of the highest numbers of thalassemia major patients in the country (Bayu Rahardja et al., 2017), making hemosiderosis a significant long-term complication in clinical practice.

The primary management of hemosiderosis is iron chelation therapy using agents such as deferoxamine, deferiprone, and deferasirox. These agents bind free iron, allowing it to be excreted through urine or feces. Although effective in reducing body iron levels and preventing organ damage, iron chelation therapy has several limitations, including adverse effects, high treatment costs, and low patient adherence due to the need for long-term or lifelong therapy (Keputusan Menteri Kesehatan Republik Indonesia Nomor 1076/Menkes/SK/VII/2003, n.d.). In addition, the unequal availability of chelation drugs across healthcare facilities limits optimal treatment for some patients. Therefore, safe, effective, and accessible adjuvant therapies are needed.

One promising approach is the use of natural products with antioxidant activity and iron-binding potential. Turmeric rhizome (Curcuma longa) and green tea leaves (Camellia sinensis) are rich in polyphenolic compounds with known biological effects. Curcumin, the primary active compound in turmeric, exhibits antioxidant properties and has the ability to chelate free iron through its phenolic and β-diketone groups. Meanwhile, catechins in green tea, particularly epigallocatechin gallate (EGCG), can bind iron in the gastrointestinal tract, reduce iron absorption, and contribute to the regulation of hepcidin (Settakorn et al., 2022a; Venita Badan Pengawas Obat dan Makanan Republik Indonesia, 2023). The independent variables in this study were the administration of turmeric rhizome extract and green tea leaf extract, given either as single extracts or in combination at different doses. The treatment groups received either a single extract at a dose of 100 mg/kg body weight or a combination of both extracts at doses of 100 mg/kg body weight (50 mg/kg each) and 200 mg/kg body weight (100 mg/kg each). The dependent variable was the serum iron level in male Wistar rats after the induction of hemosiderosis. Rats were considered to have hemosiderosis when elevated serum iron levels were observed following iron dextran induction. Serum iron concentration was measured using a spectrophotometric method, and the results were expressed in µg/dL. All measurements were performed under standardized laboratory conditions to minimize the influence of external variables. This study has obtained an ethical clearance number 050/UH2.12/2025 from the Institutional Animal Ethics Committee of Jenderal Achmad Yani University. All experimental procedures involving animals were conducted in accordance with the ethical guidelines for the care and use of laboratory animals.

The mechanisms of curcumin and catechins are complementary. Curcumin primarily acts by binding circulating free iron and reducing oxidative stress, whereas catechins mainly inhibit intestinal iron absorption and modulate iron metabolism (Koonyosying et al., 2020). This complementary mechanism suggests a potential synergistic effect in reducing iron overload and protecting tissues from oxidative damage (Slavova-Kazakova et al., 2021).

Previous studies have demonstrated that turmeric and green tea extracts are safe in experimental animals and may reduce free radicals and redox-active iron levels. However, the most effective combination dose for reducing iron levels in hemosiderosis has not been clearly established. Many studies have used relatively high single doses, while the concept of a minimum effective dose as an adjuvant therapy remains underexplored. Previous studies have also shown that curcumin and green tea polyphenols individually possess antioxidant and iron-chelating properties that may influence iron metabolism. Curcumin has been reported to bind free iron and reduce oxidative stress, while catechins such as epigallocatechin gallate (EGCG) may inhibit intestinal iron absorption and modulate iron-regulatory pathways. However, most previous investigations have evaluated these compounds separately, and limited research has explored their combined effects in experimental models of iron overload.

The combination of turmeric and green tea extracts may provide complementary mechanisms of action. Curcumin mainly acts through systemic iron chelation and antioxidant activity, whereas green tea catechins may reduce iron absorption and influence iron homeostasis. These distinct mechanisms suggest the possibility of a synergistic interaction that may enhance the reduction of circulating iron levels. Nevertheless, evidence regarding the optimal combination dose and its effectiveness in hemosiderosis models remains limited.

Therefore, this study aims to evaluate the effectiveness of a combination of ethanol extracts of turmeric rhizome (Curcuma longa) and green tea leaves (Camellia sinensis) in reducing iron levels in a hemosiderosis model and to determine the minimum effective dose as a potential adjuvant therapy for iron overload due to repeated blood transfusions. This study is expected to provide a scientific basis for the development of safe, effective, and affordable natural adjuvant therapies.

Turmeric rhizome (Curcuma longa) and green tea leaves (Camellia sinensis) contain bioactive polyphenolic compounds that may act through complementary mechanisms in reducing iron overload. Curcumin, the main active compound in turmeric, possesses strong antioxidant properties and has the ability to chelate free iron through its phenolic and β-diketone functional groups, thereby reducing the formation of reactive oxygen species generated through iron-catalyzed reactions. In addition to its iron-binding capacity, curcumin may also attenuate oxidative stress and protect tissues from iron-induced cellular damage. Meanwhile, green tea contains catechins, particularly epigallocatechin gallate (EGCG), which are known to interact with iron in the gastrointestinal tract and inhibit its absorption, thereby limiting the entry of excess iron into systemic circulation. Catechins have also been reported to influence iron metabolism by modulating regulatory pathways such as hepcidin expression and iron transport processes. These distinct but complementary mechanisms suggest a potential synergistic interaction between turmeric and green tea extracts, in which curcumin primarily reduces circulating free iron and oxidative stress, while green tea catechins limit intestinal iron uptake and contribute to the regulation of iron homeostasis. Through these combined actions, the use of both extracts together may provide a more comprehensive approach to reducing iron overload and protecting tissues from oxidative damage compared with the use of a single compound alone.

Despite the growing interest in natural products as potential adjuvant therapies for iron overload, several limitations remain in the existing literature. Previous studies have shown that curcumin from turmeric and catechins from green tea possess antioxidant and iron-chelating properties that may influence iron metabolism. Curcumin has been reported to bind free iron and reduce oxidative stress, while green tea catechins such as epigallocatechin gallate (EGCG) may inhibit intestinal iron absorption and regulate iron homeostasis. However, most previous studies have evaluated these compounds individually, and only limited research has examined their combined effects in experimental models of iron overload.

Furthermore, available studies often employ relatively high single doses of these extracts, while the concept of determining a minimum effective dose for combination therapy remains insufficiently explored. Consequently, scientific evidence regarding the optimal combined dosage and its effectiveness in reducing iron levels in hemosiderosis conditions is still limited. This gap highlights the need for further investigation to evaluate the potential synergistic effect of turmeric and green tea extracts and to identify an effective minimum dose that could be used as a safe and accessible adjuvant therapy for iron overload.

METHOD

Participant characteristics and research design

This study employed an experimental method to evaluate serum iron levels in Wistar rats that had been induced to develop hemosiderosis. A post-test with control group design was used, in which measurements were conducted after the administration of the respective treatments. The experimental model was selected to allow controlled induction of iron overload and to assess the effects of the treatment under standardized conditions.

Eligibility and exclusion criteria

The experimental animals were healthy Wistar rats weighing 200–250 grams, characterized by active movement, normal responses to external stimuli, and the absence of visible wounds or physical abnormalities. Rats that experienced significant weight loss or gain during the study were excluded, and those that became ill or died during the experimental period were considered drop-outs. The plant materials included turmeric rhizomes (*Curcuma longa*) with a bright yellow color and green tea leaves (*Camellia sinensis*) in the form of fresh young shoots that were light green, intact, and free from signs of wilting, mold, or decay. Samples were excluded if the turmeric had turned brown or black, the tea leaves were dark green, or if the materials were contaminated, foul-smelling, excessively dry or moist, or damaged during storage.

Sampling procedures
The experimental groups were formed by selecting Wistar rats that fulfilled the predefined inclusion criteria. All eligible animals were enrolled consecutively until the required sample size of 24 rats was obtained.

Sample size, power, and precision
The subjects were divided into six groups: Group A as the negative control, Group B as the positive control, Group C receiving turmeric rhizome ethanol extract at a dose of 100 mg/kg body weight, Group D receiving green tea leaf ethanol extract at a dose of 100 mg/kg body weight, Group E receiving a combination of turmeric rhizome and green tea leaf ethanol extracts at a dose of 100 mg/kg body weight, and Group F receiving the combination at a dose of 200 mg/kg body weight. The number of subjects in each group was determined using the Federer formula(M.Sopiyudin Dahlan, 2010):

$$(n-1)(t-1) \geq 15$$

Description:
n = number of animals per group
t = number of groups/treatments

Based on the Federer formula, the minimum number of animals required per group was four (n = 4). Therefore, the total number of rats required for the study was 24 animals.

Measures and Covariates

The independent variables in this study were the administration of turmeric rhizome extract and green tea leaf extract, given either as single extracts or in combination at different doses. The treatment groups received either a single extract at a dose of 100 mg/kg body weight or a combination of both extracts at doses of 100 mg/kg body weight (50 mg/kg each) and 200 mg/kg body weight (100 mg/kg each). The dependent variable was the serum iron level in male Wistar rats after the induction of hemosiderosis. Rats were considered to have hemosiderosis when elevated serum iron levels were observed following iron dextran induction. Serum iron concentration was measured using a spectrophotometric method, and the results were expressed in µg/dL. All measurements were performed under standardized laboratory conditions to minimize the influence of external variables.

The treatment doses used in this study were selected based on previous experimental studies evaluating the pharmacological effects of turmeric and green tea extracts in animal models. A dose of 100 mg/kg body weight has commonly been used in experimental studies investigating the antioxidant and iron-chelating activities of plant extracts. The higher dose of 200 mg/kg body weight was included to evaluate whether an increased dose would produce a stronger therapeutic effect and to explore the possible dose–response relationship of the combined extracts.

Materials and Extract Preparation

Turmeric rhizomes (*Curcuma longa*) were obtained from the Manoko Plantation, Lembang, and green tea leaves (*Camellia sinensis*) were obtained from the Walini Tea Plantation, West Java. The plant materials were processed into dried simplicia and extracted using 70% ethanol to obtain active compounds such as curcumin and polyphenols. Other materials used in this study included standard rat feed, distilled water, and iron dextran for the induction of hemosiderosis. Serum iron levels were measured using a colorimetric spectrophotometric method with Ferrozine reagent at a wavelength of 562 nm.

Extraction was performed using the maceration method with 70% ethanol. The plant materials were washed, cut into small pieces, and dried in an oven at 60°C to obtain dried simplicia. The dried materials were then ground into a fine powder and macerated with 70% ethanol. The filtrate was subsequently concentrated using a rotary evaporator to obtain thick extracts, and the extraction yield was calculated.

Experimental Procedures

This study used male Wistar rats weighing 200–250 g that were active, responsive, and free from visible injuries. Rats were acclimatized for fourteen days under standardized laboratory conditions, housed in cages with up to five animals per cage, and provided standard pellet feed and water ad libitum.

After acclimatization, iron overload was induced in the positive control and treatment groups (Groups B–F) by intravenous injections of iron dextran on days 8 and 11. The negative control group received no induction. A three-day stabilization period was allowed after the final injection.

On day 15, treatments were administered as follows: Group C received turmeric extract at 100 mg/kg body weight, Group D received green tea extract at 100 mg/kg body weight, Group E received a combination of both extracts at 100 mg/kg body weight, and Group F received the combination at 200 mg/kg body weight. After forteen days of treatment, blood samples were collected on day 29 to measure serum iron levels.

After acclimatization, iron overload was induced in the positive control and treatment groups (Groups B–F) using intravenous injections of iron dextran. The total dose administered was 40 mg/kg body weight, divided into two injections of 20 mg/kg body weight on day 8 and day 11 of the experiment. This induction protocol has been widely used to establish experimental models of iron overload in laboratory animals.

Following induction, a stabilization period was allowed before treatment administration. Turmeric rhizome extract and green tea leaf extract were administered orally using an oral gavage technique once daily for fourteen consecutive days. The oral route was selected to simulate the potential administration of herbal preparations in clinical practice.

Induction of Hemosiderosis

Hemosiderosis was induced using iron dextran administered intravenously to all treatment groups, except the negative control group, on day 8 of the experiment. The total dose of iron dextran was 40 mg/kg body weight, divided into two administrations of 20 mg/kg body weight each, with a three-day interval between injections. This intravenous administration was intended to increase serum iron levels and establish an iron overload condition.(Khristian et al., 2024)

Blood Sampling and Iron Measurement

Blood samples were collected through the orbital sinus using microhematocrit capillary tubes. Approximately 1.5 mL of blood was obtained from each rat and placed into non-anticoagulant (non-EDTA) (Li et al., 2024)tubes to avoid iron chelation that could affect serum iron measurements. The samples were centrifuged at 3000 rpm for 10 minutes to separate the serum, and the clear serum (approximately 0.5–0.8 mL) was stored at 2–8°C until analysis.

Serum iron levels were measured using a colorimetric spectrophotometric method with Ferrozine reagent. In this method, ferric ions (Fe^{3+}) are reduced to ferrous ions (Fe^{2+}) by sodium ascorbate in an acidic medium. The ferrous ions then react with Ferrozine to form a stable purple complex, which is measured at a wavelength of 562 nm. The absorbance values were converted into serum iron concentrations (µg/dL) according to the kit instructions.(Settakorn et al., 2022b)

Data analysis

The data obtained in this study were analyzed using statistical software to evaluate serum iron levels in Wistar rats. The analysis aimed to assess the relationship between the independent variable (combined doses of turmeric rhizome and green tea leaf ethanol extracts) and the dependent variable (serum iron levels in rats with induced hemosiderosis).

Normality of the data was tested using the Shapiro–Wilk test due to the sample size of fewer than 50 subjects. Data were considered normally distributed if $p > 0.05$. Homogeneity of variance was then assessed using Levene's test. If the data met the assumptions of normality and homogeneity ($p > 0.05$), differences in serum iron levels among the control and treatment groups were analyzed using one-way ANOVA.

A p-value ≤ 0.05 was considered statistically significant. When the ANOVA showed significant results, a post hoc Tukey test was performed to identify significant differences between groups.

RESULTS AND DISCUSSION

Results

A total of 24 male Wistar rats were included in this experimental study and were divided into six groups consisting of four animals in each group. Serum iron levels were measured after the treatment period to evaluate the effect of turmeric rhizome extract, green tea leaf extract, and their combination on iron overload conditions induced by iron dextran.

Descriptive Analysis of Serum Iron Levels

The mean serum iron levels in each experimental group are presented in Table 1. The negative control group showed the lowest mean serum iron level (215.50 ± 4.20 µg/dL), while the positive control group showed the highest level (312.75 ± 9.74 µg/dL). This finding indicates that the induction of iron dextran successfully increased serum iron levels in the experimental animals.

All treatment groups demonstrated lower serum iron levels compared with the positive control group. Among the single extract treatments, the group receiving green tea extract showed a lower mean serum iron level (239.75 ± 9.91 µg/dL) compared with the turmeric extract group (255.00 ± 8.76 µg/dL). The combination treatment groups demonstrated a greater reduction in serum iron levels compared with the single extract groups. The lowest serum iron level among treatment groups was observed in the combination group receiving 100 mg/kg body weight (219.50 ± 2.89 µg/dL).

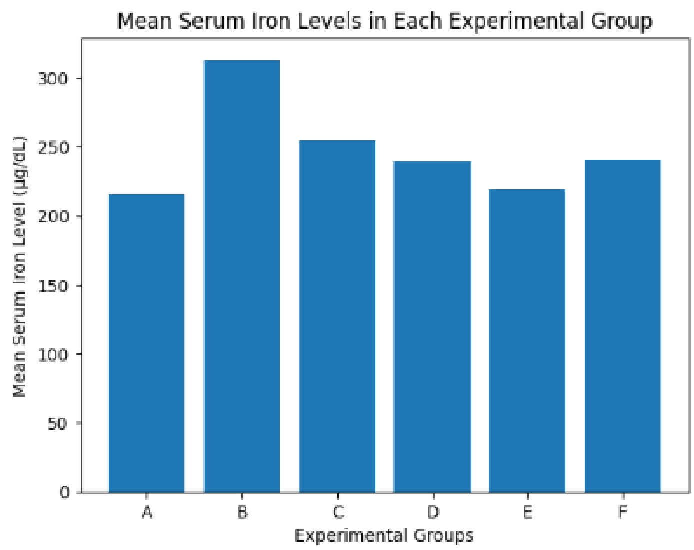
To improve the visualization of differences between groups, the mean serum iron levels are also presented graphically in Table 1.

Table 1 Mean serum iron levels

Group	n	Serum Fe Level (µg/dL) Mean ± SD
A	4	215,50 ± 4,20
B	4	312,75 ± 9,74
C	4	255,00 ± 8,76
D	4	239,75 ± 9,91
E	4	219,50 ± 2,89
F	4	240,75 ± 18,84

Negative control group	:	The mice were fed only standard pellets
Positive control group	:	The mice were only induced with hemosiderosis
Group C	:	The mice were given ethanol extract of turmeric rhizome at a dose of 100 mg/kgBW
Group D	:	The mice were given ethanol extract of green tea leaves at a dose of 100 mg/kgBW
Group E	:	The mice were given a combination of turmeric rhizome extract and ethanol extract of green tea leaves at 100 mg/kgBW
Group F	:	The mice were given a combination of turmeric rhizome extract and ethanol extract of green tea leaves at 200 mg/kgBW

The negative control group showed the lowest mean serum iron level (215.50 ± 4.20 µg/dL), while the positive control group showed the highest level (312.75 ± 9.74 µg/dL), indicating successful induction of hemosiderosis. All treatment groups demonstrated lower serum iron levels compared to the positive control group. The greatest descriptive reduction was observed in the combination group receiving 100 mg/kg body weigh



Statistical Analysis of Serum Iron Levels

Prior to statistical analysis, a normality test was performed using the Shapiro–Wilk test to determine the distribution of serum iron data after treatment with turmeric rhizome and green tea leaf ethanol extracts**.**

Table 2 Serum Fe Normality Test with Shapiro Wilk

Group	Normality Test	
	p-value*)	Data Distribution

Fe Serum	Negative control	0,899	Normal
	Positive control	0,103	Normal
	C	0,177	Normal
	D	0,513	Normal
	E	0,962	Normal
	F	0,630	Normal

Negative control group	:	The mice were fed only standard pellets
Positive control group	:	The mice were only induced with hemosiderosis
Group C	:	The mice were given ethanol extract of turmeric rhizome at a dose of 100 mg/kgBW
Group D	:	The mice were given ethanol extract of green tea leaves at a dose of 100 mg/kgBW
Group E	:	The mice were given a combination of turmeric rhizome extract and ethanol extract of green tea leaves at 100 mg/kgBW
Group F	:	The mice were given a combination of turmeric rhizome extract and ethanol extract of green tea leaves at 200 mg/kgBW

The results of the Shapiro–Wilk test showed that all groups had p-values greater than 0.05, including the negative control group ($p = 0.899$), positive control group ($p = 0.103$), Group C ($p = 0.177$), Group D ($p = 0.513$), Group E ($p = 0.962$), and Group F ($p = 0.630$). These findings indicate that the serum iron data in all groups were normally distributed and met the assumption of normality. Prior to inferential statistical analysis, the normality of serum iron data was evaluated using the Shapiro–Wilk test due to the relatively small sample size. The results showed that all groups had p-values greater than 0.05, indicating that the serum iron data were normally distributed.

Table 3 One Way ANOVA Test Results

Parameter	Value
F value	45,93
p-value	0,000 ($p < 0,05$)

Negative control group	:	The mice were fed only standard pellets
Positive control group	:	The mice were only induced with hemosiderosis
Group C	:	The mice were given ethanol extract of turmeric rhizome at a dose of 100 mg/kgBW
Group D	:	The mice were given ethanol extract of green tea leaves at a dose of 100 mg/kgBW
Group E	:	The mice were given a combination of turmeric rhizome extract and ethanol extract of green tea leaves at 100 mg/kgBW
Group F	:	The mice were given a combination of turmeric rhizome extract and ethanol extract of green tea leaves at 200 mg/kgBW

Based on the one-way ANOVA results shown in Table 2, there was a statistically significant difference among the groups, as indicated by a p-value < 0.001 ($p < 0.05$). This finding suggests that the administration of turmeric rhizome and green tea leaf ethanol extracts had a significant effect on serum iron levels in the rats. Because the assumptions of normality were satisfied, differences in serum iron levels among groups were analyzed using a one-way ANOVA test. The results demonstrated a statistically significant difference in serum iron levels among the experimental groups ($F = 45.93$; $p < 0.001$). This finding indicates that the administration of turmeric rhizome extract, green tea leaf extract, and their combinations significantly affected serum iron levels in Wistar rats with induced iron overload.

To determine which groups differed significantly, a post hoc Tukey test was performed, and the results are presented in Table 3.

Table 4 Post Hoc Tukey Test for Serum Iron Levels

Variables	Perlakuan (I)	Perlakuan (J)	*p*
Fe Serum	Negative Control	Positive Control	0,000*
		D	0,371
		E	0,999
		F	0,017*
	Positive Control	Negative Control	0,000*
		C	0,002*
		D	0,000*
		E	0,000*
		F	0,004*
	C	Negative Control	0,040*
		Positive Control	0,002*
		D	0,798
		E	0,078
		F	0,998
	D	Negative Control	0,371
		Positive Control	0,000*
		C	0,798
		E	0,558
		F	0,558
	E	Negative Control	0,999
		Positive Control	0,000*
		C	0,078
		D	0,558
		F	0,034*
	F	Negative Control	0,017*
		Positive Control	0,004*
		C	0,998
		D	0,558
		E	0,034*

Negative control group	:	The mice were fed only standard pellets
Positive control group	:	The mice were only induced with hemosiderosis
Group C	:	The mice were given ethanol extract of turmeric rhizome at a dose of 100 mg/kgBW
Group D	:	The mice were given ethanol extract of green tea leaves at a dose of 100 mg/kgBW
Group E	:	The mice were given a combination of turmeric rhizome extract and ethanol extract of green tea leaves at 100 mg/kgBW

Group F	:	The mice were given a combination of turmeric rhizome extract and ethanol extract of green tea leaves at 200 mg/kgBW

Post hoc Tukey analysis revealed that the positive control group differed significantly from the negative control and all treatment groups ($p < 0.05$). These results indicate that both single and combined extract treatments were effective in reducing serum iron levels in rats with induced hemosiderosis. Although the comparison between the combination doses of 100 mg/kg and 200 mg/kg showed a statistically significant difference ($p = 0.034$), both doses produced meaningful reductions compared to the positive control. The higher dose did not provide a substantially greater effect, suggesting that the 100 mg/kg dose may represent the minimum effective dose. To determine which groups differed significantly from each other, a Tukey post hoc test was conducted. The analysis showed that the positive control group differed significantly from the negative control group and all treatment groups ($p < 0.05$), indicating that both single extract and combination treatments were effective in reducing serum iron levels.

Comparisons among treatment groups showed that the combination treatments produced greater reductions in serum iron levels than single extract treatments. However, the difference between the combination doses of 100 mg/kg body weight and 200 mg/kg body weight was relatively small, suggesting that the lower dose may already provide an optimal therapeutic effect.

Discussion

The significant difference in serum iron levels between the negative and positive control groups indicates that iron dextran induction successfully increased serum iron levels in the experimental rats (Khristian et al., 2024). This finding is consistent with previous studies reporting that intravenous administration of iron dextran effectively induces iron overload. Under iron overload conditions, the amount of circulating iron exceeds the binding capacity of transferrin, resulting in elevated serum iron levels and increased deposition in tissues such as the liver and the reticuloendothelial system.

Administration of turmeric and green tea extracts as single treatments reduced serum iron levels. Descriptively, the green tea extract group showed lower mean serum iron levels compared to the turmeric extract group. This finding suggests that bioactive compounds in green tea, particularly polyphenols such as epigallocatechin gallate (EGCG), may exert a stronger effect on circulating iron at the tested dose. Previous studies have reported that green tea polyphenols possess strong iron-chelating and antioxidant properties, contributing to improved iron parameters and reduced oxidative stress.

Biologically, the reduction in serum iron levels may be explained by the activity of bioactive compounds present in both extracts. Curcumin and green tea polyphenols, especially catechins such as EGCG, are capable of chelating redox-active iron in the plasma, including non-transferrin-bound iron (NTBI) and labile plasma iron (LPI). This mechanism reduces the availability of reactive iron involved in Fenton reactions and free radical formation. The resulting iron complexes are more stable and less reactive, thereby reducing oxidative stress associated with iron overload (Kose et al., 2019).

The combination of turmeric and green tea extracts demonstrated a greater reduction in serum iron levels compared to single-extract treatments. This effect is likely due to the synergistic interaction between curcumin and green tea polyphenols. Synergism occurs when two compounds with different mechanisms of action complement each other, resulting in a greater biological effect than either compound alone (Kyaw et al., 2025). Curcumin acts by chelating free iron and reducing redox-active iron activity, while green tea polyphenols function as potent antioxidants that neutralize free radicals.

Together, these mechanisms may reduce toxic circulating iron fractions such as NTBI and improve systemic antioxidant capacity.

Comparison between the two combination doses showed no meaningful advantage of the higher dose over the lower dose. Both the 100 mg/kg and 200 mg/kg doses produced effective reductions in serum iron levels. According to pharmacological principles, when two doses provide similar efficacy, the lower dose is preferred as the optimal dose. This concept is known as the minimum effective dose (MED), which refers to the lowest dose capable of producing the desired therapeutic effect. Therefore, the 100 mg/kg combination dose may be considered the optimal dose in this study (Zhou et al., 2017).

Overall, the results indicate that turmeric and green tea ethanol extracts, either as single treatments or in combination, effectively reduced serum iron levels in Wistar rats with induced hemosiderosis. Green tea extract showed a greater reduction compared to turmeric extract alone, while the combination provided the most optimal effect through a synergistic mechanism. Among the tested doses, the combination dose of 100 mg/kg body weight appeared to be the most effective, as it achieved maximal therapeutic effect without additional benefit at higher doses. This combination has potential as an adjuvant therapy for conditions associated with iron overload.

From a clinical perspective, the findings of this study suggest that the combination of turmeric and green tea extracts may have potential as a complementary strategy for managing iron overload conditions, particularly in patients with transfusion-dependent thalassemia. However, this approach should not be considered a replacement for standard iron chelation therapy. Instead, the combination may serve as an adjunct therapy that supports antioxidant defense and helps regulate iron metabolism. Further clinical studies are required to evaluate the safety and therapeutic efficacy of this combination in human subjects.

LIMITATION OF THE RESEARCH

This study has several limitations. First, toxicity testing of the combination of turmeric rhizome and green tea leaf ethanol extracts was not conducted. Second, the parameter used in this study was limited to serum iron levels, which is not the primary indicator of total body iron status and does not fully reflect tissue iron stores or organ iron accumulation. Third, the duration of treatment was relatively short; therefore, the long-term effects of the combined extracts could not be evaluated. Another limitation of this study is the relatively small sample size in each experimental group, which may reduce statistical power. Although significant differences were observed, studies with larger sample sizes would provide more robust statistical evidence. In addition, baseline serum iron levels prior to treatment were not measured. Consequently, the analysis focused on post-treatment comparisons between groups rather than within-subject changes. Future studies should include baseline measurements to better evaluate treatment effects.

CONCLUSIONS AND SUGGESTIONS

Conclusions

The administration of turmeric rhizome ethanol extract and green tea leaf ethanol extract, both as single treatments at a dose of 100 mg/kg body weight per day, effectively reduced serum iron levels in Wistar rats with induced hemosiderosis. The combination of both extracts at doses of 100 mg/kg and 200 mg/kg also significantly decreased serum iron levels. Among the tested regimens, the combination dose of 100 mg/kg body weight per day showed the most effective result, indicating its potential as a minimum effective dose and as a promising adjuvant therapy for iron overload conditions.

Suggestions

Future studies are recommended to conduct acute and subchronic toxicity tests of the combination of turmeric rhizome and green tea leaf ethanol extracts to ensure the safety of their use. In addition, further research should include additional parameters that more comprehensively reflect body iron status, such as serum ferritin levels, transferrin saturation, non-transferrin-bound iron (NTBI), as well as histopathological examination and iron staining in target organs such as the liver and spleen. Moreover, longer treatment durations are recommended to evaluate the long-term effects of the combination of turmeric rhizome and green tea leaf ethanol extracts.

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ETHICAL CONSIDERATION

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Conflict of Interest Statement

The authors declare no conflict of interest. This publication represents an academic achievement and is part of the author’s undergraduate research output.

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