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EFFECTS OF NICKEL CHLORIDE ON HEMATOLOGICAL AND DEVELOPMENTAL PARAMETERS IN *Wistar albino* PREGNANT RATS

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ABSTRACT:

Several reports have suggested that soluble salts nickel may affect on hematopoiesis and development. In this study female Wistar albino rats (180-300g) received $NiCl_2$, $6H_2O$, subcutaneously (25, 50 and 100 mg/kg body weight (b w) or in drinking water (20 mg/100 ml). Selenium (0.3 mg/kg b w, s.c.) was combined to $NiCl_2$ (100 mg/kg b w, s.c.). Control groups received NaCl 0.9% (0.3 ml s.c) or drinking distilled water. All groups of rats were injected on day 4 of pregnancy in pre-implantation period. Haematological parameters were recorded on day 6 and 21 of pregnancy. Developmental parameters were assessed on day 21 of pregnancy. 25 mg/kg b w, of $NiCl_2$ s.c, induced on day 6 an immediate and significant decrease in erythrocyte counts, hematocrit values, and haemoglobin concentrations. This depletion was maintained on day 21 of pregnancy compared to control values.On other hand 50 mg/k b w, $NiCl_2$, s.c, reduced on day 6 of pregnancy the erythrocyte counts, hematocrit values and platelets counts. Inversely, on day 21 this dose elevated the erythrocyte counts, hematocrit values and haemoglobin concentrations ,with depletion of the platelets counts compared to control values. In group introduced 100 mg/kg b w, of $NiCl_2$, s.c had no effect on all haematological parameters studied. $NiCl_2$, significantly reduced the maternal body weight on day 6 and 21 of pregnancy in a dose – dependent manner in rats treated subcutaneously and in drinking water compared with control values. $NiCl_2$, s.c. (100 mg/kg b w) markedly reduced the number of live fetuses and elevated the number of abortions on day 21 of pregnancy compared to control values. $NiCl_2$, s.c or in drinking water had no effect on fetal body weight. Selenium (0.3 mg/kg b w, s.c.) combined to $NiCl_2$ (100 mg/kg b w, s.c.) did not improve the effect of $NiCl_2$.

INTRODUCTION:

Nickel, a major environmental pollutant, was potentially produce carcinogenic, genotoxic, immunotoxic and teratogenic (Bencko *et al.*,1986 , Grimsrund *et al.*, 2003; Danadevi *et al.*, 2004;and Montanaro *et al.*,2005). It was extensively used in electroplating, manufacture of batteries and steel to release into the atmosphere during mining, smelting and refining operations (Caplat, 2001). Therefore, nickel had a disastrous impact on human and animal health. Also, it found to be harmful for hematopoiesis and female reproduction of laboratory rodents (Weischer *et al.*, 1980; Dieter *et al.*, 1988 ; and Käkelä *et al.*, 1999). Selenium has been previously found to counteract the deleterious effects on nickel on the reproduction

rats (Käkelä *et al*., 1999). Adequate selenium status was known to be essential for fertility in man and animal (Hensen and Deguchi, 1996). In this study, we compared the effects of $NiCl_2$ in drinking water and subcutaneous injection administration on day 4 of gestation, in pre-implantation period, on development and haematological parameters in pregnant Wistar rats using variations in the dose, route of administration and duration of exposure.

MATERIALS AND METHODS:

Animals:

Adult Female albino Wistar rats (Pasteur Institute, Algiers) were kept in a lighting schedule of 12 h light: 12 h darkness at 23±1°C with free access for food and water. Animals were used at day 21 of pregnancy. The average length of gestation in breeding colony in our department was 22 days. Females (180-300g) were caged with males overnight and the vaginal smear examined for the presence of spermatozoa. The day on which spermatozoa were found in the smear was designated day 1 of pregnancy and animals were used on days 6 and 21. Pregnant females were housed at five rats per cage. The average of gestation in the breeding colony was 22 days (Adjroud 1995).

Chemicals:

Nickel chloride hexahydrate ($NiCl_2$, $6H_2O$) was purchased from sigma Aldrich Laborchemikalien Gmbh; Selenium (Se) was purchased from we prolab; NaCl was purchased from panacreac Qu mica Sa, diethyl ether Ficher scientific (UK).

Experiments:

Each animal was anaesthetized with diethyl ether s.c., and was weighed before each experiment. The controled groups and treated groups were injected s.c with 0.3 ml/rat of NaCl 0.9%, or drinking distilled water.

Nickel chloride hexahydrate ($NiCl_2$, $6H_2O$) was dissolved in sterile saline (NaCl 0.9%) and was given as a single s.c. at 25, 50 and 100 mg/kg body weight or 20 mg/100 ml in drinking distilled water. Selenium (Se) was dissolved in sterile saline and was given s.c. at 0.3 mg/kg body mass in association with the higher dose (100 mg/kg body mass) of Nickel chloride. Selenium was used to block the effects of the $NiCl_2$. The exposed rats or control groups were injected on day 4 of pregnancy during pre-implantation period and blood sample was collected on EDTA from jugular vein on day 6 and 21 of pregnancy for haematological study. The determination of haematological parameters was performed by coulter Erma Inc PCE-21-ON. On day 21 of pregnancy maternal body weight was recorded, uterus were excised to evaluate the number of live foetuses and abortion; the fetuses were removed and weighed.

Statistical analysis:

Data for each group of experiments (n=6) were statistically analysed by analysis of variance and expressed as mean ±S.E.M. Significant differe-nces between the treated group mean and its control group were performed by Student's "t" test. Differences were considered to be significant if $P<0.05$. Data were analysed with Excel for windows, version 5.1, USA.

RESULTS:

1-Effects of $NiCl_2$ on haematological parameters:

Subcutaneous administration of nickel chloride, 25 mg/kg b w, on day 4 during pre implantation period induced 2 days after

treatment an immediate and significant decrease in the erythrocyte counts, hematocrit values and haemoglobin concentrations from day 6 to 21 of pregnancy. While $NiCl_2$, at 50 mg/kg b w, produced a significantly reduction on day 6 of pregnancy for erythrocyte counts and hematocrit values and platelets counts but significantly increased on day 21 of pregnancy to erythrocytic counts, hematocrit values and haemoglobin concentrations. On the contrary $NiCl_2$, at 50 mg/kg b w, produced a significantly elevation on the platelets counts on day 21 of pregnancy. However, the highest dose of $NiCl_2$, 100 mg/kg b w, did not significantly affect these haematological parameters during observation period compared to control. Adding Selenium by 0.3 mg/kg b w associated with higher dose of $NiCl_2$, did not alter the effect of 100 mg/kg b w administered alone on the erythrocytic counts, hematocrit values, haemoglobin concentrations, and platelets counts on day 6 and 21 of pregnancy, compared to control values.

On the other hand, rats having received $NiCl_2$, 20 mg/100 ml in drinking water showed an immediate and significant decrease in the erythrocyte counts and hematocrit values from day 6 to day 21 of pregnancy. This dose of nickel Chloride added to drinking water had no significantly effect on haemoglobin concentrations from day 6 to day 21 of pregnancy, but slightly increased blood platelet counts on day 6 of pregnancy.

2-Effects of $NiCl_2$ on maternal body weight:

$NiCl_2$, 25, 50, and 100 mg/kg b w administered s.c., reduced progressively and significantly the maternal body weight on day 6, this fall was spectacular on day 21 of pregnancy compared to control values. Selenium (0.3 mg/kg b.w.) combined with 100 mg/kg, b.w of nickel chloride produced a slight but no significant increase in maternal body weight compared to 100 mg/kg b w of $NiCl_2$ alone. Rats exposed to 20 mg/100 ml in drinking water showed a notable decrease in maternal body weight particularly on day 6 of pregnancy compared to control and to subcutaneous route.

3-Effects of $NiCl_2$ on developmental parameters:

When rats had been treated subcutaneously on day 4 of pregnancy by $NiCl_2$, 25, 50 and 100 mg/kg, b w the fetal body weight decreased slightly on day 21 of pregnancy. Addition of selenium to $NiCl_2$ - exposed rats did not improve, the fetal body weight on day 21 of pregnancy. Similarly, rats drinking water supplemented with 20 mg/100 ml of $NiCl_2$, were showed a slight but not significant reduction of fetal body weight on day 21 of pregnancy.

A-Effects of $NiCl_2$ on live fetuses number:

Our results showed that $NiCl_2$, induced on day 21 of pregnancy a progressive diminution of the number of live fetuses with 25 and 50 mg /kg, b w, s.c.; this diminution was reached to maximum with 100 mg/kg, in comparison with control. Selenium did not prevent the decrease in the number of live fetuses induced by the highest dose (100 mg/kg) of $NiCl_2$. However, 20 mg/100 ml of $NiCl_2$ added to the drinking water had no effect on the number of live fetuses.

B-Effects of $NiCl_2$ on number of abortions:

25 and 50 mg/kg, b.w., of $NiCl_2$ slightly elevated the number of abortions; while with 100 mg/kg b w the elevation was reached to maximum in comparison with control. Selenium combined with 100 mg/kg of $NiCl_2$ did not block these abortions. On the contrary, 20 mg/100 ml of $NiCl_2$ in the drinking water did not affected significantly on the number of aborted foeti in comparison with control.

Table (1): Effects of subcutaneous nickel chloride alone or combined with selenium on haematological parameters and maternal body weight in *Wistar albino* pregnant rats

Parameters		Control (N=6)	25 mg/kg (s.c)	50 mg/kg (s.c)	100mg/kg (s.c)	Ni 100 mg/kg + Se 0.3 mg/kg
Erythrocytes counts ($x10^6/mm^3$)	day 6	6.2±0.51	3.54±0.38 **	4.3±0.15 **	6.1±0.54	5.66±0.71
	day 21	4.57±0.45	2.96±0.4 *	6±0.3 *	5.49±0.77	5.32±0.94
Hematocrit values (%)	day 6	35.62±3	20.25±2.41 **	23.1±0.85 **	32.84±3.22	30.56±3.57
	day 21	26±3	15.96±2.53 *	33.36±1.89	30.26±4.8	28.62±5
Haemoglobin concentrations (g/100 ml)	day 6	12.28±0.8	9.82±1.07 *	11.2±0.9	12±0.54	10.82±1.04
	day 21	9.64±0.76	7.5±0.7 **	11.08±0.52	10.9±1.3	10.4±1.57
Platelets counts/mm^3	day 6	646200±61551	473090±137064	391180±108234	611800±41542.7	582400±51952.6
	day 21	724000±63277.8	639400±42562.6	501200±63277.7 *	777200±101941	657400±57562.6
Maternal body weight (g)	day 6	247.4±9.3	202±9.4 *	214±12.710.15 *	194.2±19.95*	209±7.18
	day 21	294±10.8	245±32.74 *	239±24.5**	220±3.4**	226±6

Each value haematological parameters or body weight represents the mean±SEM 6 rats per group
****p<0.01, *p<0.05 compared with control value, student's *t* test.**

Table (2): Effects of oral nickel chloride alone or combined with selenium on haematological parameters and maternal body weight in pregnant *Wistar albino* rats

Parameters		Control (N=6)	20 mg/100 ml
Erythrocytes counts (x $10^6/mm^3$)	day 6	5.944±0.43	3.88±0.65*
	day 21	21:5.13±0.13	3.36±0.13**
Hematocrit values (%)	day 6	34.44 ±3	21.42±4**
	day 21	29.5±2	19.7±2*
Haemoglobin concentrations (g/100ml)	day 6	11.4 ±1	8.82 ±1
	day 21	10.24 ±0.5	9.42 ±0.37
Platelets counts /mm^3	day 6	561280±156980	629080 ±167156.3
	day 21	627200±8870.5	612600±15676
Maternal body weight (g)	day 6	220 ± 6	170±14.45***
	day 21	259±12.8	237±18*

Each value haematological parameters or body weight represents the mean±SEM 6 rats per group
*****p<0.001, **p<0.01, *p<0.05 compared with control value, student's t test.**

Table (3): Effects of subcutaneous nickel chloride alone or combined with selenium on fetal body weight; number of fetuses and number of abortions in pregnant *Wistar albino* rats (day 21)

Parameters	Control (N=6)	25 (mg/kg)	50 (mg/kg)	100 (mg/kg)	Ni 100 (mg/kg) +Se 0.3 (mg/kg)
Foetal body weight (g)	2.82±0.31	2.6±0.41	2.12±0.62	2.82±0.31	2±1.223.57
Number of fetuses	21: 8±1.76	7.6±1.5	5±2	3.2±1.64*	3.6±2
Number of abortions	21: 2.2±0.8	4±1.5	4.4±1.7	7.4±1.7*	6.8±1.95

Each value represents the mean ± SEM 6 rats per group
***p<0.01 compared with control value, student's *t* test.**

Table (4): Effects of oral nickel chloride on fetal body weight; number of fetuses and number of abortions in pregnant *Wistar albino* rats (day 21)

Parameters	Number of experiments	Control (N=6)	20 mg /100 ml
Foetal body weight (g)	6	3.12±0.34	2.48±0.88
Number of fetuses	6	7.6 ±0.75	6 ±2.6
Number of abortions	6	2±0.35	4.4±1.25

Each value represents the mean ± SEM 6 rats per group

DISCUSSION:

The immediate anaemia observed with the lower dose of $NiCl_2$ administered subcutaneously or with 20 mg/100 ml solution given in the drinking water was also observed in rats by Weischer *et al*., (1980) and Nielsen (1980). The decrease in haematological parameters seemed to be related to impaired iron absorption in rats deprived of nickel in diet (Nielsen, 1980). Furthermore, $NiCl_2$ also acted on metabolism and in particularly the decrease in the erythrocyte glutathione peroxydase with consequent increase in oxidative stress in human and fish (De Luca *et al*., 2007). On the other hand, the deficiency of nickel in di*et al*tered the fatty acids composition of total lipids and phospholipids in rats erythrocytes (stangi and Kirehess 1997). On the contrary, the middle dose of $NiCl_2$ only reduced on day 6 of gestation the erythrocyte counts, hematocrit values and the platelets counts, and had no effect on haemoglobin concentrations. Inversely, on day 21 of pregnancy, the middle dose increased the erythrocyte counts, the hematocrit values, and haemoglobin concentrations. This result was also obtained in rats (Weischer *et al* 1980) and was consistent with increase in erythropoietin synthesis in vitro in human and fish. On the other hand, the increase in haematological parameters obtained 18 days after treatment might be due to the accumulation of nickel in blood on day 21 of gestation (Dem *et al*., 2005) and then increased the iron content (Cempel, 2004) which induced an increase in the haematological parameters in our pregnant rats. The highest dose had not altered haematological parameters. In addition, selenium combined to the highest dose failed to modify the effects of the highest dose.

Reports suggested that soluble nickel salts might affect development. Indeed, our result showed that nickel chloride administered s.c., or in drinking water in the early gestation during pre-implantation induced a harmful effect particularly on the number of live fetuses and embryonic resorption with the highest dose s.c. These results agrees with embryotoxicity and fetal toxicity of $NiCl_2$ observed in rats by Sundermann *et al*., (1977), Saillenfait *et al*., (1991) and smith *et al*., (1993), also, in mice by Chernoff & Kawlock (1982) and Berman &Rehnberg (1983) and in women . The toxic effect of nickel seemed to appear in mice during the passage through the oviduct (Storeng & Jonsen, 1981) and during organogenesis (Mas *et al*., 1985) with subsequent effect on the development after implantation inducing cytotoxicity and teratogenic effect. Injection of $NiCl_2$ during early gestation crossed the feto-maternal barriers and entered the foetus during late gestation (Sundermann *et al*., 1977). In another study by Lu *et al*., 1981) reported that the concentration of nickel in the maternal blood and the placenta were found maximum level at 2 hours after injection of nickel chloride in pregnant mice. In addition, the yolk sac and placenta accumulated Ni in mice (Mas *et al*., 1985) inducing in mid pregnancy in cultured rat embryos a significant decrease in yolk sac diameter and high incidence of poor yolk sac circulation (Saillenfait *et al*., 1991) but did not prevent the transportation of metal to embryo or foetus. $NiCl_2$, in drinking water or in subcutaneous administration had no significant effect on fetal body weight looses. This result disagrees with Storeng and Jonson, (1981); Mas *et al*., (1985) and Georges *et al*.; (1989), or in rats (Saillenfait *et al*., (1991). Which reported a decrease in fetal body weight obtained .

Maternal body weight was progressively and significantly decreased in dose-related manner after treatment with $NiCl_2$, subcutaneous or in the drinking water. This

result agrees with those obtained in rats by weischer *et al*., (1980), Georges *et al*., (1985), (Seidenberg *et al*., 1986) and smith *et al*., (1993) or in mice treated by nickel sulfate in drinking water (Dieter *et al*., (1988). This decrease in maternal body weight was probably related to the decreased metabolic organs weights such as kidney and liver (Lu *et al*., 1981, Novelli *et al*., 1998, and Pari & Prasath, 2008). In conclusion, our results demonstrated nickel chloride in drinking water or in the lower dose administered subcutaneous induced immediately anaemia. The developmental parameters were only altered by the highest dose of nickel administered subcutaneous. Selenium did not antagonise the effects of nickel.

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تأثير كلوريد النيكل على المؤشرات الدموية و التطور الجنيني لإناث جرزان ويستار أثناء مرحلة التعشيش

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لقد أثبت العديد من التقارير أن أملاح النيكل المذابة قد تؤثر على تنشئة الدم والتطور الجنيني. وفي هذه الدراسة تم إعطاء إناث جرذان ألبينو وستار (١٨٠-٣٠٠) جرعات من كلوريد النيكل عن طريق الحقن (٢٥-٥٠، ١٠٠ ملليجرام/كجم) أو في ماء الشرب (٢٠ ملليجرام/١٠٠ مل)، كما تلقت السيلينيوم (٠.٣ ملليجرام/كجم وقد مزج مع (١٠٠ ملليجرام/كجم) من كلوريد النيكل، أما المجموعة الضابطة عولجت بكلوريد الصوديوم ٠.٩% (٠.٣مل) عن طريق الحقن تحت الجلد أو بالماء المقطر عن طريق الشرب.

تم حقن كل مجموعات الجرذان في اليوم الرابع من الحمل خلال فترة التعشيش، كما تم اخذ عينات دم في اليوم السادس والواحد والعشرين من الحمل، أما بالنسبة لمؤشرات التطور الجنيني فقد تم معاينتها في اليوم الواحد والعشرين من الحمل.

أظهرت النتائج أن ٢٥ ملليجرام/كجم من كلوريد النيكل أحدثت انخفاضاً سريعاً ومعنوياً في عدد كرات الدم الحمراء، نسبة الهيماتوكريت وتركيز الهيموجلوبين. واستمر هذا الانخفاض حتى اليوم ٢١ من الحمل مقارنة مع ضابط التجربة (الشاهد). أما بالنسبة لجرعة ٥٠ ملليجرام/كجم فقد أحدثت انخفاضاً في اليوم السادس من الحمل فى عدد كرات الدم الحمراء وقيم الهيماتوكريت وتركيز الهيموجلوبين، أما في اليوم الحادي والعشرين فقد ارتفعت أعداد كرات الدم الحمراء، قيم الهيماتوكريت وتركيز الهيموجلوبين، كما انخفض تركيز الصفائح الدموية مقارنة مع ضابط التجربة وبالنسبة للجرعة ١٠٠ ملليجرام/كجم من كلوريد النيكل فلم تؤثر على المؤشرات الدموية.

وقد أدى إضافة كلوريد النيكل إلى انخفاض معنوي وملحوظ فى وزن الأمهات في اليوم السادس واليوم الواحد والعشرين من الحمل سواء بعد استعمال كلوريد النيكل وحده أو مع الإضافات عن طريق الحقن أو في ماء الشرب.

وقد أدت إضافة الجرعة ١٠٠ ملليجرام/كجم إلى انخفاض ملحوظ في عدد الأجنة مع زيادة نسبة الإجهاض في اليوم الواحد والعشرين من الحمل مقارنة بضابط التجربة. هذا ولم تؤثر إضافات كلوريد الزنك سواء بالحقن أو عن طريق الماء على وزن الأجنة، كما أن إضافة السلينيوم مختلطاً مع كلوريد النيكل (١٠٠ ملليجرام/ كجم) لم يحسن من الآثار السلبية لكلوريد النيكل.