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ORIGINAL PAPER

# Nanocellulose conjugated with retinoic acid: its capability to adsorb aflatoxin B1

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**Abstract** The main aim of this study was to evaluate the adsorption of aflatoxin B1 by nanocellulose conjugated with retinoic acid (NCRA). First, aflatoxin B1 and NCRA were modeled in the Hyperchem software, and then the adsorption of aflatoxin B1 was simulated by the molecular dynamics technique. Second, nanocellulose was synthesized and conjugated with retinoic acid by cross-linker. Third, the adsorption of aflatoxin B1 and NCRA and its release were evaluated at different conditions. Fourth, its adsorption was evaluated in different foodstuffs. Sixth, its toxicity was evaluated on mouse esophageal cells (MECs). Computer-based simulation showed the adsorption of aflatoxin B1 by NCRA. This study showed that the adsorption and release were less affected by temperature and incubation time but highly affected by pH and concentration. Moreover, it was found that NCRA could adsorb aflatoxin B1 in different foodstuffs. Importantly, NCRA had no remarkable toxicity on the MECs.

**Keywords** Nanocellulose · Retinoic acid · Adsorption · Aflatoxin B1

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## Introduction

Aflatoxins are secondary metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* (Magnussen and Parsi 2013). Importantly, 4.5 billion people from developing countries are chronically exposed to high amounts of aflatoxins (Gnonlonfin et al. 2013). The most important member of aflatoxins is aflatoxin B1, whose mutagenic, carcinogenic, teratogenic, and immunosuppressive activity have been widely studied (Madrigal-Santillan et al. 2010). The carcinogenic effects of aflatoxin B1 correspond intimately with its active intermediate, aflatoxin B1-exo-8,9-epoxide (Moudgil et al. 2013). Additionally, intake of aflatoxin B1 causes paleness, hepatomegaly, bile duct hyperplasia, periportal fibrosis, and hydropic degeneration (Ortatatli et al. 2005). On the other hand, aflatoxin B1 can generate reactive oxygen species that lead to oxidation of DNA, proteins, and lipids (Mary et al. 2012).

To detoxify aflatoxins, different approaches have been proposed (Tripathi and Mishra 2009). The use of chemical agents is one of them. Some oxidizing agents

(e.g., NaOCl, benzoyl peroxide, and $H_2O_2$), some bases (e.g., ammonia, calcium hydroxide, and methylamine), and aldehydes (e.g., formaldehyde and acetaldehyde) have been investigated (Natarajan 1991). The use of physical methods including solvent extraction, adsorption, heat treatment, and irradiation is another way (Rustom 1997). However, the application of biological elements such as probiotics is a very efficient method to reduce aflatoxin in foodstuffs (Zoghi et al. 2014). It must be mentioned that each strategy has some advantages and disadvantages, and there is not one unique method for all situations (Tripathi and Mishra 2009).

Nowadays, clay additives such as aluminosilicate, zeolite, sepiolite, Na-bentonite, Novasil plus, and Astra Ben 20A are used to remove aflatoxin in animal foodstuffs (Magnoli et al. 2011; Marroquin-Cardona et al. 2009; Wang et al. 2008). Moreover, these additives can improve the physical and chemical properties of various foods (Magnoli et al. 2011; Marroquin-Cardona et al. 2009; Wang et al. 2008). Although these adsorbents bind to aflatoxin, they cannot be used in human foods. Non-specific binding is the first problem, i.e., other molecules can bind to them, and this problem may decrease the food value and efficacy of the adsorbent (Magnoli et al. 2011; Marroquin-Cardona et al. 2009; Wang et al. 2008). The second problem is the toxicity of the adsorbents, i.e., the clay additives have toxic molecules such as metal oxide ($TiO_2$, $SiO_2$, $Al_2O_3$, etc.) (Baek et al. 2012). Here, our aim was to design a non-toxic adsorbent for use in animal and human foodstuffs. Our hypothesis is that nanocellulose conjugated with retinoic acid (NCRA) can adsorb aflatoxin B1.

## Materials and methods

### Materials

To synthesis nanocellulose, raw cellulose was purchased from My Baby Co., Iran (with purity of 99.9 %). *N*-(2-aminoethyl)-3-aminopropylmethyldimethoxysilane (APTES), *N*-ethyl-*N*-(dimethylaminopropyl) carbodiimide (EDC), 5-diphenyl-tetrazolium bromide (MTT), and retinoic acid were provided by Sigma-Aldrich Co. (St Louis, MO, USA). Other chemicals including nitric acid, sulfuric acid, sodium hydroxide (NaOH), aflatoxin B1 (with purity of 97 %), methanol, acetonitrile, and dimethylsulfoxide (DMSO) were sourced from Merck, Germany.

### Molecular dynamics (MD) simulation

First, the chemical model of NCRA and aflatoxin B1 was performed by HyperChem Professional 8.0.3 software, and then MD simulations were carried out on an Acer laptop CPU B960 (Patel et al. 2013). It must be mentioned that the NCRA model was a cellotriose that bonded to APTES and retinoic acid.

In this study, the force field and topology parameters were assigned from AMBER 99. Also, a TIP3P water model with a cubic box (10 Å) was used to solvate NCRA and aflatoxin B1. The cutoff distance of electrostatic interaction was 10 Å, and van der Waals interactions were turned off between 12 and 14 Å. Briefly, the energy of the whole system was first minimized with a hybrid Lui Storey and Conjugate Descent (gradient = 0.001 and iteration = 100) with 1,000 steps, and then a simulation was made on an NVT ensemble at 300 K for 100 picoseconds (ps). In this study, both NCRA and aflatoxin B1 interacted. Finally, the image of adsorption or de-adsorption was captured at the end of the simulation.

### Nanocellulose synthesis

To synthesize nanocellulose, acid hydrolysis was used according to our previous work (Jebali et al. 2013). Briefly, 0.1 g of raw cellulose was treated with 5 M NaOH at 37 °C for 1 h and washed with distilled water (DW). Then, NaOH-treated cellulose was treated with 1 M DMSO at 37 °C for 1 h and rinsed with DW. In the next step, 1 ml of sulfuric acid (70 % w/w) was added to the rinsed cellulose and then shaken for 1 min. In the next step, 1 ml of 5 M NaOH was gently added to hydrolyzed cellulose, shaken for 5 min, centrifuged at 5,000 rpm for 5 min, washed by DW, and stored in 4 °C.

### Modification of nanocellulose by APTES and conjugation with retinoic acid

For its modification, 10 ml of nanocellulose at a concentration of 500 μg/ml was added to 5 ml of 5 % APTES (v/v), incubated for 30 min at 100 °C (Edwards et al. 2011). Then, APTES-modified nanocellulose was centrifuged at 5,000 rpm for 5 min and

washed with DW. In the next step, 1 ml of retinoic acid (100 mg/ml) was incubated with EDC (100 mg/ml) at 37 °C for 30 min. Then, 1 ml of APTES-modified nanocellulose was added to EDC-activated retinoic acid and incubated at 37 °C for 1 h. Finally, NCRA was centrifuged at 5,000 rpm for 5 min and washed with DW. The concentration of 500 μg/ml of NCRA was prepared in DW and stored at 4 °C.

## Characterization of NCRA

The structure, size distribution, and surface composition of NCRA were studied by transmission electron microscopy (TEM) (Hitachi, Japan), dynamic light scattering (DLS) (ZFS Co.), and Fourier transform infrared spectroscopy (FTIR) (Bruker, UK), respectively.

## The effect of different parameters on adsorption

In this study, different parameters (concentration, temperature, incubation times, and pH) were investigated.

*First group* In the first group, 1 ml of serial concentrations of NCRA (500, 250, 125, 62, and 31 μg/ml) was separately added to 1 ml of aflatoxin B1 at a concentration of 500 ng/l at pH 7 and separately incubated for 30 min at 4, 25, and 37 °C.

*Second group* In the second group, 1 ml of serial concentrations of NCRA (500, 250, 125, 62, and 31 μg/ml) was separately added to 1 ml of 500 ng/l aflatoxin B1 at pH 7 and separately incubated at 37 °C for 10 min, 20 min, and 30 min.

*Third group* In the third group, 1 ml of serial concentrations of NCRA (500, 250, 125, 62, and 31 μg/ml) was separately added to 1 ml of 500 ng/l aflatoxin B1 and separately incubated at pH 5, pH 7, and pH 9 for 30 min at 37 °C.

*Fourth group* In the fourth group, 1 ml of NCRA (500 μg/ml) was added to 1 ml of aflatoxin B1 (500 ng/l) and separately incubated at pH 5, pH 7, and pH 9 for 30 min at 4, 25, and 37 °C.

*Fifth group* In the fifth group, 1 ml of NCRA (500 μg/ml) was added to 1 ml of aflatoxin B1 (500 ng/l) and separately incubated at pH 5, pH 7, and pH 9 at 37 °C for 10, 20, and 30 min.

*Sixth group* In the sixth group, 1 ml of NCRA (500 μg/ml) was added to 1 ml of aflatoxin B1 (500 ng/l) and separately incubated at 4, 25, and 37 °C for 10, 20, and 30 min at pH 7.

*Seventh group (negative control)* In the seventh group (negative control), 1 ml of DW was added to 1 ml of aflatoxin B1 (500 ng/l) and incubated at different conditions (same as in the other groups).

*Eighth group (positive control)* In the eighth group (positive control), 1 ml of NCRA (500 μg/ml) was added to 1 ml of DW and incubated at different conditions (same as in the other groups).

After incubation, all tubes were centrifuged at 5,000 rpm for 5 min, and the optical density (OD) of the supernatant was read by a UV-visible spectrophotometer (ELICO, India) at 254 nm. The adsorption percent (%) was measured by Formula 1.

$$\text{The adsorption percent}\,(\%) = \left(\text{OD}_{\text{before adsorption}} - \text{OD}_{\text{after adsorption}}\right) \times 100/\left(\text{OD}_{\text{before adsorption}}\right). \quad \text{(Formula 1)}$$

## The effect of different parameters on the release of aflatoxin after adsorption

First, 1 ml of 500 μg/ml NCRA was added to 1 ml of 500 ng/l aflatoxin B1 and incubated for 30 min at 37 °C. Then, it was centrifuged at 5,000 rpm for 5 min and washed in DW to remove any free aflatoxin B1. In the next step, 1 ml of DW was added to it and separately incubated for 1, 2, and 3 h at 37 °C. In another experiment, the release of aflatoxin was separately evaluated after incubation for 1 h at 4, 25, and 37 °C. Also, in the final experiment, the tubes were separately incubated for 1 h at 37 °C, and their pH was 5, 7, and 9, respectively. After incubation, all tubes were centrifuged and the quantity of aflatoxin B1 in the supernatant was measured. As negative control, all steps were done, but NCRA was not used. As positive control, all steps were done, but aflatoxin B1 was not applied.

## The adsorption of aflatoxin by NCRA in different foodstuffs

To evaluate the aflatoxin adsorption at actual conditions, different foodstuffs (hazelnut, pistachio, almond,

walnut, wheat flour, and rice flour) were prepared from different Iranian shops. Briefly, 1 ml of aflatoxin B1 at a concentration of 500 ng/l was separately mixed into 1 g of each foodstuff (ground and unground form). Then, 1 ml of 500 µg/ml NCRA was added to them and incubated at the following condition (pH 5, temperature = 37 °C, and incubation time = 30 min). Then, all tubes were centrifuged and the concentration of aflatoxin B1 in the supernatant was measured by HPLC; the adsorption percent was calculated by Formula 2.

In the negative control tube, all steps were done, but NCRA was not used. In the positive control tube, all steps were done, but aflatoxin B1 was not used.

$$\text{The adsorption percent } (\%) = \left(\text{aflatoxin concentration}_{\text{before adsorption}} - \text{aflatoxin concentration}_{\text{after adsorption}}\right) \times 100/\left(\text{aflatoxin concentration}_{\text{before adsorption}}\right). \quad \text{(Formula 2)}$$

### Analytical procedures

First, 1 ml of supernatant of each sample was added to 2 ml of 60 % acetonitrile/water (v/v) (Merck, Germany), and stirred for 2 min at high speed. Then, 2 ml of extract was diluted with 48 ml of phosphate-buffered saline (PBS) at pH 7.4. Next, the diluted mixture was filtered through immunoaffinity columns (EASI-EXTRACT Aflatoxin from R-Biopharm Rhone) at a flow rate of 5 ml/min. Then, the column was first washed with 20 ml of PBS, then rinsed with 60 % methanol (v/v) (Merck, Germany). In the next step, 100 µl of the samples was injected into the HPLC column. In this study, the mobile phase was 40 % methanol (Merck, Germany), and the flow rate was 1 ml/min. Here, the detection of aflatoxin was carried out by a fluorescent detector. Its excitation wavelength was 362 nm, and the emission wavelength was 425 nm. Finally, the concentration of aflatoxin for each sample was measured according to the value of the aflatoxin standard and calibration curve (Sheijooni-Fumani et al. 2011).

### The evaluation of NCRA toxicity

First, 100 µl of serial concentrations (500, 250, 125, 62, and 31 µg/ml) of NCRA was separately added to 100 µl of mouse esophageal cells (MECs), obtained from Balb/C mice, then incubated for 24 h at 37 °C. After incubation, the cells were washed with normal saline to remove any NCRA. Then, 25 µl of 5 mg/ml MTT and 100 µl of RPMI1640 were added and incubated for 3 h at 37 °C. In the next step, 100 µl of 70 % v/v isopropanol was added, and the OD of each sample was read at 490 nm. Then, the cell viability of each sample was measured according to Formula 3. In the negative control, the cells were not exposed to NCRA (Jebali and Kazemi 2013).

$$\text{Cell viability} = (\text{OD}_{\text{test}}) \times 100/(\text{OD}_{\text{control}}) \quad \text{(Formula 3)}$$

### Statistical analysis

All data are shown as the mean ± standard deviation (SD). Then, the ANOVA test was applied to detect significant differences. This test was carried out using SPSS software (V.16.0 for Windows; SPSS Inc., USA), and $P < 0.05$ was considered as a significant difference.

## Results

### MD simulation

Figure 1a, b shows the chemical model of NCRA and aflatoxin B1, respectively. As mentioned, the NCRA model was a cellotriose that bonded to APTES and retinoic acid. Figure 1c demonstrates the adsorption of NCRA and aflatoxin B1 at the end of the simulation.

### Characterization of NCRA

Figure 2a, b shows the TEM image and DLS graph of NCRA, respectively. Figure 2a demonstrates that the nanostructure is rod-like and has different sizes. The approximate size of each nanostructure is near 100 nm height and 5 nm width. Figure 2b shows that the size distribution of NCRA is approximately 100–150 nm with an average of 120 nm. The FTIR spectrum of nanocellulose (I), retinoic acid (II), and NCRA (III) is demonstrated in Fig. 2c. Sharp peaks were seen at 940 and 3,000 $cm^{-1}$ for nanocellulose, but retinoic acid had specific peaks at 1,255, 1,572, 1,657, and 2,933 $cm^{-1}$. As seen, the sharp vibration peaks at 940, 1,535 (amide band I), 1,635 (amide band II), 2,933, and 3,400 for the spectrum of NCRA indicated good conjugation between retinoic acid and nanocellulose.

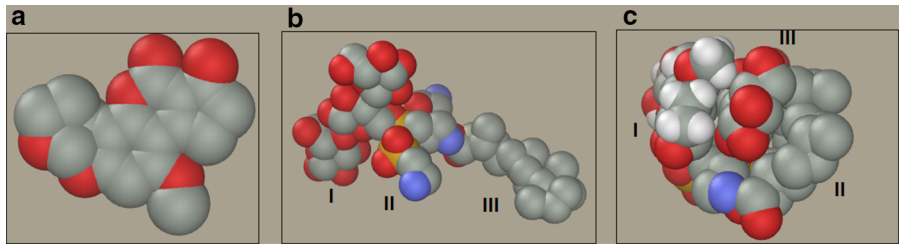


**Fig. 1** The chemical model of aflatoxin B1 (**a**) and NCRA (**b**). The final structure of NCRA and aflatoxin B1 at the end of the simulation (**c**). *I*, *II*, and *III* are cellotriose, APTES, and retinoic acid, respectively

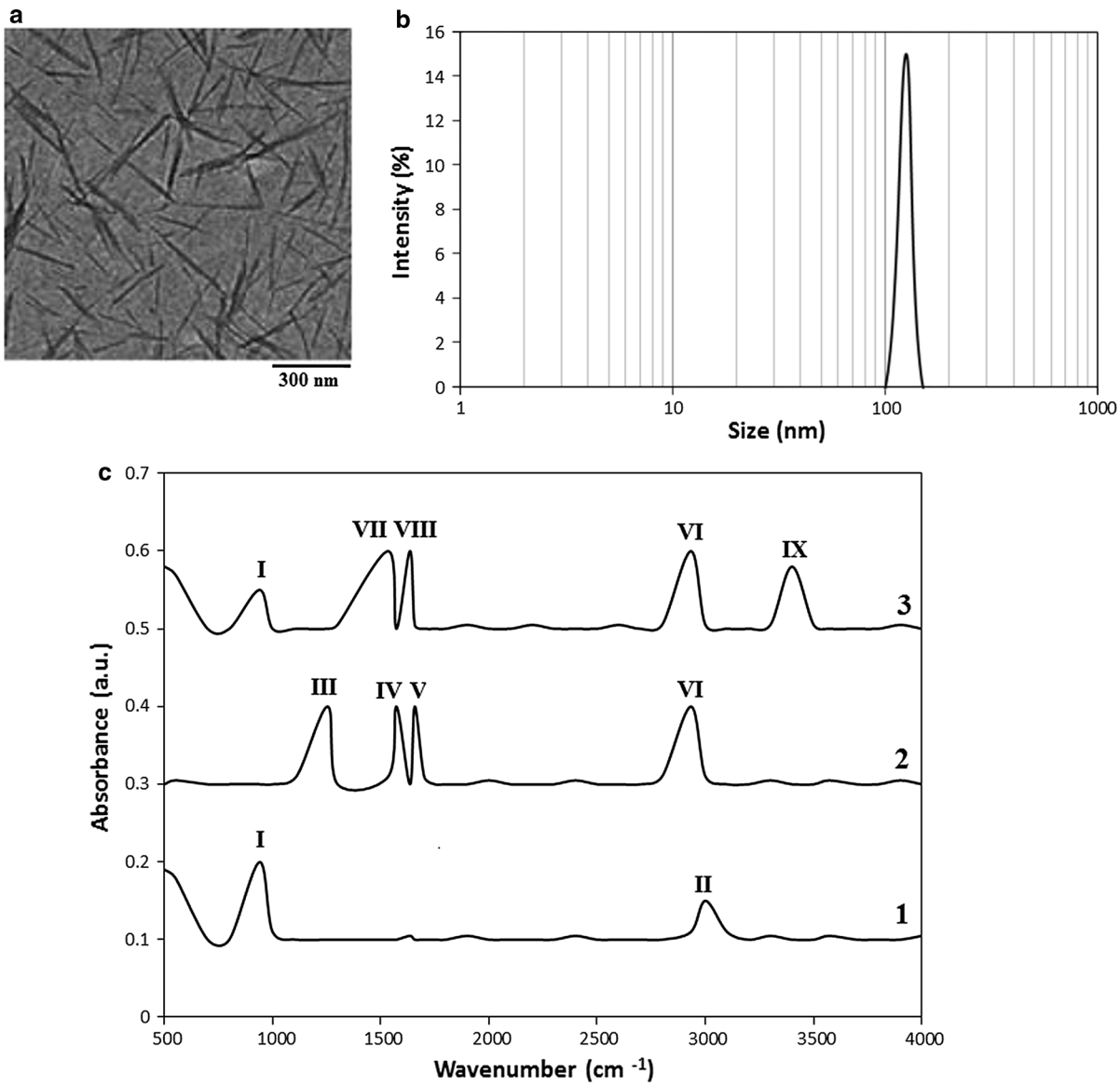


**Fig. 2** The TEM image (**a**) and DLS graph (**b**) of NCRA. The FTIR spectrum of nanocellulose (*1*), retinoic acid (*2*), and NCRA (*3*) is shown in the (**c**). *I* (940 $cm^{-1}$), *II* (3,000 $cm^{-1}$), *III* (1,235 $cm^{-1}$), *IV* (1,572 $cm^{-1}$), *V* (1,657 $cm^{-1}$), *VI* (2,933 $cm^{-1}$), *VII* (1,535 $cm^{-1}$), *VIII* (1,635 $cm^{-1}$), and *IX* (3,400 $cm^{-1}$) are presented in (**c**)

The effect of various parameters

Figure 3a–c shows the effect of concentration-temperature, concentration-incubation time, and concentration-pH on adsorption, respectively. Concerning temperature and incubation time, there was a direct relationship between an increase in these parameters and an increase in adsorption, i.e., the more temperature and incubation time, the more adsorption. In the same concentration of NCRA, there were significant differences between adsorption at 4 versus 25 °C, and between adsorption at 4 versus 37 °C ($P \leq 0.05$). At a concentration of 125 and 500 µg/ml, there were significant differences between adsorption at 30 versus 20 and 30 versus 10 min ($P \leq 0.05$). Concerning pH, the adsorption was decreased with an increase of pH. In the same concentration of NCRA, significant differences were observed between adsorption at pH 9 versus pH 7 and at pH 9 versus pH 2 ($P \leq 0.05$). Moreover, we observed a dose-dependent pattern for adsorption of NCRA and aflatoxin B1.

Figure 4a–c shows the effect of temperature-pH, pH-incubation time, and temperature-incubation time on the

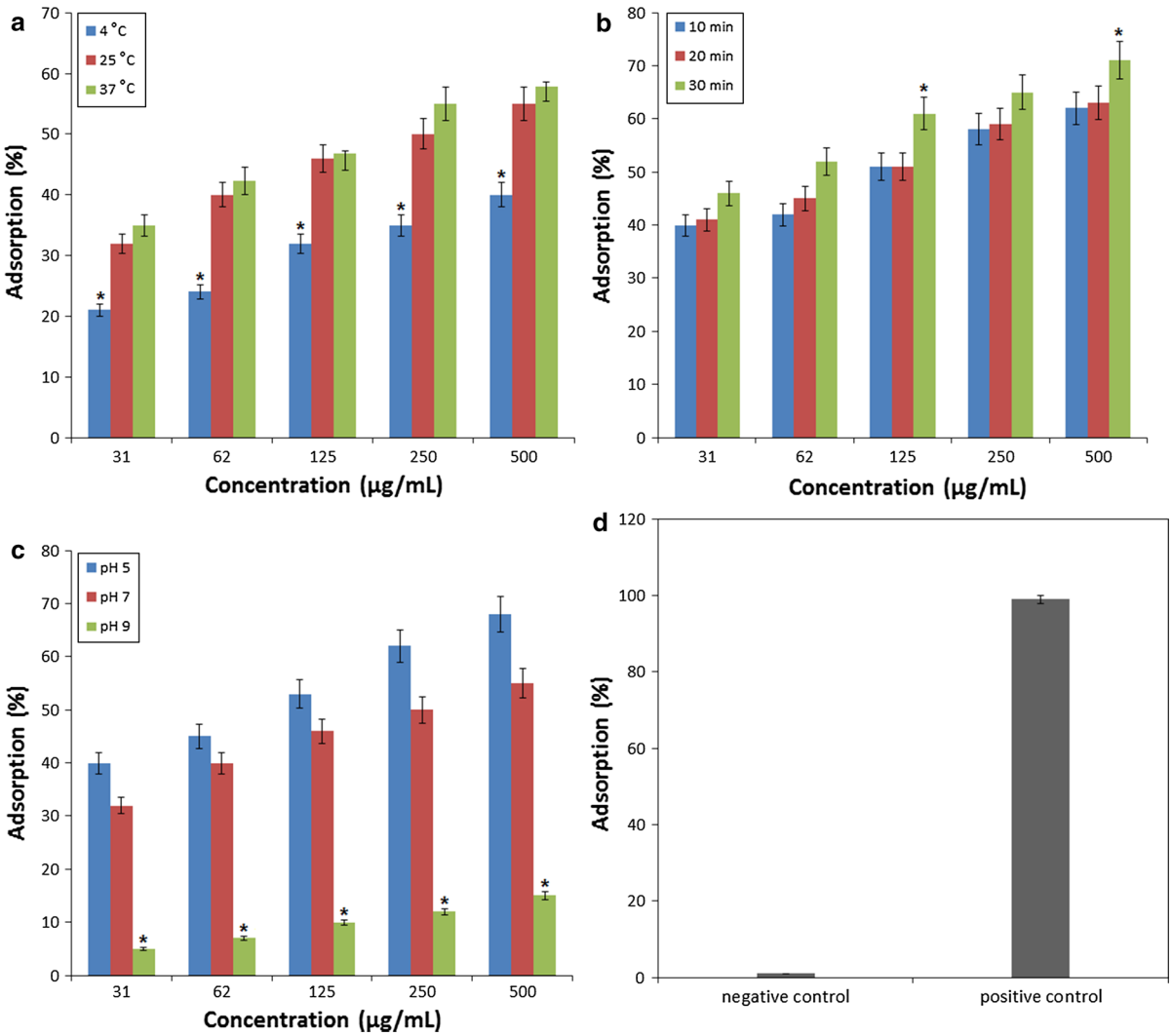


**Fig. 3** The effect of concentration-temperature (**a**), concentration-incubation time (**b**), and concentration-pH (**c**) on adsorption. **d** The results of negative and positive controls. In the negative control, DW was used instead of NCRA. In the positive control, DW was used instead of aflatoxin B1. In part of (**a**), *significant difference ($P \leq 0.05$) compared with the adsorption at 25 and 37 °C ($n = 13$). In part of (**b**), *significant difference ($P \leq 0.05$) compared with the adsorption after 10 and 20 min ($n = 13$). In part of (**c**), *significant difference ($P \leq 0.05$) compared with the adsorption at pH 7 and pH 5 ($n = 13$)

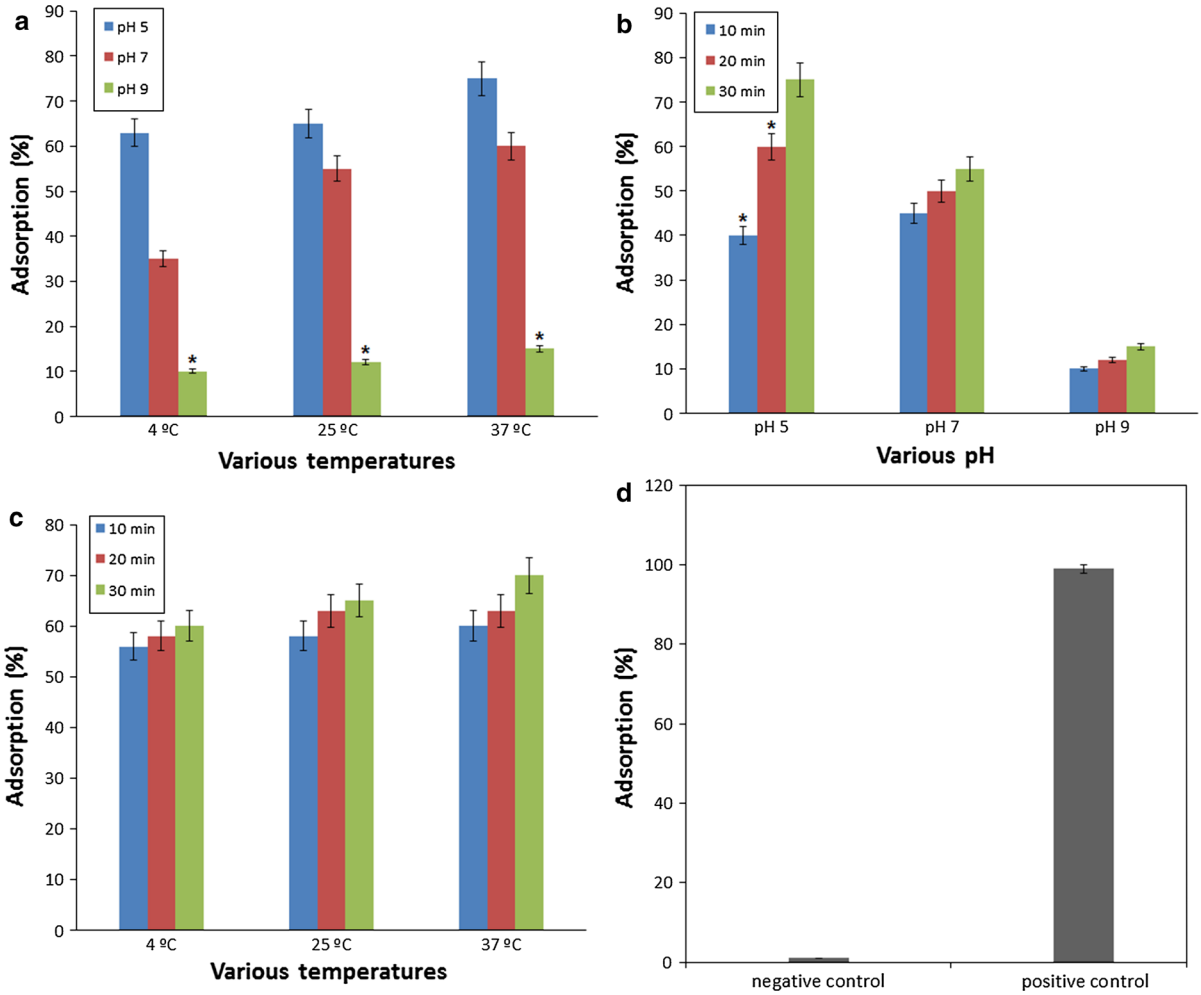


**Fig. 4** The effect of temperature-pH (**a**), pH-incubation time (**b**), and temperature-incubation time (**c**) on adsorption. **d** The results of negative and positive controls. In the negative control, DW was used instead of NCRA. In the positive control, DW was used instead of aflatoxin B1. In part of (**a**), *significant difference ($P \leq 0.05$) compared with the adsorption at pH 7 and pH 5 ($n = 13$). In part of (**b**), *significant difference ($P \leq 0.05$) compared with the adsorption after 30 min ($n = 13$)

adsorption, respectively. Concerning temperature-pH, significant differences were observed between adsorption at pH 9 versus pH 7 and pH 9 versus pH 2 at all incubation temperatures ($P \leq 0.05$). As seen, although adsorption was increased with an increase of incubation time, a significant difference was observed at pH 5 ($P \leq 0.05$). Also, both temperature and incubation time led to an increase in adsorption.

## The release of aflatoxin after adsorption

The effect of incubation time, temperature, and pH on the release of aflatoxin after adsorption is shown in the Table 1. As seen, when the incubation time was increased, no remarkable change in adsorption was seen. Also, temperature could slightly affect the release of aflatoxin, and the maximum release was observed when tube was incubated at 37 °C. This test showed that an increase in pH from 7 to 9 increased the release of aflatoxin from 6 to 20 ng/l. Also, the release of aflatoxin at pH 5 was 2 ng/l.

## Aflatoxin adsorption in different foodstuffs

Table 2 shows the percentage of aflatoxin adsorption in different foodstuffs by NCRA. This test showed that the pattern of adsorption was dose-dependent. As seen, the highest adsorption was 85 % for walnuts.

**Table 1** The effect of time, temperature, and pH on the release of aflatoxin after adsorption

| | Time (h) | | | Temperature (°C) | | | pH | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 25 | 37 | 5 | 7 | 9 |
| Aflatoxin concentration (ng/l) | 6 ± 0.03 | 7 ± 0.02 | 7 ± 0.04 | 4 ± 0.02 | 5 ± 0.03 | 7 ± 0.02 | 2 ± 0.04 | 6 ± 0.02 | 20 ± 1 |

**Table 2** The percentage of aflatoxin adsorption in different foodstuffs by conjugated nanocellulose (pH 5, temperature = 37 °C, incubation time = 30 min, concentration of NCRA = 500 μg/ml)

| | Hazelnut | Pistachio | Almond | Walnut | Wheat | Rice | Negative control[d] | Positive control[e] |
|---|---|---|---|---|---|---|---|---|
| Adsorption at G[a] (%) | 74 ± 4[c] | 74 ± 2 | 75 ± 3 | 85 ± 5 | 71 ± 5 | 72 ± 2 | 2 ± 1 | 99 ± 1 |
| Adsorption at UG[b] (%) | 74 ± 3 | 74 ± 4 | 75 ± 4 | 85 ± 3 | 71 ± 2 | 72 ± 5 | 1 ± 1 | 99 ± 1 |

[a] Ground = G

[b] Unground = UG

[c] Each test was done 13 times ($n = 15$)

[d] In the negative control tube, all steps were done, but NCRA was not used

[e] In the positive control tube, all steps were done, but aflatoxin B1 was not used

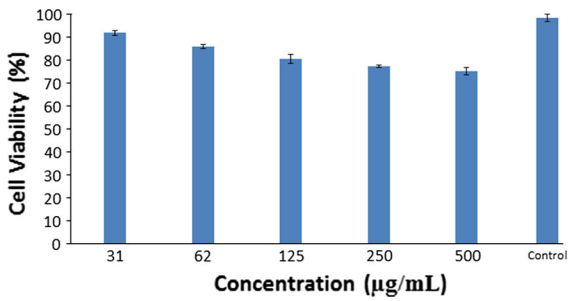


**Fig. 5** The toxicity of NCRA at serial concentrations, obtained by MTT assay ($n = 10$ for each concentration). In the control, DW was used instead of NCRA

### Toxicity of NCRA

Figure 5 shows the toxicity of NCRA, obtained by MTT assay. As shown, the toxicity of NCRA was dose-dependent. The highest toxicity (or minimum cell viability) was near 75 % for a concentration of 500 μg/ml, and the least toxicity (or maximum cell viability) was near 92 % for a concentration of 31 μg/ml.

## Discussion

Here, nanocellulose was synthesized, modified by APTES, and then conjugated with retinoic acid by EDC cross-linker. The schematic of these reactions is shown in Fig. 6. As shown in the FTIR spectrum of NCRA, the amide bands at 1,535 and 1,635 $cm^{-1}$ confirmed the covalent binding of retinoic acid and APTES-modified nanocellulose. As seen, EDC cross-linked the carboxyl group of retinoic acid and the amine group of APTES-modified nanocellulose. Here, it was found that the quantity of adsorption and release of aflatoxin was less affected by temperature and incubation time and more affected by pH. Regarding the mechanism, the high affinity of aflatoxin B1 and NCRA may be one reason. Concerning pH, it seems that the $H^+$ ion can directly affect noncovalent forces and leads to high adsorption. Since there are no data about NCRA in the scientific databases, further studies must be done in the future to describe NCRA behaviors. It was found that NCRA could adsorb aflatoxin B1 in the different foodstuffs. Interestingly, the adsorption was increased in walnuts compared with other foodstuffs. The authors hypothesize that NCRA can adsorb other substances in hazelnuts, pistachios, almonds, wheat flour, and rice flour, and this phenomenon decreases the adsorption capability. The authors hypothesize that there are some biomaterials (perhaps different oils) in walnut that increase adsorption. It is an important finding that NCRA has different adsorption capabilities in different foodstuffs, and this must be studied in future research.

Based on previous studies, aflatoxin adsorbents are activated carbon, aluminosilicates (clay, bentonite,

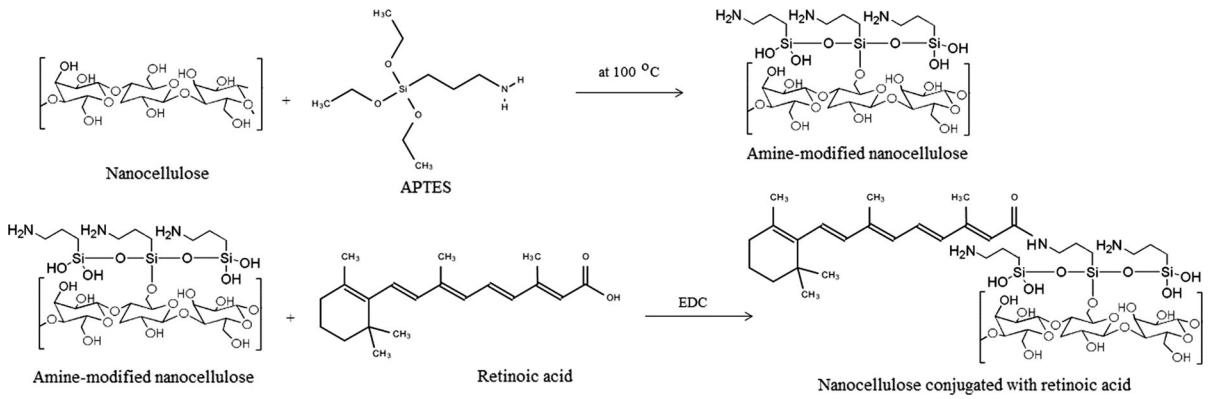


**Fig. 6** The reaction between nanocellulose and APTES as well as between amine-modified nanocellulose and retinoic acid

montmorillonite, zeolite, phyllosilicates, etc.), and the cell walls of yeast and bacteria (glucomannans, peptidoglycans, etc.) (Magnoli et al. 2011; Marroquin-Cardona et al. 2009; Wang et al. 2008). Although these materials can adsorb aflatoxin, they have two problems. First, they are general adsorbents and adsorb other molecules. Second, they have some molecules ($TiO_2$, $SiO_2$, $Al_2O_3$, inorganic monomer) that are not suitable for use in foodstuffs (Baek et al. 2012). Compared with other adsorbents, NCRA was not toxic for MECs.

In this study, the adsorption of aflatoxin B1 was confirmed by MD simulation software, too. MD simulation is the best way to find some suitable adsorbents before any experiments in the laboratory (Patel et al. 2013). In this way, many molecules are allowed to interact for a period of time, and their trajectories are determined by numerically solving Newton's equations of motion (Patel et al. 2013). It must be noted that the selection of NCRA was based on several MD simulations.

The use of MD simulation for adsorption of aflatoxin was first studied by Denga and Szczerbab (2011) to evaluate the adsorption mechanism of smectite.

They declared that the carbonyl groups of aflatoxin B1 are very important for binding to smectite. Gauden et al. (2013) worked on adsorption of some organics in carbon slit-like pores. Also, Riccardi et al. (2009) studied porous polymer adsorbents using MD software.

From the current studies, we propose that NCRA can be applied to remove aflatoxin B1 in foodstuffs. The adsorption capability of NCRA in the intestinal tract of higher animals is yet to be investigated.

## Conclusion

It can be concluded that NCRA is a novel adsorbent to remove aflatoxin B1. Importantly, the adsorption and release of aflatoxin B1 were less affected by temperature and incubation time and more affected by pH. We propose that NCRA (at a concentration of 500 μg/ml) can be used in different foodstuffs to remove aflatoxin B1. No remarkable toxicity was seen at this concentration.

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**Conflict of interest** The authors declare no conflict of interest.

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