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Research Article

## Assessment of Immunotoxic response in Albino Rats following Nickel Nitrate Treatment

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**Abstract:** Nickel nitrate affects body physiology and immunology following its absorption through food, water, air. Predetermined doses of nickel nitrate ( $\text{Ni}(\text{NO}_3)_2$ ) in acute (1 d) and subacute (7, 14, 21 ds) treatments revealed a significant increase in IgG concentration and lymphocyte number, whereas, neutrophils and eosinophils registered significant fall. These alterations indicated heavy metal stress in Immunological parameters that become targets.

**Keywords:** Nickel nitrate, Albino rats, IgG, DLC, Granulocytes.

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### 1. Introduction

Heavy metals contribute a variety of adverse health effects. There are over 20 different heavy metal toxins that can impact health effects and each toxin produces different behavioral and physiological misconduct in an exposed individual. Heavy metals have bio-importance as trace element, but the biotoxic effect of many of them in human biochemistry is of great concern. Hence, there is a need for understanding of the conditions such as concentration and oxidation states which make them harmful and how biotoxicity occurs.

Heavy metals, when ingested, combine with the body's biomolecules like proteins and enzymes to form stable compounds and become toxic when they are not metabolized by the body and accumulate in the soft tissues. These heavy metals enter the human body *via* food, water, air or absorption through the skin in agriculture, manufacturing, pharmaceutical, industrial or residential settings. Industrial exposure is common in adults, while ingestion is the most common route in children (Roberts, 1999).

Nickel (Ni) is silvery white metal and is the 24<sup>th</sup> most abundant element in the earth's crust, comprising about 3% of the composition of the earth. It is the 5<sup>th</sup> most abundant element by weight after iron, oxygen, magnesium and silicon, a member of the transition series and belongs to group VIII B on the periodic table along with iron, cobalt, palladium, platinum and five

other elements. Nickel being a naturally occurring element, existing in various mineral forms, is a mixture of five stable isotopes. Besides Ni exists in several different oxidation states, the prevalent oxidation state under environmental conditions is Ni (II), nickel in the +2 valence state. Other valences (-1, +1, +3 and +4) are also encountered though less frequently.

Heavy metals are the source of various environmental and health problems producing different behavioral and physiological misconduct in the exposed individual. Further, heavy metals do possess bio-importance as trace element in various biological processes concerned with DNA, RNA stabilization, enzymatic and hormonal activities. Nickel, silvery white transition element, abundantly used in making alloys, coins, hairpins, jewelry, prosthetic joints, heart valves, nickel plating and Ni-Cd batteries. Inhalation, absorption, food and water are the preferred routes for the entry of nickel in the organisms, has been reported to be one of the most common causes of allergic contact dermatitis and hepatotoxicity. The Immune system is a complex system of cell with various functions which in turn are regulated by soluble glycoproteins and immunoglobulins with most important immunoglobulin G has longer lifetime (23ds). An appropriate antibodies rise after an anti-allergic challenge is dependent on the functional integrity of the antigen processing cells and regulatory T cells, as well as on the capacity of the B cells to produce antigen-specific antibody. Heavy

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metals exert adverse effects on the immune system. Effect of heavy metals on IgG, IgM and IgE has been assessed stronger in females than in males. Leucocytes give the more explanatory information for immune disturbances. So, differential leucocyte count was estimated whose numeral values have an edge over total leucocyte counting's. The present studies highlight the relationship between heavy metals and immune markers.

## 2. Materials and Methods

### 2.1 Animal

Randomly selected albino rats from inbred colony of almost equal size and weight ( $100 \pm 20\text{g}$ ) at room temperature ( $27 \pm 0.5^\circ\text{C}$ ) and relative humidity ( $55 \pm 3\%$ ) with 12 hours light/dark cycle were given standard laboratory pellet feed (Golden Feed, New Delhi) and water *ad libitum*.

### 2.2 Experimental design

Nickel nitrate [ $\text{Ni}(\text{NO}_3)_2$ ] was used as an experimental chemical, and its toxicity was determined by OECD, 425(2006). The  $\text{LD}_{50}$  of [ $\text{Ni}(\text{NO}_3)_2$ ] was estimated at  $170.5\text{mg/kg b. wt.}$ , calculated by log-dose probit regression line method (Finney, 1971). The rats were divided into 4 experimental groups, one acute (1d) and three subacute (7, 14, 21 ds) groups consisting of 3 rats each. The controls were run simultaneously for both acute and subacute experimental groups. The sublethal dose of nickel nitrate for acute (1 d) treatment was  $17.05\text{mg/kg b. wt.}$ , while for subacute (7, 14, 21 ds) the doses were  $2.436\text{mg/kg b. wt.}$ ,  $1.218\text{mg/kg b. wt.}$ ,  $0.812\text{mg/kg b. wt.}$  respectively.

The albino rats were etherised and blood samples were collected and centrifuged to separate serum for evaluating serum IgG concentration (Melik and Fogler, 1983), and differential leucocyte count (Wintrobe, 1981) in anticoagulated blood, for both control and experimental (acute and subacute) sets. Changes were observed after predetermined time intervals following oral administration of nickel nitrate.

### 2.3 Statistical Analysis

Results have been expressed as the mean values  $\pm$  the standard error, and statistical differences between groups were assessed by two ways ANOVA and were signified at the levels  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ .

## 3. Results and Discussion

A significant increase in IgG concentration, lymphocytes and significant decrease in neutrophils and eosinophils has been observed after acute (1 d) and subacute (7, 14, 21 ds) treatments compared to controls (Fig. 1 & 2).

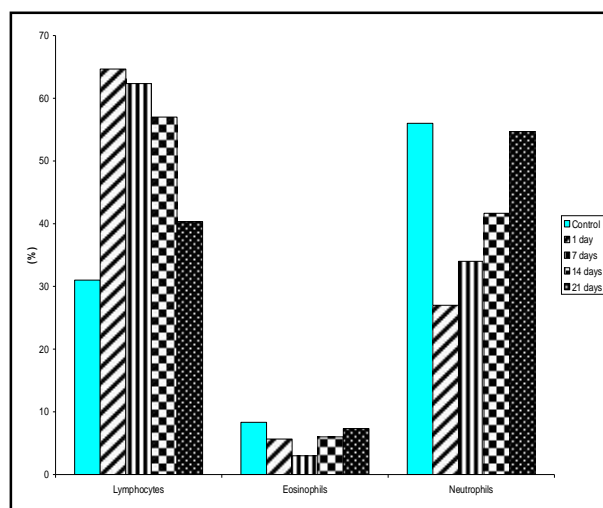


Fig. 1. Effects of nickel on differential leucocyte count in albino rats.

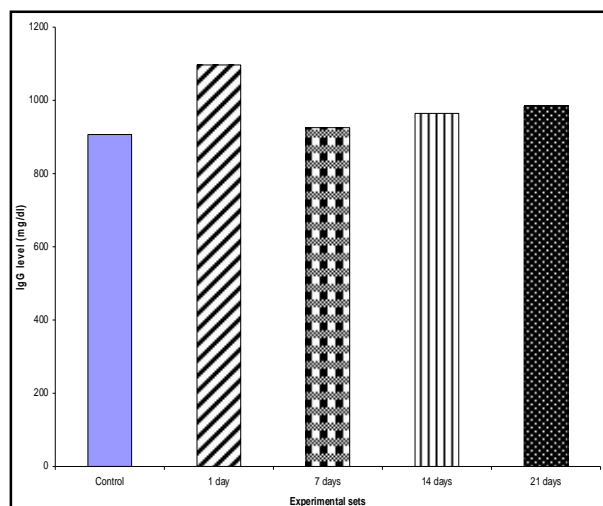


Fig. 2. Effects of nickel on IgG level in albino rats.

Nickel is silvery white transition element which is used abundantly in making alloys, coins, hairpins, jewelry, prosthetic joints, heart valves, nickel plating and Ni-Cd batteries. It has been described as a trace mineral and is present in many cells in the human body and small amount of nickel is useful in certain bodily functions like DNA and RNA stabilization, the metabolism of glucose and hormonal functions and also helps to activate certain important enzymes, such as trypsin and arginase.

Nickel plays an important role in production of red blood cells and is required for metabolism of glucose, lipids, hormones and cell membranes (Flyvholm *et al.*, 1984). Further, it becomes necessary to note that the way in which Ni is consumed, greatly affects its bioavailability (Von Burg, 1997; Barceloux, 1999; Haber, 2000). An enhancement in the IgG level (+100) and (+60) after (1d) and subacute (7, 14 and 21ds) treatment respectively has been recorded in the present study. The hyperimmunoglobulinemia so triggered is an

outcome of stress in bone marrow induced by heavy metal (Salnikow *et al.*, 2003). Subsequently, body's second line of defense gets activated leading to lymphocytosis as a result of which number of T and B cells get enhanced which provoke the generation of immunoglobulins to a greater extent. The raised IgG level is thus exhibited. The nickel related rise in IgG levels has earlier been reflected in the disruption of the offspring's immune system. It seems reasonable to assume that alterations in immunoglobulins G could be a key factor in assessing Ni toxicity.

Differential leucocyte counts (DLC), though, include many granulocytic and non granulocytic cells, neutrophils, eosinophils and monocytes and lymphocytes respectively, however, lymphocytes, eosinophils and neutrophils considered in the present investigation have been useful tools in identifying or diagnosing many types of diseases, anemia, leukaemia and heavy metal poisoning in particular. The enhanced lymphocytes is an outcome of triggering of haematopoietic system, resulting in the activation of myeloid and lymphoid cell progenitors which activate more and more lymphocytes as per the number of antigens.

Further, Nickel intoxication leads to oxidative stress, which is responsible for the significant reduction in granulocytes (eosinophils and neutrophils) (Jadhav *et al.*, 2007), once again affirms involvement of haematopoietic system. Immunological disturbance so induced can be extrapolated to higher mammalian species.

## References

- [1]. Agency for Toxic Substances and Disease Registry (ATSDR) (1997). Toxicological profile for nickel (update). Public Health Service, U.S. Department of Health and Human Services, Atlanta, G.A.
- [2]. Barceloux, D.G. (1999.) Nickel. *J. Toxicol. Clin. Toxicol.*, 37: 239 – 58.
- [3]. Finney, D.J. (1971). Probit Analysis, Cambridge University Press, 303 pp.
- [4]. Flyvholm, M.A., G.D. Nielsen and Andersen, A. (1984). Nickel content of food and estimation of Dietary intake. *Z. Lebensm. Unters. Forsch.*, 179 (6): 427-31.
- [5]. Gawkrödger, D.J., Lewis, F.M. and Shah, M. (2000). Contact sensitivity to nickel and other metals in jewelry reactors. *J. Am. Acad. Dermatol.*, 43(1): 31-6.
- [6]. Haber, L.T., Erdreich, L., Diamond, G.L., Maier, A.M., Ratney, R., Zhao, Q., Dourson, M.L. (2000). Hazard identification and dose response of inhaled nickel – soluble salts. *Regul. Toxicol. Pharmacol.*, 31(2 Pt 1): 210 – 30.
- [7]. Jadhav, S.H., Sarkar, S.N., Ram, G.C., Tripathi, H.C. (2007). Immunosuppressive effect of subchronic exposure to a mixture of eight heavy metals, found as groundwater contaminants in different areas of India, through drinking water in male rats. *Arch. Environ. Contam. Toxicol.*, 53(3): 450 – 8.
- [8]. Melik, D.H. and Fogler, H.S. (1983). Turbidimetric determination of particle size distributions of colloidal systems. *J. Colloid and Interface Science*, 92(1): 161 – 180.
- [9]. Nielsen, G.D., Søderberg, U., Jørgensen, P.J., Templeton, D.M., Rasmussen, S.N., Andersen, K.E., and Grandjean, P. (1999). Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. *Toxicol. Appl. Pharmacol.*, 154: 67-75.
- [10]. Roitt, I., Brostoff, J. and Male. D. (1996). Immunology, Dianne Zack, 4. izdaja, London.
- [11]. Salnikow, K., Li, X., and Lippmann, M. (2004). Effect of nickel and iron co-exposure on human lung cells. *Toxicol. Appl. Pharmacol.*, 196(2): 258 – 65.
- [12]. Von Burg, R. (1997). Toxicology update: Nickel and some Nickel compounds. *J. Appl. Toxicol.*, 17: 425-431.
- [13]. Wintrobe, M.M. (1981). Clinical hematology. 8th ed. Philadelphia: Lea & Febiger.