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Modification of nanocellulose by poly-lysine can inhibit the effect of fumonisin B1 on mouse liver cells

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ABSTRACT

Fumonisin B1 is an important mycotoxin, mainly produced by *Fusarium verticillioides*. It has toxic effects on liver, brain, and kidney cells. The first aim of this study was to synthesize nanocellulose modified with poly-lysine (NMPL), and the second aim was to evaluate the adsorption of fumonisin B1 by NMPL. As third aim, the function of mouse liver cells was investigated after exposure to fumonisin B1, and fumonisin B1+ NMPL. In this study, NMPL was prepared using cross-linker, and then incubated with fumonisin B1 at controlled conditions. After incubation, the adsorption and release of fumonisin B1 were evaluated in each condition. Next, mouse liver cells were separately exposed to fumonisin B1, NMPL, and (fumonisin B1+NMPL). Then, the level of aniline aminotransferase (ALT) and aspartate aminotransferase (AST) was evaluated. It was found that both adsorption and release of fumonisin B1 were not affected by temperature and incubation time, but affected by pH and concentration of NMPL. Also, this study showed NMPL could adsorb fumonisin B1 in different foodstuffs. Importantly, although the levels of ALT and AST were increased when the cells were treated with fumonisin B1 alone, they were not affected when exposed to NMPL or (fumonisin B1+NMPL). The authors suggest that NMPL is a good adsorbent to remove and inhibit fumonisin B1.

1. Introduction

Fumonisin B1 is an important mycotoxin which mainly produced by *Fusarium verticillioides*. The fungus is able to grow on a variety of foodstuffs and causes food spoilage [1,2]. Nowadays, food contamination with fumonisin B1 is a global problem. The experiments which have been done on animals and human show that fumonisin B1 has toxic effects on liver, brain, and kidney [3,4]. Moreover, fumonisin B1 can inhibit ceramide synthase (sphingosine N-acyltransferase) in liver and nerve cells [5]. Notably, the inhibition of ceramide synthase leads to increase of sphingosine and sphinganine, and decrease of sphingolipid complexes [6]. Importantly, the toxin can cause neural tube defects and esophageal cancer [7,8].

Based on previous studies, several procedures have been reported to remove fumonisin B1, including: (1) the use of chemical inhibitors (for example, organic acids, organic acid salts, copper sulfate, etc.), (2) the use of physical treatments (for example, drying, heating, etc.), and (3) the use of adsorbents (for example, aluminosilicates, montmorillonite, etc.) [9–11]. Among them, toxin adsorbents are the best choice, but it must be mentioned that all of them decrease the quality of foodstuffs. Moreover, they are not specific, and may be saturated by other chemical compounds. On the other hand, they are not completely safe, because some of them contain heavy metal oxide particles (e.g., aluminum oxide and titanium oxide) [12].

The aim of this study was to design a novel adsorbent, based on nanocellulose modified with poly-lysine (NMPL), to adsorb and inhibit fumonisin B1. First, the adsorption of fumonisin B1 by NMPL and its release were evaluated at different conditions. Then,

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the function of mouse liver cells was evaluated after exposure to fumonisin B1, NMPL, and (fumonisin B1+NMPL).

2. Materials and methods

2.1. Materials

The raw cellulose was purchased from My Baby Company, in order to synthesis of nanocellulose. RPMI1640 medium, N-ethyl-N-(dimethylaminopropyl) carbodiimide (EDC), citric acid were provided from Sigma–Aldrich Company (St Louis, MO, USA). Sulfuric acid, sodium hydroxide (NaOH), and dimethylsulfoxide (DMSO) were purchased from Merck, Germany. In this study, poly-lysine (PL) and fumonisin B1 were provided from Zyst Fannavar Shargh Company, Iran.

2.2. Synthesis of nanocellulose and conjugation with PL

Acid hydrolysis was used to synthesize nanocellulose, according to our previous work [13]. Briefly, 100 mg of raw cellulose was treated with 5 M NaOH at 37 °C for 1 h, and then washed with distilled water (DW). Next, washed cellulose was treated with 1 M DMSO at 37 °C for 1 h, and rinsed with DW. Then, one mL of 70% sulfuric acid was added to the washed cellulose, and hardly mixed, in order to complete hydrolysis. In the next step, one mL of 5 M NaOH was gently added to hydrolyzed cellulose. Then, synthesized nanocellulose was centrifuged at 5000 rpm for 5 min, and washed by DW. For conjugation, 10 mL of nanocellulose at concentration of 500 μg/mL was added to 10 mL of 5% (w/w) citric acid, and incubated for 15 min at 100 °C. After incubation, carboxy-nanocellulose was rinsed with DW by centrifugation at 5000 rpm for 5 min. In the next step, 10 mL of 100 mg/mL PL and 1 mL of 100 mg/mL EDC were added to 0.1 g of carboxy-nanocellulose, and incubated at 37 °C for 30 min. Finally, NMPL was centrifuged at 5000 rpm for 5 min, washed by DW. The final concentration was adjusted to 2000 μg/mL, and stored at 4 °C. The characterization of NMPL was done by scanning electron microscopy (SEM) (Hitachi, S-2400) and Fourier transform infrared spectroscopy (FTIR) (Bruker, UK) [13]. To obtain size distribution of NMPL, 5 SEM images were observed, and the average of particles was recorded.

2.3. Adsorption of fumonisin B1 by NMPL at controlled conditions

Different conditions (concentration of NMPL, temperature, incubation time, and pH) were investigated. In this study, 7 groups were considered as following:

Group 1: one mL of serial concentrations of NMPL (2000, 1000, 500, 250, and 125 μg/mL) was separately added to one mL of 1000 ng/L fumonisin B1 at pH 7, and separately incubated for 60 min at 4 °C, 25 °C, and 37 °C.

Group 2: one mL of serial concentrations of NMPL (2000, 1000, 500, 250, and 125 μg/mL) was separately added to 1 mL of 1000 ng/L fumonisin B1 at pH 7, and separately incubated at 37 °C for 1 h, 2 h, and 3 h.

Group 3: one mL of serial concentrations of NMPL (2000, 1000, 500, 250, and 125 μg/mL) was separately added to 1 mL of 1000 ng/L fumonisin B1 at 37 °C and separately incubated at for 60 min at pH 2, pH 7, and pH 9.

Group 4: one mL of NMPL (500 μg/mL) was added to one mL of 1000 ng/L fumonisin B1, and separately incubated at pH 5, pH 7, and pH 9 for 60 min at 4 °C, 25 °C, and 37 °C.

Group 5: one mL of NMPL (500 μg/mL) was added to one mL of 1000 ng/L fumonisin B1, and separately incubated at pH 5, pH 7, and pH 9 at 37 °C for 1, 2, and 3 h.

Group 6: one mL of NMPL (500 μg/mL) was added to one mL of 1000 ng/L fumonisin B1, and separately incubated at 4 °C, 25 °C, and 37 °C for 1, 2, and 3 h at pH 7.

Group 7: one mL of serial concentrations (1000, 500, 250, 125, 62.5 ng/L) of fumonisin B1 was separately mixed to 1 g of each foodstuff including maize, wheat, rice, and cucumber. After incubation (30 min at 37 °C), one mL of 2000 μg/mL of NMPL was added to each tube, mixed, and incubated at 37 °C for 1 h.

Note, each group had separate negative and positive controls. In the negative control, DW was used instead of NMPL. But, DW was applied instead of fumonisin B1, in positive control. The other incubation conditions were similar to others.

After incubation, all tubes were centrifuged at 5000 rpm for 5 min, and the optical density (OD) of supernatant was measured by UV–Visible spectrophotometer (Clinic II, USA) at 340 nm. Then, the percentage of adsorption was calculated by the adsorption equation formula [14,15].

2.4. Adsorption equation formula

The percentage of adsorption (%) = (A − B) × 100/(A).where *A* is the OD of negative control tube, and *B* is the OD in test tube.

2.5. The release study

To find the effect of different parameters on the release of fumonisin B1 after adsorption, the release study was done. First, one mL of 1000 μg/mL NMPL was added to one mL of 1000 ng/L fumonisin B1, and incubated for 1 h at 37 °C. After incubation, NMPL was centrifuged at 5000 rpm for 5 min, and then washed three times by DW. This step was to remove any free fumonisin B1. Then, one mL of DW was added to tube containing NMPL and adsorbed fumonisin B1, and separately incubated for 1, 2, and 3 h at 37 °C. In the second experiment, the release was separately evaluated after incubation for 1 h at 4 °C, 25 °C, and 37 °C. In the third experiment, the tubes were separately incubated for 1 h at 37 °C, and their pH was adjusted to 5, 7, and 9. Finally, all tubes were centrifuged and the OD of supernatant was read. As negative control, all steps were done, but NMPL was not used. As positive control, all steps were done, but fumonisin B1 was not applied.

2.6. Preparation of mouse liver cells

In this study, two male Balb/c mice, weight 18–20 g, were enrolled. After general anesthesia, their liver was isolated, cut, and rinsed in normal saline. Then, 10 mL of 1 mg/mL trypsin-5%EDTA was added to liver pieces, and incubated at 37 °C for 10 min. After incubation, the liver pieces were crushed with a mortar and pestle. Then, the crushed tissues were centrifuged at 5000 rpm for 15 min, and removed their supernatant. Next, 10 mL fresh RPMI1640 was added, mixed, and centrifuged at 1500 rpm for 15 min. At end, the density of cells was adjusted to 10^4 cells/mL by adding RPMI1640. Note, the experiments related to mice were in compliance with the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

2.7. The effect of NMPL on liver cell enzyme

Three groups were included for this section:

(1) 100 μL of serial concentrations (125, 250, 500, 1000, and 2000 ng/L) of fumonisin B1 was separately added to 100 μL of Balb/C mouse liver cells (10^4 cells/mL), and then incubated for 24 h at 37 °C.
(2) 100 μL of serial concentrations (125, 250, 500, 1000, and 2000 μg/mL) of NMPL was separately added to 100 μL of Balb/C

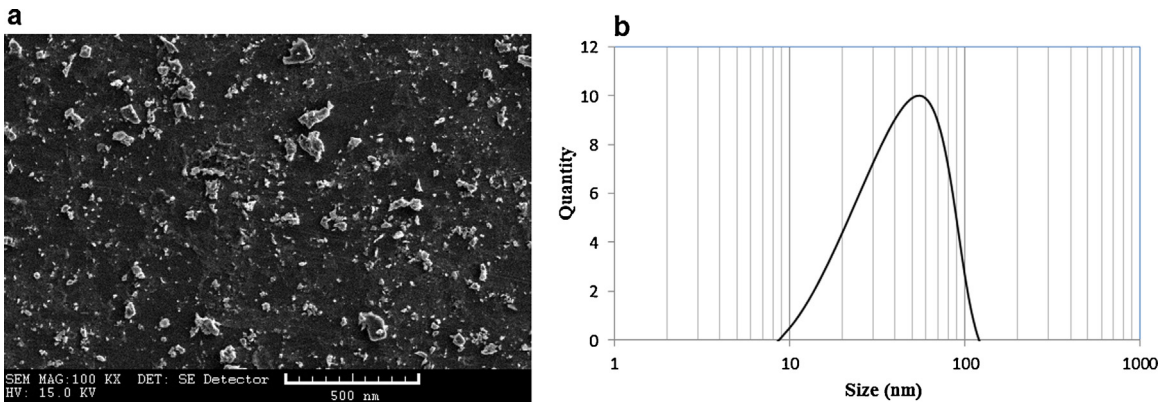

Fig. 1. (a) The SEM image and (b) the size distribution of nanocellulose modified with poly-lysine (NMPL).

mouse liver cells (10^4 cells/mL), and then incubated for 24 h at 37 °C.

(3) 50 μL of serial concentrations (250, 500, 1000, 2000, and 4000 ng/L) of fumonisin B1 was separately added to 50 μL of 1000 μg/mL NMPL, and incubated for 1 h at 37 °C. Then, 100 μL of Balb/C mouse liver cells (10^4 cells/mL) was added to each tube, and then incubated for 24 h at 37 °C.

After incubation, the tubes were centrifuged at 3000 rpm for 5 min. Finally, the level of aniline aminotransferase (ALT) and aspartate aminotransferase (AST) in supernatant was measured by Pars Azmoon kit, Iran. In the negative control, the cells were not exposed to NMPL and fumonisin B1 [16].

2.8. Statistical analysis

All tests were done 3 times, and the results are shown as the mean ± standard deviation (SD). Parametric test (ANOVA) was applied to detect the significant difference. This test was carried out by SPSS software (V.16.0 for Windows; SPSS Inc., USA), and $P < 0.05$ was considered as a significant difference.

3. Results

3.1. Characterization of NMPL

Fig. 1a shows the SEM image of NMPL. As seen, NMPL has different shapes and sizes. Based on Fig. 1b, its size distribution is about 10–120 nm. The FTIR peaks of nanocellulose (I), PL (II), and NMPL (III) are demonstrated in Fig. 2. The sharp peaks are seen at 3553, 3432, 3311, 3000, 2921, 1683, 1611, 1531, 1403, 1039, 929, and 570 cm^{-1} for nanocellulose. But PL has some peaks at 3534, 3386, 2936, 2905, 1652, 1463, 1433, 1342, 1138, 1096, 1032, 903, 778, and 608 cm^{-1}. As seen, sharp peaks at 3526, 3386, 2928, 2902, 1652, 1452, 1342, 1141, 1088, 876, 782, 604 cm^{-1} are seen for NMPL. As demonstrated, PL and NMPL are approximately similar which it indicates the good conjugation.

Fig. 3a shows the chemical model of NMPL (I), fumonisin B1 (II), and the adsorption of fumonisin B1 by NMPL. These models were built by HyperChem Professional 8.0.3. Fig. 3b demonstrates the schematic reaction between carboxy-nanocellulose and PL, catalyzed by EDC.

3.2. The effect of various parameters on the adsorption

Fig. 4a and b shows the effects of (incubation time-concentration) and (temperature-concentration), respectively. Fig. 5a and b shows the effects of (pH-concentration) and (pH-temperature), respectively. The effects of (pH-incubation time) and (temperature-incubation time) on the adsorption are shown in Fig. 6a and b, respectively. As seen, the increase of incubation time and temperature does not lead to increase of adsorption. Only, there is a significant difference between 1 h and 3 h at concentration of 2000 μg/mL (P value = 0.05). In case of pH, the adsorption is decreased with increase of pH. In same concentration of NMPL, significant differences are observed between pH 9 vs. pH 7, and between pH 9 vs. pH 5 (P value = 0.01). Table 1 shows the adsorption of fumonisin B1 by NMPL in different foodstuffs. As shown, the quantity of adsorption is related to concentration of fumonisin B1. It means that the more concentration of fumonisin B1, the more adsorption. As seen, the highest adsorption was 95% for cucumber.

3.3. Release of fumonisin B1 after adsorption

This part of study was designed to investigate the effect of incubation time, temperature, and pH on the release of fumonisin B1. It was found that the increase of incubation time and temperature did not change the release of fumonisin B1. On the other hand, the increase of pH from 7 to 9 increased the release of fumonisin B1, up to 21 ng/L.

3.4. Liver cell enzymes

Table 2 shows the level of ALT and AST after exposure to fumonisin B1, NMPL and (fumonisin B1+NMPL). As seen, the concentration of ALT and AST is increased when the cells are treated with fumonisin B1. Moreover, this effect is dos-dependent. Interestingly, the levels ALT and AST are not change when the cells exposed to NMPL alone or (fumonisin B1+NMPL). There are significant differences between levels of enzymes when the cells treated with fumonisin B1 vs. NMPL and (fumonisin B1+NMPL) ($P = 0.05$).

Table 1
The adsorption of fumonisin B1 by nanocellulose modified with poly-lysine (NMPL) in different foodstuffs.

	Tube 1[a]	Tube 2	Tube 3	Tube 4	Tube 5
Wheat	80 ± 5[b]	75 ± 2	62 ± 3	44 ± 2	20 ± 2
Rice	81 ± 4	74 ± 5	62 ± 4	42 ± 1	22 ± 2
Maize	85 ± 5	74 ± 2	65 ± 2	44 ± 1	22 ± 1
Cucumber	95 ± 4	80 ± 3	73 ± 2	62 ± 2	50 ± 1

[a] The concentration of fumonisin B1 in tube 1, 2, 3, 4, and 5 is 1000, 500, 250, 125, 62.5 ng/L.
[b] Data are M ± SD, $n = 3$ for each experiment.

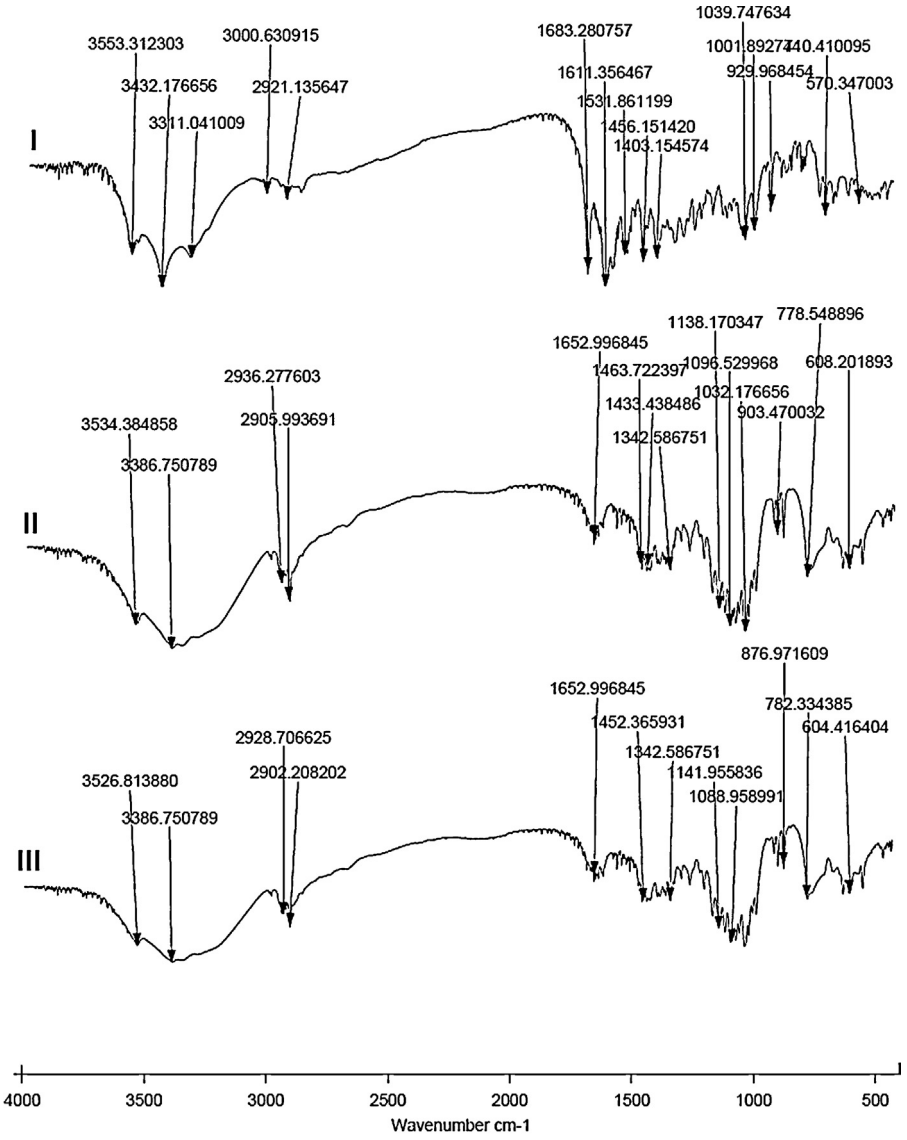

Fig. 2. The FTIR spectrum of nanocellulose (I), poly-lysine (PL) (II), and nanocellulose modified with poly-lysine (NMPL) (III).

4. Discussion

In this study, nanocellulose was synthesized, modified by citric acid, and then conjugated with PL. As seen, EDC links the carboxyl groups of carboxy-nanocellulose and amine groups of PL (Fig. 3b). IUPAC name of PL is Poly[imino(2S)-2-amino-1-oxo-1,6-hexanediyl. Its molecular formula is $(C_6H_{12}N_2O)_n$, and its molar mass is 4700 g/moL (degree of polymerization = 30). Melting point of PL is 172.8 °C or 445.9 K. In this study, molecule weight of PL was near 70,000 Dalton.

Table 2
The level of aniline aminotransferase (ALT) and aspartate aminotransferase (AST) after exposure to fumonisin B1, nanocellulose modified with poly-lysine (NMPL), and (fumonisin B1+NMPL).

	0	62.5	125	250	500	1000
Exposed to NMPL (μg/mL)						
ALT (U/L)	10 ± 1[a]	10 ± 2	11 ± 1	10 ± 2	10 ± 1	11 ± 1
AST (U/L)	50 ± 2	51 ± 2	50 ± 3	50 ± 2	51 ± 3	51 ± 2
Exposed to NMPL (μg/mL)						
ALT (U/L)	10 ± 1[a]	52 ± 3*	230 ± 20*	518 ± 25*	618 ± 25*	754 ± 27*
AST (U/L)	50 ± 2	84 ± 3*	91 ± 5*	185 ± 6*	253 ± 18*	326 ± 21*
Exposed to (fumonisin B1+NMPL) (ng/L)[b]						
ALT (U/L)	10 ± 1	10.1 ± 1	11 ± 1	10 ± 1	11 ± 1	11 ± 1
AST (U/L)	50 ± 1	51 ± 3	51 ± 3	50 ± 2	52 ± 3	51 ± 1

[a] Data are M ± SD, $n = 3$ for each experiment.
[b] Final concentration of fumonisin B1.
* $P < 0.05$ when compared with NMPL and (NMPL+fumonisin B1).

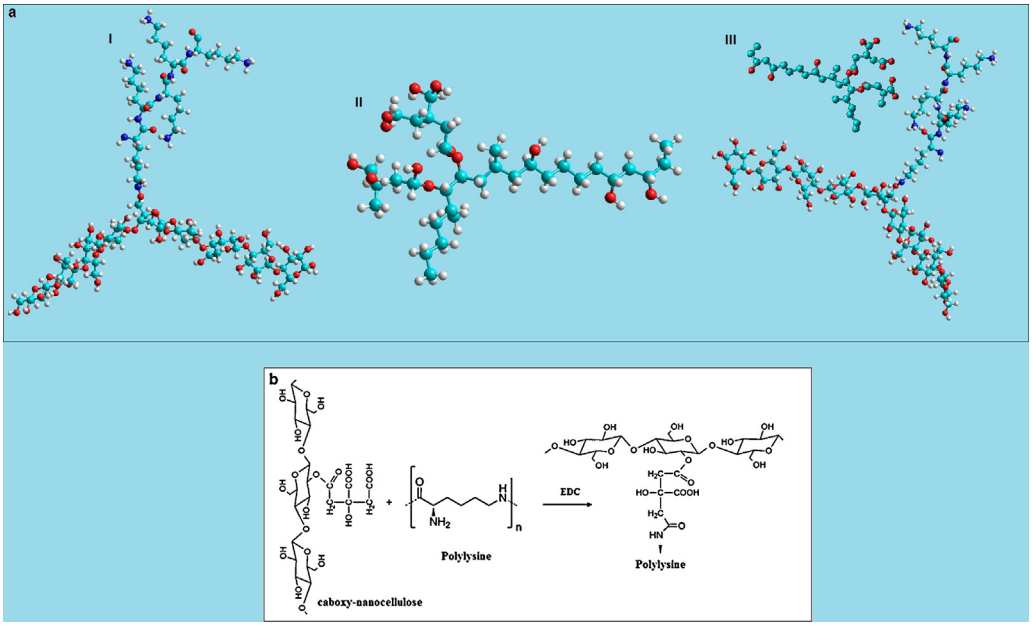

Fig. 3. (a) The chemical model of nanocellulose modified with poly-lysine (NMPL) (I), fumonisin B1 (II), and adsorption of fumonisin B1 by NMPL (III). These models were designed by Hyperchem Professional 8.0.3. (b) The schematic of reaction between carboxy-nanocellulose and poly-lysine, catalyzed by EDC.

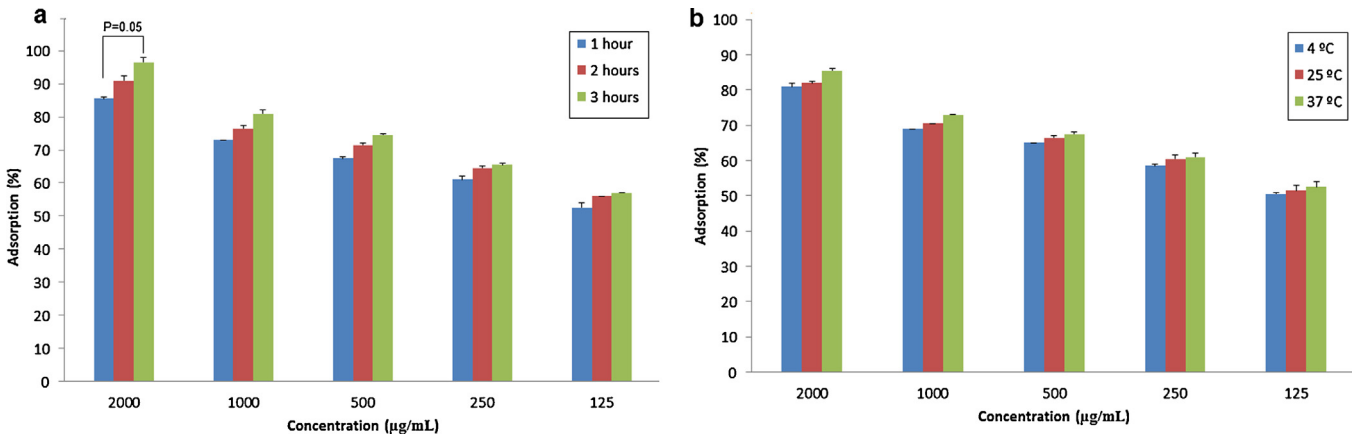

Fig. 4. The effect of (a) (incubation time-concentration) and (b) (temperature-concentration) on the adsorption of fumonisin B1 by nanocellulose modified with poly-lysine (NMPL). The results are shown as mean ± standard deviation (SD), and $n = 3$ for each experiment.

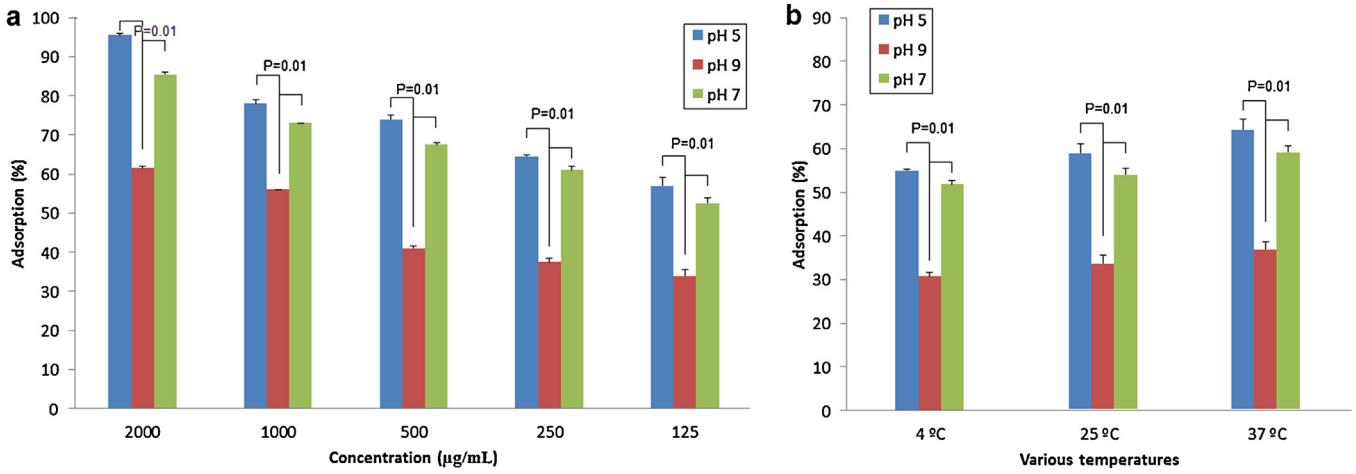

Fig. 5. The effect of (a) (pH-concentration) and (b) (pH-temperature) on the adsorption of fumonisin B1 by nanocellulose modified with poly-lysine (NMPL). The results are shown as mean ± standard deviation (SD), and $n = 3$ for each experiment.

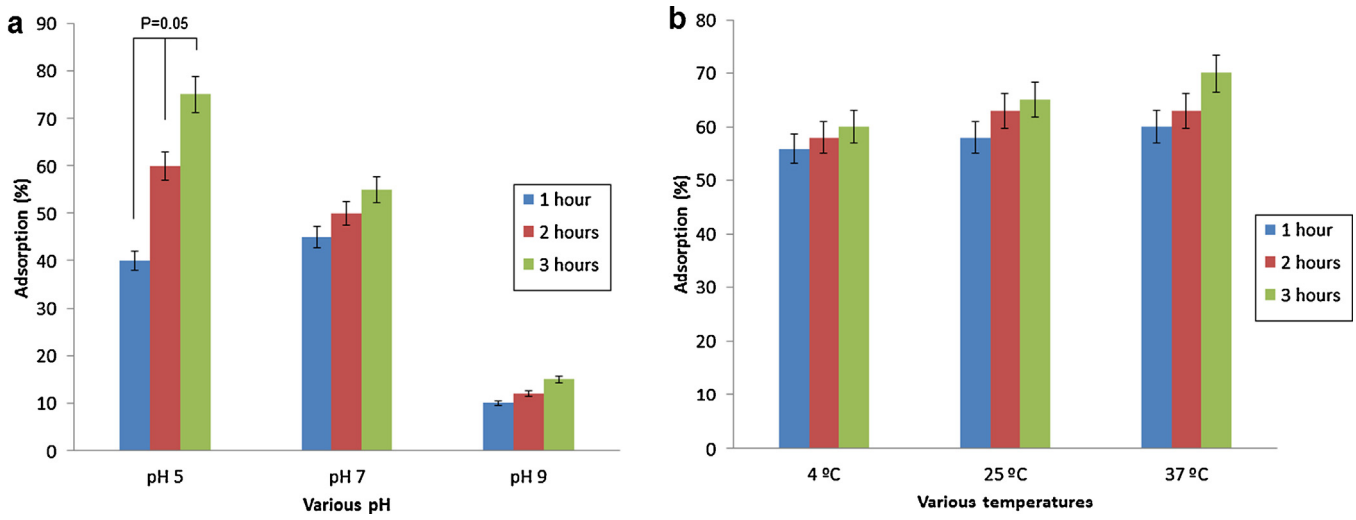

Fig. 6. The effect of (a) (pH-incubation time) and (b) (temperature-incubation time) on the adsorption of fumonisin B1 by nanocellulose modified with poly-lysine (NMPL). The results are shown as mean ± standard deviation (SD), and $n = 3$ for each experiment.

In this study, the fumonisin B1 adsorption was evaluated at different controlled conditions. Theoretically, fumonisin B1 can be adsorbed by ploy-amines, because fumonisin B1 has four carboxyl groups. This study showed that although the increase of incubation time and temperature did not affect the adsorption and release, the increase of pH led to decrease of adsorption and increase of release. The high affinity of fumonisin B1 and NMPL led to high speed of adsorption. In case of pH, H+ ions can directly affect the non-covalent binding (hydrophobic force, hydrogen force, and electrostatic force, etc.). It is fact that temperature, pH, incubation time, concentration of adsorbent, ionic strength, etc. are the main parameters of adsorption kinetics [17]. It was better that both isotherm and kinetic of adsorption were being investigated. These can help us to predict the behavior of NMPL, and must be scanned in the future works. Note, different isotherms and kinetic models (e.g., Weber and Morris model, external mass transfer equation, Elovich's model, first-order equation of Bhattacharya and Venkobachar, and Ritchies's equation) have been previously introduced for different adsorbents [18].

As an important finding, NMPL could adsorb fumonisin B1 in presence of different foodstuffs (cucumber, maize, rice, and wheat). Importantly, the more concentration of fumonisin B1 led to more adsorption. Moreover, NMPL had the highest adsorption in cucumber, near 95%. The authors suggest that there are some biomolecules in cucumber that can bind to fumonisin B1. This result is an important finding, and must be more studied in future.

No data was observed in database about adsorption property of NMPL, and this study was the first report. In this section, some related studies are reported. Aly et al. applied hydrated sodium calcium aluminosilicate and the Egyptian montmorillonite to remove aflatoxin B1 and fumonisin B1 [9]. Belajová et al. compared retention of ochratoxin A and fumonisin B1 and B2 from beer by some common adsorbents. They declared the best retentive percentage was 74–100% on the modified silica gels [10]. Solfrizzo et al. assessed the effectiveness of some adsorbents in vitro and in vivo. They showed that the adsorption capacity of cholestyramine, activated carbon, and bentonite was 85%, 62%, and 12%, respectively [11]. Daković et al. worked on the adsorption of fumonisin B1 by natural clinoptilolite-rich zeolitic tuff, and clinoptilolite modified with octadecyldimethylbenzyl ammonium (ODMBA). The results showed that the presence of ODMBA improved adsorption, especially at pH 7 and pH 9 [19]. Niderkorn et al. demonstrated the binding of *Fusarium* mycotoxins such as fumonisin B1 by fermentative bacteria [20].

Another important finding was that NMPL could inhibit the effect of fumonisin B1 on mouse liver cells. As found, fumonisin B1 could increase the level of ALT and AST. But, NMPL or (fumonisin B1+NMPL) could not change their levels. Since fumonisin B1 can be adsorbed by NMPL, it cannot transfer into liver cells, and cannot damage cell enzymes. The authors hypothesize that the adsorption of fumonisin B1 may be an important mechanism. The uptake of NMPL into liver cells should be investigated, but it was out of our scope.

5. Conclusion

Taken together, NMPL could inhibit the effect of fumonisin B1, i.e., the levels of ALT and AST enzymes were not changed, when liver cells exposed to (fumonisin B1+NMPL). It seems that the phenomenon is due to adsorption of fumonisin B1 by NMPL. Further studies must be done to use NMPL for fumonisin B1 removal.

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Conflicts of interest

No conflict of interest was addressed.

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