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IN VIVO EVALUATION OF ANTI-THALASSAEMIC ACTIVITY OF *TERMINALIA CATAPPA* CRUDE EXTRACTS IN SWISS ALBINO MICE

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ABSTRACT

Iron overload disorders, such as thalassemia, pose significant health challenges worldwide. In this study, we evaluated the efficacy of *Terminalia catappa* ethyl acetate crude extract (TCEACE) in reducing serum Fe^{3+} levels. Leaves of *Terminalia catappa* were collected, dried, and sequentially extracted using chloroform, acetone, and ethyl acetate. In this animal study, male Swiss Albino mice were used to evaluate the effects of *T. catappa* ethyl acetate crude extract (TCEACE) on iron overload. The mice received intraperitoneal injections of iron dextran to induce iron overload, mimicking thalassemia-related chronic iron accumulation. TCEACE was administered at different dosages, and serum iron levels were assessed. Our results demonstrate that TCEACE significantly outperforms the standard drug (DFO) and effectively mitigates iron overload. Both group-5 (50 mg/kg TCEACE) and group-6 (100 mg/kg TCEACE) exhibited marked reductions in serum iron levels, emphasizing a dose-dependent response. These findings highlight TCEACE as a promising natural alternative for managing iron-related conditions, warranting further investigation for clinical applications in patients with iron overload disorders.

Key words: *T. cattappa*, TCEACE, Thalassemia, DFO, Ethyl acetate, Fe^{3+}

INTRODUCTION

Thalassaemia is a group of inherited blood disorders that result from mutations in the genes responsible for hemoglobin production, leading to abnormal hemoglobin synthesis. This condition impairs the body's ability to produce adequate amounts of functional hemoglobin, which is crucial for oxygen transport in the bloodstream. The resultant ineffective erythropoiesis leads to chronic anemia, characterized by fatigue, pallor, and various complications stemming from insufficient oxygen delivery to tissues (Sobotka et al 1996). Thalassaemia primarily affects populations in the Mediterranean region, the Middle East, and Southeast Asia, where genetic predisposition is prevalent. According to the Aalikhani et al., (2022), over 300,000 affected births occur annually worldwide, highlighting the significant public health challenge posed by this disorder. The clinical

manifestations of thalassaemia can vary widely, depending on the specific type and severity of the condition, necessitating tailored approaches to management and treatment.

The increasing burden of thalassaemia has prompted researchers to explore alternative therapeutic strategies, particularly those derived from natural products. This shift is largely driven by the limitations and side effects associated with conventional treatments, such as blood transfusions and iron chelation therapy. Traditional medicine has a rich history of utilizing various plant extracts for their medicinal properties, particularly in modulating hematological parameters. For example, many cultures have relied on herbal remedies to treat anemia, leveraging the bioactive compounds present in these plants to enhance erythropoiesis and improve overall blood health. Recent studies have highlighted the potential of specific herbal extracts, such as Moringa oleifera and Hibiscus sabdariffa, which have shown significant promise in improving hemoglobin levels and other hematological indices (Kim et al., 2019; Bami et al., 2017).

Numerous studies have reported the anti-anemic effects of various herbal extracts, suggesting their utility in managing conditions like thalassaemia. For instance, Ferula assa-foetida, commonly known as asafoetida, has been shown to enhance iron absorption and promote red blood cell production in animal models (Jahanshahi et al., 2021). Similarly, Cichorium intybus (chicory) has demonstrated the ability to increase hemoglobin levels and improve overall blood parameters, making it a potential candidate for incorporation into thalassaemia management protocols (De et al., 2005; Vijayagiri et al., 2012). The pharmacological properties of these plants are often attributed to their high content of flavonoids, phenolic compounds, and vitamins, which are known to play crucial roles in iron metabolism and erythropoiesis (Kaur et al., 2003; Gujjeti et al., 2013). Such findings underscore the need for further investigation into the mechanisms through which these natural products exert their effects, paving the way for more targeted therapeutic applications. Building on the promising results of preliminary studies, this research has decided to further evaluate the *in vivo* anti-thalassaemic activity of crude extracts from *T. catappa* using Swiss Albino mice as the experimental model. The primary objective of this investigation is to assess the potential therapeutic effects of *T. catappa* ethyl acetate crude extracts (TCEACE) in mitigating the symptoms and progression of thalassaemia.

MATERIALS AND METHODS

Plant Collection

The leaves of *T. catappa* were gathered from the forested region of Utnoor Mandal, located in the Adilabad district of Telangana state. To ensure accurate identification, the plant voucher specimens were examined and confirmed by Dr. Sreenivas, a taxonomist from the Department of

Botany at Government Degree & PG College, Adilabad. This meticulous process of collection and identification was crucial for the subsequent research and analysis of these plant species.

Preparation of Plant Extracts

The *T. catappa* leaves were collected from the forest area of Utnoor Mandal, Adilabad district, Telangana State, India, thoroughly washed, and then shade-dried at room temperature until all moisture was eliminated. Once dried, the leaves were ground into a coarse powder, weighing 200 grams. This powder was stored in a clean, dry, airtight container to maintain its integrity. The extraction process was carried out using the sequential maceration method, employing solvents of increasing polarity: chloroform, acetone and ethyl acetate.

Initially, 200 grams of the powdered leaves were treated with 400 milliliters of chloroform and left to macerate for 24 hours. After this period, the mixture was filtered to separate the chloroform extract. The remaining residue was then subjected to a second extraction using 400 milliliters of ethyl acetate, again for 24 hours. Following filtration, the residue underwent a final extraction with 400 milliliters of ethanol for another 24 hours. Each of these extracts was filtered using Whatman filter paper #41 to ensure purity. The collected filtrates were placed in a beaker and allowed to undergo solvent evaporation.

Animal study and treatment:

In this study, twenty-six male Swiss Albino mice, aged 6–7 weeks and weighing between 20–25 g, were sourced from the animal house of Jeeva Life Sciences, Hyderabad. The mice were housed in groups of six in standard cages, maintained under controlled environmental conditions with a light cycle of 12 hours of light and 12 hours of darkness, at a temperature of 24 ± 25°C and a humidity level of 45–55%. All animal experiments were conducted in accordance with ethical guidelines approved by the Ethics Committee of Jeeva Life Sciences, Hyderabad (Approval number: CCSEA/IAEC/JLS/21/04/24/025).

Iron overload Induction:

To induce iron overload, all experimental mice, except those in the negative control group, received intraperitoneal (i.p.) injections of iron dextran at a dosage of 100 mg/kg/day for six weeks, administered four times a week. This regimen was designed to systematically increase iron levels within the mice, mimicking the chronic iron accumulation observed in thalassemia patients. Following the injection period, the mice were allowed to equilibrate for one month to stabilize their iron levels. Throughout the study, the health and behavior of the mice were closely monitored to ensure ethical treatment, and any adverse effects led to the removal of affected individuals from the experiment. This carefully structured approach was essential for establishing a reliable animal model for evaluating the therapeutic effects of T. catappa extracts on managing iron overload and its associated complications.

Treatment:

The mice were randomly divided into six experimental groups, with each group containing four mice:

- G1: Negative control group (received normal saline)
- G2: DFO-treated group (received 25 mg/kg/day of Deferoxamine via i.p. injection)
- G3: Positive control group (iron-overloaded)
- G4: DFO-treated iron-overload group (received 25 mg/kg/day of DFO)
- G5: TCEACE extract-treated iron-overloaded group (received 50 mg/kg/day of *Terminalia catappa* ethyl acetate crude extract)
- G6: TCEACE extract-treated iron-overloaded group (received 100 mg/kg/day of TCEACE)

Where, DFO=Deferoxamine; TCEACE=*Terminalia catappa* ethyl acetate crude extract.

The dosages of TCEACE were determined based on previous studies indicating that the extract is safe for administration up to 100 mg/kg/day. The treatments were administered intraperitoneally four times a week for a total duration of four weeks. Additionally, groups G1 and G2 received normal saline during the third month of the study.

Assessment of serum ferric cation:

To assess serum ferric cation levels, the method described by Khalili and Ebrahimzadeh (2015) was employed. Initially, 100 µl of serum collected from the experimental mice was mixed with 1,000 µl of Solution 1, which consisted of an acetate buffer (800 mM, pH 4.5) and thiourea (90 mM). This mixture was allowed to incubate for 10 minutes, after which the absorbance was measured at 600 nm using a spectrophotometer to establish a baseline reading. Following this, 250 µl of Solution 2, containing ascorbic acid (45 mM), Ferene (0.6 mM), and thiourea (20 mM), was added to the mixture. Another incubation period of 10 minutes ensued before a second absorbance reading was taken to determine the presence of ferric ions. The serum ferric cation level was then calculated using the formula:

$$\text{Iron } (\mu\text{mol}-1) = (\Delta A_{\text{Sample}} / \Delta A_{\text{Cal}} \times 187) \times 0.1791$$

where ΔA represents the change in absorbance between the two readings. This method allowed for the quantification of serum iron levels, providing crucial data for evaluating the iron status of the experimental mice and the effectiveness of the treatments administered.

Statistical analysis:

Data analysis was performed using a one-way analysis of variance (ANOVA) followed by the T-test and a p-value < 0.05 was considered the significant level in all tests. The data analysis was carried out using Microsoft office excel software.

RESULTS

Total Iron Content of Serum

The study measured serum Fe3+ levels in six different groups of treated mice, each receiving distinct treatments. Notably, significant differences in serum iron levels were observed, particularly when comparing specific groups to the positive control group (G3), which was intentionally iron-overloaded.

When analyzing serum iron content, it's crucial to recognize that G3 serves as the positive control, representing the highest iron overload level. The mean serum iron level in G1 (315.6 µmol/L) is significantly lower than that in G3 (581.32 µmol/L). This finding indicates that the negative control effectively reduces iron levels compared to the iron-overloaded state, demonstrating the impact of normal saline in preventing excessive iron accumulation.

In G2, the mean value is 285.21 µmol/L, which is also significantly lower than G3. This suggests that treatment with Deferoxamine (DFO) effectively reduces serum iron levels in the treated mice, highlighting DFO's efficacy in chelating excess iron from the body.

However, in G4, the mean serum iron content is 551.5 µmol/L, only slightly lower than G3. This implies that while DFO is administered, persistent iron overload still leads to elevated Fe3+ levels, indicating that DFO alone may not fully counteract the effects of severe iron overload in this specific context .

During the assessment of serum iron levels, a comparison between the TCEACE extract-treated groups (G5 and G6) and the positive control group (G3) revealed significant differences in iron content. Group G5, administered TCEACE at a dosage of 50 mg/kg, exhibited a mean serum Fe3+ level of 389.44 ± 5.16 µmol/L. This value was markedly lower than that of G3, which recorded a mean of 581.32 ± 2.86 µmol/L. Statistical analysis yielded a p-value of < 0.001, indicating a highly significant reduction in serum iron levels due to TCEACE treatment, suggesting its effectiveness in mitigating iron overload.

Similarly, Group G6, treated with a higher TCEACE dosage of 100 mg/kg, showed an even more pronounced mean serum Fe3+ level of 368.4 ± 7.82 µmol/L. This result also demonstrated a significant difference compared to G3, with a p-value of < 0.001. The lower iron levels in both G5 and G6 not only highlight the efficacy of TCEACE extract in reducing serum Fe3+ but also suggest

a dose-dependent response, where increasing the dosage leads to further reductions in iron levels (Table-1 and Figure-1). These findings underscore the potential of TCEACE as a therapeutic agent for managing conditions related to iron overload, making it a promising candidate for further research and clinical application.

Table-1. Serum Fe3+ content in the treated mice

Group Name	Treatment	Serum Fe3+ content (µmol/L)
G1	Negative control group (received normal saline)	315.6 ± 3.03
G2	DFO-treated group (received 25 mg/kg/day of Deferoxamine	285.21 ± 4.33
G3	Positive control group (iron-overloaded)	581.32 ± 2.86
G4	DFO-treated iron-overload group (received 25 mg/kg/day of DFO)	551.5 ± 4.54
G5	TCEACE extract-treated iron-overloaded group (received 50 mg/kg/day)	389.44 ± 5.16***
G6	TCEACE extract-treated iron-overloaded group (received 100 mg/kg/day)	368.4 ± 7.82***

(Mean ± SD; ***significant, $p \leq 0.001$).

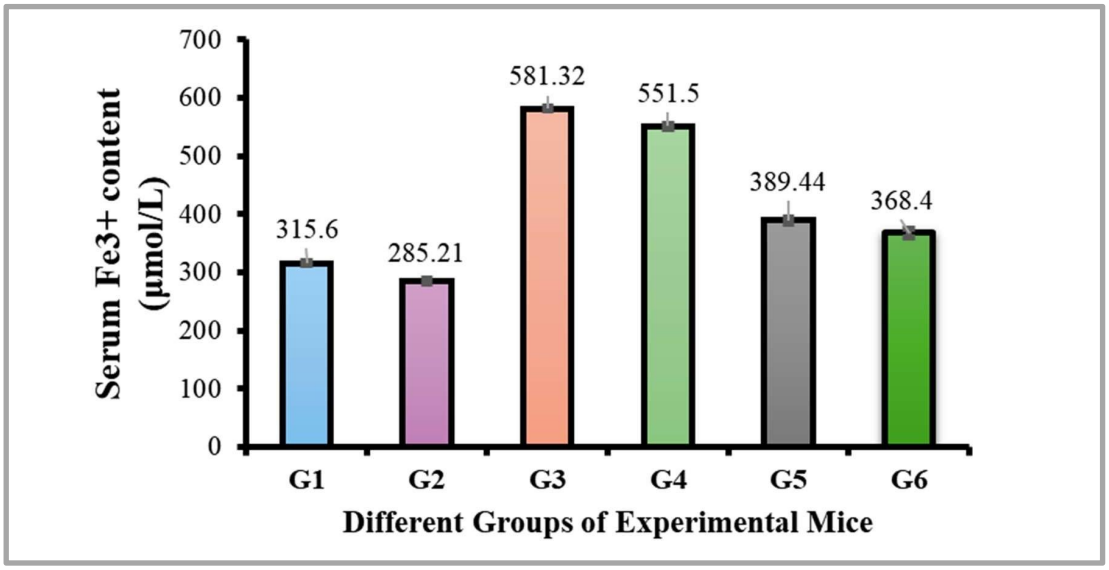

Figure-1. Serum iron content in Experimental Mice

When comparing serum Fe3+ levels among groups G4, G5, and G6, distinct differences emerge regarding treatment effectiveness. Group G4, which received the standard drug (DFO), had a mean serum Fe3+ level of 551.5 ± 4.54 µmol/L, showing no significant reduction compared to the positive control group (G3). In contrast, Group G5, treated with TCEACE extract at 50 mg/kg, exhibited a significantly lower mean of 389.44 ± 5.16 µmol/L, with a p-value of < 0.001 when compared to G4, indicating a strong effect of the extract. Furthermore, Group G6, receiving TCEACE at 100 mg/kg, showed even lower serum Fe3+ levels at 368.4 ± 7.82 µmol/L, also significantly different from G4 with a p-value of < 0.001. These results suggest that TCEACE extract is more effective than the standard drug in reducing serum iron levels.

Overall, both DFO and TCEACE extracts demonstrate effectiveness in lowering serum iron levels, with TCEACE showing promising results at both tested dosages. This highlights the potential of these treatments in managing conditions associated with iron overload, such as thalassemia and other related disorders.

DISCUSSION

The results of our current study on TCEACE's efficacy in reducing serum Fe3+ levels align with and build upon previous research in the field of iron overload management. Notably, studies by Yun et al. (2020) and Badria et al. (2015) demonstrated that various plant extracts could significantly lower serum iron levels in animal models, suggesting that phytochemicals may act as effective iron chelators. Their findings indicated a marked decrease in serum iron after treatment with specific botanical extracts, which resonates with the significant reductions observed in Groups G5 and G6 in our study. Similarly, research by Loizzo et al. (2012) and Alikhani et al. (2022) reported promising results from herbal formulations containing different phytochemical constituents in managing iron overload, further supporting our findings that TCEACE effectively reduces serum Fe3+ levels compared to standard treatments like DFO.

Furthermore, the work of Fasae et al. (2021) and Truong et al. (2020) emphasized the importance of dosage in the effectiveness of herbal treatments for iron overload. Their studies highlighted that higher concentrations of certain extracts led to more significant reductions in serum iron levels—a trend also observed in our results, where Group G6 (100 mg/kg TCEACE) exhibited lower serum Fe3+ levels than Group G5 (50 mg/kg). This dose-dependent effect underscores the potential of TCEACE as a therapeutic alternative, particularly in cases of severe iron overload. The contrasting efficacy of TCEACE compared to DFO in our study suggests that further research is warranted to explore the mechanisms behind TCEACE's action and its potential for clinical applications.

The significant reductions in serum Fe3+ levels observed in our study support the hypothesis that TCEACE extract is an effective treatment for iron overload, surpassing traditional therapies such as DFO. These findings contribute to the growing body of literature advocating for the use of herbal remedies in managing iron-related disorders. Future studies should focus on isolating the active components of TCEACE, understanding their mechanisms, and conducting clinical trials to

establish their efficacy and safety in human populations. Integrating such natural treatments could provide a valuable addition to existing therapeutic options for patients suffering from iron overload conditions.

CONCLUSION

The results of the in vivo study indicate that TCEACE extract significantly reduces serum Fe^{3+} levels compared to both the standard drug (DFO) and the positive control group. Groups G5 and G6 exhibited substantial reductions in iron levels, with p-values of <0.001, highlighting the efficacy of TCEACE in managing iron overload. This suggests that TCEACE not only surpasses the standard treatment but also offers a promising alternative for therapeutic interventions in conditions related to excess iron. Furthermore, the study demonstrates that TCEACE extract effectively reduces iron overload in both spleen and liver tissues, showing a dose-dependent efficacy comparable to Deferoxamine (DFO). These findings align with previous research on the antioxidant and iron-chelating properties of Terminalia catappa and Trichosanthes kirilowii, supporting their use in reducing iron-induced oxidative stress. Overall, TCEACE extract holds promise as a natural and potentially less toxic alternative to conventional iron chelation therapies, warranting further investigation for clinical application in thalassemia patients.

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