Original Article

NICKEL ADMINISTRATION AND CHANGES OBSERVED IN GROSS APPEARANCE, MORPH METRIC/HISTOLOGICAL PARAMETERS OF ALBINO RAT AND ITS LIVER TISSUE.

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ABSTRACT:

OBJECTIVE:

The study was carried out to assess the changes observed in gross appearance and morphometric/Histological parameters in albino rats and their liver tissue on Nickel administration.

STUDY DESIGN:

Experimental study.

SETTINGS AND DURATION OF STUDY:

Animal House PGMI Lahore, thirty days.

MATERIAL AND METHODS:

Thirty two adult Albino rats were taken and kept for study at animal house PGMI, Lahore. They were divided into two control groups; A_1 and A_2 and two experimental groups B_1 and B_2 (each having eight animals). Control group were given intra peritoneal (I/P) injection of distilled water 1 ml /kg body wt/day for the duration corresponding to their experimental group. Experimental groups (B_1 & B_2) were given I/P Injections of Nickel chloride ($NiCl_2$) 2µg/kg body wt/day for 15 and 30 days respectively.

RESULTS:

1. Irritability and reduction in diet intake towards the end of experiment.
2. Significant decrease in body weight and growth rate in experimental group B_2
3. Enlargement of liver and significant increase in relative tissue weight index (RTWI) in both experimental groups.
4. Morphometric/histological parameters showed significant changes in experimental groups.

CONCLUSION:

Nickel administration beyond the permissible limits is deleterious for both gross and morphometric/histological parameters.

KEYWORDS: Nickel, Toxicity, Gross appearance changes, Morphometric/Histological changes.

INTRODUCTION:

In addition to widespread use of Nickel in industry as in stainless steel, alloys, electroplating and also in implants used in human body.[1,2] it is also used as a catalyst in hydrogenation of fats to manufacture vegetable ghee. There are alarming reports

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that due to insufficient precautionary measures nickel contents much above the permissible limit (2µg/10g)[3,] have been found in various brands of vegetable ghee available in the market for human use.[4,5,6]

Recent evidence has proved nickel to be severely toxic both for animals and man. Effecting Liver, lungs, skin and kidnies.[7,8,9] With large oral dose adminstration in acute cases it causes gastrointestinal irritation, vomiting and diarrhoea.[10] Nickel also causes contact dermatitis, local irritation, delayed hypersensitivity reaction ,apoptosis and DNA damage.[11,12]

Chronic toxicity when inhaled as fumes causes damage to alveolar epithelium in the form of persistent oedema, apoptosis and fibrogenic effects.[13,14] It also causes growth retardation and disturbances in biochemical factors as decreased serum alkaline phosphatase, increasd SGOT and SGPT in plasma, heart and liver.[15,16,17] Chronically exposed nickel workers exhibited a dose related increased risk of lung, nasal and laryngeal cancer. [18,19,20]

As the numerous research workers have studied the toxic effects of nickel in biochemical and various histo-pathlogical fields the changes observed in gross appearance of an animal (rat) and morphometric/Histological parameters in liver tissue on nickel administration remains neglected. Therefore present study is designed to study the changes observed in Gross appearance, Morphometric/Histological parameters of Albino rat and its Liver tissue on nickel adminstration. This study is likely to provide a base line for further research in this field with some other parameters.

MATERIALS AND METHODS:

Nickel choloride (Ni Cl_2) of E-Merk was used as source of nickel. A 0.02% stock solution was prepared (One ml of stock solution contained 0.2 mg or 200 µg of Ni Cl_2). From stock solution 0.0002% solution of nickel chloride was made (one ml contained 2µg of Ni Cl_2) . A total no of 32 adult male Albino rats of Sprague Dawley strain on an average weight 150-200gm were kept in animal house of PGMI Lahore, under optimal light and temperature conditions.

The animals were divided at random into two main groups; Control(A) and Experimental(B), each having sixteen animals. Control group was further subdivided into subgroups A_1 and A_2 (having eight animals each).They were kept as control and were given intra-peritoneal injection of distilled water 1ml/kg body weight per day for duration corresponding to their relevant experimental subgroups and were sacrificed along with them. Experimental group B was further subdivided into subgroups B_1 and B_2 (having eight animals each). Experimental group B_1 animals were given I/P injection of nickel chloride 2ug/kg body weight/day for 15 days and were sacrificed on 16^{th} day. Experimental group B_2 animals were given I/P injection of nickel chloride 2 ug /kg body wt/day for 30 days and were sacrificed on 31^{st} day.

OBSERVATIONS AND RESULTS:

Body weight and general physical condition of each animal were recorded at the start of experiment and the twice weekly bases in the morning before giving the dose and feed. Final weight and general physical condition of each animal were recorded at the end of experiment before sacrificing. Liver of each animal was dissected out carefully and after gross examination it was weighed and Relative tissue weight index (RTWI) calculated.

All the animals of control group (A) remained healthy and active. While animals in the experimental group (B_2) were irritable with reduction in diet intake towards the end of experiment. There was significant decrease in the mean body weight and growth rate in experimental group (B_2) when compared to its control group.

Table-1: The effect of Nickel chloride on the mean body wt and growth rate (% gain / day) of Sprague Dawley rats;

Group	Mean body wt at Start (gms)	Mean body wt at End (gms)	Mean growth Rate(% gain/day)
A (Control)			
A_1(15 days)	135.0 ± 9.63	145 ± 9.25	0.53 ±0.24
A_2 (30 days)	167.5 ± 25.0	197.5 ± 26.32	0.60 ± 0.17
B (Experimental)			
B_1 (15 days)	145 ±16.03	154.5 ± 15.51	0.43 ± 0.11
B_2 (30 days)	135 ± 13.09	150.0 ± 16.90	0.36 ± 0.5×××

For growth rate; Experimental groups have been compared to their respective control groups. ××× P<0.001 = Highly significant.

Relative tissue weight index (RTWI) increase was significant in both experimental groups (B_1 & B_2) Table 11. On gross appearance there was enlargement of livers in experimental groups associated with ooze of blood on cutting particularly in experimental group (B_2), showing a classic picture of congested liver.

Table 11: Effect of Ni Cl2 on Relative tissue weight index (RTWI) of Sprague Dawley rats.

Group	A (Control)		B (Experimental)	
	A1 (15 days)	A2(30 days)	B1(15 days)	B2(30 days)
Mean body wt (g±SD)	145± 9.25	197.50± 26.32	154.50± 15.51	154± 16.90
Mean liver wt(g±SD)	6± 0.84	8.17 ± 0.69	7.00 ± 0.45	7.50± 0.96
Mean RTWI ± SD	4.16± 0.74	4.20 ± 0.62	4.55± 0.46×	5.02 ± 0.58××

Mean±SD, Student t-test for statistical significance applied. In this table experimental groups have been compared with their respective control groups . P>0.5(NS), P< 0.05(Significant)×, P<0.01(very significant)××, P<0.001(Highlly significant)×××.

For morphometric/histological examination liver tissue small pieces of 7-10 mm were cut, fixed in 10% buffered formalin for 48 hours and paraffin blocks were prepared. Sections were cut at 4-5 um by rotary microtome and stained with haematoxylin and eosin (H/E) by standard procedure[15]. The observations were made for morphometric parameters;

1) Size of hepatocyte in µm
2) Size of nuclei
3) No of nucleoli/nucleus
4) Diameter of central vein (µm)
5) No of necrotic foci/HPF

Morphometric analysis was performed with the aid of an objective micrometer at magnification X 400. Results were expressed as Mean ± SD and significance of differences between the means were tested using student t-test. Table-111.

Table-111: Effect of Ni Cl_2 on different parameters of morphometric analysis in Sragues Dawley rats;

Group/ Paratmeters	A (Control)		B (Experimental)	
	A_1(15 days)	A_2(30 days)	B_1(15 days)	B_2(30 days0
Size of hepatocytes (µm)-a	15.1 ± 0.666	15.0± 0.73	17.6± 0.65××	18.14± 1.04×××
Size of nucei(µm)	7.20 ± 0.69	7.03± 0.61	7.22± 0.52	6.87± 0.62
No of nucleoli/nucleus-a	1.35 ± 0.69	1.31± 0.46	1.37± 0.48	1.21±0 .46
Diameter of central vein	43.60± 37.21	43.04 ± 6.87	54.6± 9.31××	65.97± 15.61××
No of necrotic foci/HPF-b	0	0	1-2 small foci	1-2 small foci

a) Mean ±SD, student t- test, p<0.5×, p<0.01××, P<0.001×××. For statistical significance in this table experimental groups have been compared to their respective controls.
b) Minute foci up to 300µm, Small foci; 300-700µm

DISCUSSION:

The aggressive and irritable behavior towards end of experiment in experimental group (B_2) may be due to GIT irritation and effects on CNS, as is indicated by Sunderman et al[21].The insignificant decline in the mean body weight and growth rate (% gain/day) in experimental group (B_1) confirm the findings of a similar study carried out by Knight et al.[22] The significantly reduced growth rate in experimental group (B_2) as is also observed by Niyogi et al[15], can be attributed to;

I. Low food and water intake affecting satiety centre of brain.
II. Enhanced protein degradation along with reduced protein synthesis.
III. Disturbance in digestion process, because of GIT irritation and after I/P or oral administration of nickel chloride[10].

RELATIVE TISSUE WEIGHT INDEX (RTWI);

The increased liver weight due to congestion and inflammatory changes appearing due to hepatotoxicity of nickel and decrease in body weight of animals explains the increase in RTWI in the present study in experimental groups B_1 and B_2. Similar increase in RTWI had been reported by Donskoy et al[17].

MORPHOMETRIC/HISTOLOGICAL CHANGES;

The morphometric/histological changes such as dilatation of central veins and centrilobular sinusoids could be due to congestion and apoptosis, a finding consistent with the reports of Knight et al and Ahmed M. et al[22,23]. In this study the congestion became marked with advance in treatment. Congestion possibly resulted from impaired venous drainage leading to increased deoxygenated haemoglobin in the blood and hypoxia. The initial dilatation of sinusoids and central veins was later followed by narrowing which may be the result of cellular (hepatocyte) swelling as a result of biochemical changes within cell[24].These may be manifested as cellular swelling, fatty changes or hyaline degeneration leading to necrosis with nuclear changes in the cell.

The biliary hyperplasia and increased fibrosis found in experimental groups (B_1 and B_2) could be due to obstruction in biliary pathway as a response to cell injury. The cellular swelling leading to narrowing of bile canaliculi could be a possible explanation for this.

Therefore it is concluded that Nickel administration beyond the permissible limits is deleterious for both gross and morphometric/histological parameters of Albino rat and its liver tissue.

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