Turmeric (*Curcuma longa*) Rhizome Extract and Selenium Protect Effects of *Nerium indicum* Leaf Extract on Serum Calcium and Phosphate of Male Wistar Rats

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Abstract: Male Wistar rats were divided into 6 groups (A-F) and treated as -- Group A: Control; Group B: *Nerium indicum* leaves extract (NLE; 25 mg/kg b wt); Group C: *Nerium indicum* leaves extract (NLE; 25 mg/kg b wt) + selenium extract (Se; 0.5 mg/kg b wt); Group D: *Nerium indicum* leaves extract (NLE; 25 mg/kg b wt) + *Curcuma longa* rhizome extract (CRE; 200 mg/kg b wt); Group E: Selenium extract (Se; 0.5 mg/kg b wt); Group F: *Curcuma longa* rhizome extract (CRE; 200 mg/kg b wt). Serum calcium and phosphate levels were analyzed on 15 and 30 days after exposure of above mentioned treatments.

Rats from group B showed a progressive decrease in serum calcium level as compared to group A (control) from day 15 to day 30. In group C and group D the serum calcium levels showed a decrease on day 15 as compared to the control (group A). On day 30, the levels in group C and group D were increased as compared to group B but it is still hypocalcemic as compared to control. This shows that the serum calcium levels were slightly recovered as compared to group B after administration of selenium and *Curcuma longa* rhizome extract after 30 days. There is no alteration in serum calcium levels of group E and F as compared to group A.

Exposure of *Nerium indicum* leaves extract (group B) to rats showed a decrease in serum phosphate level at days 15 and 30 as compared to control (group A). There is increase in serum phosphate levels of group C and group D on day 15 and 30 as compared to group B. This clearly indicated that the phosphate levels which were decreased by exposure to *Nerium indicum* leaves extract recovered after administration of selenium and *Curcuma longa* rhizome extract. No alteration was noticed in serum phosphate levels of group E and group F.

Keywords: *Nerium indicum*, *Curcuma longa*, Selenium, Botanical pesticide, Calcium, Phosphate, Toxicity


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Introduction

Botanical pesticides are plant evolved active chemical which protect them against insects, fungi, bacteria and rodents. In recent years the uses of synthetic pesticides in the agriculture and industrial areas are increasing continuously, which cause ecological disturbance in environment (Prasad et al., 2009). Herbals are safe when uses brilliantly. They are biodegradable, eco-friendly, easily available in nature and considered as good alternative for synthetic pesticides. *Nerium indicum* (Kaner) belong to the family Apocynaceae. These plants are found all over the world mainly India, China, Bangladesh, Nepal, United States, Australia (Sykes et al., 2022; Shridhar, 2022). All parts of plants like stem, leaves, flowers and roots have cardiac glycosides, flavonoids and alkaloids, carbohydrates, tannins, phenolics and steroids. Leaves contain mostly neriiin, oleandrin (Prasad et al., 2009; Al-Snafi, 2020; Shrirao et al., 2023). In south India 150 cases of botanicals poisoning are reported where 65% cases are oleander poisoning alone. Total 4% to 10% death of people reported by Behravan et al. (2017) because of *Nerium indicum* poisoning. India is known as medicinal hub and called as Botanical garden of the world (Totade et al., 2013). Total 65% village population relies on botanicals for their daily lives. Consumption of *Nerium indicum* leaves and seeds shows symptoms of vomiting, Nausea, Diarrhea, abdominal pain and hyperkalemia in rabbit (Hassan and Kadhim, 2020), cow (Ceci et al., 2020), guinea pig (Thomas et al., 2022) and human (Beniwal et al., 2023).

Endocrine disrupting substances are active chemicals which affect endocrine or hormonal function in mammals and animals (Mandava and Mandava, 2021). Endocrine disrupter chemicals may be present in pesticides, water bottles, detergents, toys (phthalates), cosmetics and drugs (parabens) (Monneret, 2017). It is reported that *Nerium oleander* have properties of rodenticide (Bari et al., 2020), piscicides (Tiwari and Singh, 2004) and larvicides (Behravan et al., 2017). Toxicity of these plants can block heart function and nervous system of living organisms because of irregular function of Na/K-ATPase channel (Maarauf et al., 2022). *Nerium* have antiulcer (Patel et al., 2010), anti-inflammatory and antipyretic properties (Kumar and Anand, 2010).

Selenium (Se) is necessary for organisms’ life which is consumed through diet as selenomethionine and selenocystein (organic) or selenite and selenate (inorganic). Its inclusion in selenoproteins such as glutathione peroxidase and thioredoxin reductase is primarily responsible for its physiological effects (Castel et al., 2023). Selenium is required as a necessary component of many enzymes, some of which have antioxidant properties. Deficiency of selenium in animals may harm them causing oxidative stress (Burk, 2002). Being a vital mineral selenium may be considered as a dietary supplement for health benefits. Selenium is necessary for many processes including cellular redox homeostasis, defense, antioxidant, and metabolic cycling (Ozturk Kurt et al., 2023).

Curcumin is a water repellent flavonoids obtained from plant *Curcuma longa* (Turmeric, Haldi) which belong to the family Zingiberaceae (Kumar et al., 2009). In India and Bangladesh it is used as spice in different dishes to increase taste, flavor and color (Hossen et al., 2017). In Ayurveda, Unani and Chinese medicine, turmeric is used as medicines to treat liver disease, gastrointestinal and hypercholesterolemia (Hasan, 2020). It is used as traditional medicines for treatment of wound, skin illness (Khorsandi and Orazizadeh, 2008), inflammation and antioxidants (Al-Eisa et al., 2017).

Calcium is the element found in large amount in human body (Fan et al., 2016). In vertebrates, calcium plays vital role in variety of biological processes – membrane permeability, neuronal excitability, cell adhesion, essential for clotting of blood, muscle contraction, responses of organism to fluctuations in acid-base balance and is also essential for ultimate initiation of many endocrine events, such as hormone release and responses of
their definitive effectors (Srivastav et al., 2000; Veldurthy et al., 2016). It also contributes to the structural integrity of the proteins and the lipids of which the cell is composed. Phosphorus is also required for genetic information (DNA and RNA), membrane structure, enzyme/protein components (phosphohistidine, phosphoserine), intermediary metabolism (phosphorylated intermediates), and phospholipids (Norman and Litwack, 1987).

There is no report regarding the ameliorative effects of Curcuma longa extract and selenium against Nerium indicum induced alteration in serum calcium and phosphate levels. Hence, the present study was designed to investigate the protective effects of Curcuma longa rhizome extract and selenium against Nerium indicum leaves extract induced alterations in serum calcium and phosphorus levels of male Wistar rats.

Materials and Methods

Male Wistar rats (40-50 g) were purchased from Asia Scientific Emporium, Varanasi, India. Prior to treatment, rats were acclimatized for at least two weeks in polypropylene cages. Rats were given standard diet and water ad libitum throughout the acclimatization and treatment. Experimental design was approved by the Research degree Committee, DDU Gorakhpur, University, Gorakhpur, India.

(A) Experimental Design:

Rats were randomly divided into 6 groups- A, B, C, D, E and F and each group contained 20 animals. Following treatments were given orally to each group daily at 8:00 AM throughout the experiment.

Group A [Control]: No treatment was given to these rats.

Group B [Nerium indicum leaves extract (NLE) treated rats]: These rats received daily Nerium indicum leaves extract only (25 mg/kg b wt).

Group C [Nerium indicum leaves extract and Selenium extract (NLE+Se)]: These rats were treated with NLE (25 mg/kg b wt) and selenium extracts (0.5 mg/ kg b wt) simultaneously.

Group D [Nerium indicum leaves extract + Curcuma longa rhizome extract (NLE+ CRE)]: These rats were treated with NLE (25 mg/kg b wt) and Curcuma longa rhizome extract (200 mg/kg b wt) simultaneously.

Group E [Selenium extract (Se)]: These rats were treated only with Selenium extract (0.5 mg/kg b wt) only.

Group F [Curcuma longa rhizome extract (CRE)]: These rats were treated only with Curcuma longa rhizome extract (200 mg/kg b wt).

10 rats from each group were sacrificed 24 h after the last treatment on 15 to 30 days. Rats were fasted overnight and sacrificed under mild ether anesthesia.

(B) Technical Procedures:

Preparation of Nerium indicum leaves extract:

The fresh leaves of Nerium indicum were collected from Gorakhpur, Uttar Pradesh (India). These leaves were washed with tap water and dried under shade at room temperature. Dried leaves were ground in powdered form. These powdered leaves were mixed with 90% ethanol in 1:20 ratio (w/v) and kept on orbital shaker for 48 h and then filtered with the Whatman No. 1 filter paper and kept in rotatory evaporator at 35 °C. The dried materials were kept at -20 °C for future experiment. Soxhlet apparatus was not used in this process because of heat labile compound present in leaves may be denatured. During the treatment, rats received dose of extract mixed with distilled water.

Preparation of extract of Curcuma longa rhizome:

Curcuma longa rhizomes were purchased from the local market of Gorakhpur, India. The rhizomes were washed with fresh water to remove dirt and other particles. Rhizomes were dried at 40 °C in an oven for 24 h and powdered with the help of grinder. The powder was passed through a sieve to get fine particles free from
impurities. Turmeric powder mixed with 90% ethanol (1:20 w/v) and kept for 48 h with occasional stirring, then filtered with Whatman No.1 paper, supernatant was collected and dried. The dried supernatant were weighted and kept at -20 °C for future use.

**Preparation of Selenium:**

Sodium selenite (Na$_2$SeO$_3$) (Mol Wt 172.95 g/mol) was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India.

**Biochemical determination of blood serum:**

Rats (10 from each group) from all groups were sacrificed under light ether anesthesia 24 h after last dose on 15$^{	ext{th}}$ and 30$^{	ext{th}}$ day after initiation of experiment. Before sacrifice rats were fasted overnight. Blood samples were collected and sera were separated by centrifugation (at 3,000 rpm) and kept at -20 °C and analyzed for serum calcium and serum inorganic phosphate by using Beacon kit (Beacon Diagnostic Private Ltd). Each sample was analyzed in duplicate.

**Statistical Analysis:**

Data are presented as Mean ± SE of 6 specimens and Student’s t test was used to determine significance level. In all studies, the treatments group are compared with specific control group. Analysis of Variance (ANOVA) was used to observe the significant difference among all treated groups.

**Results**

Rats treated with *Nerium indicum* leaves extract (NLE; group B; P<0.0001), *Nerium indicum* leaves extract and selenium extract (NLE + Se; group C; P<0.0001) and *Nerium indicum* leaves extract and *Curcuma longa* rhizomes extract (NLE + CRE; group D; P<0.0004) showed a decrease in serum calcium levels after 15 days as compared to serum calcium level of control (group A) (Fig. 1). Calcium level remained unaffected in rat after treatment with Selenium extract (Se; group E) and *Curcuma longa* rhizome extract (CRE; group F). After 15 days the calcium level of group B (NLE) is not significant as compared with group C (NLE + Se; P<0.1245) and group D (NLE + CRE; P<0.8191) which clearly indicates that although there is slight increase in serum calcium levels in group C and group D (as compared to group B) but the treatment with Se and CRE is not completely able to normalize the decrease in serum calcium level induced by *Nerium* leaves extract treatment. Group C (NLE + Se) compared with group F (*Curcuma longa*; P<0.0016) and group D (NLE + CRE) compared with group E (Selenium; P<0.0070) showed the decrease in serum calcium level on 15 days of treatment. Analysis of Variance (ANOVA) indicated that the treatment was significant (F= 10.378; P<0.0001).

After day 30 serum calcium level decreased further after treatment with NLE (group B; P<0.0001) (Fig. 1). The serum calcium level in group C (P<0.0001) and group D (P<0.0001) decreased on day 30 as compared to group A (control). Also, on day 30 the levels in group C (P<0.0001) and group D (P<0.0001) were noticed to be increased as compared to group B. This clearly indicates that the treatment with selenium and curcumin is effective in ameliorating the serum calcium levels which were decreased by treatment with NLE. Analysis of variance (ANOVA) indicated that the treatment was significant (day 30; F= 45.96, P<0.0001).

Rats treated with *Nerium indicum* (NLE; group B; P<0.0001), *Nerium indicum* leaves extract and selenium extract (NLE + Se; group C; P<0.0056) and *Nerium indicum* leaves extract and *Curcuma longa* rhizomes extract (NLE + CRE; group D; P<0.0007) exhibited decrease in serum phosphate level on days 15 as compared to group A (control) (Fig. 2). The serum phosphate levels of group E and group F remained unaffected when compared to control. The phosphate level of group C (NLE + Se; group C; P<0.0011) and group D (NLE + CRE; P<0.0001) exhibited significant increase as compared to group B (NLE). Phosphate level of group C (compared with group F; P< 0.0197) and group D (compared with group E; P<0.0048) showed significant decrease. ANOVA indicated that the treatment was significant (F=8.015;
Fig. 1: Serum calcium levels (mg/100 ml) of Wistar rat treated with Nerium indicum leaves extract (group B), Nerium indicum leaves extract + selenium extract (group C), Nerium indicum leaves extract + Curcuma longa rhizome extract (group D), Selenium extract (group E) or Curcuma longa rhizome extract (group F). Each value indicates mean + S.E. of six specimens.

After 30 days rats treated with NLE (group B; P<0.0001), NLE + Se (group C; P<0.0006) and NLE + CRE (group D; P<0.0002) exhibited a decrease in serum phosphate level as compared to control (group A) (Fig. 2). The serum phosphate levels of group E and group F remained unaltered. The phosphate level of group C (P<0.0001) and group D (P<0.0001) were increased as compared to group B (NLE). Phosphate levels of group C (NLE + Se) compared with group F (CRE; P<0.0035) and group D (NLE + CRE) compared with group E (Se; P<0.0001) showed significant decrease on 30 days. ANOVA indicated that the treatment was significant (F=176.27; P<0.0001).

Discussion

In this study the rats treated with Nerium indicum exhibits hypocalcaemia which is in conformity with the observations of other investigators who have also noticed hypocalcemia in rabbit (Salih and Alkhayyat, 2016) and fish Heteropneustes fossilis (Prasad et al., 2011a) after exposure with Nerium leaves extract. In rabbit serum calcium levels showed insignificant decrease after 2 weeks oral administration of Nerium oleander (Farkhondeh et al., 2020). The observed hypocalcemia in the present study after Nerium indicum leaves extract treatment derives support from other studies in which hypocalcemia has
Fig. 2: Serum phosphate levels (mg/100 ml) of Wistar rat treated with *Nerium indicum* leaves extract (group B), *Nerium indicum* leaves extract + selenium extract (group C), *Nerium indicum* leaves extract + *Curcuma longa* rhizome extract (group D), Selenium extract (group E) or *Curcuma longa* rhizome extract (group F). Each value indicates mean ± S.E. of six specimens.
support from the observations of other workers as they also recorded decreased level of inorganic phosphate levels in-- (i) rats- cypermethrin (Srivastava et al., 2021); chlorpyrifos (Tripathi et al., 2013), cadmium (Tripathi and Srivastav, 2011b), microcystin LR (Moreno et al., 2003; Srivastava et al., 2020), and (ii) in chickens-gamma benzene hexachloride and quinalphos (Agarwal et al., 2009). Srivastav et al. (2018) reported hypophosphatemia in frog after treatment with chlorpyrifos. Toxicant induced hypophosphatemia has also been reported in fish after exposure to microcystin LR (Prakash et al., 2015, 2016), lead (Srivastav et al., 2013), Euphorbia royleana (Prasad et al., 2011 b), Euphorbia tirucalii (Kumar et al., 2011b), azadirachtin (Kumar et al. 2011 a), cadmium (Rai and Srivastav, 2003), cypermethrin (Mishra et al., 2001), chlorpyrifos (Srivastav et al., 1997 a) and deltamethrin (Srivastav et al., 1997 b). Hyperphosphatemia has been reported in heroin administered rats (Barai et al., 2009) and Nerium oleander treated rabbit (Al-Farwachi et al., 2008). No alteration has been noticed in blood phosphate of rats treated with heptachlor (Rangoonwala et al., 2004), diazinon (Rangoonwala et al., 2005) and mipc (Rangoonwala et al., 2007).

In the present study Nerium indicum leaves extract provoked hypocalcemia in rats which may be due to the degeneration of glomeruli and renal tubules in Nerium exposed rats (our unpublished data). In mammals exposure of various toxicants caused degeneration of kidney (Chmielnicka et al., 1989; Prozialeck et al., 2009; Tripathi and Srivastav, 2010, 2011 a). Chertok et al. (1981) observed decreased intestinal calcium absorption. Observed hypocalcemia in present study could be attributed to the kidney damage by toxicants which may have increased efflux of calcium (Chmielnicka et al., 1989; Prozialeck et al., 2009). Few investigators have attributed hypocalcemia in toxicant treated fishes to the degenerative changes in the renal tubules (Koyama and Itazawa, 1977; Roch and Maly, 1979; Larsson et al., 1981; Haux and Larsson, 1984).

In the present study treatment with selenium and Curcuma longa rhizome extract in rats caused improved serum calcium and phosphate which were decreased by exposure to Nerium indicum leaves extract. This could be due to the phytochemicals present in these extracts which caused these electrolytes to recover.

**Conclusion**

It can be concluded that treatment with Nerium indicum leaves extract decreased calcium and phosphate levels in the rats. The fluctuation in calcium and Phosphorus level in serum could be protected by supplementation of selenium and Curcuma Longa rhizome extract. Selenium and Curcuma Longa rhizome extract supplements may help to prevent the effects of toxicants on these electrolytes. It is advised that to reduce the effects of toxicants dietary supplementation of selenium and Curcuma Longa rhizome extract should be given to organisms exposed to toxicants.

**References**


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