PROPHYLACTIC EFFECT OF VITAMIN B\textsubscript{12} AGAINST SILICON DIOXIDE NANOPARTICLES- INDUCED HEPATOTOXICITY IN ADULT MALE RATS

Shadia M. Kadry, Amany A. Osman, Aml S. Saleh* and Wafaa A. Morsy

Zoology Department, Women’s College for Arts, Science and Education – Ain Shams University, Egypt.

ABSTRACT

Silicon dioxide nanoparticles are increasingly used in various applications including agriculture, industrial, medical and cosmetics despite of their toxicity. Vitamin B\textsubscript{12} is used as an antioxidant. The aim of this work is to explore the protective effect of vitamin B\textsubscript{12} against the hepatotoxic potency of silicon nanoparticles in adult male rats. There was an increase in ALT, AST, ALP and MDA degrees. Also, there were a decrease in albumin, SOD and GSH levels. Histological, histochemical and immunochemical studies of hepatic tissue were evaluated. Silicon nanoparticles treatment increased the oxidative stress and induced hepatocytes degeneration, apoptosis and disturbance in polysaccharide, protein and collagen fibers contents when compared to the control group. However, Vitamin B\textsubscript{12} administration relieved these side effects when compared to the silicon nanoparticles- treated group. It is concluded that vitamin B\textsubscript{12} could reduce silica-induced hepatic toxicity as a potential antioxidant supplement.

KEYWORDS: Silicon dioxide nanoparticles- Hepatotoxicity- Vitamin B12- Collagen fibers-P53.

INTRODUCTION

Nanoparticles are allocated as minor particles that are proficient and formed to have structural features with at least one dimension in the range of 1-100 nm.[1] Silicon dioxide nanoparticles (SiO\textsubscript{2} NPs) are used in a wide diversity of applications. SiO\textsubscript{2} NPs are one of the top five ordinarily used nanoparticles in the nanotechnology consumer stuffs.[2]
Silicon dioxide NPs are presently being used in chemical- mechanical polishing, cosmetics, foodstuffs, printer toners and biomedical appliances.\cite{3,4} The toxic effects of SiO$_2$ NPs created on their numerous physiochemical properties, which may include lipid peroxidation, oxidative stress, and disruption of cell membrane, oxidative DNA damage, apoptosis induction, mitochondrial damage and antiproliferative activity.\cite{5}

Vitamin B$_{12}$ (cyanocobalamine) has principle roles in the treatment of different pathological conditions.\cite{6} It has anti-inflammatory, immunomodulatory, antioxidant and antioxidative stress potential actions.\cite{7} The objective of the present study is to investigate the prospective prophylactic influence of vitamin B$_{12}$ in attenuating the hepatotoxicity induced by silicon nanoparticles in adult male rats.

**MATERIALS AND METHODS**

1- **Animals**
Sixty adult male albino rats of the strain *Rattus norvegicus*, weighing 150-180g, were obtained from animal house at El- Salam Farm Giza, Egypt. They were housed in well-ventilated cages and received a standard diet and water ad libitum during the experiment. Animals were kept for one week prior to the beginning of the experiment for acclimatization.

2- **Chemicals**
SiO$_2$ nanopowder was purchased from Sigma Chemical Co., St. Louis Mo., USA. Vitamin B$_{12}$ (Vit. B$_{12}$) was obtained from Sigma Pharmaceutical Industries, Egypt.

3- **Experimental design**
Sixty adult male rats were randomly divided into six groups, ten animals each: the rats in group (1) served as control, received 0.5 ml of 0.9% saline. Animals in group (2) were treated with Vit. B$_{12}$ (0.6 mg/ kg b.w.); rats in group (3) were treated with saline for 4 weeks then given SiO$_2$ (500 mg/ kg b.w., twice a week) for another 4 weeks. In group (4) animals received SiO$_2$ along with Vit. B$_{12}$ (SiO$_2$+ Vit.B$_{12}$). Rats of group (5) were treated with SiO$_2$ for 4 weeks then Vit. B$_{12}$ for another 4 weeks (SiO$_2$ then Vit.B$_{12}$); group (6) served as protective group and rats received prior Vit. B$_{12}$ for 4 weeks then treated with SiO$_2$ accompanied by Vit. B$_{12}$ for 4 weeks. All rats were treated orally for 8 weeks, and then sacrificed at the end of the eighth week of the treatment.
Rats of different groups were weighed prior to the treatment, once weekly and again prior to sacrifice. After sacrifice, liver tissues were dissected, washed in saline, patted dry then weighed than calculated both absolute and relative liver weights.

4- **Biochemical study**

Blood samples were collected and clotted then centrifuged at 3000 r.p.m. for 10 minutes. The supernatant sera used for measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and albumin according to these methods\(^8\)\(^{-11}\) respectively. Livers were fixed in 10% phosphate-buffered, homogenized and the supernatant was collected and stored at 80\(^\circ\)C for malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione reduced (GSH) levels were determined according to these methods\(^12\)\(^{-14}\) respectively.

5- **Histological study**

At the end of experiment, liver from each sacrificed rat were dissected out; trimmed of excess fat. All tissues were fixed in 10% neutral buffered formalin and were processed for paraffin sectioning by dehydration in different concentrations of alcohol, cleared with xylol and embedded in paraffin blocks. Sections of about 5\(\mu\)m thickness were stained with hematoxylin and eosin.\(^{15}\)

6- **Histochemical and immunohistochemical studies**

For demonstration of polysaccharide contents, total protein and collagen fibers in the hepatic tissue were stained with Periodic Acid Schiff (PAS), bromophenol blue and Masson’s trichrome according to the methods,\(^{16}\)\(^{-18}\) respectively.

For immunohistochemical study, both caspase-3 and P53 were demonstrated according to the methods.\(^{19,20}\)

7- **Statistical analysis**

All values were expressed as mean ± SE for ten animals (n = 10) in each group. Significant differences between the groups were determined with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) by performing one-way ANOVA with post hoc comparisons.
RESULTS
Data for all studied parameters showed non-significant differences among the control and Vit. B_{12} treated groups. SiO_{2} group showed marked decrease in the mean final body weight, the average decrease was very highly significant (P< 0.001) in comparison with the control. On the other hand, the recorded values showed a slight and moderate increase in the mean final body weights of (SiO_{2} then Vit.B_{12}) and (SiO_{2}+ Vit.B_{12}) groups, respectively. A very highly significant increase in the mean final body weight was observed in the protective group as compared to the SiO_{2} group (Table 1).

The absolute liver weight of SiO_{2} group revealed a very highly significant increase as compared to the control. SiO_{2} then Vit.B_{12} group exhibited a slight decrease (P< 0.05) in liver weight. However, SiO_{2}+ Vit.B_{12} and protective groups displayed restrained and obvious decrease in this weight (Table 1).

It is clear from Tables (1 & 2), that there was a very highly significant increase (P < 0.001) in sera ALT, AST, ALP and tissue MDA and very highly significant decrease in serum albumin and tissue SOD and GSH levels in SiO_{2} group as compared to the control. The statistical analysis presented that there was a slight decrease in ALT, AST, ALP and MDA. While there was a significant increase in albumin, SOD and GSH levels (P< 0.05) in SiO_{2} then Vit.B_{12} group. The data presented in Tables (1&2) indicated that there was a significant decrease in ALT, AST, ALP and MDA and a significant increase in albumin, SOD and GSH in SiO_{2}+ Vit.B_{12} (P< 0.01) and protective group (P< 0.001) when compared to the SiO_{2} group.

Table 1: Effect of SiO_{2} and Vit. B_{12} on the mean final body weight, liver weight and liver function parameters in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Body wt. (g)</th>
<th>Liver wt. (g)</th>
<th>ALT (U/I)</th>
<th>AST (U/I)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>212±13.3</td>
<td>6.4±0.4</td>
<td>34±3.3</td>
<td>24.2±2.4</td>
<td>114.2±2.2</td>
</tr>
<tr>
<td>SiO_{2}</td>
<td>166±4.9</td>
<td>10.9±0.4</td>
<td>73±4.2</td>
<td>63.8±6.2</td>
<td>179.3±5.6</td>
</tr>
<tr>
<td>SiO_{2}+ Vit.B_{12}</td>
<td>195±12.8</td>
<td>7.1±0.2</td>
<td>40±2.5</td>
<td>43.8±3.9</td>
<td>140.8±7.7</td>
</tr>
<tr>
<td>SiO_{2} then Vit.B_{12}</td>
<td>187±10.6</td>
<td>8.2±0.4</td>
<td>51±5.1</td>
<td>50.2±3.4</td>
<td>152.2±5.8</td>
</tr>
<tr>
<td>Protective</td>
<td>220±22.8</td>
<td>6.5±0.3</td>
<td>25±1.9</td>
<td>25.7±1.6</td>
<td>125.3±2.9</td>
</tr>
</tbody>
</table>

a: significant value compared to the control group. b: significant value compared to SiO_{2} group.

1, 2, 3: significant values at level (p<0.05), (p<0.01), (P< 0.001) respectively.
Table 2: Effect of SiO$_2$ and Vit. B$_{12}$ on the mean albumin level, MDA and antioxidative markers in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Albumin (g/dL)</th>
<th>MDA (nmol/ g tissue)</th>
<th>SOD (U/ mg tissue)</th>
<th>GSH (nmol/ g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.83±0.2</td>
<td>1.8±0.5</td>
<td>35.8±1.8</td>
<td>0.97±0.2</td>
</tr>
<tr>
<td>SiO$_2$</td>
<td>1.93±0.1 $^a$</td>
<td>7.08±0.8 $^a$</td>
<td>18.6±0.9 $^a$</td>
<td>0.14±0.1 $^a$</td>
</tr>
<tr>
<td>SiO$<em>2$+ Vit.B$</em>{12}$</td>
<td>3.2±0.7 $^b$</td>
<td>4.75±0.9 $^b$</td>
<td>29.4±2.6 $^b$</td>
<td>0.8±0.02 $^b$</td>
</tr>
<tr>
<td>SiO$<em>2$ then Vit.B$</em>{12}$</td>
<td>2.1±0.1 $^b$</td>
<td>6.4±0.4 $^b$</td>
<td>22.7±1.7 $^b$</td>
<td>0.51±0.1 $^b$</td>
</tr>
<tr>
<td>Protective</td>
<td>3.6±0.3 $^a$</td>
<td>1.8±0.4 $^a$</td>
<td>33.6±1.9 $^a$</td>
<td>1.9±0.5 $^a$</td>
</tr>
</tbody>
</table>

a: significant value compared to the control group. b: significant value compared to SiO$_2$ group.

1, 2, 3: significant values at level (p<0.05), (p<0.01), (P< 0.001) respectively.

Histological examination of the hepatic tissue of control group revealed the normal hepatic architecture (Fig.1). Liver sections of rats treated with SiO$_2$ exhibited disorganization of the hepatic lobules, necrosis, pyknotic nuclei and congestion indicated by dilatation of the central vein (Figs. 2&3). Meanwhile, treatment with Vit.B$_{12}$ along with SiO$_2$ relatively ameliorated the histological alterations induced in the hepatic tissue more than the group was treated SiO$_2$ then treated with Vit.B$_{12}$ (Figs. 4&5), respectively. Co-administration of Vit.B$_{12}$ with SiO$_2$ caused a reduction in the degenerated hepatocytes and necrotic areas (Fig. 4). On the other hand, the protective group exhibited restoration of the hepatic configuration (Fig. 6).

(1): Control group showing branching hepatic strands (H) radiating from the central vein (CV) and separating by hepatic sinusoids (HS).

(2): Liver section of SiO$_2$ group showing dilated and congested portal vein (PV), slight inflammation (arrow head) and necrotic hepatocytes (arrows).
(3): Liver section of SiO₂ group showing necrotic areas (*), highly vacuolar degeneration hepatocytes (thick arrows), pyknotic nuclei (thin arrow), karyorrhexis nuclei (arrow heads) and karyolysed nuclei (curved arrows) could be seen.

(4): Liver section of SiO₂+ Vit.B₁₂ group showing dilated central vein (CV) with necrotic area (arrow) and few small darkly stained pyknotic nuclei (arrow heads).

(5): Liver section of group treated with SiO₂ then Vit.B₁₂ showing dilated central vein (CV) with detached epithelium, pyknotic nuclei (arrow heads) and many necrotic hepatocytes (arrows).

(6): Liver section of the protective group showing marked restoration of hepatic design.

**Figs. (1-6): Liver sections stained with H&E. (x400).**

From the histochemical study, the liver of the control group showed normal distribution of polysaccharide and total protein contents in the hepatocytes (Figs 7 & 12), respectively. Liver of rats of SiO₂ group exhibited clear decrement of polysaccharide and total protein contents (Figs 8 & 13), correspondingly. SiO₂+ Vit.B₁₂ exhibited a moderate polysaccharide decrease and total protein contents in some of the hepatocytes (Figs 9 & 14), respectively. In SiO₂ then Vit.B₁₂ group rats liver disclosed a varying degree of polysaccharide and total protein contents diminution in the hepatocytes (Figs 10 & 15), harmoniously. Finally, the protective group showed nearly normal restoration of liver polysaccharide and total protein contents distribution (Figs 11 & 16), individually.
(7): Control group showing uniform distribution of polysaccharides in the hepatic tissue around central vein (arrows).

(8): Liver section of SiO₂ group showing severe decrease in PAS +ve material in the vacuolated cytoplasm of impacted hepatocytes (arrow).

(9): Liver section of SiO₂+ Vit.B₁₂ group showing slight reduction of polysaccharide material in hepatic tissue (arrows).

(10): Liver section of group treated with SiO₂ then Vit.B₁₂ showing moderate diminution of polysaccharide contents in cytoplasm of hepatic cells (arrows).

(11): Liver section of the protective group showing nearly restoration of polysaccharide material in hepatic tissue.

**Figures (7-11): Liver sections stained with PAS (X400).**
(12): Control group showing normal distribution of hepatic proteins around central vein (CV).

(13): Liver section of SiO₂ group showing distinct reduction in protein content predominantly in damaged area (arrows), respectively.

(14): Liver section of SiO₂+ Vit.B₁₂ group showing moderate to strong reaction in protein content of hepatic cells (arrows).

(15): Liver section of group treated with SiO₂ then Vit.B₁₂ showing weak to moderate reaction of the protein content in the impacted cells around the central vein (arrows).

(16): Liver section of the protective group showing. Virtually restoration of protein content in hepatic tissue.

Figures (12-16): Liver sections stained with bromophenol blue (X400).
The control and Vit.B\textsubscript{12} groups showed normal distribution of collagen fibers around the liver central vein (Fig. 17). In SiO\textsubscript{2} group exhibited marked increase in the distribution of collagen fibers (Fig. 18). In SiO\textsubscript{2} + Vit.B\textsubscript{12} exhibited a slight increase in the amount of the collagen fibers (Fig. 19). On the other hand, liver tissue of SiO\textsubscript{2} then Vit.B\textsubscript{12} group showed moderate increase of collagen fibers (Fig. 20). Lastly, liver of the hepatic tissue of the protective group showed nearly normal restoration distribution of the collagen fibers (Fig. 21).

(17): Control group showing normal distribution of collagen fibers around central vein.

(18): Liver section of SiO\textsubscript{2} group showing marked increment in collagenous fibers around portal vein (arrows).

(19): Liver section of SiO\textsubscript{2} + Vit.B\textsubscript{12} group showing slight increase in collagen fibers (arrow).

(20): Liver section of group treated with SiO\textsubscript{2} then Vit.B\textsubscript{12} showing mild increase in collagen fibers (arrow).

(21): Liver section of the protective group showing almost normal distribution of collagen fibers.

**Figures (17-21):** Liver sections stained with Masson’s trichrome (X400).
The expression of caspase-3 in the hepatic tissue was perceived as trivial or none in the control group as seen in (Fig. 22). Positive caspase immunoreactivity was detected within the damaged hepatocytes in SiO\(_2\) group (Fig. 23). Groups (SiO\(_2\)+ Vit.B\(_{12}\)& SiO\(_2\) then Vit.B\(_{12}\)) liver presented slight and moderate pale caspase immunoreactivity as seen in (Figs 24&25). While, there was a pale immunoreactivity for caspase-3 in liver tissue of the protective group (Fig. 26).

(22): Control group showing none or minimal immunoreactivity for caspase-3 positive stain.

(23): Liver section of SiO\(_2\) group showing marked increase of caspase-3 immunoreactivity in the cytoplasm of the most damaged hepatocytes (arrows).

(24): Liver section of SiO\(_2\)+ Vit.B\(_{12}\) group showing slight increase of caspase-3 immunoreactivity mainly in damaged hepatocytes (arrows).

(25): Liver section of group treated with SiO\(_2\) then Vit.B\(_{12}\) showing moderate increase of caspase-3 immunoreactivity in many injured hepatocytes (arrows).
(26): Liver section of the protective group showing more improvement, marked decrease in caspase-3 expression immunoreactivity in hepatocytes.

**Figures (22-26): Liver sections stained for caspase-3 expression (x400).**

The expression of P53 in the hepatic tissue was perceived as slight or none in the control group as seen in (Fig. 27). The deepest expression of P53 was distinguished in SiO₂ group (Fig. 28). Groups (SiO₂+ Vit.B₁₂ & SiO₂ then Vit.B₁₂) presented minor and mild P53 expression in liver as seen in (Figs 29&30). While, it was lower expressed in the protective group (Fig. 31).

(27): Control group showing negative immunoreactivity for P53 positive stain.

(28): Liver section of SiO₂ group showing distinct increase of P53 immunoreactivity in the most impaired hepatocytes (arrows).

(29): Liver sections of SiO₂+ Vit.B₁₂ group showing marked decrease in p53 immunoreactivity in hepatocytes (arrows).

(30): Liver section of group treated with SiO₂ then Vit.B₁₂ showing moderate increase in p53 immunoreactivity in hepatocytes (arrows).
Liver section of the protective group showing faint P53 expression immunoreactivity in hepatocytes.

**Figures (27-31):** Liver sections stained for P53 expression (X400).

**DISCUSSION**

The liver is the main organ elaborated in the human being metabolism and detoxification; therefore, it is more threatened than the other organs.\[^1\] In the present study, SiO$_2$NPs administration induced hepatotoxicity in rats, demonstrated by the significantly decreased body weight and increased liver weight. This decrease may be due to food intake decrement and gastrointestinal impairment. Similarly, many studies demonstrated that the hepatotoxic substances led to severe hepatic injury with anorexia, poor appetite, vomiting or may be due to gastrointestinal disorders. That may lead to impaired food absorption in the gastrointestinal tract.\[^{21,22}\] In this study, the results indicated that there is a significant increase in absolute and relative liver weights that may be due to liver encephalopathy which led to liver hypertrophy. Also, the increase in liver weight in proportion to the body weight following streptozotocin administration to rats may be attributed to the increase in triglycerides accumulation leading to enlarged liver.\[^{23}\]

The present work also confirmed significantly increased levels of ALT, AST, ALP and MDA and decreased levels of albumin, SOD and GSH. The alterations in the liver function biomarkers may be due to accumulation of SiO$_2$NPs inside hepatocytes which led to their damage and the enzymes leak out from the necrotic hepatocytes into blood serum as previous studies.\[^{24,1}\] Also, that may be due to lipid peroxidation occurring after SiO$_2$NPs administration which is indicated by MDA increase and SOD and GSH levels decrease.\[^{25,26}\] There was a significant decrease in serum albumin that may be due to oxidative damage of SiO$_2$NPs to macromolecules as proteins.\[^{27,28}\]
Our results revealed that the exposure to SiO\textsubscript{2}NPs caused pathological alterations in the hepatic tissue as swelling, necrosis, congestion and inflammatory infiltrations\textsuperscript{[1]} Also, SiO\textsubscript{2}NPs caused decrease in polysaccharide contents which may be attributed to mitochondrial dysfunction and perturbation of the glucose homeostasis which also reflected the lost capacity of the hepatocytes to metabolize glycogen storage\textsuperscript{[29]}

SiO\textsubscript{2}NPs administration caused a significant decrease in total protein content that was reflected by low serum albumin level\textsuperscript{[21]} Also, SiO\textsubscript{2}NPs may modify mitochondrial activity leading to the strong disruption of tricarboxylic acid cycle perturbation which resulted in a reduction of protein synthesis\textsuperscript{[29]} Reactive oxygen species (ROS) are responsible for a variety of physiological and cellular events including inflammation, DNA damage and apoptosis\textsuperscript{[30,31]} Our results indicated that SiO\textsubscript{2}NPs increased the activity of P53 and pro-apoptotic caspase-3 according to their oxidative damage\textsuperscript{[32]} Previous results reported caspase activated during apoptosis in many cells and known to play a vital role in both initiation and execution of apoptosis\textsuperscript{[33]}

Concerning the effect of Vit.B\textsubscript{12}, the current study results displayed an elevation in albumin level. Vit.B\textsubscript{12} found to attenuate the liver functions and oxidative stress, decreases ALT, AST, ALP and MDA levels and increases SOD and GSH levels. Administration of vitamin B\textsubscript{12} with SiO\textsubscript{2}NPs alleviated the hepatotoxicity induced by these particles. This role was more effective in the protective group as verified in this study. This beneficial impact obtained by Vit.B\textsubscript{12} may be attributed to its ability to protect and stabilize cellular membranes by manipulating the SiO\textsubscript{2}NPs oxidative injury\textsuperscript{[34]} Also, this may be accredited to the acute antioxidant and anti-inflammatory effects. The present study indicated that Vit.B\textsubscript{12} improved the histological and histochemical alterations induced by SiO\textsubscript{2}NPs in the livers of rats. Treatment with Vit.B\textsubscript{12} alleviated the apoptotic effects of SiO\textsubscript{2}NPs when compared to SiO\textsubscript{2}NPs alone, as it partially inhibited the effect of caspase-3 and P53 levels with the preservation of cell viability caused by silica nanoparticles\textsuperscript{[35]}
REFERENCES


15. **Harris HF.** After Bruce Casselman W.C. (1959): Histochemical Technique, by Methuen and Co. LTD., 1900.


17. **Bonhag PF.** Histochemical studies of the ovarian nurse cells, tissues and oocytes of the milkweed bug, Oncopltus fasciatus (Dallas) I. Cytology, nucleic acid and carbohydrates. J Morph., 1955; 96: 381.


35. **Yadav MK, Manoli NM, Madhunapantula S.** Comparative Assessment of Vitamin-B12, Folic Acid and Homocysteine Levels in Relation to p53 Expression in Megaloblastic Anemia. PLOS., 2016; 11(10): 1-17.