# Nickel Nitrate induce apoptosis in liver of mice

Khalid H. Gathwan
Collage of Dentistry / Baghdad university

## Abstract:

Liver is a vital organ of the body engaged in various metabolic, secretary and biotransformation activities. Any damage to liver can impair any all of these functions. In the present study the toxic effects of nickel (Ni) on the liver structure of male of mice . Nickel (Ni) is an industrial and environmental pollutant of aquatic system has attracted the attention of research's all over the world. Male Balb /c mice weighing 30-32 gm, 50 days old, was treated orally with (1-12 ) mg/kg body wt., $NiNo_3$. The liver weight, histological examination of liver, along with DNA ladder for apoptosis was studied . Nickel induced both a time, and dose dependent increase in apoptotic, severity of necrosis. Liver weight, decreased with increase of dose. It has been concluded that nickel caused necrotic effect in liver and apoptotic as well as decrease liver weight.

**Key words**: Nickel , necrosis , mice, apoptosis.

## Introduction

Liver is an important organ as it performs multifarious activities like organic metabolism, cholesterol metabolism, digestive functions via bile production and secretion, clotting functions, endocrine functions, excretory and degradative functions. It biotransforms many endogenous and foreign organic molecules[1]. Nickel is present in many foods (e.g., cocoa, hydrogenated fats, ground nuts, soybeans and tea [2]. Small amounts of nickel are present in fossil fuels, and their combustion is a major source of ambient nickel levels[3,4]. The tobacco used in one cigarette contains about 1 to 4 μg of nickel[5]. Some workers have reported hepatic toxicity in animals caused by nickel salts[6-9]. In liver, areas of focal necrosis and altered bile ducts were observed. Significant increases in lipid peroxide were also observed[10]. Comparative study of toxicity of nickel salts on the liver has not been made by any earlier worker. Hence the present studies have been undertaken to see the liver weight , histopathological and apoptosis after $NiNO_3$ treated

## Material and Methods

Adult male mice, weighing 30-32 gm of Balb /c, 50 days old , were oral administered different doses of ( $NiNo_3$ ) daily for 30 days through gavages i.e. 1 mg/kg b.wt ., 6 mg/kg b.w. and 12 mg/kg of NiNo3. The animals were divided into four groups each group having 5 mice (one control group on normal diet and water).The liver weight from treated groups was taken along with control after 30 days .. Liver samples of the control and treated groups , was cut into small pieces . Fixation of liver tissue was done in Bouins Fixative , and then the samples were processed for routine wax histological evaluation . Sections of 7μm were done and stained with haematoxylin /eosin (H/E) stains as described by[ 11- 12 ]. The method[13 ], used for apoptosis, which appears as a ladder in agarose gel electrophoresis, was modified from[14] .

Statistical analysis: Results are reported as mean $\pm$ SE ,data was analyzed by using student's "t" test.

## Results

The intake of feed and water by treated mice reduced as compared to control. Moreover, the decrease was dose dependent. The liver weight significant decrease with increased of doses. (Table .1 ).

Table1: Effect of nickel nitrate on liver weight of male mice

| Group | Liver Weight (gm) | Percentage Change |
|---|---|---|
| Control | 1.65±0.05 | |
| Low dose | 1.59±0.1* | -0.62 |
| Mod. Dose | 1.4±0.11** | -12.5 |
| **High dose** | **1.33±0.12**** | **-16.87** |

Value represents mean ± S.D., n=5 in each group.
The p-value was calculated between the test group and control group.
* Non-significant different from the control value p>0.05.
** Significant different from the control value p<0.05.

No histological changes were observed in the liver of control group ( Fig-1) . With low dose of nickel nitrate the central canal increased in its area. The vacuoles and the spaces in parenchymatous

tissue showed an increase. A few granules of chromatin took dark color in haematoxylin as compared to control andhaemolysis was observed (Fig. 2 )

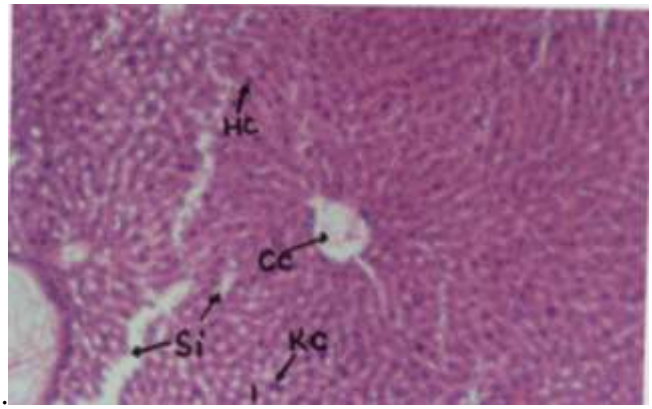


**Fig -1** : Transverse section of liver of mice treated with different doses of $NiNO_3$ .Showing Sinusoid (Si ), central canal (CC ),] hepatic cells ( HC )and kupffer cells (KC) for the **control** animals. with (H / E ) . X 100 .

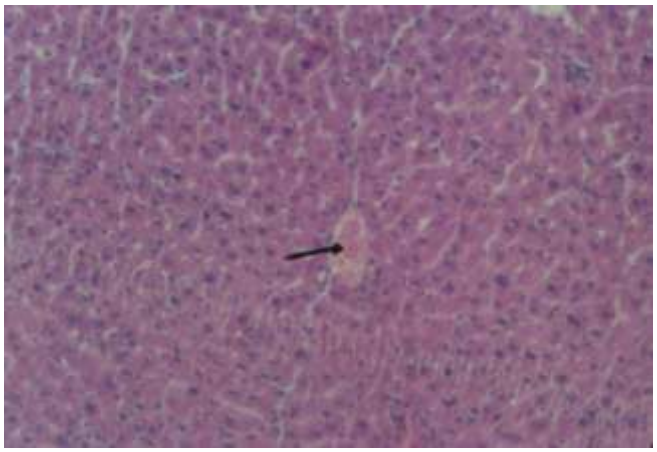

**Fig -2** : Transverse section of liver of mice treated with low doses of $NiNO_3$ . Showing damaged hepatocycles , hypertrophy of nuclei and blood in central canal (H / E ) . X 100 .

With moderate and high doses , the spaces formed after treatment had either remnants of nuclei or number of nuclei of cells. The cells became hypertrophic. At places the nuclei of blood cells aggregated in parenchymatous tissue , the spaces increased in the parenchymatous tissue of liver. The blood vessels were full of blood. These were surrounded by nuclei of broken cells (Fig. 3-4 ). The damage of hepatic cells increased with increase of dose were observed. Apoptosis was observed at 12 mg/kg body wt., Ni No3 administration (Fig.5).

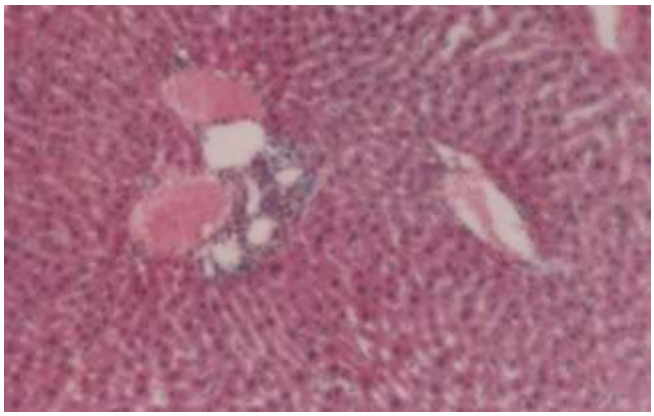

**Fig -3** : Transverse section of liver of mice treated with moderate doses of $NiNO_3$ .Showing broken cell walls, pycnotic nuclei and spaces .(H / E ) . X 100 .

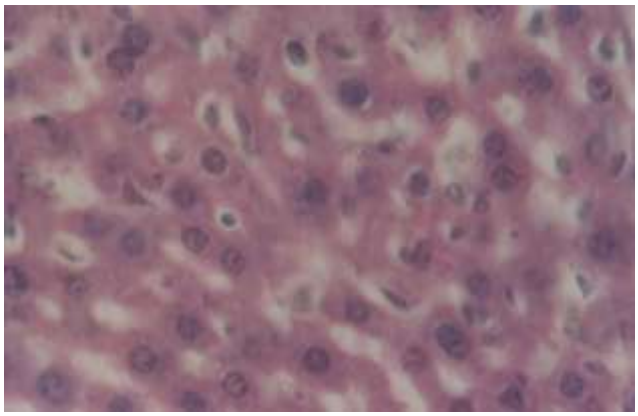

**Fig -4** : Transverse section of liver of mice treated with high doses of $NiNO_3$ .Showing broken cell walls and blood in central canal , hemolysis and severe damage to liver , pycnotic nuclei and spaces .(H / E ) . X 100 .

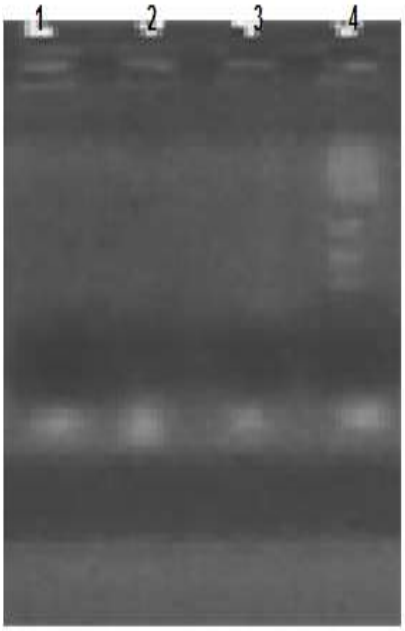


Fig.5. agarose gel analysis of liver DNA fragmentation after NiNo3 injection . DNA ladders were evident at 12mg /kg lane **4** . **lane1**, **control** mice, **lane2** ,1mg/kg, **lane3** , 6mg/kg, showed no ladder.

## Discussion

. Liver is a target organ and primary site of detoxification and is generally the major site of intense metabolism and is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms. Liver plays important role in metabolism to maintain energy level and structural stability of body[15 ] . It is also site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity[16 ] . However, this will damage the liver cells and produce hepatotoxicity.

Necrosis as a feature of acute Ni hepatotoxicity is well established. This report characterizes apoptosis in the liver, and its relationship to liver necrosis, following acute cdmium poisoning. Several approaches were applied to investigate apoptosis in the liver following ip injection of Ni into mice. The result demonstrate that apoptosis is an important mode of eliminating damaged cells in Ni hepatotoxicity. With different doses of $NiNO_3$ the sinuses broadened, some pycnosis of nuclei and necrosis of cytoplasmic contents of hepatic cells was observed. Kupffer cells showed increase in number and haemolysis along with a mass of damaged hepatic cells and nuclei were also observed. These observations are in conformity with those of Donskoy[6], who reported microvesicular fatty metamorphosis, mild hydropic degeneration and foci of inflammation. Ishimatsu[17] observed 10 times nickel concentration in liver of rats exposed to nickel in drinking water at concentration of 100 mg/litre for 6 months. RTI[18] reported low concentration of nickel in liver as compared to kidney. The histopathological changes in liver caused by nickel salts will interfere in the functions of liver as elaborated under the head ‘introduction’ and will also be responsible for decrease in liver weight as observed during present observation. These observations are in agreement with the observations made by[19] who reported a decrease in liver to body weight after oral intake of nickel with diet.

Nickel (II) induced apoptosis has been reported but findings are not consistent. An inhibitory effect of $Ca^{2+}$ and $Mg^{2+}$ induced apoptosis was observed in bovine brain-derived S-100 cells exposed to 1 μM nickel (II) [20 ] . Damaged cells stop DNA replication at $G_1$ and $G_2$ phases, presumably

allowing the repair systems to function before the next round of cell cycle damages [21 ] . Nickel (II) may induce genotoxic effects such as DNA protein crosslinking, DNA strand breaks, sister chromatid exchange and oxidative DNA base damage [22 ] .

In summary, nickel– induced liver necrosis is sustained for at least 12 mg/kg and also induced apoptosis. The early occurrence of apoptosis in acute Ni hepatotoxicity suggests a role for apoptosis in the elimination of critically injured liver cells while attempting to preserve the structural and functional integrity of the liver. From the study of effect of nickel compounds on the liver of mice it can be concluded that the toxicity of $NiNO_3$ was dose dependent

## References


1. Vander, A.J., Sherman, J.H. and Luciano, D.S.(1990 ) . The mechanisms of body function. In: Human Physiology. McGraw-Hill Pubt. Co., New York, p.580.
2. Schroeder, H.A., Balassa, J.J. and Vinton ,W.H.(1964 ) . Chromium, lead, cadmium, nickel and titanium in mice: Effect on mortality, tumors and tissue levels. *J. Nutr.*, **83**: 239-250.
3. National Research Council (NRC).(1975 ) . *Nickel*, Committee on Medical and Biological Effects of Environmental Pollutants, National Academy of Sciences, National Research Council, Washington, DC.
4. Bencko ,V.(1983 ) . Nickel: A review of its occupational and environmental toxicology, *J. Hyg. EpidemiolMicrobiolImmunol*, **27**: 237-247.
5. ATSDR.(1987 ) . *Toxicological profile for nickel,* Atlanta, 1987, Agency for Toxic Substances and Disease Registry, Department of Health and Human Services.
6. Donskoy, E., Donskoy, M., Forouhar, F., Gillies, C.G., Marzouk ,A., Reid, *et al*.,(1986 ) . Hepatice toxicity of nickel chloride in rats. *Ann. Clin. Lab. Sci.* 1986. **16**: 108.
7. Athar, M., Hasan, S.K. and Srivastava, R.C.(1987) . Evidence for the involvement of hydroxyl radicals in nickel mediated enhancement of lipids peroxidation: implications for nickel carcinogenesis. *Biochem. Res. Commun.*, **147(3)**: 1276-1281.
8. Nielsen, F.H., Myron, D.R., Givand ,S.H., Zimmerman, T.J. and Ollerich ,D.A.(1975 ) . Nickel deficiency in rats. *J. Nutr.*, **105**: 1620-1630.
9. Nielsen, F.H., Shuler, T.R., McLeod, T.and Zimmerman, T.J. (1984 ) .Nickel influences iron metabolism through physiologic, pharmacologic and toxicologic mechanisms in the rat. *J. Nutr.*, **114**: 1280-1288.
10. Yawets, A., Alter ,A. and Oron, U.(1984) . Biochemical and histological anomalies in the rat hepatic tissue following administration of bichromate and nickel in ionized form. *Toxicology*, **33**:145-155.
11. Luna ,G.L.( 1968). Manual of Histopathological Staining Methods ofthe Armed Force ( Institute Instiute of Pathology, 3rd edition. McGraw–HillCo, New York )
12. Bernet, D., Schmidt, H., Meier ,W., Burkhardt-Holm, P.andWahli , T . (1999). Histopathology in Fish: proposal for a protocol to assess aquatic pollution.
13. Kerr, J.F., winterford, C.M. and Harmon, B.V .( 1994 ). Apoptosis its significance I and cancer therapy. Cancer, 73: 2013-2026.
14. Subramanian, T., Tarodi, B. and clinadurai, G.(1995). P53- independent apoptotic and necrotic cell deaths induced by adenovirus infection suppression by EIB 19k and bcl-2 Protein . Cell growth Differ., 6: 131-137.
15. Guyton , A .C . and Hall , J .E. (2008 ) . Text book of Medical Physiology, 9th ed. Prism Book (Pvt) Ltd., Bangalore, India. pp XLiii+1148.
16. Hodgson , E . (2004 ). A textbook of modern toxicology. 3rd edition. John Wiley and Sons, Inc, New Jersey.pp 203-211..
17. Ishimatsu, S., Kawamoto, T., Matsuno, K. and Kodama, Y.(1995 ). Distribution of various nickel compounds in rat organs after oral administration. *Biol. Trace Elem. Res.*, **49**: 43-52.

18. RTI (Research Triangle Institute).(1987 ). Two generation reproduction and fertility study of nickel chloride administered to CD rats in drinking water, report submitted to Office of Solid Waste, EPA, Washington, DC.
19. Ambrose, A.M., Larson, P.S., Borzelleca, J.F. and Hennigar ,G.R. (1976 ).Long term toxicologic assessment of nickel in rats and dogs. *J. Food Sci. Technol*. **13**: 181-187.
20. Smith, M.L. and Fornace, A.J. Jr (1996). Mammalian DNA damage-inducible genes associated with growth arrest and apoptosis. *Mutat. Res.***340**: 109-124.
21. Kasprzak, K.S. (1995). Possible role of oxidative damage in metal-induced carcinogenesis. *Cancer Invest.*, **13**: 411-430.
22. Mariggio, M.A., Fulle, S., Calissano, P., Nicoletti, I. and Fano, G. (1994). The brain protein s-100 ab induces apoptosis in pc12 cells.Neurosciences ,60:2.