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From Cell Biology to Tissue Engineering

Damage to pancreatic acinar cells and preservation of islets of Langerhans in a rat model of acute pancreatitis induced by *Karwinskia humboldtiana* (buckthorn)

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Summary. *Karwinskia humboldtiana* (Kh) is a poisonous plant that grows in some regions of the American continent. Consuming large amounts of Kh fruit results in acute intoxication leading to respiratory failure, culminating in death within days. There is evidence of histological damage to the lungs, liver, and kidneys following accidental and experimental Kh intoxication. To date, the microscopic effect of Kh consumption on the pancreas has not been described. We examined the early effects of Kh fruit on pancreatic tissue at different stages of acute intoxication in the Wistar rat. We found progressive damage confined to the exocrine pancreas, starting with a reduction in the number of zymogen granules, loss of acinar architecture, the presence of autophagy-like vesicles, apoptosis and inflammatory infiltrate. The pancreatic pathology culminated in damaged acini characterized by necrosis and edema, with a complete loss of lobular architecture. Interestingly, the morphology of the islets of Langerhans was conserved throughout our evaluations. Taken together, our results indicate the damage induced by a high dose of Kh fruit in the Wistar rat is consistent with an early acute necrotizing pancreatitis that exclusively affects the exocrine pancreas. Therefore, this system might be useful as an animal model to study the treatment of pancreatic diseases. More importantly, as the islets of Langerhans were preserved, the active compounds of Kh fruit could be utilized for the treatment of acinar pancreatic cancer. Further studies might provide insight into the severity of acute Kh intoxication in humans and influence the design of treatments for pancreatic diseases and acinar pancreatic cancer.

Key words: Acute pancreatitis, Necrotizing pancreatitis, Pancreatic acini, Acute intoxication, *Karwinskia humboldtiana*

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Introduction

Karwinskia humboldtiana (Kh), commonly known as buckthorn, is a poisonous plant of the *Rhamnaceae* family that grows in the southern United States, the entire Mexican territory, and in some regions of Central and South America (Fernandez-Nava, 1992). Kh fruit is accidentally consumed by animals and humans, particularly children (Padron-Puyou, 1951).

Copious intake of Kh fruit results in an acute intoxication characterized by severe respiratory failure culminating in death within 3 to 5 days (Bustamante-Sarabia et al., 1978; Puertolas et al., 1984; Bermudez-de Rocha et al., 1995). Low intake results in chronic intoxication leading to a paralysis similar to that of Guillain-Barre (Padron-Puyou, 1951; Lopez-Clares et al., 1960; Garcia-Ramos and Cacho-Diaz, 2005).

The seeds of Kh fruit contain several anthracenones that are responsible for the symptoms associated with the intoxication (Dreyer et al., 1975; Bermudez et al., 1986; Waksman et al., 1989; Pineyro and Waskman, 2000).

As paralysis is the most striking sign of Kh

intoxication, most experimental studies with Kh fruit focus on the peripheral nerves, in which segmental demyelination and axonal degeneration are hallmarks of Kh-induced histological damage (Escobar-Izquierdo and Nieto, 1965; Charlton and Pierce, 1970; Salazar-Leal et al., 2006). In many species, damage to other organs has also been reported, including hyperchromasia, chromatolysis, gliosis, pycnosis and the loss of Purkinje cells in the central nervous system (CNS) (Charlton et al., 1970; Ortiz et al., 1992; Becerra-Verdin et al., 2009); hemorrhage and inflammatory infiltrate in the lungs; liver steatosis; swelling of the convoluted tubules and glomerular retraction in the kidneys; and necrosis in all affected organs (Marsh et al., 1928; Bustamante-Sarabia et al., 1978; Weller et al., 1980; Bermudez et al., 1992). Studies report renal and ATP-related metabolic alterations in the renal cortex, blood and hemoglobin (Jaramillo-Juarez et al., 1995, 2005). Furthermore, weight loss in experimental animals is evident (Bermudez et al., 1992; Jaramillo-Juarez et al., 2005; Salazar-Leal et al., 2006; Garcia-Juarez et al., 2012). However, microscopic damage to the pancreas has not been reported, although vascular congestion has been described after gross examination of the pancreas in goats and cattle (Marsh et al., 1928).

The aim of this study was to analyze pancreatic histology during acute intoxication with Kh fruit; the results may improve the understanding of the severity of acute Kh intoxication in humans.

Materials and methods

Fruit preparation

Ripe Kh fruit was collected from Hidalgo, Nuevo Leon, in July and August. The fruit was air-dried and light-protected, as previously described (Bermudez et al., 1986), ground and sifted through a sieve No. 50 mesh (U.S. Standard Sieve Series. Dual Manufacturing Co). An aqueous suspension was prepared from the ground fruit for administration to the animals.

Animals and Kh dosage

Animals were obtained from the biotherium of the Monterrey Institute of Technology and Higher Education (ITESM). Experiments were performed on eighteen female Wistar rats weighing 250±25 g. The animals were maintained on a 12-hour light-dark cycle, with free access to food (LabDiet #5P14 - Prolab RMH 2500) and water. The weights of all the animals were recorded daily, at the same hour, before sacrifice.

All experiments were conducted in accordance with the Mexican Official Norm NOM-062-ZOO-1999 (De Aluja, 2002) and the Guide for Care and Use of Laboratory Animals, and they were approved by the Ethical Committee of our University (registration number HT14-004).

Acute intoxication of rats was induced by oral administration of the aqueous Kh suspension with an esophageal tube (5 g/Kg of body weight in 5 mL of water), as previously described (Bermudez et al., 1992).

Experimental groups

Eighteen Wistar rats were distributed into six groups (n=3 each). Five experimental groups were intoxicated with Kh fruit with the established dosage. One control group (n=3) were administered water only. Experimental groups were sacrificed at 24, 48, 72, 96 and 120 h after Kh administration respectively. The rats from the control group were sacrificed at 120 h.

Sacrifice and tissue preparation

Fasting was avoided before sacrifice in all groups. The animals were euthanized under anesthesia with intramuscular Xylacine (10 mg/Kg) followed by intraperitoneal injection of sodium pentobarbital (88 mg/Kg).

The macroscopic appearance of the pancreas was evaluated *in situ* following abdominal dissection. The pancreases were removed *en bloc* from the duodenum to the spleen. Pancreatic preparation was as follows: the pancreases were briefly washed in phosphate buffer, and the head was dissected and immediately fixed by immersion in 4% formaldehyde (pH 7,3). Tissue samples were processed (Excelsior™ AS Tissue Processor) using gradual concentrations of isopropyl ethanol and paraffin (Histoplast IM Thermo-Scientific # 8331) and then fixed in paraffin blocks (HistoStar™ Embedding processor). Tissue sections (5 μm thick) were obtained with the HM 355S Automatic Microtome and fixed to slides, after which they were deparaffinized and gradually re-hydrated. The slides were stained with hematoxylin and eosin (H-E) and then gradually dehydrated, clarified and mounted with Entellan for observation via light microscopy.

Morphological analysis

For macroscopic evaluation, the color, aspect, vascularity, and hemorrhagic state of the pancreases were evaluated *in situ* and in photographs obtained after abdominal dissection.

For histological evaluation, H-E stained slides were evaluated with the Nikon Eclipse 50i light microscope in 6 random fields at 40x, according to previous reports of pancreatic damage in experimental models (Spormann et al., 1989; Nakamichi et al., 2005; Laukkarinen et al., 2007; Ning et al., 2013). The severity of pancreatic damage was evaluated based on a scoring system for edema, inflammatory cell infiltrate, hemorrhage and necrosis, as shown in Table 1. Slides were examined under triple-blind conditions by three morphologists. Score charts from the three examiners were processed in Microsoft Excel 2010 for the means and standard deviations. The severity of pancreatic damage was

established by the sum of all scores obtained from each individual parameter, where the highest damage score is 20, as previously reported (Ning et al., 2013).

Immunohistochemistry assay

Tissue sections (5 µm thick) were incubated for 5 minutes with 10 mg/mL sodium borohydride (452882, Sigma-Aldrich) to suppress auto-fluorescence. Next, the slides were rinsed twice in PBS (pH 7.37) and incubated in a blocking solution (0.1% Triton X-100 and 5% lactose-free non-fat milk powder (Nestle total digest)) for 1 hour. Following blocking, the slides were rinsed 4 times in PBS (5 min/rinse). Manual immunostaining was performed with the primary antibody Anti-active + pro Caspase 3 (2.5 µg/mL, rabbit polyclonal #cat ab13847, Abcam) and the secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (1:500, goat polyclonal #cat ab150081, Abcam). The slides were mounted with DAPI (Vectashield® mounting medium H-1400, Vector) and observed on a Carl Zeiss LSM 710 confocal microscope.

Statistical analysis

The data were first subjected to a normal distribution comparison with the Kolmogorov-Smirnov test, then analyzed by Student's t-test for independent samples with a normal distribution. For the pancreatic histological damage score, the differences between groups were calculated by the analysis of variance (one-way ANOVA) and a Tamhane's post hoc test. All data were processed using IBM SPSS version 20 (SPSS, Inc., Armon, NY). Significance was assigned to p-values <0.007 for the pancreatic histological damage score, and <0.05 for the animals' weight loss.

Results

Acute intoxication with Kh drastically decreases rat body weight

To determine the effect of acute intoxication with Kh fruit on the pancreas, rats were treated as previously described. During treatment, the body weight of all of the rats was monitored daily.

During treatment, the average weight of the control group was maintained between 226.7±2.9 and 230.7±9.0 g. At 48 h, the mean weight of the control group was 229.7±10.8 g, and the Kh group was 222.8±13.5 g, resulting in a 3.0% difference between the groups. At 120 h after administration of Kh, the mean weight of the control group was 230.7±9.0 g, and the mean weight of the Kh group was 200.3±8.1 g, resulting in a statistically significant weight loss of 12.1% ($p<0.05$) (Fig. 1).

Acute intoxication with Kh induces progressive pancreatic disruption

We wanted to determine whether acute intoxication with Kh has an effect on the rat pancreas. After Kh intoxication, the pancreases were collected and evaluated for color, aspect, vascularity and hemorrhagic state. The pancreases of the control rats showed a light pink color, a homogeneous appearance, soft consistency, and normal vascularity with no evidence of hemorrhage. After 24 h, the pancreases of rats exposed to Kh were a slightly purplish color with minor edema, a soft consistency and slight vascular congestion. After 48 h, the pancreases of rats exposed to Kh were reddish and slightly edematous, with a soft consistency and non-detectable blood vessels, despite being slightly hemorrhagic. The pancreases of rats treated for 72 h were a reddish-purple color and edematous, with a soft consistency, and a high level of congestion. At 96 and 120 h, the pancreases of Kh treated rats were a reddish color and very edematous with a mucinous consistency and were hemorrhagic. These results indicate that acute

Table 1. Score rubric for the severity of pancreatic damage.

Edema	Score	Inflammatory cell infiltrate	Score	Hemorrhage	Score	Necrosis	Score
Absent	0	Absent	0	Absent	0	Absent	0
Mild	1	Mild	1	<2 foci	3	<5%	3
Moderate	2	Moderate	2	3 to 5 foci	5	5 to 20%	5
Severe	3	Severe	3	>5 foci	7	>20%	7

Taken from Spormann et al., 1989; Nakamichi et al., 2005; Laukkarinen et al., 2007 and Ning et al., 2013.

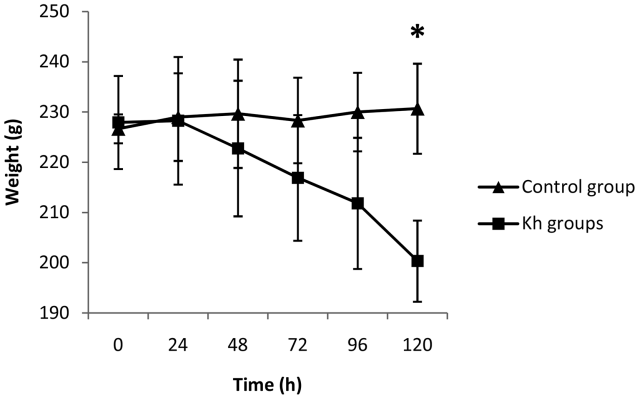

Fig. 1. Weight loss in rats after acute intoxication with *Karwinskia humboldtiana*. Female rats were orally administered an aqueous suspension of Kh fruit (5 g/Kg of body weight in 5 mL of water). Body weight was monitored after 24 (n=15), 48 (n=12), 72 (n=9), 96 (n=6) and 120 h (n=3). Control group (n=3) was monitored daily, and sacrificed at 120 h. Significant weight loss of 12.1% was recorded at 120 h, Student's t-test, *($p<0.05$).

intoxication with Kh produces time-dependent pancreatic damage.

Histological damage of the exocrine pancreas is progressive and culminates in necrosis, and the histology of the islets of Langerhans is preserved

Morphological analysis was performed to determine the extent of the pancreatic damage. The pancreases of the rats in the control group (Figs. 2, 3) showed normal morphology; the exocrine portions had pancreatic acini with pyramidal shaped acinar cells containing round, basally located basophilic nuclei and apical cytoplasm occupied by acidophilic granules, corresponding to zymogen granules. The endocrine portion had islets of Langerhans with polyhedral cells with clear cytoplasm and nuclei that were centrally located, round, and slightly basophilic.

The pancreases of the rats that were treated with Kh for 24 h showed an acinar architecture similar to the appearance of the control group; however, an overall reduction in the size of the acinar cells and a decrease in the number of zymogen granules was observed (Figs. 2, 3). Additionally, small autophagy-like vesicles began to appear in the cytoplasm of some acinar cells, and this effect was more evident in groups with extended treatment times.

The pancreases of the rats treated with Kh for 48 h showed disruption of the pancreatic acinar architecture. Acinar cells had a reduction in both the overall size and the nuclear size, they displayed chromatin fragmentation, and they had heterogeneous cytoplasm containing autophagy-like vesicles of different sizes, many of which contained intracytoplasmic bodies with basophilic staining, resembling apoptotic bodies (Figs. 2, 3). Positive immunofluorescence assay with an anti-activated-caspase-3 antibody confirmed that these intracytoplasmic bodies corresponded to apoptosis in the group treated for 48 h (Fig. 4), with a strong signal located in the cytoplasm of acinar cells. In contrast, the control rats were negative.

Pancreases of rats treated with Kh for 72 h showed extensive areas of necrosis with several apoptotic acinar cells and autophagy-like vesicles in the cytoplasm. The pancreases were edematous with macrophages, neutrophils, and lymphocytes in interlobular septa, and a notable destruction of lobular architecture was observed (Figs. 2, 3).

The pancreases of rats treated with Kh for 96 h had large areas where the lobular architecture was

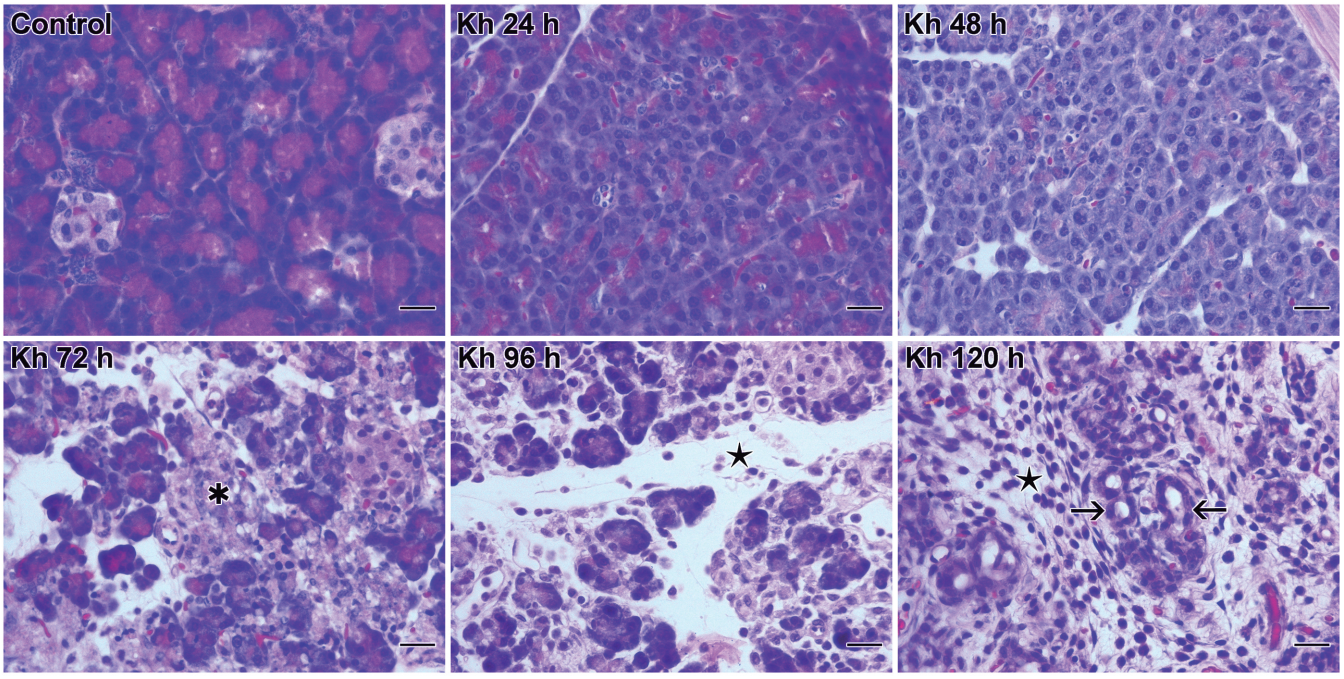

Fig. 2. Progressive pancreatic disruption in response to acute intoxication with *Karwinskia humboldtiana*. Rat pancreatic tissue was analyzed at different times following Kh fruit administration. Micrographs from the control and experimental groups are depicted as follows. The control group displays normal pancreatic histology. At 24 h after Kh administration, the overall acinar structure is conserved. At 48 h post Kh administration, fewer acinar structures are observed. At 72 h after Kh administration, the lobular architecture is disorganized, interstitial edema in the interlobular septa is observed, and necrosis is evident (*). At 96 h after Kh administration, loss of the lobular architecture and interstitial edema (star) with inflammatory infiltrate are evident. At 120 h after Kh administration, the pancreases show inflammatory infiltrate (star) and a complete loss of acini, with only a few pancreatic ducts visible (arrows). Scale bar: 20 µm.

disorganized, with only a few conserved acini. The pancreatic tissue was very edematous, and mononuclear cell infiltrate was present in the interstitium. The duct system showed an apparently well-preserved epithelium (Figs. 2, 3).

Finally, rats intoxicated with Kh for 120 h showed a complete loss of lobular architecture, and the interstitium had marked edema accompanied by numerous inflammatory cells (Figs. 2, 3).

Interestingly, throughout our evaluation time-points, the islets of Langerhans displayed a normal appearance in all experimental groups, similar to the control group. Mild hyperchromasia of the nuclei and increasingly eosinophilic cytoplasm were noted from 72 h onwards, but no signs of apoptosis or necrosis appeared in any of the experimental groups in this study (Fig. 5).

In addition to macroscopic observations, we quantitatively scored the pancreatic histological damage in all groups, as mentioned above (Table 2, Fig. 6). The control group score was 0.00±0.00 for all evaluated

Table 2. Pancreatic damage score per group (mean and standard deviation).

Parameters evaluated	Control group	Kh treated groups (time after Kh administration)				
	Mean ± SD	24 h Mean ± SD	48 h Mean ± SD	72 h Mean ± SD	96 h Mean ± SD	120 h Mean ± SD
Edema	0.00±0.00	0.35±0.39	1.04±0.61	2.35±0.39	2.69±0.61	2.81±0.26
Inflammatory cell infiltrate	0.00±0.00	0.52±0.34	1.52±0.77	2.67±0.50	3.00±0.00	3.00±0.00
Hemorrhage	0.00±0.00	1.39±0.86	3.35±1.13	3.09±1.56	4.63±2.34	5.26±1.43
Necrosis	0.06±0.17	0.70±0.25	1.22±0.76	5.31±0.84	6.74±0.57	6.35±1.20
Total score	0.06±0.17	2.96±1.53*	7.13±2.42*	13.43±1.05*	17.06±2.63*	17.43±2.23*

Evaluation of edema, inflammatory infiltrate, hemorrhage and necrosis was performed in 6 random fields (40x) for each rat (n=3 rats per group). The severity of pancreatic damage was established by the sum of all scores obtained from each individual parameter, where the highest damage score is 20. Significant difference was tested by one-way ANOVA and a Tamhane's post hoc test, *(p<0.007).

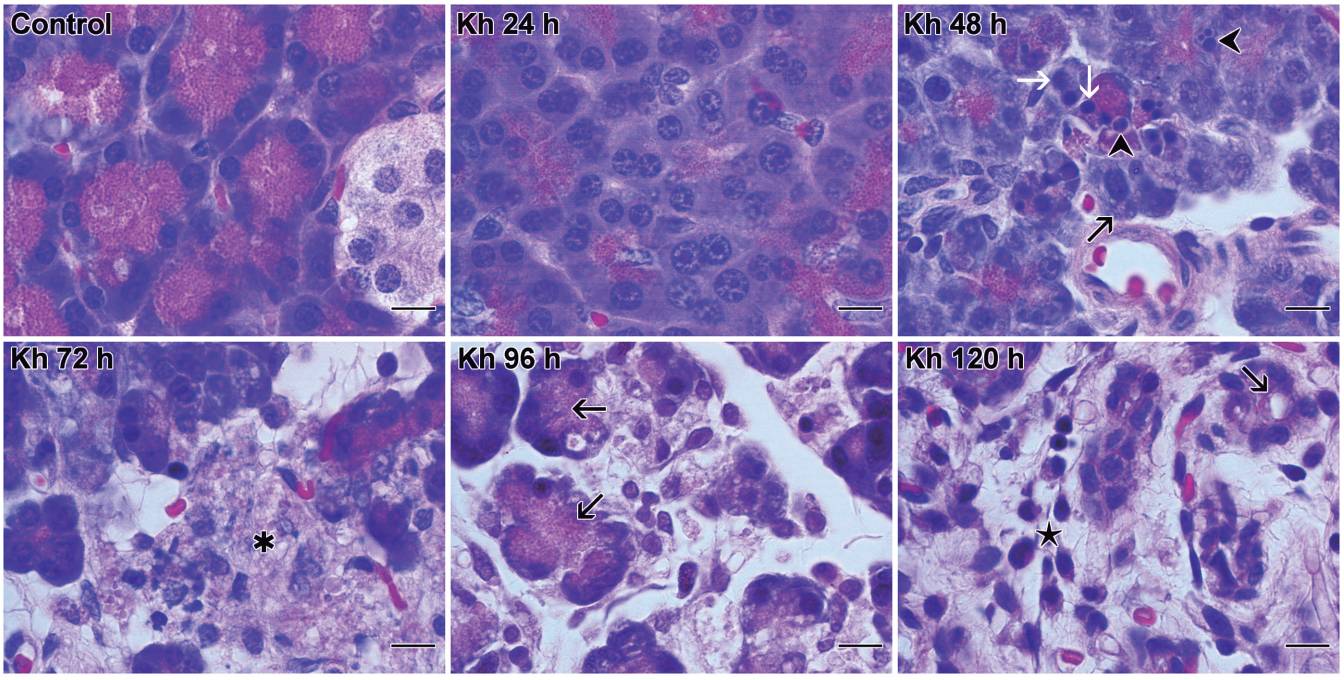

Fig. 3. Detailed progression of pancreatic disruption in response to acute intoxication with *Karwinskia humboldtiana*. The control group shows normal pancreatic histology. Kh administration: an overall reduction of acinar cell size and the amount of zymogen granules was observed at 24 h. Chromatin condensation (white arrows), vesicles suggestive of apoptotic bodies (arrowheads), and the presence of vesicular cytoplasm (black arrow) were evident at 48 h. At 72 h, interstitial edema in the interlobular septa and necrotic areas (*) were visualized. A loss of lobular architecture and interstitial edema with inflammatory infiltrate were observed at 96 h, when there was a notable paucity of structures that resemble acini (arrows). At 120 h, the pancreatic tissue was characterized (star) by a complete loss of acini, edema and abundant inflammatory infiltrate (neutrophils, leucocytes and macrophages) with a few well-preserved ducts (arrow). Scale bar: 10 µm.

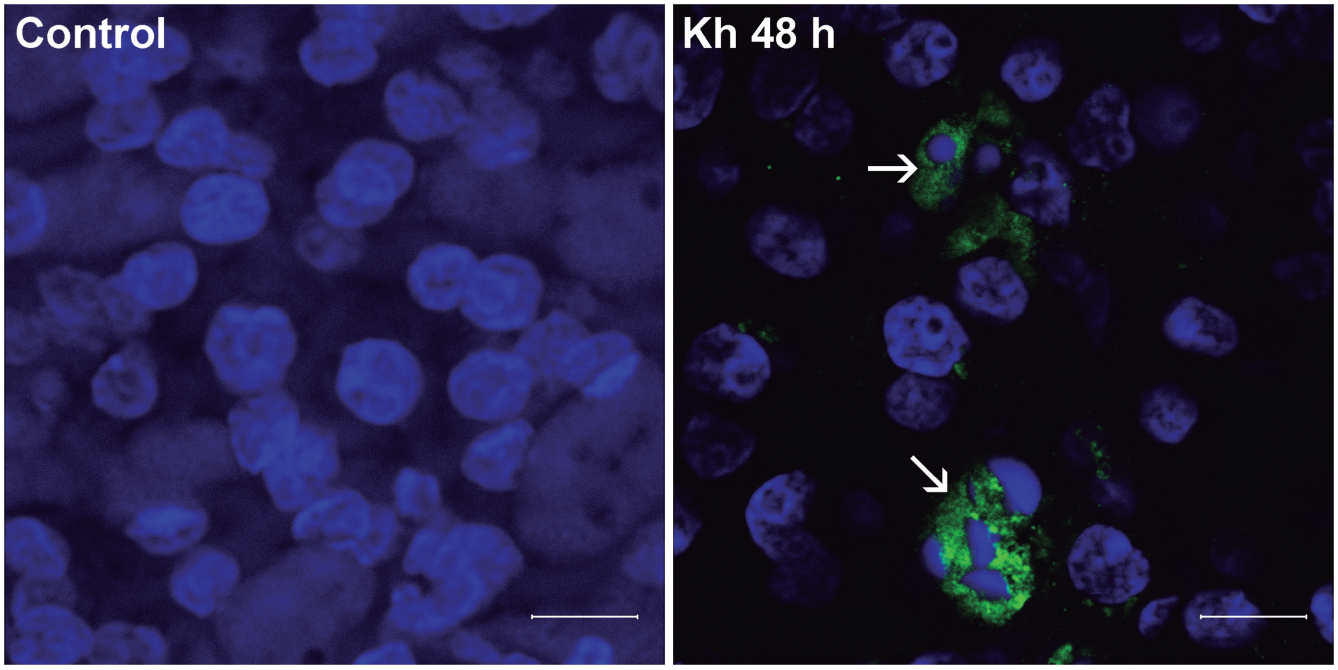

Fig. 4. Imunocytochemistry assay to determine caspase-3 pathway involvement in apoptotic cell death induced by *Karwinskia humboldtiana* in the Wistar rat. Pancreases of rats from the control group had nuclei with a normal appearance upon DAPI staining, and the cytoplasm was negative for caspase-3. Pancreases of rats intoxicated with Kh for 48 h had a positive caspase-3 signal in the cytoplasm (arrows). The nuclei were condensed and fragmented. Confocal microscopy. Scale bar: 10 µm.

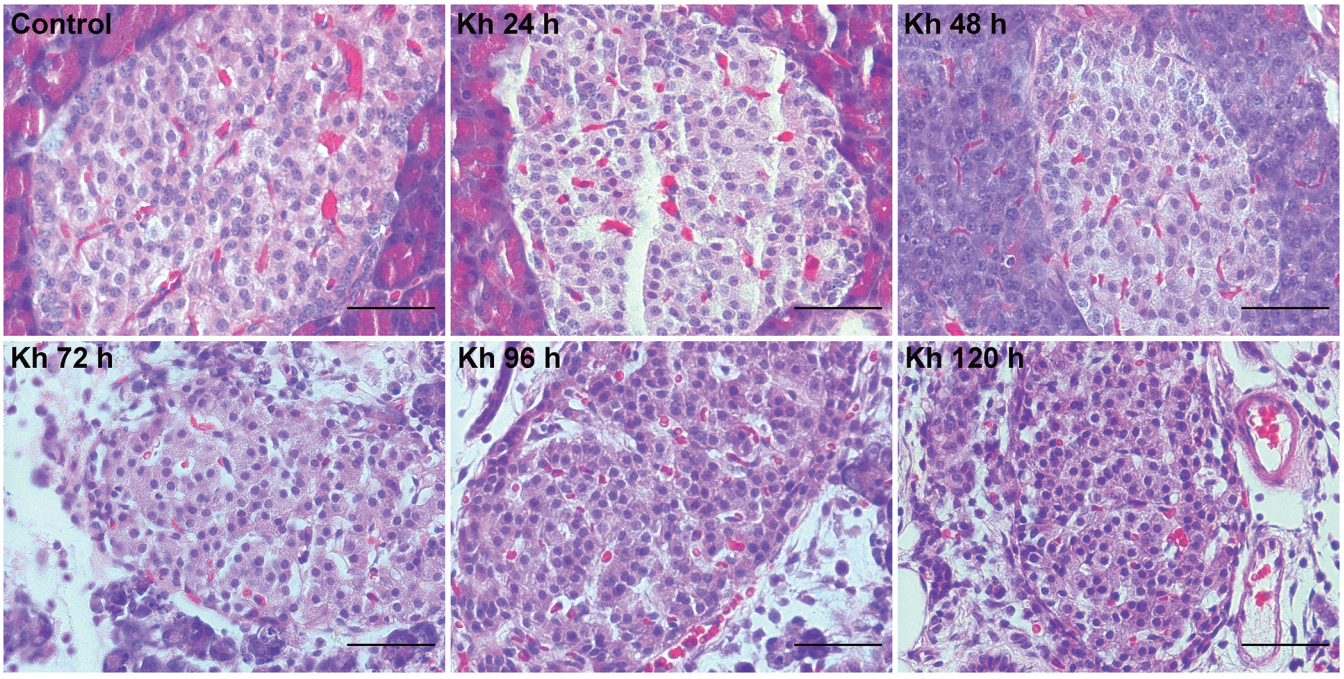

Fig. 5. The morphological integrity of the islets of Langerhans is well preserved in response to acute intoxication with *Karwinskia humboldtiana.* During the first 48 h of Kh treatment, the islets of Langerhans displayed a well-preserved morphological integrity. At 72 h, they appeared normal, but heterochromatic nuclei with prominent nucleoli were observed. From 96 h onwards, the islets of Langerhans had more acidophilic cytoplasm. No signs of apoptosis or necrosis were observed at any of the times examined in this study. H-E. Scale bar: 50 µm.

parameters, except for a score of 0.06±0.17 for necrosis. For Kh treated groups: for edema, the score increased from 0.35±0.39 at 24 h to 2.81±0.26 at 120 h; for inflammatory cell infiltrate, the score increased from 0.52±0.34 at 24 h to 3.00±0.00 at 120 h; for hemorrhage, the score increased from 1.39±0.86 at 24 h to 5.26±1.43 at 120 h; and for necrosis, the score increased from 0.70±0.25 at 24 h to 6.35±1.20 at 120 h. These results indicate that progressive damage was evident after Kh intoxication, with a remarkable presence of necrosis in pancreatic acini at 72 h (score 5.31±0.84) compared with the control group (score 0.06±0.17). Pancreatic damage increased in a time-dependent manner. The highest damage score was reached at 120 h after Kh intoxication (Total score 17.43±2.23 for the treatment group compared with 0.06±0.17 for the control rats). Notably, all characteristics observed are compatible with a diagnosis of acute necrotizing pancreatitis.

Discussion

It has been known for decades that accidental and experimental Kh poisoning in both humans and animals produces signs and symptoms that can lead to rapid death with respiratory distress (Puertolas et al., 1984; Bermudez-de Rocha et al., 1995). Moreover, there is evidence of histopathological damage to several organs, including the lungs, liver, kidneys, and nervous system, which might yield a multi-organ dysfunction syndrome (Weller et al., 1980; Bermudez et al., 1992). However, the connection between these systems, or how the severity of this intoxication ensues, has not been fully elucidated. As the pancreas is an organ that has not been evaluated in Kh intoxication, we examined whether there was any evidence of histological damage to help us understand if the pancreatic damage could be related to the multiple organ failure observed with Kh intoxication. Herein, we evaluated the histological damage to the rat pancreas in response to Kh intoxication. Weight loss was observed as rapidly as 48 h after Kh administration, and it was statistically significant at 120 h after treatment. These results are consistent with the weight loss observed in other reports, where whole fruit or isolated toxins were administered to experimental animals (Bermudez et al., 1986; Salazar-Leal et al., 2006; Garcia-Juarez et al., 2012). Additionally, weight loss has been observed in humans intoxicated with Kh fruit (Padron-Puyou, 1951; Bustamante-Sarabia et al., 1978).

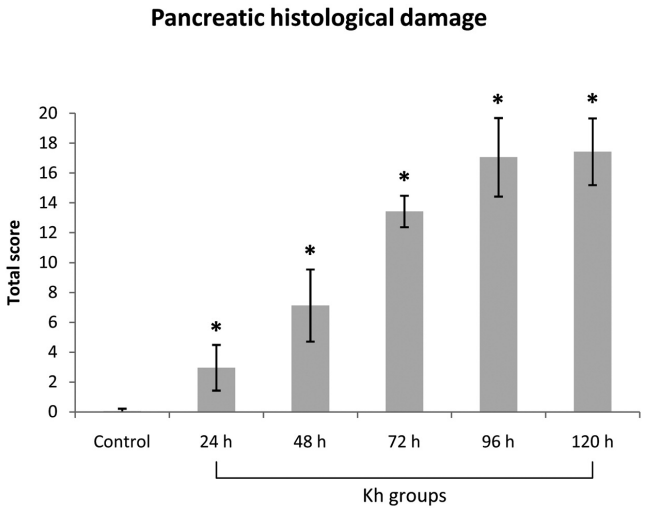

Fig. 6. Pancreatic histological damage score in response to acute intoxication with *Karwinskia humboldtiana*. Each bar represents the total score with its standard deviation. A one-way ANOVA and a Tamhane's post hoc test, revealed significant difference between Kh and control groups, *($p<0.007$). There was no difference between Kh groups of 96 and 120 h.

Progressive pancreatic damage in our model appeared 24 h after Kh administration. Histologically observed alterations included a reduction of acinar cell size and zymogen granules, as well as the presence of small cytoplasmic vesicles, apoptotic bodies, necrosis, edema, and inflammatory cell infiltrate. At the time of the final evaluation, the pancreases showed a complete loss of lobular architecture. We concluded that caspase-dependent apoptosis and necrosis have a significant role in the pancreatic damage induced by acute Kh intoxication. No evidence of ductal and/or vascular changes was found in any treatment group. Interestingly, the islets of Langerhans were well preserved throughout our evaluations, with no signs of damage.

Our findings are consistent with those described in several rodent models of acute pancreatitis (Ziegler et al., 2011; Cao and Liu, 2013; Zhang and Rouse, 2013, 2014; Usborne et al., 2014). For instance, the cholecystokinin analog caerulean induces a severe, acute edematous and necrotizing pancreatitis; the cellular alterations were dose- and time-dependent, and they included autophagic changes, apoptosis and necrosis that progressively damaged the pancreatic tissue, most notably in areas of mast cell degranulation. Further observations included the presence of numerous eosinophils in the interstitium, epithelial cell proliferation of the ducts, and periductal fibrosis (Zhang and Rouse, 2014). An additional study with caerulean noted progressive pancreatic damage that included edema, inflammation and adipocyte necrosis, a decrease in zymogen granules, apoptosis and pancreatic atrophy (Usborne et al., 2014). Retrograde infusion of Na-taurocholate through the pancreatic duct induced severe pancreatic damage (edema, inflammation and necrosis) in a concentration-, volume- and time-dependent manner, confined to the head of the pancreas. Moreover, damage in the acinar cells occurred 10 minutes after infusion, was multifocal rather than confluent, and persisted and expanded throughout the lobule (Laukkarinen et al., 2007). In a ductal ligation rat model, the pathological features observed were characteristic of acute necrotizing and/or hemorrhagic pancreatitis, which increased in a time-dependent manner (Zhang and

Rouse, 2014). Arginine intraperitoneal injection induced a mildly edematous acute pancreatitis in a time-dependent manner, with features of autophagy, apoptosis, and necrosis related to edema, inflammation and fibrosis. At late stages, these changes were accompanied by acinar cell atrophy, ductal changes (dilatation, fibrosis and inflammation) and hyperplasia of the centroacinar cells (Zhang and Rouse, 2014). Cyanohydroxybutene produced pancreatic damage in a dose- and time-dependent manner, with interstitial edema, inflammation, vacuolization, and cellular degeneration and/or necrosis (Usborne et al., 2014).

Interestingly, in our study autophagy-like vesicles appeared 24 h post Kh administration (concurrent with acinar cell shrinking and the reduction of zymogen granules) and remained throughout all times of evaluation. These observations suggest that autophagy, which is a well-known cell survival mechanism, could be the first visible sign of cellular defense against Kh intoxication, even before apoptosis.

The death of pancreatic acinar cells is a characteristic response in pancreatitis (Kaiser et al., 1996; Gukovskaya and Gukovsky, 2012). Some reports suggest that apoptosis may be a defense mechanism by which damaged acinar cells are removed, preventing the inflammation and cell necrosis that would occur if their contents leaked into the extracellular space (Logsdon and Ji, 2013). In our study, there was a rapid progression from the appearance of apoptotic bodies to extensive necrosis and edema. It has been reported that high intracellular concentrations of active trypsin induce acinar cell death via apoptosis (Ji et al., 2009), but massive or chronic overproduction of active trypsin initiates apoptosis on a large scale, resulting in necrosis (Dawra et al., 2011; Gaiser et al., 2011). Although the mechanism of Kh damage has not been elucidated, the pathological changes that occur in acinar cells rapidly overwhelm their homeostatic capacity. Pancreatic damage arises when compensatory mechanisms are overwhelmed by exposure to increasing physiological stress, including a high-fat diet and alcohol, which ultimately leads to pancreatic injury (Logsdon and Ji, 2013). Copious ingestion of Kh fruit could exceed the capacity of autophagy and apoptosis to provide pancreatic protection, with rapid deterioration towards edema and necrosis. Importantly, one of the main prognostic indicators in acute pancreatitis patients is the amount of necrosis; higher amounts of necrosis correlate with a worse prognosis. When 50% of the pancreas is necrotic, the mortality rate may reach 20%. Similarly, in experimental models, the severity of pancreatitis is directly correlated to the extent of necrosis and inversely proportional to apoptosis (Kaiser et al., 1995; Saluja et al., 1996; Bhatia, 2004; Mareninova et al., 2006).

In contrast to other studies, we observed no changes in the ducts, vascular system, and islets of Langerhans at our evaluation times.

The inflammatory cell infiltrate included neutrophils, macrophages, lymphocytes, and scattered mast cells in a non-degranulating state. No eosinophils were observed, contrasting with previous studies in which eosinophils were predominant (Zhang and Rouse, 2014).

The "systemic" determinants need to be considered when evaluating the severity of acute pancreatitis. Although pancreatic necrosis and organ failure can coexist, they are not necessarily simultaneous (Sanchez-Lozada et al., 2005b). Intra- and extrapancreatic inflammation is generally accompanied by a Systemic Inflammatory Response Syndrome (SIRS) (Schepers et al., 2013), with approximately 30% of patients developing SIRS within 48 h of hospital admission (Modifi et al., 2006). Excessive SIRS leads to Multiple Organ Dysfunction Syndrome (MODS), the leading cause of morbidity and mortality in acute pancreatitis (Bhatia et al., 2005; Gaisano and Gorelick, 2009; Gukovskaya and Gukovsky, 2012; Kylänpää et al., 2012), with respiratory failure being one of the most important causes of death (Sanchez-Lozada et al., 2005a; Pandol et al., 2007; Akbarshahi et al., 2012).

Acute intoxication with Kh fruit has commonly been associated with renal and liver failure and Acute Respiratory Distress Syndrome (ARDS). Because pancreatic damage occurs within hours of Kh fruit administration, the pancreas could be the first target of damage, prior to SIRS being triggered. Death in accidental and experimental Kh intoxication could then result from SIRS causing the deterioration of other organs, including the lungs, liver, and kidney. Integrative studies on organs in the same individual are required to understand how Kh compounds interact at the clinical-histological-immune-physiological level and to further explain the rapid progression of patients intoxicated with Kh fruit.

In summary, our results indicate that the damage induced by a high dose of Kh fruit in the Wistar rat is compatible with an early acute necrotizing pancreatitis confined to the exocrine pancreas. This system could be used as an animal model for the study of pancreatic disease treatments. More importantly, as the islets of Langerhans were histologically preserved, the active compounds of the *Karwinskia humboldtiana*'s fruit might help to treat acinar pancreatic cancer. Further studies might shed light on the severity of acute Kh intoxication in humans, as well as aid the design of treatments for pancreatic diseases and acinar pancreatic cancer.

Conclusion

The administration of a high dose of Kh fruit causes progressive destruction of pancreatic acinar structures in the Wistar rat, sparing the endocrine pancreas and the islets of Langerhans. The histological damage is compatible with necrotic acute pancreatitis.

The pancreas may be a primary target organ in Kh fruit poisoning, contributing to the development of SIRS that ultimately leads to MODS in the Wistar rat.

Acute intoxication with Kh fruit in the Wistar rat could be a novel experimental model to study the early events occurring in acinar cells after toxic disturbances and to explore how the islets of Langerhans are conserved. Ultimately, this system might be used as an animal model to study the treatment of human pancreatic diseases.

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