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Fiber supplements from agro-industrial waste: Effect on valproic acid hepatotoxicity in rats

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Abstract:
The agro-industrial sector is one of the biggest organic waste producers. Instead of being discarded into the environment, fruit and vegetable waste could be processed to obtain added value. This article introduces pomace fiber powder made from agro-industrial waste of fruits and vegetables. Its protective potential against hepatotoxicity induced by valproic acid was studied in Wistar rats, both as preventive and curative treatments.

In the preventive test, valproic acid (250 mg/kg/day) was administered orally together with the pomace fiber powder (0.3 and 0.15 mg/kg/day) for 14 days. In the curative test, the rats received valproic acid for 14 days, followed by pomace fiber powder another 14 days, at the same amounts. Physicochemical analysis revealed that the experimental pomace fiber powder contained 15.2 ± 0.5% and 22.0 ± 1.2% of insoluble and soluble dietary fiber, respectively. This ratio made it possible to classify the new supplement as functional. The rats administered with valproic acid gained body weight and demonstrated a significant increase in serum enzyme activities, alanine aminotransferase, and alkaline phosphatase ($p < 0.05$).

These results were confirmed by histopathological examination. In both preventive and curative treatments, the supplementation normalized body weight, improved liver biomarkers, and attenuated the hepatic injury induced in rats by valproic acid. The new pomace fiber powder made of agro-industrial waste proved to be an effective raw material that attenuates the side effects associated with prolonged valproic acid administration.

Keywords: Food waste, waste minimization, pomace, sustainable production, bioeconomy, fiber, hepatotoxicity

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INTRODUCTION

Industrial production of fruits and vegetables offers a number of economic benefits: it extends the shelf-life of products while facilitating its transportation and storage. However, the sorting procedure has to follow consumer demands; as a result, it produces a lot of waste that generates contamination. Fruits and vegetables that do not meet consumer requirements are discarded or used as livestock feed [1]. Horticulture waste causes serious environmental problems such as unpleasant odors or proliferation of insects and rodents. For example, the central market of Santa Fe, Argentina, is one of the largest waste generators in the city. Every month, it sends about 150 tons of unsorted organic (food) and inorganic (plastic, paper, wood, cardboard) wastes to the landfill. The environmental costs are not the only costs associated with the final disposal of agro-industrial waste: it also includes the missed profit associated with the food waste, the costs of transportation to the landfill, the fee for its overturn, etc.

Other large market hubs face a similar situation, as do companies that wash and package fruits and vegetables. Argentina needs new technologies that would provide sustainable economic solutions since industrial fruit production is one of its fundamental industries.

Although fruit waste possesses economic value, it is currently undervalued [2]. An optimized system for treating fruit and vegetable waste could turn it into a high-quality and low-cost source of fiber and bioactive compounds. Fruit and vegetable waste could even be revalued as raw materials with good prospects in the food industry and medicine, where it could be used as a source of food additives [3]. In particular, dietary fiber and phytocompounds play a key role in the prevention and modulation of host response to certain diseases, infections, and adverse drug reactions [4–6]. Drug-induced adverse reactions account for about 6% of hospital admissions [7]. Valproic acid is a medical preparation that is usually well-tolerated in humans. However, its chronic use may lead to adverse reactions, ranging from increased body weight and asymptomatic elevation of liver enzymes (transaminases) to microvesicular steatosis with diffuse necrosis [6, 8].

This effect seems to be related to carnitine depletion, with the consequent mitochondrial alteration. Consequently, concomitant administration of L-carnitine is recommended as a preventive treatment of hepatotoxicity given decompensation in carnitine shuttle during fatty acid metabolism following the accumulation of valproic acid metabolites [8]. In turn, animal models of valproic acid toxicity show an alteration of fatty acid metabolism associated with accumulation of hepatic lipid content within 2-4 h after valproic acid administration. Molecular studies suggest that hepatic accumulation of 4-en-valproic acid and its b-oxidation products trigger a cascade of reactions culminating in liver injury [8]. Histopathological and biochemical assays also revealed that valproic acid causes inflammation, oxidative stress, and hepatocyte damage in rats [8, 9]. Another research focused on supplementation with such antioxidants as postbiotic butyrate. It is a short-chain fatty acid produced by intestinal bacterial fermentation of dietary fibers. It normalized liver biomarkers, improved fatty acid metabolism, and reduced inflammation and fibrosis induced by valproic acid [10]. Ellagic acid, polyunsaturated fatty acid, and ω-3 docosahexaenoic acid also prevent hepatotoxicity associated with valproic acid [10, 11].

However, no attempt has been made so far to mitigate the toxicity of valproic acid through the intake of natural fiber. Dietary fiber is known for its numerous health benefits against various diseases [12]. Fiber consumption was reported to improve liver biochemistry in cases of necroinflammatory and fibrotic changes associated with steatohepatitis [13]. This study was designed to reuse fruit and vegetable discards in order to investigate whether the administration of the raw material obtained would attenuate adverse effects induced by valproic acid in rats.

STUDY OBJECTS AND METHODS

Raw materials. The possibility of recovering a waste material depends on its composition and availability over time. The first step was to quantify waste by type to define an appropriate processing strategy. In this study, fruit and vegetable discards were obtained from the Fruit and Vegetable Concentrator Market in Santa Fe, Argentina. The wastes were sorted into three groups. Group 1 included carrots, pumpkins, tomatoes, grapes, watermelons, pink grapefruits, peppers, etc. Group 2 included potatoes, sweet potatoes, cassava, zucchini, squash, peas, corn, beans, bananas, apples, etc. Group 3 included artichoke, chard, celery, cauliflower, asparagus, chicory, lettuce, broccoli, cucumbers, beans, cabbage, garlic, etc.

Figure 1 shows the temporal distribution of the wastes in April 2023. Group 1 and, to a lesser extent, Group 2 were the main contributors to waste production throughout the year. Group 3, on average, represented less than 10% of the total waste and became significant only during November. This study focused on the valorization of Group 1 discards generated on a sales day in April 2023. Regarding handling and storage, the discards were subjected to visual inspection: the parts affected by microorganisms were extracted and stored at 4°C until use.

Preparing pomace fiber powder from fruit and vegetable waste. Group 1 wastes were processed in a JU655 juice extractor to obtain pomace and juice, which were separated by filtration. Then, the pomace was dried in a stove at 110°C overnight and milled in a micronizer into a fine powder (Fig. 2).

Analytical methods. We used standard methods 934.01, 942.05, 922.06, and 991.43, as recommended by the Association of Official Analytical Chemists, to determine the moisture, ash, fats, and total dietary fiber (soluble and insoluble), respectively. The nitrogen content was determined by the Kjeldahl method 2001.11 [14]. The protein content was estimated as the nitrogen content multiplied by 6.25. The concentration of total sugar was measured by the 3.5-dinitrosalicylic acid (DNS) method after acid hydrolysis with 1.2 M HCl at 65°C for 15 min and neutralization with 1 M NaOH [3]. To define the contents of phosphorus, iron, and heavy metals (Hg, Pb, As, Cd), we appealed to the inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer, Optima 2100 DV). This procedure was preceded by the digestion in a nitric and perchloric acid solution (1:5, v/v).

The water- and oil-holding capacities were determined in line with the methodology described in [3]. The fiber density was determined by measuring the volume and weight of a representative sample of the fiber and combining these values to calculate density.

The microbiological profile of the pomace was described using the manufacturer's instructions for a bacteriological diagnostic kit, ZT Lab, Argentina. It involved total plate count, total coliforms, and Salmonella [15].

Quantifying total flavonoids. We used spectrophotometry to define the total flavonoids as described in [4]. The aluminum chloride colorimetric method was used to determine the flavonoid content in the waste extract: absorption readings were taken at 425 nm after 30 min of incubation in the dark. With the standard curve of quercetin, the total flavonoid contents were expressed as milligram quercetin equivalent per gram of extract, mg QE/g.

Animal treatments. The Wistar male rats (155 and 160 g) were acquired from the biotherium of the Veterinary Medicine Department, National University of Santa Fe, Argentina. The experimental animals lived in an animal house in a room with controlled temperature and humidity, with a constant 12 h:12 h light and dark cycle and free access to water and food of Ganave brand, Argentina.

Ethical approval. This work was approved by the Ethics Committee of the Medical Department, National University of the Northeast (UNNE, Corrientes), Permit Number: Res N° 0017 CICUAL 23.

Experimental procedures. After a week of clinical observation, the rats were randomly divided into eight groups of four rats in each. During the first seven days of the trial, they followed a control diet. The rats that passed this acclimatization and quarantine period were randomly divided into four groups, four rats in each. For 14 days, they received food according to the scheme described below. All in all, the animals underwent two trials, a preventive and a curative one:

During preventive trial, the animals were subjected to preventive treatment to induce hepatotoxicity and evaluate the protection potential of the fiber from fruit and vegetables waste during valproic acid (TEVA-Argentina) treatment.

This stage included the following groups of experimental animals:

– Control group animals that received distilled water (Group I);
– Animals that received 250 mg/kg body weight of valproic acid orally for 14 days to induce hepatotoxicity (Group II);
– Control group that received orally 0.3 mg/kg body weight of experimental fiber (Group III);
– Animals administered concomitantly with valproic acid and fiber at a low dose of 0.15 mg/kg body weight for 14 days (Group IV); and
– Animals administered concomitantly with valproic acid and fiber at a high dose of 0.3 mg/kg body weight for 14 days (Group V).

During curative trial, the animals were assessed for possible reversal of hepatotoxicity.

This stage included the following groups of experimental animals:

– Rats that received 250 mg/kg body weight of valproic acid for 14 days to induce hepatotoxicity before being sacrificed 14 days after the end of the valproic acid treatment (Group VI);
– Rats administered with valproic acid for 14 days, then supplemented with fiber at a low dose of 0.15 mg/kg body weight (Group VII); and
– Rats administered with valproic acid for 14 days, then supplemented with fiber at a high dose of 0.3 mg/kg body weight (Group VIII).

The bioavailability of orally administered drugs depends on the absorption and plasma clearance processes that can be affected by some dietary components in the gastrointestinal tract. Thus, food can decrease, delay, increase, or accelerate the absorption of different drugs. However, these studies are scarce, and their results are variable. An *in-vivo* study in healthy individuals evaluated the influence of citrus pectin (14 g) on the pharmacokinetics of valproic acid (500 mg; single dose) [16]: the fiber affected neither the absorption rate nor the amount of drug absorbed.

However, it is not easy to assess the extent to which they contribute to changes in drug bioavailability, therapeutic failures, and toxicity. For this reason, in this study, the administration of valproic acid was carried out in the morning (8 a.m.); and the pomace fiber was given in the late afternoon (6 p.m.), that is, with a difference of 10 h between the administrations. The doses of fiber supplemented were in line with our previous research [3]. In the case of valproic acid, the dose and time of administration came from Abdelkader *et al*. [10].

Biochemical Assay. At the end of each experimental period, four rats from each group were weighed and then anaesthetized after fasting for 8 h. We collected heart, livers, kidneys, and blood samples in heparinized tubes by cardiac puncture. The blood was centrifuged immediately after collection at 4°C and 4000 g for 15 min. The plasma obtained was processed in a spectrophotometer (Biotraza), using colorimetric and kinetic techniques distributed by Wiener Laboratories (Santa Fe, Argentina). The parameters assessed in each of them included total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, aspartate amino transferase, alanine amino transferase, and alkaline phosphatase [3, 4].

Total cholesterol. Through the action of the enzyme cholesteryl esterase, cholesterol esters broke down to free cholesterol and fatty acids. In turn, the enzyme cholesterol oxidase catalyzed the oxidation of cholesterol to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide caused the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinoneimine dye. The intensity of the dye was proportional to the concentration of cholesterol, which was measured by the absorbance increase at 512 nm.

High-density lipoprotein cholesterol (HDLC). The test relied on the adsorption of synthetic polyanions on the lipoprotein surface. The combined action of the polyanions and the detergent solubilized the high-density lipoprotein cholesterol. In the presence of peroxidase, the hydrogen peroxide formed reacts with N, N-bis(4-sulphobutyl)-m-toluidine and 4-aminoantipyrine to produce a quinoneimine red dye. Its intensity was proportional to the high-density lipoprotein cholesterol concentration, which was determined by the absorbance increase at 552 nm.

Low-density lipoprotein cholesterol (LDLC). In this method, high-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and chylomicrons were hydrolyzed with Detergent 1. Consequently, cholesterol from these lipoproteins was affected by the enzymatic action of cholesteryl esterase and cholesterol

oxidase enzymes to generate hydrogen peroxide. The latter was then consumed by peroxidase in the presence of 4-aminoantipyrine, thus generating a colorless product. Detergent 2 added together with N,N-bis(4-sulphobutyl)-m-toluidine as a conjugating agent released cholesterol from the low-density lipoprotein cholesterol particles to produce a colored compound. Its intensity was proportional to the concentration of low-density lipoprotein cholesterol, which was determined by measuring the absorbance increase at 552 nm.

Triglycerides. Lipoprotein lipase hydrolyzed triglycerides to fatty acids and glycerol. The resulting molecule was phosphorylated to glycerol-3-phosphate by adenosine triphosphate in a reaction catalyzed by glycerol kinase. Glycerol phosphate oxidase catalyzed oxidation of glycerol-3-phosphate to form dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase enzyme activity, hydrogen peroxide carried out the oxidative coupling of 4-chlorophenol and 4-aminophenazone and formed a quinoneimine red dye, which was measured at 512 nm. The increase in absorbance was proportional to the concentration of triglycerides in the sample.

Aspartate aminotransferase. This enzyme catalyzed the transfer of an amino group between L-aspartate and 2-oxoglutarate to obtain oxaloacetate and L-glutamate. In the presence of malate dehydrogenase, oxaloacetate reacted with nicotinamide adenine dinucleotide + hidrogen (NADH) to form its oxidized and reduced form NAD+. The rate of NADH oxidation was proportional to the catalytic activity of aspartate aminotransferase assessed by measuring the absorbance decrease at 340 nm.

Alkaline phosphatase. In the presence of magnesium and zinc ions, phosphatases cleaved p-nitrophenylphosphate into phosphate and p-nitrophenol.

Alanine aminotransferase. This enzyme catalyzed the reaction between L-alanine and 2-oxoglutarate. Pyruvate formed was reduced by NADH in a reaction catalyzed by lactate dehydrogenase to form L-lactate and NAD+. Thus, the rate of NADH oxidation was proportional to the catalytic activity of alanine aminotransferase determined by the absorbance decrease at 340 nm.

Histopathological examination. After sacrifice, the hearts, livers, and kidneys were dissected, examined for color changes, and weighed to obtain a relative weight. After fixing liver and kidney samples in 10% buffered formalin for 24 h, they were dehydrated in isopropyl alcohol of increasing strength, rinsed with xylol, and embedded in paraffin. After being cut with a microtome, the histological sections were deparaffinized and stained with hematoxylin/eosin to evaluate histopathological alterations in each experimental group [3, 4]. The stained tissue sections underwent optical microscopy to obtain digital images.

Statistical analysis. The data were expressed as mean ± standard deviation, and the significance of the data between groups was tested by Infostat scientific software, Argentina. Statistical analyses relied on Tuckey's test. In all cases, we took the probability level of 95% as significant.

RESULTS AND DISCUSSION

Figure 1 describes the selection and temporal classification of the original fruit and vegetable waste obtained from the central market in Santa Fe, Argentina. The research featured colored fruits and vegetables discarded in April 2023.

The discards yielded a light brown fiber-rich powder of neutral flavor (Fig. 2).

Table 1 shows the chemical composition of the experimental pomace powder. The moisture content proved adequate for long shelf-life under suitable conditions. The total dietary fiber content was 37.20 ± 0.22% solids, where 15.20 ± 0.50% corresponded to insoluble dietary fiber and the remaining 22.0 ± 1.20% corresponded to soluble dietary fiber. The ratio made it possible to classify the pomace powder as a functional supplement [3].

The ash content was in agreement with the values reported by other researchers who studied similar fruit and vegetable wastes [17]. It can be associated with the presence of iron and phosphorus, both bio-elements

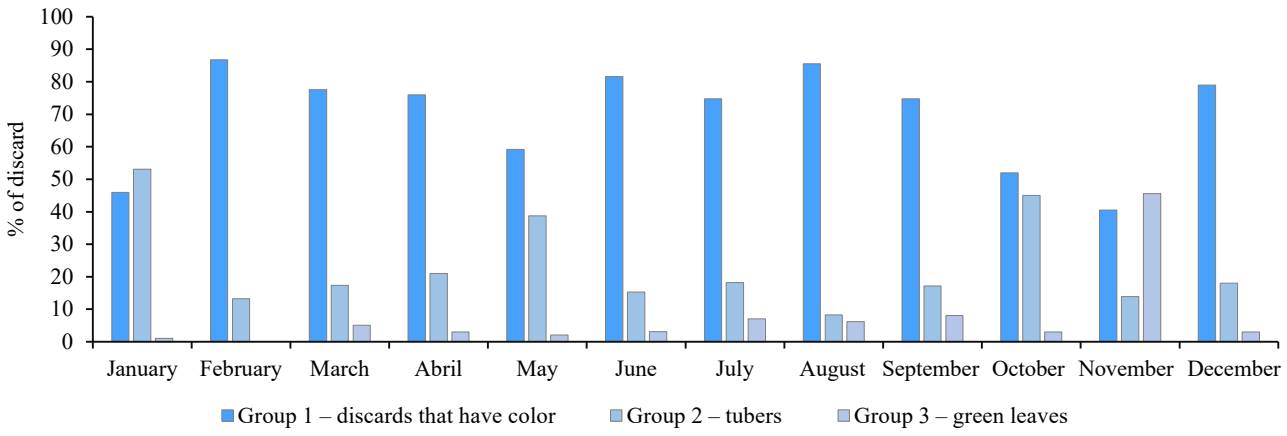


Figure 1 Fruit and vegetable waste discarded on the central market of Santa Fe, by month

Figure 2 Experimental fiber-rich powder obtained from agro-industrial waste pomace

Table 1 Chemical composition of pomace fiber powder obtained from fruit and vegetable waste

Compound	Value
Water, %	12.0 ± 0.10
Sugars, %	18.60 ± 0.15
Soluble fiber, %	22.00 ± 1.20
Insoluble fiber, %	15.20 ± 0.50
Total fiber, %	37.20 ± 0.22
Proteins, %	8.70 ± 0.25
Fats, %	5.20 ± 0.02
Ash, %	4.70 ± 0.30
Iron, ppm	13.00 ± 0.50
Phosphorus, ppm	176.00 ± 0.50
Heavy metals, ppm	< 10

Values are represented as means ± standard deviations (n = 3). Some results were expressed as % solids

Table 2 Physical composition of pomace fiber powder obtained from fruit and vegetable waste

Indicator	Value
Water-holding capacity, g/g	3.30 ± 0.40
Oil-holding capacity, g/g	1.30 ± 0.25
Density, g/cm^3	350.0 ± 10.0

Values are represented as means ± standard deviations (n=3)

Table 3 Microbiological analysis of pomace fiber powder obtained from fruit and vegetable waste

Indicator	Value
Total plate count, UFC/g	< 1000
Salmonella	not detected
Total coliforms, UFC/g	< 10

Values are means ± standard deviations (n = 3)

being important for human and animal nutrition. The heavy metal content was low, indicating that the pomace fiber powder was not toxic to humans. Insoluble dietary fiber absorbs and retains water. Water-holding capacity is the amount of water that binds to fiber without any external force but gravity and atmospheric pressure [3]. Our pomace fiber powder had a water-holding capacity value of 3.30 ± 0.40 g/g, which is relatively low compared to other natural fibers with lower amounts of fermentable sugars (Table 2). The oil holding capacity was 1.30 ± 0.25 g fat/g.

The microbiological analyses proved that the method of production guaranteed the safety of the experimental product, rendering it suitable for human and animal consumption (Table 3).

The *in-vivo* assays showed that valproic acid administration resulted in a large increase in body weight (Table 4, 5) and a significant decrease in the relative weights of both the liver and kidneys compared to the control group (Table 6) [17]. Table 4 shows the results of the preventive study.

Previous clinical and experimental studies reported a dietary inverse correlation between fiber intake and body weight, body fat and changes in body mass index [18]. In our research, the experimental pomace fiber powder in rats' diet reversed body weight gain to normal values, in both preventive and curative studies. The mechanism by which fiber reduces body weight is a subject of different hypotheses, e.g., that it increases satiety, decreases energy intake, and/or slows down nutrient absorption. Table 5 shows the results of the curative study.

In this study, periodic observations demonstrated no major change in habitual energy intake or behavioral patterns during the entire test period. These findings suggest that the experimental pomace powder had an effect on fat oxidation and storage, as reported in other publications. However, other certain factors might be considered, e.g., hormonal control and the effects of fiber in the intestines [6, 13, 18]. Similarly, the animals that received the pomace fiber powder had a lower liver weight relative to the group that received valproic acid only. The effect was probably due to the loss of white adipose tissue [19].

The relative kidney weight was lower than in the animals that received valproic acid, but this result could not be related to a possible biological significance (data not shown). The macroscopic and microscopic examinations clearly showed that neither valproic acid

Table 4 Body weight after treatment with valproic acid and pomace fiber powder during preventive study

Groups	Initial body weight, g	Final body weight, g
Group I: control (distilled water)	165.15 ± 4.60	197.09 ± 6.52^a
Group II: valproic acid	166.54 ± 7.01	242.06 ± 6.97^b
Group III: control + pomace powder	168.46 ± 6.56	200.03 ± 6.72^a
Group IV: valproic acid + pomace powder 0.15 mg/kg body weight	162.66 ± 5.13	220.47 ± 7.14^a
Group V: valproic acid + pomace powder 0.3 mg/kg body weight	167.01 ± 6.28	221.53 ± 7.08^a

Mean ± standard deviation with different superscripts in the column differ from each other ($p < 0.05$)

Table 5 Body weight after treatment with valproic acid and pomace fiber powder during curative study

Groups	Initial body weight, g	Final body weight, g
Group I: control (distilled water)	202.06 ± 7.63	220.88 ± 6.08[a]
Group VI: valproic acid	244.32 ± 5.47	256.95 ± 7.72[b]
Group VII: valproic acid + pomace powder 0.15 mg/kg body weight	236.73 ± 5.52	223.41 ± 7.75[a]
Group VIII: valproic acid + pomace powder 0.3 mg/kg body weight	234.76 ± 6.29	219.72 ± 8.03[a]

Mean ±standard deviation followed by different letters in the column differ from each other ($p < 0.05$)

Table 6 Relative organ weight from rats after treatment with valproic acid and pomace fiber powder

Groups	Heart, g	Liver, g	Kidney, g
Group I: control (distilled water)	0.37 ± 0.02[a]	3.88 ± 0.14[a]	0.91 ± 0.09[a]
Group II / VI: valproic acid	0.28 ± 0.03[b]	3.21 ± 0,09[b]	0.77 ± 0.07[b]
Group III: control + pomace powder	0.37 ± 0.03[a]	3.83 ± 0.11[a]	0.92 ± 0.10[a]
Group IV / VII: valproic acid + pomace powder 0.15 mg/kg body weight	0.39 ± 0.05[a]	3.55 ± 0.14[a]	0.93 ± 0.11[a]
Group V / VIII: valproic acid + pomace powder 0.3 mg/kg body weight	0.38 ± 0.06[a]	3.45 ± 0.12[a]	0.94 ± 0.12[a]

Mean ± standard deviation followed by different superscripts in the column differ from each other by Tukey's test, with 95% confidence (n = 4)

Table 7 Effects of valproic acid and pomace fiber powder on blood biochemistry in rats during preventive study

Variable	Group I: control	Group II: valproic acid	Group III: pomace powder	Group IV: valproic acid + 0.15 mg/kg body weight, pomace powder	Group V: valproic acid + 0.3 mg/kg body weight, pomace powder
Total colesterol, mg/dL	61.23 ± 3.37[a]	92.33 ± 4.64[b]	83.13 ± 4.89[a]	78.43 ± 3.37[a]	55.11 ± 5.03[a]
High-density lipoprotein cholesterol, mg/dL	30.41 ± 1.88[a]	19.03 ± 1.91[b]	23.25 ± 2.31[b]	19.63 ± 2.92[b]	33.93 ± 3.20[a]
Low-density lipoprotein cholesterol, mg/dL	20.81 ± 2.49[a]	35.01 ± 2.98[b]	16.07 ± 2.08[a]	32.13 ± 4.27[b]	14.14 ± 3.67[a]
Triglycerides, mg/dL	142.67 ± 14.25[a]	222.40 ± 16.75[b]	109.12 ± 15.57[a]	99.57 ± 17.40[a]	104.27 ± 16.01[a]
Aspartate aminotransferase, U/mL	73.33 ± 21.46[a]	85.41 ± 24.30[b]	69.93 ± 22.20[a]	60.43 ± 19.47[a]	52.14 ± 15.09[a]
Alanine aminotransferase, U/mL	36.71 ± 11.97[a]	104.17 ± 34.40[b]	34.55 ± 10.91[a]	39.86 ± 12.56[a]	42.57 ± 10.62[a]
Alkaline phosphatase, U/mL	322.57 ± 89.40[a]	468.23 ± 104.16[b]	329.12 ± 81.50[a]	282.37 ± 75.21[a]	288.79 ± 60.46[a]

Mean ± standard deviation followed by different superscripts in the column differ from each other ($p < 0.05$)

(250 mg/kg/day) nor the high dose (0.3 mg/kg/day) of experimental pomace fiber alone could cause obvious morphological changes in the kidney tissue [8]. In addition, the administration of valproic acid caused a significant increase in total cholesterol, low-density lipoprotein cholesterol, and triglycerides in plasma after treatment (Table 7) [20]. On the other hand, the healthy animals supplemented with the pomace fiber powder demonstrated an increase in total cholesterol while the values of low-density lipoprotein cholesterol and triglycerides went down. The increase in total cholesterol was probably due to the fact that the time elapsed between the beginning of the study and the sacrifice of the healthy animals was not long enough for the healthy rats to adapt to the supplementation, or that the effect depended on the physiological state of the animal [12, 21].

A reduction in triglycerides and total cholesterol was detected in the rats administered with valproic acid and supplemented with both doses of pomace powder (Tables 7). The animals supplemented with the high dose of pomace powder demonstrated lower low-density lipoprotein cholesterol and higher high-density lipoprotein cholesterol. Results similar to ours were reported for other types of fiber, which showed that daily intake of soluble fiber significantly reduced serum concentrations of total cholesterol and low-density lipoprotein cholesterol in rodents and humans [12, 18, 22]. The reduction in cholesterol levels was probably due to the soluble fiber fraction in the pomace fiber powder, in particular fermentable pectins and hemicelluloses [6, 18]. In contrast, our results were inconsistent with those of other studies, probably, due to the differences in processing techniques, molecular weight, viscosity, and administration method. According to the scientific literature, this finding could be due to the effect of pomace fiber powder on the expression of HMG-CoA reductase, which reduces cholesterol synthesis and increases cholesterol excretion in bile [22, 23]. It is also possible that the fermentation of pomace powder by the intestinal microflora modified the production of short-chain fatty acids, thus reducing

Table 8 Effects of valproic acid and pomace fiber powder on blood biochemical parameters in rats during curative study

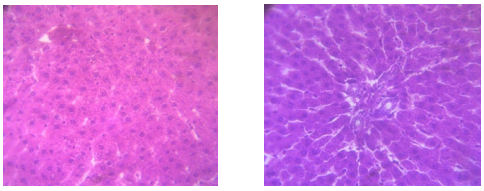
Variable	Group I: control	Group VI: valproic acid	Group VII: valproic acid + 0.15 mg/kg body weight, pomace powder	Group VIII: valproic acid + 0.3 mg/kg body weight, pomace powder
Total cholesterol, mg/dL	75.11 ± 4.53[a]	99.51 ± 3.40[b]	92.79 ± 3.43[b]	94.16 ± 4.73[b]
High-density lipoprotein cholesterol, mg/dL	33.07 ± 3.03[a]	21.44 ± 2.76[b]	21.44 ± 2.63[b]	26.63 ± 2.13[a]
Low-density lipoprotein cholesterol, mg/dL	20.27 ± 3.25[a]	20.50 ± 2.05[a]	12.87 ± 3.23[b]	16.20 ± 2.61[b]
Triglycerides, mg/dL	155.01 ± 18.02[a]	230.13 ± 15.36[b]	178.72 ± 18.50[a]	144.41 ± 17.10[a]
Aspartate aminotransferase, U/mL	76.22 ± 23.21[a]	78.59 ± 29.78[a]	52.25 ± 16.97[b]	51.43 ± 19.52[b]
Alanine aminotransferase, U/mL	45.04 ± 13.89[a]	79.96 ± 24.89[b]	33.01 ± 11.37[a]	37.60 ± 13.49[a]
Alkaline phosphatase, U/mL	357.68 ± 62.54[a]	396.70 ± 63.77[a]	293.33 ± 59.74[b]	305.14 ± 57.18[b]

Mean ± standard deviation followed by different superscripts in the column differ from each other ($p < 0.05$)

acetate production and increasing propionate synthesis. Consequently, endogenous synthesis of cholesterol, fatty acids, and low-density lipoproteins went down [22, 24].

In animals exposed to the curative study and supplemented with the experimental pomace fiber powder, plasma concentrations of low-density lipoprotein cholesterol and triglycerides decreased significantly and those of high-density lipoprotein cholesterol increased with respect to the group that received valproic acid (Table 8). This finding was consistent with the results of previous reports on the cholesterol-reducing effects of dietary fiber [3, 23]. Interestingly, both doses of pomace fiber powder significantly decreased total plasma triglyceride concentrations in both assays, i.e., preventive and curative, and, at basal level, in healthy animals supplemented with pomace powder only. This seems to indicate that the experimental fiber supplement was effective in reducing triglycerides in plasma and that its effect depended on the physiological state of the animal [21]. The reason for this finding is not fully established at present. Probably, the reduction in serum triglyceride levels is the result of decreased fat absorption in the small intestine, as well as preservation of the gastrointestinal tract due to the prebiotic activity of the fibers [12, 21, 24]. In the present work, the cytosolic integrity was estimated from the concentrations of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase in the different mice groups under study. The results showed a significant increase in alanine aminotransferase and alkaline phosphatase activity in the plasma of animals administered with valproic acid compared to the control animals (Tables 7). This increase was evident in both preventive and curative trials (Table 8). The alterations could be related to oxidative stress caused by toxic metabolites of valproic acid that damaged liver cells and consequently released these enzymes into the systemic circulation [20].

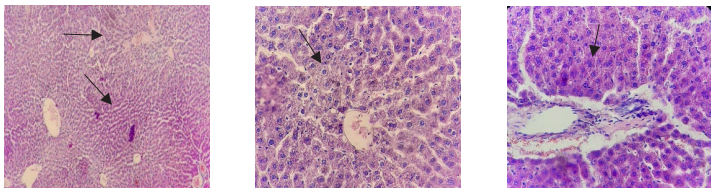
In both preventive and curative studies, the experimental pomace fiber powder affected the concentration of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase with respect to the group that received valproic acid. These results suggest that the supplement reduced hepatotoxicity induced by valproic acid. The pomace fiber powder was obtained from its original vegetable source, which was processed to obtain the fibers, i.e., it contained other bioactive substances, e.g., flavonoids, which also prevent degenerative diseases [4, 5]. The analysis of the pomace fiber powder composition showed that the extract contained 30 mg/100 g of total flavonoids (quercitrin equivalents, dry weight). Therefore, these antioxidants were likely to be able to stabilize the plasma membrane in liver cells, thus protecting them from the stress caused by valproic acid [4, 10]. The reduction in aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase observed in our research (Table 7, 8) was in agreement with some previous studies [23]. On the other hand, the liver histology of the experimental rats showed under an optical microscope that the control group rats maintained intact hepatic lobular architecture. Figure 3a illustrates sinusoids and portal channels in rat liver. These findings were similar to those observed in the group supplemented with pomace fiber powder only (Fig. 3b). Thus, both groups showed normal hepatic lobular architecture.



a b

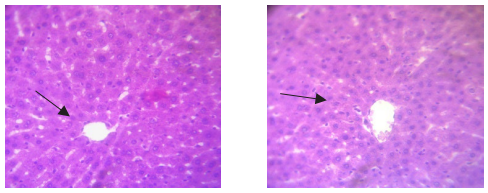
Figure 3 Photomicrographs of liver sections: (a) control group; (b) rats fed with experimental pomace powder. 40× magnification

However, the livers removed from the rats that received valproic acid were paler than normal at necropsy (data not shown). As with previous reports, the liver sections stained with hematoxylin-eosin revealed numerous optically clear or cloudy empty vacuoles in periportal hepatocytes (Fig. 4). The lobular architecture was distorted compared with the control group. Moreover, these histological changes were accompanied by moderate inflammatory cell infiltration [9–11]. These features persisted up to 14 days after the rats stopped receiving valproic acid.



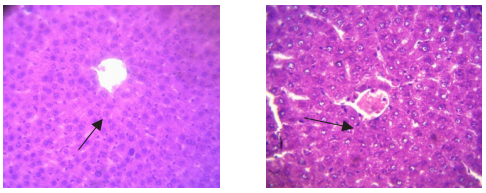
a b c

Figure 4 Photomicrographs of liver histology from rats treated with valproic acid: (a and b) after 14 days of treatment with valproic acid; (c) 14 days after final treatment with valproic acid. 10 and 40× magnification. Black arrows point at signs of distorted hepatic lobules associated with inflammatory cells infiltration



a b

Figure 5 Photomicrographs of liver sections from rats administered with valproic acid and concomitantly supplemented with: (a) 0.3 mg/kg body weight; (b) 0.15 mg/kg body weight of pomace fiber powder during preventive study. 40× magnification. Black arrows point at histological structure preservation sites



a b

Figure 6 Photomicrographs of liver sections from rats administered with valproic acid and concomitantly supplemented with (a) 0.3 mg/kg body weight; (b) 0.15 mg/kg body weight of pomace fiber powder during curative study. 40× magnification. Black arrows point at histological restoration sites

In contrast, Figures 5 and 6 clearly show that the experimental pomace fiber powder attenuated and restored thehistological abnormalities induced by valproic acid. In the current study, the histopathological examination of rats treated with valproic acid and supplemented with the experimental pomace fiber powder showed well-preserved liver architecture and absence of inflammatory processes, both in the preventive (Fig. 5a, b) and curative study (Fig. 6a, b).

Therefore, the experimental pomace fiber powder obtained from fruit and vegetable waste counteracted histopathological liver alterations related to prolonged treatment with valproic acid. This finding could be related to the anti-inflammatory and lipotropic effects of flavonoids and fibers present in the pomace powder, respectively [12, 25]. This study did not feature the pharmacokinetic parameters of valproic acid generated in the presence and absence of pomace powder. In addition, the administration of valproic acid and pomace fiber powder was performed in opposite periods and separated by about 10 h, thus ruling out any interaction between the two. Treatment with valproic acid often results in gastrointestinal side effects, e.g., constipation [26]. In this regard, previous studies reported that fiber was fermented by intestinal microorganisms to produce short-chain fatty acids (propionic acid, butyric acid, etc.), which regulated hepatic lipid metabolism [24, 25]. These components were less fermentable and could contribute significantly to fecal bulk and stool frequency, thus reducing the symptoms of constipation. This effect, probably, favors the excretion of toxic metabolites derived from valproic acid, in particular, valproyl-CoA, which is not metabolized and inhibits the hepatic carnitine palmitoyltransferase 1A [8, 20]. This enzyme regulates β-oxidation of mitochondrial fatty acids. In addition, it is associated with drug-induced hepatotoxic mechanisms and weight gain, commonly observed in patients or animals administered with valproic acid [8–10]. However, further studies might corroborate this hypothesis.

CONCLUSION

This research re-evaluated fruit and vegetable wastes as a potential source of fiber supplements, as well as defined its main components and bioactivity. The experimental pomace fiber powder administered orally to rats protected liver tissue from valproic acid toxicity by normalizing the level of hepatic biomarkers and preserving the hepatic architecture. In view of the results obtained, complementary studies might explain the mechanisms involved in the effect of pomace fiber powder on the different pathways involved in valproic acid toxicity.

CONTRIBUTION

Conceptualization, M.R. Ramirez; methodology, M.R. Ramirez and J.C. Yori; software, M.R. Ramirez; validation, M.R. Ramirez, D. Manuale and J.C. Yori; formal analysis, M.R. Ramirez, and J.C. Yori; investigation, M.R. Ramirez; D. Manuale and J.C. Yori; resources, M.R. Ramirez and J.C. Yori; data curation, M.R. Ramirez and J.C. Yori.; writing-original draft preparation, M.R. Ramirez; writing-review and editing, M.R. Ramirez and J.C. Yori; visualization, M.R. Ramirez and; supervision, M.R. Ramirez project administration, M.R. Ramirez

and J.C. Yori. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

No conflict of interest was disclosed by the authors.

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