Heliyon 9 (2023) e21560

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

# Tackling the cytotoxicity and genotoxicity of cellulose nanofibers from the banana rachis: A new food packaging alternative

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ARTICLE INFO

*Keywords:*
Cellulose nanofibers
Banana rachis
Cytotoxicity
Genotoxicity
*In vivo* toxicity

ABSTRACT

Cellulose nanofibrils from the banana rachis are a good alternative as packaging materials, food packaging, stabilizing agents, and functional food ingredients. To address the potential effects of ingested banana rachis cellulose nanofibrils (BR-CNFs), their toxicity *in vitro* and *in vivo* was evaluated using Caco-2 intestinal cells and mice, respectively. The results showed that BR-CNFs did not cause cytotoxic effects at the concentrations evaluated on Caco-2 cells. In addition to cytotoxicity tests, genotoxicity assays using comet assay indicated that Caco-2 cells showed no DNA damage at the concentrations of CNFs tested. Finally, acute *in vivo* cytotoxicity assays indicated that mice showed no sign of pathogenesis or lesions in the liver, kidney, or small intestine when treated with a single dose of BR-CNFs. Moreover, when the mice were treated daily for a month with BR-CNFs no hyperplasia or hypertrophy was observed in any of the organs evaluated. Additionally, biochemical parameters such as blood chemistry, creatinine, liver enzymes, and renal function showed that the BR-CNFs do not cause organ damage. Overall, this study shows that BR-CNFs are neither cytotoxic nor genotoxic. In conclusion, these studies are essential to guarantee the safety of this high value-added product in the food industry.

## 1. Introduction

The global environmental crisis has stimulated the design and development of new biodegradable products. Cellulose nanofibers (CNFs) are nanomaterials obtained from waste wood products, plant fibers, or by-products of agribusiness that are often added to food products to improve quality, appearance, safety, nutrition or to facilitate production processes [1–3]. In recent years, the extensive use of CNFs has increased human exposure to these new products. CNFs are obtained from these raw materials through chemical and mechanical treatments, which aim to remove as many non-cellulosic components as possible to obtain a sample with a homogeneous size distribution, good optical properties, biodegradability, and biocompatibility [4,5].

CNFs are considered an emerging raw material in the agri-food area because they have unique properties to improve different aspects related to food quality and appearance [6]. They have demonstrated a high potential as food packaging materials, as stabilizing agents, and as functional food ingredients [7]. Despite the multiple benefits of CNFs, they are not yet recognized by the U.S. Food and Drug Administration (FDA), in part because the source from which they are derived affects their composition, size distribution, and, in

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https://doi.org/10.1016/j.heliyon.2023.e21560
Received 16 August 2023; Received in revised form 21 September 2023; Accepted 24 October 2023
Available online 25 October 2023

turn, their toxicity characteristics, and their interaction with the digestive system [8]. CNFs from wood have been extensively investigated, and findings suggest that their ingestion has little direct acute toxicity. However, some studies indicate that various ingested materials and nanomaterials obtained from other plant products alter aspects of intestinal function not normally evaluated in standard toxicity tests, which could have significant health implications [9,10].

Previous research showed indications of the benefit of consuming CNFs from wood. For example, simulated digestion studies revealed reduced triglyceride hydrolysis during the small intestinal phase. *In vitro* tests using a model of the small intestinal epithelium showed significantly reduced absorption and translocation of triglycerides and free fatty acids from digested foods [11]. Finally, in single tube feeding experiments with male *Wistar Han* mice, the postprandial increase in serum triglycerides was reduced by 36 % in the presence of 1 % (w/w) CNF [11]. However, the CNFs' consumption of other plant products has yet to be discovered.

It is well known that the source of CNFs can affect the toxicity of various tissues, so any new product must be rigorously evaluated for its composition, shape, homogeneity, and toxic properties [12]. Therefore, any unused product must be rigorously assessed regarding its design, form, uniformity, and toxic properties. Colombia is one of the largest banana-producing countries in the world. The production of waste from the commercialization of this product is a local problem that merits the development of new alternatives for the environmental management of these wastes. Using agroindustrial by-products to produce CNFs is crucial to generate employment and reduce waste. Banana rachis has been previously used to obtain CNFs [13]. However, to date, no known research is dedicated to studying the toxicity or interaction of CNFs from agroindustrial by-products with the digestive system.

Because of the above factors and considering that so far, no *in vivo* toxicological evaluation has been performed on CNF derived from Colombian agroindustrial by-products and the importance of the source in the characteristics of the CNF, this study evaluated the *in vitro* and *in vivo* cytotoxicity of this type of nanofibers obtained from the banana rachis, as well as the genotoxicity of the nanofibers obtained. These studies are essential to guarantee the safety of this product and to develop the integral exploitation of Colombian agroindustrial by-products as raw materials with high added value in the food industry.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All the reagents used in this study were purchased from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany. The Exilva microfibrillated cellulose was purchased from Borregaard, Norway, and the fetal bovine serum was obtained from Microgen, Bogotá, Colombia. The LDH-cytotoxicity Assay Kit was obtained from Abcam.

### 2.2. Isolation of cellulose nanofibrils

Cellulose nanofibrils from the banana rachis (BR-CNFs), and Exilva, Borregaard's CNFs (Commercial-CNFs) were used in this research. The BR-CNFs were isolated following a chemical-mechanical process as described by Zuluaga et al. (2009) and Velásquez-Cock et al., [2,4]. Briefly, the ground banana rachis was subjected to an alkaline treatment with KOH 5 wt % for 14 h at room temperature, followed by a delignification with $NaClO_2$ at pH 4 for 1 h at 70 °C. The insoluble matter was filtered and washed until neutral pH before passing it through a second alkaline treatment for 14 h with KOH 5 wt % at room temperature, followed by a demineralization treatment with HCl 1 wt % at 80 °C for 2 h. The remaining sample was filtered and washed to a neutral pH. Finally, chemically purified cellulose fibers were passed 30 times through grinding equipment (Supermasscolloider, Masuko Sangyo) [14] and sterilized in an autoclave at 121 °C for 15 min.

Subsequently, commercial and banana rachis CNFs were transferred to a phosphate buffered saline (PBS) following a dialysis process [14]. Briefly, 250 mL of each CNFs at two wt % were introduced in a dialysis membrane of 6–8 kDa, and the membrane was subsequently sealed and submerged in a PBS solution until CNFs changed the conductivity from 30.8 mS/m to 13.34 mS/m. Subsequently, stock suspensions of the CNFs 1 wt % in PBS were prepared for *in vivo* and *in vitro* tests and finally sterilized in a LabTech brand autoclave for 20 min at 121 °C.

### 2.3. In vitro cytotoxicity

*In vitro* cytotoxicity assays were performed on human epithelial colorectal adenocarcinoma cells Caco-2 (ATCC, American Type Culture Collection - Rockville, MD, USA) exposed to the nanocelluloses suspensions at a concentration range of 0.025 %–0.75 % wt, for 24 and 48 h. For this, cells were maintained at 37 °C and 5 % $CO_2$ in T-25 flasks (Falcon) containing Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, cat 12800017) at pH 7.4, supplemented with 10 % (v/v) fetal bovine serum (FBS, LABG&M, Microgen, Bogota, Colombia) and penicillin-streptomycin at a concentration of 1000 U/mL each. Cytotoxicity of CNFs was evaluated by three methods: erythrosin vital dye, Alamar blue, and LDH enzyme activity.

#### 2.3.1. Erythrosin assay

For the first assay, $3 \times 10^5$ Caco-2 cells were exposed at 0.75 % wt concentration of BR-CNFs and Commercial-CNFs. Subsequently the cell culture was exposed to 0.05X erythrosin vital dye to determine the viability. All assays were performed in three independent biological replicates, each with three technical replicates. Subsequently, cells were counted in Neubauer chambers, and viability was quantified as the number of living cells (colorless) over the number of total cells (colorless + red cell) per hundred. Both, culture medium and PBS were used as negative controls, and cells exposed to $H_2O_2$ served as the positive control.

### 2.3.2. Alamar blue test

In the second case, $5 \times 10^4$ cells were seeded in 96-well microplates and incubated for 24 h as described above. Subsequently, the medium was removed, and the cells were exposed to the BR-CNFs or commercial-CNFs for 2, 4, and 24 h at 0.025 %, 0.05 %, 0.1 %, 0.25 %, and 0.5 % wt concentrations to evaluate if the ingestion of CNFs produced damage in the intestinal cells during the digestive process [15]. Viability was determined using Alamar blue dye, which indirectly quantifies the activity of cellular reductase enzymes as a marker of cytotoxicity. After the corresponding time, the medium was removed, and 200 μl of medium without FBS and 20 μl 0.125 mg/ml Alamar blue (Sigma) were added to each well and incubated for 24 h. The resultant color change in the presence of living cells (from blue to pink) was quantified on a plate reader at 570 and 600 nm wavelengths, following protocols previously standardized in the laboratory. Cells exposed to culture medium or PBS were used as negative controls, and cells exposed to 10 μM hydrogen peroxide ($H_2O_2$) and 0.1 % Triton X-100 were used as a positive control.

The experiments were conducted thrice in triplicate wells for each NFC sample and dose. The data were processed in Excel, and the reader measurements were converted into '% inhibition' using the equation below and reorganized for GraphPad Prism:

$$\%\ Inhibition = 1 - \frac{sample\ absorbance - medium\ absorbance}{negative\ control\ (no\ treatment) absorbance - medium\ absorbance}$$

### 2.3.3. LDH activity

Finally, $5 \times 10^4$ cells exposed and unexposed to the CNF concentrations mentioned above in section 2.2.2 were analyzed for LDH enzyme release. For this, the commercial LDH-cytotoxicity Assay Kit was used, which quantifies formazan formation from lactate oxidation to pyruvate. Quantification was performed spectrophotometrically at 500 nm after 48 h post-treatment, following the recommendations of the commercial company. The results were expressed as the percentage of cytotoxicity (LDH in culture medium/ [LDH in culture medium + LDH of lysed cells] x 100), following previously published recommendations [16].

## 2.4. In vitro genotoxicity evaluation

To evaluate the possible damage of nanocelluloses on the DNA of Caco-2 cells, cells were grown as explained above and exposed to different concentrations of CNFs. The cells were embedded in low melting point agarose and placed on Gelbond sheets. Samples were placed in the presence of lysis solution (0.23 M NaCl, nine mM EDTA, 0.9 mM Tris base; 1 % Triton X-100, 10 % DMSO, pH 10) and separated by electrophoresis at 25 V for 30 min. Cells were exposed to U.V. light as a positive control for genetic damage and to PBS and culture medium as negative controls. The slides were stained with ethidium bromide 0.02 mg/mL for 10 s, washed with distilled water, and dried for subsequent photographic recording. The reading was performed under a fluorescence microscope and analyzed using the image analyzer program IMAGE J plugin OpenComet [17]. For each cell, the Olive moment was evaluated, which refers to the tail length multiplied by the fraction of DNA in the observed comet tail. For each of the concentrations, at least 350 cells were evaluated.

## 2.5. In vivo cytotoxicity

### 2.5.1. Acute in vivo cytotoxicity assays

Female BALB/c mice (6-7-week-old) were housed in the animal facilities of the SIU research building at the University of Antioquia. The animals were maintained in cages and supplied with nutritionally adequate food (Laboratory Autoclavable Rodent Diet 5010, Labdiet, California, USA) and water ad libitum. Sterilized pine chips were utilized for bedding and changed weekly. During this trial, animals were divided into three groups of three mice each, following the recommendations of the ethics committee of the University of Antioquia. The first group was exposed to a single dose (0.1 % wt) of BR-CNFs, the second group to commercial-CNFs (0.1 % wt), and the third group of mice exposed to water to replace the CNFs, used as negative controls. Either CNFs or sterile water was delivered to the stomach via the esophagus by a single oropharyngeal aspiration using a 25G gavage needle and following previously reported procedures [18]. The mice were observed for adverse reactions immediately after treatment, 4, 24, 48, 72 h, and eight days post-treatment. All mice had their weight recorded during the 8-day follow-up. At the end of the treatment, pathogenicity tests for liver, kidney, and skeletal muscle were performed in mice from each group. The Pathology laboratory performed the pathogenicity tests at the University of Antioquia, Colombia.

### 2.5.2. Repeated dose in vivo cytotoxicity assays

Each experimental group consisted of 10 mice for this part of the study. The first group was exposed to BR-CNFs, the second was exposed to Commercial-CNFs, and the third was treated with water alone as a negative control. The mice were exposed daily for 30 days at 0.1 % wt concentration of each compound, using the same procedure mentioned above. Every day, the mice were observed for weight and behavior, and at the end of the treatment, pathogenicity tests for liver, kidney, and skeletal muscle were performed in some mice from each group. Additionally, biochemical parameters such as blood chemistry, creatinine, liver enzymes, and renal function were analyzed in these mice.

## 2.6. Statistical analysis

Statistical comparisons between treated and control cells were performed by one-way analysis of variance (ANOVA), followed by a

Tukey's multiple comparison test at a 5 % significance level ($p \leq 0.05$) after verifying the normality of the data. Analyses were performed using GraphPad PRISM 8, version 8.2.1 (GraphPad Software, San Diego, CA, USA).

### 2.7. Ethics statement

All animals were handled in strict accordance with good animal practice as defined by the Colombian code of practice for the care and use of animals for scientific purposes, established by the Law 84 of 1989. Ethical approval (Act No. 135 of 2020) for analyzing

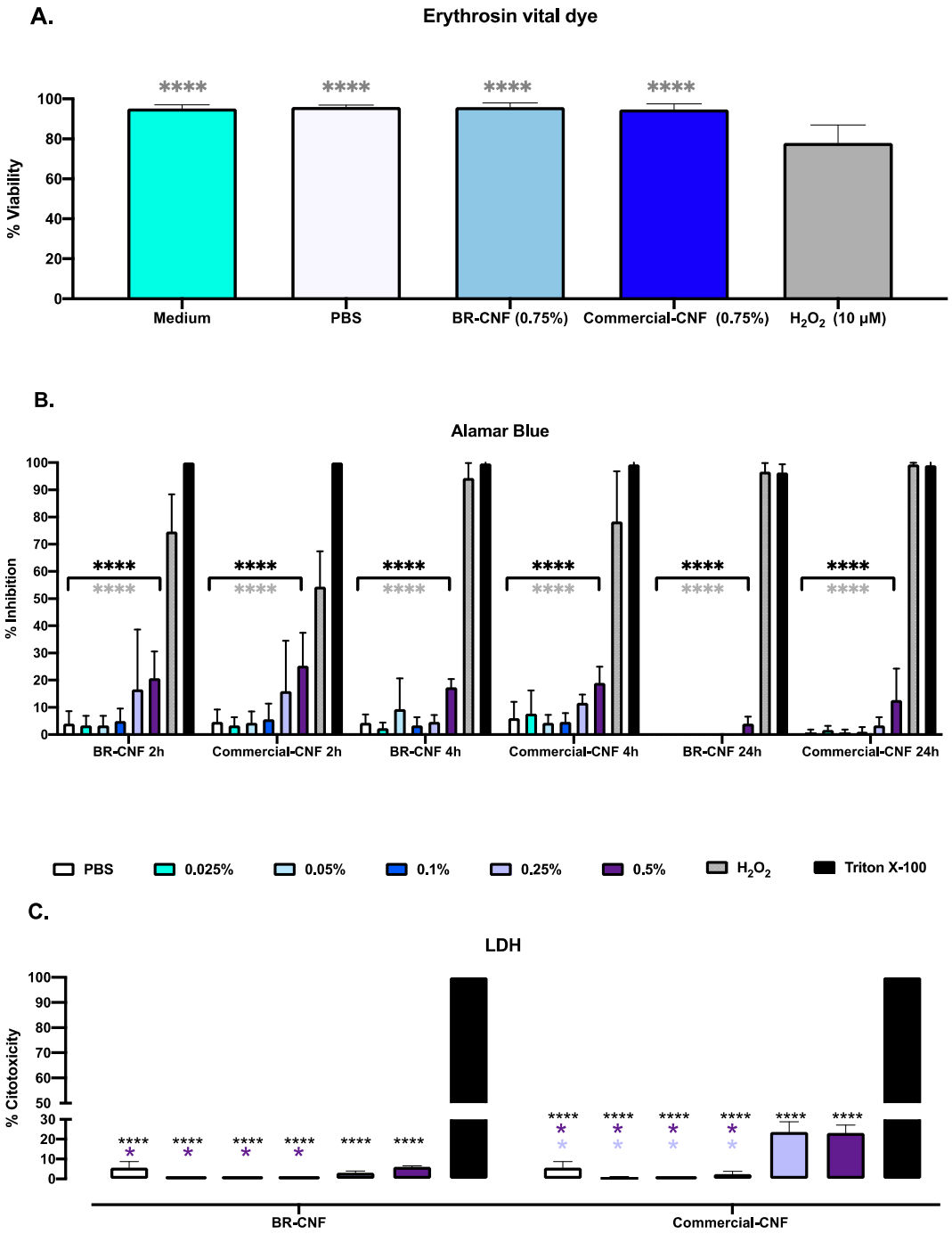


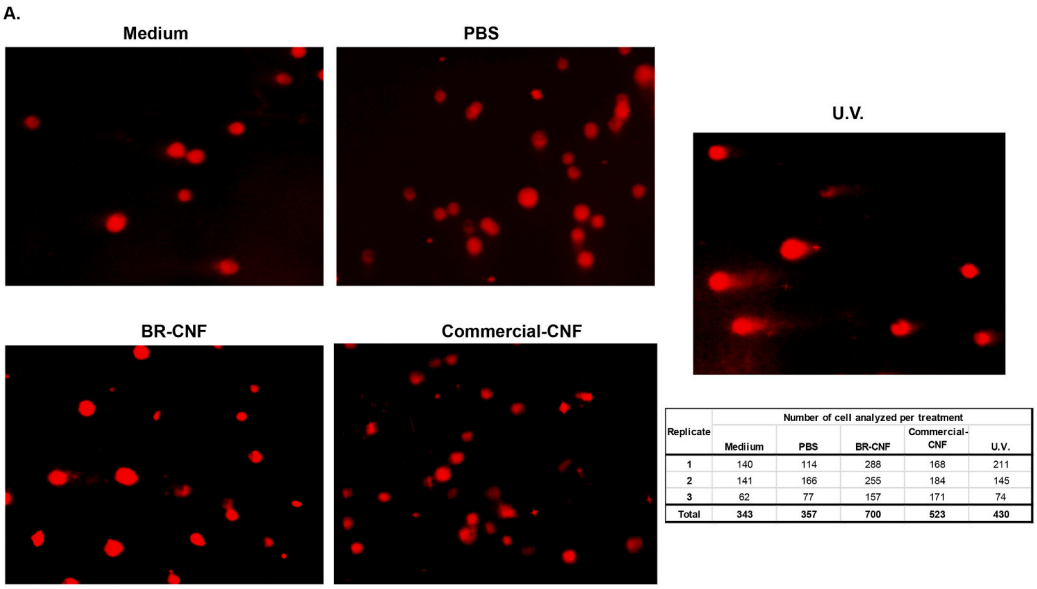

**Fig. 1.** Evaluation of CNFs *in vitro* cytotoxicity on Caco-2 intestinal cells. **A.** Vital dye erythrosin. **B:** Alamar blue. **C:** LDH enzyme activity. ANOVA followed by a Tukey's multiple comparison test was used to determine statistical differences between the treatments. *$p < 0.05$, ****$p < 0.0001$. The color of the asterisk corresponds to each treatment and indicates statistical differences with treatments where the asterisk is located.

animal specimens was obtained from the Animal Ethics Committee of the University of Antioquia.

## 3. Results and discussion

### 3.1. In vitro cytotoxicity evaluation

Cellulose nanofibers obtained from banana rachis (BR-CNFs) have considerable potential as a food source and packaging in the field of cosmetology, among many other applications. In this sense, the toxicity study of new materials designed for human consumption should be mandatory for assessing biosafety and biocompatibility before commercialization. In the current research, the cytotoxicity of BR-CNFs was evaluated by three different methodologies. In general, the multifactorial analysis of variance showed that neither the type of cellulose nor the exposure time have a statistically significant effect on cell viability (P-values $\geq$0.0) (Fig. 1). The multiple-range test indicates no statistically significant differences in cell viability between 0.025, 0.05, and 0.100 % wt concentrations. However, there is a statistically significant difference between the 0.25 and 0.5 % wt concentrations at 2 and 4 h. The results with




| Replicate | Number of cell analyzed per treatment | | | | |
|---|---|---|---|---|---|
| | Medium | PBS | BR-CNF | Commercial-CNF | U.V. |
| 1 | 140 | 114 | 288 | 168 | 211 |
| 2 | 141 | 166 | 255 | 184 | 145 |
| 3 | 62 | 77 | 157 | 171 | 74 |
| Total | 343 | 357 | 700 | 523 | 430 |

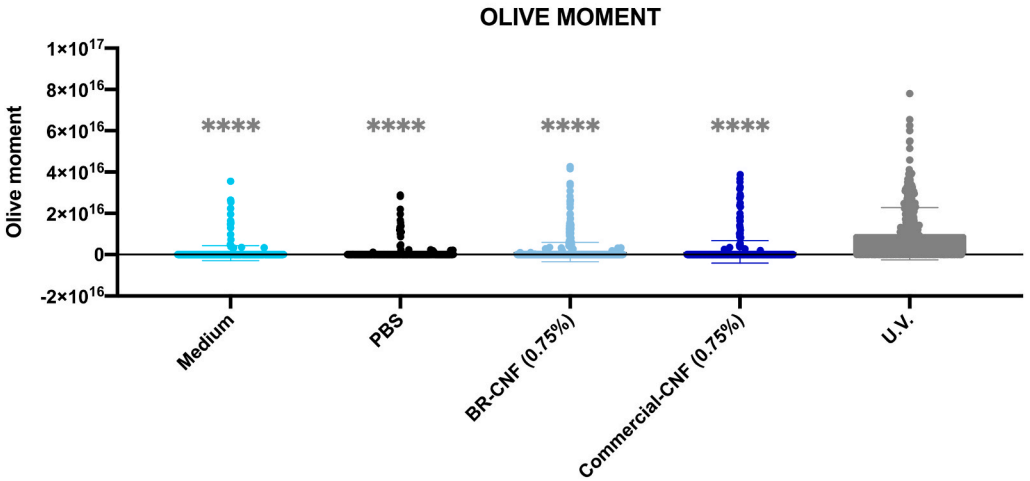



**Fig. 2.** *In vitro* genotoxicity evaluation of BR-CNF at 0.75 % wt on Caco-2 intestinal cells. **A.** Representative photos for each treatment which were used to calculate the DNA damage. The table shows the number of cells that were quantified for each replicate and treatment. **B:** Olive moment. ANOVA followed by Tukey's multiple comparison tests was used to determine statistical differences between the treatments and the positive controls (****$p < 0.0001$). The color of the circle corresponds to each treatment.

Alamar blue indicated that BR-CNF was not cytotoxic at any of the concentrations and exposure times evaluated (Fig. 1B). Cell viability in the presence of erythrosine confirmed the results obtained by the Alamar blue assay, indicating that BR-CNFs and Commercial-CNFs did not affect cell viability (Fig. 1A). Likewise, LDH release assays showed little cytotoxicity at concentrations below 0.5 % of BR-CNFs and 0.25 % for commercial ones (Fig. 1C).

Overall, the cytotoxicity results showed that CNFs obtained from banana rachis did not cause any cytotoxic effects at the concentrations evaluated on Caco-2 cells. Some previous work carried out with these same cells and banana peels as a source of CNFs have demonstrated similar results, indicating that only concentrations above 1000 μg/mL can have a toxic effect [12]. It is important to note that such high concentrations do not apply to biodegradable films. In our study, the maximum concentration evaluated was 0.75 % wt, well below the toxic concentrations previously found [19]. However, our results differ from those reported by Pereira et al., 2013, who found that CNFs at concentrations above 0.5 % wt produce plasma membrane damage, generate reactive oxygen species, and damage DNA [12]. Furthermore, Pereira et al. found that CNFs obtained from cotton were toxic in fibroblasts cultured *in vitro* at 0.2 % wt. Even at concentrations above 1 % wt, CNFs produce damage at the plasma membrane level and lead to cell death [12]. Additionally, Kisin et al. showed that short exposure of human epithelial cells to wood-derived nanocellulose induced DNA damage and increased ROS production [20]. On the other hand, Ventura et al. found an increase in the frequency of micronuclei in A549 cells and THP-1 macrophages when exposed to cellulose nanofibers obtained from *Eucalyptus globulus* [21].

### 3.2. Genotoxicity evaluation

In addition to cytotoxicity tests, genotoxicity assays are also prioritized when evaluating new products because it is necessary to complement the cytotoxicity studies. In the present study, human intestinal cells were also selected to evaluate the possible genotoxic effects of the BR-CNFs. Caco-2 cells have been previously used as a model to evaluate the toxicity of CNFs [22]. We used the comet assay to determine the genotoxic effect of the CNFs at the maximum concentration used in this study (0.75 % wt). The number of cells analyzed for each treatment (PBS, culture medium, BR-CNF, Commercial-CNF, and UV) is shown in the table inserted in Fig. 2. The olive moment indicated that only cells exposed to UV showed DNA damage. Cells treated with CNFs behaved similarly to cells exposed to PBS and culture medium (Fig. 2). In summary, Caco-2 cells showed no DNA damage at the concentrations of CNFs tested.

### 3.3. In vivo cytotoxicity evaluation

The acute *in vivo* cytotoxicity assays indicated that mice showed no sign of pathogenicity or lesions in the liver, kidney, or small intestine when treated with a single dose of BR-CNFs obtained from the banana rachis. However, for commercial nanocellulose, mild injury in the small intestine was observed (Table 1).

### 3.4. In vivo cytotoxicity at repeated doses

When the mice were treated daily for one month with CNFs, the results showed a small lesion at the level of the three organs. However, it is essential to highlight that the lesions do not correspond to the treatment with nanocellulose because the control mice treated with water presented the same type of lesions (Table 2). We observed no hyperplasia or hypertrophy in any of the organs evaluated. However, there was a degree of moderate turbid degeneration in the liver, mild vacuolar nephrosis in the kidneys, and moderate congestive splenitis in the intestine in the mice of the three groups analyzed. On the other hand, the weight of the mice remained constant throughout the experiment, with some exceptions in one of the mice treated with commercial nanocellulose and another treated with water.

Concerning the biochemical analysis, the mice treated with BR-CNFs presented values below the reference value 70 for the hepatic ALAT enzyme, with no differences between the groups. Likewise, the blood urea nitrogen analysis showed that all values are below 25, indicating that the products evaluated do not present kidney damage. Similarly, the results of creatinine and urea showed average values indicating that the kidneys of the treated mice function normally (Table 3).

Cytotoxicity and genotoxicity assays offer details on how cells respond to compounds. *In vitro* and *in vivo* results from this study

**Table 1**
*In vivo* cytotoxicity on different organs of mice exposed to single doses (0.1 % wt) of CNFs.

| Treatment | Replicate | Liver | Kidney | Small intestine |
|---|---|---|---|---|
| **Control** | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| **BR-CNF** | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| **Commercial-CNF** | 1 | 1 | 1 | 2 |
| | 2 | 1 | 1 | 2 |
| | 3 | 1 | 1 | 1 |

Injury degree: 1: without injury; 2: mild; 3: between mild and moderate; 4: moderate; 5: between moderate and severe; 6: severe. Scale proposed for the Pathology laboratory.

**Table 2**
*In vivo* cytotoxicity on different organs of mice exposed to repeated doses of CNFs (0.1 % wt) daily for 30 days.

| Treatment | Liver (n = 10) | Kidney (n = 10) | Small Intestine (n = 10) |
|---|---|---|---|
| **Control** | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 1 | 2 |
| | 2 | 1 | 2 |
| | 2 | 1 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| **BR-CNF** | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 1 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 3 | 1 | 2 |
| **Commercial-CNF** | 2 | 1 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 1 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |
| | 2 | 2 | 2 |

Injury degree: 1: No injury; 2: mild; 3: between mild and moderate; 4: moderate; 5: between moderate and severe; 6: severe.

indicate that banana rachis CNFs do not induce cell damage at the concentrations and times evaluated on Caco-2 intestinal cells. Therefore, banana rachis, as a source of cellulose nanofibers, is proposed as an excellent alternative for use in various applications in the food industry and at the biotechnological level.

Another essential aspect to highlight is the evaluated concentration of the CNFs. We evaluated concentrations up to 0.5 wt, well above those tested in other CNFs evaluated on Caco-2 cells. Mortensen et al., 2022, recently evaluated concentrations of 0.05 wt [23]. Although there is no report on the maximum dose that can be used for human consumption or what concentration is available in the intestinal tract, we evaluated concentrations well above those allowed for human consumption. Even so, at these concentrations, no cytotoxicity was found. Evaluating the cytotoxic effect of CNFs at concentrations above 1 % wt was impossible because they presented a high degree of aggregation, and solubility in the culture medium was difficult. However, as previously mentioned, these concentrations are above those used for human consumption, so using the concentration ranges to evaluate cytotoxicity is ideal. Some studies have shown that aggregated CNFs can produce more cellular damage, altering gene expression and cytotoxicity [12].

Additionally, some patents obtained for CNFs used as food additives use concentrations between 0.02 and 0.7 wt %. In this sense, the results obtained in our study support the idea that CNFs obtained from banana rachis do not present a danger to consumers.

It is noteworthy, the genotoxic effects of the BR-CNFs were also evaluated in addition to the cytotoxicity tests. All *in vitro* experiments were further verified by *in vivo* studies in the murine model. The results indicated that both a single dose and a one-month

**Table 3**
Blood chemistry in mice treated with the CNFs.

| Treatment | ALAT | BUN | Creatinin | Urea |
|---|---|---|---|---|
| **Control** | 53.47 | 22.26 | 0.52 | 47.67 |
| | 41.68 | 20.87 | 0.48 | 44.7 |
| | 78.06 | 24.76 | 0.52 | 53.03 |
| | 41.63 | 23.35 | 0.52 | 50.01 |
| **BR-CNF** | 59.5 | 22.1 | 0.44 | 47.33 |
| | 44.65 | 23.95 | 0.5 | 51.29 |
| | 52.91 | 26.8 | 0.57 | 57.39 |
| **Commercial-CNF** | 34.25 | 21.02 | 0.52 | 45.02 |
| | 67.04 | 21.68 | 0.59 | 46.43 |
| | 42.51 | 20.95 | 0.54 | 44.87 |
| | 49.31 | 21.21 | 0.54 | 45.42 |

Reference values. ALAT: 25–70; BUN: 12–25; Creatinin: 0.3–1; Urea: 25.68–53.5.

treatment at a repeated daily dose showed no effect on the health of the animals.

Finally, Colombia is considered one of the leading banana producers worldwide, so this product has the potential as a future food alternative. These results suggest that each new cellulose nanofiber produced must be evaluated to identify its cytotoxicity and genotoxicity effects.

## 4. Conclusions

Banana rachis CNFs did not significant effect on cell viability and genotoxicity in Caco-2 cells and mice. Additionally, it was found that BR-CNF did not affect the organs and mice tissues. Overall, this study shows that BR-CNFs neither cytotoxic nor genotoxic *in vivo* nor *in vitro*. However, new studies on evaluating the gut microbiome diversity in response to CNF exposure, immunomodulation, and longer treatments are necessary to validate the use of these new compounds.

## Author contributions

Omar Triana-Chavez: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ana María Mejia-Jaramillo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Catalina Gómez-Hoyos: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Ana Isabel Cañas Gutierrez: Performed the experiments; Analyzed and interpreted the data.

Natalia Correa-Hincapié: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Robin Zuluaga Gallego: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

## Data availability statement

Data will be made available on request.

## Additional information

No additional information is available for this paper.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Omar Triana reports financial support was provided by Universidad de Antioquia. Robin Zuluaga Gallego reports financial support was provided by Universidad Pontificia Bolivariana. Natalia Correa reports financial support was provided by Instituto Tecnológico Metropolitano.

## Acknowledgment

This work was supported by G8+1, a group of nine universities from Medellín that work in cooperation (Universidad de Antioquia, UdeA, grant number 617-09/20-49, Universidad Pontificia Bolivariana grant number 617-09/20-49, and Instituto Tecnológico Metropolitano grant number 617-09/20-49) and Government of Antioquia. We are very grateful to Prof. Carl Lowenberger from Simon Fraser University, Canada, for the English review and Dr. Andrés Pareja from CES University, Colombia for providing us the Caco2 cells.

## References

[1] R.J.B. Peters, G. van Bemmel, Z. Herrera-Rivera, H.P.F.G. Helsper, H.J.P. Marvin, S. Weigel, P.C. Tromp, A.G. Oomen, A.G. Rietveld, H. Bouwmeester, Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles, J. Agric. Food Chem. 62 (2014) 6285–6293, https://doi.org/10.1021/jf5011885.

[2] R. Zuluaga, J.L. Putaux, J. Cruz, J. Vélez, I. Mondragon, P. Gañán, Cellulose microfibrils from banana rachis: effect of alkaline treatments on structural and morphological features, Carbohydr. Polym. 76 (2009) 51–59, https://doi.org/10.1016/j.carbpol.2008.09.024.

[3] C. Gómez Hoyos, P. Mazo Márquez, L. Penagos Vélez, A. Serpa Guerra, A. Eceiza, L. Urbina, J. Velásquez-Cock, P. Gañán Rojo, L. Vélez Acosta, R. Zuluaga, Cocoa Shell: an Industrial By-Product for the Preparation of Suspensions of Holocellulose Nanofibers and Fat, Springer Netherlands, 2020, https://doi.org/10.1007/s10570-020-03222-6. (Accessed 15 December 2022).

[4] J. Velásquez-Cock, P. Gañán, P. Posada, C. Castro, A. Serpa, H. C. Gómez, J.-L. Putaux, R. Zuluaga, Influence of combined mechanical treatments on the morphology and structure of cellulose nanofibrils: thermal and mechanical properties of the resulting films, Ind. Crops Prod. 85 (2016) 1–10, https://doi.org/10.1016/j.indcrop.2016.02.036.

[5] F. Pinto, A.F. Lourenço, J.F.S. Pedrosa, L. Gonçalves, C. Ventura, N. Vital, A. Bettencourt, S.N. Fernandes, R.R. da Rosa, M.H. Godinho, H. Louro, P.J.T. Ferreira, M.J. Silva, Analysis of the in vitro toxicity of nanocelluloses in human lung cells as compared to multi-walled carbon nanotubes, Nanomaterials 12 (2022) 1432, https://doi.org/10.3390/nano12091432.

[6] S. Khare, G.M. DeLoid, R.M. Molina, K. Gokulan, S.P. Couvillion, K.J. Bloodsworth, E.K. Eder, A.R. Wong, D.W. Hoyt, L.M. Bramer, T.O. Metz, B.D. Thrall, J. D. Brain, P. Demokritou, Effects of ingested nanocellulose on intestinal microbiota and homeostasis in Wistar Han rats, NanoImpact 18 (2020), 100216, https://doi.org/10.1016/j.impact.2020.100216.
[7] H. C. Gómez, A. Serpa, J. Velásquez-Cock, P. Gañán, C. Castro, L. Vélez, R. Zuluaga, Vegetable nanocellulose in food science: a review, Food Hydrocolloids 57 (2016) 178–186, https://doi.org/10.1016/j.foodhyd.2016.01.023.
[8] T.S. Franco*, G.I.B. de Muniz, Nanocellulose in food science and Technology - potential, advantages and gaps of research, nov, Tech. Nutr. Food Sci. 1 (2018) 1–2.
[9] W. Mu, Y. Wang, C. Huang, Y. Fu, J. Li, H. Wang, X. Jia, Q. Ba, Effect of long-term intake of dietary titanium dioxide nanoparticles on intestine inflammation in mice, J. Agric. Food Chem. 67 (2019) 9382–9389, https://doi.org/10.1021/acs.jafc.9b02391.
[10] W. Dudefoi, Impact of food grade and nano-TiO2 particles on a human intestinal community, Food Chem. Toxicol. 106 (2017) 242–249, https://doi.org/10.1016/j.fct.2017.05.050.
[11] G.M. DeLoid, X. Cao, R.M. Molina, D.I. Silva, K. Bhattacharya, K.W. Ng, S.C.J. Loo, J.D. Brain, P. Demokritou, Toxicological effects of ingested nanocellulose in in vitro intestinal epithelium and in vivo rat models, Environ. Sci.: Nano 6 (2019) 2105–2115, https://doi.org/10.1039/c9en00184k.
[12] M.M. Pereira, N.R.B. Raposo, R. Brayner, E.M. Teixeira, V. Oliveira, C.C.R. Quintão, L.S.A. Camargo, L.H.C. Mattoso, H.M. Brandão, Cytotoxicity and expression of genes involved in the cellular stress response and apoptosis in mammalian fibroblast exposed to cotton cellulose nanofibers, Nanotechnology 24 (2013), 075103, https://doi.org/10.1088/0957-4484/24/7/075103.
[13] E.G.J. García, K. Mora, Bernal C.,Cellulose Nanofiber Production from Banana Rachis, Mater. Sci. (2020).
[14] J. Velásquez-Cock, A. Serpa, L. Vélez, P. Gañán, C. Gómez Hoyos, C. Castro, L. Duizer, H.D. Goff, R. Zuluaga, Influence of cellulose nanofibrils on the structural elements of ice cream, Food Hydrocolloids 87 (2019) 204–213, https://doi.org/10.1016/j.foodhyd.2018.07.035.
[15] M. Llana Ruiz-Cabello, M. Puerto, D. Gutiérrez-Praena, S. Pichardo, A. Jos, A.M. Cameán, Estudio in vitro de la viabilidad de células Caco-2 en presencia de componentes del aceite esencial de Allium spp, Rev. Toxicol. (2013) 144–148.
[16] J.C. del Río García, C. Moreno Ramos, P. Pinton, S. Mendoza Elvira, I.P. Oswald, Evaluación de la citotoxicidad de la aflatoxina y la fumonisina en células intestinales de porcino, Rev. Iberoam. De. Micol. 24 (2007) 136–141, https://doi.org/10.1016/S1130-1406(07)70029-3.
[17] C. Helma, M. Uhl, A public domain image-analysis program for the single-cell gel-electrophoresis (comet) assay, Mutat. Res. Toxicol. Environ. Mutagen. 466 (2000) 9–15, https://doi.org/10.1016/S1383-5718(99)00232-6.
[18] M.T. Farcas, E.R. Kisin, A.L. Menas, D.W. Gutkin, A. Star, R.S. Reiner, N. Yanamala, K. Savolainen, A.A. Shvedova, Pulmonary exposure to cellulose nanocrystals caused deleterious effects to reproductive system in male mice, J. Toxicol. Environ. Health A. 79 (2016) 984–997, https://doi.org/10.1080/15287394.2016.1211045.
[19] H. Tibolla, F.M. Pelissari, J.T. Martins, A.A. Vicente, F.C. Menegalli, Cellulose nanofibers produced from banana peel by chemical and mechanical treatments: characterization and cytotoxicity assessment, Food Hydrocolloids 75 (2018) 192–201, https://doi.org/10.1016/j.foodhyd.2017.08.027.
[20] E.R. Kisin, N. Yanamala, D. Rodin, A. Menas, M. Farcas, M. Russo, S. Guppi, T.O. Khaliullin, I. Iavicoli, M. Harper, A. Star, V.E. Kagan, A.A. Shvedova, Enhanced morphological transformation of human lung epithelial cells by continuous exposure to cellulose nanocrystals, Chemosphere 250 (2020), 126170, https://doi.org/10.1016/j.chemosphere.2020.126170.
[21] C. Ventura, A.F. Lourenço, A. Sousa-Uva, P.J.T. Ferreira, M.J. Silva, Evaluating the genotoxicity of cellulose nanofibrils in a co-culture of human lung epithelial cells and monocyte-derived macrophages, Toxicol. Lett. 291 (2018) 173–183, https://doi.org/10.1016/j.toxlet.2018.04.013.
[22] V.R. Lopes, M. Strømme, N. Ferraz, In Vitro biological impact of nanocellulose fibers on human gut bacteria and gastrointestinal cells, Nanomaterials 10 (2020) 1159, https://doi.org/10.3390/nano10061159.
[23] N.P. Mortensen, M. Moreno Caffaro, K. Davis, S. Aravamudhan, S.J. Sumner, T.R. Fennell, Investigation of eight cellulose nanomaterials' impact on Differentiated Caco-2 monolayer integrity and cytotoxicity, Food Chem. Toxicol. 166 (2022), 113204, https://doi.org/10.1016/j.fct.2022.113204.