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# Assessment of effectiveness of oral supplementation of isolated fiber of carrot on metabolic parameters in mature rats

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#### ABSTRACT

This present study was conducted to evaluate the metabolic effects of isolated fiber of carrot supplementation in rats. Physicochemical properties of fiber were determined. The groups were as follows: animals fed a standard diet, control group; high fiber supplementation (70 mg); low fiber supplementation (35 mg); for 12 weeks. Blood samples were collected at the time of sacrifice. The weights of heart, liver, kidneys and spleen of the experimental rats with respect to body weight were recorded. Commercial kits were used to determine serum glucose concentration, lipid profile (cholesterol, HDL-cholesterol, triglycerides), and the two main aminotransferases glutamic-oxalacetic transaminase (GOT)/glutamate-pyruvate transaminase (GPT). A histopathological assay was performed on the heart, liver, and spleen tissues of animals. Supplementation with fiber favors weight loss in female ( $(242.03 \pm 23.73)$ –(197.81 ± 10.45) g); and male rats ( $(262.50 \pm 32.21)$ –(213.96 ± 12.56) g and induces a decrease in glucose levels in the supplemented animals. With the exception of total high-density lipoprotein cholesterol, the other lipid fractions decrease significantly in rats supplemented. Fiber supplementation did not induce changes in the dissected organs of the supplemented animals. In conclusion supplementation of fiber, improves glucose control, lower plasma lipid concentrations and reduced body weight in normal rats.

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# 1. Introduction

Fibers are naturally occurring metabolites from plants. They are present in fruits, vegetables and roots [1,2]. These compounds are an integral part of human diet and are also taken intentionally as food preparations or fiber-rich products [3]. They act as inhibitors or activators for a large variety of mammalian enzymes systems [1], and as metal chelators and scavengers of free oxygen radicals [4,5]. As with other roots, fruits and vegetables, carrot (*Daucus carota*) contain a range of micronutrients which are essential for health.

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In particular carrots contain a high level of natural antioxidants, especially  $\alpha$ - and  $\beta$ -carotene and phenolic compounds [6,7]. However, carrots may have additional health benefits as they are also rich in dietary fibers, which can be subdivided into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) according to their solubility in water [8]. It has been hypothesized that effects of these complex mixtures of polysaccharides, oligosaccharides and phytochemicals, instead of a single component, are responsible for the health benefits derived from fibers [9,10]. Among which stand out the control of satiety, reduction energy intake, reduction of body weight, of cholesterol and the triglycerides in humans and animals [1,3-5].

However, it was not possible to identify previous studies that have evaluated the effects of isolated and purified fiber from discarded carrots. After the harvest, carrots that not meeting the quality standards and the requirements of size and shape imposed

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by the consumer market, are discarded. As a consequence, packing companies generate a big amount of discard, approximately 30% of the harvest, which is used as cattle food. Cattle consume only a part while the rest decompose producing significant environmental damage [11]. In Santa Fe, Argentina, the estimated discard volume is 80–100 tons/day during the harvest time (June to December) [12]. In order to decrease the environmental impact produced by carrot discards and increase the sustainability of this important primary crop, an integral process of extraction of valuable by-products from discarded carrots (DC) (ethanol, carotene and a fiber rich fraction), was proposed by Clementz et al. [8].

For the reasons stated, this raw material was chosen to evaluate the effect of prolonged administration in rodents. In such a context, the aim of this study was to investigate *in vivo* effects of isolated carrot fiber used as a supplement on basal metabolism, in rodents. The main focus points in this study were analyze whether the carrot fibers obtained from discards, with methodology described previously, has properties on gain weight, blood glucose, triglycerides, and the reduction of total cholesterol in rats.

# 2. Materials and methods

# 2.1 Raw materials

DC (*Daucus carota*), were collected from a packing shed in the Garay department area ( $31^{\circ}25'$  S,  $60^{\circ}20'$  W), Santa Fe, Argentina. As for handling and storage, the method described by Aimaretti and Ybalo [12] was used, which consists of extracting the areas of carrots attacked by microorganisms and then storing the discards at 4 °C until their use.

## 2.2 Preparation of carrot discard dietary fiber (CDDF).

The procedure for CDDF preparation from CD was described previously (Proccess 3) [8]. After drying step CDDF were milling in a micronizer mill up to a fine powder (Fig. 1).



Fig. 1 Fiber isolated from discarded carrots.

#### 2.3 Animals and study design

#### 2.3.1 Animals

All procedures and animal cared adhered strictly to Institutional guidelines for experimental animal health, safety, and comfort of General Directorate of Teaching and Research and Professional Development (Argentina, Res HCS No. 8469). Ninety 62-week-old

female and male rats were housed in a room controlled for temperature ((22  $\pm$  1) °C) and a 12/12 h light-dark cycle with free access to food and water for a 2-week adaptation period prior to the study. After acclimatization, animals that reached a weight equal to or greater than 200 g (inclusion criterion for the study), were used for the experiments. The mature animals were randomly assigned to three groups, to receive a standard diet or fiber-containing diets. They were fed for 12 weeks with a diet (Ganave, Argentina) containing 0 (Control), 0.035 (lower dose) or 0.070 (higher dose) g/kg (carrot isolated fiber/rats weight) [13]. The monitoring of diet consumption showed an intake within the administered dose range. In this study, the dose of fiber was one of the most important considerations, so the amount was calculated as a supplement based on the recommendations of various research works [14]. The rats had free access to water throughout the experiment. Observations were made daily on the experimental animals, mainly, changes in the skin and fur, eyes, mucous membranes, occurrence of secretions and excretions, autonomic activity (lacrimation, pupil size, unusual respiratory pattern), changes in the weight, posture, and response to handling. As well as the appearance of tonic or clonic movements, stereotypies (excessive grooming, repetitive turning) or other behavior alterations [15].

#### 2.3.2 Body weight assessment and blood collection

Body weight was assessed at baseline, every three weeks, and at the end of the experiment. After 12 weeks of supplementation, the animals were euthanized by decapitation after a 12 and 8 h fasting period; as well as 2 h, after feeding (n = 5 per group). Blood was collected in test tubes and settled for 30 min before centrifugation. It was subsequently separated into aliquots and stored at -80 °C until the completion of serum analysis, to determine the levels of glucose, total triglycerides, total cholesterol as HDL cholesterol [16-18]. Glutamic-oxalacetic transaminase (GOT) and glutamate-pyruvate transaminase (GPT) enzymes were also determined. In this study, high-density-lipoprotein (HDL) cholesterol was measured enzymatically after precipitated with heparin-manganese. Cholesterol in the low-density lipoprotein (LDL) fraction was estimated to be the difference between the cholesterol content of the infranatant and that of the HDL fraction. The kidneys, liver, stomach, spleen, and heart were then dissected to determine their weight and perform anatomopathological analyses.

#### 2.4 Analytical methods

For this study, contents of moisture, ash, fats and total dietary fiber (soluble and insoluble) were determined employing AOAC standard methods 934.01, 942.05, 922.06, 991.43, respectively (AOAC, 2000). The nitrogen content was determined by the Kjeldahl method 2001.11 (AOAC, 2000) [19]. Protein content was estimated as the nitrogen content multiplied by 6.25. The heavy metal contents (Hg, Pb, As, Cd) was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES, in a Perkin Elmer, Optima 2100 DV) after digestion in an acid solution of 1:5 (*VIV*) nitric:perchloric acid. Water-holding capacity (WHC), oil-holding capacity (OHC) of carrot fibers were determined following the methodology described

by Robertson et al. [20]. The density of the fibers was determined by a technique of weight: volume using a graduate probette. The microbiological characterization of CDDF was made using a bacteriological diagnostic kit provided for ZT Lab of Argentina. Total plate count, total coliforms and Salmonella were determined.

#### 2.5 Serum biochemical analysis

The biochemical determinations were carried out using Commercial Wiener kits (Wiener laboratory, Riobamba 2944-(S2003GSD) Rosario–Argentina). The values obtained were expressed in mg/dL. For euthanasia, the rats were sedated and then euthanized.

#### 2.6 Relative organ weight of the animals

At the time of sacrifice, the hearts, livers, spleen and kidneys of the experimental rats were identified, removed, rinsed with physiological saline solution and dried carefully with tissue paper. The weights (g) of the organs, with respect to the body weight of the rats, were immediately recorded.

# 2.7 Histological analysis

At the end of the supplementation experiments, following the sacrifice of the animals, the liver, heart, kidneys and spleen were removed, stored in 25% neutral formalin and embedded in paraffin wax. The organs were sectioned and stained with hematoxylin and eosin, according to Culling [21]. The stained slides were visualized through a light microscope (Olympus, Tokyo, Japan) ×400 magnification for histological evaluation. At least 5 sections were viewed and photographed at ×400 magnification.

#### 2.8 Statistical analysis

Statistical analyses were carried out using INFOSTAT version 2020p with the data expressed as means  $\pm$  SD. One-way ANOVA was performed using with a Duncan multiple comparison test and a Tukey test post hoc, P < 0.05 was considered to be statistically significant.

#### 3. Results

# 3.1 Characterization of CDDF

Table 1 summarized the physicochemical and biological properties of composition of CDDF indicated that the same could be a promising source of food fiber or as a low-calorie bulk ingredient in functional food applications. CDDF can be used directly as food ingredient because it contains low amounts of fat, protein and ash [22]. The total dietary fiber content in carrot discards was  $(74.00 \pm 0.22)\%$  dry sample, where  $(54.3 \pm 0.5)\%$  corresponds to IDF, and the remaining  $(19.6 \pm 1.2)\%$  to SDF. The SDF/IDF ratio is important for dietary and functional properties, structural and sensorial properties. It is generally accepted that those fiber sources suitable for use as food ingredient should have an SDF/IDF ratio close to 1:2 [19]. The SDF/IDF ratio in this investigation was 1:2.77. In this respect, carrot fiber provides a very suitable tissue for food supplementation.

WHC is defined as the quantity of water that is bound to the fibers without the application of an external force (except for gravity and atmospheric pressure) [20]. A typical function of the IDF fiber is the capability to absorb and retain water. The WHC value obtained from CDDF was  $(18.00 \pm 0.40)$  g/g which is very high for a natural fiber. In addition, CDDF is cold and hot swelling and does therefore not need any technological changes. The WHC value obtained in this research is higher than the agricultural by-product from other fibers, e.g., 10 g/g sugar beet fiber, 4.5 g/g apple fiber, 3.1 g/g wheat fiber [20]. On the basis of this value CDDF can be used as an ingredient in food products, e.g. for preventing synaeresis and modifying the viscosity and texture of some formulated foods [22]. OHC is the amount of oil retained by the fibers after mixing, incubation with oil and centrifugation [18]. The OHC value of carrot discards was higher to dehydrated carrot,  $(9.00 \pm 0.25)$  and 5.5 g/g fiber, respectively [23]. The OHC value found is however higher than those of other by-products such as apple (1.3 g/g fiber) or sugar cane bagasse (3.26 g/g fiber) [24]. This OHC value suggests the possibility of using the fibers for the stabilization of high fat food products and emulsions [18]. The results of the microbiological analyses to which CDDF was subjected indicate that the method of obtaining it used guarantees its safety and makes it a product suitable for human and animal consumption.

#### Table 1

Composition of carrot fiber obtained from discarded carrots.

Index	Result		
Sensory properties			
Texture	Powder		
Appearance/colour	Pale yellow		
Taste	Neutral		
Chemical composition			
Water (%)	$12.00 \pm 0.85$		
Soluble Fibre (%)	$19.6 \pm 1.2$		
Insolube fibre (%)	$54.3 \pm 0.5$		
Total fibre (%)	$74.00 \pm 0.22$		
Proteins (%)	$7.00 \pm 0.25$		
Fats (%)	$0.20 \pm 0.02$		
Ash (%)	$7.20 \pm 0.30$		
Heavy metals (mg/kg)	< 10		
Physical properties			
Water-holding capacity (g/g)	$18.00 \pm 0.40$		
Oil-holding capacity (g/g)	$9.00 \pm 0.25$		
Density (g/cm <sup>3</sup> )	$300.0 \pm 10.0$		
Microbiological characteristic			
Total plate count (UFC/g)	< 1 000		
Salmonella	Negative		
Total coliforms (UFC/g)	< 10		

Notes: Values are means  $\pm$  standard deviations (n = 3).

#### 3.2 Animal studies

Food intake was not significantly different among the groups throughout the experiment. During the 12 weeks' exposure period, no toxic symptoms were observed at the administered dose, both on a general physical and behavioral level. At the end of the 12 weeks of

#### Table 2

Variations in body weight (g) after 12 weeks of supplementation.

Gender	Group	Initial BW	BW 30 days	BW 60 days	Final BW
	Control	$218.50 \pm 23.73$	$233.50 \pm 27.21$	$239.50 \pm 17.28$	$242.03 \pm 23.73$
Female	Low dose	$217.02 \pm 12.68$	$215.51 \pm 14.65$	$207.12 \pm 10.21$	$197.94 \pm 11.47$
	High dose	$225.05 \pm 13.74$	$216.92 \pm 11.47$	$211.54 \pm 11.71$	$197.81 \pm 10.45^{\circ}$
	Control	$233.86 \pm 28.81$	$238.68 \pm 30.48$	$251.01 \pm 29.64$	$262.50 \pm 32.21$
Male	Low dose	$240.21 \pm 11.35$	$233.32 \pm 13.74$	$227.05 \pm 13.52$	$223.75 \pm 14.64$
	High dose	237.58 ± 12.21	$224.12 \pm 14.01$	$217.35 \pm 15.01$	213.96 ± 12.56*

Table 4

Notes: Data are expressed as the mean  $\pm$  standard deviation. \* Indicates significantly different (P < 0.05). n = 5. BW: body weight.

treatment there was no death of any animal and all the animals were sacrificed. During necropsy, no microscopic or macroscopic lesions were found, related to supplementation.

#### 3.2.1 Body weight

Experimental and epidemiological evidence suggests an association between fiber intake and weight loss [2,25,26]. The mean body weights for each group are shown in Table 2. The present study registered normal weight gain behavior and significant differences were found after 12 weeks of treatment. A decrease in body weight was detected in the treated groups and this trend was constant during the study. Likewise, it is observed that, in the groups treated with fiber, the difference ranges between 7% to 9.94% ((237.58 ± 12.21); (213.96 ± 12.56) g) in males and 8.79% to 12.10% (225.05 ± 13.74); (197.81 ± 10.45) g) in females compared to first day of supplementation. The relative weight values of the organs did not show significant differences with respect to the control. However, an increase in the relative weight is observed in the kidneys of the supplemented animals in relation to the controls (Table 3).

#### Table 3

Variations in relative organ weight (g) from rats after 12 weeks of supplementation.

Gender	Orgon	Control	Cumplomontod		
Gender	Organ	Collutor	Supplemented		
	Heart	$0.262 \pm 0.070$	$0.265 \pm 0.060$		
Female	Kidney	$0.313 \pm 0.080$	$0.353 \pm 0.110$		
Female	Liver	$3.174\pm0.120$	$3.275\pm0.140$		
	Spleen	$0.236 \pm 0.020$	$0.232\pm0.040$		
	Heart	$0.316\pm0.050$	$0.316 \pm 0.040$		
Male	Kidney	$0.383 \pm 0.090$	$0.416\pm0.120$		
Male	Liver	$3.576 \pm 0.120$	$3.434 \pm 0.100$		
	Spleen	$0.246\pm0.040$	$0.248 \pm 0.070$		

Notes: Data are expressed as the mean  $\pm$  standard deviation. \* Indicates significantly different ( $P \le 0.05$ ). The values correspond to the organs of the rats supplemented with the highest dose. n = 5.

#### 3.2.2 Laboratory measurements

The effect of the carrot fiber supplementation on cholesterol, triglyceride and glucose, concentrations are shown in Table 4. Regarding the biochemical parameters, during the 12 weeks of supplementation statistically significant differences were observed between the levels of glucose, cholesterol and triglyceride, in supplemented animals in relation to the controls.

	Biochemical parametars	Concentration (mg/dL)								
Gender		12 h			8 h		2.5 h			
		Control	LD	HD	Control	LD	HD	Control	LD	HD
	Glucose	126.58	118.30	115.50*	128.4	113.60*	114.50*	138.60	135.50	137.65
		(26.10)	(16.92)	(5.06)	(13.99)	(10.72)	(7.68)	(16.42)	(12.45)	(15.43)
	TG	183.33	141.80*	106.87*	180.6	151.35*	128.50*	167.66	104.55*	117.52*
M-1-		(15.39)	(3.65)	(8.23)	(17.23)	(12.28)	(16.34)	(13.45)	(12.47)	(15.68)
Male	TC	56.37	54.53	51.02*	54.75	51.24	46.03*	70.12	64.50*	56.06*
	IC	(3.96)	(10.63)	(3.43)	(9.78)	(5.06)	(8.02)	(3.160)	(8.75)	(12.39)
	HDL	32.05	27.25	29.05	32.08	33.50	31.05	35.05	36.08	29.50
		(3.27)	(3.43)	(8.02)	(2.08)	(13.99)	(9.78)	(2.08)	(5.64)	(9.02)
	Glucose	129.37	126.04	127.66	133.03	111.66*	113.04*	131.5	128.53	123.66*
	Glucose	(14.62)	(6.24)	(9.40)	(16.92)	(10.87)	(12.36)	(14.31)	(11.65)	(9.52)
	TG	216.53	162.36*	130.64*	196.00	156.65*	132.03*	207.50	161.00*	142.53*
Female		(2.83)	(9.07)	(11.4)	(9.47)	(12.61)	(14.35)	(13.33)	(9.46)	(15.23)
	TC	57.71	56.71	55.83	58.55	53.12	51.33*	63.52	58.52	64.04
		(12.56)	(18.2)	(11.8)	(3.86)	(3.96)	(10.63)	(3.43)	(9.78)	(10.05)

Variations in serum biochemical parameters after 12 weeks of supplementation.

Notes: Values are expressed as mean and standard deviation (numbers in brackets). The determinations were made 12 and 8 h of fasting. As well as 2.5 hours after the feeding. LD = low dose. HD = high dose (n = 5). \*Indicates significantly different ( $P \le 0.05$ ).

47.50 45.25

(2.75) (3.27)

39.65\*

(3.43)

42.04

(8.02)

41.73 40.50

(2.08) (5.50)

#### 3.2.3 Serum glucose

HDL

42.66

41.82

(14.48) (12.61)

40.66

(7.87)

Previous studies indicate that moderate increases in fiber intake from food or supplements are associated with a significant reduction in fasting plasma glucose in non-diabetic subjects (Table 4). In this work, carrot fiber supplementation decreased the glucose concentration (P < 0.05) in the male and female animals. In the supplemented males, the reduction was detected at 12 h ((126.58 ± 26.10); (115.50 ± 5.06) mg/dL) and 8 hours of fasting ((128.40 ± 13.99); (113.60 ± 10.72); (114.50 ± 7.68) mg/dL). In female, the fiber supplementation decreased the glucose concentration at 8 h of fasting ((133.03 ± 16.92); (111.66 ± 10.87)); (113.04 ± 12.36) mg/dL). Nonetheless the females that were supplemented with the high dose, also exhibited a reduced the postprandial glucose ((131.50 ± 14.31); (123.66 ± 9.52) mg/dL). Similarly, another study indicates that dietary fiber intake decreases postprandial glucose and insulinemia [39].

#### 3.2.4 Serum triglyceride

In this study was observed a decrease in the serum total triglyceride levels (P < 0.05) for each group treated with dietary

carrot fiber. The effect is dose dependent, and is independent of sex. The results are detailed in Table 4.

# 3.2.5 Total serum cholesterol

Carrots isolated fiber decrease serum cholesterol values (Table 4). In the male animals the effect was dose specific, and the decrease was observed in all determinations, after 12 h ((56.37 ± 3.96); (51.02 ± 3.43) mg/dL) and 8 h of fasting ((54.75 ± 9.78); (46.03 ± 8.02) mg/dL) and after feeding ((70.12 ± 3.16); (64.51 ± 8.75); (56.06 ± 12.39) mg/dL), the observed effect is dose specific. On the other hand, in females the decrease on the TC levels was observed only at 8 hours of fasting for the highest dose ((58.55 ± 3.86); (51.33 ± 10.63) mg/dL).

#### 3.2.6 HDL cholesterol

In relation to the effect of carrot fiber on HDL-levels, male rats supplemented with the highest dose show significant differences after feeding. However, in females the differences were significant at 8 h of fasting, only for the highest dose, as shown in Table 4. However, no explanation can be offered for the lower HDL cholesterol levels seen after fiber carrot administration between sexes. Thus perhaps, the results observed could be related to the strain of rats used.

# 3.2.7 GOT and GPT determination

It is well known that aminotransferase homeostasis is a very finely controlled system, and that it responds rapidly to changes to physiological changes. The two main aminotransferases, GOT and GPT were determined (Table 5). Among animal species there are variations in serum normal values that are related to differences in age, sex, feeding habits, and even the technique used to determine it. The serum biochemical markers for liver, GOT, and GPT, were also not affected by the intake of carrot fiber, at any dosages.

#### Table 5

Rat blood serum transaminases (IU/L) according to treatment, after 12 weeks of supplementation.

Enzyme	Samples	Sacrifice time (h)	Control	LD	HD
	Female	12	$82.51 \pm 22.13$	$94.05 \pm 23.54$	$101.21 \pm 21.65$
GOT	Male	12	$85.41 \pm 17.48$	$82.66 \pm 9.45$	$89.66 \pm 10.65$
	Female	8	$85.33 \pm 23.63$	$82.33 \pm 19.02$	$86.25 \pm 18.95$
	Male	0	$93.58 \pm 8.49$	$103.04\pm30.01$	$114.5\pm26.01$
	Female	2.5	$66.02 \pm 21.41$	$69.02 \pm 21.41$	$89.01 \pm 26.33$
	Male	2.5	$69.91 \pm 8.89$	$63.33 \pm 14.21$	$59.55 \pm 19.09$
	Female	12	$50.85 \pm 8.58$	$42.25 \pm 12.14$	$44.63 \pm 9.85$
	Male		$60.18 \pm 14.01$	$44.07 \pm 17.05$	$46.62 \pm 15.75$
GPT	Female	8	$52.01 \pm 8.89$	$44.36 \pm 14.02$	$45.25 \pm 8.98$
GP1	Male	0	$56.94 \pm 9.56$	$46.32 \pm 18.45$	$45.70 \pm 11.12$
	Female	2.5	$44.62 \pm 12.96$	$34.85 \pm 10.24$	$38.62 \pm 8.58$
	Male	2.3	$50.02 \pm 12.01$	$43.45\pm7.48$	$44.85 \pm 9.78$

Notes: Values are expressed as mean and standard deviation. The determinations were made 12 and 8 h of fasting. As well as 2.5 h after the feeding. LD = low dose. HD = high dose (n = 5).

# 3.2.8 Histopathological assessment

Figs. 2-4 presented the microscopic evaluation of the spleen, heart and liver tissues from control and supplemented rats. The heart

tissue of rats supplemented with carrot fiber for 12 weeks exhibited a structure pattern with no histopathological changes, similar to the control rats (Fig. 2). The microscopic investigation of liver tissues revealed the presence of normal hepatic parenchyma, including normal hepatic cords, central veins and portal areas (Fig. 3). Fig. 4 demonstrated the histological examination of spleen tissues of rats supplemented with fiber for 12 weeks. The features of the spleen tissue of the aforementioned animals were similar to the features of the spleen tissue of rats control.

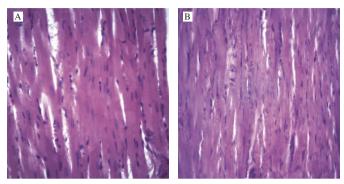


Fig. 2 Microscopic examination of heart tissues of rats control (A) and supplemented with high dose of fiber (B). Hematoxylin and eosin staining; magnification ×400.

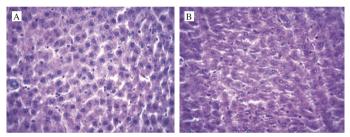


Fig. 3 Cross section of liver tissues of rats control (A) and supplemented with high dose of fiber (B). Hematoxylin and eosin staining; magnification ×400.

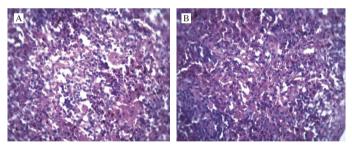


Fig. 4 Cross section of spleen tissues of rats control (A) and supplemented with high dose of fiber (B). Hematoxylin and eosin staining; magnification ×400.

# 4. Discussion

Several researchers have reported that intake of dietary fiber has beneficial effects on risk factors for developing several chronic diseases, such as coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal diseases [1,28]. However, adequate quantities of dietary fiber for elderly, children and critically ill have not yet been established [13].

In this work, the effects of fiber isolated from carrots were studied in weight management. Animals supplemented showed a decrease in body weight, and this effect was more pronounced in female animals. The higher dose of the fiber decreases around of 10%-12% body weight in both male and female after 12 weeks of supplementation, which suggests that the degree of reduction in the absorption of fat was significant in both sexes. Results similar to ours have been reported for other researchers who suggested that an additional 14 g fiber per day caused in a decrease in energy intake (10%) and a weight loss (about 1.9 kg) during the intervention period (3.8 months) [13,30].

In clinical studies, fiber consumption has been inversely associated with body weight [31], body fat [32] and with BMI changes [32,33]. This could be due to an effect of carrot fiber on fat oxidation and fat storage, as has been found on others studies, or eventually to decrease food intake by promoting satiation and/or satiety [3,30,34,]. Others factors such as hormonal control and colonic effects should also be considered [35]. Several studies reported that reasonable increases in fiber intake from food or supplements are associated with a reduction in fasting plasma glucose and insulin values and increased insulin sensitivity in non-diabetic and diabetic subjects [36-40]. Our results are in agreement with these findings as fasting serum glucose concentrations were significantly affected by the supplementation (Table 4).

The findings may arise due to an increased intestinal viscosity, or as a result of a decreased carbohydrate/caloric intake [35,39]. Colonic effects such as short chain fatty acid production by colonic bacteria fermentation and liberation of other metabolites such as phytocompounds are also been suggested. This, in turn, could influence glucose production in the liver and, thereby fasting glucose concentration [35,38,41,42].

Many studies argue that dietary fiber intake promotes decreased gastric emptying and/or slows energy and nutrient absorption, consequently to reduce postprandial glucose and lipids levels [13,36]. In this study, the higher dose of the fiber decreased postprandial glucose values in female rats, suggesting a control in plasma glucose concentration. Similar results to ours have been reported in animals or individuals with diabetes mellitus [37-41].

In this sense, D'Alessio [43] reported that glucagon-like peptide 1 exhibits multiple effects in animals and humans. Among these are the decrease of gastric emptying rates, raise of glucose uptake and elimination from peripheral tissues. It also improves insulin-dependent glucose clearance, prevents glucagon secretion, and diminishes hepatic glucose production [13]. Alterations in these metabolic pathways, therefore, as well as other factors such as carbohydrates absorption and insulin sensitivity should be studied as possible mechanisms for the effects we have found [43].

In the same way, the fiber supplementation reduced plasma total cholesterol concentrations, male rats supplemented showed a decreased in the both serum total cholesterol and HDL-cholesterol at end of experimental period. While female supplemented with the higher dose of the fiber showed a decreased in serum total cholesterol and HDL-cholesterol, only after 8 h of fasting, a finding consistent with the results of previous reports of the cholesterol-reducing effects of raw carrot [42,44]. This could be due to an effect of fiber on the expression of the enzyme HMG-CoA reductase, which reduces cholesterol synthesis and increases cholesterol excretion in the bile [45,46].

Results similar to ours have been reported for whole carrot and fibers found in carrot pomace, including the insoluble fiber fraction [47-49]. The reduction in cholesterol levels is likely due to the soluble fiber fraction of carrots, specifically the pectins and fermentable hemicelluloses, or that micronization of the carrot pomace insoluble fiber fraction [48-50]. However, no effects on serum cholesterol were detected with higher levels of carrots (raw and processed) or carrot fiber in healthy men or young women [51,52]. The authors concluded that the amount of pectins provided by the diet was too low to observe a cholesterol-lowering effect during the experimental period [52]. It is also possible that differences between studies could be due to differences in proportions of digestible carbohydrates between cultivars [4].

Finally, the potential benefits of the carrots isolated fiber were also observed in serum triglyceride concentrations. In this study, both doses of the fiber isolated decreased plasma total triglycerides concentrations in male and female in basal conditions. This seems to indicate that carrot fiber is effective over a broad spectrum of doses, confirming our expectation that both the time of supplementation time and the dose used are important. It is possible that the reduction in serum triglyceride levels may be a result of a decreased absorption of fat from the small intestine [35]. This not exclude, however, that the long-term effects of fiber supplementation could also be partly explained by gastrointestinal tract preservation due to the prebiotic activity [41,42,53].

In conclusion, prolonged supplementation with isolated carrot fiber was shown to affect body weight and certain metabolic parameters in rats. These results are consistent with the hypothesis that fibers can have effects in a number of gastrointestinal disorders, protect against development of obesity and improved glycemic control. However, the main differences with respect to other studies that use carrot fibers are the raw material used (discards) and the methodology applied to obtain it, from which three products are obtained: ethanol, carotene and a fiber rich fraction. Fiber rich fraction presents a high content of dietary fiber, which indicates an effective removal of fermentable sugars and carotenoids [8]. Consequently, it is possible to affirm that certain variables that may affect the results were eliminated, such as the chemical profile of the samples, the genetic variability of the carrots, the various forms of preparation and processing.

# **Conflict of interest**

No potential conflict of interest was reported by the authors. None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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