NAMPT INHIBITION AND INCREASED NAD-BIOAVAILABILITY ATENUATE LIVER DAMAGE IN CCl4-INDUCED MICE LIVER FIBROSIS

Juan Suarez-Cuenca[1], Ariel Gutierrez Buendia[1], Rebeca Pérez-Cabeza de Vaca[2], Eduardo Vera-Gómez[2], Gabriela Domínguez-Pérez[2], Alejandro Hernández-Patricio[2], Karen De la Vega-Moreno[2], Moisés Salamanca-García[2], Ramón Coral-Vázquez[2], Christian Toledo-Lozano[2], Silvia García[3], Paul Mondragón-Terán[2], and Rolondo Hernández-Muñóz[4]

[1]Centro Medico Nacional 20 de Noviembre
[2]Centro Médico Nacional 20 de Noviembre
[3]National Medical Centre 20 de Noviembre
[4]Universidad Nacional Autonoma de Mexico Instituto de Fisiologia Celular

February 1, 2023

Abstract

Background and Purpose: Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme within the NAD+ salvage biosynthetic pathway and also a cytokine modulator with effects on inflammation and fibrogenesis. FK866 (NAMPT inhibitor) has shown anti-inflammatory properties. However, FK866-induced depletion of NAD+ may also impair liver bioenergetic metabolism during Chronic Liver Disease (CLD). The aim of this study was to evaluate the effects of NAMPT inhibition and NAD+ restoration on experimental CLD. Experimental Approach. CCl4-induced liver cirrhosis was established after 6 weeks. Treatment groups (n=5 mice per group) included: 1) vehicle; 2) CCl4 control; 3) Silymarin + CCl4; 4) FK866 + CCl4; and 5) NMN+FK866+CCl4. At the end of the experimental induction and treatments, mice were sacrificed and liver was collected for weight, and paraformaldehyde fixed for histological characteristics of inflammation and fibrosis, as well as NAD+/NADH determination by colorimetric assay. Liver function tests were performed from blood sample. Statistical analysis was performed by T-test. Key Results. NAMPT inhibition resulted in a mild attenuation of histological and biochemical features of the CCl4-induced liver damage. NAD+ restoration, by the concomitant administration of its precursor NMN, resulted in a significant improvement of the histological characteristics; evidenced by a lower inflammatory infiltrate and fibrosis as well as lower levels of bilirubin. NAMPT inhibition and adequate NAD+ restoration were confirmed by a colorimetric assay of NADH and NAD+ and biochemical features were measured by routinary Liver Function Tests. Conclusion and implications. This study shows that NAMPT inhibition concomitant to NAD restoration significantly attenuate experimental liver damage.

NAMPT INHIBITION AND INCREASED NAD-BIOAVAILABILITY ATENUATE LIVER DAMAGE IN CCl_4-INDUCED MICE LIVER FIBROSIS

Suárez-Cuenca Juan Antonio[1], Gutiérrez-Buendía Juan Ariel[1], Pérez-Cabeza de Vaca Rebeca[1], Vera-Gómez Eduardo[1], Domínguez-Pérez Gabriela Alexandra[1], Hernández-Patricio Alejandro[1], De la Vega-Moreno Karen[1], Salamanca-García Moisés[2], Coral-Vázquez Ramón Mauricio[3], Christian Gabriel Toledo-Lozano[1], García Silvia[1], Mondragón-Terán Paul[4], Hernández-Muñoz Rolando[5].

[1] Experimental Metabolism and Clinical Research Laboratory, Clinical Research Department. Centro Médico Nacional "20 de Noviembre", ISSSTE. Mexico City, Mexico.

[2] Pathology Department, Centro Médico Nacional "20 de Noviembre", ISSSTE. Mexico City, Mexico.

[3] Subdirección de Enseñanza e Investigación, Centro Médico Nacional "20 de Noviembre", ISSSTE. Mexico City, Mexico.

[4] Regenerative Medicine and Tissue Engineering Laboratory; Coordination of Research. Centro Médico Nacional "20 de Noviembre", ISSSTE. Mexico City, Mexico.

[5] Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, UNAM, Ciudad Universitaria/Circuito Exterior, C.P. 04510, Ciudad de México, México.

***Corresponding author:** Juan Antonio Suárez-Cuenca, MD, PhD. Experimental Metabolism and Clinical Research Laboratory, Clinical Research Department, Centro Médico Nacional "20 de Noviembre". ISSSTE. Av. San Lorenzo 502, Col. del Valle, C.P. 03100, México City, Mexico. Phone: +5255-52005003 Ext: 14661. E-mail address: suarej05@gmail.com

Funding: Budget Program E015 - Support for Scientific and Technological Research. Funder: Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado.

ABSTRACT

Background and Purpose : Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme within the NAD+ salvage biosynthetic pathway and also a cytokine modulator with effects on inflammation and fibrogenesis. FK866 (NAMPT inhibitor) has shown anti-inflammatory properties. However, FK866-induced depletion of NAD^+may also impair liver bioenergetic metabolism during Chronic Liver Disease (CLD). The aim of this study was to evaluate the effects of NAMPT inhibition and NAD^+ restoration on experimental CLD.

Experimental Approach. CCl_4-induced liver cirrhosis was established after 6 weeks. Treatment groups (n=5 mice per group) included: 1) vehicle; 2) CCl_4 control; 3) Silymarin + CCl_4; 4) FK866 + CCl_4; and 5) NMN+FK866+CCl_4. At the end of the experimental induction and treatments, mice were sacrificed and liver was collected for weight, and paraformaldehyde fixed for histological characteristics of inflammation and fibrosis, as well as NAD+/NADH determination by colorimetric assay. Liver function tests were performed from blood sample. Statistical analysis was performed by T-test.

Key Results . NAMPT inhibition resulted in a mild attenuation of histological and biochemical features of the CCl_4-induced liver damage. NAD+ restoration, by the concomitant administration of its precursor NMN, resulted in a significant improvement of the histological characteristics; evidenced by a lower inflammatory infiltrate and fibrosis as well as lower levels of bilirubin. NAMPT inhibition and adequate NAD^+restoration were confirmed by a colorimetric assay of NADH and NAD^+ and biochemical features were measured by routinary Liver Function Tests.

Conclusion and implications. This study shows that NAMPT inhibition concomitant to NAD restoration significantly attenuate experimental liver damage.

KEYWORDS: Chronic Liver Disease (CLD), Nicotinamide Phosphoribosyltransferase (NAMPT), Nicotinamide Mononucleotide (NMN), Nicotinamide Adenine Dinucleotide (NAD), Liver Fibrosis, Oxidative Stress, Carbon Tetrachloride (CCl_4).

What is already known

NAMPT has been related to inflammatory and fibrogenic cytokines.

NAMPT is a rate-limiting enzyme on the NAD^+biosynthetic pathway.

What this study adds

NAMPT as a regulator of proinflammatory and profibrogenic processes within experimental chronic liver disease.

Pharmacological depletion of NAMPT and NAD-recovery *in vivo*atenuates damage response during chronic liver disease.

Clinical significance

NAMPT inhibition concomitant to NAD recovery significantly attenuated histological damage in a murine CLD model.

NAMPT may be an interesting therapeutical target for CLD.

INTRODUCTION

Liver cirrhosis is the end-stage of Chronic Liver Disease (CLD) and represents an increasing major public health problem with a high global incidence and prevalence, with a potentially fatal outcome for up to 1.5 billion persons with CLD and 2 million deaths worldwide each year, and a broad series of related diseases and complications, including liver cirrhosis and hepatocellular carcinoma [1] Main etiologies for this disease are related to alcohol consumption, viral chronic hepatitis, hepatotoxic drugs and non-alcoholic steatohepatitis (NASH) [2].

This agents, may cause liver tissue damage and dysfunction by chronic hepatocyte damage and hepatic stellate cells activation, both related to mitochondrial dysfunction [3,4]. In fact, mitochondrial abnormalities like ultrastructural lesions, depletion of mitochondrial DNA, impaired respiratory capacity, proton leakage, increased oxidative stress and impaired β-oxidation have been described during inflammatory progression in non-alcoholic fatty liver disease [5]

Mitochondrial requirements for energy production are provided by different biochemical sources such as Nicotinamide adenine dinucleotide (NAD^+) synthesis [6].

NAD^+ is a fundamental co-enzyme that catalizes cellular redox reactions in diverse processes to maintain various metabolic functions, such as glycolisis, fatty acids beta oxidation or the tricarboxylic acid cycle, among others [7].

NAD impairment may affect mitochondrial function by triggering a series of biochemical alterations, thus, causing a disequilibrium in the redox homeostasis of the cell with its various implications on metabolic processes and its pathohysiological consequeces.

Furthermore, potential regulation of oxidative stress driven by NAD is suggested from studies demonstrating that NAD precursors, such as Nicotinamide Mononucleotide (NMN) and Nicotinamide Riboside (NR), are capable to enhance oxidative metabolism through mitochondrial activation within hepatocytes, resulting in a beneficial effect for experimental CLD. [8,9]

The main biosynthetic pathway of NAD+ in mammals is the salvage pathway from which nicotinamide is "rescued" and then used again on the cycle. Nicotinamide is converted to NMN by nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in this pathway. Then, NMNATs convert NMN into NAD^+. Within this pathway, NAMPT plays a crucial role by its participation in cellular NAD^+ levels regulation (FIGURE 1) [7]

NAMPT is a protein involved in the regulation of multiple processes, including necroptosis, oxidative stress, innate immunity and cytokine expression [10,11]. At the same time, NAMPT constitutes a key enzyme during NAD biosynthesis; acting as a regulator of the intracellular NAD pool, particularly relevant for diverse functions and pathologies. [6,12,13]

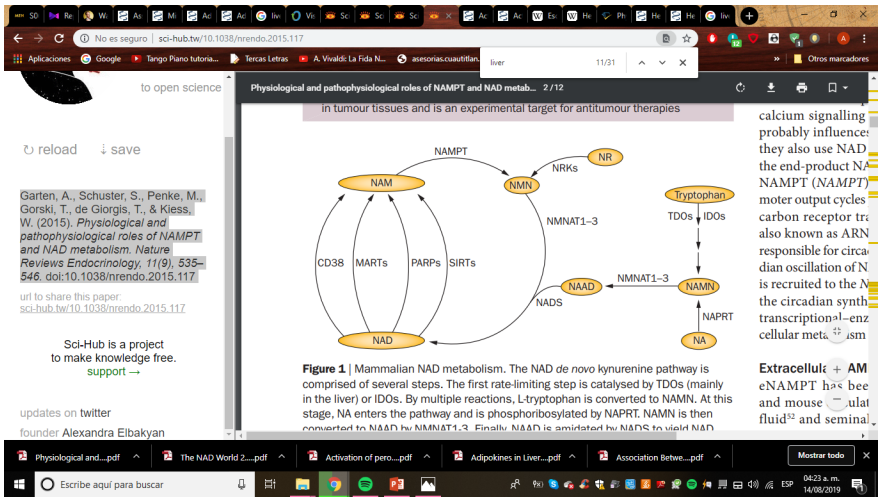


Figure 1. NAD biosynthetic pathways. Garten, A. et al. (2015). *Physiological and pathophysiological roles of NAMPT and NAD metabolism. Nature Reviews Endocrinology, 11(9), 535–546.*

Concomitant to the effects on NAD, several activities, have been attributed to NAMPT acting as a cytokine mediator, particularly on inflammatory and immunological processes, with potential effects in the pathophysiology of CLD [14].

Consistently, studies have shown an upregulated production of NAMPT during the inflammatory response of CLD, with the consecutive amplification of the inflammation through the increased production of cytokines like IL-1β, TNF-α, IL-6 and IL-10 [15,16]

NAD+ biosynthesis has been studied as a potential therapeutic target for treating some types of cancer and inflammatory diseases via NAMPT inhibition. The nicotinamide analog FK866 is a competitive inhibitor of NAMPT, and the resulting NAD+ depletion has been associated with lower levels of tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6) as well as neutrophil infiltration. [17]

However, the role of NAMPT and NAD^+ within CLD remains uncertain. Therefore, this study aimed to determine the effects of the pharmacological inhibition of NAMPT and NAD repletion *in vivo* in an experimental liver fibrosis model.

MATERIALS AND METHODS

Reagents

Carbon Tetrachrloride – CCl_4 [Sigma – Aldrich MO, USA]; FK866 [Cayman Chem. Co; MI, USA]; Nicotinamide Mononucleotide- NMN [Sigma – Aldrich MO, USA], Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, U.S.); Silymarin [Sigma – Aldrich MO, USA].

Animals

Male Balb/c mice were acquired from the Cellular Physiology Institute – UNAM certificated house breeding (SAGARPA-SENASICA B00.02.08.01.01.1955/2016). Were housed in the bioterium of the National Medical Center "20 de Noviembre"- ISSSTE and fed with regular chow and water *ad libitum* . All the experimental procedures were approved by the Bioethics and Biosafety Committee of the National Medical Center "20 de Noviembre" – ISSSTE (Authorized protocol number: 305.2017).

Treatment

The mice were randomly divided into groups (5 mice per group). Control group, CCl_4 group, Silymarin + CCl_4group; FK866+CCl_4 group and NMN+FK866+CCl_4 group.

The Control group was injected twice/week with 800uL/kg of CCl_4 vehicle (vegetable oil) for 6 weeks; CCl_4 group received 800uLg/kg of $CCl_4$10% in vegetal oil twice/week for 6 weeks, as the rest of the groups with CCl_4; Silymarin + CCl_4 group received daily doses (100 mg/kg) of silymarin for 2 weeks. [FK866+CCl_4] group was administered with CCl_4 as previously described in addition of 10 mg/kg of FK866 (10% in NaCl 0.9% solution) daily for 15 days; [NMN+FK866+CCl_4] group received FK866 and CCl_4 as previous groups as well as 120 mg/kg of NMN twice/week for 3 weeks. All administrations were performed through intraperitoneal injection.

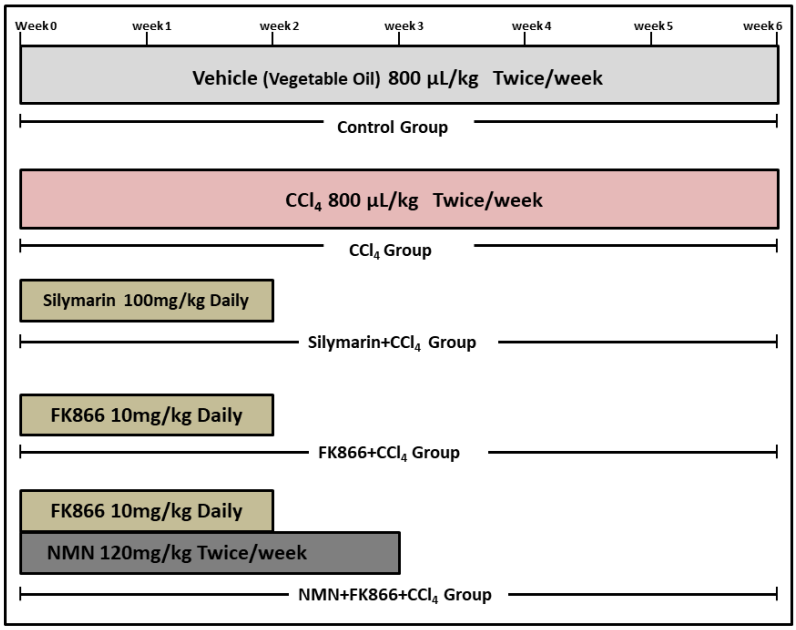


Figure 2. Schematic representation of the experimental schedule for the model induction and the pharmacological intervention.**CCl_4** : Carbon Tetrachloride; **Silymarin** : Hepatoprotective drug; **FK866** : NAMPT inhibitor; **NMN** : Nicotinamide Mononucleotide.

After treatments, all groups were sacrificed with pentobarbital overdose to collect liver and blood samples. Fresh liver was collected for weight determination, histological analysis and NAD+/NADH determination via colorimetric assay, standardized by protein concentration. Blood sample was collected via cardiac puncture; plasma was isolated to perform liver function tests.

BIOETHICS

All pharmacological interventions were performed accordingly to the Guide for the Care and Use of Laboratory Animals from the US National Institutes of Health [18] and euthanasia was based on ethical recommendations and practical specifications of the American Veterinary Medicine Association Guidelines for the Euthanasia of Animals, and all procedures were also accordingly to current Mexican regulation NOM-062-ZOO-1999 about technical specifications for production, care and use of laboratory animals.

HISTOLOGY AND LIVER FUNCTION

Liver sample was collected and fixed in paraformaldehyde 4% in 15mL conical tubes (Thermo Fisher Scientific Waltham, MA.) for H&E and Masson's trichromic staining to determine structural damage, inflammation and fibrotic tissue infiltration in the liver parenchyma by pathologic score.

Blood was obtained by cardiac puncture and collected in sterile syringes containing 1mL of 10% EDTA

solution to avoid coagulation, then plasma was obtained by centrifuging 15 min at 5,000 rpm (4°C) in a refrigerated centrifuge to remove the clot and plasma was stored at -70°C in 2mL microtubes to further determination of liver function tests determined using a standard auto-analyzer (SYNCHRON-CX9PRO Clinical System Beckman Coulter; California, USA).

INFLAMMATION AND FIBROSIS QUANTIFICATION

Both inflammation and fibrosis were quantified in Hematoxylin & Eosin and Masson's Trichrome-stained liver slices respectively by determining the amount of lymphocytes and collagen fibers infiltrates in the liver parenchyma using ImageJ software.

QUANTIFICATION OF NAD+ AND NADH LEVELS

To assess cellular NAD+ levels, the EnzyChromTM NAD/NADH Assay Kit (BioAssay Systems, Hayward, CA) was used following manufacturer recommendations. Another liver sample was homogenized with NAD and NADH buffer provided by the manufacturer and stored in 2.0mL microtubes (Eppendorf, Hamburg Germany) to further determination of NAD+ and NADH; after colorimetric determination; protein standardizations were performed by Pierce BCA Protein Assay Kit, accordingly to the specifications of the manufactu rer (Thermo Fisher Scientific; Waltham, MA).

Data and Statistical Analysis. Statistical analysis was performed with Prism Software (version 6.0; GraphPad Software Inc., La Jolla, CA). The significance of differences between two groups was determined by one-tailed T-test. Results are presented as mean & standard error of the mean (SEM). A p-value<0.05 was considered as significant.

RESULTS

Mice weight and Liver-to-body weight ratio

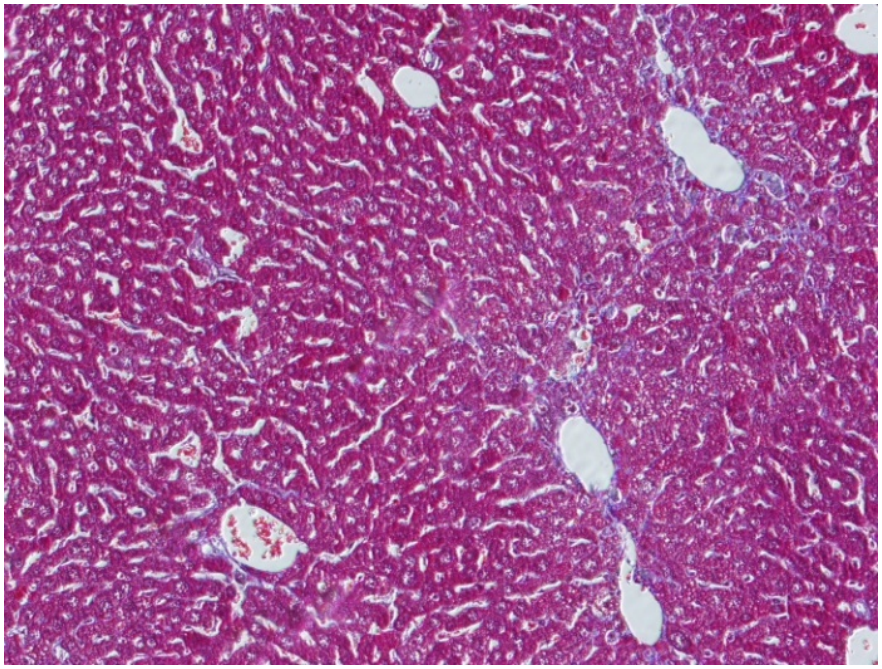
No significant variations of weight gain (Figure 3A) as well as liver-to-body weight ratio (Figure 3B) were observed during the induction of experimental CLD; although FK866-treated mice exhibited a trend for early weight loss, further recovered until the end of the induction (figure 3A).

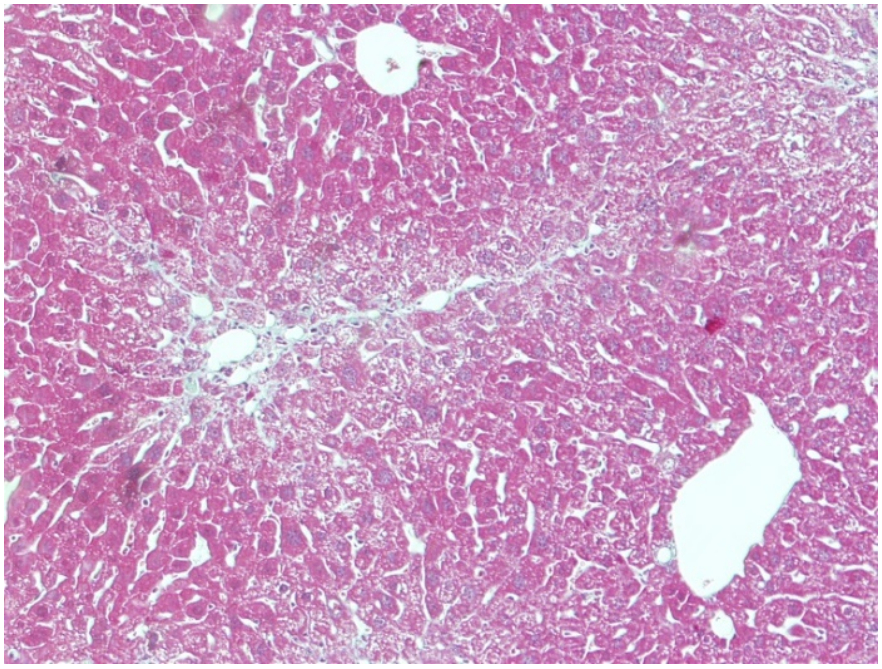
Figure 3. Weight during induction of CLD. Weight gain (A), as well as liver-to-body weigh ratio (B) during the induction of experimental CLD are shown. (*) = $p<0.05$, 2-tail T-Test.

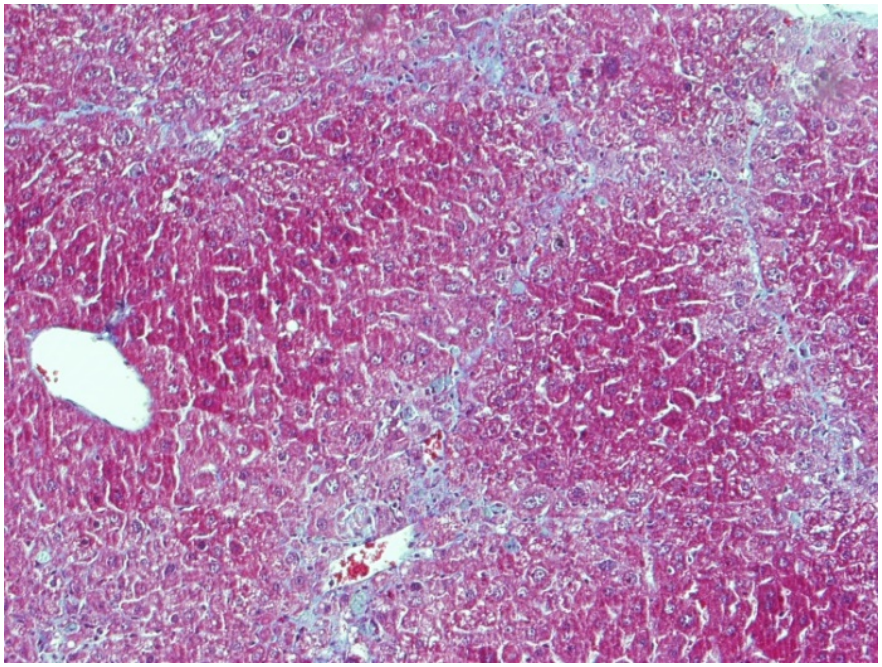
Histology and Damage Quantification

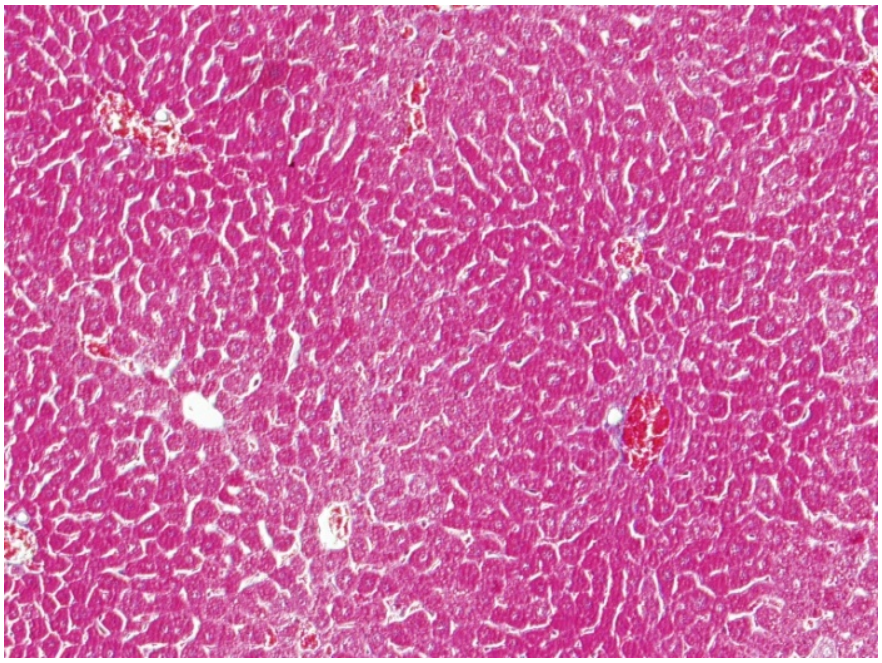
Histological liver damage induced by CCl_4 was characterized by a severe damage in the liver parenchyma, reflected by lymphocytes infiltration (Figure 3, H&E), perivascular necrosis and F3 fibrosis (Figure 2, Masson). Control groups treated with vegetal oil or silymarin showed lower lymphocytes infiltration with very low evidence of necrosis or fibrosis.

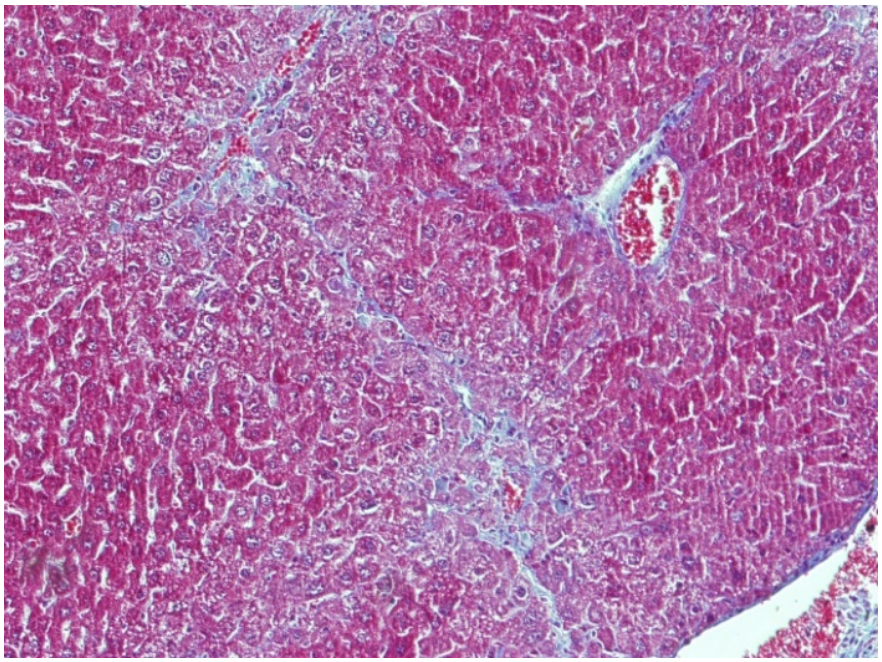
The CCl_4+FK866 group (pharmacological inhibition of NAMPT) showed significantly lower inflammation than CCl4 Group, there was also a mild reduction in the amount of fibrotic tissue, nevertheless there were no significant differences; whereas the group where NMN was added showed significantly lower lymphocyte infiltration and fibrotic tissue, similar to the effects observed on the silymarin group.

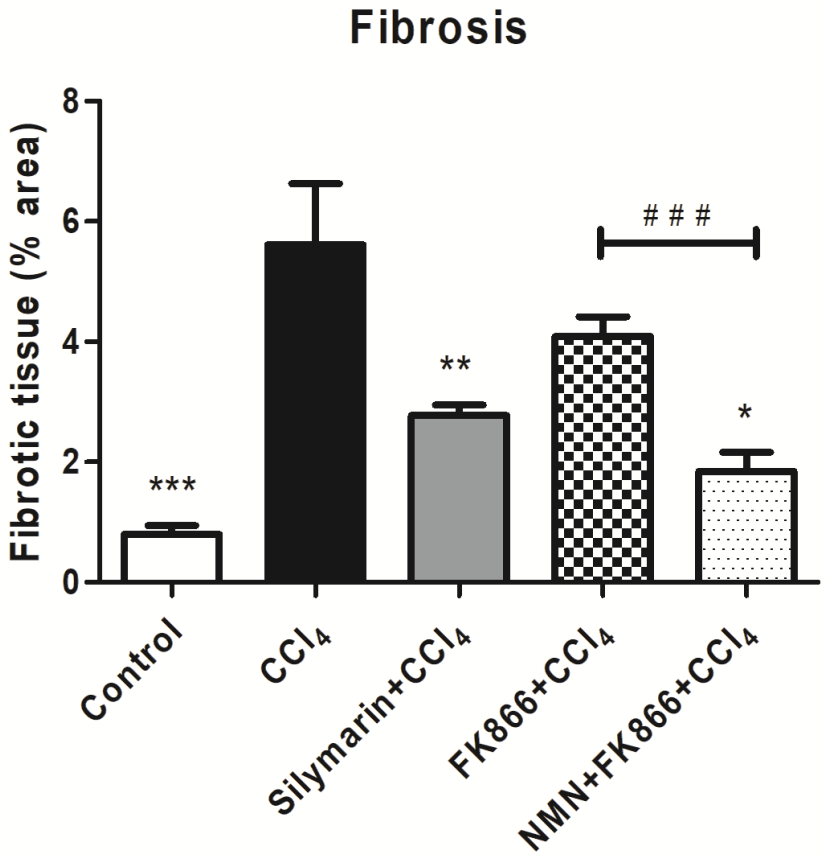


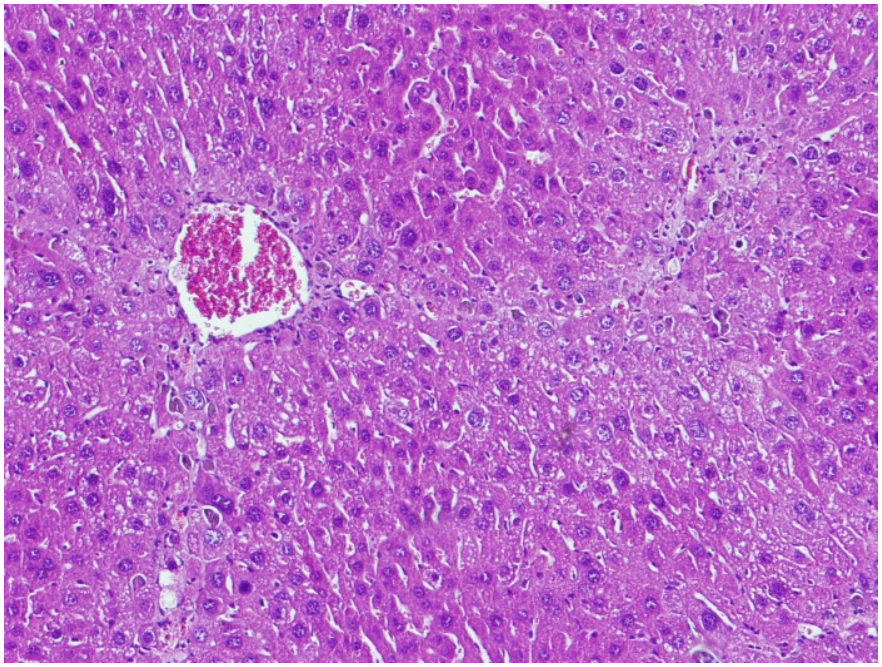


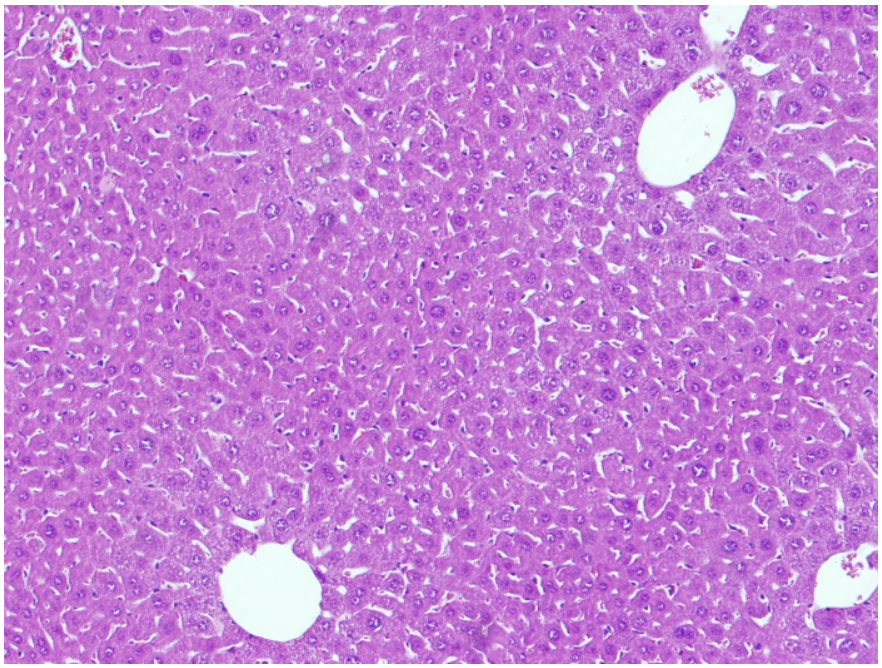


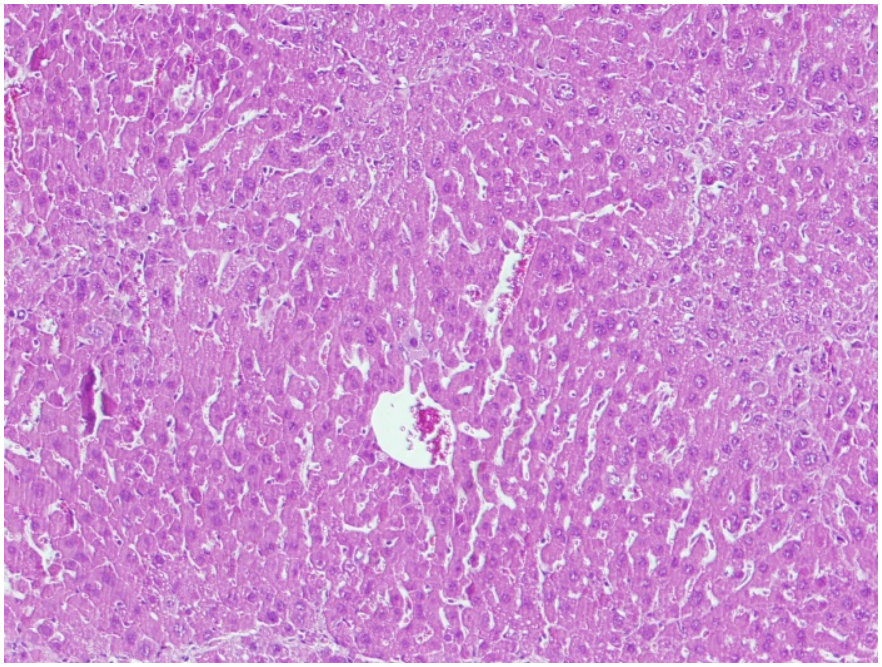


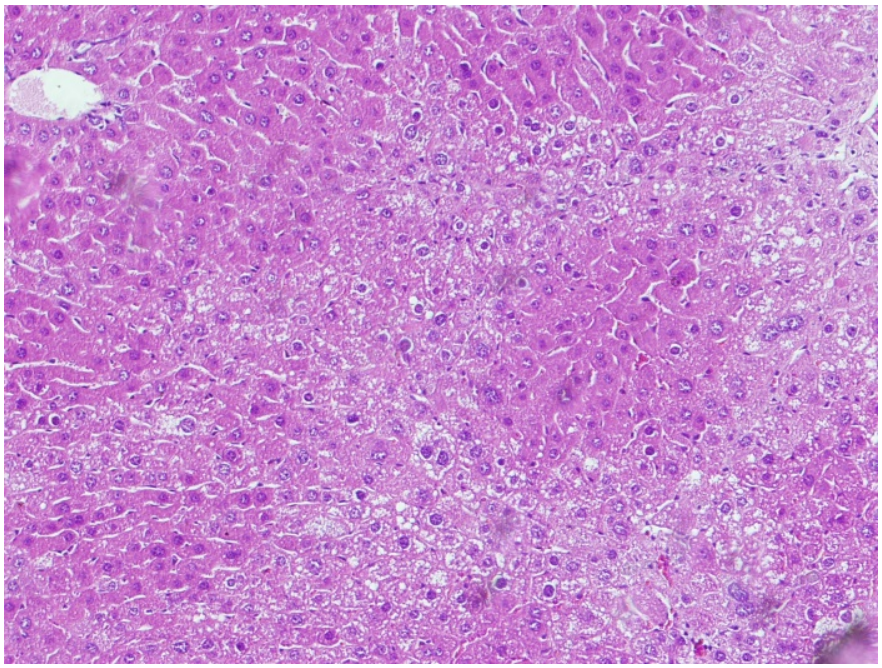


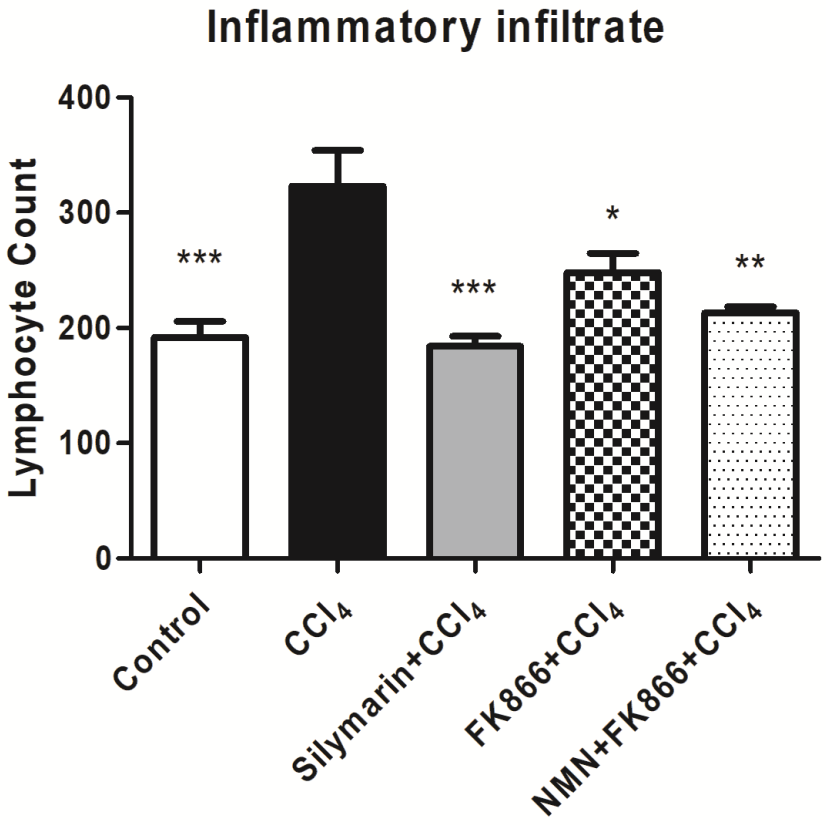


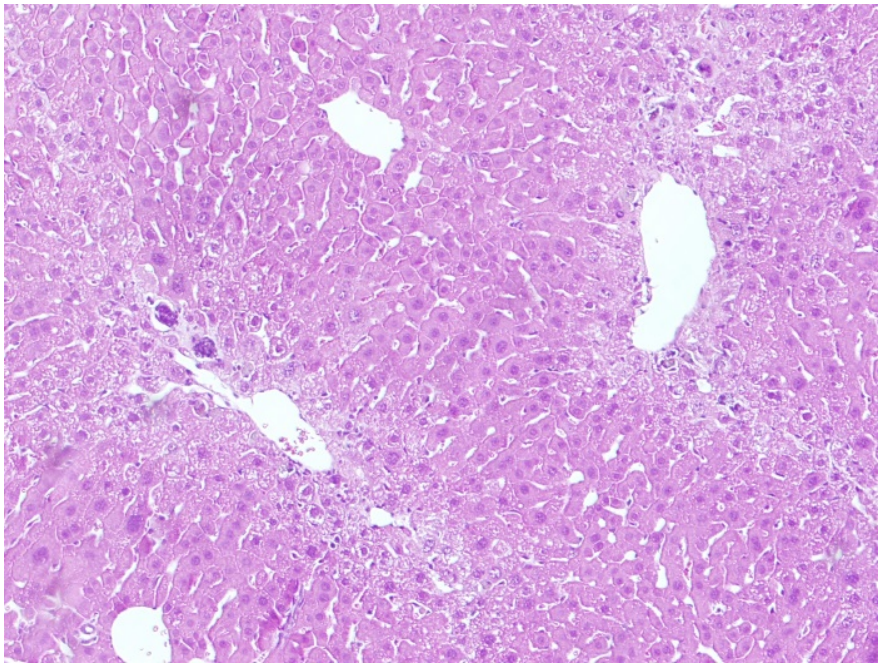






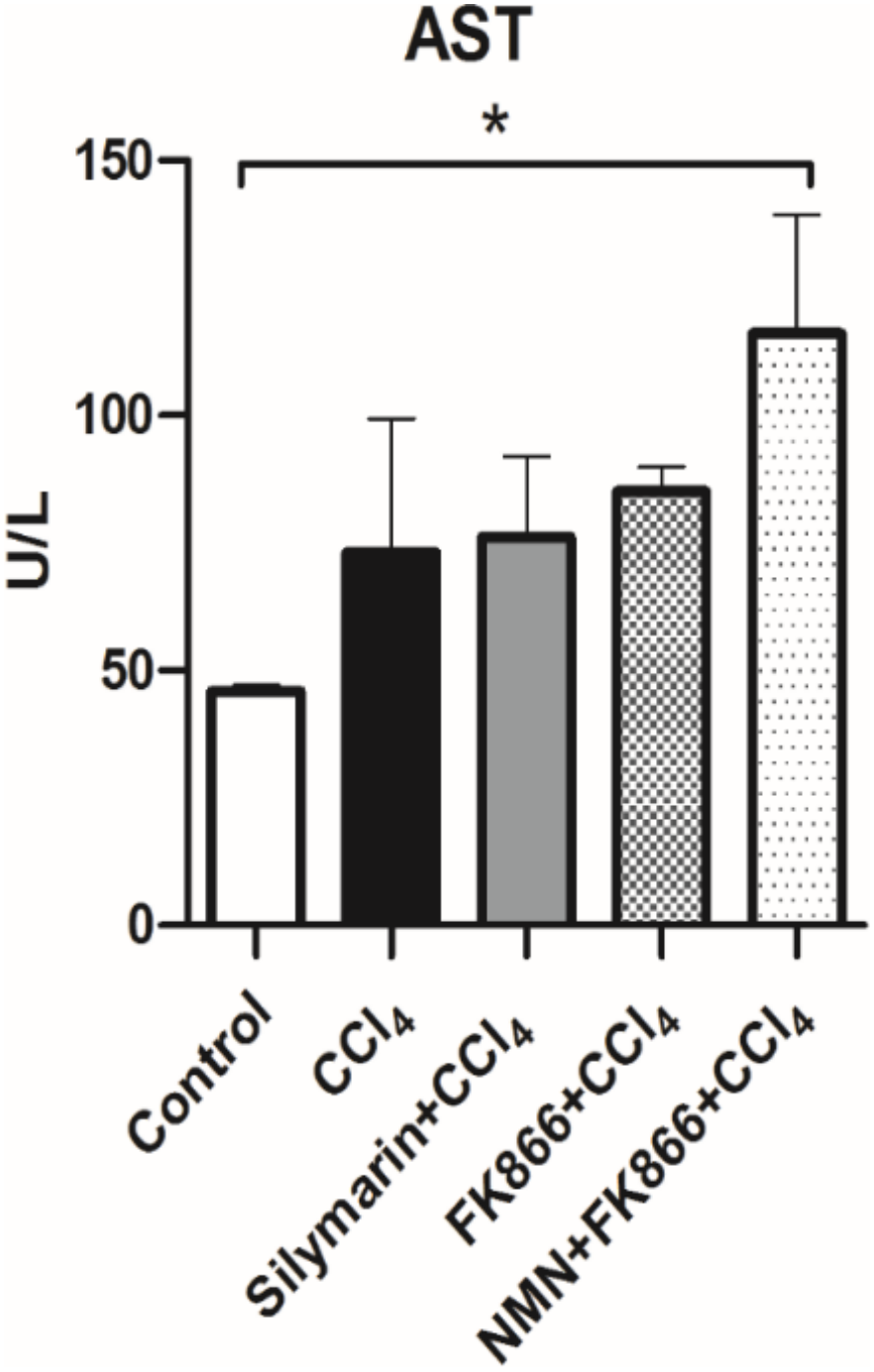






Liver Function Tests

Biochemical analysis in plasma showed an expected increase, of about 10-50 U/L, in aminotransferases in CCl_4-treated groups, with slightly higher increase associated with NMN treatment. Besides, CCl_4 significantly elevated direct bilirubin in 0.1mg/dL which was prevented by silymarin treatment. NAMPT inhibition with FK866 limited bilirubin increase, which was more significant when NMN was concomitantly used. Finally, CCl_4 treatment tended to decrease albumin levels, while no significant variations in albumin were observed as related with NAMPT inhibition (Figure 7).

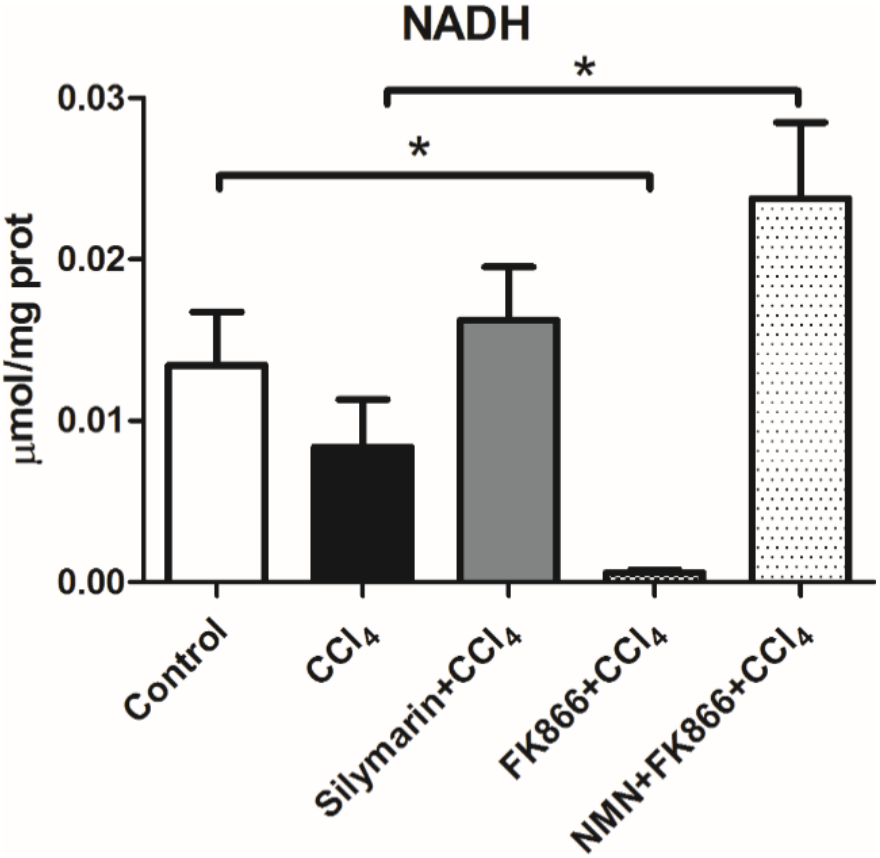


NAD+ and NADH liver determination

Treatment with CCl_4 decreased both NAD+ and NADH in liver tissue; whereas the concomitant use of Silymarin as a treatment against hepatic damage prevented hepatic NADH depletion.

Further experiments were performed to validate a reliable pharmacological inhibition of NAMPT as well as the repletion assay by increasing NAD+, NADH bioavailability by administrating NMN, which is an important precursor in the main biosynthetic pathway. As expected, significant depletion of NAD+ and

NADH was observed when FK866, the pharmacological NAMPT inhibitor was used, while NAD+ and NADH concentrations in the tissue were restored with concomitant use of NMN (Figure 3).



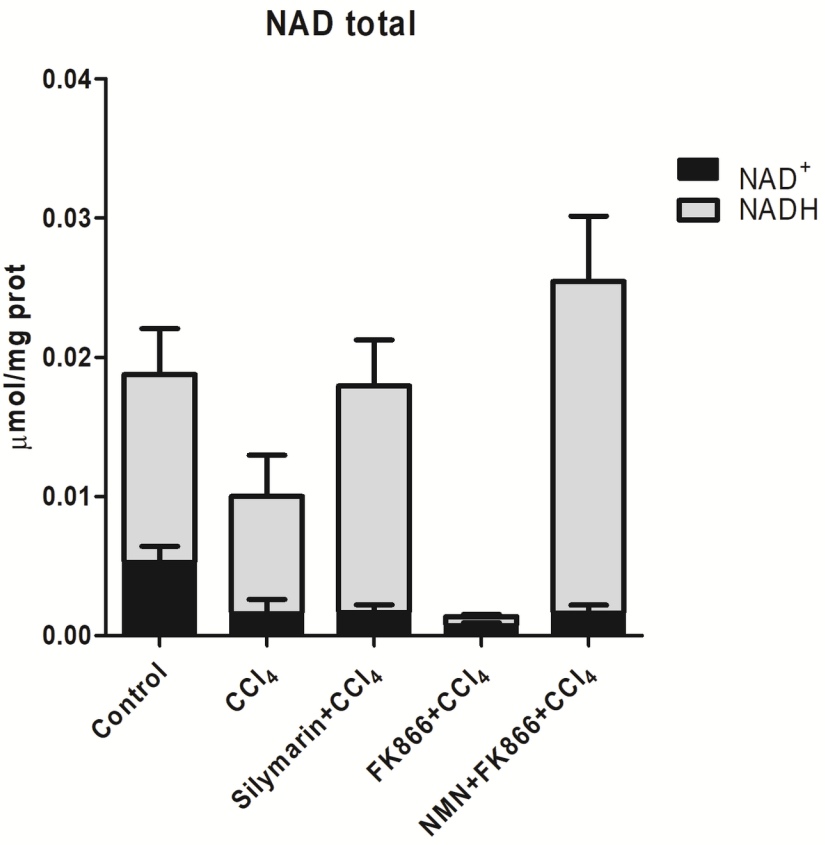


Figure 9 . Total NAD, showed as the sum of both NAD^+ and NADH contributions.

DISCUSSION

NAMPT is a pleiotropic protein known for its activity as a cytokine mediator, but also a relevant molecule implicated in NAD^+ biosynthesis. Therefore, it has a crucial biological role in cellular redox reactions and body homeostasis. NAMPT dysregulation has been related to the pathogenesis of hepatic disease; particularly cotributing to impaired NAD^+biosynthesis, dysregulated cellular oxidative stress and enzyme dysfunction. [11]

In the present study, we used an experimental model of chronic liver damage characterized by inflammation and fibrosis as well as biochemical changes reflecting significant structural and functional liver damage. In this model, the concomitant use of NAD^+ precursor NMN, prevented FK866-induced NAD depletion in the liver tissue, resulting in higher attenuation of histological and biochemical liver damage induced by CCl_4. NAMPT pharmacological inhibition and restoration strategies were reliable in this study. The validation assays performed demonstrate a significant decrease in the hepatic concentration of NAD^+ and NADH in the FK866-treated mice livers and after CCl_4-induced liver damage; as well as significant NAD^+ restoration by the administration of its precursor, NMN.

The hepatoprotective effect of NAD^+ recovery, with the use of its precursor NMN, highlights the relevance of maintaining adequate NAD^+ hepatic concentrations. This is consistent with observations that lower levels of NAD^+occur during chronic liver disease; which have been related to bioenergetic impairment, dysfunctional mitochondrial fatty-acid oxidation and higher ROS production [19]. NAD availability may show controversial effects in different organs. Consistent with our findings, increased hepatic concentration of NAD^+ has been

associated to salutary effects such as healthy aging and hepatic regeneration [20, 21]. Conversely, increased NAD^+has also been associated to the pathogenesis of cancer and other inflammatory diseases; suggesting that the final biological effect depends on the context of the cellular processes and energy use by the organism. In this sense, pharmacological control of NAD^+ synthesis represents an interesting therapeutic target in the treatment of several diseases [22]

On the other hand, NAMPT inhibition showed a mild limitation of liver damage in this model, which was lower than the effect of NMN. The responsible mechanism in this case may not be related with NAMPT's regulating effect on NAD^+ biosynthesis, but its activity as regulator of pro-inflammatory mediators, insulin resistance, obesity and/or metabolic disorders. This is suggested by the finding that the use of FK866, NAMPT inhibitor, exerted lower impact in attenuating liver damage, even though it significantly decreased NAD concentration. Consistently, FK866 has been associated with a decrease in TNFα, IL-6 levels and neutrophil infiltration, suggesting that such regulatory activity is responsible for limiting liver damage in this model [17, 23]. Moreover, there was an anti-inflammatory effect in the FK866-treated mice livers, which showed lower lymphocyte infltration than mice with CCl_4-induced damage. Accordingly, previous studies have suggested that FK866 administration may exhibit hepatoprotective effects on acute liver failure [24]. In this study, FK866 was evaluated within the context of a chronic liver damage; and it also exerted beneficial effects within CLD; proving NAMPT and NAD^+ biosynthetic pathway to be interesting approaches as potential therapeutical targets

An unexpected finding was the AST elevation in the NMN-treated group, concomitant to the improvement of histological features. One possible explanation may be attributed to a bias of measurement, since the technique to determine AST enzymatic activity involves the catalysis of transamination of aspartate and α-oxoglutarate to L-glutamate and oxalacetate, with further reduction of this last reactant to L-malate using **NADH** as co-factor increasing the chance of interference with the measurement method, and limiting the reliability of AST as marker of liver damage. However, the global effect was best reflected by other indicators like histological features and bilirrubin value.

CONCLUSION

This study demonstrated that NAMPT inhibition and concomitant NAD restoration significantly attenuate experimental liver damage. This effect is likely due to relation of NAMPT and NAD with pro-inflammatory mediators and energy homeostasis, respectively; indicating the potential of NAMPT and NAD as therapeutic target deserving future investigations. Further experiments are required to better characterize NAD-related processes and their hepatoprotective effect, such as mitochondrial function and other NAD-dependent pathways, like as PARPs and SIRTs.

REFERENCES

1. Moon, A. M., Singal, A. G., & Tapper, E. B. (2019). Contemporary Epidemiology of Chronic Liver Disease and Cirrhosis. Clinical Gastroenterology and Hepatology.
2. Ge, P.S.; Runyon, B.A. Treatment of patients with cirrhosis. N. Engl. J. Med. 2016, 375, 767–777.
3. Dall, M., Trammell, S., Asping, M., Hassing, A. S., Agerholm, M., Vienberg, S. G., Gillum, M. P., Larsen, S., & Treebak, J. T. (2019). Mitochondrial function in liver cells is resistant to perturbations in NAD^+ salvage capacity. *The Journal of biological chemistry* , *294* (36), 13304–13326. https://doi.org/10.1074/jbc.RA118.006756
4. Begriche K, Massart J, Robin MA, Bonnet F, Fromenty B. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. HEPATOLOGY 2013;58:1497-1507.
5. Koliaki C et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell Metab 2015;21:739-746.
6. Stein, L. R., & Imai, S. (2012). The dynamic regulation of NAD metabolism in mitochondria. Trends in Endocrinology & Metabolism, 23(9), 420–428. i:10.1016/j.tem.2012.06.005].
7. Johnson, S., & Imai, S. (2018). NAD+ biosynthesis, aging, and disease. F1000Research, 7, 132.
8. Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, et al. The NAD(+) precursor

nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab 2012;15:838-847.

9. Gariani, K., Menzies, K. J., Ryu, D., Wegner, C. J., Wang, X., Ropelle, E. R., ... Auwerx, J. (2015). Eliciting the mitochondrial unfolded protein response by nicotinamide adenine dinucleotide repletion reverses fatty liver disease in mice. Hepatology, 63(4), 1190–1204.
10. Zhang LQ, Heruth DP, Ye SQ: Nicotinamide phosphoribosyltransferase in human diseases. J Bioanal Biomed 2011, 3:13e25
11. Zhang, L. Q., Nsumu, M., Huang, P., Heruth, D. P., Riordan, S. M., Shortt, K., ... Ye, S. Q. (2018). Novel Protective Role of Nicotinamide Phosphoribosyltransferase in Acetaminophen-Induced Acute Liver Injury in Mice. The American Journal of Pathology, 188(7), 1640–1652.
12. Rongvaux A, Shea RJ, Mulks MH, Gigot D, Urbain J, Leo O, Andris F. Pre-Bcell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. Eur J Immunol. 2002;32:3225–34.
13. NAD biosynthetic pathways. Garten, A. et al. (2015). Physiological and pathophysiological roles of NAMPT and NAD metabolism. Nature Reviews Endocrinology, 11(9), 535–546.
14. Christa Buekler et al. Adipokines in Liver Cirrhosis. Int. J. Mol. Sci. 2017, 18, 1392.
15. Tilg H. Adipocytokines in nonalcoholic fatty liver disease: key players regulating steatosis, inflammation and fibrosis. Curr Pharm Des 2010;16:1893–1895.
16. Stofkova A. Resistin and visfatin: regulators of insulin sensitivity,inflammation and immunity. Endocr Regul 010;44:25–36.
17. Oyarzún, A. P., Westermeier, F., Pennanen, C., López-Crisosto, C., Parra, V., Sotomayor-Flores, C., ... Lavandero, S. (2015). FK866 compromises mitochondrial metabolism and adaptive stress responses in cultured cardiomyocytes. Biochemical Pharmacology, 98(1), 92–101. doi:10.1016/j.bcp.2015.08.097
18. [Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050/ doi: 10.17226/12910]
19. Dall, M., Trammell, S. A. J., Asping, M., Hassing, A. S., Agerholm, M., Vienberg, S. G., ... Treebak, J. T. (2019). Mitochondrial function in liver cells is resistant to perturbations in NAD+ salvage capacity. Journal of Biological Chemistry, jbc.RA118.006756.
20. Mukherjee, S., Chellappa, K., Moffitt, A., Ndungu, J., Dellinger, R. W., Davis, J. G., Agarwal, B., & Baur, J. A. (2017). Nicotinamide adenine dinucleotide biosynthesis promotes liver regeneration. *Hepatology (Baltimore, Md.)* , *65* (2), 616–630. https://doi.org/10.1002/hep.28912
21. Gardell, S. J., Hopf, M., Khan, A., Dispagna, M., Hampton Sessions, E., Falter, R., Kapoor, N., Brooks, J., Culver, J., Petucci, C., Ma, C. T., Cohen, S. E., Tanaka, J., Burgos, E. S., Hirschi, J. S., Smith, S. R., Sergienko, E., & Pinkerton, A. B. (2019). Boosting NAD^+ with a small molecule that activates NAMPT. *Nature communications* , *10* (1), 3241. https://doi.org/10.1038/s41467-019-11078-z.
22. [Garten, A., Schuster, S., Penke, M., Gorski, T., de Giorgis, T., & Kiess, W. (2015). Physiological and pathophysiological roles of NAMPT and NAD metabolism. Nature Reviews Endocrinology, 11(9), 535–546]. /// [Johnson, S., & Imai, S. (2018). NAD+ biosynthesis, aging, and disease. F1000Research, 7, 132. doi:10.12688/f1000research.12120.1
23. Gesing J, Scheuermann K, Wagner IV, Löffler D, Friebe D, Kiess W, Schuster V, Körner A. NAMPT serum levels are selectively elevated in acute infectious disease and in acute relapse of chronic inflammatory diseases in children. PLoS One. 2017 Aug 24;12(8):e0183027. doi: 10.1371/journal.pone.0183027. PMID: 28837586; PMCID PMC5570332.
24. Guo, E., Li, R., Yang, J., Zhang, J., Li, A., Yang, Y., ... Jiang, X. (2017). FK866 attenuates acute hepatic failure through c-jun-N-terminal kinase (JNK)-dependent autophagy. Scientific Reports, 7(1). doi:10.1038/s41598-017-02318-7.

DATA AVAILABILITY. The data that support the findings of this study are available from the corresponding author upon reasonable request.