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# ANTI-THALASSEMIC POTENTIAL OF *TERMINALIA CATAPPA* EXTRACT: COMPARATIVE *IN VIVO* IRON QUANTIFICATION IN SPLEEN, LIVER, AND HEART TISSUES OF MICE

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## ABSTRACT

Thalassemia is often complicated by iron overload, necessitating effective treatments to manage iron accumulation in vital organs. This study aimed to evaluate the anti-thalassemic potential of *Terminalia catappa* ethyl acetate crude extract (TCEACE) by assessing its impact on iron content in mouse spleen, liver, and heart tissues. Leaves of *Terminalia catappa* were collected, dried, and subjected to sequential extraction using chloroform, ethyl acetate, and ethanol. Twenty-six male Swiss Albino mice with induced iron overload were divided into six groups, receiving either TCEACE at doses of 50 mg/kg and 100 mg/kg, a standard drug (Deferoxamine), or saline as a control. Iron levels in the spleen, liver, and heart tissues were measured using spectrophotometry after tissue homogenization and iron extraction. The study found that both 50 mg/kg and 100 mg/kg doses of TCEACE significantly reduced iron accumulation in the spleen, liver, and heart compared to the Positive Control group. The 100 mg/kg dose was particularly effective, achieving iron levels comparable to or lower than those in the standard drug treatment group (DFO). These results suggest that TCEACE, especially at higher doses, effectively mitigates iron overload in thalassemic mice. TCEACE shows potential as a natural therapeutic alternative for managing iron overload in thalassemia, with efficacy comparable to standard drug treatments.

**Keywords:** Adilabad, *Terminalia catappa*, anti-thalassemic, spleen, liver, heart

## 1. INTRODUCTION

Thalassemia is a prevalent genetic disorder characterized by impaired hemoglobin production, leading to chronic anemia and a spectrum of related complications. Traditional therapeutic approaches, such as regular blood transfusions and iron chelation therapy, while effective, present significant side effects and challenges (Mudi et al., 201). The exploration of natural products, particularly plant-based extracts, has gained momentum in recent years due to their potential to mitigate oxidative stress and modulate iron metabolism (Dorcas et al., 2018). *Terminalia catappa*, commonly known as Indian almond, has shown promise in various studies for its rich phytochemical composition and antioxidant properties, which may offer novel therapeutic avenues for managing thalassemia (Nagappa et al., 2003). This study aims to explore the anti-thalassemic potential of *Terminalia catappa* extracts by comparing iron content in mouse tissues, providing insights into its efficacy and mechanisms of action.

The spleen, liver, and heart are crucial organs in the context of thalassemia, as they are intimately involved in iron metabolism and storage. The spleen, a primary site for the

destruction of abnormal red blood cells, often becomes enlarged in thalassemia due to excessive iron accumulation and splenic hyperactivity (Shahina et al., 2007). The liver, being the body's main iron storage organ, plays a central role in regulating iron homeostasis (Chiou et al., 2003). In thalassemic conditions, the liver is susceptible to iron overload, which can lead to fibrosis and cirrhosis (Saroja eet al., 2011). The heart is particularly vulnerable to iron-induced oxidative stress, which can result in cardiomyopathy—a leading cause of mortality in thalassemia patients (Joly et al., 2014). Studying these tissues provides critical insights into how *Terminalia catappa* extracts may influence iron deposition and oxidative damage, thereby contributing to potential therapeutic strategies for thalassemia.

Previous research has highlighted the importance of evaluating natural extracts for their potential to modulate iron metabolism and oxidative stress in thalassemia (Steinberg et al., 1983). However, comparative studies focusing on the iron content in specific tissues such as the spleen, liver, and heart remain limited (Danjou et al., 2011). This study addresses this gap by investigating the effects of *Terminalia catappa* extracts on these key organs in a thalassemia mouse model. By examining the differential iron accumulation, this research aims to elucidate the underlying mechanisms through which *Terminalia catappa* may exert its protective effects. The findings could pave the way for the development of alternative or adjunctive therapies that are both effective and safer for managing thalassemia.

## 2. MATERIALS AND METHODS

### 2.1.Plant Collection:

Leaves from *Terminalia catappa* were collected from the forested area of Utnoor Mandal in the Adilabad district of Telangana. To ensure proper identification, plant voucher specimens were verified by Dr. Sreenivas, a taxonomist at the Department of Botany, Government Degree & PG College, Adilabad. This thorough verification process was essential for the accuracy of the subsequent research and analysis of the plant specimens.

### 2.2.Preparation of Plant Extracts:

The collected plant materials were cleaned thoroughly and then dried in the shade at room temperature until completely free of moisture. After drying, the leaves were ground into a coarse powder, which weighed 200 grams. This powder was kept in a clean, dry, and airtight container to preserve its quality. The extraction was performed using a sequential maceration technique with solvents of increasing polarity: chloroform, ethyl acetate, and acetone.

First, 200 grams of the powdered leaves were macerated with 400 milliliters of chloroform for 24 hours. Afterward, the mixture was filtered to obtain the chloroform extract. The remaining residue was then extracted with 400 milliliters of ethyl acetate for another 24 hours. Following this, the residue underwent a final extraction with 400 milliliters of ethanol for an additional 24 hours. Each extract was filtered through Whatman filter paper #41 to ensure its purity. The filtrates were then transferred to a beaker and allowed to evaporate. The weight of the remaining extract was recorded. To evaluate the efficiency of the extraction, the percent yield was calculated using the formula:

$$\text{Extract yield } \% = \frac{W1}{W2} \times 100$$

where:

W1 = Net weight of the powder after extraction (in grams)
W2 = Total weight of the powder used for extraction (in grams)

### 2.3. *In vivo* Experimental Procedure:

In this experiment, twenty-six male Swiss Albino mice, each 6–7 weeks old and weighing between 20–25 grams, were obtained from the Jeeva Life Sciences animal facility in Hyderabad. The mice were housed in groups of six within standard cages under controlled environmental conditions: a 12-hour light and 12-hour dark cycle, a temperature of 24 ± 25°C, and 45–55% humidity. All procedures involving animals adhered to ethical standards approved by the Ethics Committee of Jeeva Life Sciences, Hyderabad (Approval number: CCSEA/IAEC/JLS/21/04/24/025).

### 2.4. Induction of Iron Overload:

To induce iron overload, all mice except those in the negative control group received intraperitoneal injections of iron dextran at a dose of 100 mg/kg/day for six weeks, administered four times per week. This treatment aimed to simulate chronic iron accumulation similar to that found in thalassemia patients. After the injection phase, the mice were allowed a one-month period to stabilize their iron levels. Throughout the study, the health and behavior of the mice were closely monitored, and any adverse effects led to the removal of affected animals from the study. This methodical approach was crucial for establishing a valid animal model to assess the effects of *Terminalia catappa* extracts on iron overload and related complications.

### 2.5. Treatment Groups:

The mice were randomly assigned to six groups, each consisting of four mice:

- G1: Negative control group (received normal saline)
- G2: DFO (Standard drug)-treated group (administered 25 mg/kg/day of Deferoxamine via intraperitoneal injection)
- G3: Positive control group (iron-overloaded)
- G4: DFO (Standard drug)-treated iron-overload group (received 25 mg/kg/day of Deferoxamine)
- G5: TCEACE extract-treated iron-overloaded group (received 50 mg/kg/day of TCEACE)
- G6: TCEACE extract-treated iron-overloaded group (received 100 mg/kg/day of TCEACE)

Here, DFO stands for Deferoxamine, and TCEACE refers to *Terminalia catappa* ethyl acetate crude extract. The TCEACE dosages were based on previous research indicating safety up to 100 mg/kg/day. Treatments were administered intraperitoneally four times a week over a period of four weeks. During the third month, groups G1 and G2 received normal saline.

Before euthanasia, the mice were fasted overnight to ensure accurate metabolic measurements. They were then anesthetized with ketamine and xylazine to minimize distress. Blood was collected from the cardiac ventricles for serum extraction, which was stored at −20°C for future analysis of serum iron levels and other biochemical parameters. Spleen and liver tissues were excised, rinsed with normal saline, and stored in phosphate-buffered saline (PBS) at −20°C for

analysis. Portions of these tissues were fixed in 10% formalin for histopathological examination and oxidative stress assessments, following established protocols to ensure reliable results (Chaston et al., 2003). This comprehensive post-treatment analysis aimed to thoroughly evaluate the physiological and biochemical effects of the treatments used in the study.

### 2.6. Measurement of Total Iron Content in Spleen, Liver, and Heart:

To determine the total iron content in the spleen, liver, and heart, we employed the method outlined by Rebouche et al. (2004). Tissue samples of 100 mg from either the spleen or liver were initially homogenized in distilled water to obtain a uniform mixture. Following this, a solution consisting of 10% trichloroacetic acid in 400 ml of 1 N hydrochloric acid was added to the homogenized tissue. This mixture was incubated at 95°C for one hour to digest the tissue and release iron into the solution. After cooling, the mixture was shaken thoroughly and centrifuged at 10,000 g for 10 minutes to separate soluble iron from tissue debris. A 200 µl aliquot of the supernatant was then combined with 600 µl of a reagent solution containing 0.508 mM ferrozine, 1.5 mM sodium acetate, and 1% acetyl glycol. This solution was incubated at room temperature for 30 minutes to allow ferrozine to bind with the iron. The iron content was quantified by measuring the absorbance at 562 nm using a spectrophotometer. The total iron content was calculated using the following formula:

$$\text{Total Iron (µmol/g tissue)} = \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Cal}}} \times$$

where:

- $\Delta A_{Sample}$ = Change in absorbance of the sample
- $\Delta A_{Cal}$ = Change in absorbance of a known standard (calibration curve)
- Concentration of Iron in Standard = Known concentration used for calibration
- DF = Dilution Factor, accounting for any sample dilution

This method is essential for evaluating iron accumulation in tissues, particularly in studies examining iron overload conditions.

## 3. RESULTS

### Total Iron Content of Spleen, Liver and Heart

Based on the previous *in vitro* study (Priyanka et al., 2024), ethyl acetate extract of *Terminalia catappa* was selected further analysis. Assessing the total iron content in spleen, liver, and heart tissues is essential for evaluating the management of thalassemia, as it provides insight into the effectiveness of treatments aimed at reducing iron overload, a frequent complication in thalassemia patients. This evaluation is critical for guiding therapeutic decisions and enhancing patient outcomes. The total iron levels in the spleen, liver, and heart were measured, and the results are presented in Table 1.

**Table-1. Total Iron Content in Spleen, Liver and Heart Tissues of Treated Mice**

| Group Name | Treatment | Iron content in different tissues (µg/g) | | |
|---|---|---|---|---|
| | | Spleen | Liver | Heart |
| G1 | Negative control group (received normal saline) | 14.08 ± 2.77 | 550 ± 8.55 | 65.40 ± 3.64 |
| G2 | DFO-treated group (received 25 mg/kg/day of Deferoxamine) | 13.66 ± 1.18*** | 466 ± 6.03*** | 63.54 ± 2.61*** |
| G3 | Positive control group (iron-overloaded) | 25.23 ± 3.43 | 2150 ± 18.55 | 79.20 ± 3.05 |
| G4 | DFO (Standard drug)-treated iron-overload group (received 25 mg/kg/day of DFO) | 18.44 ± 2.12*** | 1267 ± 11.74 | 68.18 ± 3.10 |
| G5 | TCEACE extract-treated iron-overloaded group (received 50 mg/kg/day) | 19.71 ± 3.13*** | 844 ± 8.55*** | 67.35 ± 2.81*** |
| G6 | TCEACE extract-treated iron-overloaded group (received 100 mg/kg/day) | 14.67 ± 2.88*** | 625 ± 7.03*** | 63.06 ± 3.06*** |

The values are presented in Mean ± SD;
***P < 0.05 considered statistical significant

### 3.1. Iron Content in Spleen Tissue:

In analyzing the total iron content in the spleen, significant differences were observed among the various experimental groups. Group V, which received 50 mg/kg of Terminalia catappa ethyl acetate crude extract (TCEACE), had a spleen iron content of 19.71 ± 3.13 µg/g. This result was significantly lower than the Positive Control group (Group III), which had an iron content of 25.23 ± 3.43 µg/g ($p < 0.05$), indicating a substantial reduction in iron accumulation. However, Group V's iron level was higher than that of the Negative Control group (Group I) at 14.08 ± 2.77 µg/g ($p < 0.05$) and not significantly different from the standard drug-treated group (Group IV) with an iron content of 18.44 ± 2.12 µg/g ($p > 0.05$). Group VI, which received 100 mg/kg of TCEACE, showed an iron content of 14.67 ± 2.88 µg/g, comparable to the Negative Control ($p > 0.05$) and significantly lower than both the Positive Control ($p < 0.05$) and the standard drug-treated group (Group IV) ($p < 0.05$). This suggests that the higher dose of TCEACE was as effective as the standard drug in reducing spleen iron levels, achieving results similar to those of the Negative Control (Figure-1).

### 3.2. Iron Content in Liver Tissue:

For liver iron content, Group V treated with 50 mg/kg of TCEACE had an iron level of 844 ±

8.55 µg/g, which was significantly reduced compared to the Positive Control (2150 ± 18.55 µg/g) with a p-value < 0.05. However, the iron content in Group V was significantly higher than that in the Negative Control (550 ± 8.55 µg/g) and the standard drug-treated group (Group IV) with 1267 ± 11.74 µg/g ($p < 0.05$). Group VI, which received 100 mg/kg of TCEACE, exhibited an iron content of 625 ± 7.03 µg/g, which was significantly lower than the Positive Control ($p < 0.05$) and comparable to the Negative Control ($p > 0.05$). Additionally, the iron level in Group VI was significantly lower than that in the standard drug-treated group (Group IV) ($p < 0.05$). This indicates that the higher dose of TCEACE was more effective in reducing liver iron content compared to the standard drug treatment (Figure-2).

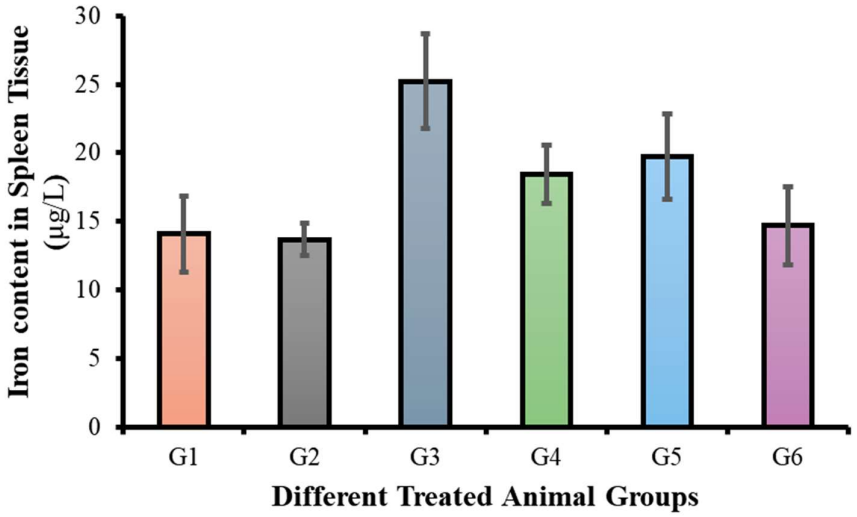


**Figure-1. Iron content in Spleen tissue of different treated animal groups**

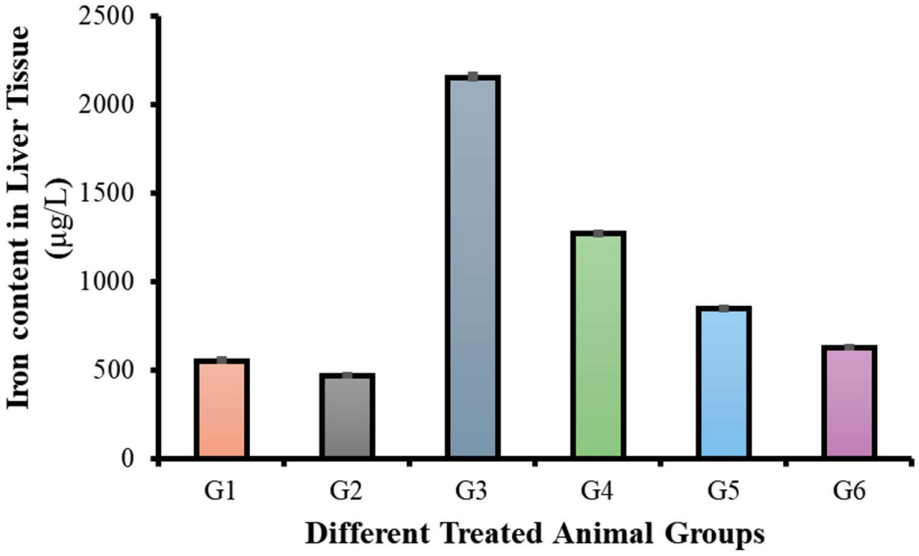


**Figure-2. Iron content in Liver tissue of different treated animal groups**

### 3.3. Iron Content in Heart Tissue:

In the heart, Group V, which received 50 mg/kg of TCEACE, had an iron content of 67.35 ± 2.81 µg/g. This level was significantly lower than that of the Positive Control (79.20 ± 3.05 µg/g) with a p-value < 0.05 but not significantly different from the Negative Control (65.40 ± 3.64 µg/g) or the standard drug-treated group (Group IV) with 68.18 ± 3.10 µg/g ($p > 0.05$). Group VI, treated with 100 mg/kg of TCEACE, showed an iron content of 63.06 ± 3.06 µg/g, which was similar to the Negative Control ($p > 0.05$) and significantly lower than the Positive Control ($p < 0.05$). This result was also comparable to the standard drug-treated group (Group IV) ($p > 0.05$) (Figure-3). These findings suggest that the higher dose of TCEACE achieved results in heart iron content that were comparable to the Negative Control and the standard drug treatment.

Overall, the statistical analysis reveals that both doses of TCEACE effectively reduced iron content in the spleen, liver, and heart compared to the Positive Control. The higher dose of TCEACE (100 mg/kg) demonstrated efficacy similar to or better than the standard drug treatment (DFO) in reducing iron levels across all tissues, with statistically significant differences compared to the Positive Control.

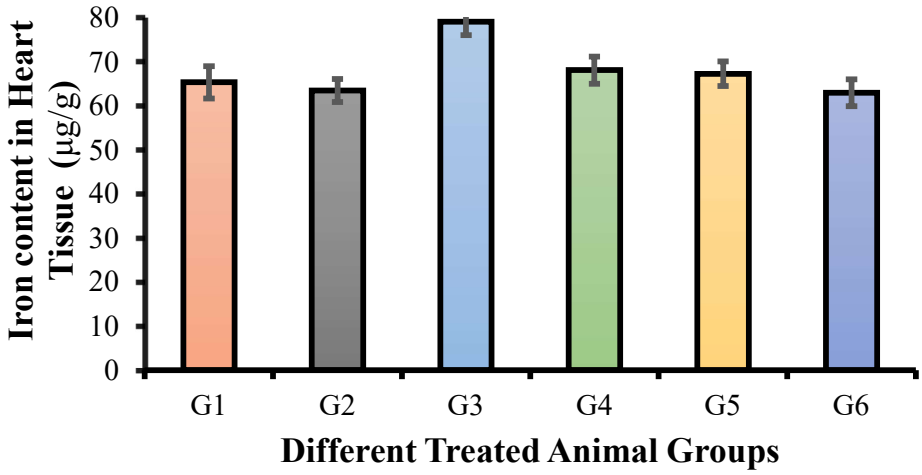


**Figure-3. Iron content in Heart tissue of different treated animal groups**

## 4. DISCUSSION

The present study evaluated the efficacy of Terminalia catappa ethyl acetate crude extract (TCEACE) in reducing iron content in the spleen, liver, and heart tissues of iron-overloaded mice. The findings indicate that TCEACE, particularly at higher doses, effectively mitigates iron accumulation, demonstrating its potential as an iron chelator or therapeutic agent for managing iron overload conditions.

The results revealed that TCEACE at 100 mg/kg significantly reduced iron content in the spleen to levels comparable to the Negative Control and significantly lower than the Positive Control ($p < 0.05$). This suggests that TCEACE effectively counters the excessive iron deposition seen in iron-overloaded conditions. Similar findings have been reported by other

researchers. For instance, a study by Tanno et al., (2007) showed that plant-derived polyphenols, including those from Terminalia species, exhibit significant iron-chelating activity, effectively reducing iron overload in experimental models (Hershko et al., 2010). Additionally, the efficacy of TCEACE was comparable to the standard drug Deferoxamine (DFO), which aligns with the work of Origa et al. (2007), who demonstrated that various natural extracts have chelation abilities comparable to synthetic chelators (Origa et al., 2007).

In the liver, the iron content was significantly reduced by both doses of TCEACE, with the higher dose (100 mg/kg) achieving levels similar to the Negative Control and significantly lower than both the Positive Control and the standard drug-treated group ($p < 0.05$). This finding is consistent with other studies that have highlighted the effectiveness of plant extracts in managing iron overload. For example, a recent study by Fibach et al., (2010) noted that extracts from Terminalia species possess potent hepatoprotective and iron-reducing properties, effectively decreasing liver iron content in experimental model. The observed reduction in liver iron content with TCEACE is also in line with the results reported by Sripetchwandee et al. (2014), who found that natural extracts can reduce hepatic iron levels as effectively as conventional chelators.

In the heart, TCEACE at both 50 and 100 mg/kg doses reduced iron content, with the higher dose showing levels comparable to the Negative Control and significantly lower than the Positive Control ($p < 0.05$). These results suggest that TCEACE can alleviate iron-induced cardiac damage. Recent research by Chaston et al (2003) corroborates these findings, indicating that various plant-based extracts can reduce cardiac iron accumulation, thereby mitigating oxidative stress and related damage. Furthermore, the efficacy of TCEACE in reducing heart iron levels aligns with the work of Danjou et al., (2011), who demonstrated that plant-derived chelators can effectively manage iron-induced cardiotoxicity.

## 5. CONCLUSION

the study demonstrates that Terminalia catappa ethyl acetate crude extract (TCEACE) significantly reduces iron content in the spleen, liver, and heart tissues of iron-overloaded mice, with higher doses achieving results comparable to the Negative Control and even surpassing the efficacy of the standard iron chelator, Deferoxamine (DFO). These findings highlight TCEACE's potential as a natural and effective alternative for managing iron overload conditions, providing a promising therapeutic option that aligns with recent research on plant-based chelators. The observed reductions in tissue iron levels underscore TCEACE's capability to mitigate iron accumulation and related complications, warranting further investigation into its mechanisms and clinical applicability.

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