

# Toxic Effects of Nickel Nanoparticles at a Subacute Oral Administration to Rats <sup>†</sup>

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**Abstract:** The toxic effects have been evaluated of Nickel nanoparticles (NiNPs) at their 92-day oral administration to male Wistar rats at doses of 0.1; 1 and 10 mg/kg body weight. An increase in glycemia, triglyceride, LDL, total protein, and its globulin fraction levels were noticed in NiNPs exposed rats. NiNPs caused a decrease in the reserves of reduced liver glutathione and excretion of selenium in the urine, an increase in serum levels of cytokines IL-1 $\beta$ , IL-2, IL-6, IL-12p70, TNF- $\alpha$ , and INF- $\gamma$  with simultaneous decrease in IL-17A as well as increased fibrosis-marking genes and Tp53 expression.

**Keywords:** nickel; nanoparticles; toxicity; rats; cytokines; fibrosis; apoptosis; gut barrier permeability

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## 1. Introduction

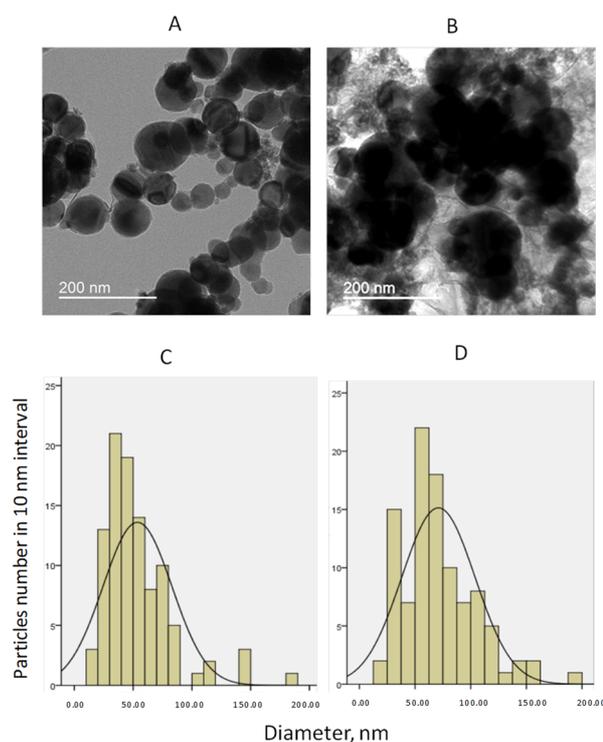
Nickel nanoparticles (NiNPs) are used in catalysts for hydrogenation of dietary fats, in cosmetics, insecticides, preparations for theranostics in medicine [1–4], and can also expose people occupationally in the metallurgy and mining industry [5]. According to multiple studies in vitro NiNPs possess pro-oxidant, pro-inflammatory, genotoxic and carcinogenic activity [6–8]. At inhalation or intratracheal instillation to experimental animals different adverse effects of Ni-containing NPs have been detected including general toxicity, immunotoxicity and reproductive toxicity [9]. However consequences of a prolonged administration of these nanomaterials with food at low doses are not well understood. The aim of the study was to evaluate the toxic effects of NiNPs at their prolonged oral administration to male Wistar rats

## 2. Materials and Methods

Ten groups of 12 male Wistar rats received during 92 days balanced semisynthetic diet (AIN93M) either without additions (Control group 1), or supplemented with Ni carbonate basic salt (Ni salt) at a dose 0.1; 1 and 10 mg/kg b.w. as Ni (groups 2–4), or NiNPs preparation 1 (NiNP1) (groups 5–7) or NiNPs preparation 2 (NiNP2) (groups 8–10) at the same doses as Ni respectively. At the end of the feeding period biochemical, immunological and morphological endpoints were studied.

### 3. Results

As it may be seen from Figure 1, NiNPs 1 and 2 preparation contained spheric particles with more than 99% of Ni (according to study by electron energy dispersion spectrometry). Mean diameters of the particles in the preparations were equal to  $53.7 \pm 2.9$  nm and  $70.9 \pm 3.3$  nm; 55.5% of the particles in the NiNP1 and 24.0% in NiNP2 were less than 50 nm in diameter.



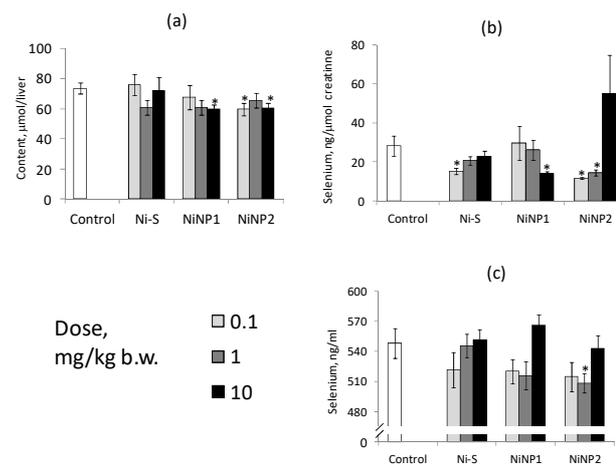
**Figure 1.** TEM images of NiNP1 (A), NiNP2 (B), their size distribution ((C,D), respectively) (data obtained by A.G. Masyutin).

Blood biochemistry study has shown, that as a result of oral exposure of animals to NiNP1, an increase in glucose, LDL, serum albumin and globulin fraction, decrease of uric acid were noticed. The main change produced by Ni salt, included the rise in triglycerides level. Corresponding changes in rats receiving NiNP2, were absent or less pronounced with exception of protein fractions levels. Serum ALAT and AsAT activities in experimental groups stayed mainly within normal range (the results are presented in details in the poster presentation)

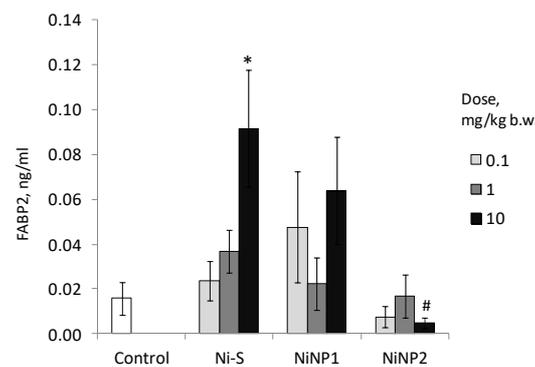
As it may be seen from Figure 2, the stores of reduced glutathione in liver and selenium reserves in rats, subjected to NiNPs, were significantly depleted and said effects were in some degree more pronounced than in Ni-salt exposed animals.

The increase of fatty acids binding protein FABP2 (Figure 3) levels in Ni salt exposed animals suggests the presence of intestinal mucosal barrier violation in these animals. Corresponding effect was less pronounced in NiNP1 exposed rats and were eventually absent in Ni-NP2 exposed.

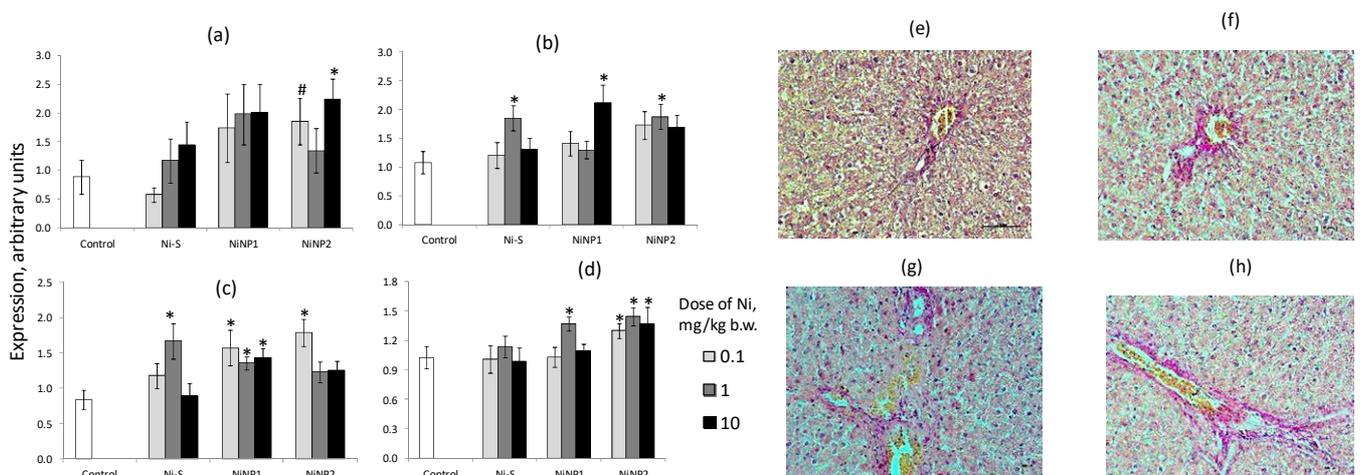
Oral exposure of rats to NiNP1 and NiNP2 resulted in increase of fibrosis and apoptosis markers expression (Figure 4). In rats receiving Ni-salt these changes were in some degree less pronounced. Light microscopy study of liver (van Gieson staining) revealed the accumulation of collagen elements in perivascular area of tissue that was somewhat more pronounced in rats receiving Ni salt and especially NiNP1 and NiNP2.



**Figure 2.** The content of thiol compounds (reduced glutathione) in rat’s liver,  $\mu\text{mol}/\text{organ}$  (a); specific urinary excretion of selenium,  $\text{ng}/\mu\text{mol creatinine}$  (b); selenium content in blood serum,  $\text{ng}/\text{mL}$  (c). On the y-axis—values ( $M \pm \text{s.e.m.}$ ) in the appropriate units. \*—difference with the control group is significant,  $p < 0.05$ , Mann-Whitney U-test. Number of animals in groups: 6 (a); 8 (b,c).



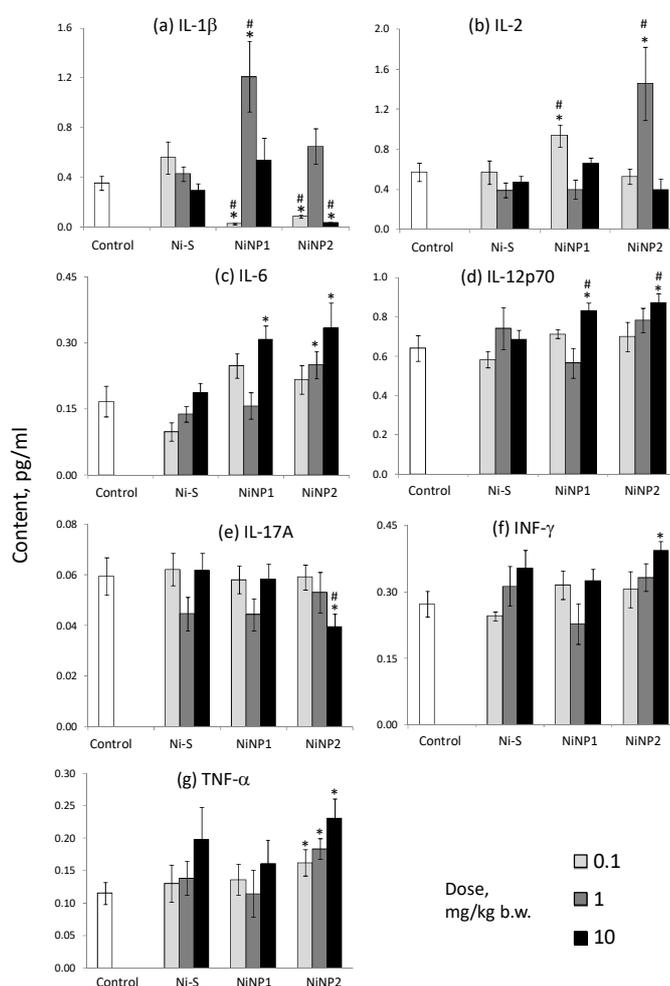
**Figure 3.** The content ( $M \pm \text{s.e.m.}$ ) of the fatty acids binding protein, FABP2, in the blood serum of rats.\*—the difference with the control group is significant; #—difference with nickel salt group is significant,  $p < 0.05$ . Mann-Whitney U-test. The number of animals—8 in each group.



**Figure 4.** Relative expression of the fibrosis genes Timp3 (a); MMP2 (b), MMP9 (c) and apoptosis factor Tp53 (d) in rat’s liver. On the y-axis - expression ( $M \pm \text{s.e.m.}$ ) in relative units. \* - difference with the control group is significant; # - difference with nickel salt group is significant,  $p < 0.05$ , Mann-Whitney U-test. The number of animals - 6 in each group. e-h. Morphology of rat’s liver: e) liver of a rat from control group; f) liver of a rat with Ni salt, 10  $\text{mg}/\text{kg}$  b.w; g) liver of a rat with

with Ni-NP1, 10 mg/kg b.w., h) liver of a rat with Ni-NP2, 10 mg/kg b.w.; staining with Fuxin-picric acid (van Gieson). Magnification  $\times 200$ .

The study showed the complex nature of the response of cytokine levels to nickel NPs consumed by rats (Figure 5). The maximum levels of IL-1 $\beta$  were achieved at the intermedial dose of Ni-NP1, IL-2 at the smallest dose of Ni-NP1 and the intermedial dose of Ni-NP2. At highest doses of both types of NPs, elevated levels of the pro-inflammatory cytokines IL-6 and IL-12p70 were observed, and in the case of Ni-NP2, also IFN- $\gamma$  and TNF- $\alpha$ . The latter was accompanied by a decrease in the levels of IL-1 $\beta$  and IL-17A. Corresponding changes under the influence of an equivalent dose of nickel salt were absent or were less pronounced. The data obtained indicate the formation of a pro-inflammatory profile of cytokines in rats exposed to nickel NPs.



**Figure 5.** The levels of cytokines in the blood serum of rats: (a) IL-1 $\beta$ ; (b) IL-2; (c) IL-6; (d) IL-12p70; (e) IL-17A; (f) INF- $\gamma$ ; (g) TNF- $\alpha$ . On the y axis—concentration, pg/mL ( $M \pm m$ ). \*—difference with the control group is significant; #—difference with Ni salt group is significant,  $p < 0.05$ , Student's  $t$ -test and/or Mann's U-test. Number of animals—8 in each group.

Morphological study of liver, small intestine and kidneys of rats demonstrated changes in liver (increase of binucleated cells and cells with disintegrated nuclei, decrease of nucleus diameter) ileal mucosa (change in villous length and villous to crypt length ratio) and kidney (glomerular edema) of rats exposed to both Ni salt and two preparations of Ni-NPs (data on the morphological examination of organs are presented in the poster presentation).

#### 4. Conclusions

The experimental data showed that the severity of the toxic effects of NiNPs depended on their size and, in some cases, were more pronounced at their low or intermediate doses than at the highest one. Some manifestations of the toxic effect of NiNPs, including immunological endpoints (cytokines levels), blood biochemistry, *Timp3* and *Tp53* expression were absent or less pronounced in animals that were subjected to a soluble Ni salt in a metal-equivalent dose. We may conclude that toxic action of NiNPs is mediated in general by Ni<sup>++</sup> ions emission from them in biological environment, but in some degree, this may be influenced by kinetic peculiarities of NPs penetration through biological barriers if compared to soluble Ni salt. The estimate for LOAEL of NiNPs is less than 0.1 mg/kg of body weight according to the endpoints studied. This indicates the need to regulate the content of nickel in nanoform in various types of products and the environment.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data available on request due to restrictions e.g., privacy or ethical.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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