Assessment of Nano Cellulose from Peach Palm Residue as Potential Food Additive: Part II: Preliminary Studies

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Abstract High consumption of dietary fibers in the diet is related to the reduction of the risk of non-transmitting of chronic diseases, prevention of the constipation etc. Rich diets in dietary fibers promote beneficial effects for the metabolism. Considering the above and recognizing the multifaceted advantages of nano materials, there have been many attempts in recent times to use the nano materials in the food sector including as food additive. However, whenever new product for human and animal consumption is developed, it has to be tested for their effectiveness regarding improvement in the health of consumers, safety aspects and side effects. However, before it is tried with human beings, normally such materials

would be assessed through biological tests on a living organism to understand its effect on health condition of the consumer. Accordingly, based on the authors' finding reported in a previous paper, this paper presents body weight, biochemical (glucose, cholesterol and lipid profile in blood, analysis of feces) and histological tests carried out with biomass based cellulose nano fibrils prepared by the authors for its possible use as food additive. Preliminary results of the study with mice have clearly brought out potential of these fibers for the said purpose.

Keywords Nano fibrils · Food-additive · Diet · Mice · Biochemical · Histology · *Bactris gasipaes*

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Introduction

It is well known that dietary fiber has a beneficial role in overall health of healthy adults only at adequate levels (25–38 g/day) (Slavin et al. 2009). Dietary fibers are designated following Hipsley's proposal as 'those containing carbohydrate and components resistant to the digestion or the non-digestible constituents that make up the plant cell wall'; dietary fiber has been considered as a natural food component preventing the development of digestive tract diseases and arteriosclerosis (DeVries et al. 1999; Sundberg et al. 1998). In view of this, the terminology has been given higher status in human diet, which is commonly called as "crude fiber" for the diets derived for the nutrition. On the other hand, Trowell and coworkers adopting Hipsley's definition further refined it as 'those components of the cell wall of the vegetables (cellulose, hemicelluloses, pectin and lignin) that resist the digestion of enzymes of the human digestive secretions' (DeVries et al. 1999). The terminology was later modified by including non-digestible components that are not part of the cell wall, such as gums, mucilage and storage polysaccharides (Sundberg and

Xue 1998). This definition has been in vogue for more than 30 years now. Further, these fibers have been classified as ‘soluble’ and ‘insoluble’ with respect to its solubility in water. The soluble fibers include the pectin, beta-glucans, gums, mucilage and polysaccharides, while the insoluble fibers include cellulose, hemicelluloses and lignin (Mira et al. 2009). The dietary fibers have common characteristics of not digesting in the small intestine, but are fermented in the large intestine. High consumption of fibers in the diet is still related to the reduction of the risk of non-transmitting chronic diseases, involving mechanisms such as improvement of lipid profile or prevention of the constipation. (Mira et al. 2009). Further, rich diets in dietary fibers promote beneficial effects for the metabolism with soluble fibers increasing the time of movement in intestines, reducing the speed of removal of gas as well as the glucose in food and serum cholesterol. They are highly fermentable; contribute to producing acids, residues in the large intestine. On the other hand, the insoluble fibers reduce the time of movement in intestine while increasing the fecal volume and reduce the glucose absorption. In fact, their role in improving the function of intestines reduces the absorption of triglycerides and serum cholesterol (Newmann et al. 1998). However, these fibers do not usually lower the blood glucose levels, while the soluble fiber becomes viscous when mixed with water, increasing the time of intestinal travel and leading to delay in the absorption of the glucose (Shils et al. 2003). Considering all the good effects of dietary fibers in food, gradual addition of these fibers in small amounts to the diet is considered as a good strategy to minimize the side effects such as gastroenteritis and other diseases (Wood 1977). However, there is no unanimous recommendation regarding the amount of dietary fibers required for human beings although American Diabetic Association recommends its increased ingestion such as 20 to 35 g/day for adults are available (Franz et al. 2004). World Organization of Health recommends 16 to 24 g/day fiber for the prevention of intestinal constipation in children and adults and the Council of Nutrition and Feeding (Food and Nutrition Board - FNB) (Donatto et al. 2006) indicates 38 g for adult men and 25 g for adult women.

It has been observed that the cellulose provides retention of water in the feces up to 40 % of body weight and increases the weight and the volume of fecal material in addition to increasing the peristalsis and reduces the time of transit through colon while decreasing the intraluminal pressure (Wood 1977). Recognizing the multifaceted advantages of nano materials including cellulose nano fibrils, attempts have been made for their use as food additive (Weiss et al. 2006; Chaudhry et al. 2008; Xun et al. 2012). It is well known that whenever new product for human and animal consumption is developed, be it medicine, food or additives to it, they have to be tested for their effectiveness with regard to the improvement in the

health of consumers, safety aspects and any other side effects. At certain stages of research, it is necessary to use the biological tests to verify new product within a living organism. The main model adopted for metabolism studies is the mouse (Kennedy et al. 2010; King et al. 2006) due to its high genetic variability and of the dietary habits of the human beings, the model experiments with mice is considered appropriate for experimental studies (Hernández 2006). In addition, there is also a general similarity among cardiovascular system, and other physiological systems, of mice with some of the mammals, including human beings (Menendez 1985).

The American Institute of Nutrition (AIN) has published the formula of an experimental diet for rodents, designated as AIN-76A in 1977 and 1980. This formulation was substituted and published in 1993 as AIN-93 (Reeves et al. 1993), which is used thoroughly in biological tests with mice. Biological, biochemical and histological tests would throw light on various aspects. These include: (i) body weight to know about quantity of diet taken, (ii) blood analysis for glucose, lipid profile through determination of total cholesterol, HDL and LDL, (iii) analysis of feces (excreta) to know about the quantity of minerals excreted and (iv) study of liver to know about any variation occurring between various groups of animals due to the diets consumed by them. In fact, the functions of various minerals in the body of living beings are given elsewhere (American Medical Association 1979; Cormark 1994; Soetan et al. 2010; Junqueira and José 2013; Reeves et al. 1993; Schweizer and Edwards 1992; Slavin et al. 2009; Teixeira 2001; Weaver and Heaney 1999; Zhao et al. 1995).

It is now well known that due to the rheological behavior of nano cellulose gels, nano cellulose can be used as a food additive of low calorie replacement for the conventional carbohydrate additives used as thickeners, chemical contaminants and residues, flavor carriers and suspension stabilizers in a wide variety of food products (www.fsai.ie/WorkArea/DownloadAsset.aspx?id=7858). It is useful for producing a variety of food items such as chips, wafers, soups, gravies, puddings etc., and thus such applications have been early recognized as a highly interesting application field for nano cellulose. Cellulose nano fibrils have been produced by the present authors using heart of peach palm residues and characterized for their chemical constituents and toxicological aspects (Andrade et al. 2013). As indicated in that paper, the objective of this study is to study the effect of addition of these nano fibers in diets through animal trials. Accordingly, this paper presents the results of animal trials carried out with mice with controlled diet and those prepared using different amounts of these cellulose nano fibrils in it. In view of the reasons mentioned in the foregoing paragraphs, mice for biological tests and the standard diet AIN-93 have been chosen in the present study. Biological, biochemical and histological tests have been carried out to find the effect of

addition of cellulose nano fibrils on health condition of these animals. It is hoped that the results of this preliminary study would throw light for the subsequent studies including human trials for the use of cellulose nano fibrils derived from biomass.

Experimental

Materials

Cellulose nano fibrils prepared by the authors using the Heart-of-peach palm was used in this study. Details of its preparation and characterization are reported (Andrade et al. 2013).

The diet, being the most important factor in the experimental animal nutrition, was prepared following the criteria used for the AIN-93 formulation. It includes: (i) the diet should be made starting from pure ingredients, which should be easily available at a reasonable cost, (ii) it should be in accordance with the requirement of nutrients as per National Research Council (NRC 1978, 1995; Reeves et al. 1993), (iii) its composition should be stable and reproducible, and of course, (iv) it can be used in a wide range of applications. Hence, the components for such diet were obtained from market (Rhoister Industry and Trade Ltd., São Paulo-SP).

Male mice (*Rattus norvegicus albinus*) used in this study for biological tests were adults of Wistar lineage, weighing between 150 and 200 g, obtained from the biotery, at the University of Blumenau, in the state of Santa Catarina, Brazil.

Methods

Diets

The final diets were prepared only once in the Non-Wood Products Laboratory, EMBRAPA Forestry, Colombo-PR (Brazil). Four different types of diets were prepared as follows: Type I -AIN-93 (Control); Type II- AIN-93+ 7 % of cellulose nano fibrils suspension; Type III-AIN-93+14 % of cellulose nano fibrils suspension and Type IV- AIN-93+ 21 % of cellulose nano fibrils suspension. Amounts of vitamins, minerals and other components of the diet were balanced according to the recommendations of AIN-93 M for mice. These diets were divided into appropriate portions and transferred to the biotery, University of Blumenau, where further experiments were carried out.

Characterization of the diets

The diet prepared was characterized for moisture, ash, lipids, proteins, acid contents, and the pH and calorific value. Chemical analysis for the above mentioned constituents were carried out as per the methods of Analysis of Food from

Analytical Standard of Adolfo Lutz Institute (Brasil 2005). Moisture content was determined by gravimetric method by taking 5 g of the sample and dried overnight in a hot air oven at 105 °C. Ash content in the nano fibrils was determined by heating about 2 g of the sample kept at 550 °C for 4 h, to remove all the carbon. Lipid content was extracted directly using fat extractor using ethyl ether as solvent. Quantification of protein content was made from the nitrogen determination following the classical Kjeldahl method. Amount of total fibers present in the sample was determined by modified enzymatic-gravimetric method (Dantas et al. 2006). For the determination of the acid content in the diets, titration method was followed and the pH analysis was carried out with a mixture of 10 g of the sample in 100 mL of pure water using a calibrated pH meter (Hanna Instruments, model pH211).

Feed of diets to mice

The experimental procedures followed for the feed of diets to mice were as per the standards prescribed by the Commission of Teaching of the Brazilian School of Animal Experimentation (COBEA) and the Commission of Ethics in the Use of Animals (CEUA) of the University of Blumenau/FURB/SC, Second Edition- Protocol No. 15/2012.

Biological tests

The animals were divided into four groups of eight mice each as required to feed 4 different types of diets prepared. They were fed with *ad libidum* food and water for 30 days before starting further tests on them.

The animals received a dose of rodent anesthetic conforming to Fiocruz handbook (Melo et al. 2012) (xylazine + ketamine + barbiturate) at the end of 30 days of experiment. Immediately after this, samples were collected for the histological analysis (liver) and as blood for lipidogram analysis.

Biochemical analysis

The samples of blood for the biochemical analysis were collected by puncturing the heart of mice and stored in test tubes, which were kept in refrigerator without any anticoagulant. Then, to carry out biochemical tests, the samples were centrifuged for 10 min with 3500 rpm using a Fanem Excelsa Baby Centrifuge to separate the clot (or the figurative elements of the blood) from the plasma.

Diagnostic kits were used to analyze total cholesterol and triglycerides (American Diabetes Association 2009; Analisa 2010a; Analisa 2010b; Franz et al. 2004; Tomas and Curtis 1986). All the determinations were done using blanks. Trinder enzymatic colorimeter method was used for the analysis of the total cholesterol and triglycerides, producing reddish color as observed in a spectrophotometer.

Analysis of minerals in the feces

All the samples of feces was processed in a Waring bench crusher (Model: 334BL97), and then dried at 60 °C for 24 h to remove all the residual moisture.

Approximately 0.5 g dried sample was taken in appropriate tubes to which 4 mL of nitric acid of 65 % concentration was added. Then the tubes were kept in a block digester whose temperature was gradually increased from 50 to 150 °C. After, samples have undergone digestion, as observed by its colorless nature, about 0.5 mL of perchloric acid was added and the temperature of the block was increased to 180 °C. The total digestion of the samples took about six hours. When the samples were totally digested, the tubes containing the remaining approximately 0.5 ml of the colorless mineral extract were cooled and 15 ml of pure water was added.

The micronutrients (Cu, Fe, Mn, and Zn) and the macronutrients (Ca and Mg), were determined using a Perkin Elmer Atomic Absorption Spectrometer (Model: Analyst 200). The minerals [sulfur and phosphorus] were determined using a Shimadzu UV–vis spectrophotometer (Model 1800), while, other materials (sodium and potassium) were determined using a Photometer (Model: 910 MS).

Weight of mice and glucose (blood sugar) analysis

The body weight of all the mice was taken every week using OHAUS precision balance. Glucose content in the blood samples was determined using True Read blood sugar (HOME Diagnostics).

Histological analysis

The livers of the animals were fixed with 10 % formaldehyde for 72 h. After fixation, the biological material has undergone stages of dehydration, diafanization, and paraffinization and then sliced using the microtome. The cut material was then hydrous and stained with hematoxylin and eosin. The glass slide was used for observation under an optical microscope to find the integrity or lesion in the liver, which would help in evaluating the tissue architecture and presence of any alteration or hepatic steatosis.

Statistical analysis

The statistical analysis of all the results was carried out by the variance analysis (ANOVA: double factor without repetition) with Tukey post test. The value of $p < 0.05$ in the all the experimental groups were considered as having statistically significant difference. Values of $p > 0.05$ indicated that the

obtained data were repetitive and can be reproducible. For this statistical analysis was used the GraphPad Prism version 5.01 software.

Results and discussions

Diets

The composition of the standard diet is shown in the Table 1. Distinct photographs of this standard diet and the other 3 diets containing different amounts of cellulose nano fibrils are shown in the Fig. 1.

Biological tests in rats [*Rattus norvegicus albinus*]

Composition of diets

The composition of the diets is shown in the Table 2. It can be seen that the content of lipids is below prescribed value (4 g/L) at AIN – 93 M.

Table 1 Composition of standard Diet (AIN-93) as per the formulation (12 → Reeves et al. 1993)

Corn Starch (g/Kg)	465.69
Casein (85 % de protéin) (g/Kg)	140
Dextrinized corn starch (g/Kg)	155
Saccharose (g/Kg)	100
Bean oil (g/Kg)	40
Fibers (g/Kg)	50
Mineral Mixture (AIN-93 M) (g/Kg) *	35
Vitamins Mixture (AIN-93-M) (g/Kg) **	10
L-Cistin (g/Kg)	1.80
Choline bitartrate (41.1 % Choline) (g/Kg)	2.50
Terc-butile-hydroquinine (TBHQ) (mg/Kg)	8

*The mixture (in g/kg) consists of: Anhydride calcium carbonate (357.00); Monobasic potassium phosphate (250.00); Potassium citrate (28.00); Sodium chloride (74.0); Potassium sulfate (46.60); Magnesium oxide (24.00); Ferric citrate (6.06); Zinc carbonate (1.65); Sodium silicate (1.45); Manganese carbonate (0.63); I Copper carbonate (0.30); Chromium-potassiumsulfate (0.275); Boric acid (81.50) (mg/Kg); Sodium fuoret (63.50) (mg/Kg); Nickel carbonate (31.80) (mg/Kg); Ammonium lithium chloride (17.40) (mg/Kg); Anhydride sodium selenate (10.25) (mg/Kg); Potassium iodide (10.00) (mg/Kg); Ammonium Paramolibdate (7.95) (mg/Kg); Ammonium Vanadate (6.66) (mg/Kg); Sucrose powder (209.806)

**Vitamin Mix (in g/kg): Nicotinic acid (3.00); Calcium Pantionate (1.60); HCl-pyroxoxin (0.70); HCl-thiamine (0.60); Riboflavine (0.60); Folic acid (0.20); Biotine (0.02); Vitamin B-12 (2.50); Vitamin E (15.00); Vitamin A (0.80); Vitamin D-3 (0.25; Vitamin k-1 (0.07); Sucrose powder (974.655)

Fig. 1 Photographs of Diets Prepared in This Study: (a)- Control [AIN-93]; (b)- AIN+7 % NCF; (c)-AIN+14 % NCF and (d)- AIN+21 % NCF

Analysis of body weight of mice

Figure 2 shows the plots of variation of weight of mice versus time for all the 4 diets. It can be seen that in all the cases, the body weight increases with time feeding after the first week, although in different proportions except during the third week, when slight decrease (about 0.42–0.88 %) is observed. Also, the final weight gain is between 9–10 % in all cases, the highest being in the control group. Statistical analysis by regression analysis of the results revealed values of $R^2=0.9388$, 0.882, 0.8495 and 0.8942 respectively for control and groups I, II, III.

Further, the values of p for the groups were 0.0065, 0.0164, 0.0260 and 0.0151, all of them being <0.05 , suggest that there is significant difference in these values with increasing time in the same group. These data also indicate that the mice in all groups were healthy and usually have ate the food, rather than rejecting it, corroborating the experimental observation of absence of abnormal manifestations such as vomiting and / or diarrhea.

When these values were analyzed using ANOVA tests to find out whether there were correlation between the different feeding times and mice groups, the value of p was found to be 1.0 (>0.5), suggesting that there was no significant variations in the correlation between the two analyzed variables (time and different groups).

Biochemical analysis

Analysis of blood glucose in the rats

Blood glucose level is essential for verification of homeostatic processes of metabolism. These rates were determined for different groups of animals throughout the experiment, and the results are shown in Fig. 3.

For the control group, the animals showed glucose values of 107.37 mg/dL at the beginning of the experiment, and this value reached 110.25 mg/dL at the end of 28 days, showing an increase corresponding to 2.88 mg/dL, ie., overall increase of approximately 5 %.

Group 1 was fed with standard chow plus the addition of 7 % cellulose nano fibrils gel the glycemic rate increased from

Table 2 Composition of diets prepared for feeding the rats (in g/100 g, on dry basis)

Constituent	Control
Moisture	8.81
Ash	3.16
Fibers	10.85
Protein	6.47
Lipids	1.09
Acids	19.36
pH	6.16
Carbohydrates	78.43
Total Calorific Value (kcal/100 g)	349.37

Fig. 2 Plots of Body Weight of Rats having Different Diets Vs. Time [Duration of Tests]

Fig. 3 Plots of Glucose Content (mg/dL) in Rats having Different Diets Vs Time

103.37 mg/dL to 129.0 mg/dL, a value about 25 % higher than at the beginning of the experiments.

The second group fed with 14 % cellulose nano fibrils gel added to standard chow, started the tests with the lowest values of glucose, 97 mg/dL, and reached at the end, an average of 111.12 mg/dL, representing a total increase of 14.55 %.

The third and last group, which was fed with standard feed, added a 21 % cellulose nano fibrils gel, the experiment started with an average of 100 mg/dL, with mean values of 106.00 mg/dL. The difference in glucose values for this group was 6 %. The statistical analysis by ANOVA test showed that there was no significant difference between the four groups; furthermore, there are not statically different from glucose contents with feed time.

The results of this study showed that the supplement provided to the animals did not affect glycemic rate, indicating the proper maintenance of homeostasis in animal experimentation. Further, these findings do not agree with those reported by the Vivarium of USP (Melo et al. 2012), showing blood glucose levels in rats between 150.7 and 207.5 mg/dL, but corroborate data of Guimarães and Mazaro 2004, 116.1 mg/dL and approach the data recorded

by Dantas et al. 2006 108.0 mg/dL. The differences between the values found in the literature and reported in this study may have resulted from experimental variations, and the stress suffered by the animals during the period of confinement.

Analysis of lipids profile

Triglyceride levels (Fig. 4 a) found for the control group were 87 ± 19 mg/dL. For the animals of group 1 value recorded was 100 ± 16 mg/dL, group 2 was 98 ± 12 mg/dL, and the animals of group 3 was 117 ± 23 mg/dL, this last group was fed with the largest fraction of nanofibrils suspension. These values did not show significant differences when compared among groups. The amounts of triglyceride for all groups, were lower than reported by the USP's biotery (Melo et al. 2012), which are 110 to 175 mg/dL.

On the other hand, all groups had similar values to those found in studies of Guimarães and Mazaro 2004, which reported rates of 83.7 mg / dL, and close to the results of Dantas et al. 2006, with 82.0 mg / dL. Besides, it is suggested increased samples number should be taken for future studies to reduce the standard deviation of the measured values.

The total cholesterol values found for the animals after completion of the experiment are shown in Fig. 4 b. None of the groups showed statistically significant variations.

All total cholesterol values measured for healthy animals without subjected to any treatment are found to be lower than 84 mg/dL, reported by Guimarães and Mazaro 2004, or 98.9–110.2 mg/dL found by Melo et al. 2012. Among others, adequate intake of dietary fiber has the advantage of lowering cholesterol, which was not observed in the data obtained (Botelho et al. 2002; Franz et al. 2004).

It is noteworthy that the biochemical parameters may vary according to sex, strain, genotype, and are influenced by age, diet, handling and environment, among other factors

Fig. 4 Variation of Biochemical Parameters in Rats Fed with Different Diets (a): Triglycerides, (b): Total Cholesterol

(Menendez 1985). Nevertheless, these results strongly suggest that the amount of fiber used in the experiment was not enough to cause significant biochemical effects in animal metabolism.

Analysis of minerals in excreta

The water retention capacity of the fiber can influence the digestion and absorption of minerals (Zhao et al. 1995). All

Fig. 5 Results of Statistical Analysis of Minerals in Excreta of Rats of Different Groups Values of Variation <0.05 as per ANOVA Software

the elements analyzed with important role in several metabolic pathways (Slavin et al. 2009).

Phosphorus, potassium, copper, iron and zinc contents did not show significant differences among groups statistically analyzed by ANOVA tests (Fig. 5).

Sodium showed a p-value of 0.0006 (less than 0.5), showing significant variation for this mineral. This mineral has vital functions such as osmotic pressure, acid–base balance and control of water metabolism (Lopes 1998).

Calcium binds to many cellular proteins, resulting in their activation (Weaver and Heaney 1999). This mineral showed a significant difference only when the control group was compared with others. These data show that a small addition of nano fibrils did not cause any significant effect; however, in

higher concentrations the changes were significant suggesting that nano fibrils might have been responsible for the increased release of this mineral.

Manganese showed significant variations from the control group to groups 2 and 3; also from group 1 to groups 2 and 3, which indicates that this element is possibly affected by nano fibrils when added in higher concentrations.

As for nitrogen, the ANOVA showed a p-value of 0.0004, which is much lower than 0.5, showing significant variation in this element. Significant differences were found by Tukey test when control was compared to the other groups, suggesting that nitrogen has been affected by nano fibrils. According to Tomas and Curtis 1986, nitrogen absorption is affected by diets with high dietary fiber

Fig. 6 Microphotographs of Different Liver Rats Showing the Histological Analysis. (a & c): Control Diet; (b & d): Diet with 7 % Cellulose Nano Fibrils; (e-f): Diet with 14 % Cellulose Nano Fibrils; (g-h): Diet with 21 % Cellulose Nano Fibrils [Magnifications: Fig. a-d are of $\times 100$ and Figures e-h are of $\times 400$]

content, thus the amount of nano fibrils was enough to see a small change.

Schweizer and Edwards 1992 have also reported that the effects of the fibers or utilization of nutrients is not dependent on chemical composition, structure, viscosity and particle size of the food. The intake of soluble fibers such as pectins and gums delays and decreases the absorption of nutrients, while insoluble sources such as wheat bran, produce little effect on the absorption of nutrients in the small intestine. This would be the same situation for the chow addition with insoluble cellulose nano fibrils suspension.

Histological analyses

Figure 6 shows the histological analysis of livers of all the groups of mice as observed by optical microscope at two magnifications ($\times 100$ and $\times 400$). These samples are transverse cuts of hepatic and neighboring lobes. It can be seen that samples from mice fed with the control diet are similar to those reported in the literature (Junqueira and José 2013). At higher magnification, it can be seen that there are small openings and the hepatic lobes are towards the center of lobular vein. In the latter case, one can see some red blood cells together with endothelial cells that constitute vein wall. They are equally sinusoidal visible capillary in the hepatocyte strands, converging towards the center of lobular vein. Besides, normal morphologic aspect of the liver of the mice fed with diets containing nano fibrils can yet be seen in Fig. 6 wherein no structural alteration of any type in the organ can be observed with the optical microscope even after increasing amounts of the nano fibrils addition in the diet.

Examples of alterations in the hepatic parenchyma in the case of harmful effects of the cellulose nanofibrils would be seen by the presence of fine periportal hepatocyte vacuolation, indicating an increased storage of the triglycerides in their interiors, or with changes in the limit cytoplasm, hindering perfect visualization, or still, the chromatin in more condensed nuclei of the same. Equally, it would be an abnormality sign of an increase for macrophages present in the periphery of the sinusoid. However, none of these characteristics showing any changes from that of control fed mice could be seen in the present study, indicating that added cellulose nano fibrils did not cause any perceptible histological damages in the livers of the animals.

In the extremities of the analyzed organs, independent of the experimental conditions, some hepatocytes with the swollen cytoplasm were observed, indicating the presence of holes. This was found in all the four groups of mice, the highest amount observed being in the mice fed with control. Probably, this could be due to the preparation of samples and hence may require further experiments to confirm this using other fixatives, like ALFAC and BOUIN. Similarly, further studies may be required to evaluate the effect of addition of higher amount of nano fibrils to the diets on other organs, such as stomach

and intestine for finding out the abnormalities, if any, that might occur due to the addition of cellulose nano fibrils.

Conclusions

- With the addition of nano fibrils to the diet given to the mice, there was a trend for an increased weight of the animals over experimental time.
- The blood sugar of all the mice did not show any significant statistical difference over the whole duration of the experiment, indicating the maintenance of homeostasis of the animals in experimentation.
- The lipid profile of all the mice neither showed statistically any significant increase in the concentration of serum triglycerides with increasing amount of cellulose nano fibrils in the diet, nor significant difference in the levels of total cholesterol.
- There was no relevant physiological loss of mineral nutrients with increasing amount of cellulose nano fibrils.
- The supplemented diet with cellulose nano fibrils did not cause hepatic damages as observed in the images of histological studies of all the mice.
- The amounts of fibers used in the present work did not cause harmful effects in the animal metabolism, indicating that in small concentrations the bleached cellulose pulp from peach palm and processed to prepare cellulose nano fibrils could be used as dietary supplement.
- Future studies would be necessary for the evaluation of the effect of larger amounts of nano fibrils in the animal diets followed by more selective biochemical analyses, such as cholesterol HDL, LDL and HDL and histological studies of other important organs for toxicological evaluation such as the stomach and the intestines of the animals.

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