Short Communication

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Effect of Frusemide on Serum and Urinary Zinc levels in Rabbits

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ABSTRACT

Introduction: Zinc is an essential trace element playing a vital role in the maintenance of health. Frusemide, a loop diuretic, is known to deplete sodium, potassium, magnesium and calcium levels. But, very few reports exist on its effect on Zinc levels. Hence, this study was performed on rabbits to investigate the same in detail. **Material & Methods:** Healthy adult New Zealand White rabbits (n=6 per group) were treated with Normal saline (2 ml kg^{-1}) and Frusemide (5, 10 or 15 mg kg^{-1} each) intraperitoneally. Their serum and urinary zinc levels were studied by Atomic Absorption Spectrophotometry. Analysis of serum Zinc levels was also done after dietary Zinc supplementation for 20 days. **Results:** A dose-dependent decrease in serum Zinc levels owing to increased urinary Zinc excretion was observed. Both the urinary volume and urinary Zinc concentration increased dose-dependently. The percent fall in serum Zinc occurred in proportion to the pre-treatment serum values. Administration of Zinc in the diet restored the serum Zinc levels to normal. **Conclusion:** Frusemide may be responsible for Zinc depletion. Supplementation of Zinc may help to prevent the same in patients on prolonged Frusemide therapy.

Key words: Dose-dependent, Frusemide, Frusemide-induced complications, Hypozincaemia, Hyperzincuria, Rabbits.

INTRODUCTION

Zinc, atomic number 30, is a metallic transition element is an important trace element in the body. Nearly 10% of the proteins encoded in the mammalian genome require zinc for their proper structure and function.[1,2] Over 70 zinc metalloenzymes are required for carbohydrate, protein, lipid and nucleic acid metabolism.[3] Other zinc metalloproteins are involved in diverse processes such as cell signalling, gene expression, membrane structure and function, modulation of the redox state of the cell and cellular respiration.[4,5] Mild zinc deficiencies can cause chronic metabolic derangement leading to or exacerbating immune deficiency, gastrointestinal problems, endocrine disorders, neurologic dysfunction, cancer, accelerated aging and degenerative disease.[6]

Loop diuretics are used in numerous conditions such as CHF, HTN, renal diseases and other oedematous conditions. Frusemide causes a decrease in the serum sodium, potassium, calcium and magnesium levels and an increase in serum uric acid and glucose levels.[7] But, there are sparse and inconclusive reports on its effect on serum Zinc levels.[8,9] Thus, we have investigated the effect of Frusemide on serum and urine Zinc levels in rabbits.

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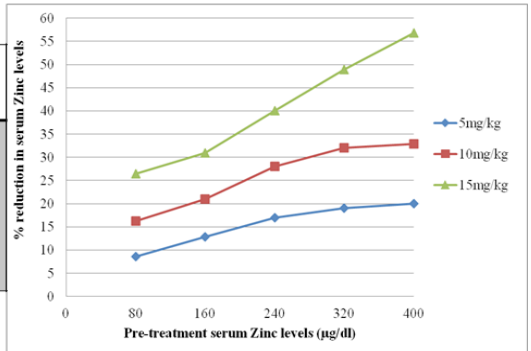
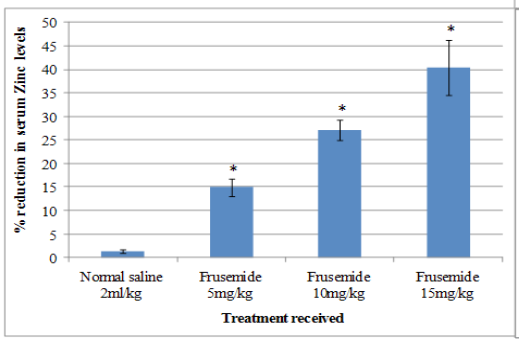
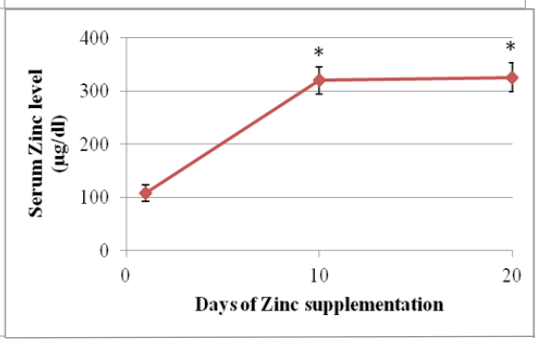
TABLE 1: Effect of Frusemide on urinary Zinc excretion in rabbits

Group (n=6)	NS (2ml kg^{-1})	Frusemide (5mg kg^{-1})	Frusemide (10mg kg^{-1})	Frusemide (15mg kg^{-1})
Urine volume (ml)	24.2 ± 1.3	64.0 ± 6.4^{c}	68.0 ± 5.5^{c}	82.0 ± 10.3^{c}
Zinc conc. (µg dl^{-1})	32 ± 0.4	49 ± 3.3^{a}	57 ± 4.5^{c}	58 ± 5.3^{b}
Total Zinc (µg)	7.5 ± 2.1	26.5 ± 3.2^{b}	39.0 ± 5.1^{c}	48.1 ± 9.2^{c}

Data is expressed as Mean ± S.E.M. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$.







Graphical Abstract

MATERIALS AND METHODS

Experimental animals

The study was approved by the Institutional Animal Ethics Committee. Healthy, adult, male New Zealand White rabbits (1.5-2 kg) were obtained from the Institutional Central Animal House and housed individually in wire bottomed cages, maintained under standard conditions (Temperature=27 ± 2^{0}C, Humidity=30-70%, 12 hr light-dark cycle). Standard laboratory pellet diet & water ad libitum was provided and were acclimatized to laboratory conditions for 7 days prior to experimentation. All animal experiments were conducted in accordance with the CPCSEA Guidelines, 2003.[10]

Experimental design

The animals were randomly divided into 4 groups of 6 animals each. They were administered single intraperitoneal injection of N. saline (control) and Frusemide (5, 10 & 15 mg/kg) in groups 2, 3 and 4 respectively on 1st, 10th and 20th day. Diet was supplemented with Zinc sulphate (Elemental Zinc = 6mg/rabbit) for 20 days.

SAMPLES

Blood samples from marginal ear vein of each rabbit before and 4 hours after drug administration on 1st, 10th and 20th day were obtained and centrifuged (4000 rpm for 15 min). The serum obtained was stored in vials at -4^{0}C till further usage. The urine was also collected from rabbits placed in metabolic cages till 4 hours after drug administration on the same days, filtered and stored at -4^{0}C till further usage.

ATOMIC ABSORPTION SPECTROPHOTOMETRY

Serum and urine samples were analysed for Zinc levels on an Atomic Absorption Spectrophotometer (4139-ECIL) and values were obtained in triplicate.

STATISTICAL ANALYSIS

Data is expressed as Mean ± S.E.M. and analyzed using Paired Sample t-test and one-way ANOVA with post hoc Dunnett's test where required. P values <0.05 were considered significant.

RESULTS

EFFECT OF FRUSEMIDE ON SERUM ZINC LEVELS

As shown in Figure 1, the control group showed insignificant change in serum Zinc levels with a minute reduction by 1.25%, whereas, the test groups (2,3 and 4) showed significant ($p<0.001$) and dose-dependent reduction in serum Zinc levels by 14.9%, 27.1% and 40.4% after 5, 10 and 15 mg/kg Frusemide injection respectively when compared to their pre-test values.

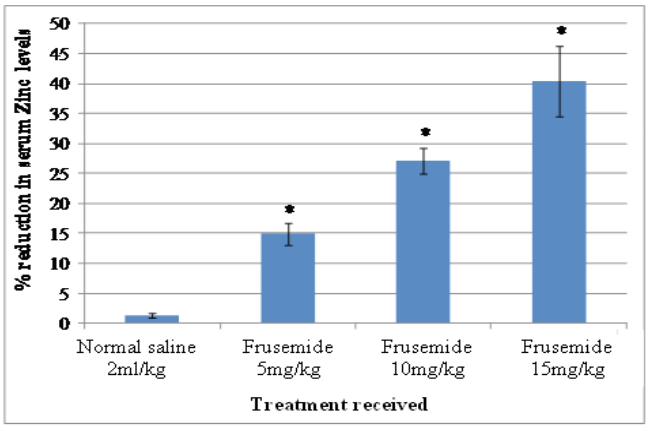


Figure 1: % reduction in Serum Zinc levels following frusemide injection (i.p.)

The figure represents % reduction in serum Zinc levels following Frusemide injection (5, 10 and 15 mg kg^{-1} i.p.). Data presented: Mean ± S.E.M. *p<0.001. Normal saline = 1.25 ± 0.4%, Frusemide 5 mg kg^{-1} = 14.9 ± 1.8%, Frusemide 10 mg kg^{-1} = 27.1 ± 2.1%, Frusemide 15 mg kg^{-1} = 40.4 ± 5.9%.

Table 1: Effect of Frusemide on urinary Zinc excretion in rabbits

Group (n=6)	NS 2 ml kg^{-1}	Frusemide 5 mg kg^{-1}	Frusemide 10 mg kg^{-1}	Frusemide 15 mg kg^{-1}
Urine volume (ml)	24.2±1.3	64.0±6.4[c]	68.0±5.5[c]	82.0±10.3[c]
Zinc conc (μg dl^{-1})	32±0.4	49±3.3[a]	57±4.5[c]	58±5.3[b]
Total Zinc (μg)	7.5±2.1	26.5±3.2[b]	39.0±5.1[c]	48.1±9.2[c]

Data is expressed as Mean ± S.E.M. [a]p<0.05, [b]p<0.01, [c]p<0.001

It was also observed that the reduction by Frusemide injection in serum Zinc levels was dependent on the pre-treatment values. With lower serum Zinc values on Day 1, the reduction was less which increased on 10th and 20th day following Zinc supplementation with higher initial Zinc levels. The change had a direct proportion as shown in Figure 2.

EFFECT OF FRUSEMIDE ON URINARY ZINC EXCRETION

As shown in Table 1, the mean urine volume in control group was 24.2 ± 1.3 ml. Frusemide injection caused a significant (p<0.001) and dose-dependent increase in urine volume (64.0 ± 6.4, 68.0 ± 5.5, 82.0 ± 10.3 ml) with 5, 10 and 15 mg/kg doses respectively when compared to the control groups. Urinary Zinc concentration in control was 32 ± 0.4 μg/dl which increased to 49 ± 3.3 (p<0.05), 57 ± 4.5 (p<0.001) and 58 ± 5.3 (p<0.001) μg/dl in 5, 10 and 15 mg/kg Frusemide treated rabbits respectively. The changes were statistically significant. Total amount of urinary Zinc was also increased significantly following Frusemide injection; 26.5 ± 3.2 (p<0.01), 39.0 ± 5.1 (p<0.001) and

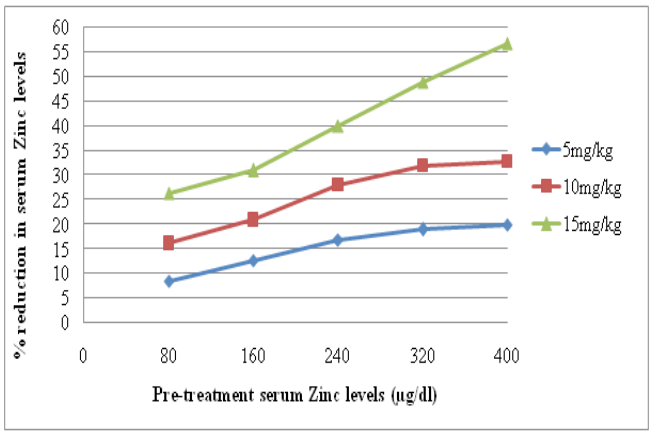


Figure 2: Effect of 5, 10 and 15 mg kg^{-1} Frusemide on Serum Zinc levels following Zinc supplementation in diet

Each curve represents the distribution of % reduction in 18 pre-treatment Serum Zinc levels of 6 rabbits obtained on 1st, 10th and 20th day of Zinc supplementation.

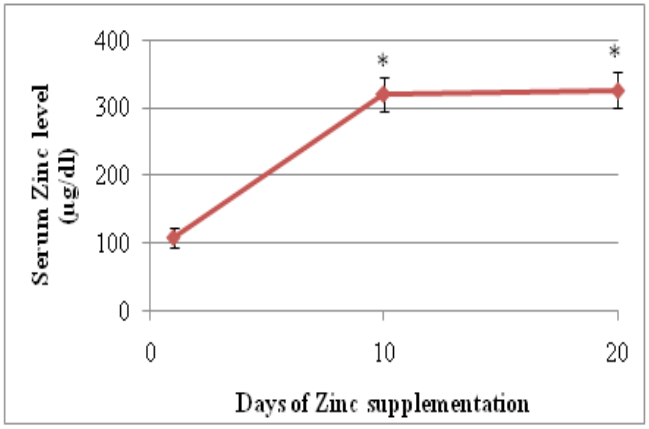


Figure 3: Change in serum zinc levels (μg dl^{-1}) following zinc supplementation in diet

The curve represents rise in Serum Zinc levels (μg dl^{-1}) following zinc supplementation in diet as measured on days 1, 10 and 20 in 24 rabbits. *p<0.01. Day 0 = 108.2 ± 15.7 μg dl^{-1}, Day 10 = 320.2 ± 25.4 μg dl^{-1}, Day 20 = 326.0 ± 26.7 μg dl^{-1}.

48.1 ± 9.2 (p<0.001) μg with 5, 10 and 15 mg/kg doses respectively when compared to control (7.5 ± 2.1 μg).

CHANGE IN SERUM ZINC LEVELS (μg/dl) FOLLOWING ZINC SUPPLEMENTATION IN DIET

As shown in Figure 3, the normal mean serum Zinc levels in pre-treated rabbits (n=24) ranged from 89 to 159 μg/dl (Mean ± S.E.M., 108.2 ± 65.7 μg/dl) on the 1st day of study. Zinc supplementation in diet caused a gradual rise in serum Zinc levels which reached 268-396 (Mean ± S.E.M., 320.2 ± 25.4) μg/dl on 10th day; the increase 196% (p<0.01) was significant as compared to 1st day values. Supplementation for next 10 days further increased the Zinc levels slightly in all the rabbits on 20th day, ranging from 271 to 420 μg/dl (326.0 ± 26.7 μg/dl, Mean ± S.E.M.). the increase (202%, p<0.01) was statistically similar to the 10th day.

DISCUSSION

This is the first study to be dealing with the relationship between Frusemide and Zinc in such detail. Although Zinc is excreted mainly by the gastrointestinal route,[11] it is obvious from the study that Frusemide leads to a highly significant and dose-dependent fall in serum Zinc levels through urinary excretion. The significant rise in urinary excretion of Zinc is inclusive of both the total Zinc content and the urinary Zinc concentration. The regulation of urinary zinc losses is not well understood. Possibly, the shifts in urinary zinc excretion are mediated by adjustments in renal tubular zinc transport.[12] Experiments with cysteine infusion in anesthetized dogs have demonstrated that the nephron is capable of both proximal secretion and distal reabsorption of Zn.[13] Thus, Frusemide might be causing an increase in the proximal tubular secretion of Zinc. The fall in serum Zinc levels is more with initially high serum Zinc levels as compared to initially low serum Zinc levels. Hence, the body tries its best to maintain a constant level of serum Zinc by adjusting the percentage of urinary excretion according to the pre-treatment values.

There are some reports of Zinc toxicity due to chronic exposure in metal industries,[14] excessive supplementation and food poisoning due to storage of food items in galvanised containers.[15-17] Hence, Frusemide may be used as a therapeutic agent in hyperzincaemia & zinc poisoning.

It is an established fact that loop diuretics can cause hyperglycemia and hyperuricemia.[7] Since, the role of Zinc in insulin synthesis and secretion has been established over the years,[18- 21] it is possible that hypozincaemia caused by chronic Frusemide administration may lead to hyperglycemia in normal individuals and deteriorate the condition further in diabetics.

Both Zinc and Copper must be present in order to minimize oxidative damage in arthritis and in proper functioning of enzymes like Xanthine oxidase and Superoxide dismutase.[22] These enzymes play the prime role in gout. Thus, zinc deficiency might lead to precipitation of an attack of gout and might possibly be one of the mechanisms behind frusemide-induced hyperuricemia. Apart from this, Zinc depletion causing decreased testosterone production may lead to infertility[23,24] and increased inflammatory response.[25] And, as shown here, administration of Zinc in diet restored the normal serum Zinc levels indicating that supplementation of Zinc with Frusemide might help to prevent these complications.

With respect to the importance of zinc as an essential element and frequency of diuretic treatment, the observed increased urinary losses of zinc deserve further attention. Moreover, it would be advisable to supplement Zinc along with Frusemide therapy to prevent/reduce the latter's adverse effects.

CONCLUSION

Frusemide may be responsible for Zinc depletion. Supplementation of Zinc may help to prevent the same in patients on prolonged Frusemide therapy.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

SOURCE OF SUPPORTS

R.A.K. designed the study, analyzed the data and supervised the study. S.P.B. administered the experiment, collected output data and wrote the manuscript.

Author Profile

- Dr. Sangeeta P. Bhat is a final year MD Pharmacology resident at Jawaharlal Nehru Medical College, Aligarh whose main objectives are to cultivate good research skills and gain sound knowledge in the field of drug development and to pursue a career/PhD in the development of targeted drugs from medicinal plants.

- Dr. Rahat Ali Khan is presently serving as Professor Department of Pharmacology J. N. Medical College Aligarh Muslim University Aligarh India. he is involved in teaching and research for more than 3 decades. Thrust areas of his research are Neuropharmacology, Clinical Pharmacology, Immunopharmacology, Trace element studies, Autacoids-Histamine and Indigenous drugs.

Highlights of Paper

- Zinc is an essential trace element playing a vital role in the maintenance of health.
- Frusemide administration causes a dose-dependent decrease in serum Zinc levels.
- The decrease in serum Zinc is due to the increased urinary volume and urinary Zinc concentration dose-dependently.
- The percent fall in serum Zinc occurred in proportion to the pre-treatment serum values.
- Administration of Zinc in the diet restored the serum Zinc levels to normal.

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