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Selenium Nanoparticles Ameliorative Effect on Acetaminophen Hepatotoxicity in Male Mice

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ABSTRACT

Selenium nanoparticles (SeNPs) have been widely used as anti-inflammatory and anti-toxic agent. The present study used *Bacillus tequilensis* for biosynthesizing SeNPs from sodium selenite (Na_2SeO_3) and investigated its ameliorative effects on acetaminophen (APAP) hepatotoxicity in male mice. The results indicated that Alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) significantly elevated in mice treated with APAP, while other liver markers (total proteins and albumin) did not change. SeNPs either alone or in combination with APAP showed ameliorative effects on liver enzymes to some extents where their activities decreased to be insignificant with those of normal group. A slight variation was shown in total antioxidant capacity (TAC). Histopathologically, the hepatocytes of the mice treated with APAP showed cloudy swelling and vacuolar degeneration, while those treated with SeNPs or both SeNPs and APAP appeared more or less histologically normal. In conclusion, SeNPs can be used to improve or replace today's therapies of APAP hepatotoxicity.

1. INTRODUCTION

Selenium nanoparticles (SeNPs) have been produced by many bacterial species like *Pantoea agglomerans*, *Zooglea ramigera*, *Pseudomonas alcaliphila*, *Bacillus subtilis*, *Bacillus cereus* and *Duganella* sp. [1-3]. Selenium respiring bacteria *Sulfurospirillum barnesii*, *Bacillus selenitireducens* and *Selenihalanaerobacter shriftii* also produced SeNPs in anaerobic conditions [4]. However, production in aerobic condition is much easier than in the anaerobic condition. Anaerobic biogenesis is a tiresome and difficult process [1].

Acetaminophen (APAP) is well-known drug inducing severe oxidative stress allowing mitochondrial permeability transition in liver cells. Liver cells eliminate free radicals and face apoptosis as a result of the drug hepatotoxicity [5]. Several studies tackled the hepatotoxic effect of APAP in male and female mice and rat [6-13].

In male albino rats, SeNPs have been used as an antioxidant against the hepatic damage caused by parasitism [14] and neural and renal APAP toxicity. SeNPs also showed anti-inflammatory and analgesic effects in irradiated rats [15]. It is therefore, necessary to investigate its ameliorative role against APAP hepatotoxicity in male mice.

2. MATERIALS AND METHODS

2.1. Experimental Design and Biochemical Assays

Twenty male mice weighed 30 to 40 g were primarily divided into 4 groups (5 individuals per group). The animals were put in plastic cages, fed normally and left for acclimatization for 10 days in good ventilated room at Taif University of Western Saudi Arabia. The control mice administrated tap water. The second received APAP dissolved in water with the human therapeutic dose (75 mg/kg body weight). The third group was taken SeNPs (0.001 mol of 50 nm SeNPs dispersed in liter ddH_2O) while the fourth group was subjected to a mixture of APAP and SeNPs. After 21 days administration, blood samples were obtained from the anesthetized animals while liver tissues were taken immediately after dissection. Liver enzymes; ALT, AST and ALP and the antioxidants super oxide dismutase (SOD), total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured by kits prepared by authors cited in Dakrory *et al.* [16].

2.2. Production and Description of SeNPs

SeNPs were prepared by reducing selenium ions with *Bacillus tequilensi*. Bacterial strain was grown up in 100 ml nutrient broth incubated at 37˚C. After 18 hrs, the incubated culture was centrifuged at 7000 rpm for 10 min and washed three times. It was dispersed in 10 ml ddH_2O at pH 7.0. Addition of sodium selenite (Na_2SeO_3) was undertaken in order to make Selenium concentration to be 1 mM. Further incubation was happened for 24 hrs. Observation of the biomass turning red was a marker of convenient formation of SeNPs [17]. Centrifugation at the same conditions followed by washing was conducted to collect the bacterial biomass. The precipitate was resuspended in 5 mL Phosphate Buffer at pH 7.4 followed by ultrasonication (130 W, 10 min, Vibracell VCX-130; Sonics and Materials Inc., CT, USA). The suspension was filtered with 0.45 mm and 0.2 mm pore size cellulose acetate filters (Advantec, Tokyo, Japan). Again, centrifugation of the filtrates was done at 10,000 rpm, 4˚C for 30 min and the pellet was suspended in 5 mL of ultrapure water for further study. Characterization of the formed particles was conducted [3, 18]. The produced SeNPs were described by UV-Vis spectroscopy (Perkin Elmer, Lambda 25) with scanning range of 200 - 900 nm at 1nm resolution.

2.3. Transmission EM (TEM) and X-Ray Diffraction (XRD) Analysis

After centrifugation, a drop of the synthesized SeNPs was placed on the carbon coated copper grids and kept under vacuum desiccation overnight before loading onto a specimen holder. Size, morphology and composition were recorded by TEM operated at 120 k accelerating voltage (JTEM 1230, Japan, JEOL) with selected area electron diffraction. XRD analysis was conducted by an automated diffract meter (Philips type: Pw1840) at 0.02 step size, 20 in 2^{θ}/min scanning rate and a 2^{θ} range from 100 to 700. Powder patterns indexing and unit cell parameters least squares fitting using the software X'Pert High score Plus was undertaken.

2.4. Liver Histology

For the histological preparations of liver tissues, fixation, preservation, embedding, dehydration,

deparaffinization, rehydration was conducted as described by Amer *et al.* [19]. H&E staining was undertaken according to protocols of Bancroft *et al.* [20]. Photomicrographing [19] was also done.

2.5. Statistics

The results were statistically manipulated by ANOVA packaged in SPSS version 11.0. LSD was conducted for the paired comparisons.

3. RESULTS AND DISCUSSION

Observation of the culture incubated with (Na_2SeO_3) showed a change from yellow to red (**Figure 1**) which is characteristic for SeNPs. The red color [3] which was due to the excitation of surface plasmon vibrations in SeNPs provided a convenient spectroscopic signature of the nanoparticles formation [1, 4]. No color change could be demonstrated in cultures without selenium dioxide as negative control. The UV-Vis absorption spectra recorded a strong resonance at 335 nm (**Figure 1**). However, no absorption peak appeared after addition of salts indicating that a constituent of the culture medium was not involved in SeNPs.

The biosynthesized SeNPs shown in TEM image were spherical and polydispersed with average diameter range of 10 - 55 nm (**Figure 2(a)**). *Bacillus megaterium* and *Bacillus cereus* reduced selenite to SeNPs after 40 hrs [1, 21].

Bacillus sp. MSh-1 produced spherical SeNPs with 80 nm diameter [3, 18]. We consider this study the first that reported the formation of small SeNPs by *Bacillus tequilensis*. The XRD analysis for the biosynthesized selenium indicated three intense peaks in the whole spectrum of 2q values ranging from 5 to 80 (**Figure 3**). The diffractions peak at 2q value of 23.780, 29.797 and 43.878 can be indexed to the (100), (101) and (102) planes of the face-centered cubic (fcc) red elemental selenium, respectively [21].

As AL-Harabi *et al.* [22] concluded, liver was the main tissues clearly influenced significantly by nonoparticles treatment. ALP, AST and ALT were increased significantly ($p < 0.01$) in the group received APAP indicating the toxic effect of the drug. Several investigations accumulated similar findings [6-13].

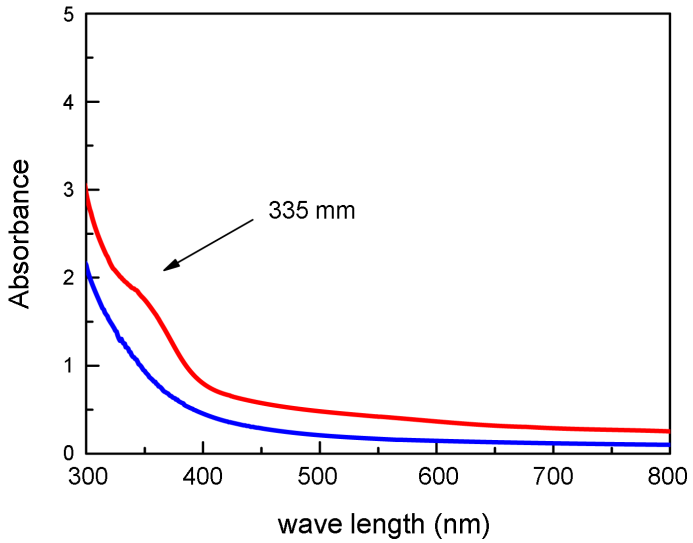
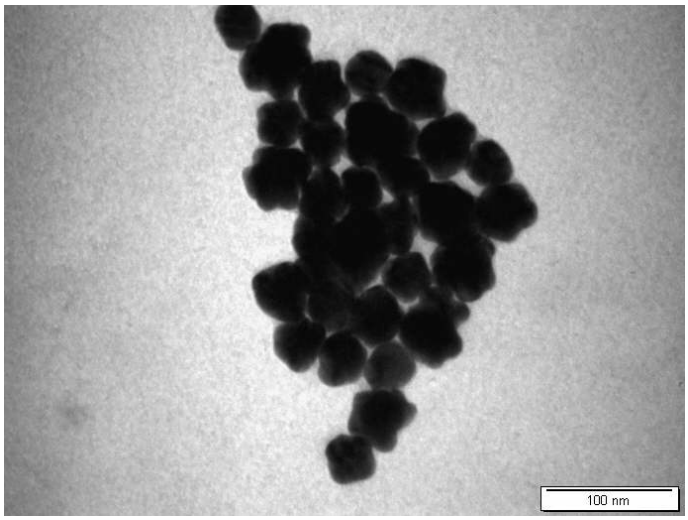
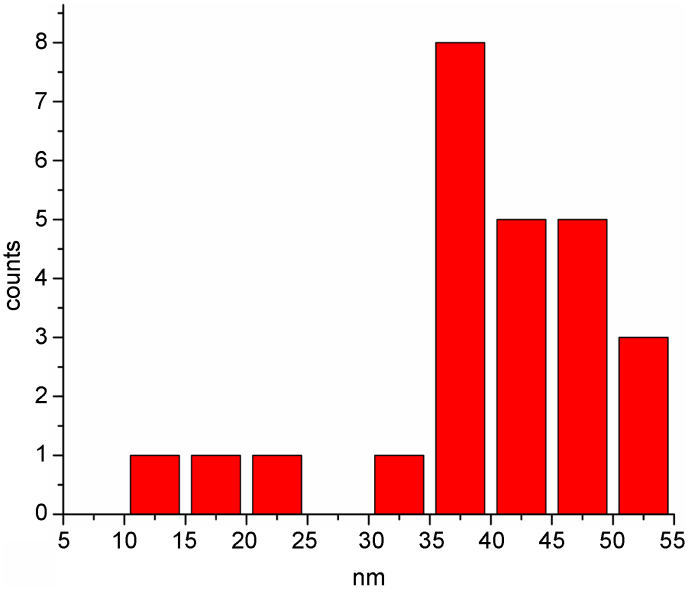


Figure 1. UV-Visible absorption spectra of bacterial synthesized SeNPs. The typical surface plasmon resonance (SPR) band is shown at 335 nm (red line). The blue line refers to the spectrum of the negative control.



(a)



(b)

Figure 2. TEM micrographs recorded from drop-cast films of bacterial synthesized SeNPs (a) and its particle size distributions (b).

The enzymes activity in the groups treated with SeNPs immediately after APAP supplementation or in combination with APAP showed normal enzyme activity with no significant difference to that of the control (Table 1). Unfortunately, the two main liver markers (albumin and total proteins) did not exhibit significant variations in either control or treated groups. Similar findings were revealed by Oleaga *et al.* [23] where, doxorubicin which is considered hepatotoxic, did not show any effect on albumin. No significant influence in the liver antioxidants has been recorded, except slight changes ($p > 0.05$) in the group treated with APAP and SeNPs in MDA and in SeNPs group for TAC. This can be interpreted with the toxicity of SeNPs that enhances the liver cells to eliminate free radicals [24]. In conclusion SeNPs, with very low concentration, generally protected liver against oxidative damage induced by APAP in mice.

Liver histology of the control group appeared more or less normal (Figure 4). The organ was divided

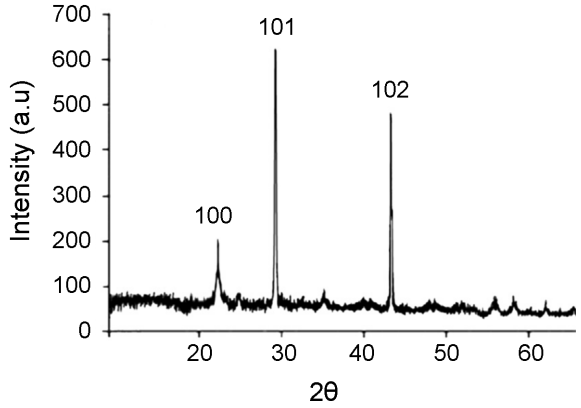


Figure 3. XRD pattern of the bacterial SeNPs. The characteristic strong diffraction peak located at 29.79 u is ascribed to the (101) facets of the face-centered cubic elemental Se structure.

Table 1. Mean ± SE of hepatic enzymes and antioxidant activities in male mice supplemented APAP, SeNPs, APAP + SeNPs for 21 days. Significant differences between was (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) among the different treatments according to LSD of ANOVA test. Symbols for significant differences were as follows: ♦♦ (between control and APAP groups), ++ (between APAP and SeNPs groups), •• (between APAP and APAP + SeNPs groups), $$ (between Control and APAP + SeNPs groups) and ¥ (between APAP and SeNPs groups). Symbols for significant differences of antioxidants (malondialdehyde = MDA, total antioxidant = TAC and superoxide dismutase = SOD) were as follows: ‡ (between APAP and SeNPs groups), ♥ (between control and APAP + SeNPs groups). *refers to significance difference estimated by ANOVA among groups.

Variable	C	APAP	SeNPs	APAP + SeNPs	f-value
ALP (U/L)	23.03 ± 3.2	36.06 ± 3.3, ++, ••	26.3 ± 1.2	17.8 ± 2.2	7.3**
AST (U/L)	86.1 ± 16.3	226.4 ± 8.9, ++, ••	123.9 ± 27.2	84.9 ± 3.5	14.5***
ALT (U/L)	75.6 ± 15.9	153.3 ± 6.9, ++, ¥	87.7 ± 11.2	117.1 ± 9.4$$	14.4***
Albumin (g/dl)	4.1 ± 0.5	4.2 ± 0.1	3.5 ± 0.4	4 ± 0.1	0.74
Protein (g/dl)	11.7 ± 1.4	10.2 ± 1.2	11.5 ± 0.7	10.4 ± 0.2	0.48
MDA (nmol/g)	1289.5 ± 164.2	1965.8 ± 149.3	1658.8 ± 120.7	2229 ± 432♥	2.9
TAC (mM/g)	1.5 ± 0.08	1.4 ± 0.05	1.6 ± 0.0‡	1.6 ± 0.01	3.6*
SOD (U/g)	1904.7 ± 47.6	7642.8 ± 829.7	6499.9 ± 4647.2	4071.5 ± 928.5	0.47

into hepatic lobules in which the central vein was centrally located and surrounded by hepatic columns. The hepatocytes appeared polyhydral with centrally located vesicular nuclei. Hepatic sinusoids were seen between the hepatic columns. Van kupffer cells were frequently seen. The hepatocytes of the mice treated with APAP had cloudy swelling and vacuolar degeneration (Figure 5(a)). Pericentral necrosis was seen in most cases in which the hepatocytes showed strongly eosinophilic cytoplasm with pyknotic nuclei (Figure 5(b)). Centrolobularly, solitary cells underwent pyknosis in some areas. The blood vessels were dilated and engorged with blood. The hepatic sinusoids were dilated in some areas with mild proliferation of Von

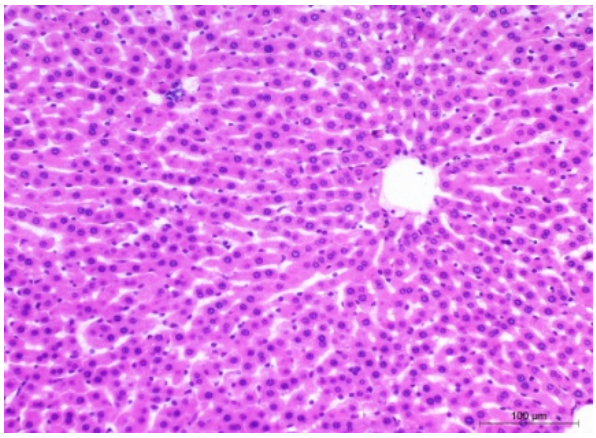
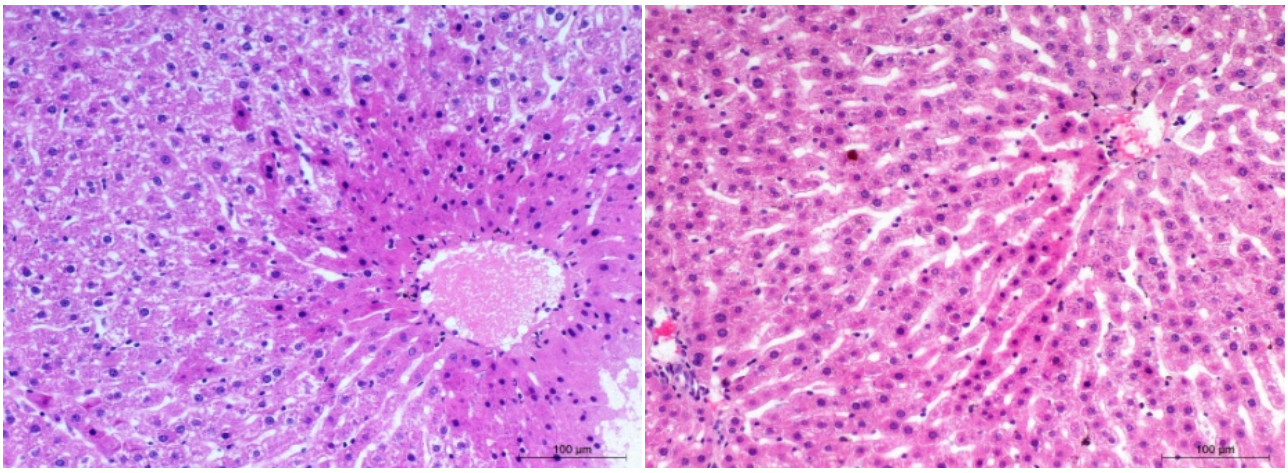


Figure 4. H&E stained photomicrograph of liver from a control group rat.



(a) (b)

Figure 5. H&E stained photomicrographs of rat liver treated with APAP that are showing degenerative changes (a) and hepatocytes with pyknotic nuclei (b).

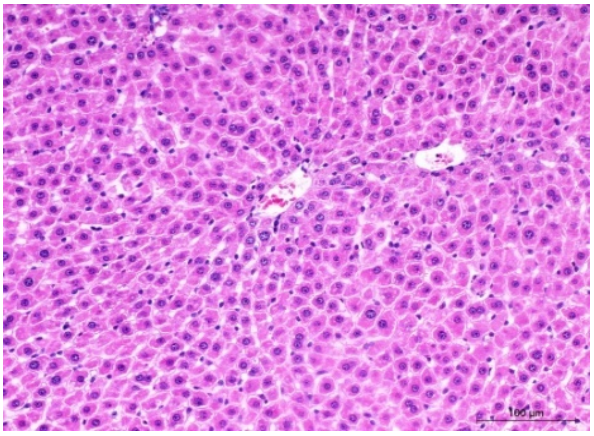


Figure 6. H&E stained photomicrograph of liver for rat treated with SeNPs.

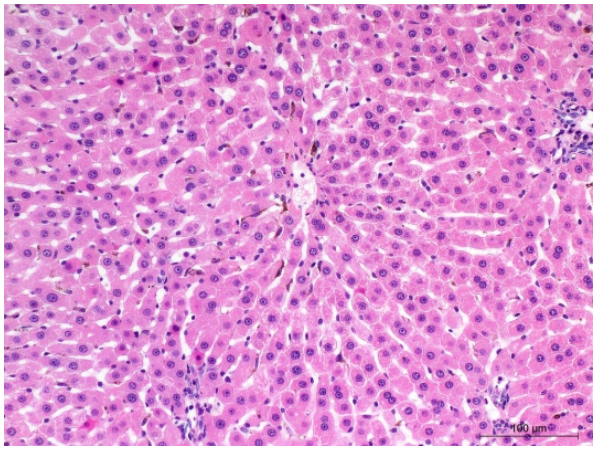


Figure 7. H&E stained photomicrograph of normal liver for rats treated with both APAP and SeNPs.

kupffer cells. The liver of mice treated with SeNPs appeared more or less histologically normal (**Figure 6**). No necrotic changes had been recorded. The hepatic lobules were well organized. In some areas, a few cells were very mildly degenerated but without toxic changes. Compared to APAP group, the liver of mice treated with both APAP and SeNPs were markedly improved and the liver appeared normal to more extent. Few cells were still showing very mild degenerative changes and pyknosis (**Figure 7**). The blood vessels were mildly dilated and congested. No necrotic changes could be seen. Similar damage for liver tissues by APAP has been recorded [25-28]. SeNPs showed also ameliorative effect, histologically, on hepatotoxicity induced by drugs or parasites in several investigations [14, 29].

4. CONCLUSION

In conclusion, SeNPs can be used to replace today's therapies of APAP hepatotoxicity as the liver enzymes become normal after treatment with the synthesized nanoparticles. Meanwhile, liver histology showed an improvement by SeNPs administration.

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