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Duodenal wall injury with destruction of Brunner's glands in experimental acute poisoning with *Karwinskia humboldtiana* fruit

Nallely Esparza-Rodríguez[1], Juan Carlos Segoviano-Ramírez[2], Emilio Hasbun-Pacheco[3], Juan Pablo Martínez-Silva[4], Katya Carcaño-Díaz[5], Jaime García-Juárez[3]

[1] *Instituto Mexicano del Seguro Social (IMSS), Hospital General de Zona No. 50, Servicio de Medicina Interna, San Luis Potosí, C.P. 78397, México.*
[2] *Universidad Autónoma de Nuevo León (UANL), Facultad de Medicina, Departamento de Patología, Madero y Dr. Aguirre Pequeño, Mitras Centro, C.P. 64460, México.*
[3] *Universidad Autónoma de Nuevo León (UANL), Facultad de Medicina, Departamento de Histología, Madero y Dr. Aguirre Pequeño, Mitras Centro, C.P. 64460, México.*
[4] *Instituto Mexicano del Seguro Social (IMSS), Unidad Médica de Alta Especialidad No. 71, Servicio de Medicina Interna, Torreón, Coah., C.P. 27000, México.*
[5] *Universidad Marista de Mérida Yucatán, Laboratorio de Fisiología, Progreso, C.P. 97300, México*
***Corresponding Author**: Jaime García-Juárez, Universidad Autónoma de Nuevo León (UANL), Facultad de Medicina, Departamento de Histología, Madero y Dr. Aguirre Pequeño, Mitras Centro, C.P. 64460, México.*

Abstract
Aims: During acute experimental intoxication with Karwinskia humboldtiana (Kh) fruit, an acute necrotic pancreatitis that destroys the exocrine portion of the pancreas is produced. This inflammatory process does not injure the islets of Langerhans, and is accompanied by weight loss. Although weight loss has been attributed to liver injury, until before this work, no histological alterations had been described in the wall of the small intestine. Our objective was to evaluate the histopathological characteristics of the duodenal wall during acute intoxication with Kh fruit by using a semiquantitative tissue injury scale to correlate it with the weight loss.
Methods: To evaluate the microscopical morphology of the intestinal wall, the parameters of Erben´s scale for murine models were used. The following parameters and scores were taken into account: inflammatory infiltrate, extent of infiltrate, epithelial hyperplasia, decrease in goblet cells per crypt, presence of neutrophils, epithelial erosion and villous blunting.
Results: Experimental intoxication with Kh progressively damages the duodenum wall with apoptotic evidence injures on the Brunner´s submucosal glands.
Conclusion: Acute intoxication with Kh fruit shows a progressive toxic effect on the tissue components of the duodenum wall, which can more directly explain the weight loss reported on experimental model. The apoptotic lesions found on Brunner's submucosal glands occurs in a manner similar to that reported in the pancreas. Therefore, the toxic components of the fruit could be useful in the treatment of Hamartomas of the Brunner´s glands.

***Key words**: Karwinskia humboldtiana, Duodenal inflammation, Brunner's glands, Hamartoma, Apoptosis*

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I. Introduction

Karwinskia humboldtiana (*Kh*) produces a fruit that contains a highly toxic seed that affects organs such as the lung, liver and kidney (Bermúdez et al., 1992).

Likewise, during acute experimental intoxication with *Kh* fruit, an acute necrotic pancreatitis that destroys the exocrine portion of the pancreas is produced. This inflammatory process does not injure the islets of Langerhans, and is accompanied by weight loss (Bermúdez et al., 1986; Carcano et al., 2016; Segoviano et al., 2023).

Although weight loss has been attributed to liver injury (Jaramillo et al., 2009; García et al., 2016), until before this work, no histological alterations had been described in the wall of the small intestine, which can be correlated directly with weight loss.

During acute pancreatitis, damage to the intestinal barrier occurs leading to increased permeability and translocation of bacteria and toxins (Zhou et al., 2010).

Furthermore, the Brunner´s submucosal glands located in the duodenum, which secrete: mucins, bicarbonate, epidermal growth factor and bactericides that protect the duodenal mucosa, as well as other factors that promote pancreatic secretion (Krause, 2000) could be affected.

Therefore, the joint destruction of the exocrine pancreas and the duodenal submucosal glands would leave the organism deprived of its main source of bicarbonate (Beaume et al., 2018; Rana et al., 2019).

By other hand, it is important to remark that alterations in these submucosal glands of the duodenum had not previously been described in acute *Kh* poisoning.

Brunner's glands are classified as compound tubulo-alveolar glands, they are mucosecretory type (Krause, 1998). The cells that form the secretory units have a cylindrical or slightly pyramidal shape, acidophilic cytoplasm and abundant pale secretory granules; their nuclei are basal, basophilic and they have a prominent nucleolus. The glands are located between the irregular dense connective tissue of the duodenal submucosa (Jawad et al., 2019).

Although in previous studies reporting pancreatic damage, the death of the animals has been attributed to respiratory failure and lung injury (Bermudez et al., 1986; Bermudez et al., 1992). Acute pancreatitis could be complicated by metabolic acidosis, which may explain the apparent respiratory distress in experimental animals (Rumbus et al., 2018).

Therefore, our objective was to evaluate the histopathological characteristics of the duodenal wall during acute intoxication with *Kh* fruit by using a semiquantitative tissue injury scale and determine if there is histological evidence of injury in the Brunner´s submucosal glands to correlate it with respiratory manifestations and the weight loss.

II. Material And Methods

18 Wistar rats weighing 125 ± 25 g were used, 15 from they were treated with a single dose of 5 g/kg of dried *Kh* fruit, ground and sieved to 50x mesh, while the other 3 rats were used as healthy controls. The treated animals were sacrificed in groups of 3 rats every 24 hours after the administration of the fruit until completing 5 groups as follows: 24, 48, 72, 96 and 120 hours. The control group was sacrificed at the same time as the last experimental group. All procedures were carried out in accordance with NOM-062-ZOO-1999 and samples were obtained as part of the HT14-004 protocol that was approved by our Ethics Committee. The macroscopic appearance of the duodenum was photographically documented with an iPhone 3® cell phone camera.

Samples from the proximal segment of the duodenum were fixed in buffered formalin and processed until embedded in paraffin. Subsequently, histological sections 5 microns thick were made and then stained with H&E.

To evaluate the microscopical morphology of the intestinal wall, the parameters of Erben´s scale for murine models were used (Erben et al., 2014). The following parameters and scores were taken into account: inflammatory infiltrate (1-4), extent of infiltrate (1-3), epithelial hyperplasia (1-5), decrease in goblet cells per crypt (1-4), presence of neutrophils (1-5), epithelial erosion (1-5) and villous blunting (1-5). The maximum score for this scale was 31 points.

5 fields per sample were evaluated with the 40x objective by triplicate, the score averages of each group with their standard deviation were obtained. To analyze statistical differences between the treated and control groups, the T-student test was applied.

All histopathological findings found in the intestinal villi, in the crypts of Lieberkhün and in the Brunner's glands of the duodenal submucosa were photodocumented with the 40x objective using a Carl Zeiss microscope with integrated HD camera.

III. Results

Macroscopic description of duodenum and pancreas

Macroscopically, the duodenum and pancreas of the rats in the control group, without treatment, showed a pale pink color with a homogeneous superficial appearance, soft consistency and normal vascularity, without evidence of hemorrhages or other alterations (Figure 1A). In the 24 h group, after *Kh* administration, both duodenum and pancreas presented a slightly violet color, with mild edema, soft consistency, and presence of vascular congestion (Figure 1B). After 48 h post administration of *Kh* fruit, the duodenum and pancreas presented a reddish color, with mild edema, soft consistency, and a hemorrhagic appearance (Figure 1C) in the peritoneal surface. At 72 h, the duodenum and pancreas presented a red-violet color, with edema, soft consistency and marked vascular congestion (Figure 1D). Finally, at 96 and 120 h, the duodenum and pancreas presented a red color, with marked edema, mucinous consistency and a frank hemorrhagic appearance (Figure 1E and F).

Microscopic description of the duodenal mucosa

In the duodenum of the control group, a normal histological appearance of the intestinal villi and Lieberkhün´s crypts were observed (Figure 2A and 3A). Also, normal-appearing enterocytes and goblet cells (arrows) were observed in the intestinal villi (Figure 2A). Similarly, in the Lieberkhün´s crypts (Figure 3A) goblet cells with normal aspect (*) and some mitoses (arrowhead) were observed.

In the lamina propria of the Lieberkhün´s crypts of the *Kh*-treated groups (Figure 3B-F), vascular congestion was observed, which was more evident at 120 hours after treatment with *Kh* fruit (Figure 3F). Although, 48 hours after the administration of *Kh* it was observed that the intestinal villi presented edema (*) located in the lamina propria, it was more notable from 72 hours (Figure 2C-F) and greater secretion of mucus was observed on the epithelial surface (arrowheads). Furthermore, an apparent decrease in goblet cells was observed at 24 and 48 hours after fruit administration (Figure 2B and C) followed by proliferation of these cells and increased mitotic activity after 72 hours and especially in the Lieberkhün´s crypts (Figure 3D-F). Also, the presence of some neutrophils was observed in the lamina propria.

Experimental intoxication with Kh injures the Brunner´s submucosal glands

In the unintoxicated, control group, it was observed that the Brunner's glands had their normal morphology. The secretory cells showed their tipical cylindrical shape, acidophilic cytoplasm with abundant pale secretory granules, basal and basophilic nucleus with a prominent nucleolus (Figure 4A).

In rats intoxicated with *Kh*, Brunner's glands were observed with progressive tissue damage, which consisted of: retraction of some secretory cells at 24 h after administration of the fruit (Figure 4B) with apparent reduction of the adenomere lumen in the 48 h group (Figure 4C). Subsequently, the presence of apoptotic bodies, necrosis and inflammatory infiltrate in the surrounding submucosa was observed at 72 h (Figure 4D). In the 96 h group, inflammatory cells were observed within the lumen of the secretory ducts (Figure 4E). Finally, at 120 h, only the ducts surrounded by inflammatory cells could be observed in the absence of recognizable adenomeres (Figure 4F).

Experimental intoxication with Kh progressively damages the duodenum wall

The averages obtained using the histopathological assessment scale of the duodenal wall were: 0.9 ± 0.4 for the control group; while for the treated rats groups they were 6.9 ± 0.7 at 24 hours, 9.6 ± 0.2 at 48 hours, 9.4 ± 1.1 at 72 hours, 10.8 ± 0.2 at 96 hours and 14.2 ± 1.4 at 120 hours after *Kh* fruit administration. These averages were plotted together with their standard deviation (Figure 5). The statistical comparison demonstrated a significant difference starting 24 hours after treatment with *Kh* compared to the control group ($p<0.010$). The score values obtained for each parameter can be seen in Table I.

It is important to note that in the experimental groups that received *Kh* fruit, we found that the inflammatory infiltrate extended from the lamina propria to the submucosal layer, over all in the latter groups, especially after the appearance of apoptotic bodies on the Brunner's glands (shown in Figure 4D).

In the category of cryptitis (presence of neutrophil in the lamina propria and submucosa), we found an increase of this parameter in the groups 72, 96 and 120 hours after the administration of *Kh* fruit. Although these cells were not indicated in the figures, they were counted as part of the evaluation parameters.

In the epithelium of the Lieberkün´s crypts, mitosis was observed at 24 hours after the administration of *Kh* fruit, but they were more noticeable in the *Kh* groups from 48 to 120 hours (as seen in Figure 3C -F).

Likewise, we observed that counting of the goblet cells was reduced in the groups 24 and 48 hours after treatment with *Kh* fruit (Figure 3B-C), but the presence of these cells was increased afterwards (Figure 3D-F). Therefore, this parameter increased the result of the score only in the groups where fewer cells were found.

The edema present in the lamina propria of the intestinal villi slightly increased the damage score, but only in the groups 72, 96 and 120 hours after the administration of the *Kh* fruit.

IV. Discussion

In experimental models of poisoning with *Kh* fruit, weight loss is commonly reported and has been correlated with tissue damage in the liver (Jaramillo et al., 2009; García et al., 2016). However, using this acute intoxication model in Wistar rats, we found damage to the exocrine pancreas accompanied by progressive weight loss after the administration of a dose of 5g/kg of *Kh* fruit (Carcano et al., 2016).

Until today, none report mentioning histopathological alterations in duodenum had been published. After a bibliographic revision, only one report mentioning a macroscopic erythematous appearance of the pancreas, inflammation of the stomach and jejunum caused by intoxication with *Kh* fruit, in various animal species, was found (Marsh, 1928).

Some works, that was subsequently carried out using the *Kh* fruit and toxins extracted from it, were focused mainly on histopathological analysis of nervous tissue, liver, lung and kidney (Bermúdez et al., 1986; García et al., 2013; Gómez et al., 2005; Martínez et al., 1997). Although it has also been mentioned lesions on the large intestine (Jaramillo et al., 2020). However, previously to this study, evidence of tissue injury in the small intestine, as a consequence of experimental poisoning with *Kh* fruit, had not been reported.

The injury reported in the liver corresponds to centrilobular necrosis and vascular congestion (Bermúdez et al., 1992) and has also been evidenced to cause alterations in blood coagulation (Jaramillo et al, 2009).

It is possible that weighting loss may be correlated with liver damage, such as that resulting after the administration of phomopsin, a metabolite of the fungus *Phomopsis* (Allen et al., 2004). However, it can also be related to the consumption of medicinal plants; such is the case of *Garcinia cambogia* which besides has been related with development of acute pancreatitis (Iqbal, 2019).

In addition, there are plants that cause adverse gastrointestinal effects, such as *Abrus precatorius*, which is consumed in India as an aphrodisiac adjuvant; or *Colchicum autumnale* for therapeutic applications in acute gout, arthritis, familial Mediterranean fever and amyloidosis; or *Jatropha curcas*, which was used in traditional medicine for malaria and edema, as well as *Jatropha multifidi*, which in Africa was used for parasitic infestations, rheumatic conditions and as an abortifacient; or *Rhododendron simsii* used as an antitussive, just to mention a few whose gastrointestinal adverse effects range from vomiting, diarrhea, abdominal pain; to hematuria, decrease in body temperature, organ failure and death (Farzei, 2020).

In this work we evidenced histopathological changes in the duodenum wall. It is possible correlate these changes with weight loss in the animals of our experimental model; since the small intestine carries out multiple functions of the digestive tract, such as the absorption of HCO^{-3} ions and nutrients (Rønnestad et.al., 2014).

Furthermore, Brunner's tubular glands, which are located mainly in the proximal portion of the mucosa and submucosa of the duodenum, normally secrete mucins and provide 50 mmol per day of HCO^{-3}, protecting the duodenal mucosa from the acidic pH of the chyme (Gao et al., 2004; Beaume et al., 2018; Al Hariri et al., 2023).

Thus, the destruction of Brunner's glands found during this work, together with the destruction of the exocrine pancreas, also reported by our searching group (Carcano et al., 2016), significantly decreases the supply of HCO^{-3} which could lead to metabolic acidosis (Kraut and Madias, 2010).

Metabolic acidosis is one of the complications of acute pancreatitis and manifests itself, among other things, with a compensatory increase in respiratory rate (Zhou et al., 2010). Although this was attributed to lung damage in previous studies (Bermudez et al., 1986; Bermudez et al., 1992), metabolic acidosis may also contribute to the manifestations of respiratory distress in this experimental model.

On the other hand, besides that the toxin T-514 (Peroxisomicine A1) contained in the fruit of *Kh*, has been used as a probable antineoplastic agent (Piñeyro et al. 1994; Jaramillo et al., 2020); the damage we found on the Brunner's glands, in our model, indicates that this fruit could have utility for the treatment of hamartoma or hyperplasia of the Brunner's glands, since today there is no definitive treatment for this disease (Lee et al., 2008).

Although Brunner's gland hamartoma is rare and usually benign, in some cases it could develop malignancy and cause duodenal obstruction (Peloso et al., 2017). Therefore, it would be useful to have a therapeutic option derived from this fruit.

For all of the above, we can affirm that the apoptotic alterations observed in the Brunner´s glands, at 72 hours after the administration of *Kh* fruit, similar to those previously reported in pancreas (Carcano et al., 2016) reflect that these glands together with pancreas show common susceptibility to *Kh* fruit. For this reason, it is consistent that some researchers consider to the Bruner´s glands as a secondary pancreas (Gao et al., 2004).

Furthermore, evidence of damage to the duodenal wall and Brunner's glands supports the hypothesis that during acute poisoning with *Kh* fruit, an inflammatory lesion is firstly caused in the pancreas (Carcano et at., 2016) and small intestine. Subsequently, acute pancreatitis can cause systemic inflammation, multiple organ failure, and death (Yuan et al., 2011).

V. Conclusion

Acute intoxication with *Kh* fruit causes the destruction of the Brunner's submucosal glands in the duodenum in a manner similar to that reported in the pancreas. Likewise, we can say that *Kh* shows a toxic effect on the tissue components of the duodenum wall, which can more directly explain the weight loss experienced by experimental animals.

The histological lesion in the duodenal wall is progressive and consists of edema of the intestinal villi; erosion of the intestinal epithelium accompanied by initial loss of goblet cells followed by hyperplasia; in addition to vascular congestion in the lamina propria and apoptotic lesions in the Brunner's glands.

In this work we report, for the first time, evidence of tissue damage to the Brunner's glands in acute experimental poisoning with ripe fruit of *Karwinskia humboldtiana*.

The lesion in Brunner's glands, reported here for the first time; which, together with the injury to the exocrine pancreas that was reported in previous studies, can be correlated with the development of a possible metabolic acidosis that, in turn, explains the respiratory alterations reported in this *in vivo* experimental model.

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Conflicts of interests

The principal researcher and the collaborating researchers declare that there is no conflict of interest regarding the publication of this paper.

ORCID

Nallely Esparza-Rodriguez, ID: https://orcid.org/0000-0001-9455-8125;
Juan Carlos Segoviano-Ramirez, ID: https://orcid.org/0000-0001-9169-2118;
Emilio Hasbun-Pacheco, ID: https://orcid.org/0009-0004-2950-332X;
Juan Pablo Martínez-Silva, ID: https://orcid.org/0009-0003-5034-6771;
Katya Carcano-Diaz, ID: https://orcid.org/0000-0002-9927-0363;
Jaime Garcia-Juarez, ID: https://orcid.org/0000-0003-4544-2913.

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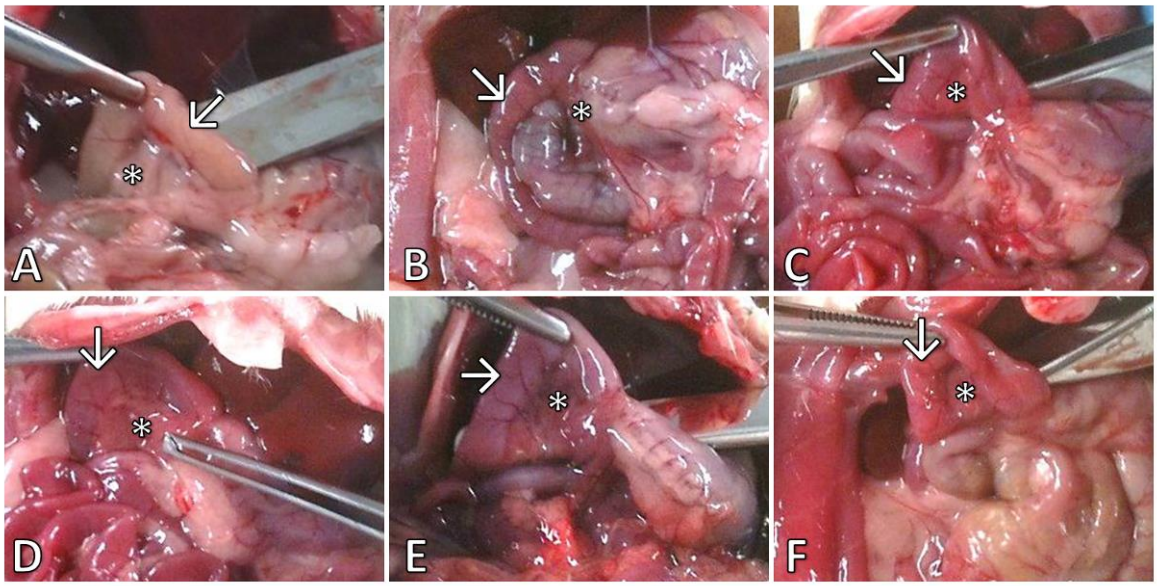

Figure 1: Macroscopic analysis of the duodenum.

A) Duodenum of a rat from the control group in which we can see the pale pink surface of the organ with its normal-appearing vasculature (arrow). Likewise, the pancreas (*) shows its normal appearance.
B) At 24 hours after admministration of *Kh* fruit, the duodenum (arrow) and pancreas (*) show a congestive appearance.
C) In the duodenum (arrow) and pancreas (*) of rats sacrificed 48 hours after administration of *Kh* fruit, they continue to show congestive vasculature and a reddish color.
D) 72 hours, the duodenum (arrows) and pancreas (*) are present a similar appearance to the previous group.
E) Congestive and edematous appearance of the duodenum (arrow) and pancreas (*) at 96 hours, in this photo a violet color can be seen in both organs.
F) In the duodenum (arrow) and pancreas (*) at 120 hours, an evident hemorrhagic appearance can be seen.
Crops of images obtained with the camera of an iPhone 3® cell phone at a distance of 30 cm immediately after the abdominal dissection.

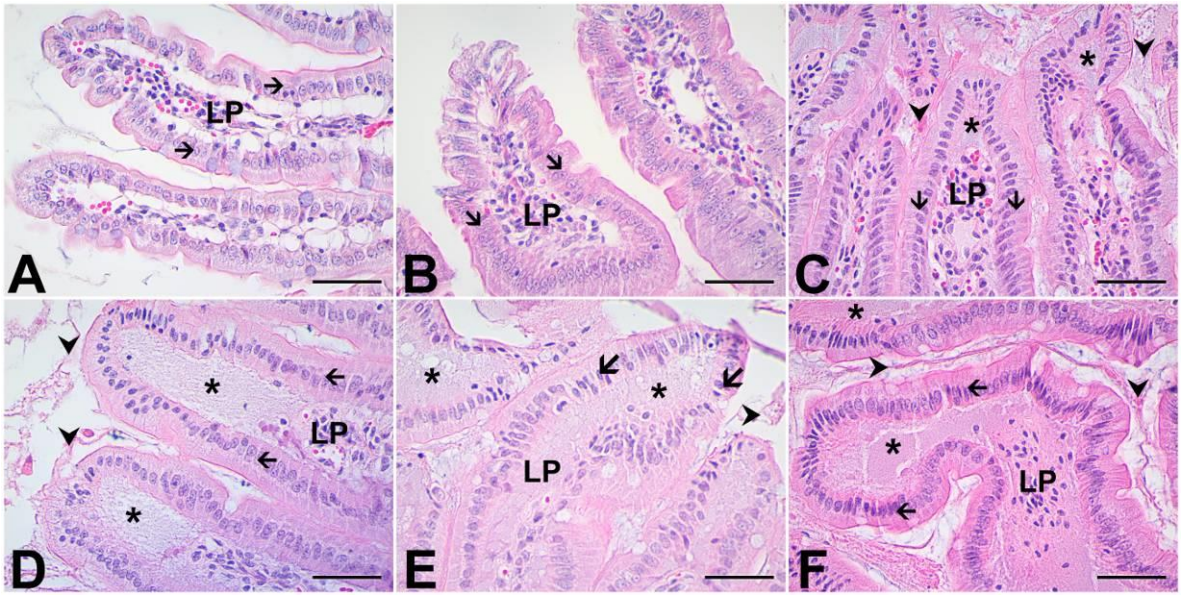

Figure 2: Duodenal villi.

A) Duodenal villi of a rat, from the control group, in which we can see the normal morphology of the simple columnar epithelium with microvilli alternating with goblet cells (arrow), and loose connective tissue of the lamina propria (LP), forming part of the duodenal mucosal layer projecting into the intestinal lumen. A thin film of mucus can be observed above the epithelium.
B) At 24 hours after administration of *Kh* fruit, the epithelium (arrows) and lamina propria (LP) of the intestinal villi show a similar appearance to the control group.
C) In the lamina propria of the duodenal villi (LP) at 48 hours, the presence of edema can be distinguished (*), while above the epithelium (arrow) mucus and acidophilic fibrinoid material can be seen (arrowheads).
D) At 72 hours, a notable presence of edema (*) is observed in the lamina propria (LP) of the duodenal villus. In addition, thickening of the film of mucus and acidophilic fibrinoid material (arrowhead) is seen above the epithelium (arrows).
E) The doudenal villi show an appearance similar to those from the previous group at 96 hours after administration of *Kh* fruit. The epithelium (arrow), the mucus film with fibrin (arrowheads) and the edema present (*) in the lamina propria (LP) have been marked.
F) In the duodenal villi of the group sacrificed 120 hours after the administration of *Kh* fruit, a thick film of mucus with fibrinoid material (arrowheads) is observed above the epithelium (arrow) and in the lamina propria (LP) shows edema (*).
Photomicrographs of histological sections stained with H&E, obtained with a 40x objective, bar 50 micrometers long.

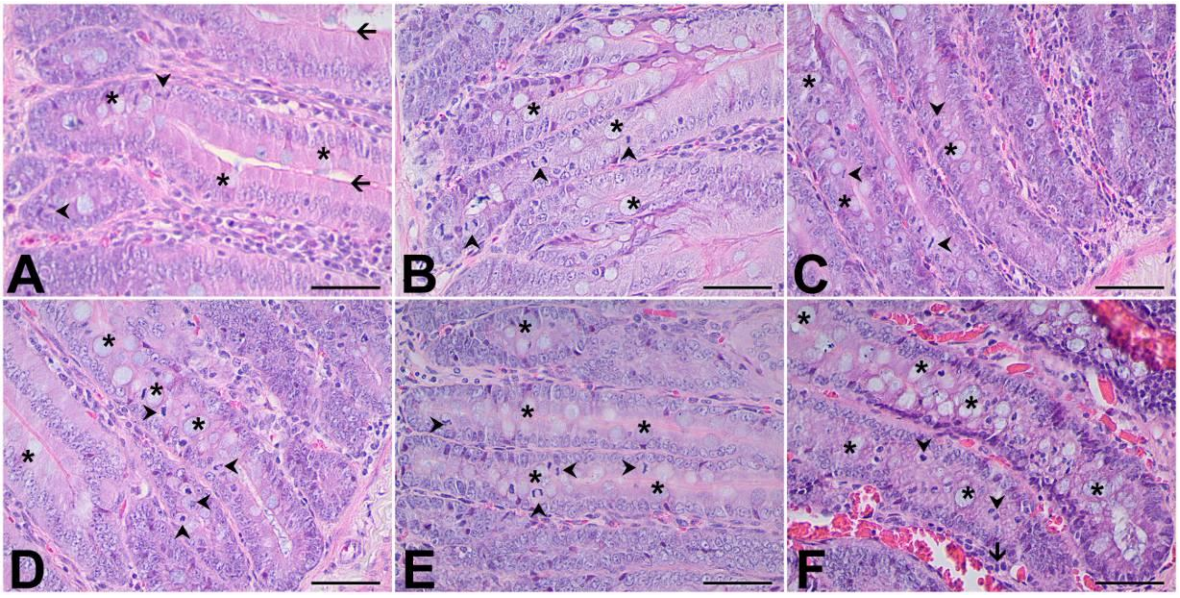

Figure 3: Lieberkhün´s duodenal crypts.

A) Control group demonstrating the normal-looking morphology of simple straight tubular glands called Lieberkhün´s crypt. The simple cylindrical epithelium composed predominantly by enterocytes with "striated plate" like microvilli on the apical edge (arrows), in addition to some goblet cells (*) and few mitosis (head of arrow) are showed.
B) Lieberkhün´s duodenal crypts from a rat sacrificed 24 hours after administration of *Kh* fruit. In this image goblet cells (*) and mitosis (arrowheads) are marked.
C) In the duodenum samples from rats sacrificed at 48 hours, an appearance similar to the previous group can be seen. Vascular congestion is observed in the lamina propria (arrowhead).
D) At 72 hours, a greater proportion of goblet cells (*) and mitosis (arrowheads) present in the Lieberkhün´s duodenal crypts is evident, compared to the previously described groups.
E) In the duodenal crypts of samples obtained at 96 hours, the proportion of goblet cells (*) and mitosis (arrowheads) is similar to those shown in the previous group.
F) At 120 hours, it can be seen that the Lieberkhün´s crypts have more goblet cells (*) as well as cells in mitosis (arrowheads) compared to the control group. In this field, vascular congestion in the lamina propria that surrounding the crypts and some cells that look like neutrophils (arrow) are showed.
Photomicrographs of histological sections stained with H&E, obtained with a 40x objective, bar 50 micrometers long.

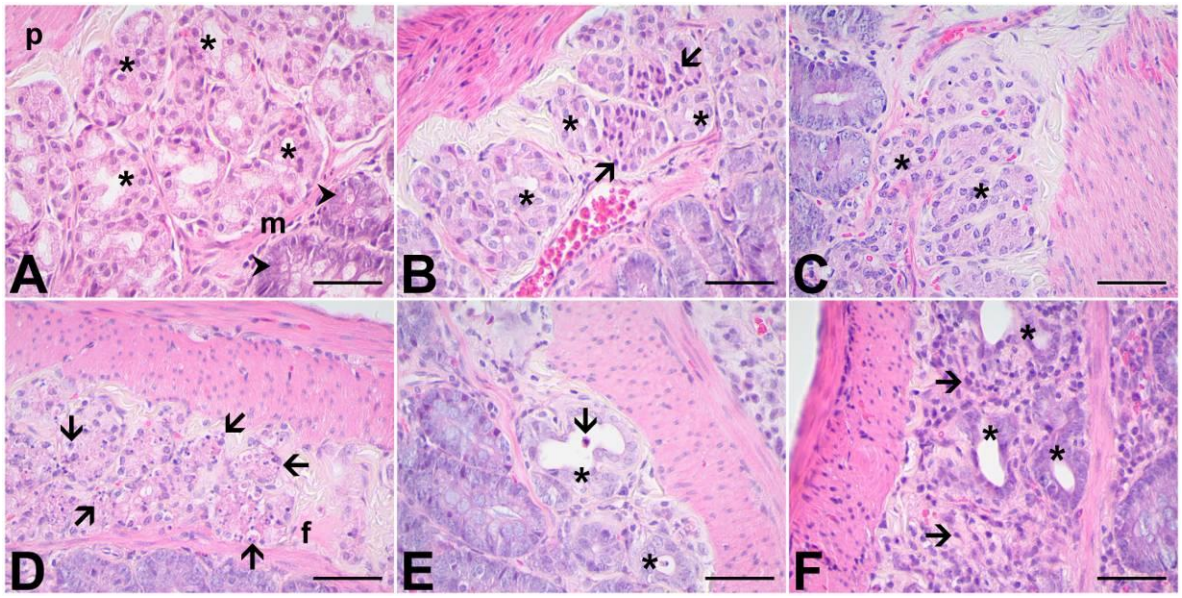

Figure 4: Brunner's glands.

A) Control group demonstrating normal-looking morphology of Brunner's submucosal glands. The simple cubic epithelium of adenomers (*) has basophilic, euchromatic nuclei with a prominent nucleolus, while the cytoplasm is acidophilic. The tubuloalveolar appearance of these glands is evident, since they have a wide lumen. Smooth muscle from the muscularis mucosa (m) and muscularis propria (p) layers flank the submucosa. In the lower right corner, the bottom of the Lieberkhün crypts located in the duodenal mucosa (arrowheads), can be seen.
B) Brunner's glands (*) of a intestinal sample from rat sacrificed 24 hours after administration of *Kh* fruit showing a similar appearance to the control group. However, in this image some adenomer´s nuclei presenting pyknotic appearance (arrows) are showed.
C) Duodenal submucosal glands from a rat sacrificed 48 hours. Although the glands (*) still appear normal, it can be seen that their size is slightly smaller and the lumen is not as wide as observed in the control group.
D) Brunner's glands that present cells with histological evidence of cell fragmentation (apoptotic bodies) at 72 hours after administration of *Kh* fruit (arrows). Likewise, fibrinoid material (f) can be seen between the collagen fibers of the submucosa.
E) At 96 hours, few adenomers with simple squamous epithelium (*) and infiltrates of inflammatory cells (eosinophils) in its lumen can be observed (arrow).
F) At 120 hours, few adenomers, whose epitelial cells with basophilic cytoplasm (*), surrounded by abundant mononuclear inflammatory cells (arrows) can be seen.
Photomicrographs of histological sections stained with H&E, obtained with a 40x objective, bar 50 micrometers long.

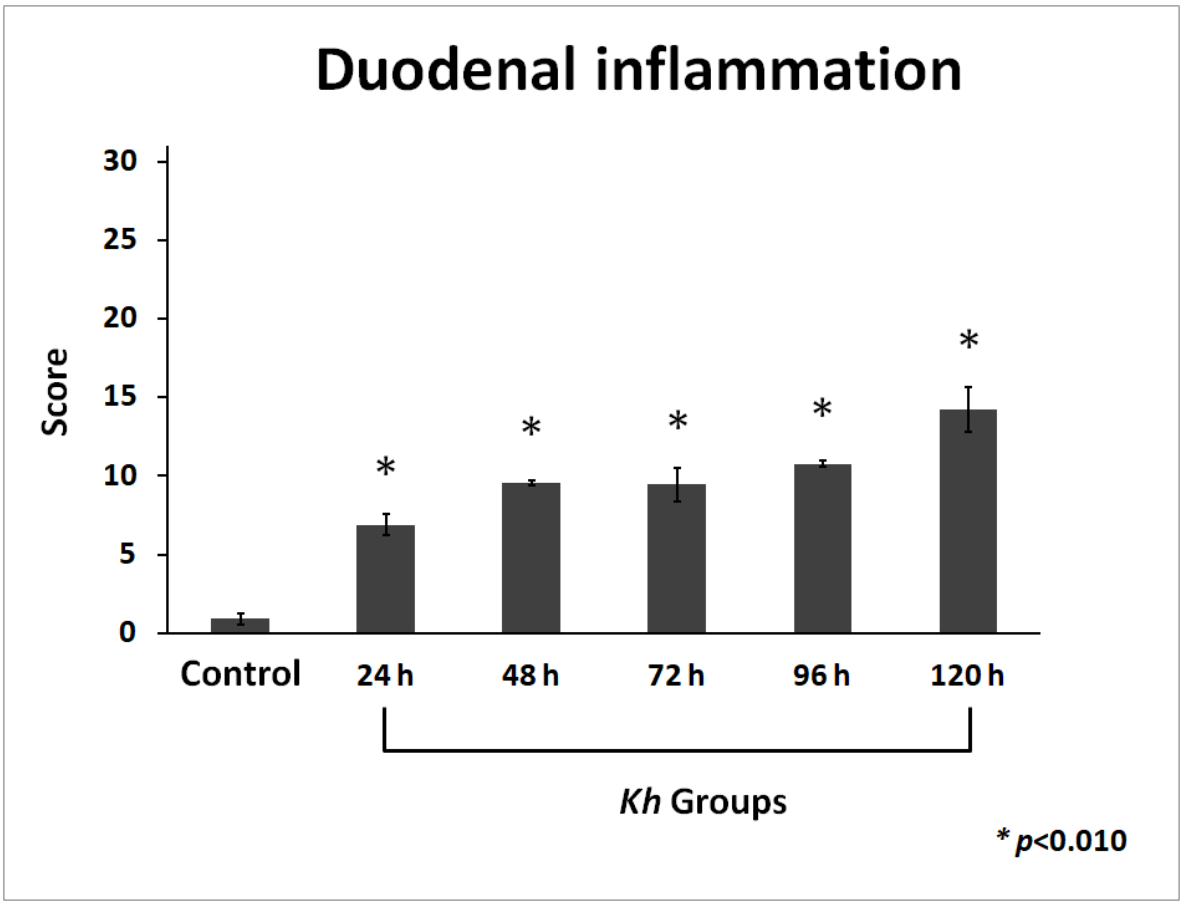

Figure 5: Score of duodenal inflammation.

This graph shows the progressive degree of inflammation of the duodenal wall in the groups that received *Karwinskia humboldtiana* fruit. All groups show a significant statistical difference (*) in relation to the control group ($p < 0.010$).

Table I

Category	Criterion	Control group	*Kh* Groups 24 h	48 h	72 h	96 h	120 h
Inflammatory cell infiltrate	Severity	0.4 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	1.2 ± 0.4	1.2 ± 0.4	1.8 ± 0.4
	Extent	0.4 ± 0.2	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.3 ± 0.0	1.9 ± 0.2
Epithelial changes	Hyperplasia	0.0 ± 0.0	2.2 ± 0.5	4.9 ± 0.2	5.0 ± 0.0	4.8 ± 0.2	5.0 ± 0.0
	Globet cell loss	0.0 ± 0.0	2.9 ± 0.2	2.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mucosal archictecture	Cryptitis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 0.5	2.9 ± 0.2	2.8 ± 0.2
	Erosion	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.8
	Villous Blunting	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	0.6 ± 0.2	0.6 ± 0.2
	SCORE	0.9 ± 0.4	6.9 ± 0.7	9.6 ± 0.2	9.4 ± 1.1	10.8 ± 0.2	14.2 ± 1.4

Table I: Score calculated of each one of evaluated criteria according to Erben´s scale (Erben et al., 2014). The averages with its standard deviation were obtained from the evaluation of 5 fields by triplicate with the 40x objective in each sample.