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Pharmacological Study

Bioavailability study of calcium sandoz-250 by atomic absorption spectroscopy in albino rats

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Abstract

Background: Calcium sandoz-250 is an Ayurvedic calcium supplement, containing *Khatika Churna*. Bioavailability study of the formulation is essential for estimation of peak plasma concentration (C_{max}), time to C_{max} and rate of absorption. **Aim:** To evaluate the absorption parameters of calcium sandoz-250 in albino rats by atomic absorption spectroscopic (AAS) method. **Materials and Methods:** Study was carried out as a single dose, open-label, randomized study. Estimation of calcium was carried out by AAS, after validating the method for a few parameters for the estimation. Pharmacokinetic parameters such as C_{max}, time to peak concentration (T_{max}), area under the plasma concentration - time curve were calculated for calcium on administration of calcium sandoz-250. **Results:** Linearity curve was plotted for 0.5-2.5 ppm, given R^2 value 0.9975. The C_{max}, i.e. C_{max} after administration of calcium sandoz-250 was 0.793 µg/ml at 90 min (T_{max}). Measurable calcium-blood levels were noticed in all subjects up to 3.0 h after administration of calcium sandoz-250. **Conclusion:** Calcium sandoz-250, consisting of *Khatika Churna*, increases the blood calcium level in albino rats.

Key words: Ayurvedic formulation, bioavailability study, calcium sandoz-250

Introduction

Calcium (Ca) is one of the most important extracellular cation.[1] Approximately, 99% of the body's Ca is stored in the bones and teeth.[2] Apart from supporting the skeletal integrity, Ca plays an important role in blood clots, muscular contractility and nervous excitability.[3] Although serum Ca levels can be maintained in the normal range by bone resorption, dietary intake is the only source by which the body can replenish stores of Ca in bone. Ca is absorbed almost exclusively within the duodenum, jejunum and ileum. Each of these intestinal segments has a high absorptive capacity for Ca, with their relative Ca absorption being dependent on the length of each respective intestinal segment and the transit time of the food bolus. The calcium intake is critical during the 1st year of life for the anatomical structure.[4] Prospective studies demonstrated that the biggest increase of the bone mass occurs principally during the age of puberty.[5] An inadequate calcium intake during the growth period may cause a failure to reach the increase in bone mass. This situation produces different bone and illnesses such as osteopenia, osteoporosis, decreased skeletal integrity, increasing the fracture risk in later life.[6] Some of the researchers have reported that the increase in calcium intake attenuates the bone mass loss during the age of menopause.[7-9] It appears that all the calcium in food or other source is available for absorption, but the absolute amount is determined by physiological factors such as efficiency of absorption, calcium need, vitamin D, age and hormonal status, etc.[10,11]

Calcium sandoz is a popular brand of calcium supplement, used widely throughout the world; mainly by children and women. Calcium sandoz contains calcium carbonate as the main ingredient.[12] However, calcium sandoz-250 contains *Khatika Churna*, as it is an ayurvedic preparation and it is claimed to have 250 mg calcium equivalent in each chewable tablet.[13,14] Bioavailability of calcium from calcium sandoz-250 was studied in animal model as a preclinical trial. There have been no studies in humans about the bioavailability of calcium from calcium sandoz-250. As calcium sandoz-250 is an Ayurvedic formulation, the absorption might shows variations from the data of the allopathic formulation of calcium supplements or food.[15-17]

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Materials and Methods

Animals

Adult male wistar albino rats (n = 12) weighing 120-150 g were procured from central animal facility and housed in cages under standard laboratory conditions (10 h dark/14 h light, temperature 20-25°C, relative humidity 65%) for 7 days.

Reagents and chemicals

Ultra-pure water, metal standard solutions of calcium (sigma Aldrich) were prepared by appropriate dilutions of 1000 mg/L stock solutions.

Instrumentation

Shimadzu model AA 6300 flame atomic absorption spectrometer (AAS) (Tokyo–Japan) equipped with a deuterium background corrector and a hydride vapor generator for analysis of arsenic. Hollow cathode lamps of specific metals were used as a radiation source.

Study design

The study was randomized single dose study, included two equal groups of animals (n = 6 in each). The protocol was duly approved by the Institutional animal ethics committee (No. JSSCP/IAEC/M.PHARM/PHARM.ANALYSIS/05/2009-2010).

Drug assignment

Two equal groups were made from all albino rats. First group received calcium sandoz-250 (dose was calculated according to the body weight of the animal) and the second group kept as control.

Single dose of calcium sandoz was given to each of the six rats after an overnight fasting. Blood sample was collected from the tail vein of rats after 0.0, 0.15, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0 and 36.0 hrs of drug administration. The amount of calcium sandoz was measured by a suitable flame AAS method.

Preparation of standard calcium solution

Standard calcium solutions were prepared from 1000 mg/L stock solution. A serial dilution containing 0.5, 1.0, 1.5, 2.0 and 2.5 ppm of calcium were prepared and used as working standards.

These solutions were then subjected to analysis by flame AAS under the following instrumental conditions:

Instrument parameters	Calcium
Optical parameters	
Element	Ca
Lamp current (mA)	10
Wavelength (nm)	422.7
Slit width (nm)	0.7
Lamp mode	BGC-D_2
Atomizer/gas flow rate setup	
Fuel gas flow rate (L/min)	2.0
Support gas flow rate (L/min)	15.0
Flame type	Air-C_2H_2
Burner height (mm)	7

The absorbances of the standard solutions were recorded and the calibration curve was plotted by taking concentration in X-axis and absorbance in Y-axis.

Estimation of calcium in plasma samples

Optimization of the extraction procedure

For the estimation of calcium in plasma, animal plasma was spiked with standard calcium solution. To precipitate the plasma protein as they may coagulate on heating, the following methods were tried:

- Precipitation of plasma proteins with perchloric acid (10%)
- Precipitation of plasma proteins with trichloroacetic acid (10% and 20%)
- Precipitation of plasma proteins with nitric acid
- Dilution of the plasma samples 50 fold with deionized water.

Methods

A total volume of 0.2 ml plasma was taken, to this appropriate amount of standard calcium solution (2.0 ppm) and 0.2 ml of precipitating agents (perchloric acid, trichloroacetic acid and nitric acid) were added. The volume was made to 1 ml with deionized water. The resulting solutions were centrifuged at 4000 rpm for 5 min and supernatant was taken and diluted to 10 ml with deionized water.

However in all cases recovery were found to be less than 50%, so finally direct estimation was done by diluting the plasma by 50 fold using deionized water. The resulting samples on analysis gave good recovery (above 95%).

Calibration curve was plotted for the concentration range of 0.5 ppm to 2.5 ppm in plasma and the method was validated for following parameters

- Linearity
- Range
- Precision
- Accuracy.

The samples obtained from the animal study were analyzed by the same method.

Results and Discussion

Linearity curve was plotted for the solutions of calcium solutions ranging from 0.5 µg/ml to 2.5 µg/ml [Table 1], giving R^2 value 0.9975 and slope 0.0163 [Figure 1].

Extraction method was optimized for extraction of calcium from the plasma. Recovery with protein precipitation was less than 50% for almost all precipitating agent, while direct determination of calcium with 50 fold dilution gave

Table 1: Linearity range of calcium standard solution by AAS

Concentration of calcium solution (µg/ml)	Absorbance
0.5	0.0078
1.0	0.0170
1.5	0.0260
2.0	0.0330
2.5	0.0400

AAS: Atomic absorption spectroscopy

98.5% recovery [Table 2]. Recovery was studied for all the concentration of the calibration curve giving the result between 94.28% and 107.14% [Table 3].

Calibration curve was plotted for the plasma samples spiked with the known amount of calcium, with R^2 value 0.995, slope 0.014 and intercept 0.001 [Table 4, Figure 2].

Plasma samples collected from the animals at different intervals were analyzed for the calcium content after blank was introduced, detectable amount measured after 15 min of administration. Pharmacokinetic parameters such as peak plasma concentration (C_{max}), Time to peak concentration (T_{max}), Area under the plasma concentration-time curve (AUC_{0-t} and $AUC_{0-\infty}$) were calculated for calcium on administration of calcium sandoz-250 [Table 5].

Figure 3 shows bioavailability curve of the calcium after oral administration of calcium sandoz-250. A summary of different pharmacokinetic parameters was depicted in Table 6.

Pharmacokinetics data of calcium sandoz-250 obtained from bioavailability study

- Pharmacokinetic parameters such as C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were calculated for calcium on administration of calcium sandoz-250
- Measurable calcium-blood levels were noticed in all subjects up to 3.0 h after administration of calcium sandoz-250
- The C_{max}, i.e. C_{max} after administration of calcium sandoz-250 was 0.793 μg/ml at 90 min (T_{max})
- AUC_{0-t} and $AUC_{0-\infty}$ were found to be 79.28571 and 80.82454 respectively
- K_{eli} and half-life for the calcium sandoz-250 (Ayurvedic formulation) were found to be 0.030172 and 22.97 min respectively.

Table 2: Optimization of method

Method	% Recovery
Protein precipitation by trichloroacetic acid	48.5
Protein precipitation by perchloroacetic acid	50.4
Protein precipitation by nitric acid	35.6
Direct plasma by 50 fold dilution	98.5

Table 3: Linearity and recovery study

Concentration of calcium (μg/ml)	Absorbance	Concentration of calcium found (μg/ml)	% Recovery
0.5	0.0076	0.471428571	94.28
1.0	0.0160	1.071428571	107.14
1.5	0.0260	1.500000000	100.00
2.0	0.0300	2.071428571	103.57
2.5	0.0340	2.357142857	94.28

Table 4: Calibration curve of calcium in plasma by AAS

Concentration (μg/ml)	Mean absorbance	SD
0.5	0.00775	0.000354
1.0	0.0159	0.000141
1.5	0.02385	0.000495
2.0	0.02995	0.000212
2.5	0.0363	0.001414

AAS: Atomic absorption spectroscopy, SD: Standard deviation

Conclusion

On the basis of the results obtained from the study, conclusion can be drawn that calcium Sandoz-250, consisting of *Khatika Churna*, increases the blood calcium level in albino rats. Hence, further investigation should be carried out to evaluate the effect of calcium sandoz-250 on human blood calcium level.

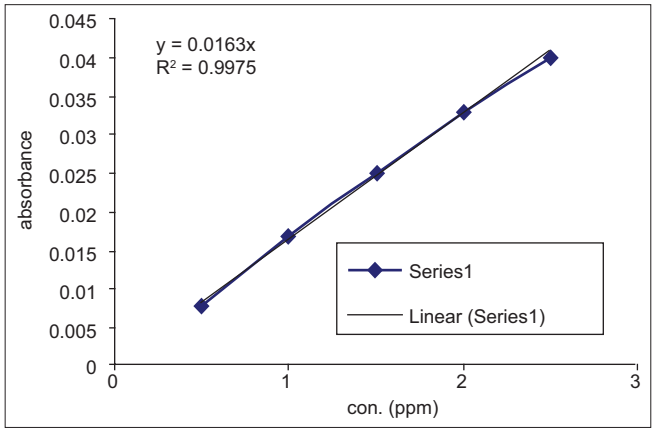

Figure 1: Linearity plot of calcium standard solution by atomic absorption spectroscopic

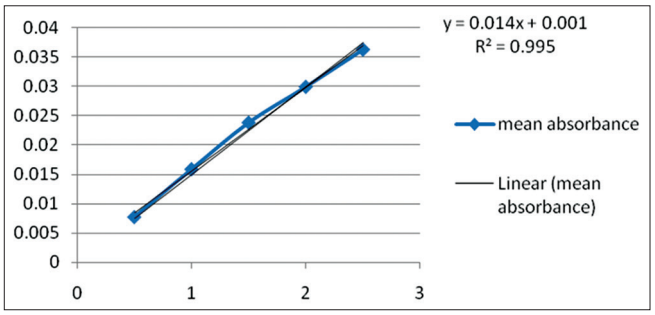

Figure 2: Calibration curve of calcium in atomic absorption spectroscopic

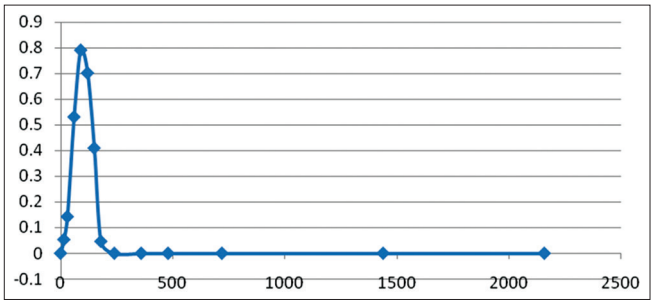

Figure 3: Mean plasma concentration of calcium from animals (administered calcium sandoz-250)

Table 5: Mean plasma concentration of calcium from animal (administered calcium sandoz-250)

Time (min)	Mean absorbance	Mean plasma concentration of calcium from group 3 (µg/ml)
0	0.00000	0.000000
15	0.00175	0.053571
30	0.00300	0.142857
60	0.00845	0.532143
90	0.01210	0.792857
120	0.01085	0.703571
150	0.00675	0.410714
180	0.00165	0.046429
240	0.00030	0.000000
360	0.00000	0.000000
480	0.00000	0.000000
720	0.00000	0.000000
1440	0.00000	0.000000
2160	0.00000	0.000000

Table 6: Pharmacokinetic parameters

Parameters	Calcium
C_{max} (µg/ml)	0.792857
T_{max} (min)	90
Half-life (min)	22.97356
K elimination	0.030172
AUC_{0-t}	79.28571
$AUC_{0-\infty}$	80.82454

AUC: Area under the plasma concentration, T_{max}: Time to peak concentration

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हिन्दी सारांश

परमाणु अवशोषण स्पेक्ट्रोस्कोपी से कैल्शियम सैंडोज २५० की जैविक उपलब्धी पर अध्ययन

अबिमलकुमार एन. पटेल, एन. कृष्णावेणी, निरूद्दीन पी. जिवानी, आकृति एस. खोडकिया, शाश्वत के. परिडा

कैल्शियम सैंडोज २५० यह एक खटीका चूर्णयुक्त कैल्शियम पूरक आयुर्वेदिक औषधि है। पिक प्लाझमा मात्रा (C_{max}), पिक प्लाझमा मात्रा के लिये लगने वाला समय एवं औषधि का अवशोषण दर जानने के लिये औषधि का जैविक उपलब्धी अध्ययन करना जरुरी है। प्रस्तुत अध्ययन चूहों में कैल्शियम सैंडोज २५० नामक औषधि का अवशोषण के मापदंडों को जानने हेतु किया गया। कैल्शियम सैंडोज २५० के एकल खुराक से रक्त मे पिक प्लाझमा मात्रा (C_{max}) ९० मिनट (T_{max}) में ०.७९३ µg/ml पायी गयी। कैल्शियम सैंडोज २५० की खुराक देने से रक्त मे कैल्शियम कि औसतम मात्रा ३ घंटों तक पायी गयी।