



Article

The Protective Effect of Nutraceuticals on Hepatic Ischemia-Reperfusion Injury in Wistar Rats

Carlos Andrés Pantanali ¹, Vinicius Rocha-Santos ¹, Márcia Saldanha Kubrusly ¹, Inar Alves Castro ², Luiz Augusto Carneiro-D'Albuquerque ¹ and Flávio Henrique Galvão ^{1,*}

- ¹ Liver and Gastrointestinal Transplant Division, Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo 05403-900, Brazil; cpantanali@gmail.com (C.A.P.); vinicius.rocha@hc.fm.usp.br (V.R.-S.); msk@alumni.usp.br (M.S.K.); profluizcarneiro@gmail.com (L.A.C.-D.)
- ² LADAF, Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo 01246-000, Brazil; inarcastro@gmail.com
- * Correspondence: fgalvao@usp.br; Tel.: +55-11-26613323

Abstract: Nutraceuticals are bioactive compounds present in foods, utilized to ameliorate health, prevent diseases, and support the proper functioning of the human body. They have gained attention due to their ability to hit multiple targets and act as antioxidants, anti-inflammatory agents, and modulators of immune response and cell death. Therefore, nutraceuticals are being studied to prevent and treat liver ischemia–reperfusion injury (IRI). This study evaluated the effect of a nutraceutical solution formed by resveratrol, quercetin, omega-3 fatty acid, selenium, ginger, avocado, leucine, and niacin on liver IRI. IRI was performed with 60 min of ischemia and 4 h of reperfusion in male Wistar rats. Afterward, the animals were euthanized to study hepatocellular injury, cytokines, oxidative stress, gene expression of apoptosis-related genes, TNF- α and caspase-3 proteins, and histology. Our results show that the nutraceutical solution was able to decrease apoptosis and histologic injury. The suggested mechanisms of action are a reduction in gene expression and the caspase-3 protein and a reduction in the TNF- α protein in liver tissue. The nutraceutical solution was unable to decrease transaminases and cytokines. These findings suggest that the nutraceuticals used favored the protection of hepatocytes, and their combination represents a promising therapeutic proposal against liver IRI.

Keywords: nutraceuticals; liver; ischemia–reperfusion injury; apoptosis



Citation: Pantanali, C.A.; Rocha-Santos, V.; Kubrusly, M.S.; Castro, I.A.; Carneiro-D'Albuquerque, L.A.; Galvão, F.H. The Protective Effect of Nutraceuticals on Hepatic Ischemia-Reperfusion Injury in Wistar Rats. *Int. J. Mol. Sci.* **2023**, *24*, 10264. <https://doi.org/10.3390/ijms241210264>

Academic Editor: Kouichi Yoshinari

Received: 4 May 2023

Revised: 9 June 2023

Accepted: 10 June 2023

Published: 17 June 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Nutraceuticals are natural bioactive or chemical compounds which, in addition to playing a nutritional role, enhance health, cure illnesses, or have preventive properties [1,2]. They are dietary supplements, and from the nutritional point of view, nutraceuticals are a source of nutrients (lipids, carbohydrates, vitamins, proteins, minerals) and non-nutrients (prebiotics, probiotics, phytochemicals, enzymatic regulators) [1,3]. Nutraceuticals can be extracted from both vegetal and animal foods, concentrated, and administered in a suitable pharmaceutical form, with the aim of improving health, in dosages that exceed those obtainable from normal foods [4,5].

In vitro and in vivo studies have provided evidence that nutraceuticals have antioxidant, anti-inflammatory, antibacterial, antiviral, and antifungal activities, as well as evidence that they act as modulators of immune response, angiogenesis, and cell death [4,6–8]. These effects are possible due to the multi-target reach of nutraceuticals: endogenous glutathione, interleukins, cytokines, tumor necrosis factor, transcription factor nuclear factor- κ B, growth factors, caspases, hepatocyte intracellular neutral lipids, etc. [9–11].

By reaching all these targets, nutraceuticals are able to prevent several diseases, such as diabetes mellitus, obesity, cardiovascular diseases, cancer, eye disorders, neurologic diseases, and liver IRI [12,13]. The latter is caused by a limited blood supply and subsequent

blood supply recovery during surgical procedures, including management of liver trauma, hepatic resection, and liver transplantation [14–16]. It represents the main underlying cause of primary graft dysfunction or non-function and liver failure post-transplantation, in addition to being an important risk factor for acute and chronic rejection [15,17].

Hepatic IRI remains a major unresolved problem in clinical practice [15]. Nutraceuticals are a rising therapy due to their nutritional and therapeutic benefits, as well as safety profile [12]. Some of them have already been studied and exhibited promising results, such as resveratrol, quercetin, omega-3 fatty acids, selenium, ginger, and avocado [18–24].

Currently, attention has been focused on the synergistic effects of nutraceutical combinations [25,26]. The “synergism concept” was introduced by Liu et al. [27,28]. In this regard, the combination of polyphenols and vitamins is extremely effective in preventing osteoporosis, cardiovascular diseases, cancer, diabetes mellitus, and neurodegenerative diseases [29].

This study aimed to formulate a nutraceutical solution comprising resveratrol, quercetin, omega-3 fatty acids, selenium, ginger, avocado, leucine, and niacin to target the various signaling pathways of liver IRI and decrease its effects. We studied hepatocellular injury, inflammatory mediators, apoptosis by TUNEL, gene expression of apoptosis-related genes, TNF- α and caspase-3 proteins in liver tissue, and histology. We compared the results between five groups: CONTROL—no intervention; IR—rats submitted to liver IRI; NUTRACEUTICALS + IR (NUT + IR)—rats that received the nutraceutical solution by gavage for 7 days and underwent liver IRI; NUTRACEUTICALS (NUT)—rats that received the nutraceutical solution for 7 days; and SHAM—rats submitted only to hepatic manipulation.

2. Results

2.1. Hepatocellular Injury

The rats of the IR group exhibited a significant increase in serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) compared to the CONTROL and NUT groups. The NUT + IR group presented a significant increase in serum levels of AST and ALT compared to the CONTROL group (Figure 1).

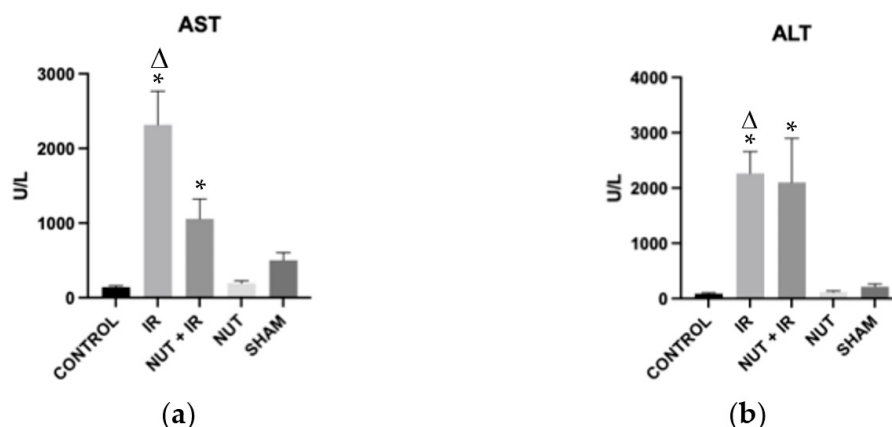


Figure 1. Serum levels of transaminases of each group: (a) AST—aspartate aminotransferase; (b) ALT—alanine aminotransferase. The data shown are mean $\pm$ SEM; * $p < 0.05$ vs. CONTROL group; $\Delta p < 0.05$ vs. NUT group.

2.2. Inflammatory Mediators

There was no difference in terms of IL-1 β , IL-6, and IL-10 among the groups. The serum TNF- α level was significantly increased in the IR group compared with the SHAM group (Figure 2).

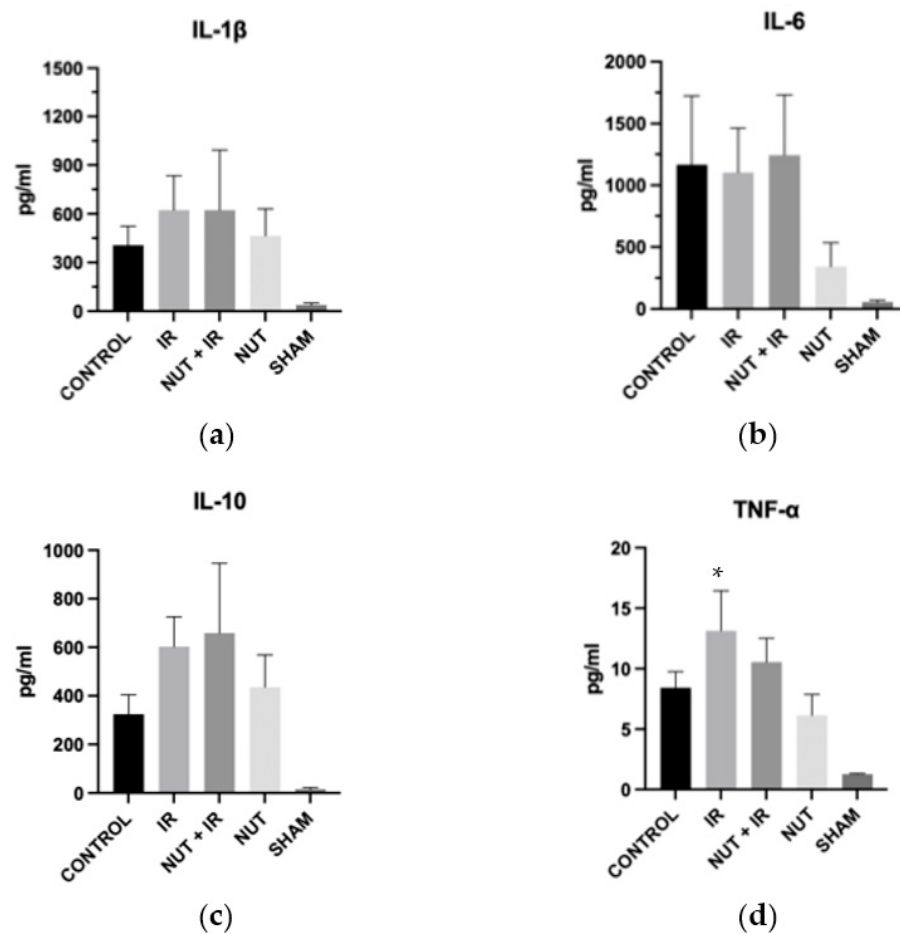


Figure 2. Inflammatory mediators: (a) IL-1 β , (b) IL-6, (c) IL-10, and (d) TNF- α . The data shown are mean $\pm$ SEM; * $p < 0.05$ vs. SHAM group.

2.3. Lipid Peroxidation

The CONTROL group exhibited a significantly higher level of MDA in the liver tissue when compared to the IR, NUT + IR, and SHAM groups. The same was also observed in the NUT group compared to the NUT + IR group (Figure 3).

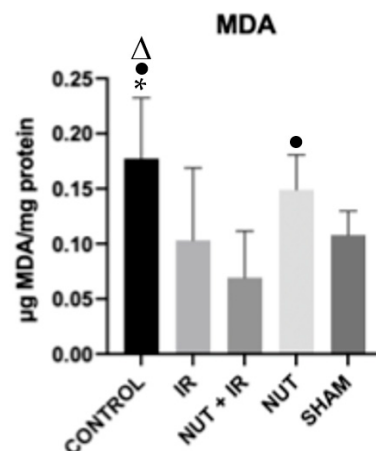


Figure 3. MDA in the liver tissue of each group. The data shown are mean $\pm$ SEM; * $p < 0.05$ vs. IR group; • $p < 0.05$ vs. NUT + IR group; Δ $p < 0.05$ vs. SHAM group.

2.4. Gene Expression of Apoptosis: BAX, BCL-2, CASPASE 8, and CASPASE 3

The gene expression of BAX and BCL-2 was significantly higher in the NUT + IR group compared to the CONTROL and SHAM groups and similar to the NUT and IR groups. The latter group exhibited a significant increase in the gene expression of BAX compared to the CONTROL, NUT, and SHAM groups. Among the gene expression of CASPASES, there was only one difference with CASPASE 3. The gene expression of CASPASE 3 was significantly lower in the NUT + IR group than in the IR group, which in turn had a significantly higher gene expression compared to the CONTROL and SHAM groups (Figure 4).

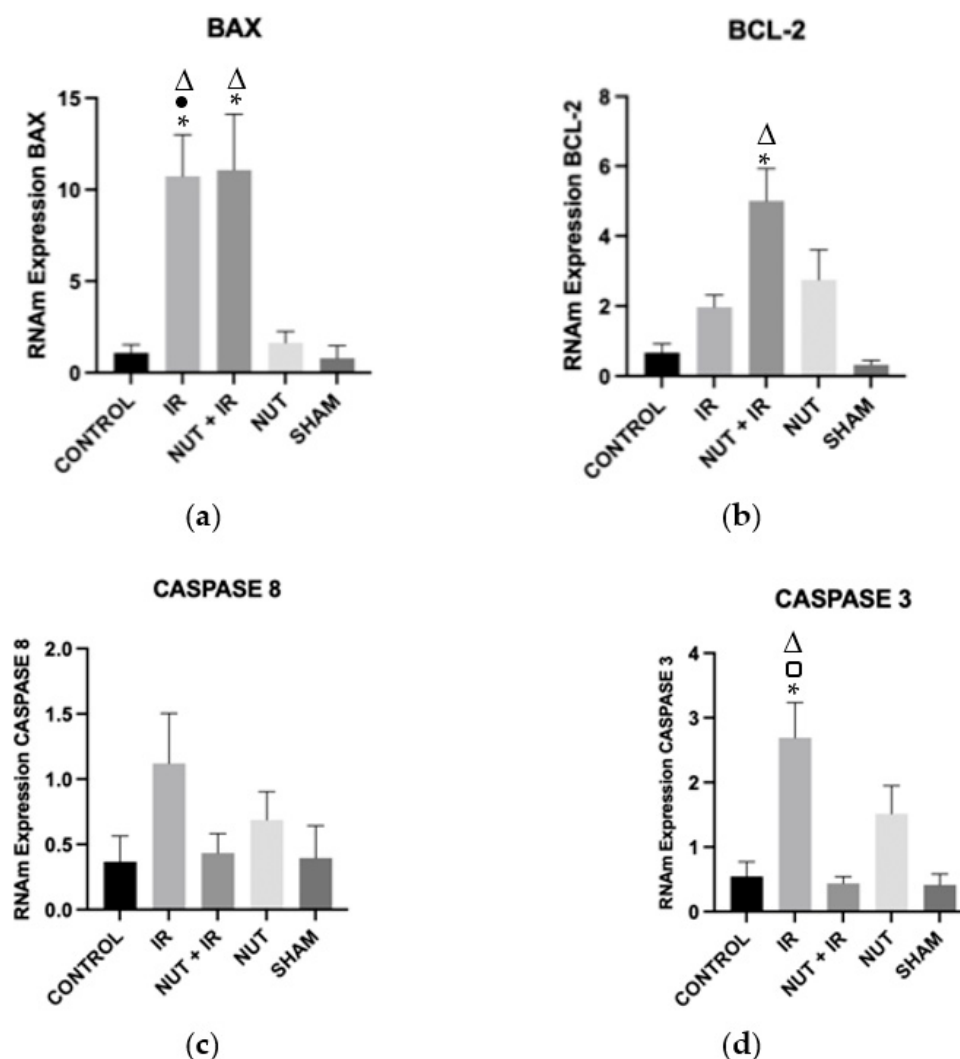


Figure 4. Gene expression of apoptosis-related genes in all groups: (a) BAX, (b) BCL-2, (c) CASPASE 8, and (d) CASPASE 3 genes. The data shown are mean $\pm$ SEM; * $p < 0.05$ vs. CONTROL group; $\square$ $p < 0.05$ vs. NUT + IR group; $\bullet$ $p < 0.05$ vs. NUT group; Δ $p < 0.05$ vs. SHAM group.

2.5. Immunohistochemistry: Apoptosis, Cleaved Caspase-3, and TNF- α Proteins in the Liver

2.5.1. Apoptosis—TUNEL Assay

The TUNEL assay was used to determinate the apoptosis of the liver cells. The NUT + IR group exhibited a significant decrease in percentage of apoptosis compared to the IR group. Moreover, the IR group had a significantly higher percentage of apoptosis than the CONTROL, NUT, and SHAM groups (Figure 5).

2.5.2. Cleaved Caspase-3 Protein

The immunohistochemistry analysis showed that the cleaved caspase-3 protein in liver tissue was significantly lower in the NUT + IR group than in the IR group. This means that the nutraceutical solution was able to decrease both gene expression and caspase-3 protein in IR injury. The IR group had a significantly higher cleaved caspase-3 protein level compared to the CONTROL and SHAM groups (Figure 6).

2.5.3. TNF- α Protein

In relation to TNF- α protein in liver tissue, the NUT group presented the highest significant percentage compared to all other groups. The IR group had a significantly higher TNF- α percentage compared to the NUT + IR and SHAM groups (Figure 7).

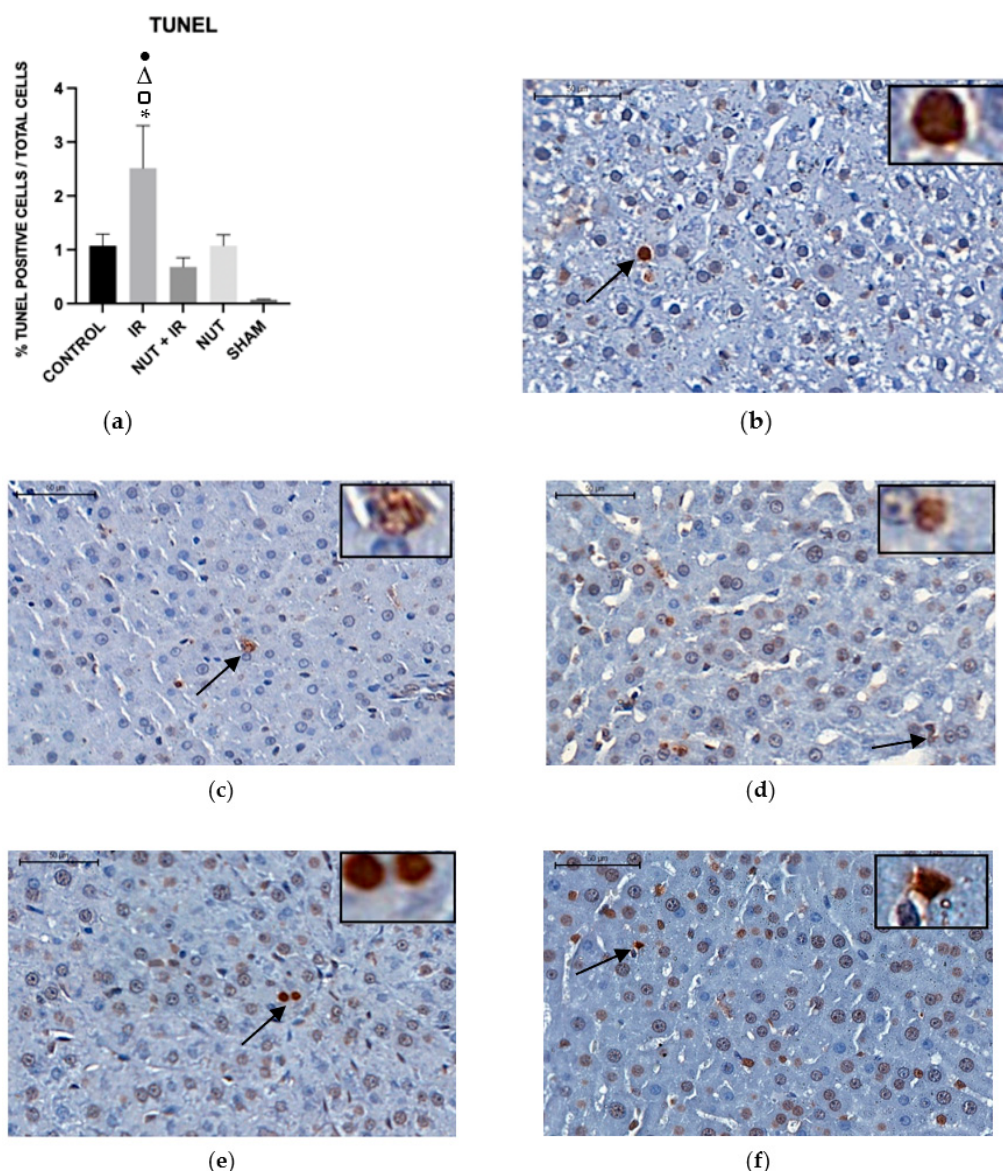


Figure 5. TUNEL assay of hepatic tissues from the different groups: (a) data shown are mean $\pm$ SEM; * $p < 0.05$ vs. CONTROL group; Δ $p < 0.05$ vs. NUT + IR group; $\square$ $p < 0.05$ vs. NUT group; $\bullet$ $p < 0.05$ vs. SHAM group. Arrows and highlighted boxes indicate TUNEL positive cells in each group: (b) CONTROL; (c) IR; (d) NUT + IR; (e) NUT; and (f) SHAM. All images were obtained with $50\times$ magnification and highlighted boxes with $400\times$ magnification, with scale bars of $500\ \mu\text{m}$ and $50\ \mu\text{m}$, respectively.

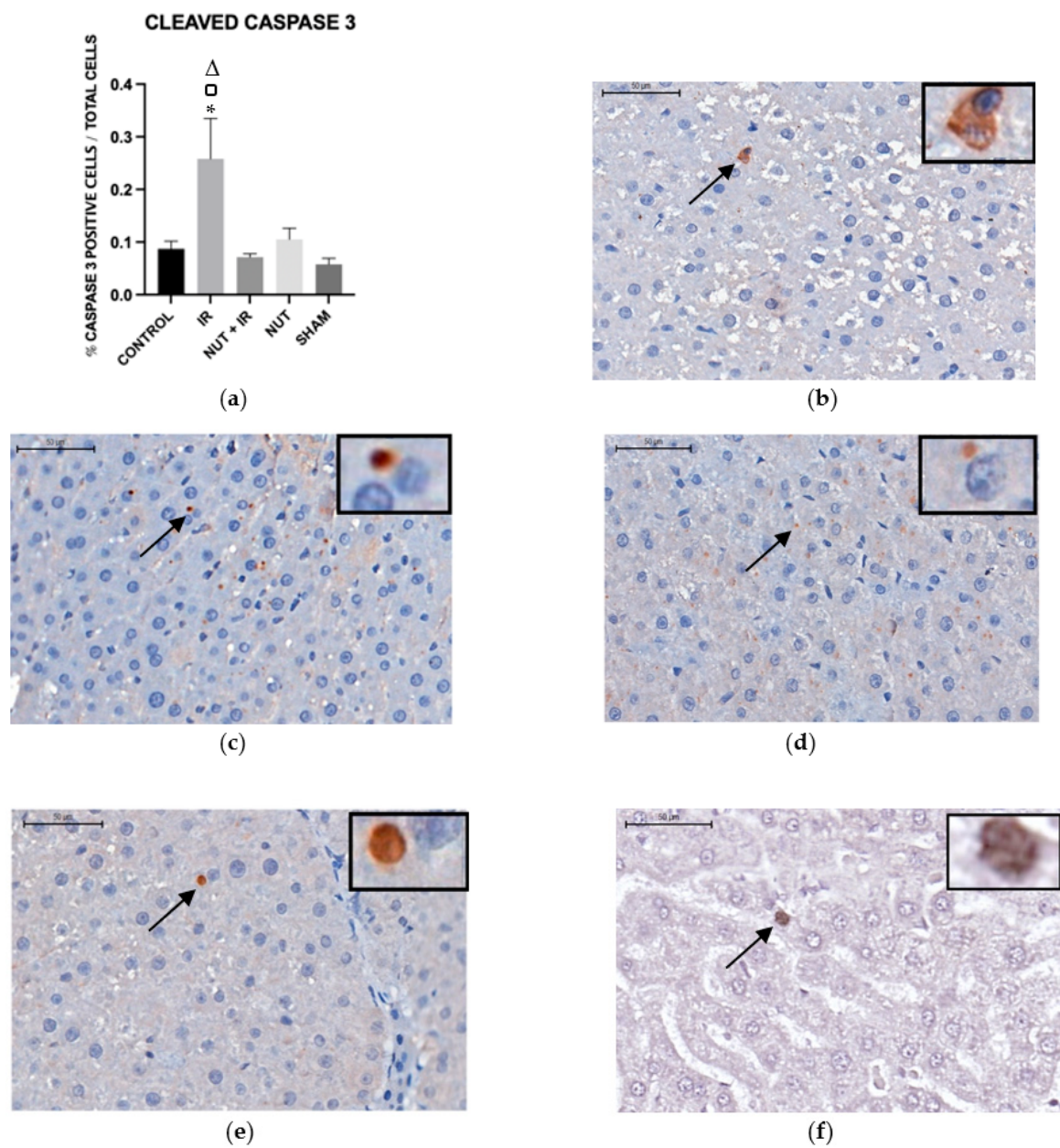


Figure 6. Immunohistochemistry. Cleaved caspase-3 protein in liver tissue from the different groups: (a) data shown are mean $\pm$ SEM; * $p < 0.05$ vs. CONTROL group; $\square$ $p < 0.05$ vs. NUT + IR group; Δ $p < 0.05$ vs. SHAM group. Arrows and highlighted boxes indicate cleaved caspase-3 positive cells in each group: (b) CONTROL; (c) IR; (d) NUT + IR; (e) NUT; and (f) SHAM. All images were obtained with $50\times$ magnification and highlighted boxes with $400\times$ magnification, with scale bars of $500\text{ }\mu\text{m}$ and $50\text{ }\mu\text{m}$, respectively.

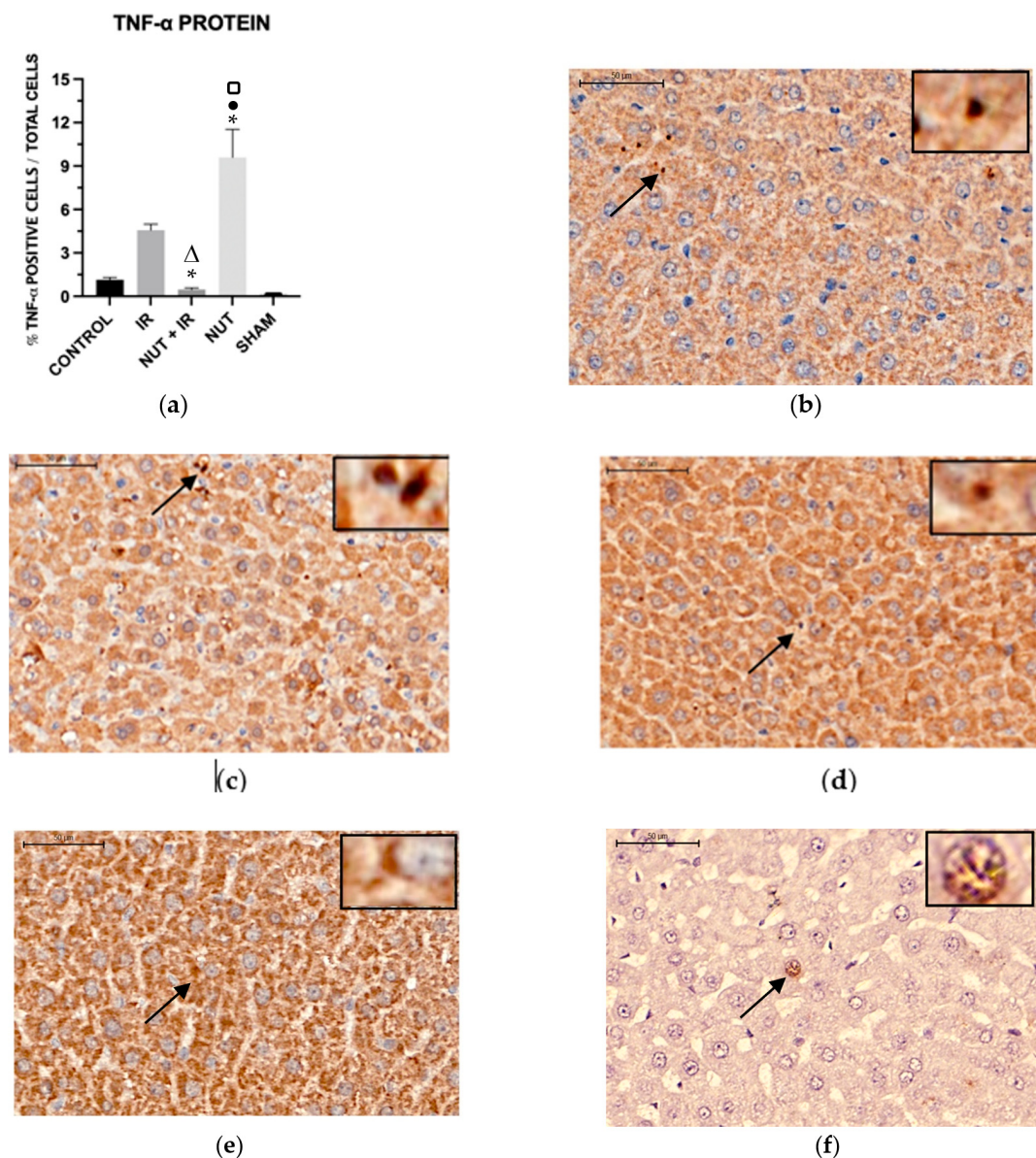


Figure 7. Immunohistochemistry. TNF- α protein in liver tissue from different groups: (a) data shown are mean $\pm$ SEM; * $p < 0.05$ vs. IR group; Δ $p < 0.05$ vs. NUT group; $\bullet$ $p < 0.05$ vs. CONTROL group; $\square$ $p < 0.05$ vs. SHAM group. Arrows and highlighted boxes indicate TNF- α positive cells in each group: (b) CONTROL; (c) IR; (d) NUT + IR; (e) NUT; and (f) SHAM. All images were obtained with $50\times$ magnification and highlighted boxes with $400\times$ magnification, with scale bars of $500\ \mu\text{m}$ and $50\ \mu\text{m}$, respectively.

2.6. Liver Histological Injury

According to the liver histological score used, the IR group (score 37) had a significantly higher level of liver injury when compared to the NUT + IR group (score 25). It was also observed that the immunohistochemical analysis of caspase-3 showed a marked presence in the related field to IR injury by hematoxylin–eosin (HE), demonstrating a correlation between histological and immunohistochemical findings (Figure 8).

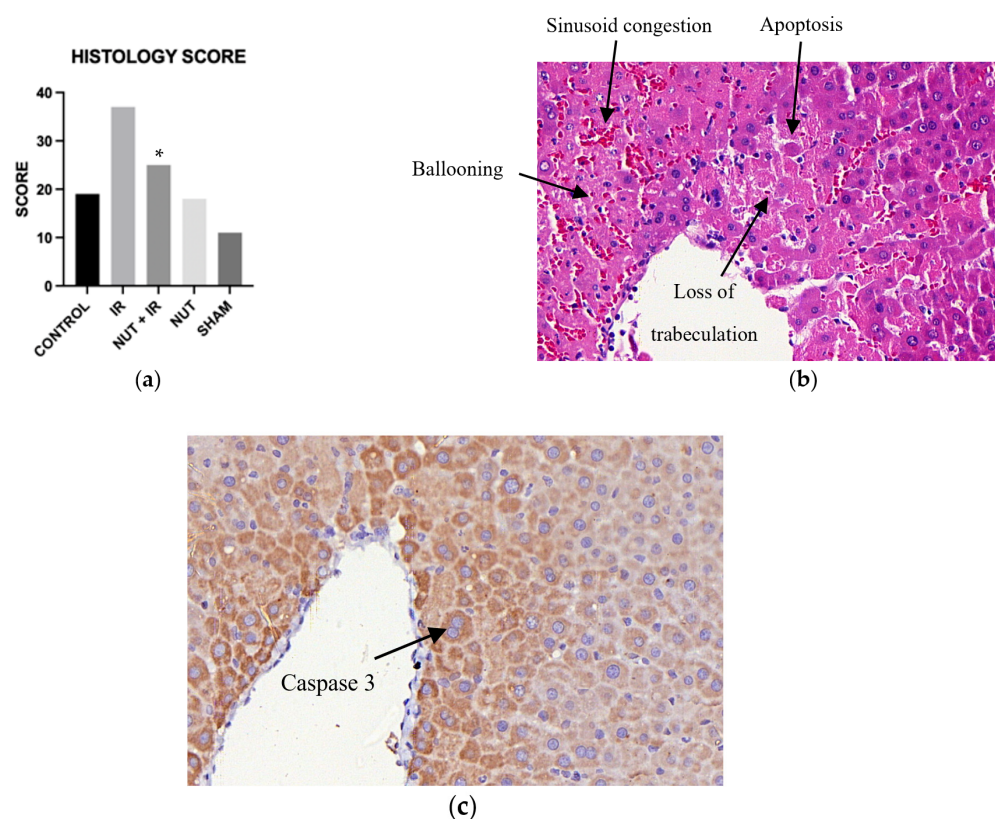


Figure 8. Histological and immunohistochemical analysis: (a) total liver histology score from different groups; * $p < 0.05$ vs. IR group. (b) HE (Hematoxylin-eosin—600×): photomicrograph of hepatic parenchyma showing ischemic changes (ballooning, apoptosis, destrabeculation, and sinusoid congestion). (c) Caspase-3 (Immunohistochemistry—600×): photomicrograph of hepatic parenchyma showing positivity in the cytoplasm (intracytoplasmic brown granular pattern).

3. Discussion

Preexisting nutritional status affects post-operative metabolism, liver function, inflammation, and liver regenerative capacity [30,31]. Therefore, the patient's condition plays an important role in predicting postoperative complications [13]. Particularly in hepatic IRI, preexisting nutritional status is a major determinant of hepatocyte injury [32].

Several dietary components significantly benefit health, presenting antioxidant or anti-inflammatory properties [31,33]. Hence, the re-establishment and maintenance of correct nutritional status by these nutraceuticals before, during, and/or after surgery could lead to improvements in complications related to IRI. Thus, they represent a potential approach alone or in combination with other therapies to improve patient outcomes [13].

Our nutraceutical solution was unable to decrease the transaminases and cytokines released by liver IRI. One of the probable reasons for that result is the fact that this study was conducted only in the early phase of IRI, not in the late phase when the peak of transaminases and necrosis occur [34,35]. Moreover, the inflammatory mediators reach their peaks at different moments: TNF- α peaks between 30 min and 2 h after reperfusion; IL-1 β after 8 h; IL-6 after 12 h; and IL-10 between 30 min and 3 h after reperfusion [36–40]. Therefore, it is necessary to conduct further work to study the effect of nutraceutical solutions on the inflammatory process of hepatic IRI on a timeline.

During liver IRI, Kupffer cells produce reactive oxygen species (ROS) [41]. ROS play a dual role in IRI: they promote apoptosis and stimulate inflammatory mediators, as well as facilitate cell survival under hypoxic conditions and induce antioxidant defenses [42]. In a healthy liver, in response to IRI, levels of PGC-1 α , which is a transcriptional co-activator that controls the expression of metabolic pathways, which allow for cellular adaptation to limited nutrient availability, are stimulated, and this stimulation results

in increased antioxidant defenses of the cell [43,44]. Supporting this fact, Fukai et al. demonstrated increased total glutathione and reduced glutathione after IRI [45]. This increased antioxidant capacity in non-lethal oxidative stress is one of the mechanisms of the protective effect of ischemic preconditioning [46] and may be the reason why rats in the IR and NUT + IR groups exhibited lower levels of MDA than those in the CONTROL and NUT groups, respectively.

Following the line of not altering the inflammatory process, the nutraceutical solution was able to decrease apoptosis, which is a non-inflammatory subtype of cell death during hepatic IRI [47]. Apoptosis is a form of cell death that is critical in regulating tissue homeostasis, and it is considered the key mechanism of injury during the early phase of hepatic IRI in both experimental and human grafts [48–50]. During liver transplantation, apoptosis is involved in cellular injury in acute rejection, as well as in ductopenia seen in chronic rejection [51]. In addition, apoptosis of the donor's liver is an important predictive factor for early graft dysfunction, and its high rate is associated with shorter graft survival [52].

Furthermore, the histological evaluation adapted from Quireze et al.'s score [53] showed that the nutraceutical solution was also able to significantly reduce tissue injury caused by hepatic IRI. This finding is in accordance with the literature, which has demonstrated that inhibition of apoptosis can decrease IRI in liver grafts [50,54].

After seeing the effect of the nutraceutical solution on apoptosis, we investigated its possible mechanism of action and found that it causes a decrease in gene expression and the caspase-3 protein in liver tissue. These facts may characterize the nutraceutical solution as a *caspase inhibitor*, which involves a novel target to protect the liver from IRI [55]. In this regard, there are some compounds being studied such as IDN-6556 and F573. The pan-caspase inhibitor IDN-6556 inhibits caspase-3 activation and reduces sinusoidal endothelium cell apoptosis when used as an additive in the University of Wisconsin storage solution during the preservation period of rat livers [56]. Moreover, when it is administered in cold storage and flush solutions during human liver transplantation, it provides local therapeutic protection against IR injury and apoptosis [48]. IDN-6556 also protects both murine and human islets in culture and after transplantation, slows down the aminotransferase activity in HCV patients, and lowers portal pressure in patients with compensated cirrhosis and severe portal hypertension [57–59].

The other pan-caspase inhibitor F573, in turn, also mitigates liver IRI by reductions in the cytokine TNF- α , apoptosis, and the ALT level [60]. Another application of this caspase inhibitor was shown with the reduction in apoptosis of human and mouse pancreatic islets in vitro and an improvement in their function when they are transplanted into the portal vein [61].

Besides our nutraceutical solution working as a caspase inhibitor, it was also able to decrease the TNF- α protein level in the liver tissue, which is another mechanism of action that may have contributed to decreasing apoptosis. In this regard, Ben-Ari et al. showed that treatment with an anti-TNF- α monoclonal antibody before ischemia is able to mitigate apoptosis by inhibiting the activity of caspases -9 and -3 [62].

There are many other diseases such as inflammatory disorders (psoriasis, arthritis, sepsis), neurologic diseases (Alzheimer's, epilepsy), metabolic diseases (obesity, diabetes, nonalcoholic liver fatty disease), and cancer that are strongly associated with abnormal activity of caspases and apoptosis [63–66].

All these studies broaden our horizons and make us think about other possibilities for the clinical use of our nutraceutical combination, in addition to liver IRI. However, before that, there is a need for further studies to establish all the mechanisms of action and the effects of this nutraceutical combination.

Despite the fact that nutraceuticals often pose a challenging pharmacological profile [67], their pharmacokinetic and pharmacodynamic properties are being extended, allowing interpolating results from animals to humans. In this regard, some nutraceutical combinations have already been studied to treat liver steatosis in patients with nonalcoholic liver fatty diseases, showing promising results [68,69].

In addition, these clinical trials are also showing that nutraceutical combinations are being well tolerated and safe [68,69]. Even so, their real efficacy and safety need to be confirmed in future randomized clinical trials.

4. Materials and Methods

4.1. Animals

Thirty-seven male Wistar rats (245–345 g) were obtained from the Institute of Medical Sciences of the University of São Paulo and housed in LIM37 from the University of São Paulo Medical School. They were placed at room temperature between 20 °C and 22 °C, in a 12 h light/dark cycle. The rats were fed with commercial Nuvilab CR-1 Irradiated feed (Nuvital Nutrientes, Colombo, Paraná, Brazil) and hydrated with filtered water ad libitum. The experimental protocol was approved by the Ethics Committee on the use of animals at our institution (909).

4.2. Preparation of the Nutraceutical Solution

For the nutraceutical formulation, carboxymethyl cellulose (CMC) syrup 0.5% (Bio Idêntica Manipulation Pharmacy, São José, Santa Catarina, Brazil) was used as a vehicle [70,71].

In the preparation of the nutraceutical solution, omega-3 powder (Natural Products & Technologies, São Bernardo, São Paulo, Brazil) was weighed and transferred to a porcelain mortar, in which 0.5 mL of sunflower oil was added for its solubilization. Then, each remaining component was weighed separately, in the following order: resveratrol, quercetin, chelated selenium, dry ginger extract, avocado powder, leucine, and nicotinamide (Infinity Pharma, Campinas, São Paulo, Brazil). They were mixed with the solubilized omega-3 (Table 1).

Table 1. Amount of each nutraceutical.

Nutraceuticals	mg/kg	mg/mL	Amount (g) in 100 mL
Resveratrol	2.96	0.74	0.074
Quercetin	3.56	0.89	0.0908
Chelated selenium	1.76	0.44	2.6831
Omega-3	2.0	0.50	0.05
Ginger extract	3.24	0.81	0.081
Avocado powder	5.08	1.27	0.127
Leucine	4.44	1.11	0.111
Nicotinamide	20.0	5.0	0.5

The concentration of each nutraceutical ingredient was calculated for a rat with an average weight of 250 g and in a 100 mL solution, without exceeding the toxic limits of each one (Table 1).

The administration of the nutraceutical solution was made at a dose of 1.0 mL, once a day, by gavage (with a BD-12 stainless steel curved gavage needle of 1.2 mm diameter × 41.2 mm length; Ciencor Scientific Ltd., São Paulo, Brazil) for seven consecutive days before the experiment [72–75].

4.3. Anesthesia and Surgical Procedures

The rats were anesthetized with 5% ketamine hydrochloride (Ketalar® Cristália, São Paulo, Brazil) 100 mg/kg and 2% xylazine hydrochloride (Rompum® Bayer, Leverkusen, Germany), at a dose of 10 mg/kg, intraperitoneally. The animals were submitted to oro-tracheal intubation with a Jelco 16 catheter (Jelco® Descarpac, São Paulo, Brazil) and ventilated with a tidal volume of 0.08 mL/g of weight, a respiratory rate of 60 cycles/min, and a FiO₂ of 0.21 (Small Animal Ventilator model 683, Harvard Apparatus, Holliston, MA, USA).

A midline laparotomy was performed, and the pedicles of the left lateral and median hepatic lobes were occluded with a 2.5 mm microvascular clamp, inducing the ischemia

of approximately 70% of the total liver volume for 60 min [76,77]. The abdominal incision was closed with a continuous 4.0 nylon suture during this period of ischemia to prevent dehydration and hypothermia in animals.

After 60 min of ischemia, the abdomen of the rats was opened again and the clamp was removed to allow for 4 h of liver reperfusion [76,77]. The incision was closed again, and the animals returned to individual cages.

Following the reperfusion time, rats were anesthetized again, and a new midline laparotomy with median thoracotomy was performed. A blood sample was collected through cardiac puncture. Then, the left ventricle was punctured with a Jelco 16 catheter and connected to a 250 mL 0.9% saline solution; the rats were euthanized, and their organs were carefully washed with saline solution [78].

After the liver had been washed homogeneously, a partial hepatectomy of previously ischemic lobes was performed.

4.4. Experimental Design

The rats were allocated into five groups. In the CONTROL group (n = 8), the rats did not undergo any surgical procedure. In the IR Group (n = 8), the animals were submitted to hepatic IR. In the NUT + IR group (n = 8), the rats received a nutraceutical solution for seven consecutive days before hepatic IR was performed. In the NUT group (n = 8), the animals received a nutraceutical solution for seven consecutive days. Additionally, in the SHAM group (n = 5), the rats underwent midline laparotomy, and the liver was manipulated without pedicled clamping.

4.5. Serum Biochemical Analysis

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were used as indicators of liver injury. AST and ALT activities were assayed 4 h after reperfusion by ultraviolet kinetic method (COBAS C111, Roche, Indianapolis, IN, USA) according to the International Federation of Clinical Chemistry. The results are expressed in units per liter (U/L).

The following inflammatory mediators were also evaluated: interleukin 1 beta (IL1- β), interleukin 6 (IL-6), interleukin 10 (IL-10), and TNF- α . Plasma specimens were prepared for analysis in a 96-well plate utilizing a kit of 13-cytokine Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Corp., Billerica, MA, USA) following the manufacturer's recommendations.

Concentrations of cytokines were determined from a standard curve of the mean fluorescence intensity versus pg/mL.

4.6. Oxidative Stress

The MDA concentration was determined by reverse-phase High-Performance Liquid Chromatography (HPLC) according to Hong et al. [79]. Liver tissue homogenate (1/40 PBS *v/v*) (0.05 mL) was submitted to alkaline hydrolysis with 12.5 μ L of 0.2% butylated hydroxytoluene in ethanol and 6.25 μ L of a 10 M sodium hydroxide aqueous solution. This mixture was incubated at 60 °C for 30 min, and 750 μ L of 7.2% TCA aqueous solution containing 1% KI was added. The samples were kept on ice for 10 min and centrifuged at $10,000 \times g$ for 10 min. The supernatant (500 μ L) was mixed with 250 μ L of 0.6% TBA and heated at 95 °C for 30 min. After cooling, the MDA was extracted from the solution with 750 μ L of *n*-butanol and analyzed by HPLC (Agilent Technologies 1200 series; Santa Clara, CA, USA). The TBA–MDA conjugate derivative (50 μ L) was injected into a Phenomenex reverse-phase C18 analytical column (250 $\times$ 4.6 mm; 5 μ m, Phenomenex, Torrance, CA, USA) with an LC8-D8 pre-column (Phenomenex AJ0-1287) and was quantified using fluorometric detection at excitation and emission wavelengths of 515 and of 553 nm, respectively [79].

The analysis was run under isocratic conditions, using a mobile phase of 60% phosphate-buffered saline (PBS) (50 mmol, pH 7.1) + 40% methanol at a flow rate of 1.0 mL/min. A standard curve (15–80 μ mol MDA, $r = 0.9981$) was prepared using 1,1,3,3-tetraethoxypropane.

The protein concentration was measured by the BCA method using the Pierce BCA kit (Thermo Fisher Scientific, Waltham, MA, USA) as per the manufacturer's instructions and a solution of bovine serum albumin as standard for the calibration curve (0.025–2.00 mg ptn/mL, $r = 0.9969$) [79]. Samples were analyzed using a Synergy HT Spectrophotometer (BioTek, Winooski, VT, USA) with Gen5 software version 3.0 (BioTek). The results are expressed as $\mu\text{g MDA/mg protein}$.

4.7. Gene Expression of Apoptosis

Liver tissue from animals in each group was collected, immediately frozen in liquid nitrogen, and stored at -80°C until RNA extraction was performed. For the extraction of total RNA, TRIZOLTM reagent (Life Technologies Carlsbad, Carlsbad, CA, USA) was used according to the protocol proposed by the manufacturer.

The RNA concentration was determined by the NanoDrop ND-1000 spectrophotometer. The degree of RNA purity was evaluated by a 260/280 nm ratio, using only those whose ratio was ≥ 1.8 . The integrity profile of extracted RNA was evaluated by electrophoresis to verify the presence of bands corresponding to 18S and 28S ribosomal RNAs. The quantified RNA was stored at -80°C until use.

The design of oligonucleotides was conducted with the Primer 3 program (<http://primer3.ut.ee> accessed on 22 September 2022). Analysis of the expression of mRNA levels of BAX, Bcl-2, CASPASE 3, and CASPASE 8 genes was performed on a Rotor-Gene RG-3000 thermocycler (Corbett Research, Sydney, Australia). The commercial kit SuperScriptTM III Platinum[®] SYBR Green One-Step qRT-PCR (Life Technologies Corporation, Carlsbad, CA, USA) was used. The beta-actin gene was used as a normalizer of qRT-PCR reactions. The 2-Delta Delta CT method was used for relative quantification of gene expression (Livak & Schmittgen, 2001).

4.8. Immunohistochemistry

4.8.1. Apoptosis

For the in situ detection of apoptosis in a single cell, the final identification deoxynucleotidyl transferase (TdT) test was used (TUNEL; Boehringer Mannheim, Germany) [80,81]. According to the standard established by the Laboratory of Histomorphometry and Lung Genomics at the University of São Paulo Medical School, 3–4 μm thick sections of liver tissue were made and placed on silanized slides (Sigma Chemical Co.; St. Louis, MO, USA) on a suitable support, as previously described by Souza et al. [82].

4.8.2. Cleaved Caspase-3 and TNF- α Proteins

Subserial sections from paraffin blocks were used for immunohistochemistry. The antibodies used were caspase-3 and TNF- α (Table 2). Immunohistochemistry was performed according to the manufacturer's instructions.

Table 2. Immunohistochemical markers.

Antibody	Concentration	Brand	Code	Clone
Caspase 3	1:200	Novocastra	NCL-CPP32	-
TNF- α	1:200	Santa Cruz	sc-1348	-

Briefly, after the deparaffinization process and the hydration of the liver tissue sections, the recovery of antigenic sites was performed at high temperature in citrate pH 6 for caspase-3 and TRIS-EDTA pH 9 for TNF- α . Endogenous peroxidase blocking was performed with 10 v (3%) oxygenated water four times for 5 min for caspase-3 and for TNF- α with methanol and oxygenated water, volume by volume, two times for 10 min. In the latter two antibodies, protein blots were made, and then the slides were washed in tap water, followed by distilled water, and left in TBS buffer at pH 7.4.

The antibodies were diluted at concentrations shown in the table below (Table 2). The slides were incubated overnight at 4 °C in a humid chamber. Subsequently, incubation was performed with the secondary antibody (ABC Elite, Vector Laboratories Inc, Newark, CA, USA) specific for each species, and the antibody was produced for 30 min in an incubator at 37 °C. Diaminobenzidine (DAB) (Sigma-Aldrich Chemie, Steinheim, Germany) was used as the chromogen. Then, counterstaining was performed with Harris' Hematoxylin (Merck, Darmstadt, Germany).

4.9. Histology

Samples from the median and left anterolateral liver lobes were collected 4 h after reperfusion and fixed in 10% formalin for standard hematoxylin and eosin (HE) staining. A single-blinded pathologist performed the histologic evaluation.

The histologic injury was evaluated according to the scoring system proposed by Quireze et al. [53], which was adapted based on the presence and intensity of the following alterations: ballooning, steatosis, apoptosis, loss of hepatic trabeculae, and necrosis. Those lesions were graded according to the absence (grade 0) or presence of minimal (grade 1), moderate (grade 2), or severe (grade 3) alterations, as determined by the pathologist.

4.10. Data Processing

Data were statistically analyzed using GraphPad Prism software (version 9.5.1). One-way analysis of variance (ANOVA) was used to assess differences between tested groups, followed by Tukey's multiple comparison tests. The non-parametric results of transaminases and gene expression were analyzed by the Kruskal–Wallis test, followed by Dunn's test. Categorical data of liver histological injury were analyzed using the Chi-square statistic. The results are presented as means $\pm$ standard errors of means (SEM). A *p*-value less than 0.05 was considered statistically significant.

5. Conclusions

In summary, we proposed a nutraceutical solution that was able to decrease apoptosis and histologic injury caused by liver IRI. Its suggested mechanisms of action are a reduction in gene expression and the caspase-3 protein, as well as a reduction in TNF- α protein in liver tissue. These findings suggest that the nutraceutical combination used favors the protection of hepatocytes and represents a promising therapeutic proposal against liver IRI.

Besides transplantation, hepatocyte apoptosis also occurs in chronic liver diseases that affect 1.5 billion persons globally. All these patients can be helped by controlling apoptosis.

Author Contributions: Conceptualization, F.H.G. and V.R.-S.; methodology, F.H.G. and I.A.C.; investigation, C.A.P. and M.S.K.; writing—original draft preparation, C.A.P.; writing—review and editing, M.S.K. and V.R.-S.; visualization, M.S.K.; supervision, L.A.C.-D.; project administration, F.H.G.; funding acquisition, L.A.C.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This research and the APC were funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and registered at 00690623909, an institutional quota.

Institutional Review Board Statement: The animal study protocol was approved by the Comitê de Ética no Uso de Animais (CEUA) of University of São Paulo Medical School (registration number 909/2017 and date of approval 13 September 2017).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: This work was developed in the Medical Research Laboratories (LIM 37 and 03) at the University of São Paulo Medical School. Special thanks to our colleagues Sandra Nassa Sampietri, Cinthia Lanchotte, Regina Célia Teixeira Gomes, Ângela Batista Gomes dos Santos, Esmeralda Miristeni Eher, and Sandra de Moraes Ferneztian.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sachdeva, V.; Roy, A.; Bharadvaja, N. Current Prospects of Nutraceuticals: A Review. *Curr. Pharm. Biotechnol.* **2020**, *21*, 884–896. [[CrossRef](#)] [[PubMed](#)]
2. Brower, V. Nutraceuticals: Poised for a Healthy Slice of the Healthcare Market? *Nat. Biotechnol.* **1998**, *16*, 728–731. [[CrossRef](#)] [[PubMed](#)]
3. Bergamin, A.; Mantzioris, E.; Cross, G.; Deo, P.; Garg, S.; Hill, A.M. Nutraceuticals: Reviewing Their Role in Chronic Disease Prevention and Management. *Pharmaceut. Med.* **2019**, *33*, 291–309. [[CrossRef](#)]
4. D’Angelo, S.; Scafuro, M.; Meccariello, R. BPA and Nutraceuticals, Simultaneous Effects on Endocrine Functions. *Endocr. Metab. Immune Disord. Drug Targets* **2019**, *19*, 594–604. [[CrossRef](#)]
5. Espín, J.C.; García-Conesa, M.T.; Tomás-Barberán, F.A. Nutraceuticals: Facts and Fiction. *Phytochemistry* **2007**, *68*, 2986–3008. [[CrossRef](#)] [[PubMed](#)]
6. Prasad, S.; Gupta, S.C.; Tyagi, A.K. Reactive Oxygen Species (ROS) and Cancer: Role of Antioxidative Nutraceuticals. *Cancer Lett.* **2017**, *387*, 95–105. [[CrossRef](#)] [[PubMed](#)]
7. Song, J.-M.; Lee, K.-H.; Seong, B.-L. Antiviral Effect of Catechins in Green Tea on Influenza Virus. *Antiviral Res.* **2005**, *68*, 66–74. [[CrossRef](#)] [[PubMed](#)]
8. de Mejia, E.G.; Dia, V.P. The Role of Nutraceutical Proteins and Peptides in Apoptosis, Angiogenesis, and Metastasis of Cancer Cells. *Cancer Metastasis Rev.* **2010**, *29*, 511–528. [[CrossRef](#)]
9. Aggarwal, B.B.; Van Kuiken, M.E.; Iyer, L.H.; Harikumar, K.B.; Sung, B. Molecular Targets of Nutraceuticals Derived from Dietary Spices: Potential Role in Suppression of Inflammation and Tumorigenesis. *Exp. Biol. Med.* **2009**, *234*, 825–849. [[CrossRef](#)]
10. Liu, B.; Cheng, Y.; Zhang, B.; Bian, H.; Bao, J. Polygonatum Cyrtonema Lectin Induces Apoptosis and Autophagy in Human Melanoma A375 Cells through a Mitochondria-Mediated ROS–P38–P53 Pathway. *Cancer Lett.* **2009**, *275*, 54–60. [[CrossRef](#)]
11. Mirarchi, A.; Mare, R.; Musolino, V.; Nucera, S.; Mollace, V.; Pujia, A.; Montalcini, T.; Romeo, S.; Maurotti, S. Bergamot Polyphenol Extract Reduces Hepatocyte Neutral Fat by Increasing Beta-Oxidation. *Nutrients* **2022**, *14*, 3434. [[CrossRef](#)] [[PubMed](#)]
12. Puri, V.; Nagpal, M.; Singh, I.; Singh, M.; Dhingra, G.A.; Huanbutta, K.; Dheer, D.; Sharma, A.; Sangnim, T. A Comprehensive Review on Nutraceuticals: Therapy Support and Formulation Challenges. *Nutrients* **2022**, *14*, 4637. [[CrossRef](#)] [[PubMed](#)]
13. Cornide-Petronio, M.E.; Álvarez-Mercado, A.I.; Jiménez-Castro, M.B.; Peralta, C. Current Knowledge about the Effect of Nutritional Status, Supplemented Nutrition Diet, and Gut Microbiota on Hepatic Ischemia-Reperfusion and Regeneration in Liver Surgery. *Nutrients* **2020**, *12*, 284. [[CrossRef](#)]
14. Eltzschig, H.K.; Eckle, T. Ischemia and Reperfusion—From Mechanism to Translation. *Nat. Med.* **2011**, *17*, 1391–1401. [[CrossRef](#)]
15. Jiménez-Castro, M.B.; Cornide-Petronio, M.E.; Gracia-Sancho, J.; Peralta, C. Inflammasome-Mediated Inflammation in Liver Ischemia-Reperfusion Injury. *Cells* **2019**, *8*, 1131. [[CrossRef](#)] [[PubMed](#)]
16. de Oliveira, T.H.C.; Marques, P.E.; Proost, P.; Teixeira, M.M.M. Neutrophils: A Cornerstone of Liver Ischemia and Reperfusion Injury. *Lab. Invest.* **2018**, *98*, 51–62. [[CrossRef](#)]
17. Zhai, Y.; Petrowsky, H.; Hong, J.C.; Busuttill, R.W.; Kupiec-Weglinski, J.W. Ischaemia–Reperfusion Injury in Liver Transplantation—From Bench to Bedside. *Nat. Rev. Gastroenterol. Hepatol.* **2013**, *10*, 79–89. [[CrossRef](#)] [[PubMed](#)]
18. He, D.; Guo, Z.; Pu, J.-L.; Zheng, D.-F.; Wei, X.-F.; Liu, R.; Tang, C.-Y.; Wu, Z.-J. Resveratrol Preconditioning Protects Hepatocytes against Hepatic Ischemia Reperfusion Injury via Toll-like Receptor 4/Nuclear Factor-KB Signaling Pathway in Vitro and in Vivo. *Int. Immunopharmacol.* **2016**, *35*, 201–209. [[CrossRef](#)]
19. Su, J.-F.; Guo, C.-J.; Wei, J.-Y.; Yang, J.-J.; Jiang, Y.-G.; Li, Y.-F. Protection against Hepatic Ischemia-Reperfusion Injury in Rats by Oral Pretreatment with Quercetin. *Biomed. Environ. Sci.* **2003**, *16*, 1–8.
20. Calder, P.C. Mechanisms of Action of (n-3) Fatty Acids. *J. Nutr.* **2012**, *142*, 592S–599S. [[CrossRef](#)]
21. Zapletel, C.; Heyne, S.; Breitzkreutz, R.; Gebhard, M.M.; Golling, M. The Influence of Selenium Substitution on Microcirculation and Glutathione Metabolism after Warm Liver Ischemia/Reperfusion in a Rat Model. *Microvasc. Res.* **2008**, *76*, 104–109. [[CrossRef](#)] [[PubMed](#)]
22. Abdel-Azeem, A.S.; Hegazy, A.M.; Ibrahim, K.S.; Farrag, A.-R.H.; El-Sayed, E.M. Hepatoprotective, Antioxidant, and Ameliorative Effects of Ginger (*Zingiber Officinale* Roscoe) and Vitamin E in Acetaminophen Treated Rats. *J. Diet Suppl.* **2013**, *10*, 195–209. [[CrossRef](#)]
23. Maghsoudi, S.; Gol, A.; Dabiri, S.; Javadi, A. Preventive Effect of Ginger (*Zingiber Officinale*) Pretreatment on Renal Ischemia-Reperfusion in Rats. *Eur. Surg. Res.* **2011**, *46*, 45–51. [[CrossRef](#)] [[PubMed](#)]
24. Eser, O.; Songur, A.; Yaman, M.; Cosar, M.; Fidan, H.; Sahin, O.; Mollaoglu, H.; Buyukbas, S. The Protective Effect of Avocado Soybean Unsaponifiables on Brain Ischemia/Reperfusion Injury in Rat Prefrontal Cortex. *Br. J. Neurosurg.* **2011**, *25*, 701–706. [[CrossRef](#)] [[PubMed](#)]
25. De Araujo, Q.R.; Gattward, J.N.; Almoosawi, S.; Parada das Costa Silva, M.G.C.; Dantas, P.A.D.S.; Araujo Júnior, Q.R., Jr. De Cocoa and Human Health: From Head to Foot—A Review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1–12. [[CrossRef](#)] [[PubMed](#)]
26. Singh, C.K.; Siddiqui, I.A.; El-Abd, S.; Mukhtar, H.; Ahmad, N. Combination Chemoprevention with Grape Antioxidants. *Mol. Nutr. Food Res.* **2016**, *60*, 1406–1415. [[CrossRef](#)] [[PubMed](#)]

27. Liu, R.H. Health Benefits of Fruit and Vegetables Are from Additive and Synergistic Combinations of Phytochemicals. *Am. J. Clin. Nutr.* **2003**, *78*, 517S–520S. [\[CrossRef\]](#)
28. Liu, R.H. Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. *J. Nutr.* **2004**, *134*, 3479S–3485S. [\[CrossRef\]](#)
29. Hegazy, A.M.; El-Sayed, E.M.; Ibrahim, K.S.; Abdel-Azeem, A.S. Dietary Antioxidant for Disease Prevention Corroborated by the Nrf2 Pathway. *J. Complement. Integr. Med.* **2019**, *16*, 3. [\[CrossRef\]](#)
30. Saïdi, S.; Abdelkafi, S.; Jbahi, S.; van Pelt, J.; El-Feki, A. Temporal Changes in Hepatic Antioxidant Enzyme Activities after Ischemia and Reperfusion in a Rat Liver Ischemia Model. *Hum. Exp. Toxicol.* **2015**, *34*, 249–259. [\[CrossRef\]](#)
31. da Silva, R.M.; Malafaia, O.; Torres, O.J.M.; Czczeko, N.G.; Marinho Junior, C.H.; Kozłowski, R.K. Evaluation of Liver Regeneration Diet Supplemented with Omega-3 Fatty Acids: Experimental Study in Rats. *Rev. Col. Bras. Circ.* **2015**, *42*, 393–397. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Domenicali, M.; Vendemiale, G.; Serviddio, G.; Grattagliano, I.; Pertosa, A.M.; Nardo, B.; Principe, A.; Viola, A.; Trevisani, F.; Altomare, E.; et al. Oxidative Injury in Rat Fatty Liver Exposed to Ischemia-Reperfusion Is Modulated by Nutritional Status. *Dig. Liver Dis.* **2005**, *37*, 689–697. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Chandrasekara, A.; Josheph Kumar, T. Roots and Tuber Crops as Functional Foods: A Review on Phytochemical Constituents and Their Potential Health Benefits. *Int. J. Food Sci.* **2016**, *2016*, 3631647. [\[CrossRef\]](#)
34. Hasegawa, T.; Ito, Y.; Wijeweera, J.; Liu, J.; Malle, E.; Farhood, A.; McCuskey, R.S.; Jaeschke, H. Reduced Inflammatory Response and Increased Microcirculatory Disturbances during Hepatic Ischemia-Reperfusion Injury in Steatotic Livers of *Ob/Ob* Mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2007**, *292*, G1385–G1395. [\[CrossRef\]](#)
35. Fang, H.; Liu, A.; Dahmen, U.; Dirsch, O. Dual Role of Chloroquine in Liver Ischemia Reperfusion Injury: Reduction of Liver Damage in Early Phase, but Aggravation in Late Phase. *Cell Death Dis.* **2013**, *4*, e694. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Perry, B.C.; Soltys, D.; Toledo, A.H.; Toledo-Pereyra, L.H. Tumor Necrosis Factor- α in Liver Ischemia/Reperfusion Injury. *J. Invest. Surg.* **2011**, *24*, 178–188. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Kato, A.; Gabay, C.; Okaya, T.; Lentsch, A.B. Specific Role of Interleukin-1 in Hepatic Neutrophil Recruitment after Ischemia/Reperfusion. *Am. J. Pathol.* **2002**, *161*, 1797–1803. [\[CrossRef\]](#)
38. Cui, L.-Z.; Wang, B.; Chen, L.-Y.; Zhou, J. The Effect of Ischemic Precondition to IL-6 on Rat Liver Ischemia-Reperfusion Injury in Transplantation. *Asian Pac. J. Trop. Med.* **2013**, *6*, 395–399. [\[CrossRef\]](#)
39. Oreopoulos, G.D.; Wu, H.; Szaszi, K.; Fan, J.; Marshall, J.C.; Khadaroo, R.G.; He, R.; Kapus, A.; Rotstein, O.D. Hypertonic Preconditioning Prevents Hepatocellular Injury Following Ischemia/Reperfusion in Mice: A Role for Interleukin 10. *Hepatology* **2004**, *40*, 211–220. [\[CrossRef\]](#)
40. Guimarães Filho, M.A.C.; Cortez, E.; Garcia-Souza, É.P.; de Soares, V.M.; Moura, A.S.; Carvalho, L.; de Maya, M.C.A.; Pitombo, M.B. Effect of Remote Ischemic Preconditioning in the Expression of IL-6 and IL-10 in a Rat Model of Liver Ischemia-Reperfusion Injury. *Acta Circ. Bras.* **2015**, *30*, 452–460. [\[CrossRef\]](#)
41. Kaltenmeier, C.; Wang, R.; Popp, B.; Geller, D.; Tohme, S.; Yazdani, H.O. Role of Immuno-Inflammatory Signals in Liver Ischemia-Reperfusion Injury. *Cells* **2022**, *11*, 2222. [\[CrossRef\]](#)
42. Prieto, I.; Monsalve, M. ROS Homeostasis, a Key Determinant in Liver Ischemic-Preconditioning. *Redox Biol.* **2017**, *12*, 1020–1025. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Sánchez-Ramos, C.; Prieto, I.; Tierrez, A.; Laso, J.; Valdecantos, M.P.; Bartrons, R.; Roselló-Catafau, J.; Monsalve, M. PGC-1 α Downregulation in Steatotic Liver Enhances Ischemia-Reperfusion Injury and Impairs Ischemic Preconditioning. *Antioxid Redox Signal* **2017**, *27*, 1332–1346. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Summermatter, S.; Handschin, C. PGC-1 α and Exercise in the Control of Body Weight. *Int. J. Obes.* **2012**, *36*, 1428–1435. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Fukai, M.; Hayashi, T.; Yokota, R.; Shimamura, T.; Suzuki, T.; Taniguchi, M.; Matsushita, M.; Furukawa, H.; Todo, S. Lipid Peroxidation during Ischemia Depends on Ischemia Time in Warm Ischemia and Reperfusion of Rat Liver. *Free Radic. Biol. Med.* **2005**, *38*, 1372–1381. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Rüdiger, H.A.; Graf, R.; Clavien, P.-A. Sub-Lethal Oxidative Stress Triggers the Protective Effects of Ischemic Preconditioning in the Mouse Liver. *J. Hepatol.* **2003**, *39*, 972–977. [\[CrossRef\]](#)
47. Hirao, H.; Nakamura, K.; Kupiec-Weglinski, J.W. Liver Ischaemia-Reperfusion Injury: A New Understanding of the Role of Innate Immunity. *Nat. Rev. Gastroenterol. Hepatol.* **2022**, *19*, 239–256. [\[CrossRef\]](#)
48. Baskin-Bey, E.S.; Washburn, K.; Feng, S.; Oltersdorf, T.; Shapiro, D.; Huyghe, M.R.; Burgart, L.; Garrity-Park, M.; Van Vilsteren, F.G.I.; Oliver, L.K.; et al. Clinical Trial of the Pan-Caspase Inhibitor, IDN-6556, in Human Liver Preservation Injury. *Am. J. Transplant.* **2007**, *7*, 218–225. [\[CrossRef\]](#)
49. Sindram, D.; Porte, R.J.; Hoffman, M.R.; Bentley, R.C.; Clavien, P. Platelets Induce Sinusoidal Endothelial Cell Apoptosis upon Reperfusion of the Cold Ischemic Rat Liver. *Gastroenterology* **2000**, *118*, 183–191. [\[CrossRef\]](#)
50. Kuo, P.C.; Drachenberg, C.I.; de la Torre, A.; Bartlett, S.T.; Lim, J.W.; Plotkin, J.S.; Johnson, L.B. Apoptosis and Hepatic Allograft Reperfusion Injury. *Clin. Transplant* **1998**, *12*, 219–223.
51. Parolin, M.B.; Reason, I.J.M. Apoptose Como Mecanismo de Lesão Nas Doenças Hepatobiliares. *Arq. Gastroenterol.* **2001**, *38*, 138–144. [\[CrossRef\]](#)

52. Zhu, Z.; Tang, Y.; Huang, S.; Zhao, Q.; Schroder, P.M.; Zhang, Z.; Zhang, Y.; Sun, C.; Wang, L.; Ju, W.; et al. Donor Liver Apoptosis Is Associated with Early Allograft Dysfunction and Decreased Short-Term Graft Survival after Liver Transplantation. *Clin. Transpl.* **2018**, *12*, e13438. [\[CrossRef\]](#)
53. Quireze, C.; de Souza Montero, E.F.; Leitão, R.M.C.; Juliano, Y.; Fagundes, D.J.; Poli-de-Figueiredo, L.F. Ischemic Preconditioning Prevents Apoptotic Cell Death and Necrosis in Early and Intermediate Phases of Liver Ischemia-Reperfusion Injury in Rats. *J. Invest. Surg.* **2006**, *19*, 229–236. [\[CrossRef\]](#)
54. Zhang, Y.; Ye, Q.-F.; Lu, L.; Xu, X.-L.; Ming, Y.-Z.; Xiao, J.-S. Panax Notoginseng Saponins Preconditioning Protects Rat Liver Grafts from Ischemia/Reperfusion Injury via an Antiapoptotic Pathway. *Hepatobiliary Pancreat. Dis. Int.* **2005**, *4*, 207–212.
55. Mao, X.; Cai, Y.; Chen, Y.; Wang, Y.; Jiang, X.; Ye, L.; Li, S. Novel Targets and Therapeutic Strategies to Protect against Hepatic Ischemia Reperfusion Injury. *Front. Med.* **2022**, *8*, 757336. [\[CrossRef\]](#)
56. Natori, S. The Caspase Inhibitor IDN-6556 Prevents Caspase Activation and Apoptosis in Sinusoidal Endothelial Cells during Liver Preservation Injury. *Liver Transplant.* **2003**, *9*, 278–284. [\[CrossRef\]](#) [\[PubMed\]](#)
57. McCall, M.; Toso, C.; Emamaullee, J.; Pawlick, R.; Edgar, R.; Davis, J.; Maciver, A.; Kin, T.; Arch, R.; Shapiro, A.M.J. The Caspase Inhibitor IDN-6556 (PF3491390) Improves Marginal Mass Engraftment after Islet Transplantation in Mice. *Surgery* **2011**, *150*, 48–55. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Pockros, P.J.; Schiff, E.R.; Shiffman, M.L.; McHutchison, J.G.; Gish, R.G.; Afdhal, N.H.; Makhviladze, M.; Huyghe, M.; Hecht, D.; Oltersdorf, T.; et al. Oral IDN-6556, an Antiapoptotic Caspase Inhibitor, May Lower Aminotransferase Activity in Patients with Chronic Hepatitis C. *Hepatology* **2007**, *46*, 324–329. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Garcia-Tsao, G.; Fuchs, M.; Shiffman, M.; Borg, B.B.; Pyrsopoulos, N.; Shetty, K.; Gallegos-Orozco, J.F.; Reddy, K.R.; Feyssa, E.; Chan, J.L.; et al. Emricasan (IDN-6556) Lowers Portal Pressure in Patients with Compensated Cirrhosis and Severe Portal Hypertension. *Hepatology* **2019**, *69*, 717–728. [\[CrossRef\]](#)
60. Bral, M.; Pawlick, R.; Marfil-Garza, B.; Dadheech, N.; Hefler, J.; Thiesen, A.; James Shapiro, A.M. Pan-Caspase Inhibitor F573 Mitigates Liver Ischemia Reperfusion Injury in a Murine Model. *PLoS ONE* **2019**, *14*, e0224567. [\[CrossRef\]](#)
61. Pepper, A.R.; Bruni, A.; Pawlick, R.; Wink, J.; Rafiei, Y.; Gala-Lopez, B.; Bral, M.; Abualhassan, N.; Kin, T.; Shapiro, A.M.J. Engraftment Site and Effectiveness of the Pan-Caspase Inhibitor F573 to Improve Engraftment in Mouse and Human Islet Transplantation in Mice. *Transplantation* **2017**, *101*, 2321–2329. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Ben-Ari, Z.; Hochhauser, E.; Burstein, I.; Papo, O.; Kaganovsky, E.; Krasnov, T.; Vamichkim, A.; Vidne, B.A. Role of Anti-Tumor Necrosis Factor-Alpha in Ischemia/Reperfusion Injury in Isolated Rat Liver in a Blood-Free Environment. *Transplantation* **2002**, *73*, 1875–1880. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Dhani, S.; Zhao, Y.; Zhivotovsky, B. A Long Way to Go: Caspase Inhibitors in Clinical Use. *Cell Death Dis.* **2021**, *12*, 949. [\[CrossRef\]](#)
64. Ghavami, S.; Shojaei, S.; Yeganeh, B.; Ande, S.R.; Jangamreddy, J.R.; Mehrpour, M.; Christoffersson, J.; Chaabane, W.; Moghadam, A.R.; Kashani, H.H.; et al. Autophagy and Apoptosis Dysfunction in Neurodegenerative Disorders. *Prog. Neurobiol.* **2014**, *112*, 24–49. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Sassmann-Schweda, A.; Singh, P.; Tang, C.; Wietelmann, A.; Wetschurck, N.; Offermanns, S. Increased Apoptosis and Browning of TAK1-Deficient Adipocytes Protects against Obesity. *JCI Insight* **2016**, *1*, 7. [\[CrossRef\]](#)
66. Pfeffer, C.; Singh, A. Apoptosis: A Target for Anticancer Therapy. *Int. J. Mol. Sci.* **2018**, *19*, 448. [\[CrossRef\]](#)
67. Benić, M.S.; Nežić, L.; Vujić-Aleksić, V.; Mititelu-Tartau, L. Novel Therapies for the Treatment of Drug-Induced Liver Injury: A Systematic Review. *Front. Pharmacol.* **2022**, *12*, 785790. [\[CrossRef\]](#)
68. Ferro, Y.; Pujia, R.; Mazza, E.; Lascala, L.; Lodari, O.; Maurotti, S.; Pujia, A.; Montalcini, T. A New Nutraceutical (Livogen Plus®) Improves Liver Steatosis in Adults with Non-Alcoholic Fatty Liver Disease. *J. Transl. Med.* **2022**, *20*, 377. [\[CrossRef\]](#)
69. Ferro, Y.; Montalcini, T.; Mazza, E.; Foti, D.; Angotti, E.; Gliozzi, M.; Nucera, S.; Paone, S.; Bombardelli, E.; Aversa, I.; et al. Randomized Clinical Trial: Bergamot Citrus and Wild Cardoon Reduce Liver Steatosis and Body Weight in Non-Diabetic Individuals Aged Over 50 Years. *Front. Endocrinol.* **2020**, *11*, 494. [\[CrossRef\]](#)
70. Rani, M.; Rudhzhiah, S.; Ahmad, A.; Mohamed, N. Biopolymer Electrolyte Based on Derivatives of Cellulose from Kenaf Bast Fiber. *Polymers* **2014**, *6*, 2371–2385. [\[CrossRef\]](#)
71. Javanbakht, S.; Shaabani, A. Carboxymethyl Cellulose-Based Oral Delivery Systems. *Int. J. Biol. Macromol.* **2019**, *133*, 21–29. [\[CrossRef\]](#) [\[PubMed\]](#)
72. Brai, B.; Adisa, R.; Odetola, A. Hepatoprotective Properties of Aqueous Leaf Extract of Persea Americana, Mill (Lauraceae) ‘Avocado’ Against Ccl4-Induced Damage In Rats. *Afr. J. Tradit. Complement. Altern. Med.* **2014**, *11*, 237. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Iwasaki, W.; Kume, M.; Kudo, K.; Uchinami, H.; Kikuchi, I.; Nakagawa, Y.; Yoshioka, M.; Yamamoto, Y. Changes in the Fatty Acid Composition of the Liver with the Administration of N-3 Polyunsaturated Fatty Acids and the Effects on Warm Ischemia/Reperfusion Injury in the Rat Liver. *Shock* **2010**, *33*, 306–314. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Zúñiga, J.; Venegas, F.; Villarreal, M.; Núñez, D.; Chandía, M.; Valenzuela, R.; Tapia, G.; Varela, P.; Videla, L.A.; Fernández, V. Protection against in Vivo Liver Ischemia-Reperfusion Injury by n-3 Long-Chain Polyunsaturated Fatty Acids in the Rat. *Free Radic. Res.* **2010**, *44*, 854–863. [\[CrossRef\]](#)
75. Miyauchi, T.; Uchida, Y.; Kadono, K.; Hirao, H.; Kawasoe, J.; Watanabe, T.; Ueda, S.; Jobara, K.; Kaido, T.; Okajima, H.; et al. Preventive Effect of Antioxidative Nutrient-Rich Enteral Diet against Liver Ischemia and Reperfusion Injury. *J. Parenter. Enter. Nutr.* **2019**, *43*, 133–144. [\[CrossRef\]](#) [\[PubMed\]](#)

76. Rocha-Santos, V.; Figueira, E.R.; Rocha-Filho, J.A.; Coelho, A.M.; Pinheiro, R.S.; Bacchella, T.; Machado, M.C.; D'Albuquerque, L.A. Pentoxifylline Enhances the Protective Effects of Hypertonic Saline Solution on Liver Ischemia Reperfusion Injury through Inhibition of Oxidative Stress. *Hepatobiliary Pancreat. Dis. Int.* **2015**, *14*, 194–200. [[CrossRef](#)] [[PubMed](#)]
77. Figueira, E.R.R.; Bacchella, T.; Coelho, A.M.M.; Sampietre, S.N.; Molan, N.A.T.; Leitão, R.M.C.; Machado, M.C.C. Timing-Dependent Protection of Hypertonic Saline Solution Administration in Experimental Liver Ischemia/Reperfusion Injury. *Surgery* **2010**, *147*, 415–423. [[CrossRef](#)]
78. Fernandes, D.C.; Gonçalves, R.C.; Laurindo, F.R.M. Measurement of Superoxide Production and NADPH Oxidase Activity by HPLC Analysis of Dihydroethidium Oxidation. *Methods Mol. Biol.* **2017**, *1527*, 233–249.
79. Hong, Y.-L.; Yeh, S.-L.; Chang, C.-Y.; Hu, M.-L. Total Plasma Malondialdehyde Levels in 16 Taiwanese College Students Determined by Various Thiobarbituric Acid Tests and an Improved High-Performance Liquid Chromatography-Based Method. *Clin. Biochem.* **2000**, *33*, 619–625. [[CrossRef](#)]
80. Gavrieli, Y.; Sherman, Y.; Ben-Sasson, S.A. Identification of Programmed Cell Death in Situ via Specific Labeling of Nuclear DNA Fragmentation. *J. Cell Biol.* **1992**, *119*, 493–501. [[CrossRef](#)]
81. Wijsman, J.H.; Jonker, R.R.; Keijzer, R.; van de Velde, C.J.; Cornelisse, C.J.; van Dierendonck, J.H. A New Method to Detect Apoptosis in Paraffin Sections: In Situ End-Labeling of Fragmented DNA. *J. Histochem. Cytochem.* **1993**, *41*, 7–12. [[CrossRef](#)] [[PubMed](#)]
82. Souza, P.; Rizzardi, F.; Noleto, G.; Atanazio, M.; Bianchi, O.; Parra, E.R.; Teodoro, W.R.; Carrasco, S.; Velosa, A.P.P.; Fernezlian, S.; et al. Refractory Remodeling of the Microenvironment by Abnormal Type V Collagen, Apoptosis, and Immune Response in Non-Small Cell Lung Cancer. *Hum. Pathol.* **2010**, *41*, 239–248. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.