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# A Study on the Quantitative Estimation of Ironin the leaves of *Centellaasiatica* (*Thankuni*) along with its effect on the Blood Hemoglobin Level in Rat Model.

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***Abstract:***
***Background:*** *Centella asiatica (CA) or Thankuni, in Bengali, is a perennial plant native to Asian countries. It is mainly used for wound healing, improvement of nervous function, skin problems including leprosy, ulcers, diarrhoea, arthritis, hypertension etc. It maybe possible that this herb is beneficial anaemia also. Not enough evidence is available regarding the effect of CA on blood hemoglobin level. Thus, the current article targets to estimate the iron content of the herb and study its effect of blood hemoglobin.*
***Materials and Methods:*** *The herb was collected from different markets of Kolkata and iron estimation was performed using atomic absorption spectrophotometry. Male albino rats were then divided in two groups: Control (C) and Treated (OT). The OT group received CA paste and stock diet whereas C group received only stock diet for 15 days. The study was conducted in three sets at summer, monsoon and winter.*
***Results:*** *The total iron content of CA was 14.5±2.3 mg/100g. CA treatment significantly increased the blood hemoglobin levels of OT group as compared to the C group in all three sets.*
***Conclusion:****CA is a rich source of dietary iron and it has potential role to prevent and treat anaemia.*
***Key Words:*** *Centella asiatica, Iron, Hemoglobin, Anaemia*

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## I. Introduction

*Centella asiatica* or *Thankuni*, in Bengali, is a perennial plant native to India, Japan, China, Indonesia, South Africa, Srilanka and South pacific. It is tasteless, sometimes little bitter, odour less plant that thrives in and around water. It has small fan shaped green leaf with white or light purple to pink flower and bear small oval fruit. The leaves and stems are used for therapeutic purpose. *Centella asiatica* has been used as medicinal herb for thousands of years in India, China, and Indonesia. It is mainly used for wound healing, improvement of nervous function, skin problems including leprosy, ulcers, diarrhoea, arthritis, hypertension etc. [1] Anaemia is a condition characterised by a decreased level of Red Blood Cells or Haemoglobin in the blood leading to an impaired oxygen transport. It may be due to nutritional, genetic, physiological and other causes. [2] As per the National Family Health Survey-4 (NFHS-4) the all India prevalence of anaemia is 58.6%, 53.1% and 22.7% among children, women and men respectively. [3]It is thus essential to continuously screen and monitor the vulnerable population for anaemia and design newer strategies for its prevention and treatment. Since *Centella asiatica* has potent medicinal properties it maybe assumed that it can show beneficial effects in anaemia also.Thus, the current study targets to find out the iron content of *Centella asiatica* and study its effect on serum haemoglobin level.

The objectives of the present study are:

- To quantify the iron content of *Centella asiatica*.
- To find out the effect of the aqueous extract of the herb on serum hemoglobin level of experimental rats.
- To compare the effects on experimental rats with the controls.
- To validate the traditional knowledge regarding the herb.

## II. Materials and Methods

***Iron analysis****:*

Required quantities of Centella asiatica leaves were purchased from different markets of Kolkata(Dumdum, Kalighat, Nagerbazar and Shyambazar) to maintain randomness of the sample. The leaves were washed and then dried by keeping on the blotting paper. 10 gm of leaves was taken in a previously

weighed crucible. The crucible was made moisture free by drying in an oven at 110°C. The leaves were then incinerated in a muffle furnace at 550°-600°C for 4 hours. It was then kept in a desiccator, cooled & weighed to ensure completion of incineration. The crucible was again heated in a muffle furnace for 1 hour. This was repeated until a constant weight was obtained. [4] The ash was digested with 5ml cone. HN03 and filtered. Distilled water was then added to it in a volumetric flask where the volume was made up to 50ml. This was then treated as working stock solution for further analysis. [5] Atomic absorption spectrophotometry was used for iron quantification.

***Experiment on Animals****:*

Young healthy male rats of Wistar strain having body weight ranging 70-80g were purchased from authorised animal supplier. These animals were acclimatized in the laboratory for 7 days prior to the experiments. In this period rats were fed on a stock diet [6] and tap water ad libitum (22±2°C, 12-h light/dark cycle).

It was found in different studies that extraction of *Centella asiatica* and also the whole leaves were used in different scientific research works. In the present study it is therefore chosen to use *Centella asiatica* fresh leaves to emphasize the nutritional aspect of the leaf. The leaves of *Centella asiatica* were washed thoroughly and made a smooth paste with mortar pestle and then calculated amount of water was added to the paste. Then forced feeding was done with the help of feeding needle every day in a particular time of the day throughout the experimental period.

The rats were divided in two groups namely, Control Group (C) and Treated Group (OT). Sample size was calculated using the following formula,

Number of rats in each experimental group (n)= (10/K) +1 [K= number of experimental groups] [7]

6 rats were added to each group. Total 3 sets were prepared for summer, monsoon and winter. The C group was maintained on stock diet and OT group received stock diet and *Centella asiatica* paste (150 mg/ kg body weight) for 15 days. Animals were made unconscious using ether anaesthesia. Blood was drawn with a 5ml syringe, fitted with hypodermic needle directly from the heart. Serum haemoglobin level was estimated by Cyanmethemoglobin method. [8]

***Statistical Analysis:***

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) programming software. Student's t-test test was performed for making comparison between two groups. Results having *P*-value <.05 were considered statistically significant.

## III. Results

The iron content of them samples collected from different markets of Kolkata is given in Table-1. The total mean iron content was 14.5±2.3 mg/100g. It shows that the herb is a quite good source of dietary iron.

**Table-1:** Iron content of the samples of *Centella asiatica* collected from different markets of Kolkata

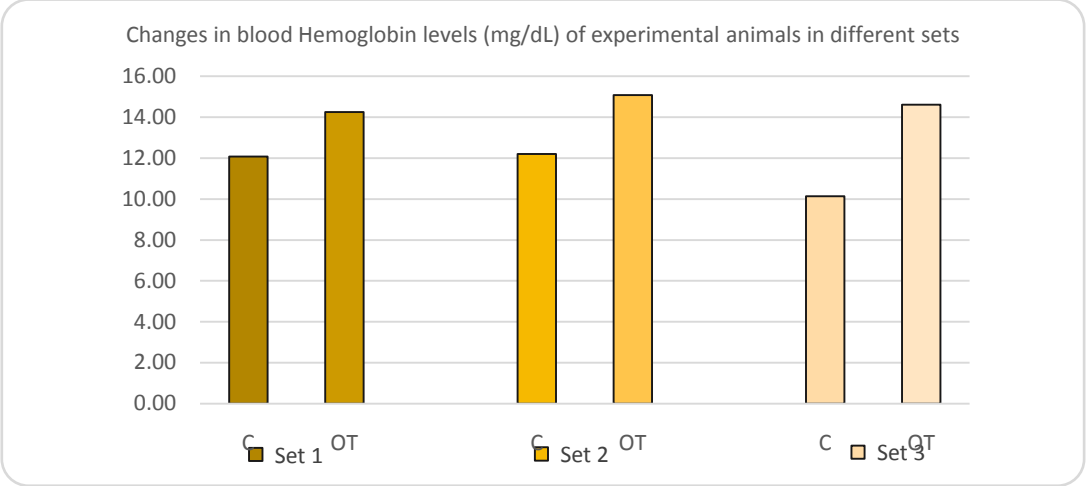
| Location of Market | Iron Content mg/100g (Mean ± SD) |
|---|---|
| Dumdum (N=6) | 16.875± 1.5138 |
| Kalighat (N=5) | 18.266± 4.0365 |
| Nagerbazar (N=6) | 17.6467± 0.9818 |
| Shyambazar (N=6) | 17.26± 2.3197 |
| Total (N=23) | 17.4791± 2.2774 |

*Centella asiatica*, at a dose of 150 mg/kg, significantly increased the blood hemoglobin levels of the OT group as compared to the C group in all three sets. The data is shown in Table-2 and Figure-1.

**Table-2:** Hemoglobin levels of experimental rats (mg/dL)

| Set No. | Group | Haemoglobin mg/dL (Mean ± SD) | t-Value[a] | *P*-Value |
|---|---|---|---|---|
| Set I (Summer) | C (n=6) | 12.080±0.046 | 3.08 | <.01* |
| | OT (n=6) | 14.249±0.526 | | |
| Set II (Monsoon) | C (n=6) | 12.202±0.705 | 2.81 | <.01* |
| | OT (n=6) | 15.077±0.742 | | |
| Set III (Winter) | C (n=6) | 10.132±0.557 | 3.50 | <.001* |
| | OT (n=6) | 14.605±1.152 | | |
| Foot Note: a Student's t-test, *Significant at 5% level, **Significant at 1% level | | | | |

**Figure-1:**Changes in blood Hemoglobin levels (mg/dL) of experimental rats in different sets




## IV. Discussion

In the present study, it was observed that *Centella asiatica* is a rich source of dietary iron and there is a sharp increase in concentration of hemoglobin (Hb) in blood across all the sets of experimental animals treated at different times with *Centella asiatica* paste, as compared to their controlled counter parts. 'Heme' which is an integral part of haemoglobin, is synthesized in a complex series of reactions involving enzymes in mitochondria and cytosol.

The first step of reaction occurs in mitochondria where in succinyl CoA and glycine is condensed by the enzyme ALA synthase (δ-aminolevulinic acid synthase) to form δ-aminolevulinic acid or δ-aminolevulinate. This molecule is transported to the cytosol where a series of reactions producing a ring structure called coproporphyrinogen III. After this, the molecule returns to the mitochondrion where an additional reaction produces protoporphyrin IX. [9] The enzyme ferrochelatase inserts iron into the ring structure of protoporphyrin IX to produce heme. Heme binds as a co-repressor to an apo-repressor protein to form a holo-repressor which represses the transcription of ALA synthase gene. A fall in cellular heme concentration prevents the holo-repressor formation and consequently depresses the ALA synthase gene, enhancing the synthesis of enzyme. [10] Lead is a known toxic substance which can lead to aneamia. Saxena *et al* [11] established in their study that ALA synthase was increased in lead poisoning rats as a result of decrease in aminolevulic acid in blood and in brain.From the pathway of heme bio-synthesis, we know that amino-levulinic acid is a precursor of hemoglobin. Researchers suggested that after administration of *Centella asiatica* (200mg/kg B.W) on lead poisoned rats, there is a decrease in amino-levulinic acid synthase enzyme and increase in amino-levulinic acid production.This in turn increases heme synthesis in experimental animals.

Another research supported the above fact that treatment with aqueous extract of *Centella asiatica* provides significant protection against delta-amino-levulinic acid dehydratase (ALAD) activity. [12]Amino-levulinate dehydratase synthesizes porphobilinogen through the asymmetric condensation of two molecules of amino-levulinic acid. Then porphobilinogen is converted to uroporphyrinogen and then coproporphyrinogen I in the pathway of heme biosynthesis. *Centella asiatica* also provides significant recovery in the inhibited liver ALAD(delta-aminolevulinic acid dehydratase) activity. [13] In addition to these, various other researches also indicate that oxidative stress is increased in iron deficiency anaemia[14] and this herb has the capacity to reduce that oxidative stress burden. [15,16] In fact, the flavonoid quercetin and rutin which are plenty in *Centella asiatica*[17] has a capacity to reduce oxidative stress[18] as well as improves haemoglobin concentration of blood.

From the observations made in different research works and also from the analysis of minerals of the leaves (Table I) it may be concluded that *Centella asiatica* has a good amount of iron. It is also a very good source of vitamin C which is necessary for iron absorption from the gut. [19]All these factors together have a significant positive influence on the pathway of haemoglobin synthesis and thus helps in the formation of haemoglobin. More large-scale studies are required to understand its effects on human subjects.

## V. Conclusion

The current study highlights that *Centella asiatica* or *Thankuni* can significantly increase serum hemoglobin levels of experimental animals. It is also a good source of dietary iron. Thus, it maybe used as a preventive as well as therapeutic herb in anemia. This also supports the traditional knowledge regarding this herb.

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