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Structural and functional integrity of endocrine pancreas post administration of *Karwinskia humboldtiana* fruit to Wistar rats: a possible therapeutic application for cancer of exocrine origin

Juan Carlos Segoviano-Ramirez[1,3], Nallely Esparza-Rodriguez[2,6], Katya Carcano-Diaz[4], Rosa Nelly Diaz-Perez[2], Jose Prisco Palma-Nicolas[5], Romel Hernandez-Bello[5] and Jaime Garcia-Juarez[1,2]

[1]Center for Research and Development in Health Sciences, Bioimaging Unit, Autonomous University of Nuevo Leon (UANL), [2]Department of Histology, [3]Department of Pathology, Faculty of Medicine, Autonomous University of Nuevo Leon (UANL), Monterrey, Nuevo Leon, [4]Laboratory of Physiology, Marista University of Merida, Mérida, Yucatán, [5]Department of Microbiology, Faculty of Medicine, Autonomous University of Nuevo Leon (UANL), Monterrey, Nuevo León, and [6]General Directorate of Quality and Health Education (DGCES), Secretary of Health, México City, México

Summary. Aims. Pancreatic adenocarcinoma represents a therapeutic challenge due to the high toxicity of antineoplastic treatments and secondary effects of pancreatectomy. T-514, a toxin isolated from *Karwinskia humboldtiana* (*Kh*) has shown antineoplastic activity on cell lines. In acute intoxication with *Kh*, we reported apoptosis on the exocrine portion of pancreas. One of the mechanisms of antineoplastic agents is the induction of apoptosis, therefore our main objective was to evidence structural and functional integrity of the islets of Langerhans after the administration of *Kh* fruit in Wistar rats.

Methods. TUNEL assay and immunolabelling against activated caspase-3 were used to detect apoptosis. Also, immunohistochemical tests were performed to search for glucagon and insulin. Serum amylase enzyme activity was also quantified as a molecular marker of pancreatic damage.

Results. Evidence of toxicity on the exocrine portion, by positivity in the TUNEL assay and activated caspase-3, was found. On the contrary, the endocrine portion remained structurally and functionally intact, without apoptosis, and presenting positivity in the identification of glucagon and insulin.

Conclusions. These results demonstrated that *Kh* fruit induces selective toxicity on the exocrine portion and establish a precedent to evaluate T-514 as a potential treatment against pancreatic adenocarcinoma without affecting the islets of Langerhans.

Key words: Adenocarcinoma, *Karwinskia humboldiana*, Apoptosis, Islets of Langerhans, Diabetes, Selective toxicity

Corresponding Author: Jaime García-Juárez, Bioimaging Unit, Center for Research and Development in Health Sciences, Autonomous University of Nuevo Leon (UANL), Gonzalitos y Dr. Carlos Canseco, Mitras Centro, C.P. 64460, Mexico. e-mail: jaime.garciajr@uanl.edu.mx
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Introduction

Pancreatic cancer is a health problem that ranks tenth in incidence in men and eighth in women, occupying fourth place as a cause of death in the United States (Siegel et al., 2022). The pancreatic ductal adenocarcinoma is the most common of all malignant pancreatic tumours and accounts for more than 85% of all pancreatic tumours (Luchini et al., 2016; Schawkat et al., 2020).

Today, the most effective medical treatment for advanced pancreatic cancer is combined chemotherapy, but it is hard to find the correct combination of drugs with minimal side effects for each patient (Chin et al., 2018). In addition, although surgical treatment is only available to 15 to 20% of patients with pancreatic adenocarcinoma (Grossberg et al., 2020), often pancreatectomy causes secondary endocrine insufficiency that triggers type 3c diabetes mellitus (Scholten et al., 2019). This endocrine insufficiency increases the incidence of postsurgical diabetes mellitus up to 100% (Scavini et al., 2015). Because of that, the search for some effective therapeutic alternatives that do not trigger all mentioned medical

complications is a challenge.

On the other side, T-496, T-514, T-516 and T-544 are some anthracenonic compounds extracted from *Karwinskia humboldtiana* (*Kh*), a plant whose fruit has a highly venomous seed (Dreyer et al., 1975; Fernández Nava, 1992). One of those toxins, T-514, showed cytotoxic effects on cancer cells from hepatoma, colonic adenocarcinoma, lung adenocarcinoma, non-differenced bronchogenic carcinoma, and small cell carcinoma (Piñeyro-López et al., 1994). Nevertheless, the usefulness of T-514 over cell cultures from pancreatic cancer has not been evaluated.

The induction of apoptosis is relevant as a potential mechanism of action of some antineoplastic agents to eliminate transformed cells (Naeimi et al., 2019). Regardless of the pathway leading to the activation of caspase-3, structured and orderly chromatin degradation is one of the morphological characteristics of apoptosis. The TUNEL assay is the technique to demonstrate this molecular process (Tang et al., 2019).

In a previous study carried out by our research group, using Wistar rats as an animal model of acute intoxication with the fruit of *Kh*, we evidenced the destruction of the exocrine glandular tissue of the pancreas, characterized by the appearance of apoptotic bodies, activation of caspase-3, chromatin fragmentation, inflammation and necrosis (Carcano-Diaz et al., 2016).

Following this search line, in the present study, we decided to evaluate whether cell death is limited to the exocrine portion of the pancreas without damaging the islets of Langerhans by two different techniques: 1) TUNEL assay to show the nuclear chromatin fragmentation into injured cells (Lu et al., 2020), and 2) the immunolabelling of activated caspase-3, an enzyme that participates in the pathway of apoptosis (Forika et al., 2021).

In addition to the above, because the blood levels of serum amylase tend to increase or decrease when the pancreas presents inflammatory or neoplastic processes respectively (Hansen et al., 2021), the seric activity of this biomarker of acute pancreatitis (Ismail and Bhayana, 2017) was quantified.

Finally, to improve the characterization of our model of acute pancreatitis, we evaluated the production of insulin and glucagon by cells of the islets of Langerhans by immunolabelling.

Thus, our main objective was to identify, as evidence of functionality of the islets of Langerhans, the presence of such molecules and to check that these cells retain a morphologically intact aspect despite the intense destruction of the exocrine portion of the pancreas, previously reported.

Materials and methods

Model of acute intoxication with the fruit of Kh

For this investigation, eighteen female Wistar rats weighing 225±25 g were used. Animals were maintained under environmentally controlled conditions in the animal facility of our research centre. This project was evaluated and approved by the Institutional Scientific and Ethics Committee (Folio HT14-004). All procedures were performed following NOM-062-ZOO-1999.

Fruits of *Kh*, collected during July and August, were allowed to dry, then they were ground and sieved in a 50x mesh. A group of fifteen rats (treated group), using oral gavage, were administered with a single dose (5 g/kg) of fruit powder mixed with water according to previous reports (Bermudez et al., 1992; Carcano-Diaz et al., 2016). The control group (non-treated group) was composed of three rats that received only water. The animals were killed following protocols (in groups of 3 each) at 24, 48, 72, 96 and 120h after administration of fruit.

Collecting and processing tissue samples

Tissue samples of the proximal pancreas from intoxicated rats (treated group) were obtained at established times. Samples from the control (non-treated group) were collected at the end of the experiment. All tissue samples were fixed in buffered formalin at pH 7.4 for 48h and subsequently processed until embedded in paraffin.

Collecting serum samples to the determination of amylase

In 1 ml Eppendorf tubes, blood samples obtained from the right ventricle were allowed to clot. Once they were coagulated, the serum was separated by centrifugation at 14,000 rpm for 10 minutes at 4°C. Serum samples were recovered and stored at -80°C until used for the amylase assay.

TUNNEL test

To search apoptosis in pancreas samples, Trevigen's TACS 2 TdT-DAB in situ apoptosis detection kit (4810-30-K), was used following the manufacturer's instructions. Five-micron histological sections were deparaffinized and rehydrated until taking them to PBS at pH 7.4 for 10 minutes. Subsequently, to permeabilize tissues, they were incubated with Proteinase K for 15 minutes and washed with ultra-purified water. Endogenous peroxidase was neutralized immersing slides in bleaching solution for 5 minutes and washing them with PBS for 1 minute. One pancreas sample from the non-treated group, used as a positive control, was incubated with 50 µl of TACS Nuclease enzyme in a humid chamber for 30 minutes at room temperature and subsequently washed with PBS. All samples, including the positive control, were immersed in the TdT labelling buffer for 5 minutes and subsequently incubated in a humid chamber with the labelling reaction mixture for 60 minutes at 37°C.

Slides were immersed in the stopping buffer for 5 minutes to stop the reaction. After washing them with ultra-purified water, the samples were incubated at 37°C in a humid chamber with 50 µl of streptavidin-horseradish peroxidase solution for 10 minutes, washed with PBS, and immersed in DAB solution for 5 minutes. Finally, they were washed with ultra-purified water and contrasted with 1% methyl green for 30 seconds. Once slides were washed, they were mounted and analyzed under brightfield light microscopy.

Immunohistochemical staining

Immunolabelling was carried out also on 5 µm thickness pancreas sections. A rabbit anti-activated caspase-3 primary antibody (ab2302), in a concentration of 5 µg/ml in PBS, was used to detect the cells with apoptosis.

Alpha cells (A cells) were labelled with a rabbit anti-glucagon primary antibody (ab92517) diluted 1:500 in PBS. On the other hand, a rabbit anti-insulin primary antibody (ab63820), diluted 1:500 in PBS, was used for labelling beta cells (B cells). A horseradish peroxidase-conjugated donkey anti-rabbit secondary antibody (ab6802), diluted 1:1000 in PBS, also was used for DAB immunoprecipitation.

Briefly, all pancreas sections were deparaffinized at 60°C for 40 minutes. Subsequently, they were immersed in xylene for 5 minutes twice and then in absolute and 96% alcohol for 5 minutes each. Antigen retrieval was performed in HIER H buffer (pH 9.0) at 98°C for 20 minutes in a PT Module (Thermo Scientific). The samples were immersed in a 3% hydrogen peroxide solution in methanol for 30 minutes to deactivate endogenous peroxidase. Next, they were washed twice with PBS for 5 minutes each. They were then blocked with 0.1% Triton X100 in 3% donkey serum in PBS for 90 minutes. They were then incubated with the primary antibody overnight at 4°C in a humid chamber. The next day, the samples were tempered, washed with PBS, and incubated with the secondary antibody for 2 hours at room temperature in a humid chamber. After that, they were washed with PBS, incubated with DAB solution for 3 minutes, and washed with PBS 4 times for 5 minutes each. One washing with distilled water, previous contrasting staining with Gill's Hematoxylin for 30 seconds, was done. Finally, they were washed with distilled water, dehydrated, cleared, and mounted with Entellan© resin.

Determination of serum amylase

For determination of serum amylase activity, the α-amylase assay colorimetric kit (DAMY-100) Quanti-Chrom™ was used according to the manufacturer's instructions.

First, the serum samples from treated and non-treated rats were thawed and placed on ice. 10 µl of serum from each sample was placed separately in Eppendorf tubes. In another tube, 10 µl of ultra-purified water was used as blank control. Samples were incubated with 190 µl of the preheated substrate at 37°C for 5 minutes. 80 µl of stop reagent was added to each tube to stop the reaction. Subsequently, all tubes were centrifuged at 14,000 rpm for 5 minutes. Then, 200 µl of supernatants from each of the tubes was transferred separately to a clear bottom 96-well box to obtain the optical density (OD) readings from samples, blank control, and the calibrator provided by the manufacturer. The OD readings were obtained using a plate reader at 595 nm.

With the OD data, the α-amylase activities were calculated using the formula provided by the manufacturer, as follows:

Activity = [Sample OD - Blank OD x n x 550 (U/L)] / [Calibrator OD - H_2O OD].

(It was not necessary to dilute the samples for which the value of n=1).

Plotting (average values and standard deviation along with the P value) of the α-amylase activities from experimental groups were obtained using the Student´s t-test.

Results

Identification of fragmented chromatin by TUNEL assay

In the non-treated group, nuclei of the cells in both the endocrine and the exocrine portions showed negative result to TUNEL assay.

In the treated group, negative results of TUNEL assay were shown by nuclei of the cells into the islets of Langerhans from those samples collected at 24, 48, 72, 96 and 120 hours after administration of the fruit of *Kh* (Fig. 1).

Concerning the acinar cells of the exocrine portion, we only found negative results in the samples collected at 24 hours. On the contrary, positive results at 72 hours were markedly observed (Fig. 1D). Finally, in the samples obtained at 96 and 120 hours, we only could observe few positive marks (Fig. 1E,F).

In the sample (positive control) incubated with TACS nuclease (Fig. 1G), all nuclei of the cells from both exocrine and endocrine portions showed positive marks as we expected.

Identification of activated caspase-3 in pancreatic tissue

Some positive marks to immunolabelling against activated caspase-3 were found in those samples of pancreas obtained at 24 hours from intoxication (Fig. 2B). Nevertheless, those marks were more evident in the samples obtained at 48 and 72 hours (Fig. 2C,D). In the same way, the presence of positive marks was notably diminished in the samples obtained at 96 and 120 hours after intoxication (Fig. 2E,F). It is important to note that in samples obtained at 72 hours (Fig. 2D), remarkable

destruction of the exocrine portion of the pancreas, together with data of inflammation were appreciated. This tissue destruction was so intense that the islets of Langerhans were found so prominent in the samples from 96 hours (Fig. 2E).

None of the samples showed any activated caspase-3 positive signal over the cells of the isles of Langerhans, nor were apoptotic bodies observed. Non-treated group (Fig. 2A) did not show any positivity in either the endocrine or the exocrine portion.

Proving the functional integrity of the A and B cells

In samples, at all studied times, from the treated group, positive marks to insulin in the cytoplasm of the B cells were identified, as well as in the non-treated group. The positive marks were identified in most cells distributed throughout the whole islet of Langerhans.

The endocrine tissue, in all slides from the treated group, showed an aspect very similar to those from the non-treated group, even in those cases where surrounding exocrine tissue showed an intense inflammation characterized by edema, necrosis and inflammatory cellular infiltrated at 96 and 120 hours (Figs. 3E,F, 4E,F).

No inflammatory cells, in the blood capillary of the islets of Langerhans from any of the treated rats were found. Neither was presence of edema observed in this portion (Figs. 3, 4).

In the same way, in all samples of the pancreases from intoxicated rats (at all times), positive marks to immunolabelling against glucagon in the cytoplasm of the A cells were identified, as well as in the non-treated group (Fig. 4). These A cells were localized in peripheral areas of the islets of Langerhans and their histological aspects were similar to those shown by the non-treated

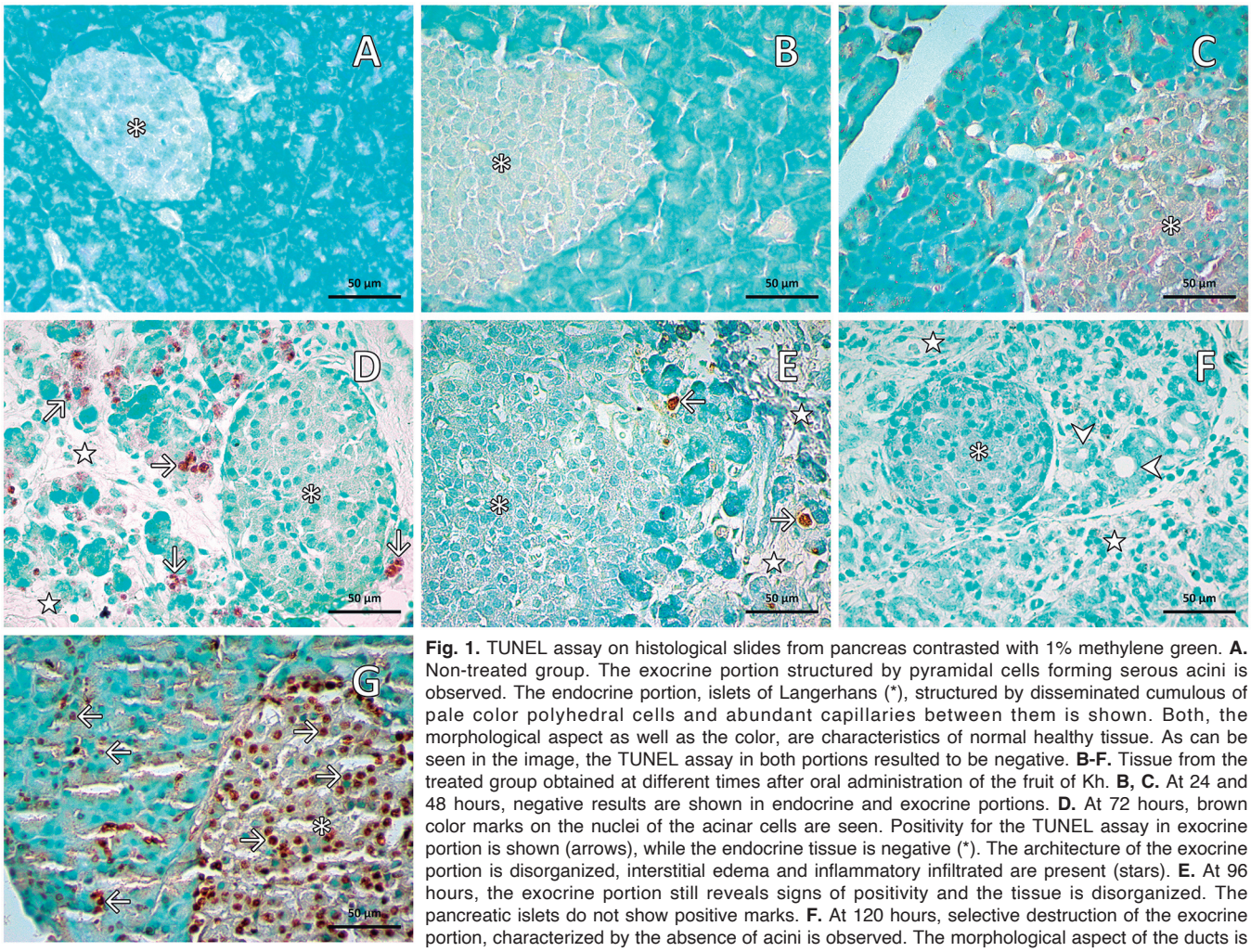

Fig. 1. TUNEL assay on histological slides from pancreas contrasted with 1% methylene green. **A.** Non-treated group. The exocrine portion structured by pyramidal cells forming serous acini is observed. The endocrine portion, islets of Langerhans (*), structured by disseminated cumulous of pale color polyhedral cells and abundant capillaries between them is shown. Both, the morphological aspect as well as the color, are characteristics of normal healthy tissue. As can be seen in the image, the TUNEL assay in both portions resulted to be negative. **B-F.** Tissue from the treated group obtained at different times after oral administration of the fruit of Kh. **B, C.** At 24 and 48 hours, negative results are shown in endocrine and exocrine portions. **D.** At 72 hours, brown color marks on the nuclei of the acinar cells are seen. Positivity for the TUNEL assay in exocrine portion is shown (arrows), while the endocrine tissue is negative (*). The architecture of the exocrine portion is disorganized, interstitial edema and inflammatory infiltrated are present (stars). **E.** At 96 hours, the exocrine portion still reveals signs of positivity and the tissue is disorganized. The pancreatic islets do not show positive marks. **F.** At 120 hours, selective destruction of the exocrine portion, characterized by the absence of acini is observed. The morphological aspect of the ducts is apparently preserved (arrow heads) despite the intense surrounding inflammatory phenomenon (stars). The endocrine portion retains intact morphological appearance (*). **G.** Pancreas from non-treated group incubated with TACS Nuclease (positive control for staining). Brown color marks in the nuclei, both in the exocrine as well as in the endocrine portions, are shown (arrows).

group.

Determination of serum amylase

The mean serum amylase activity for the non-treated group was 161±12.91 U/L. In the treated groups it was 156±34.25 U/L for the 24-hours, 128±17.89 U/L for the 48-hours, 168±68.45 U/L for the 72-hours, 135±27.56 U/L for the 96-hours and 164±25.20 U/L for the 120 hours after *Kh* administration. According to Student´s t test, no significant differences between the treated groups concerning the non-treated group ($p>0.05$) were found (Fig. 5).

Discussion

The correct selection of the antineoplastic drug in treatment of patients with cancer is one of the most critical issues. The goal is to choose a drug capable of killing the transformed cells which blocks their proliferation with minimal deleterious effect on healthy tissues (Naeimi et al., 2019) and few collateral effects.

In a previous report from our working team, using an experimental acute intoxication model with the fruit of *Kh*, we described the histopathological aspect of the pancreas characterized by apoptotic bodies, activation of caspase-3, chromatin fragmentation, necrosis and inflammation in the exocrine portion, but without damage to the islets of Langerhans. Although the activated caspase-3 was detected in pancreatic acinar cells by immunofluorescence, it was unclear whether this signal was limited only to the exocrine portion (Carcano-Diaz et al., 2016). It is known that apoptosis is triggered by, among others, activation of caspase-3, which induces chromatin fragmentation and the formation of apoptotic bodies (Tang et al., 2019).

In the present study, we could see that the cells of the islets of Langerhans remained intact and did not present apoptosis despite the intense destruction of the exocrine portion of the pancreas. We arrive at this conclusion because in all experimental groups studied, the immunoreaction against activated caspase-3 in the cells of islets of Langerhans was negative. Furthermore, these cells showed a negative result in the TUNEL assay, discarding any chromatin fragmentation. With this, we also corroborate that *Kh* only affected the acinar cells (from the exocrine portion) which showed positive marks.

Both assays showed that nuclear chromatin fragmentation, one of the characteristics of apoptosis,

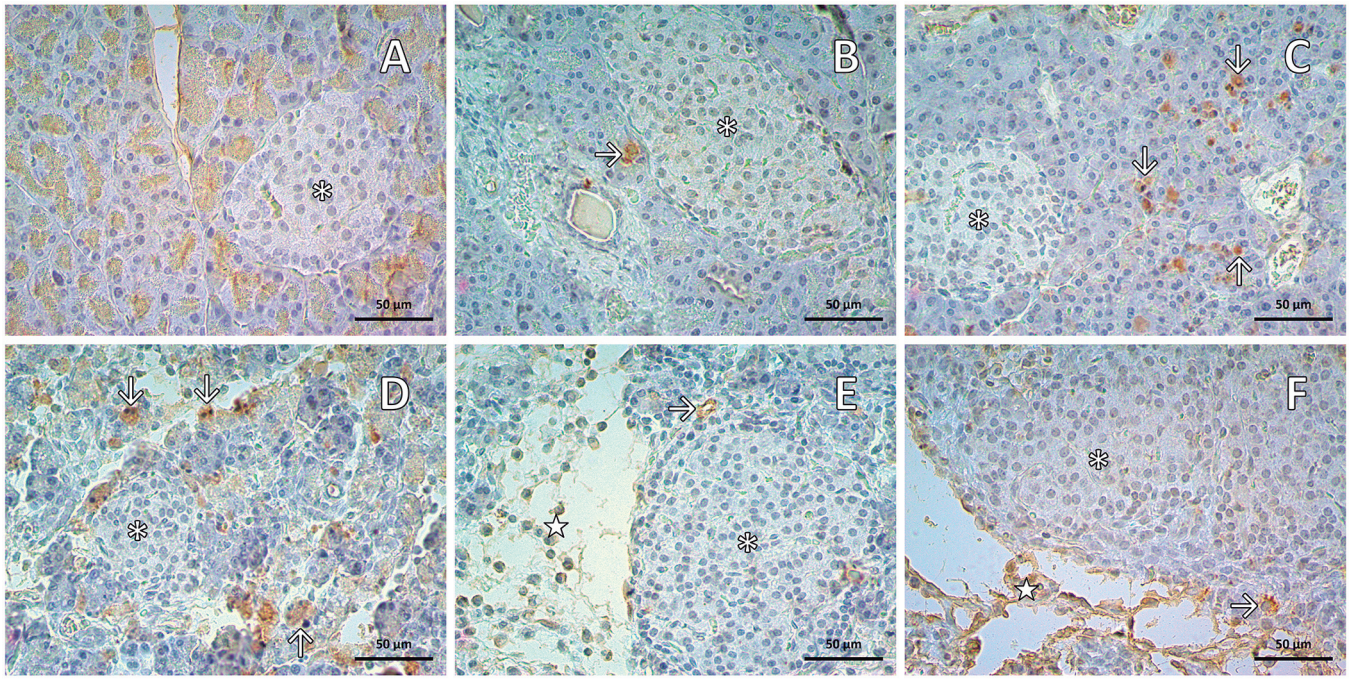

Fig. 2. Indirect immunolabelling against activated caspase-3 by DAB precipitation contrasted with Gill´s haematoxylin. **A.** Non-treated group showing negative result in both portions of the pancreas. **B.** At 24 hours after intoxication, the exocrine portion of the pancreas has few positive marks for immunolabelling (arrow). The retraction of acini concerning the non-treated group is evident. **C.** At 48 hours, abundant positive marks for activated caspase-3 on pancreatic acini are shown (arrows). **D.** At 72 hours, a whole loss of the morphological architecture of the exocrine portion is shown. Labelling of this portion is shown positive (arrows), while endocrine tissue remains negative (*). **E.** At 96 hours after administration of the fruit, some positive marks of immunolabelling still can be observed over the, evidently destroyed, exocrine tissue (star). It is evident the morphological preservation of the endocrine tissue where the immunolabelling to activated caspase-3 is negative. **F.** At 120 hours, the preservation of the morphological integrity in the endocrine portion (*) is remarkable. On the contrary, the selective destruction of the exocrine portion is evident.

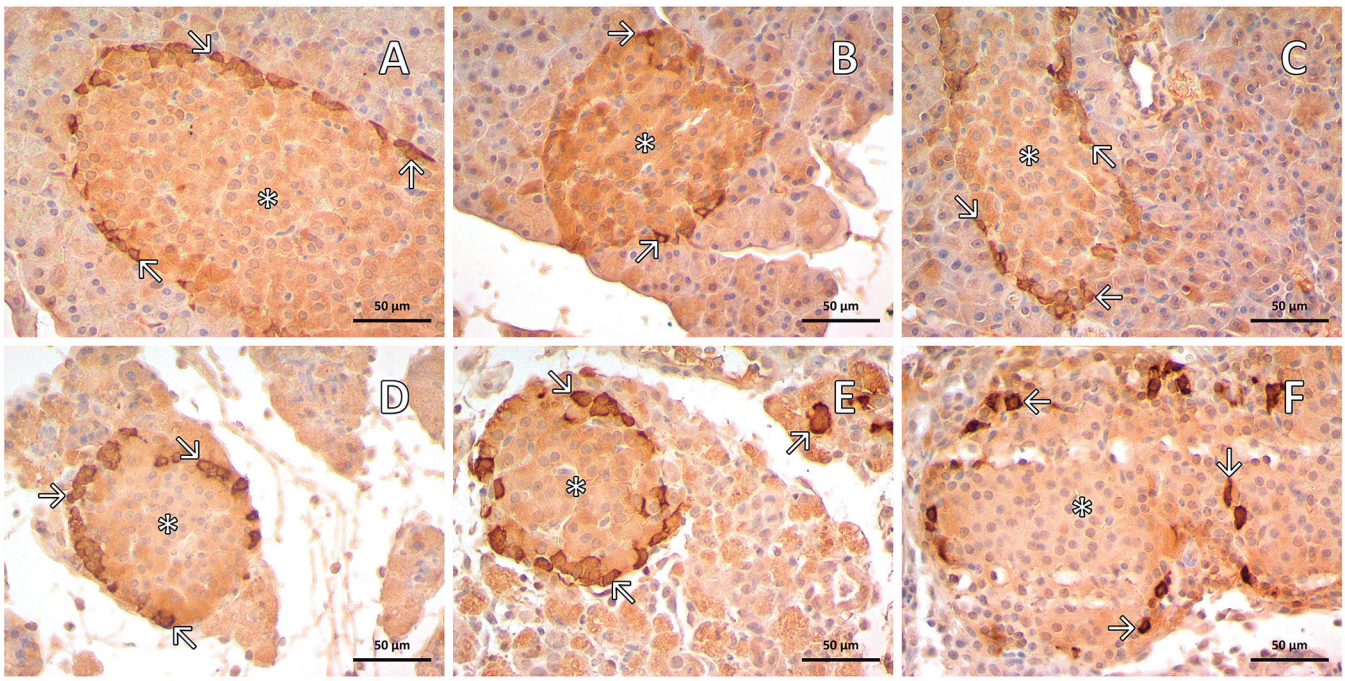

Fig. 3. Indirect immunolabelling against glucagon by DAB precipitation contrasted with Gill´s haematoxylin. **A.** Non-treated group that shows positivity to the immunoassay on the cytoplasm of the A cells (arrows) located on the periphery of the islets of Langerhans (*). **B-F.** Treated group. In all the times analyzed, the positivity to the glucagon immunoassay (dark brown color marks) can be observed only on the A cells (arrows) of the endocrine islets.

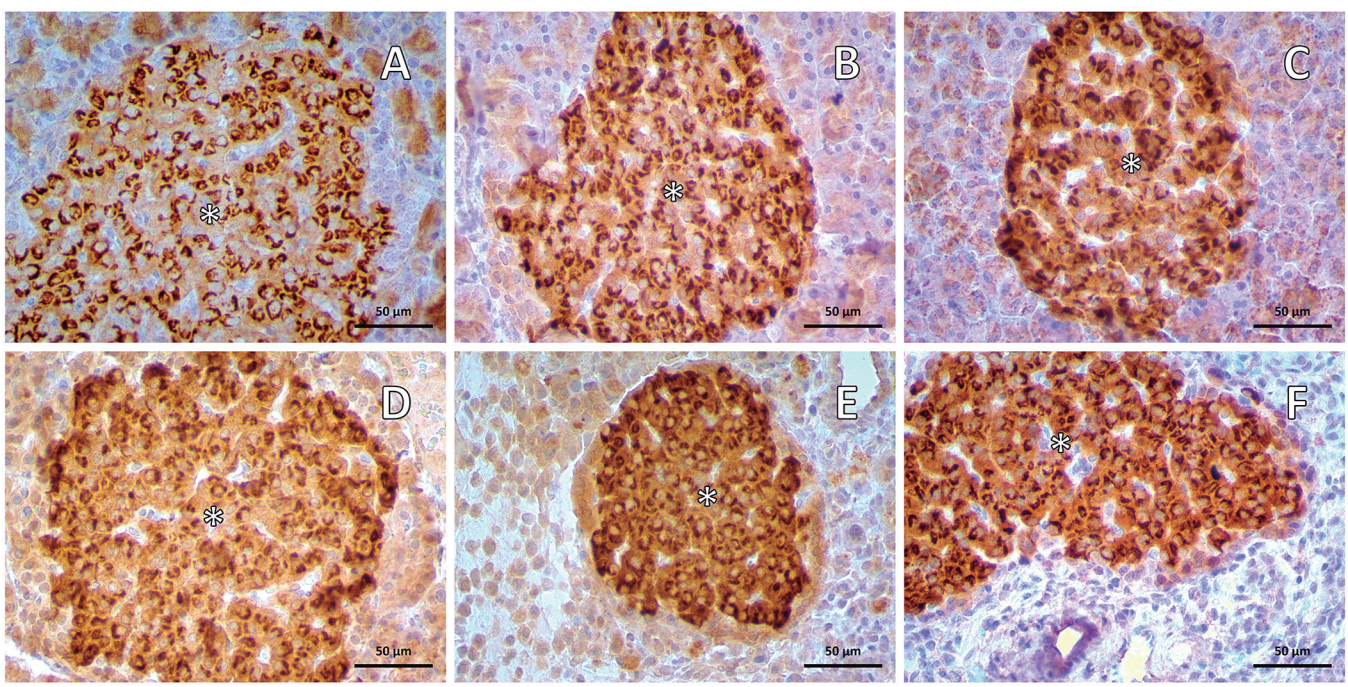

Fig. 4. Indirect immunolabelling against insulin by DAB precipitation contrasted with Gill´s haematoxylin. **A.** Non-treated group showing positivity on the cytoplasm of the B cells that are distributed throughout the islet of Langerhans (*). **B-F.** Treated group, at different times, also showing positivity to the insulin immunoassay (dark brown color marks) on most pancreatic islet cells (B cells).

was limited only to exocrine tissue, while cells of the endocrine tissue remained morphologically intact. Therefore, now we can affirm that in the studied model, there was a selective lesion to the exocrine pancreatic tissue due to the confinement of the positive immunohistochemical mark for activated caspase-3 and the TUNEL assay on acinar cells.

The selective effect observed in this model of acute intoxication by *Kh* correlates with some results previously reported where the authors demonstrated that T-514, a toxin extracted from the *Kh* fruit, had a selective cytotoxic activity against some carcinogenic cellular lines from hepatoma, colonic adenocarcinoma, lung adenocarcinoma, non-differentiated bronchogenic carcinoma, and small cell carcinoma (Piñeyro-López et al., 1994). This correlation establishes a precedent to evaluate its possible cytotoxic effect on cellular lines from pancreatic cancer.

Some compounds with a promising anticancer effect exist, such as luteolin, which induces apoptosis in pancreatic cancer cells by suppressing the K-ras/GSK-3β/NF-kB signalling pathway *in vivo*. Also, there is a release of cytochrome c, activation of caspase-3 and reduction of Bcl-2/Bax apoptosis ratio, which arrests the cell cycle and suppresses the phosphorylation of proteins and signalling pathways (Imran et al., 2019). Other agents, such as polyphenol Nevadensin, produce an inhibitory effect over topoisomerase II *in vitro* (Müller et al., 2021). Now it is known that topoisomerase II plays a role in the transcription and replication of DNA by catalyzing reactions leading to genomic stability (Austin et al., 2018). Because of that, its affectation imbalances the dynamics of the cell cycle inducing apoptosis (Riccio et al., 2020). One of the compounds producing this effect is ciclopirox olamine (Yin et al., 2022).

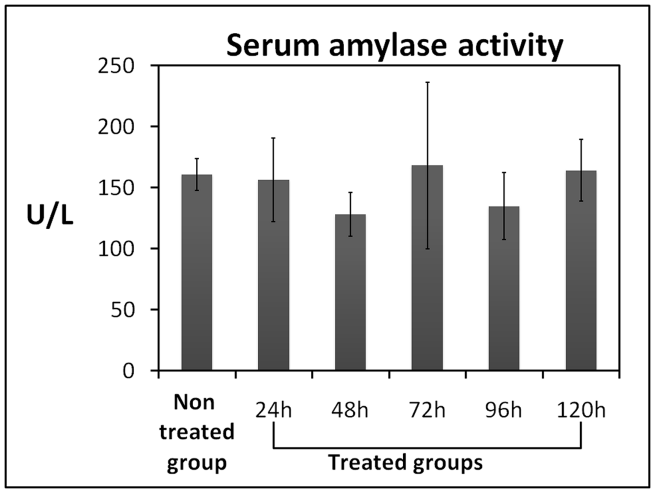

Fig. 5. Determination of seric amylase activity. Each bar represents the average and standard deviation of three determinations of serum amylase enzyme activity. The non-treated group and the treated groups (at 24, 48, 72, 96 and 120 hours) don´t show statistically significant differences between them (p>0.05).

Although in this model of intoxication with the fruit of *Kh*, the specific pathway that leads to apoptosis is not known, other studies evidenced that T-514, named Peroxisomicine A1, induces apoptosis by inhibition of topoisomerase II (Jaramillo-Rangel et al., 2020). Therefore, both our results as previously published in other *in vitro* studies using purified T-514 (Lansiaux et al., 2001), would allow us to think that the possible pathway of activation of apoptosis (in our model) could be through inhibition of topoisomerase II.

Another relevant result was to demonstrate that glucagon and insulin producing cells remained functional in the islets of Langerhans of all experimental groups. Immuno-histochemical assays, identifying the presence of glucagon in peripheral cells of the islets of Langerhans and insulin inside of cells distributed throughout the cluster, evidenced it. The location of the positive immunolabelling to glucagon and the location of A cells, previously reported for murine models (Titova et al., 2020), as well as the detection of insulin and the distribution of insulin-secreting B cells throughout the pancreatic islet (Dalgaard, 2019), were coincident.

The immunohistochemical evidence demonstrated that, in this model of acute experimental intoxication, the A and B cells secreting insulin and glucagon continued being functional. Therefore, we can conclude that the endocrine function of the pancreatic islets did not stop. This means that, if we use the purified T-514 in cultures of cells from pancreatic cancer, some selective toxicity like that shown in other cancerogenic lines could be observed. Ductal pancreatic adenocarcinoma is the most common among malignant pancreatic tumours and corresponds to more than 85% of all tumours in the pancreas (Luchini et al., 2020; Schawkat et al., 2020). Furthermore, it is still one of the malignant neoplasms with increasing incidence, high resistance to chemotherapy (Adamska et al., 2017), and the most mortality, so there is an urgent need to develop newer and more effective treatments.

That is how, when facing the current adverse therapeutic scenario against pancreatic cancer (Siegel et al., 2022), our obtained results are promising in the search for therapeutic alternatives.

Moreover, selective toxicity of fruit on the exocrine portion turns out to be advantageous, since it would avoid the endocrine insufficiency caused by the drugs currently used against pancreatic adenocarcinoma that affect the cells of the islets of Langerhans (Yeh et al., 2018).

Therefore, we could expect the same selective toxicity when the isolated toxin shall be administered to a model of pancreatic cancer. If so, it would be very fortunate for patients to have an effective and selective alternative without the known side effects.

On the other hand, the incidence of diabetes mellitus secondary to pancreatic antitumoral therapy is as diverse as the available therapeutic options. Even the pre-

existing diabetes could be aggravated due to these therapies (Wu et al., 2020). However, after total pancreatectomy, a 100% incidence has been reported (Scavini et al., 2015).

Serum amylase is one of the most used biomarkers in the diagnosis of pancreatic disorders, in addition to being a cheap test and being available in most clinical laboratories (Hansen et al., 2021); in humans, according to international guides like the INSPIRE consortium, its determination constitutes one of the main criteria for diagnosis of pancreatitis (Abu-El-Haija et al., 2018). Because of this, we considered determining the seric concentration of amylase in our model.

The obtained results did not show statistically significant changes between the seric concentrations found in the treated groups concerning the non-treated group; furthermore, we observed a direct correlation between its concentrations in blood with the reduction of the size of acini, data previously reported (Carcano-Diaz et al., 2016).

Some authors have said that during most cases of acute pancreatitis, seric levels of amylase are very high. When they are found to be low, they might reflect the presence of some obstructive phenomenon (Hansen et al., 2021). Nevertheless, in our model, we found low levels of amylase which reflect the decreasing size of acini and their consequent destruction. Because of that, we could conclude that there is a direct cytotoxic effect on the exocrine portion of the pancreas.

Conclusions

In conclusion, in our acute intoxication model, results show that the fruit of *Kh* induces selective toxicity on the exocrine portion of the pancreas because: 1) the cells of the islets of Langerhans, in addition to keeping the morphological integrity already described previously, do not show fragmentation of the chromatin; 2) A and B cells keep producing glucagon and insulin respectively, and 3) the activated caspase-3 is present only in the acinar cells from the exocrine portion. Finally 4) in this model, the activity of seric amylase remains constant despite extensive destruction of the exocrine tissue.

These findings establish a precedent to evaluate the toxins from *Kh* as a potential treatment against pancreatic adenocarcinoma without affecting the islets of Langerhans.

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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. Juan Carlos Segoviano-Ramirez, ID: https://orcid.org/0000-0001-9169-2118; Nallely Esparza-Rodriguez, ID: https://orcid.org/0000-0001-9455-8125; Katya Carcano-Diaz, ID: https://orcid.org/0000-0002-9927-0363; Rosa Nelly Diaz-Perez, ID: https://orcid.org/0000-0001-5964-4802; Jose Prisco Palma-Nicolas, ID: https://orcid.org/0000-0003-3508-9941; Romel Hernandez-Bello, ID: https://orcid.org/0000-0002-0744-5948; Jaime Garcia-Juarez, ID: https://orcid.org/0000-0003-4544-2913

References

Abu-El-Haija M., Kumar S., Quiros J.A., Balakrishnan K., Barth B., Bitton S., Eisses J.F., Foglio E.J., Fox V., Francis D., Freeman A.J., Gonska T., Grover A.S., Husain S.Z., Kumar R., Lapsia S., Lin T., Liu Q.Y., Maqbool A., Sellers Z.M., Szabo F., Uc A., Werlin S.L. and Morinville V.D.. (2018). Management of acute pancreatitis in the pediatric population: A clinical report from the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Pancreas Committee. J. Pediatr. Gastroenterol. Nutr. 66, 159-176.

Adamska A., Domenichini A. and Falasca M. (2017). Pancreatic ductal adenocarcinoma: Current and evolving therapies. Int. J. Mol. Sci. 18, 1338.

Austin C.A., Lee K.C., Swan R.L., Khazeem M.M., Manville C.M., Cridland P., Treumann A., Porter A., Morris N.J. and Cowell I.G. (2018). TOP2B: The first thirty years. Int. J. Mol. Sci. 19, 2765.

Bermudez M.V., Martinez F.J., Salazar M.E., Waksman N. and Piñeyro A. (1992). Experimental acute intoxication with ripe fruit of Karwinskia humboldtiana (Tullidora) in rat, guinea-pig, hamster and dog. Toxicon 30, 1493-1496.

Carcano-Diaz K., Garcia-Garcia A., Segoviano-Ramirez J.C., Rodriguez-Rocha H., Loera-Arias M. de J. and Garcia-Juarez J. (2016). Damage to pancreatic acinar cells and preservation of islets of Langerhans in a rat model of acute pancreatitis induced by *Karwinskia humboldtiana* (buckthorn). Histol. Histopathol. 31, 1001-10.

Chin V., Nagrial A., Sjoquist K., O'Connor C.A., Chantrill L., Biankin A.V., Scholten R.J. and Yip D. (2018). Chemotherapy and radiotherapy for advanced pancreatic cancer. Cochrane Database Syst. Rev. 3, CD011044.

Dalgaard L.T. (2019). Assessment of β-Cell replication in isolated rat islets of Langerhans. Methods Mol. Biol. 2029, 25-35.

Dreyer D.L., Arai I., Bachman C.D., Anderson W.R. Jr, Smith R.G. and Daves G.D. Jr (1975). Toxins causing noninflammatory paralytic neuronopathy. Isolation and structure elucidation. J. Am. Chem. Soc. 97, 4985-4990.

Fernández Nava R. (1992). Nombres comunes, usos y distribución geográfica del género Karwinskia (Rhamnaceae) en México. Anales del Instituto de Biología. Serie Botánica. 63, 1-23.

Forika G., Kiss E., Petovari G., Danko T., Gellert A.B. and Krenacs T. (2021). Modulated electro-hyperthermia supports the effect of Gemcitabine both in sensitive and resistant pancreas adenocarcinoma cell lines. Pathol. Oncol. Res. 27, 1610048.

Grossberg A.J., Chu L.C., Deig C.R., Fishman E.K., Hwang W.L., Maitra A., Marks D.L., Mehta A., Nabavizadeh N., Simeone D.M., Weekes C.D. and Thomas C.R. Jr (2020). Multidisciplinary standards of care and recent progress in pancreatic ductal adenocarcinoma. CA Cancer J. Clin. 70, 375-403.

Hansen S.E.J., Langsted A., Varbo A., Madsen C.M., Tybjærg-Hansen A. and Nordestgaard B.G. (2021). Low and high pancreatic amylase

is associated with pancreatic cancer and chronic pancreatitis. Eur. J. Epidemiol. 36, 975-984.

Imran M., Rauf A., Abu-Izneid T., Nadeem M., Shariati M.A., Khan I.A., Imran A., Orhan I.E., Rizwan M., Atif M., Gondal T.A. and Mubarak M.S. (2019). Luteolin, a flavonoid, as an anticancer agent: A review. Biomed. Pharmacother. 112, 108612.

Ismail O.Z. and Bhayana V. (2017). Lipase or amylase for the diagnosis of acute pancreatitis? Clin. Biochem. 50, 1275-1280.

Jaramillo-Rangel G., Chávez-Briones M.D., Niderhauser-García A. and Ortega-Martínez M. (2020). Toxicity and anticancer potential of Karwinskia: A review. Molecules 25, 5590.

Lansiaux A., Laine W., Baldeyrou B., Mahieu C., Wattez N., Vezin H., Martinez F.J., Piñeyro A. and Bailly C. (2001). DNA topoisomerase II inhibition by peroxisomicine A(1) and its radical metabolite induces apoptotic cell death of HL-60 and HL-60/MX2 human leukemia cells. Chem. Res. Toxicol. 14, 16-24.

Lu J., He Y., Cui L., Xing X., Liu Z., Li X., Zhang H., Li H., Sun W., Ji A., Wang Y., Yin H. and Li C. (2020). Hyperuricemia predisposes to the onset of diabetes via promoting pancreatic β-cell death in uricase-deficient male mice. Diabetes 69, 1149-1163.

Luchini C., Capelli P. and Scarpa A. (2016). Pancreatic ductal adenocarcinoma and its variants. Surg. Pathol. Clin. 9, 547-560.

Luchini C., Grillo F., Fassan M., Vanoli A., Capelli P., Paolino G., Ingravallo G., Renzulli G., Doglioni C., D'Amuri A., Mattiolo P., Pecori S., Parente P., Florena A.M., Zamboni G. and Scarpa A. (2020). Malignant epithelial/exocrine tumors of the pancreas. Pathologica 112, 210-226.

Müller L., Schütte L.R.F., Bücksteeg D., Alfke J., Uebel T. and Esselen M. (2021). Topoisomerase poisoning by the flavonoid nevadensin triggers DNA damage and apoptosis in human colon carcinoma HT29 cells. Arch. Toxicol. 95, 3787-3802.

Naeimi M., Shafiee M., Kermanshahi F., Khorasanchi Z., Khazaei M., Ryzhikov M., Avan A., Gorji N. and Hassanian S.M. (2019). Saffron (*Crocus sativus*) in the treatment of gastrointestinal cancers: Current findings and potential mechanisms of action. J. Cell. Biochem. 120, 16330-16339.

Piñeyro-López A., Martínez de Villarreal L. and González-Alanís R. (1994). *In vitro* selective toxicity of toxin T-514 from *Karwinskia humboldtiana* (buckthorn) plant on various human tumor cell lines. Toxicology 92, 217-227.

Riccio A.A., Schellenberg M.J. and Williams R.S. (2020). Molecular mechanisms of topoisomerase 2 DNA-protein crosslink resolution. Cell. Mol. Life Sci. 77, 81-91.

Scavini M., Dugnani E., Pasquale V., Liberati D., Aleotti F., Di Terlizzi G., Petrella G., Balzano G. and Piemonti L. (2015). Diabetes after pancreatic surgery: Novel issues. Curr. Diab. Rep. 15, 16.

Schawkat K., Manning M.A., Glickman J.N. and Mortele K.J. (2020). Pancreatic ductal adenocarcinoma and its variants: Pearls and perils. Radiographics 40, 1219-1239.

Scholten L., Stoop T.F., Del Chiaro M., Busch O.R., van Eijck C., Molenaar I.Q., de Vries J.H., Besselink M.G. and Dutch Pancreatic Cancer Group (2019). Systematic review of functional outcome and quality of life after total pancreatectomy. Br. J. Surg. 106, 1735-1746.

Siegel R.L., Miller K.D., Fuchs H.E. and Jemal A. (2022). Cancer statistics, 2022. CA Cancer J. Clin. 72, 7-33.

Tang D., Kang R., Berghe T.V., Vandenabeele P. and Kroemer G. (2019). The molecular machinery of regulated cell death. Cell Res. 29, 347-364.

Titova A.A., Mavlikeev M.O., Kaligin M.S., Suleymanova D.M., Chekmaryeva I.A., Kiyasov A.P. and Deev R.V. (2020). Early ultra- and microstructural alterations in rat pancreas in alloxan-induced diabetes mellitus. Ultrastruct. Pathol. 44, 61-70.

Yeh H.C., Golozar A. and Brancati F.L. (2018). Cancer and diabetes. In: Diabetes in America. 3rd ed. Chapter 29. Cowie C.C., Casagrande S.S., Menke A., Cissell M.A., Eberhardt M.S., Meigs J.B., Gregg E.W., Knowler W.C., Barrett-Connor E., Becker D.J., Brancati F.L., Boyko E.J., Herman W.H., Howard B.V., Narayan K.M.V., Rewers M. and Fradkin J.E. (eds). National Institute of Diabetes and Digestive and Kidney Diseases (US). Bethesda (MD).

Yin J., Che G., Jiang K., Zhou Z., Wu L., Xu M., Liu J. and Yan S. (2022). Ciclopirox olamine exerts tumor-suppressor effects via topoisomerase II alpha in lung adenocarcinoma. Front. Oncol. 12, 791916.

Wu L., Nahm C.B., Jamieson N.B., Samra J., Clifton-Bligh R., Mittal A. and Tsang V. (2020). Risk factors for development of diabetes mellitus (Type 3c) after partial pancreatectomy: A systematic review. Clin. Endocrinol. (Oxf). 92, 396-406.

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