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Research Article

Toxicity Assessment of Nickel Nitrate and Effect on Total Leucocyte Count in Albino Rat

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Abstract: 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. Heavy metals have bioimportance as trace element but the biotoxic effects of many of them in human biochemistry are 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. Nickel nitrate affects body physiology following its absorption through food, water and air. Predetermined doses of nickel nitrate ($Ni(NO_3)_2$] in acute (1d) and subacute (7, 14, 21, 28ds) treatments revealed significant increase in total leucocyte count (TLC). The results indicate extent of toxicity and enhancement in total leucocyte count under toxic stress of nickel nitrate in albino rat.

Keywords: Toxicity Assessment, LD_{50}, Nickel Nitrate, TLC, Albino Rat.

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 behavioural and physiological misconduct in an exposed individual. 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. 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). Inhalation exposure in occupational settings is a primary route for nickel-induced toxicity and may cause toxic effects in the respiratory tract and immune system. Human nickel exposure originates from a variety of sources and is highly variable, is a known haematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive, toxic, pulmonary toxic nephrotoxic, hepatotoxic and carcinogenic agent.

Immunotoxicity is an important health hazard of heavy metal exposure. It is an established fact that certain environmental chemicals, pesticides and heavy metals can alter the immune response of laboratory animals. In some instances, the immune system appears to be exclusively sensitive to these agents compared to other toxicological parameters. Both stimulation and suppression of immune responses could be suggestive of specific mode of action in contaminated exposed animals. Although the majority of data accumulated till date pertains to effects in small laboratory rodents, there is little reason to believe that similar quantifiable effects do not occur in domestic and food-producing animals due to basic functional similarities of the immune system of mammals in general. In the present investigation, an effort has been made to highlight the effect of different doses of nickel nitrate on albino rats in terms of toxicity assessment and total leucocyte count.

2. Material and Methods

2.1 Experimental Animal

Randomly selected albino rats 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.*

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2.2 LD_{50} Determination

The albino rats were divided into 5 groups each consisting of 5 individuals. Standard solution of experimental test compound, nickel nitrate was prepared by dissolving the crystals 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 (Finney, 1971). 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.

2.3 Experimentation

Nickel nitrate [$Ni(NO_3)_2$] was used as an experimental chemical, and its toxicity was determined by OECD TG 425 (2006). The LD_{50} of [$Ni(NO_3)_2$] was estimated as 170.5mg/kgb.wt., calculated by log-dose probit regression line method (Finney, 1971). The rats were divided into 4 experimental groups, one acute (1d) and four subacute (7, 14, 21, 28ds) 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 (1d) treatment was 17.05mg/kgb.wt., while for subacute (7, 14, 21, 28ds) the doses were 2.436mg/kgb.wt., 1.218mg/kgb.wt., 0.812mg/kgb.wt. and 0.608mg/kgb.wt. respectively. The albino rats were etherized and blood samples were collected for total 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.

2.4 Statistical Analysis

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

3. Results and Discussion

LD_{50} value has been calculated as 1705mg/kgb.wt. (Table 1-3) and total leucocyte count show enhancement after nickel nitrate treatment for 1, 7, 14, 21 and 28ds. Results are non-significant to significant with increased duration (Table-4; Fig. 1).

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

S. No.	Dose (mg/kgb.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

Table 2. Determination of LD_{50} of nickel nitrate by regression analysis in albino rats.

Dose (mg/kgb.wt.)	No. of rats (N)	% mor.	Log Dose (X)	Empirical probit	Expected probit (Y)	Working probit (y)	Weighting coefficient (n)	Weight w = n x N	wx	wy	wxy	WX^2	wy^2
400	5	0	2.6	–	–	–	–	–	–	–	–	–	–
800	5	20	2.9	4.16	4.09	4.160	0.471	2.35	6.81	9.77	28.35	19.76	40.66
1600	5	40	3.2	4.75	4.85	4.747	0.627	3.13	10.01	14.85	47.54	32.05	70.53
3200	5	80	3.5	5.84	5.51	5.808	0.581	2.90	10.15	16.84	58.95	35.52	97.82
6400	5	100	3.8	0.0	6.10	6.723	0.405	2.02	7.67	13.58	51.60	29.16	91.54
								ΣW = 10.4	**ΣWX = 34.64**	**ΣWy= 55.04**	**ΣWXy = 186.44**	**ΣWX^2 = 116.49**	**Σwy^2 = 300.55**

Table 3. LD_{50} value, fiducial limits, variance and regression equation for albino rats after nickel nitrate treatment.

Experimental animal	Compound	Regression Equation	LD_{50} (mg/kg b.wt.)	Variance	Fiducial limits
Rattus norvegicus	Nickel nitrate	Y = 5.29 ± 2.77 (m – 3.33)	1705	0.06	m_1 = (+) 3.6137 m_2 = (-) 3.5862

Table 4. TLC (/cc) of albino rats following treatment of nickel nitrate.

S. No.	Days of intoxication	No. of Rats	Total leucocyte count		Significance level
			Control	Treated	
			Mean ± S.Em.	Mean ± S.Em.	
1.	**Acute (1d)**	3	5433.33±120.15	5700.0±152.75	P > 0.05
2.	**Subacute (7ds)**	3	5433.33±120.15	5866.66±145.29	P < 0.05
3.	**Subacute (14ds)**	3	5433.33±120.15	5733.33±145.29	P < 0.05
4.	**Subacute (21ds)**	3	5433.33±120.15	6100.0±115.47	P < 0.01
5.	**Subacute (28ds)**	3	5433.33±120.15	6200.0±152.75	P < 0.001

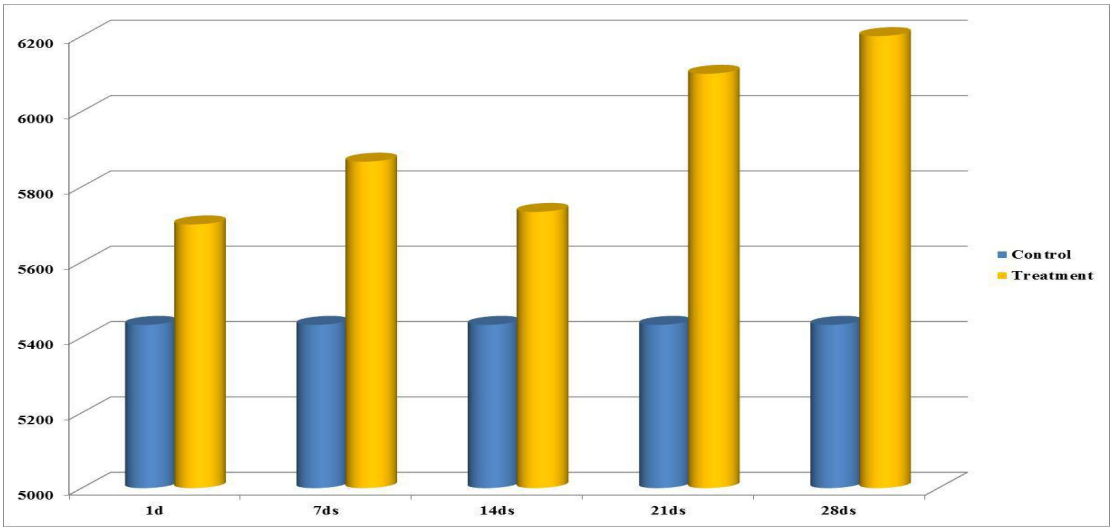

Fig. 1. TLC (/cc) of albino rats following treatment of nickel nitrate.

Mammals get exposed to nickel through air, water and diet. Nickel is a co-factor in several enzymes of plants and vegetarian diet. Diets rich in nuts and soy product may lead to intake of nickel nearly 1gm/day. Some researches show extent of toxicity of nickel in animals (ATSDR, 1997; Barceloux, 1999). Flyvholm *et al.,* (1984) also reported nickel content of food and estimation of dietary intake. Nickel is the most common immunosensitizer of all metals. In the past nickel allergies mainly at the workplace, however, allergies also occur non-occupationally 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 disturbs the physiology besides immunity.

The present investigation highlights the effect of nickel nitrate on TLC which has a say in immune response. 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 important role in phagocytosis and immunity and are therefore crucial in defense against infection (Blumenreich, 1990). Elevated white blood cells count is an indicator of heavy metal poisoning. Further high white blood cells count indicates an infection, hypersplenism, bone marrow depression (drugs, radiation or heavy metal poisoning) or primary bone marrow disorders. WBC plays an important role in defense against infection they search and embrace xenobiotic substance in form of ions or particles and destroy them. Leukocytosis in response to nickel intoxication has been observed after all treatments schedules. Leukocytosis indicates an infection, hypersplenism and bone marrow depression due to heavy metal poisoning.

The studies conducted *vide supra* can be extrapolated to higher mammalian species. It is thus concluded that nickel contamination be taken seriously with regard to target and non–target species as the repercussions may be alarming.

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