

## Comparative Evaluation of Flaxseed (*Linum usitatissimum*) and Ferrous Sulphate in Tannic Acid-Induced Iron Deficiency Anaemia in Wistar Rats

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**This study assessed the hematinic effect of *Linum usitatissimum* (flaxseed) on iron-deficiency anaemia in Albino Wistar rats. Anaemia was induced using tannic acid, and the rats were divided into five groups for 30 days. Group one received a standard diet, group two a standard diet with ferrous sulphate and the remaining groups received a standard diet with different concentrations of flaxseed extract. Hematological parameters, including Hemoglobin, MCV, MCHC, and MCH levels, were measured before and after treatment. The results showed that flaxseed extract showed dose-dependent improvement in hematological parameters, compared to ferrous sulphate, suggesting its potential as a natural treatment for iron-deficiency anaemia. Higher doses of flaxseed extract may further enhance the therapeutic benefits in patients with iron-deficiency anemia.**

**Keywords:** Als; Anaemia; Hematinic Effect; Iron Deficiency Anaemia; *Linum Usitatissimum*; Mean Cell Volume; Tannic Acid; Wistar Rats.

Iron deficiency anaemia (IDA) is a pathology that occurs when the body does not form sufficient healthy red blood cells (RBCs). Globally, it affects more than 1.2 billion individuals. Increased physiological iron requirements, iron absorption defects, reduced intake, or chronic blood loss in adolescents, children, young adults, and pregnant women are the causes of iron deficiency. To maintain a normal iron balance, approximately 1 mg of iron must be consumed daily in the diet. Impaired iron absorption occurs because of fat malabsorption and chronic diarrhea. Common symptoms of IDA are pale skin, weakness,

koilonychia (spoon-shaped nails), cheilitis (lips inflammation), glossitis (tongue inflammation) and Plummer-Vinson syndrome (rare condition that presents with difficulty in swallowing, IDA, glossitis, cheilosis and oesophageal webs).

*Linum usitatissimum*, commonly known as flaxseed (Als, Jawas, Aksebija), is a nutritionally rich annual herb of the Linaceae family. <sup>1</sup> Its seeds are abundant in fiber, omega-3 fatty acids, copper, lignans, iron and thiamine, among other minerals. These components offer bioactivity that is advantageous to both human and animal health because of their antioxidative, anti-inflammatory

and lipid-regulating properties. A nutrient found in plants called lignans is a type of phytoestrogen that resembles the female hormone, estrogen. Because of this similarity, lignans may have estrogenic effects on the body. Estrogen deficiency prevents iron release from adipocytes by increasing iron levels in the surrounding adipose tissues and upregulating hepcidin, which inhibits intestinal iron absorption and lowers serum iron levels. Thus, flax seeds might increase iron levels by increasing estrogen levels, but no studies have been performed to prove this theory.

In India, women of reproductive age are affected by iron deficiency. This is due to cultural norms, inadequate diet (less intake of vegetables and meat), illiteracy, socioeconomic status, birth spacing of less than two years between previous two pregnancies, heavy menstrual bleeding, and limited awareness of anaemia.<sup>2</sup>

#### **Aim and objectives**

- To induce anaemia in albino wistar female rats using tannic acid
- To evaluate the improvement of haemoglobin levels, after giving *Linum usitatissimum* seeds (LU) extract
- To compare the hematinic effect of *Linum usitatissimum* seeds (LU) extract, at various doses, with standard oral formulation Ferrous sulphate

#### **MATERIALS AND METHODS**

This study was approved by the Institutional Animal Ethics Committee of the Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar (KIIT/KIMS/IAEC/12/21). The study period was 2 months (60 days). This study was conducted at the Animal House, Department of Pharmacology.

#### **Collection and preparation of seed extract**

*Linum usitatissimum* seeds were purchased from a nearby local market and authenticated. The seeds were removed, dried in the shade, and grinded using a mortar and pestle. After that, they were soaked in distilled water. To accomplish this, approximately 100 g of the flax seed powder was submerged in 500 ml of distilled water and blended for 48 h using an incubator shaker. To produce concentrated extracts, solvents were discarded using a Rotary Vacuum Evaporator

set at 40 °C. The extract was stored in a refrigerator until use.

#### **Animals used**

- a. Species / common name: Albino Wistar Rats
- b. Weight- 180 – 200 gm
- c. Gender- Female
- d. Number- 30
- e. Source of animal- Animal House, Department of Pharmacology, Kalinga Institute of Medical Sciences, KIIT University, Bhubaneswar, Odisha

In this study, adult female albino rats weighing 180 – 200 g were used. The animals were kept at acclimation for a week before initiation of the study. The standard conditions for keeping animals include: humidity 35 – 60 percent, room temperature  $\pm$  25°C, and 12 h of dark as well as light. All animals were provided with food and water ad libitum. The animals were divided into five groups with six animals in each group as shown in Table 1.

#### **Induction of Anaemia**

Tannic acid was added to the basal diet of all 30 albino Wistar rats (Group I to Group V) to induce anaemia. For three weeks, the rats were given this diet. These rats developed anaemia after 21 days which was confirmed with investigation. From day 22, Group II rats were given ferrous sulphate (standard therapy), while Group III, Group IV, and Group V received *linum* seed extract at dose of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively, coupled with a normal diet for the next 30 days. Blood samples were collected on day 1 (before the initiation of tannic acid in the diet), day 21 (for confirmation of anaemia), and day 52.

#### **Acute toxicity studies**

An acute oral toxicity study was conducted according to Organization for Economic Cooperation and Development (OECD) guideline No. 423. Six albino Wistar rats were administered a high dose of the extract (2000 mg/kg). These rats were observed for a period of 14 days for any clinical signs of morbidity, mortality, or other factors. Acute toxicity assessment at 2000 mg/kg showed no mortality or significant adverse effects, indicating a safe dosage range. Therefore, doses of this plant extract of 100, 200, and 400 mg/kg were chosen and considered safe for research purposes.

In albino Wistar rats, the normal haemoglobin level ranges from 13.0-15.0g/dL. In

experimental settings, anaemia is defined when Hb levels drop below 10.0 g/dL. Therefore, from the above table (Table 2), it can be seen that there is a major decrease in blood haemoglobin levels in all the groups after induction of anaemia from baseline (day 1 to day 21). It is further observed that there is rise in haemoglobin levels after 30 days of initiation of extract in anaemic rats (Group III to Group V). The maximum elevation is observed with high-dose extract (400 mg/kg), which is comparable to the standard drug for iron deficiency anaemia.

From the above table (Table 3), it is observed that there is a major decline in blood MCV levels in all the groups after the induction of anaemia from baseline (day 1) up to day 21. It is further found that there is a major increase in MCV levels 30 days after extract initiation in anaemic rats (Group III to Group V). The maximum elevation is observed with high-dose extract (400 mg/kg), which is comparable to the standard drug for iron deficiency anaemia.

From the above table (Table 4), it is observed that there is a significant decline in blood MCH levels in all the groups after induction of anaemia from baseline (day 0) upto day 21. A major increase in MCH levels is observed 30 days after the initiation of the extract in anaemic rats (Group III to Group V).

From the above table (Table 5), it is observed that there is a major decline in blood mean cell haemoglobin concentration (MCHC) levels in all groups after induction of anaemia from baseline (day 0) upto day 21. Furthermore, there is a major rise in MCHC levels after 30 days of initiation of extract in anaemic rats (Group III to Group V).

## DISCUSSION

The primary focus of iron deficiency anemia (IDA) prevention strategies in India is the distribution of iron supplements and iron-supplemented meals derived from inorganic

**Table 1.** Shows the grouping of animals

Groups	Number of Animals	Drugs used
Group I	6	Control group - Basal diet + Tannic acid (normal diet started after day 22)
Group II	6	Standard group-Ferrous sulphate (Normal diet + FeSo4 40 mg/kg P.O)
Group III	6	Test group 1 (Normal diet + Extract 1 - 100 mg/kg)
Group IV	6	Test group 2 (Normal diet + Extract 2 - 200 mg/kg)
Group V	6	Test group 3 (Normal diet + Extract 3 - 400 mg/kg)

**Table 2.** Shows changes in the haemoglobin levels of rats before induction (day 1), after the development of anaemia (day 21), and 30 days after initiation of *Linum* extract (day 52)

Groups	Day 1 Haemoglobin (g/DL)	Day 21 Haemoglobin (g/DL)	Day 52 Haemoglobin (g/DL)
Group I	13.55 ± 0.21	9.75 ± 0.25	9.22 ± 0.45
Group II	12.89 ± 0.71	8.27 ± 0.37	13.84 ± 0.58***&
Group III	13.21 ± 0.57	8.62 ± 0.72	12.34 ± 1.23*
Group IV	13.22 ± 1.26	8.89 ± 1.29	12.85 ± 0.87*
Group V	12.92 ± 0.56	8.13 ± 0.94	13.98 ± 0.69***&

Data are presented as mean ± SD, and statistical significance was determined using one-way ANOVA with post hoc analysis. When compared to day 21, i.e. induction of anaemia, \* denotes a p-value of <0.05, which is significant, and \*\* denotes a p-value of <0.01, which is greatly significant. & shows that two groups are comparable.

**Table 3.** Shows changes in the mean cell volume (MCV) levels of rats before induction, after the development of anaemia, and 30 days after initiation of *Linum* extract

Groups	Day 1 MCV (fL)	Day 21 MCV (fL)	Day 52 MCV (fL)
Group I	61.8±0.76	49.2± 0.98	49.8 ± 1.36
Group II	62.3±0.54	50.2± 0.23	59.6 ± 1.65***&
Group III	61.5±0.72	49.2± 1.47	57.6 ± 2.67*
Group IV	61.6±0.98	49.8± 1.54	58.6 ± 1.68*
Group V	62.2±0.46	50.3 ± 0.45	59.4± 2.53***&

Data are presented as mean ± SD, and statistical significance was determined using one-way ANOVA with post hoc analysis. When compared to day 21, i.e. induction of anaemia, \* denotes a p-value of <0.05, which is significant, and \*\* denotes a p-value of <0.01, which is greatly significant. & shows that two groups are comparable.

**Table 4.** Shows changes in the Mean Cell Haemoglobin (MCH) levels of rats before induction, after the development of anaemia and 30 days after initiation of *Linum* extract

Groups	Day 1 Mean Cell Haemoglobin (pg)	Day 21 Mean Cell Haemoglobin(pg)	Day 52 Mean Cell Haemoglobin(pg)
Group I	19.4 ± 0.54	16.6 ± 0.54	16.4±0.85
Group II	19.2 ± 0.38	15.4 ± 0.89	19.2 ± 0.25**
Group III	18.9 ± 0.61	16.2 ± 0.38	18.7 ± 0.53*
Group IV	18.8 ± 1.26	16.1 ± 0.97	18.9 ± 0.45*
Group V	19.3 ± 0.67	15.7 ± 0.59	19.1 ± 0.56*

Data are presented as mean ± SD, and statistical significance was determined using one-way ANOVA with post hoc analysis. When compared to day 21, i.e. induction of anaemia, \* denotes a p-value of <0.05, which is significant, and \*\* denotes a p-value of <0.01, which is greatly significant

**Table 5.** Shows changes in the Mean Cell Haemoglobin concentration (MCHC) levels of rats before induction, after the development of anaemia, and 30 days after initiation of *Linum* extract

Groups	Day 1 Mean Cell Haemoglobin Concentration(g/DL)	Day 21 Mean Cell Haemoglobin Concentration(g/DL)	Day 52 Mean Cell Haemoglobin Concentration(g/DL)
Group I	37.5 ± 1.68	31.5 ± 0.86	31.4 ± 1.12
Group II	38.1 ± 0.88	30.8 ± 1.64	37.4 ± 0.65**
Group III	37.3 ± 0.54	31.2 ± 0.45	35.4 ± 0.53*
Group IV	37.6 ± 1.58	31.6 ± 0.86	36.3 ± 0.76*
Group V	38.2 ± 0.34	30.9 ± 1.74	37.1 ± 1.32*

Data are presented as mean ± SD, and statistical significance was determined using one-way ANOVA with post hoc analysis. When compared to day 21, i.e. induction of anaemia, \* denotes a p-value of <0.05, which is significant, and \*\* denotes a p-value of <0.01, which is greatly significant

bivalent iron salts with low absorption rates (10–15%).<sup>3</sup> Anaemia is a nutritional deficiency disorder in which red blood cells (RBCs) and their oxygen-carrying capacity are reduced. Haemoglobin plays a crucial role in delivering oxygen to various tissues, and iron is required for its synthesis.<sup>4</sup> Iron deficiency is the most common type of anaemia globally, and additional causes include deficiency of nutrients like iron, folate, vitamin B12, vitamin A, parasitic infestations, inflammatory disorders, or genetic defects in haemoglobin synthesis. It is more prevalent in children and women with low socioeconomic status and health conditions. Decreased dietary intake of iron and other nutrients remains a prominent cause of IDA.<sup>5</sup> Symptoms of IDA include dyspnea, fatigue, palpitations, tachycardia, and angina. This insufficiency results in hypoxemia, which triggers a compensatory decrease in the intestinal blood flow. This in turn causes motility issues, malabsorption, nausea, weight loss, and pain abdomen.<sup>6</sup> Ultimately, it progresses to central hypoxia, causing headaches, lethargy, cognitive impairment, and decreased quality of life.<sup>7</sup>

*Linum usitatissimum* is an annual herb belonging to the Linaceae family that requires moist soil for growth. The common growing regions for this plant are the Eastern Mediterranean, Western Asia, the Middle East, and India. The seeds of these plants are commonly known as flax seeds and are available in two varieties: yellow, brown, or golden.<sup>8</sup> It was previously considered a neglected food, but recently, the nutritional value of these seeds has gained attention. It is a great source of dietary fiber, protein, and fat.<sup>9</sup> Flaxseeds contains lignans such as secoisolariciresinol diglucoside (SDG),  $\alpha$ -linolenic acid (ALA), and omega-3 fatty acids, all of which have been shown to provide nutritional benefits to humans. Linolenic acid is crucial for the development of brain functions in children and to lower the risk of cardiovascular illnesses, linolenic acid is crucial.<sup>10</sup> The proteins in flax seeds are rich in arginine, glutamic acid, and aspartic acid, while they also contain dietary fibers that are effective in reducing constipation, improving bowel movements, and acting as a hypocholesterolemic agent. The lignans present in these seeds have antioxidant, antidiabetic, anticancer, and free oxygen radical-scavenging

activities. Some studies have suggested that these lignans are helpful in bone development.<sup>11</sup>

Tannins are a class of compounds found in plants and are secondary metabolites belonging to the phenolic class. These substances exhibit diverse physiological effects and activities. Tannins are associated with several unfavorable nutritional effects. Studies have shown that feeding these compounds to growing animals results in a number of biochemical and physiological effects<sup>12</sup>, such as reduced intestinal absorption of amino acids as well as carbohydrates, growth suppression, negative nitrogen balance, lowered immune response, and raised liver as well as protein catabolism.<sup>13</sup> Therefore, it was used in this study to induce IDA.

Iron is an essential component of haemoglobin synthesis. In IDA, haemoglobin levels decrease as a result of iron deficiency. In the current study, after receiving tannic acid, the haemoglobin levels decreased in all rats. However, after receiving continuous treatment for four weeks, recovery progressed and haemoglobin levels in the treated groups were noticeably higher than those in the control group (Table 2). It was also observed that recovery was dose-related, as better outcomes were observed in rats receiving higher concentrations. Similar results have been reported by Kooshki A<sup>14</sup> where daily consumption of flaxseed oil was responsible for the elevation of haemoglobin levels, even in dialysis patients.

Known as red cell indices, Wintrobe initially presented (MCV), (MCH), and (MCHC) in 1929. These values are helpful in distinguishing between different kinds of anaemia that arise from different sources. Iron deficiency anaemia usually causes microcytic hypochromic anaemia where the red cell indices are low. In the current research also, it has been seen that there was a decrease in MCV, MCH, and MCHC levels after induction of anaemia (Table 3,4,5). The levels were progressively increased after giving the extract in the deficient rats. This improvement was significant as well as comparable with the standard drug. The improvement was better in rats receiving higher doses. Similar observations were seen in Tabibi<sup>15</sup>, where daily intake of flaxseed oil improved the red cell indices in anaemia and dialysis patients.

*Linum usitatissimum* is a complete dietary supplement and is also referred to as “Superfood.”

It contains protein, carbohydrates, and dietary fibers. The highest dietary concentration of lignans, a form of phytoestrogen derived from plants that resembles the female hormone estrogen, is found in flax seeds. This hormone is specific to women and has an adverse effect on hepcidin, a small polypeptide that is produced by hepatocytes, which decreases the absorption of iron from the gut as well as the release of iron from storage tissues. Therefore, *Linum usitatissimum* seeds possess estrogenic properties which help in increasing iron absorption by decreasing hepcidin. However, there are very few studies that have been done on this hypothesis. This is a short pilot study, and further research is necessary to strengthen the role of flax seeds in anaemia.

### CONCLUSION

Iron deficiency is a condition resulting from increased physiological iron demand, reduced iron intake, pathological malabsorption, and chronic blood loss in adolescents, children, pregnant women, and young adults. *Linum usitatissimum* contains lignans that have estrogenic effects in the body, which tend to increase iron absorption by reducing hepcidin. In the present study, the use of flaxseeds showed improvement in iron deficiency anaemia, which is an area of concern especially in women. Flax seeds are considered as “Superfood” are cheaper, more easily available to the general public, and can be explored further for usage as a dietary supplement in iron deficiency anaemia.

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#### Conflict of Interest

The author(s) do not have any conflict of interest.

#### Data Availability Statement

This statement does not apply to this article.

#### Ethics Statement

The animal study was reviewed and approved by the Institutional Animal Ethics Committee of the Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar (KIIT/KIMS/IAEC/12/21). All animal procedures were performed in accordance with the IAEC guidelines.

#### Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

#### Clinical Trial Registration

This research does not involve any clinical trials

#### Permission to reproduce material from other sources

Not Applicable.

#### Authors contribution

Vartika Srivastava- Conceptualization, visualisation, literature search, clinical studies, data analysis, statistical analysis, manuscript writing- Original Draft; Chaitali Pattanayak- Conceptualization, Visualisation, supervision, data analysis, manuscript review; Suman Supreeti- Manuscript preparation, editing and reviewing; Sougata Sarkar- Conceptualization, visualisation, literature search, clinical studies, data analysis; Swati Suchismita Sethi- Methodology writing, manuscript drafting, Data collection, data analysis

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