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ALTERATIONS IN LYMPHOCYTE, NEUTROPHIL AND EOSINOPHIL COUNT UNDER STRESS OF NICKEL NITRATE IN ALBINO RAT

REENA YADAV*, P.K. SINGH AND NEELAM UPADHYAY

Department of Zoology, School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra-282002, U.P., India

ABSTRACT

Metals are used in industry, agriculture and medicine and can exert occupational or homicidal toxicity. They interfere with the function of essential cations and cause enzyme inhibition to generate oxidative stress. As a result, multisystem signs and symptoms are a hallmark of heavy metal intoxication. In case of intoxication, chelators may be used to bind the metal and facilitate its excretion from the body. Nickel exists in our surroundings in the form of packaging, contamination, industrialization etc. It has so heavily contaminated the surrounding that exposure to it is unavoidable. This study was aimed at evaluate the possible effects of nickel nitrate exposure on blood parameters targeting to lymphocytes, neutrophils and eosinophils. Predetermined doses of nickel nitrate ($Ni(NO_3)_2$] in acute (1 day) and subacute (7, 14, 21, 28days) treatments revealed significant alterations in lymphocytes, neutrophils and eosinophils. The results indicate extent of toxicity and alterations in lymphocytes, neutrophils and eosinophils under toxic stress of nickel nitrate in albino rat. This study will imply new dimensions in heavy metal research. The study directly related to humans as albino rat are very similar to human in physiological and biochemical aspects. Further, modernization of our life with daily increase in digitalized equipment's increase the risk to exposure with heavy metals.

KEYWORDS: ***Heavy metal toxicity, Nickel nitrate, Mammalian system, Rattus norvegicus, Immunotoxicity, Differential Leucocyte Count***

Corresponding Author

REENA YADAV*

Department of Zoology, School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra-282002, U.P., India

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INTRODUCTION

Blood is promptly affected by environmental pollutants and toxicants that can cause many metabolic disorders. The high level of nickel acts as a potential pollutant, insecticide and rodenticide with very high toxicity, associated with the hematological damage[1]. This study aimed to determine the toxicity of nickel nitrate on differential leucocyte count in albino rat. Mammals get exposed to nickel through air, water and diet. Nickel is a co-factor in several enzymes of plants and vegetarian diet. Diet rich in nuts and soy product may lead to intake of nickel nearly 1 gm/day. Nickel is the most common immunosensitizer of all metals. In the past nickel allergies mainly at the workplace, however, allergies also occur non-occupationally occur in mammals particularly females. Transport of nickel to tertiary consumer is an outcome of anthropogenic activities and natural sources. Being a xenobiotic substance in the environment, it is responsible for repercussion which disturbes the physiology besides immunity. Heavy metals contribute a variety of adverse health effects[2]. 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 bioimportance as trace element but the biotoxic effect of many of them in human biochemistry are of great concern[3]. Hence, there is a need for understanding the conditions such as concentration and oxidation states which make them harmful and biotoxicity occurance. Nickel nitrate affects body physiology following its absorption through food, water, air. 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 behavioural and physiological misconduct in an exposed individual[4]. Although Ni is omnipresent and is vital for the function of many organisms, concentrations in some areas from both anthropogenic release and naturally varying levels may be toxic to living organisms.

MATERIALS AND METHODS

Experimental Animal

Randomly selected albino rats (either sex) from inbred colony of almost equal size and weight (100 ± 20g) at room temperature (27±0.5ºC) and relative humidity (55±3%) with 12 hours light/dark cycle were given standard laboratory pellet feed (Golden Feed, New Delhi) and water *ad libitum*. These were reared in departmental animal house. The ethical committee guidelines (as per departmental ethical committee instructions Reg No. 1608/CPSEA) has been followed to conduct the experiment.

LD_{50} Determination

The albino rats were divided into 5 groups of 5 individuals each (Table-1). Standard solution of experimental test compound, nickel nitrate was prepared by dissolving the powder into water. Different doses of nickel nitrate were administered orally by gavage tube. The rat mortality was recorded for each dose after 14 days. The data were analyzed statistically by log-dose/ probit regression line method[5]. Regression line was drawn on the basis of two variables, log-dose and empirical probit on a simple graph paper used to determine the expected probit, necessary for LD_{50} determination.

Table 1
Dose and Percentage mortality of albino rats on treatment with nickel nitrate.

S.No.	Dose (mg/kg b.wt.)	No. of rats	Treatment time (hrs)	Mortality Number	Percentage mortality
1.	400	5	96	0	0.00
2.	800	5	96	1	20.00
3.	1600	5	96	2	40.00
4.	3200	5	96	4	80.00
5.	6400	5	96	5	100.00

Experimentation

Nickel nitrate [$Ni(NO_3)_2$] was used as an experimental chemical and its toxicity was determined by OECD[6]. The LD_{50} of [$Ni(NO_3)_2$] was estimated as 170.5 mg/kg b.wt, calculated by log-dose probit regression line method . The rats were divided into 5 experimental groups, one acute (1d) and four sub acute (7, 14, 21, 28 ds) groups consisting of 3 rats each. The controls were run simultaneously for both acute and sub acute experimental groups. The sub lethal dose of nickel nitrate for acute (1 d) treatment was 17.05 mg/kg b.wt., while for sub-acute (7, 14, 21, 28 ds) the doses were 2.436 mg/kg b.wt, 1.218 mg/kg b.wt., 0.812 mg/kg b.wt and 0.608 mg/kg b.wt respectively. The albino rats were etherized and blood samples were collected for differential leucocyte count[7] of anticoagulated blood, for both control and experimental (acute and sub acute) sets. Changes were observed after predetermined time intervals following oral administration of nickel nitrate.

Collection of blood

The albino rats were etherized and dissected carefully. The blood samples were collected from the ventricle of heat with the help of hypodermic needle and transferred to vials.

STATISTICAL ANALYSIS

Results have been expressed as the mean values ± the standard error, and statistical differences between groups were assessed by two way ANOVA[8] and were signified at the levels $P<0.05$, $P<0.01$, $P<0.001$.

RESULTS AND DISCUSSION

The results are shown in tables and figure for illustration (Table 2-4; Fig. 1-3). The results shows significant alterations in lymphocyte count, neutrophil count and eosinophil count.

Table 2
Lymphocyte count (%) of albino rats following treatment of nickel nitrate

S.No.	Days of intoxication	No. of Rats	Lymphocyte count Control Mean ± S.Em.	Lymphocyte count Treated Mean ± S.Em.	Significance level
1.	Acute (1d)	3	49.33±1.70	54.00±2.30	P > 0.05
2.	Sub-acute (7 ds)	3	49.33±1.70	56.00±2.30	P > 0.05
3.	Sub-acute (14 ds)	3	49.33±1.70	58.66±3.17	P > 0.05
4.	Sub-acute (21 ds)	3	49.33±1.70	67.33±2.90	P < 0.05
5.	Sub-acute (28 ds)	3	49.33±1.70	77.66±4.33	P < 0.01

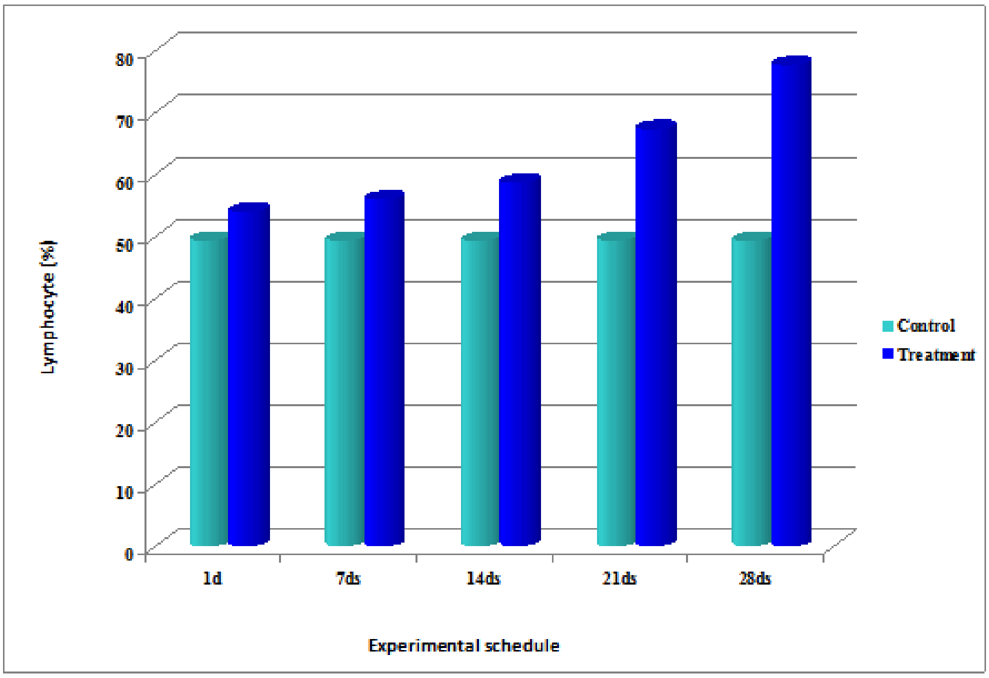

Figure 1
Lymphocyte count (%) of albino rats following treatment of nickel nitrate

Table 3
Neutrophil count (%) of albino rats following treatment of nickel nitrate

S.No.	Days of intoxication	No. of Rats	Neutrophil count Control Mean ± S.Em.	Neutrophil count Treated Mean ± S.Em.	Significance level
1.	Acute (1d)	3	38.00±2.30	44.00±2.64	P > 0.05
2.	Sub-acute (7 ds)	3	38.00±2.30	49.33±2.60	P > 0.05
3.	Sub-acute (14 ds)	3	38.00±2.30	57.00±4.35	P < 0.05
4.	Sub-acute (21 ds)	3	38.00±2.30	60.00±4.93	P < 0.05
5.	Sub-acute (28 ds)	3	38.00±2.30	64.33±4.63	P < 0.01

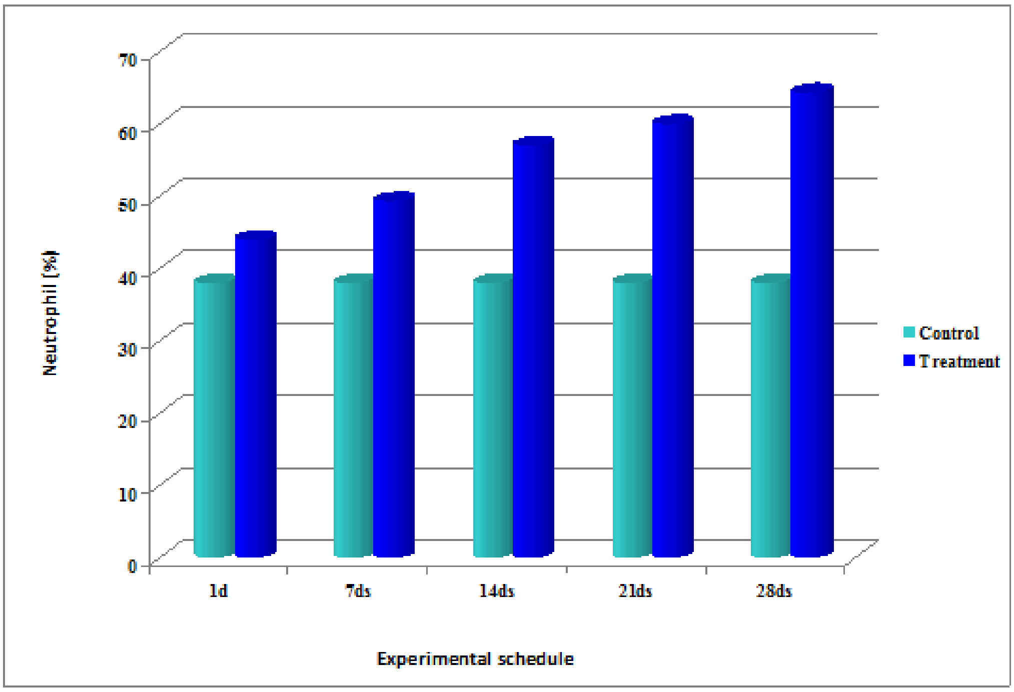

Figure 2
Neutrophil count (%) of albino rats following treatment of nickel nitrate

Table 4
Eosinophil count (%) of albino rats following treatment of nickel nitrate

S.No.	Days of intoxication	No. of Rats	Eosinophil count		Significance level
			Control	Treated	
			Mean ± S.Em.	Mean ± S.Em.	
1.	Acute (1d)	3	1.33±0.33	1.66±0.33	P > 0.05
2.	Sub-acute (7 ds)	3	1.33±0.33	2.00±0.57	P < 0.01
3.	Sub-acute (14 ds)	3	1.33±0.33	2.66±0.33	P < 0.001
4.	Sub-acute (21 ds)	3	1.33±0.33	3.0±0.57	P < 0.001
5.	Sub-acute (28 ds)	3	1.33±0.33	3.66±0.33	P < 0.001

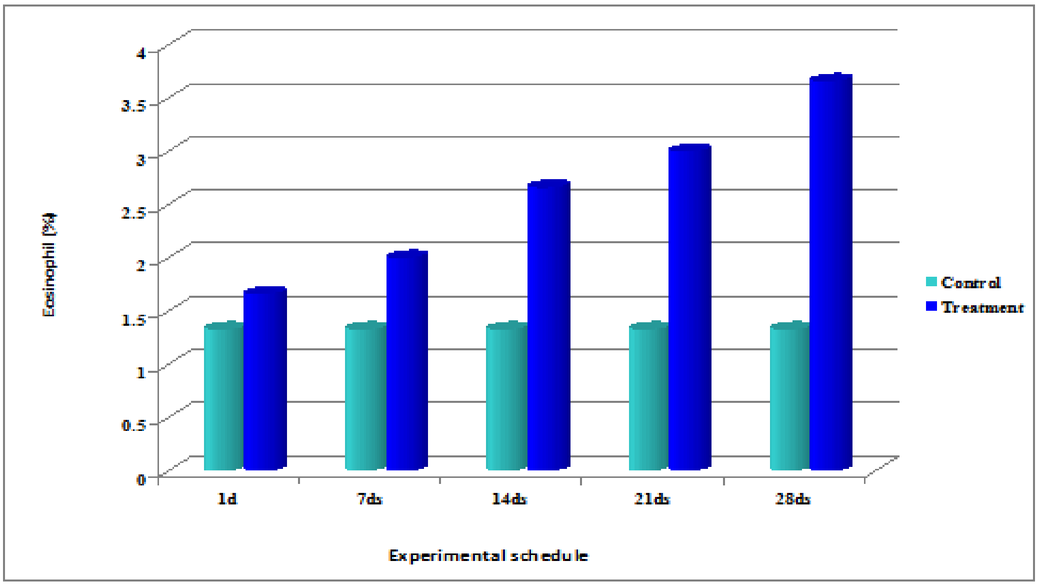

Figure 3
Eosinophil count (%) of albino rats following treatment of nickel nitrate

Nickel is a silvery white transition element which is used abundantly in making alloys, coins, hairpins, jewellery, prosthetic joints, heart valves, nickel plating and Ni – Cd batteries. It has been described as a trace mineral and is present in the human body to facilitate bodily functions like DNA and RNA stabilization, metabolism of glucose and hormonal functions. It also helps to activate important enzymes such as trypsin and arginase. Nickel also plays an important role in the production of red blood cells and is required for the metabolism of glucose, lipids, hormones and cell membranes[9]. It is important to note that the way in which Ni is consumed greatly affects its bioavailability[10-12]. Inhalation, absorption, food and water are the routes for the entry of nickel in the body. Nickel nitrate exhibits dose dependent toxicity and has a profound effect on differential leucocyte count in *Rattus norvegicus*[13]. White blood cells (WBC) are a heterogeneous group of nucleated cells that are found in peripheral circulation for at least a period of their life. They play an important role in phagocytosis and immunity and are therefore crucial in defense against infection[14]. Monocytes, neutrophils and eosinophils are the main phagocytic cells that they search for and embrace foreign particles and destroy them. The absolute number of each type of WBC, often more informative than its proportion, has also been calculated on the basis of differential and the total number of leucocytes per unit volume. Entry of xenobiotic substance leads to rise in total leucocyte count. Elevated white blood cell count is an indicator of heavy metal poisoning and is in affirmation to Beyenhof[15]. Further, high white blood cells count indicates an infection, hypersplenism, bone marrow depression (drugs, radiation or heavy metal poisoning) or primary bone marrow disorders[16]. Differential leucocyte counts include the percentage of Neutrophils, lymphocytes and eosinophils. Only the circulating neutrophils are accounted in the WBC count whereas the half-life of mature neutrophils in circulation is about 7 hours. Eosinophils possibly play a role in defence against multicellular parasites and in limiting inflammation and the percentage of neutrophils and eosinophils both decreased significantly. Lymphocytes are very useful for identifying or diagnosing many types of diseases such as anemia, malaria, syphilis, heavy metal poisoning, leukemia, appendicitis etc. [16]Lymphocytes have agranular clear cytoplasm that stains pale blue, whereas the nucleus stains dark purple are smaller than the three granulocytes and accounts for 25 – 35 % of the white blood cells. In the present study, lymphocytes percentage has been found to increase significantly, reflects Ni ions to be responsible for it. Further, a relative increase in the proportion of lymphocytes is typical of infectious mono-nucleosis or a chronic infection. In the present investigation, the lymphocytes percentage which has been significantly increased affirms nickel stress. The studies conducted *vide supra* can be extrapolated to higher mammalian species. It can be concluded that nickel contamination should be taken seriously with regard to target and non–target species as the repercussions may be alarming.

CONCLUSION

In the present research work, significant alterations has been observed after nickel exposure. The present study is helpful in knowing immunotoxic effects of nickel nitrate which is a highly used metal in ornamental and household things and daily exposure is very common. It specially affects women due to use by them. It is necessary to explore its alternative to be used in daily use to minimize effects. Hence this study will have to be considered during design of precautionary label on nickel containing materials.

AUTHORS CONTRIBUTION STATEMENT

Dr. P.K. Singh conceptualized and gave guidelines with regard to this work. Mrs. Reena Yadav collected data and conducted experiments in the laboratory. Mrs. Neelam Upadhyay helped in the analysis of our study data. All authors discussed the methodology and results and contributed to the final manuscript and gave necessary inputs towards the designing of the manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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