Elimination of excessive iron by *Terminalia chebula* in the iron overloaded rabbits

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**ABSTRACT**

Iron is important and essential for life. Humans can get iron from blood transfusions or iron pills in addition to that taken in with food. Unbound iron can trigger free radical activity, which can cause cell death, and destroy DNA. A thalassaemia patient receives 20 units of blood per year. Each unit of blood (500ml) contains 200-250mg of iron. In the present study it was planned to look at the role, if any, of *Terminalia chebula* for iron elimination when an excessive amount of iron happens to accumulate in the body. Rabbits were overloaded with iron (Jectosol injection) @ 0.5ml/day each for 4 weeks. Serum iron levels were determined in the normal, iron overloaded control and iron overloaded rabbits treated with *Terminalia chebula*. The results show a significant increase in iron concentration in serum of iron overloaded rabbits. The administration of *Terminalia chebula* powdered fruit to iron overloaded rabbits led to a significant decrease in serum iron concentration.

**Key words:** Serum iron, *Terminalia chebula*, Iron overloaded rabbits.

**INTRODUCTION**

Iron is important and essential for all life. Even the bacterial cells require iron for their survival and growth (Grieger & Kluger, 1978). It is a vital constituent of respiratory pigment haemoglobin, which delivers oxygen to the body. In addition to haemoglobin and myoglobin, iron is also an essential constituent of very important enzymes like cytochrome C oxidase and others including catalase, peroxidases and flavoprotein. Its essentiality to every form of life arises from its role as prosthetic group in a number of enzymes and proteins.

Humans can get iron from blood transfusions or iron pills in addition to that taken in with food. People consume two types of iron: non-heme iron and heme iron. Unbound or free iron is highly destructive and dangerous. However, this essentially required iron becomes toxic to body if it accumulates beyond the normal limits (Frieden, 1974). Unbound iron can trigger free radical activity which can cause cell death and destroy DNA.

Thalassaemia, in which deficient globin chain synthesis occurs, comprises a group of genetic disorders that results from inherited abnormality of globin production. Thalassaemia patient receives 20 units of blood per year. Each unit of blood (500ml) contains 200-250mg of iron. With regular transfusions there is a steady accumulation of iron in the liver, heart, pancreas, bones, gall bladder and endocrine glands. Thus, although well transfused thalassaemic children grow and develop normally, they die of iron overload unless steps are taken to remove the excess iron (Prati, 2000).
The physiological limitation that prevents the elimination of accumulated iron can be circumvented by treatment with a chelating agent capable of complexing with iron and permitting its excretion. A clinically highly effective iron chelator such as desferrioxamine B is a trihydroxamic acid produced by *Streptomycins pilosus*. It binds iron with a large preference over other metal ions such as calcium. But the expensive medical paraphernalia required for desferrioxamine administration makes the treatment expensive, and curbs its availability in areas of the world where medical resources are limited. To produce a therapeutic effect, parenteral administration of the drug in prolonged infusions is needed and, therefore, many investigators try to search for orally active iron chelator with effect comparable to desferrioxamine.

*Terminalia chebula* is called the "king of medicines" in Tibet, having extraordinary powers of healing. The pericarp of the fruit contains anthraquinone glycosides, which are responsible for the laxative effect. The presence of saponins, anthrones and anthranols has also been documented (Reddy, 1990). The fruit is used medicinally. In animals, it speeds up gastric emptying by 86 percent, compared to 76 percent for metoclopramide. Extracts of the fruit pericarp have demonstrated cardiotonic activity in isolated frog hearts (Reddy, 1990). An aqueous extract of *Terminalia chebula* has been investigated as a potential anti-caries and anti-plaque agent (Jagtap & Karkera, 1999). Crude alcoholic extracts of *Terminalia chebula* and *Terminalia bellerica* were found to lack cellular toxicity in an assay using fresh sheep erythrocytes and exhibited antibacterial activity against a number of bacterial species (Ahmad et al., 1998). It also protects epithelial cells against influenza A virus, supporting the traditional use of *Terminalia* for aiding in recovery from acute respiratory infections (Badmaev & Nowakowski, 2000).

As discussed above, *Terminalia chebula* is a widely used therapeutic agent for many ailments and diseases in human being. In the present study it was planned to look at the role, if any, of *Terminalia chebula* for iron elimination when an excessive amount of iron happens to accumulate in the body.

**MATERIALS AND METHODS**

In the present study, 32 rabbits (*Oryctolagus cuniculus*) were used. They were all males of the age between 5 and 5.5 months, with a body weight of 1-1.5 Kg approximately. Rabbits were kept in clean, spacious and well-aerated rooms. The floor of the room was kept as dry as possible, because wet warm environment provides a favorable atmosphere for the development of coccidiosis (Chapman, 1929; and Peck, 1934).

**Treatment of Rabbits**

All the rabbits were fed on normal diet consisting of fodder and grams and were divided into following 4 groups with 8 rabbits each: Group 1 (Control group), this group included the normal rabbits that were not treated with *Terminalia* or iron; Group II (*Terminalia* treated group), this group was given a
daily dose of *Terminalia* for four weeks; Group III (iron overloaded group), this group was iron overloaded by intramuscular injections of iron Jectosol for four weeks and then left untreated; Group IV (iron overloaded rabbit treated with *Terminalia*), this group was overloaded with iron for 4 weeks and then was treated with *Terminalia* for further four weeks. Rabbits of all groups were assayed for serum iron level at regular intervals.

**Drug & Dosage**

The fruits of *Terminalia chebula* were finely powdered in lab. The powder was dissolved in water and administered daily @ 250mg/Kg body weight. Rabbits were iron overloaded by using Jectosol Injection @ 0.5ml daily for four weeks.

**Blood Sampling**

5 ml syringes were used to collect blood samples of all groups from the vein at back of the ear. This procedure was carried out during 08.00 -10.00 A.M. to avoid variations due to circadian rhythm, etc. Serum was isolated and assayed for iron levels by colorimetric test (CAB Method).

**Statistical Analysis**

Results were expressed as mean±SER and statistical analysis was performed using student’s t-test. p<0.05 implied significance.

**RESULTS**

The data on the iron overloading of rabbits with extraneous iron source indicated that iron concentration in blood serum increased significantly (Table 1). The iron went on increasing in concentration with daily iron injections. After 4 weeks serum iron level reached 379.50±0.54 μg/dl (74.05% increase) as compared to the normal control rabbits (218.04±2.56 μg/dl).

*Terminalia* treatment led to a decrease in iron level of blood serum in all groups (Table 2). *Terminalia* treatment to the iron unloaded rabbits led to a decrease in serum iron level from 218.05±2.56 μg/dl to 169.11±6.88 μg/dl after four weeks (Table 2).

*Terminalia* treatment to the iron overloaded rabbits who had received extraneous iron @ 0.5ml Jectosol injection daily for four weeks led to a significant and considerable decrease in serum iron level. It decreased to 197.05±2.18 μg/dl as compared to 340.63±1.39 μg/dl in iron overloaded untreated group (Table 2).
### Table 1: Serum iron concentration, µg/dl, in control rabbits and iron overloaded rabbits.

<table>
<thead>
<tr>
<th>Period Group</th>
<th>Zero Week M±SER</th>
<th>One Week M±SER</th>
<th>Two Week M±SER</th>
<th>Three Week M±SER</th>
<th>Four Week M±SER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>218.04 ±2.56 (8)</td>
<td>214.50 ±1.37 (8)</td>
<td>211.89 ±5.62 (8)</td>
<td>209.13 ±1.14 (8)</td>
<td>206.85 ±0.56 (8)</td>
</tr>
<tr>
<td>Iron overloaded</td>
<td>218.04 ±2.56 (8)</td>
<td>335.90 ±4.32 (8)</td>
<td>348.12 ±10.90 (8)</td>
<td>360.70 ±2.64 (8)</td>
<td>379.50** ±0.54 (8)</td>
</tr>
</tbody>
</table>

**P<0.05  
M±SER denote mean and standard error.  
No. in parenthesis indicates the number of rabbits in each group.

### Table 2: Serum iron concentration, µg/dl, in control rabbits, iron overloaded rabbits (untreated) compared with *Terminalia* treated rabbits in each group.

<table>
<thead>
<tr>
<th>Period Group</th>
<th>Zero Week M±SER</th>
<th>One Week M±SER</th>
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<td>206.85 ±0.56 (8)</td>
</tr>
<tr>
<td>Iron overloaded then left untreated</td>
<td>379.04** ±2.56 (8)</td>
<td>372.02 ±1.72 (8)</td>
<td>369.70 ±0.47 (8)</td>
<td>345.55 ±2.59 (8)</td>
<td>340.63 ±1.39 (8)</td>
</tr>
<tr>
<td><em>Terminalia</em> treated</td>
<td>218.04 ±2.56 (8)</td>
<td>193.98 ±6.39 (8)</td>
<td>181.64 ±2.38 (8)</td>
<td>173.98 ±2.10 (8)</td>
<td>169.11 ±6.88 (8)</td>
</tr>
<tr>
<td><em>Terminalia</em> treated after iron overdosage</td>
<td>379.04** ±2.56 (8)</td>
<td>329.83** ±1.35 (8)</td>
<td>257.33** ±1.16 (8)</td>
<td>236.80** ±3.80 (8)</td>
<td>197.05** ±2.18 (8)</td>
</tr>
</tbody>
</table>

**P<0.05  
M±SER denote mean and standard error.  
No. in parenthesis indicates the number of rabbits in each group.
DISCUSSION

In the present study, iron overloading of rabbits with iron sorbitol citric acid complex (Jectosol) led to a significant and considerable increased accumulation of iron in serum by 74.05%. The increased iron accumulation in rabbits in the present investigation is consistent with iron overloading already reported in other animals like rats (Porter et al., 1993), gerbils (Cathew et al., 1993) and dogs (Lisboa, 1971). Symptoms of iron overloading include chronic fatigue, abdominal pain, irregular heart rhythm, loss of period, loss of interest in sex, hair loss, and skin color changes.

The molecules that bind very tightly to metal ions are known as "chelators". These are simple molecules that are easily manufactured. Others are complex proteins made by living organisms. They detoxify metal ions and prevent poisoning effect. The tight binding of chelators to iron blocks the ion's ability to catalyze redox reactions. Iron ions have six electrochemical coordination sites. Consequently, a chelator molecule that binds to all six sites completely inactivates the "free" iron. Such chelators are termed "hexidentate", e.g. desferrioxamine. In some chelators only single molecule interacts with only two of the coordination sites on iron. These chelators are called "bidentate". An example of this type of molecule is ferrichrome. Another example is deferiprone, or "L1", a chelator currently in clinical trials.

Chelators can also be classified using a number of other criteria such as their origin, their interaction with solvents such as water or their stoichiometric interaction. One key clinical feature of iron chelators is the degree to which they are absorbed from the gastrointestinal tract and their specificity.

A clinically highly effective iron chelates such as desferrioxamine B is a trihydroxamic acid produced by Streptomyces pilosus. It remains the most effective and safe iron chelator for treatment of patients with transfusional iron overload. The drug binds iron with a large preference over other metal ions such as calcium. Desferrioxamine chelates iron in a one-to-one ratio. But poor compliance with the rigours of parenteral treatment in patients limits its regular use and thus reduces life expectancy in these patients. Excessive dosage of deferrioxamine may result in growth retardation, sensorineal ototoxicity, ocular toxicity as well as bone deformities.

Diethylene triamine penta-acetic acid (DPTA) is cheaper but also is given parenterally and has the disadvantage that it also chelates substantial amount of zinc. With repeated frequent use, DPTA produces severe toxic effects as a result of zinc depletion (Hoffbrand & Wonke, 1997).

An orally active compound, ICL670A, designed by computer modeling is a potent and selective iron chelator. Its ability to mobilize tissue iron and promote its excretion has been shown in several animal models. ICL670A was well tolerated and has a good safety profile (Galanello, 2001). But the compound is currently undergoing further clinical evaluation.

Oral administration not only has the potential to be used for long term treatment but also may increase efficiency by providing a continuous supply of circulating drug. In the present study, Terminalia chebula has been selected for the removal of excessive iron from the body. This drug can be administered...
orally. In Ayurveda it is considered to destroy all diseases and eliminate all wastes from the body and it promotes tissue growth and health. Various extracts have been prepared from the powdered fruits which have a wide antibacterial and antifungal spectrum, and also inhibit growth of *E. coli*, the most common organism responsible for urinary tract infection.

The present investigation has shown that *Terminalia chebula* administered in small doses eliminates excessive iron quite efficiently. It eliminates iron from serum when administered to iron overloaded rabbits @ 250mg/Kg daily. The damage to body on excessive iron overloading seems to have been repaired. Moreover, the therapeutic drug used in this investigation is very cheap as compared to deferoxamine and other drugs (oral) being used at present to treat thalassaemic and thalassaemia-like patients. But it requires more investigation to find out the minimum daily dose for human body, before this drug can be recommended for the treatment of thalassaemic patients and others suffering from excessive iron accumulation in their bodies.

**REFERENCES**


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