

COMPARATIVE BIOAVAILABILITY STUDIES OF CALCIUM FROM DIFFERENT SOURCES

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ABSTRACT

Calcium deficiency due to inadequate intake of calcium or its poor intestinal absorption is one of several causes of osteoporosis and other skeletal diseases. Studies suggest that increased intake of calcium may promote bone health. Consumption of conventional calcium supplements has been associated with a few disadvantages such as constipation, bloating, gas and flatulence in few patients. Calcium from plant source, being balanced naturally with other minerals, may be a more suitable option to fulfill calcium needs. A study was undertaken to compare the bioavailability of calcium from plant origin *Eleusine coracana* and that from conventional calcium supplements. A suitable formulation was developed from the extract of *E. coracana*. The absorption of calcium from the extract, the formulation and a conventional calcium tablet was compared using *in vivo* evaluation by measuring serum calcium levels before and post treatment. Results from our study show an improved calcium uptake from the plant extract and formulation, suggesting the use of such supplements for the prevention of osteoporosis.

Keywords: Calcium uptake, *Eleusine coracana*, Plant calcium, Bioavailability

INTRODUCTION

Calcium is important for promotion of bone health ¹⁻³. Poor eating habits, combined with sedentary lifestyle and low exposure to daylight has led to calcium deficiency becoming very common. When supplements containing only calcium are consumed, bone is compromised of other minerals; minerals that are necessary for calcium absorption and proper delivery such as magnesium, zinc, boron and others ⁴. Also, few patients experience constipation, bloating, gas and flatulence on consuming conventional calcium supplements ⁵. Plant derived calcium (organic calcium) which is often balanced naturally with other minerals, is a good way to meet most calcium needs. This is why it is important to focus on rich organic whole foods for calcium balanced with other minerals. Vegetables are an important source of calcium and they may also provide additionally, vitamins and minerals, which exert additional beneficial effects on the bone. Calcium reduces the risk of osteoporosis and increases bone mineral density in postmenopausal women. It has been proven that a high dietary intake of naturally available calcium, especially plant calcium will be beneficial towards prevention of osteoporosis ⁶⁻⁷.

E. coracana is an important millet rich in calcium and iron. The objective of this study was to compare the bioavailability of calcium from plant source and conventional calcium supplements available in the market.

MATERIALS AND METHODS

Plant material

Eleusine coracana seeds were collected from local market and authenticated from Agharkar Research Institute, Pune. The seeds were washed and dried under the sun. The dried seeds were powdered in a mixer grinder and sieved through a 40# sieve.

Extraction

The powdered plant sample (2g) was extracted using 0.1M citric acid solution (100ml) for 10 minutes under ultrasonication ⁸. The solution was filtered under vacuum and solvent was evaporated. The dry residue obtained was used for phytochemical screening of organic and inorganic constituents. Calcium in the extract was estimated using Atomic emission spectroscopy.

Formulation

The extract was further used for development of a product in the form of ready-to-drink granules. Granules were prepared using the following formula:

Name of ingredient	Quantity (w/w)
<i>E. coracana</i> extract	86%
Lactose	10%
Sucrose	4%
Starch paste (10% w/w)	q.s.

Animals

Albino wistar rats (120-150 g) of either sex were used for the study. They were housed under standard conditions of light (12 hr light and 12 hr dark cycle) and temperature (22 ± 1 °C) and fed with water and standard diet *ad libitum*. Experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee and care of laboratory animals was taken as per CPCSEA guidelines (Reg. No. 25/1999/CPCSEA).

Bioactivity study

The rats were divided in four groups of six each: group I (Control group); group II (Extract group) was treated with *E. coracana* (1098 mg/kg p.o.); group III (Standard group) was treated with standard calcium tablet (281.1 mg/kg p.o.) and group IV (Formulation group) was treated with formulation developed from the extract (1290 mg/kg p.o.). Treatment period was for ten days.

Blood was withdrawn on day one and on the last day of treatment from retro-orbital plexus after subjecting animals to light anesthesia using ether. Serum from blood was separated by centrifuging at 10000 RPM, -20°C for 30 minutes. Serum calcium levels were estimated using a modified colorimetric method that uses *o*-Cresolphthalein complexone as the indicator ⁹.

Statistical analysis

Results were statistically analyzed using One-way ANOVA followed by Tukey-Kramer post test. At 95% confidence interval, *P* values less than or equal to 0.05 were considered significant.

RESULTS

The Phytochemical screening of *Eleusine coracana* extract revealed presence of starch, tannins, saponins, and calcium and magnesium.

The evaluation of serum showed an increase in calcium levels post treatment (Table 1).

Table 1: Serum calcium levels of before and post treatment.

Treatment group	Serum calcium level (mg %)	
	Day 1	Day 10
Control group	8.550	9.033
Extract group	8.317	11.35
Standard group	8.967	10.72
Formulation group	8.383	11.37

All values are expressed in mean.

There was no significant increase in the serum calcium levels of Control group. In the extract group, there was a significant enhancement in serum calcium levels when compared with control and standard group. Similarly, the serum calcium levels of Formulation group were improved significantly when compared with control and standard group. Standard group showed a significant rise in serum calcium levels when compared with control group only. Extract group and formulation group showed no significant elevation in serum calcium levels when compared to each other (Table 2).

Table 2: Increase and the percent increase in the serum calcium levels.

Treatment group	Serum calcium level (mg %)	
	Increase	Percent increase
Control group	0.4833 ± 0.07032	5.690 ± 0.8421
Extract group	3.033 ± 0.5340 ^{a,d}	35.94 ± 5.611 ^{a,d}
Standard group	1.750 ± 0.08466 ^b	19.53 ± 0.7701 ^b
Formulation group	2.983 ± 0.2903 ^{c,e}	35.73 ± 3.728 ^{c,e}

All values are expressed as mean ± SEM; N = 6 in each group, One-way ANOVA followed by Tukey-Kramer post test is applied for statistical analysis.

P values:

^a< 0.001 when extract was compared with control

^b< 0.01 when standard was compared with control

^c< 0.001 when formulation was compared with control

^d< 0.001 when extract was compared with standard

^e< 0.001 when formulation was compared with standard

DISCUSSION

For effective absorption, it is an advantage if calcium is in ionized and soluble form¹⁰. The test solution being an aqueous extract had higher solubility in water when compared with standard. The formulation, also, was readily water soluble. Solubility aids in making calcium more quickly available for absorption, latter being the first step towards effective bioavailability. Also, for non-metabolisable supplemental nutrients, bioavailability is effectively equivalent to absorbability¹¹. Hence, the calcium from extract and granules might be better absorbed than the calcium from standard.

It has been reported that some indigestible carbohydrates like resistant starch, increase calcium absorption. The component of dietary fibre which remains undigested until fermentation in the colon is termed resistant starch¹². The effects of such carbohydrates on calcium absorption were studied in rats¹³. It was suggested that the resistant starch increased calcium absorption because it was likely to enhance its solubility. Literature reports that *Eleusine coracana* contains resistant starch¹⁴ which may be contributing to increased absorption.

Additionally, we have used lactose as one of the excipient in formulation. Experimental studies in rats suggest that lactose stimulates calcium absorption. Lactose may aid calcium absorption in vivo, by increasing the passive, vitamin D-independent absorption of calcium in the ileum¹⁰.

Thus, the increased bioavailability of calcium from plant source of *Eleusine coracana* can be justified with above mentioned facts. From the results it can be inferred that this product could be effective in osteoporosis prevention. Further long-term clinical studies need to be carried out in this direction to support our hypotheses.

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