EFFECT OF NIMODIPINE ON SERUM CONCENTRATIONS OF ZINC, COPPER AND IRON, POSSIBLE INVOLVEMENT OF DIVALENT TRANSPORTERS

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ABSTRACT

In recent years with the widespread use of medication, the relevance of drug nutrient interactions in daily practice continues to increase.Such interactions can involve one or more nutrients, present in food and nutrition. Drug food interactions can occur mechanistically in the absorption, distribution, metabolism and excretion of intestinsas well as in the interaction of receptors with drugs. Accordingly, in an in vivo rat study, the study assesses the effect of long term use of high and low doses of nimodipine on serum levels (Zn, Cu and Fe).,Twenty four male Sprague Dawly rats were assigned to three groups of 8 rats each, the first group was treated with D.W as a control group, the second group was treated with 20mg / kg nimodipine and the third group was treated with 80mg / kg nimodipine, all groups received oral treatment for 30 days, On day 31 all groups of rats were sacrificed and blood samples were drawn and collected for later analysis of trace elements in the polyethylene tubes. The findings showed that nimodipine treatment cause a significant decrease Concentration of the serum iron and zinc compared with control with lowest serum iron level observed in group received high dose of nimodipine. However, compared with control, both low and high doses of nimodipine had no effect on serum copper concentration. In conclusion using low or high doses of nimodipine lead to significant decrease in serum concentration of iron and zinc but no effect was observed in the serum concentration of copper compared to the control group level.

INTRODUCTION

There is still a range of safe and adequate exposure for each element, within which homeostasis maintain optimal concentration and functions of the tissues . Each metal may be toxic if the normal safe exposure range is exceeded. Different mechanisms in living body maintain the optimal function in the biological system through a wide range of exposures to the environment and diet. Some trace elements

carrier molecules have buffering capacity against excess, but more important is kinetic control of the mechanisms of absorption and excretion (1).

With the wide use of medication, the relevance of drug food interactions has continued to increase. Interactions may include a single nutrient, multiple nutrients, general food, or nutrition status. Drug food interactions can occur mechanistically in the absorption, distribution, metabolism and excretion in intestines, as well as in the interaction of receptors with drugs (2).

There are numerous factors (dietary, drugs and host) can interact and affect mineral absorption in living organism. However, Micronutrient bioavailability can be enhanced by increasing concentration of nutritional substances or by decreasing of anti-nutrients concentration (3). The absorption mechanisms of trace elements, especially iron and zinc, were extensively studied but are not fully understood. The absorption process involves the absorption of nutrients from the intestinal lumen by intestinal mucosal cells, the transfer of the nutrient through the mucosal cell and the transmission from the intestinal cells to other tissues and organs. (4, 5). In fact, Zn, Cu, Fe, Se and Cr are important for growth and function and regulated precisely where excess and deficiency have resulted in various diseases (6).

Copper is an essential micronutrient required for a wide range of physiological functions including antioxidants, angiogenesis and biosynthesis of neurotransmitters (7). There are numerous transporters and variety of carrier proteins participate in regulation of absorption, distribution and excretion of trace elements and interactions on these carriers are predictable, the most important of these carriers are divalent metal transporters(DMT1), first cloned from rodents in 1995(8). The mRNA for divalent metal transporters was found in duodenum, liver and erythroid cells, but can also be easily identified throughout the kidney, brain, thymus, heart, lung and testis. (9). On the expression of divalent metal conveyors mRNA constructs in Xenopus oocytes or HEK293 cell lines suggest that divalent metal conveyors can also function in the pH - dependent transport of metals other than iron, including manganese, nickel and possibly zinc (9). Divalent metal transporter-1 mediates the absorption of dietary iron across the duodenal brush border membrane, It has been established that iron transport by DMT1 is pH dependent and that ferrous iron is the preferred form of the metal for uptake(10), On the other hand there are contradictory reports on the localization of DMT1 in the brain[11].Transporters also play a key role in excretion or reabsorption of trace elements. Recent study have clearly demonstrated that DMT1 can transport iron,zinc and cadmium along the loop of Henle and distal tubule(12).

In the last years, there are remarkable progresses in studying the metal channels specificity and possible involvement of more than one ion in the same channel, Ca- channels play important role in trace elements movement across cell membrane. They also found that nimodipine as calcium specific L-type calcium-gated channel blocker inhibits iron uptake(13). Cardiac L-type voltage-gated calcium channels provide an important means for the entry of iron into cardiac myocytes and suggested that iron overload patients could be treated with this potential therapy (14).

Nimodipine is a calcium channel blocker that is highly effective for treating classical angina and migraine, is well absorbed by oral administration, peak blood levels occur in approximately one hour, with a half-life of 1 to 2 hours and protein binding in excess of 95 %. (15). Nimodipine has been used as a standard treatment for cerebral vasospasm and cerebral ischemia in recent years (16). The current study was planned to measure the long-term effect on serum concentration of trace elements (Zn, Cu and Fe) rats of low and high doses of nimodipine.

METHODS AND MATERIALS

Reagents and Chemicals

Nimodipine tablet(Bayer company germany), Chloroform (BDH Ltd., Poole, England), Diethyl Ether(May and Baker, England), Nitric acid &Perchloric acid(Fluka-Garantie (Poch), Germany).

Preparation of drug solutions

One tablet of Nimodipine (60mg) was dissolved in distilled water to produce a standard solution with a concentration of 2mg/ml from which different doses were prepared according to study design.

Animals and Study Design

24 adult male rats of 200 - 300 g body weight were obtained from the animal house, of College of Pharmacy, University of Basrah, Iraq. The rats were housed on a 12 h light/12 h dark cycle, and received the standard pellet and water *ad libitum*. After one week acclimatization period, the animals were divided into three groups consisting of 8 rats each; first group was treated with D.W as control group, the second group was treated with nimodipine 20mg/kg and the third group was treated with nimodipine 80mg/kg. The drug was prepared as a watery solution dissolved in D.W. and given daily as a single dose orally using gavage tube for 30 days. Then consequently on day 31, all rats were sacrificed after anesthesia with ether; then blood samples were obtained and serum was frozen for later analysis(17).

Evaluation of Zn, Cu, and Fe

Concentrations of trace elements were evaluated by using atomic absorption spectrophotometer Buck Model 211- VGP (VER 3.94 C by Analyst: Gerald J. De Menna), 0.05 ppm for Fe and 0.005 ppm for Zn and Cu detection limit, respectively(18, 19). Fixed flame requirements and stabilized wave lengths for Zn, Cu and Fe, respectively (wavelengths 214, 324, 247 nm). The time of measurement was 3 seconds[20]. Standard solutions aspirated primarily for calibrating the device before the sample aspiration. 6 Standard concentrations for each element were prepared using 1000 ppm supplied by company of Buck, and their absorbance was designed to prepare the calibration curve. Based on the following formula, the concentration of each mineral was calculated (20):

Concentration of the test sample PPM = Dilution factor × Read concentration PPM

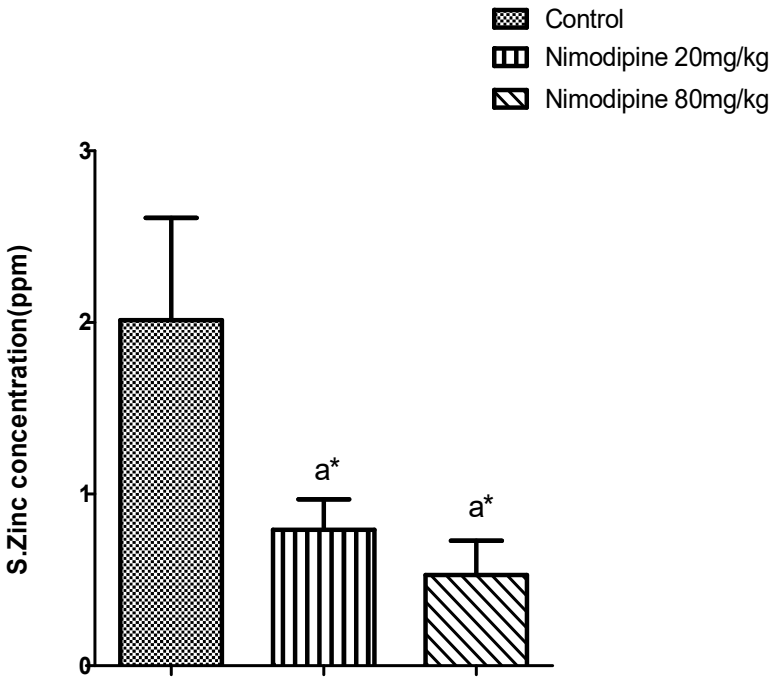
Analysis of statistics

Data were presented as mean ± S.D ; all data were statistically assessed using one way ANOVA variance analysis, supported by Tukey's post-hoc analysis. Significantly different values with $P < 0.05$ were considered. Analysis was carried out using the prism graph pad 6.

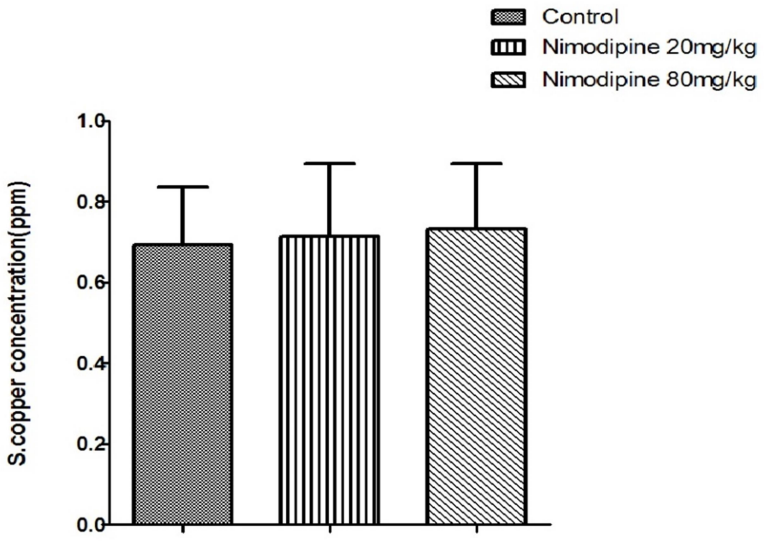
RESULTS

The expected modulatory effects of low and high doses of nimodipine on the trace element serum concentration (ZN, Cu & Fe) were evaluated in the present study ; and quantified in comparison with the control group. In figure(1), long-term administration of both low(20mg/kg) and high(80mg/kg) doses of nimodipine decrease serum zinc concentrations significantly($P<0.05$) as compared with control ; furthermore, no significant differences between groups treated with nimodipine were reported in serum Zn levels. Figure (2) clearly indicate that both low (20mg/kg) and high doses (80mg/kg) of nimodipine did not affect rat serum copper concentration compared with their levels in the control group.

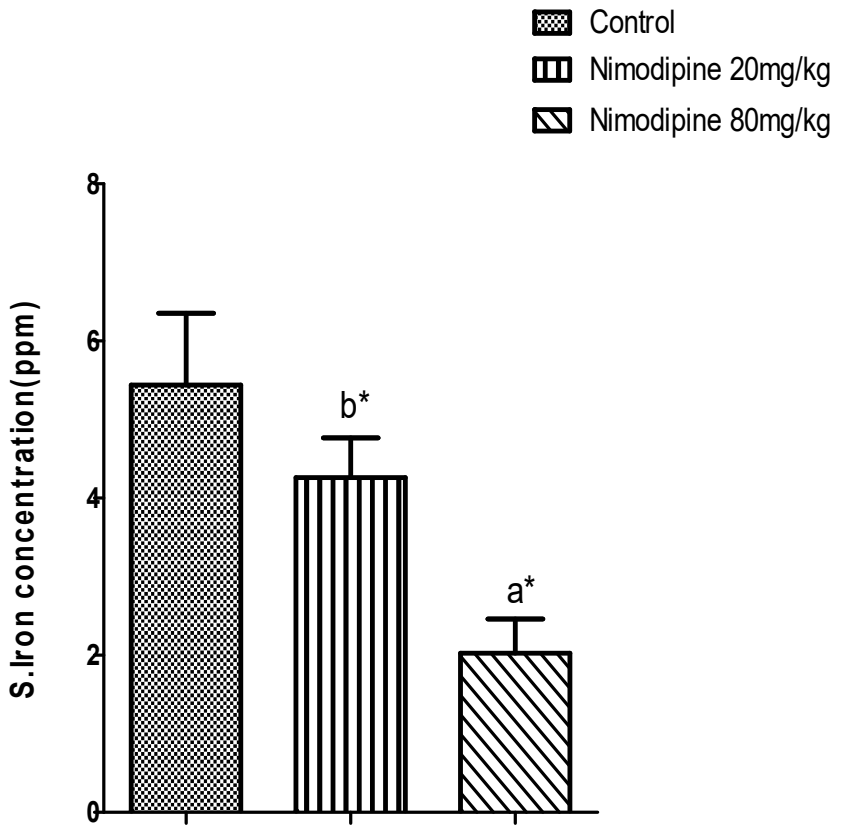
On the other hand, as shown in figure (3) the mean iron serum level in the nimodipine-treated group was significantly($P<0.05$) reduced compared to the control group with the lowest iron level in the high dose nimodipine-treated group. In all tested groups there was no significant correlation between Zn, Cu and Fe as summarized in figures(4,5 and 6).



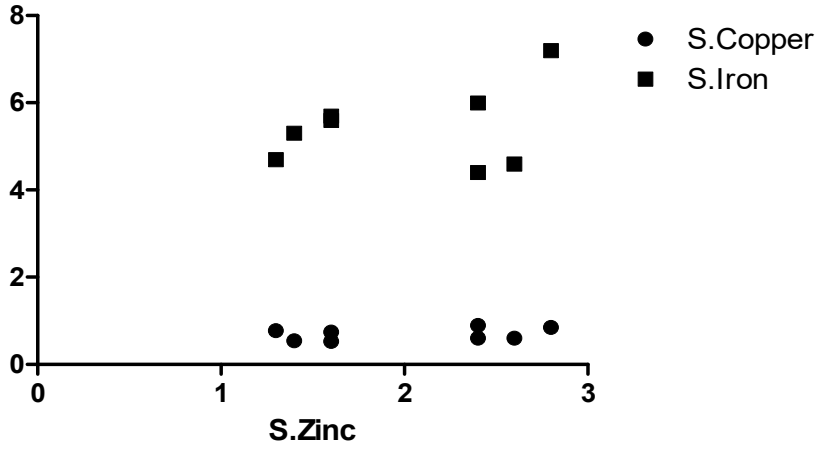
Figure(1): Effects of long-term use of nimodipine on serum zinc level; values are presented as mean±S.D; values with non-identical letters (a,b) represent significant differences among groups. * significantly different compared to control (P<0.05).



Figure(2): Effects of long-term use of low & high dose of nimodipine on serum copper level; values are presented as mean ±S.D; no significant differences among treated groups as compared with control (P>0.05).



Figure(3): Effects of long-term use of nimodipine on serum iron level; values are presented as mean±S.D; values with non-identical letters (a,b) represent significant differences among groups; * significantly different compared to control (P<0.05)



Figure(4): correlation analysis of zinc with iron and copper in the control group. No significant correlation analysis was observed within group.

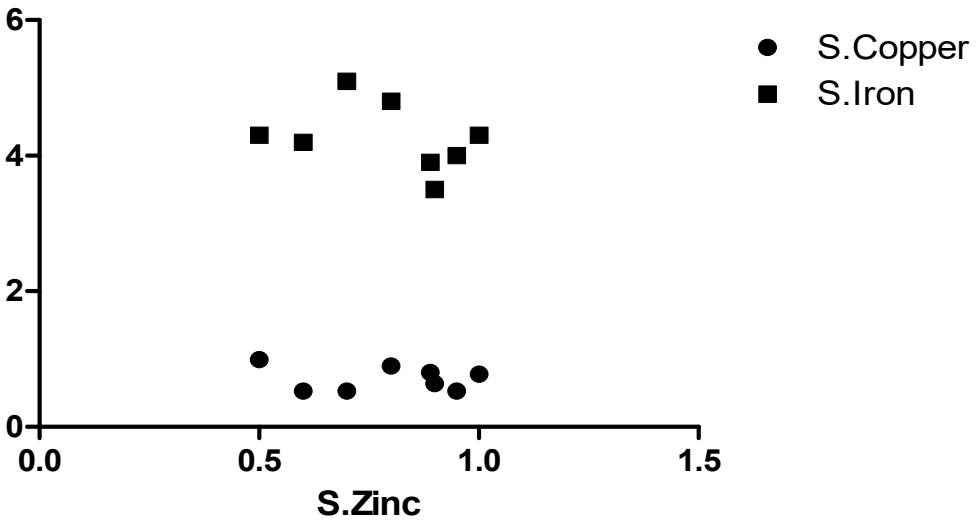
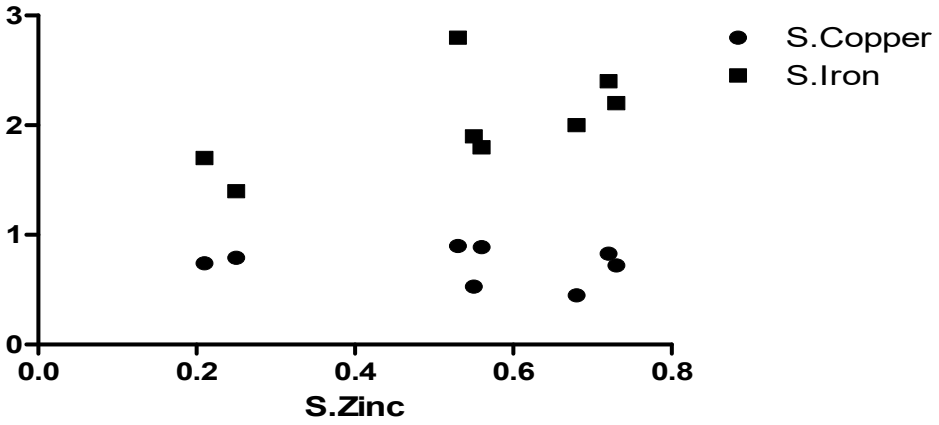


Figure (5): correlation analysis of zinc with iron and copper in the 20mg nimodipine treated group. No significant correlation analysis was observed within group.



Figure(6): correlation analysis of zinc with iron and copper in the 80mg nimodipine treated group. No significant correlation analysis was observed within group.

DISCUSSION

The drug nutrient-interaction field has exploded in recent years with the discovery of many new proteins and transporters that can be involved in drug and mineral absorption, distribution and excretion. On the other side, drugs that are used frequently, particularly for chronic disease treatment, may modulate the function of specific transporters and interact with substrates (2). In this study, 30 days of nimodipine administration significantly decreased Zn and Fe serum levels compared to control group. Interpretation of our finding was somewhat difficult as there were conflicting data on intake of Ca-channel blockers and homeostasis of trace elements.

Mechanistically, nimodipine treatment decreased the serum concentrations of iron can be explained according to the fact that it may enhances urinary iron excretion by interfering with DMT-1 activity, This result matched many of the previously reported data. Iron accumulation in organs was completely resolved after using nifedipine (a medication used to treat high blood press) by increasing iron excretion in the urine about five-fold compared to the solvent-treated control group in mice. The role of DMT-type1 is augmented by Ca2 + dihydropyridine channel blockers (21). They therefore propose a new rationale for treating primary and secondary iron overload based on appropriate mouse models. It has also been developed that cardiac calcium channels have an essential mechanism for the entry of iron into cardiac myocytes and propose that calcium channel blockers can be a potential therapeutic target for iron overload patients, this by inhibiting the entry of iron by DMT(14). The result of this study showed that highest dose of nimodipine(80mg/kg) show maximum reduction of serum iron concentration, this outcome supported by previous research, where revealed that nimodipine, a specific L-type gated calcium channel blocker, suppresses iron uptake (similar to calcium uptake) in a dose-dependent way (13).

Because DMT has been allocated in various organs, as the DMT1 mRNA has also been found in duodenum, heart, erythroid cells, lung, brain, thymus, lungs, liver, and testis, and facilitates the absorption of nutritional iron through the duodenal brush border membrane (9).

So interference with activity of such transporters may affect substrate absorption. It has also been discovered that staining of duodenal tissue by Prussian blue confirmed iron precipitation in the enterocytes of the nifedipine-treated mice group. The effect can be clarified by enhanced DMT1-mediated enterocytic iron absorption combined with non-affected iron distribute to circulation by ferroprotein anticipate results of this kind , so absorption will not increase concomitantly with increase DMT activity , this possibility is consistent with our data of decrease iron levels (21).

Our finding showed a significant($P<0.05$) reduction of serum zinc concentration with use of nimodipine compared with control group, this reduction can be explained in the same manner as in iron level reduction, where many researches demonstrated that both zinc and iron subjected to the same transporter. it obviously shown in the rat that DMT1 can carry iron across the Henle loop and distal, tubule also carries zinc and cadmium, and Ca channel plays a major role in the movement of trace elements across the cell membrane (21).

Distal cadmium permeability was strongly blocked by iron and zinc and may involve divalent transporters(22) Moreover, DMT1 has a strong affinity for zinc (9).

Unlike this idea, Mackenzie B *et al* discovered that Ca-channel blockers such as nifedipine did not influence the transport of iron or manganese in HEK293 cells transfected with human DMT1 isoform 1A/-IRE (23). In the current study, we also assess the effects of long-term nimodipine administration on the serum copper concentration, which is not known as DMT-1 substrate, to allow a more accurate determination of the effect of nimodipine on membrane transporters Compared to the control group, orally doses of nimodipine seemed to have no significant effects on serum copper levels. This finding can be attributed to the fact that copper homeostasis requires numerous chaperones and transporters, all working to preserve intracellular levels of Cu and to defend the cell from this metal's toxicity (24).

There are special copper transporters, the high-affinity of copper importer is functionally and structurally preserved in yeast, fruit flies, plants and humans and is also located in intracellular vesicles in the intestinal plasma membrane. The significance of such a carrier in severe disorders like Menkes and Wilson's Diseases is quite obvious (25, 26).

Therefore, divalent metal transporters perform just a minimal role in the absorption and excretion of copper that is not influenced by the effect of nimodipine on this transporter's activity. This previous report promotes our result that the concentration of serum copper in the group allowed to treat with nimodipine is unaltered compared to control.

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تأثير دواء النيموديبين على تراكيز الزنك والنحاس والحديد في مصل الدم مع احتمالية تورط النواقل ثنائية التكافؤ

رسول جلوب

فرع الصيدلة والسموم ، كلية الصيدلة ، جامعة البصرة ، البصره ،العراق.

الخلاصة

ازدادت في السنوات الأخيرة اهمية التداخلات بين الاغذية والادوية وذلك لانتشار استخدام الادوية بصورة مستمرة. هذه التداخلات يمكن ان تنطوي على المغذيات المنفردة، والمغذيات متعددة، والغذاء بشكل عام. ميكانيكيا، تحدث هذه التداخلات خلال عملية الامتصاص ،التمثيل الغذائي، أو توزيع الدواء داخل الجسم، والتمثيل الغذائي او خلال عملية الطرح وقد يكون التأثير ايجابيا وسلبيا. وفقا لذلك، تم تصميم هذه الدراسة لتقييم تأثير على المدى الطويل على حد سواء الجرعات المنخفضة والعالية من النيموديبين على تركيز مصل الدم من العناصر النزرة (الزنك، النحاس والحديد) في الفئران. أربع وعشرين من الذكور البالغين من فئران سبراغ دولى تم تقسيمها إلى ثلاث مجموعات كل مجموعة تتكون من ٨ فئران ، تم معاملة المجموعة الأولى بالماء المقطر وتم اعتبارها مجموعة السيطرة، تمت معالجة المجموعة الثانية بدواء النيموديبين ٢٠مغ/كغ وتمت معالجة المجموعة الثالثة بدواء النيموديبين ٨٠مغ/كغ ، كل المجاميع تلقت العلاجات عن طريق الفم لمدة ٣٠ يوما، وفي يوم ٣١ تم التضحية بالفئران وجمعت عينات الدم لتحليل العناصر النزرة. أظهرت النتائج أن دواء النيموديبين يقلل بشكل ملحوظ تراكيزكل من الحديد والزنك مقارنة مع مجموعة السيطرة مع أدنى مستوى في تركيزالحديد في الدم لوحظ في المجموعة التي تلقت جرعة عالية من دواء النيموديبين. ومع ذلك، بالمقارنة مع مجموعة السيطرة، لم يلاحظ اي تأثير لكل من الجرعات المنخفضة والعالية من دواء النيموديبين على تركيز النحاس في مصل الدم. في الختام أن الاستخدام طويل الامد للجرع العالية والمنحفضة لدواء النيموديبين يقلل بشكل ملحوظ تراكيز مصل الدم لكل من الحديد والزنك ولم يكن هناك تأثير على تراكييز النحاس مقارنة بمجموعة السيطرة.

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