AST and ALT were observed in the D+CCO group compared to D group, but not in cholesterol (COL), triglycerides (TG), creatinine (CREA) (Figure 1D-J). No significant changes were observed in the levels of malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) when comparing the D+CCO group with respect to D (Figure 2A-C), but there was a significant decrease in the expression of *Rela* and *Gpx* in the D+CCO group with respect to D (Figure 2 E and D).

Conclusions: CCO at the dose used and during the study period was not hepatotoxic, had a hypoglycemic effect from the first dose and decreased ALT, AST and BUN levels. CCO has an anti-inflammatory effect by decreasing *Rela* gene expression.

Ethical statement

The protocol was registered and approved by the Ethics Committee.

Declaration of interests

None

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

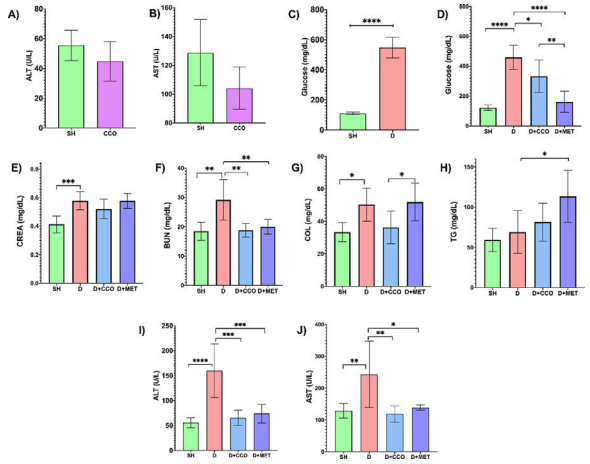

Figure 1. Results of the biochemical markers in the different study groups. (A and B) ALT and AST levels in SH groups and D. (C) Glucose levels after one week of administration with alloxane. (D-J) Glucose, CREA, BUN, COL, TG, ALT and AST levels in the various study groups after the administration period. Values are expressed as mean ± SD. P values: * p<0.05, ** p<0.01, *** p<0.001 and **** p<0.001.

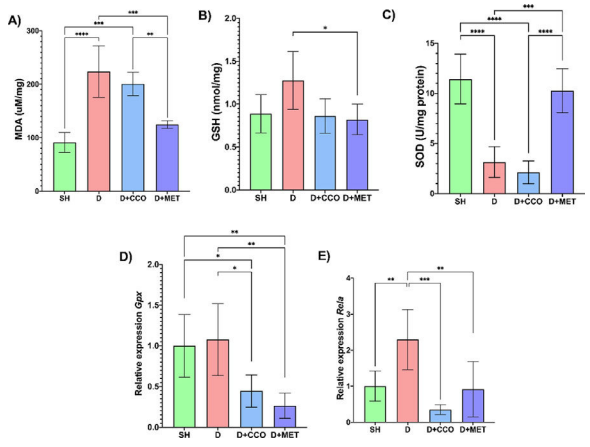

Figure 2. Results of oxidative stress and gene expression markers in the different study groups. (A, B and C) MDA, GSH and SOD oxidative stress markers. (E and D) Relative expression of *Gpx* and *Rela* in the various study groups. Values are expressed as mean ± SD. P values: * p<0.05, ** p<0.01, *** p<0.001 and **** p<0.001.

https://doi.org/10.1016/j.aohep.2024.101398

Hepatoprotective effects of N-acetylcysteine prevents hepatocellular carcinoma development induced experimentally.

Fernando Caloca-Camarena[1,2], Hugo C. Monroy-Ramirez[1], Scarlet Arceo-Orozco[1], Marina Galicia-Moreno[1], Juan Armendáriz-Borunda[1]

[1] *Instituto de Biología Molecular en Medicina y Terapia Génica, Departamento de Biología Molecular y Genómica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara 44340, Jalisco, México*
[2] *Programa de Doctorado en Farmacología, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara 44340, Jalisco, México*

Introduction and Objectives: Hepatocellular carcinoma (HCC) development involves imbalance of cellular processes such as oxidative stress, inflammation, fibrogenesis and cell proliferation. N-acetylcysteine (NAC) is an effective drug used clinically to treat drug-induced liver injury, but its ability to modulate molecular mechanisms activated during HCCestablishment is unknown.

This study aimed to evaluate antioxidant, antifibrogenic, and antiproliferative NAC properties in the HCC induced experimentally.

Materials and Patients: Male Fisher 344 rats divided into 3 groups: 1. Control (CTL); 2. HCC: Diethylnitrosamine (DEN) + 2-acetylaminofluorene (2-AAF). 3. HCC/NAC: DEN+2-AAF and NAC. Liver damage, oxidative stress, fibrogenesis and proliferation markers were evaluated by colorimetrics methods, Western blot, Dot blot, immunofluorescence, immunohistochemistry, respectively. H&E and Masson's Trichrome stains were also performed. This project was conducted in accordance with the guidelines of the University of Guadalajara under the approval number of the bioethics, research, and ethics research committees CI-01723.

Results: NAC exerts hepatoprotective effects, by preserving hepatic micro and macrostructure, slowing dysplastic nodules formation, and preventing an increase in ALT and GGT enzymatic activity. This drug also is able to exert anti fibrogenic effects by repressing extracellular matrix accumulation through to inhibition of α-SMA and TFG-β expression. Likewise, NAC demonstrated antiproliferative capacity by reducing Glypican-3 and Ki-67 expression.

Furthermore, NAC exerts its antioxidant effects by regulating Nrf2 signaling pathway, modulating CAT and SOD expression, and GSH levels. Finally, this drug prevents DNA oxidative damage through increasing enzyme 8-oxoguanine-DNA glycosylase (OGG1/2) expression, and therefore, reducing 8-oxoguanine (8oxoG) levels.

Conclusions: In this work, we demonstrate that NAC exerts antioxidant, antifibrogenic and antiproliferative effects useful in the prevention of the development of this disease. It is necessary to carry out additional analyzes that allow a more precise clarification of NAC hepatoprotective mechanisms, and that allow it to be repositioned as an adjuvant therapy in HCC treatment.

Ethical statement

All experimental procedures were approved by the Research Committee, the Research Ethics Committee, and the Biosafety Committee of the University of Guadalajara, with the approval number CI-01723.

Declaration of interests
None

Funding
This research was partially financed by Programa de Fortalecimiento de Institutos, Centros y Laboratorios de Investigación 2022, Universidad de Guadalajara to J. A.-B.; Programa de Impulso a la Investigación-2021-II (PIN-2021-II) to M. G.-M., and PhD scholarship from CONACyT to F. C.-C.

https://doi.org/10.1016/j.aohep.2024.101399

Pirfenidone slows the development of fibrosis and malignant neoplasms by modulating inflammation in an experimental model of hepatocarcinoma.

Scarlet Arceo-Orozco[1], Fernado Caloca-Camarena[1], Marina Galicia-Moreno[1], Hugo C. Monroy-Ramírez[1], Juan Armendáriz-Borunda[1,2]

[1] *Instituto de Biología Molecular en Medicina y Terapia Génica, Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Guadalajara, México*
[2] *Escuela de Medicina y Ciencias de la Salud, Tecnológico de Monterrey campus Guadalajara, Zapopan, México*

Introduction and objectives: Hepatocellular carcinoma (HCC) is the most common liver neoplasm in the world. Inflammatory, oxidative and fibrogenic processes are key in tumor development and propagation. Pirfenidone (PFD) has been shown to have hepatoprotective, anti-fibrogenic and immunomodulatory properties during hepatocarcinogenesis. However, its effect on established HCC is unknown. Our aim is to evaluate the effect of PFD administration on the tumor and inflammatory microenvironment in an experimental hepatocellular carcinoma model.

Materials and Patients: Fischer-344 rats (n=18) protocolized into three groups: CTL: control, HCC: damage group, (induced by diethylnitrosamine (DEN) 50 mg/kg and 2-acetaminofluorene (2AAF) 25mg/kg/weekly for 16 weeks), HCC/PFD: damage group + administration of PFD 300 mg/kg/daily. Subsequently, immunoassays and histological analyzes were performed to assess inflammatory patterns, fibrosis, and malignancy.

All animals received human care, and all the experiments were performed according to the Guide for the Care and Use of Laboratory Animals, under the approval of the Research, Ethics, and Biosafety committees of the CUCS whit approval number CI-03020.

Results: In the HCC/PFD group, the observed nodules were smaller in number, size, and protrusion compared to the HCC group. Additionally, there was a decrease in fibrosis development, extracellular matrix synthesis, as well as collagen and α-SMA expression. The loss of hepatic architecture was restored, and there was a decrease in the percentage of transformed hepatocytes positive for Glypican-3 expression, in contrast to the HCC group. Furthermore, there was a restoration of p53 expression. Moreover, the local secretome showed a decrease in IL-10 and an increase in IL-6 and IL-1β compared to the HCC group. Finally, the expression and localization of CD45 and CD161 were observed to be increased within the tumor niches compared to the HCC group.

Conclusions: Treatment with PFD slows the development of both macroscopic and microscopic patterns of malignancy and fibrosis, decreases the activation of hepatic stellate cells, the local inflammatory secretome, and modulates the components of the tumor microenvironment, thus improving the conditions and progression of the neoplasia. Therefore, pirfenidone could mean an improvement in the quality of life and an increase in the survival of patients with HCC in advanced stages.

Ethical statement
The protocol was registered and approved by the Ethics Committee.

Declaration of interests
None

Funding
Programa de Fortalecimiento de Institutos, Centros y Laboratorios de Investigación 2022, otorgado al Instituto de Biología Molecular en Medicina y Terapia Génica Programa para el
Desarrollo Profesional Docente, para el Tipo Superior (PRODEP), Apoyo a Nuevos
Profesores de Tiempo Completo, 511-6/2020-8586 PTC-1534. Fondo para Proyectos de
Impulso a la Investigación (PIN 2020- I).

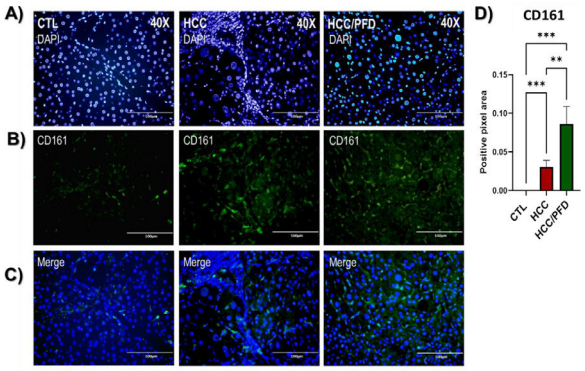

Figure 2. Immunofluorescence for CD161 detection. In the blue channel (A), cell nuclei stained whit DAPI. In the green channel (B), Alexa-green for CD161. (C) Merge. It is observed that the PFD treatment increased the infiltration of CD161-positive cells, whit co-localization in tumor nice centers, suggesting enhanced cytotoxic activity by these cells. (D) Quantification of positive pixel areas, showing a higher positive area in the PFD-treated group. One-way ANOVA analysis considered statistically significant at $p<0.05$, $^{*}p<0.5$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$.

https://doi.org/10.1016/j.aohep.2024.101400

Pirfenidone induces translocation of sirt1 to nucleus and deacetylation of histone 3 slows down the development of fibrosis and tumorigenicity in a experimental model of hepatocarcinoma.

Hugo C. Monroy-Ramírez[1], Scarlet Arceo-Orozco[1], Araceli G. Rivera-Cabral[1], Marina Galicia-Moreno[1], Juan Armendáriz-Borunda[1,2]

[1] *Instituto de Biología Molecular en Medicina y Terapia Génica, Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Guadalajara, México*
[2] *Escuela de Medicina y Ciencias de la Salud, Tecnológico de Monterrey campus Guadalajara, Zapopan, México*

Introduction and objectives: Hepatocellular carcinoma (HCC) is the most common liver neoplasm worldwide. Pro-inflammatory and