Research, Society and Development, v. 11, n. 10, e526111033047, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i10.33047

Induced arthritis, hepatocytes cytotoxicity and non-steroidal anti-inflammatory drugs (NSAIDs) – experimental study

Artrite induzida, citotoxicidade dos hepatócitos e anti-inflamatórios não esteroidais (AINES) – estudo experimental

Artritis inducida, citotoxicidad de hepatocitos y fármacos antiinflamatorios no esteroideos (AINE) – estudio experimental

Received: 07/16/2022 | Reviewed: 07/29/2022 | Accept: 08/01/2022 | Published: 08/09/2022

Felipe Misael da Silva Morsoleto
ORCID: https://orcid.org/0000-0002-4802-0399
University of Western Paraná, Brazil
E-mail: felipe_morsoletto@yahoo.com.br
Pedro Rondon Werneck
ORCID: https://orcid.org/0000-0001-8729-5259
University of Western Paraná, Brazil
E-mail: prondonwerneck@gmail.com
Juliana Aparecida Ramiro Moreira
ORCID: https://orcid.org/0000-0002-4831-4573
Herminio Ometto Foundation, Brazil
E-mail: juliana.rm@fho.edu.br
Fernando Russo Costa do Bomfim
ORCID: https://orcid.org/0000-0002-2614-3603
Herminio Ometto Foundation, Brazil
E-mail: fernando_bomfim@live.com
Marcelo Augusto Maretto Esquisatto
ORCID: https://orcid.org/0000-0002-2588-619X
Hermínio Ometto Foundation, Brazil
E-mail: marcelosquisatto@fho.edu.br
Maria Jose Misael da Silva Morsoleto
ORCID: https://orcid.org/0000-0001-7544-6224
University of Aveiro, Portugal
E-mail: mjmorsoleto@yahoo.com.br

Abstract
This study aimed to histologically evaluate the pharmacological activity of meloxicam, COX-2 inhibitor, on liver function in arthritic rats. Meloxicam is a non-steroidal anti-inflammatory drug (NSAID), COX-2 inhibitor, generally prescribed in the area of rheumatology. Thus, 18 Wistar rats were divided into 3 groups with 6 animals each: G1 (Negative Control), G2 (Arthritis induced with Zymosan [Zy]), G3 (Arthritis induced with Zymosan [Zy] and treated with meloxicam). The G3 treatment started on the 21st day after induction. The animals were sacrificed, by overdose of Ketamine/Xylazine, seven, 14 and 21 days after the beginning of the meloxicam treatment. Liver samples were collected from the animals, fixed in Millonig buffer containing 10% formaldehyde and processed for conventional histological analysis. G1 animals showed absence of inflammation and degeneration of hepatocytes. In the G2 group, a slight cytoplasmic alteration was observed at seven and 14 days. At 21 days, intense cytoplasmic and nuclear disorganization was detected, accompanied by cell necrosis. In G3, large spacing between sinusoid capillaries was detected. In some 14-day-old animals, amyloidosis was observed, with the presence of a large number of vacuolated hepatocytes involved in an amorphous mass of necrotic cells. Most of the hepatocyte nuclei had compacted chromatin, evidencing cellular degeneration. Meloxican, used as an anti-inflammatory in the synovial region, showed that, in addition to its beneficial effects for the treatment of rheumatological diseases, it is capable of, when administered orally, promoting a high level of liver damage.
Keywords: Meloxicam; Zymosan; Induced arthritis.

Resumo
Este estudo teve como objetivo avaliar histologicamente a atividade farmacológica do meloxicam, inibidor da COX-2, sobre a função hepática em ratos artríticos. O meloxicam é um anti-inflamatório não esteroidal (AINE), inibidor da COX-2, geralmente prescrito na área de reumatologia. Assim, 18 ratos Wistar foram divididos em 3 grupos com 6 animais cada: G1 (Controle Negativo), G2 (Artrite induzida com Zymosan [Zy]), G3 (Artrite induzida com Zymosan

[Zy] e tratados com meloxicam). O tratamento com G3 iniciou-se no 21º dia após a indução. Os animais foram sacrificados, por overdose de Cetamina/Xilazina, sete, 14 e 21 dias após o início do tratamento com meloxicam. Amostras de fígado foram coletadas dos animais, fixadas em tampão Millonig contendo 10% de formaldeído e processadas para análise histológica convencional. Os animais do G1 apresentaram ausência de inflamação e degeneração dos hepatócitos. No grupo G2, foi observada discreta alteração citoplasmática aos sete e 14 dias. Aos 21 dias, foi detectada intensa desorganização citoplasmática e nuclear, acompanhada de necrose celular. Em G3, foi detectado grande espaçamento entre os capilares sinusóides. Em alguns animais com 14 dias de idade foi observada amiloidose, com a presença de grande número de hepatócitos vacuolados envolvidos em uma massa amorfa de células necróticas. A maioria dos núcleos dos hepatócitos apresentava cromatina compactada, evidenciando degeneração celular. O meloxicano, utilizado como anti-inflamatório na região sinovial, mostrou que, além de seus efeitos benéficos para o tratamento de doenças reumatológicas, é capaz de, quando administrado por via oral, promover alto nível de dano hepático.
Palavras-chave: Meloxicam; Zymosan; Artrite induzida.

Resumen
Este estudio tuvo como objetivo evaluar histológicamente la actividad farmacológica del meloxicam, inhibidor de la COX-2, sobre la función hepática en ratas artríticas. El meloxicam es un fármaco antiinflamatorio no esteroideo (AINE), inhibidor de la COX-2, generalmente prescrito en el área de la reumatología. Así, 18 ratas Wistar se dividieron en 3 grupos de 6 animales cada uno: G1 (Control Negativo), G2 (Artritis inducida con Zymosan [Zy]), G3 (Artritis inducida con Zymosan [Zy] y tratada con meloxicam). El tratamiento G3 comenzó el día 21 después de la inducción. Los animales fueron sacrificados, por sobredosis de Ketamina/Xilazina, siete, 14 y 21 días después del inicio del tratamiento con meloxicam. Se recogieron muestras de hígado de los animales, se fijaron en tampón Millonig que contenía formaldehído al 10 % y se procesaron para análisis histológico convencional. Los animales G1 mostraron ausencia de inflamación y degeneración de hepatocitos. En el grupo G2 se observó una ligera alteración citoplasmática a los siete y 14 días. A los 21 días se detectó una intensa desorganización citoplasmática y nuclear, acompañada de necrosis celular. En G3, se detectó un gran espacio entre los capilares sinusoidales. En algunos animales de 14 días se observó amiloidosis, con presencia de un gran número de hepatocitos vacuolados envueltos en una masa amorfa de células necróticas. La mayoría de los núcleos de hepatocitos presentaban cromatina compactada, evidenciando degeneración celular. Meloxican, utilizado como antiinflamatorio en la región sinovial, demostró que, además de sus efectos beneficiosos para el tratamiento de enfermedades reumatológicas, es capaz, cuando se administra por vía oral, de promover un alto nivel de daño hepático.
Palabras clave: Meloxicam; Zymosan; Artritis inducida.

1. Introduction

Meloxicam, an enolcarboxamide whose chemical structure is 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a new anti-inflammatory non-steroidal drug (NSAID), its pharmacokinetics vary according to the animal species, and may have a half-life of 3 to 36 hours. It is a preferred NSAID COX 2 inhibitor due to changes in its chemical structure in relation to other NSAIDs in its group. COX inhibitors not only interfere with inflammatory mechanisms, but also disrupt various body functions. Toxicity, gastrointestinal, renal failure, and cardiovascular risks are well-known adverse effects of COX1 and COX 2 inhibitors (Nasr *et al.*, 2020). These are the drugs (NSAIDs) most used to control pain and acute and chronic inflammation. They are largely organic acids indicated for the control of pain and acute and chronic inflammation. It is an NSAID preferentially COX 2 inhibitor due to changes in its chemical structure in relation to other NSAIDs in its group. These medications, non-steroidal anti-inflammatory drugs, make up a universe of 30% of the most used drugs with medical prescription or self-medication. COX inhibitors not only interfere with inflammatory mechanisms, but also disrupt various body functions. Gastrointestinal toxicity, kidney failure, and cardiovascular risks are well-known adverse effects of COX inhibitors (Sylvester, 2019).

Although COX has been identified for more than 20 years, it has been in recent years that it has become clear that there are two homologous isoforms, being referred to as COX-1 and COX-2 (Ju *et al*., 2022). In almost all normal tissues, the structural presence of COX-1 is detected, and in low to undetectable levels of COX-2, which can be expressed in greater amounts through the presence of cytokines, growth factors and tumor stimulants, suggesting its relevance. cancer and inflammatory processes. In this way, COX-1 was given the name constitutive; to COX-2, inductive(Samra *et al*., 2020). Recently, the existence of a third

isoform of this enzyme family, called COX-3, was proposed, which, unlike COX-1 and COX-2, would not produce pro-inflammatory prostanoids, but anti-inflammatory substances. Such evidence can be seen in cases of chronic inflammatory diseases such as rheumatoid arthritis (Vane, 1996; Willoughby *et al*., 2000; Kummer & Coelho, 2002)

Zymosan is derived from the cell wall of the fungus Saccharomyces cerevisiae, its main structural component is β-glucan, which has immunostimulatory properties (Derbocio, 2005). Injection into the joint cavity causes an increase in vascular permeability leading to a cellular influx with a predominance of polymorphonuclear cells (Morsoleto, 2007). Zymosan is recognized by the dectin 1 receptor that is expressed in monocytes, macrophages, neutrophils and dendritic cells, after recognition it stimulates the production of inflammatory cytokines (Morsoleto *et al*., 2022). This polysaccharide also causes the generation of anaphylatoxin C5a, through the activation of the alternative pathway of the complement system, with subsequent activation of monocytes and accumulation of neutrophils (Derbocio, 2005).

2. Methodology

This experimental project was carried out in histology and microscopy laboratories and in the rooms integrated in the animals of the experimentation center of Fundação Hermínio Ometto (FHO) in Araras, SP. The procedures and animal studies follow CONEP's fundamentals for the use of animals in research. Being approved by the Unicamp Scientific Research and approved the methodology (protocol under number 811-1, Research Committee and Unicamp (Morsoleto, 2007). used 18 female Wistar rats with an average weight of 70 g, 60 days, fortresses in (3) groups three of six (6), which were found with balanced diet and water ad libitum, during periods of light/12 hours, ro for 12 hours For induced research, the groups implemented at room temperature warmed up (G2(induced and controlled group) and G3(induced and treated group), which were later treated with Meloxicam (Aché) The animals intervene with intraperitoneal injection of 0.3 mL of ketamine and 0.05 mL of xylazine animal body weight and protection against zymosan injection Sigma-Aldre-Aldre. , Minas so St Louis] (1 mg) diluted in 50µl of 0.9% saline solution in the right. at time 0 with application of 50µL of Zy in the right knees of animals from G2 and G3. Treatment with Meloxicam (Camplesi, 2010) started on day 21 after induction. This technique of oral administration of food or medication was performed with the animal without anesthesia. So that the animal would not lose swallowing reflex. A gavage cannula and a 1ml syringe were used in the process. The syringe was filled with 0.7ml for every 200g of animal. That was immobilized according to the description of technique I of animal restraint in the manual. The head was turned upwards and the cannula was introduced in the lateral position of the mouth, gently sliding into the oral cavity, over the animal's tongue, performing a delicate and continuous movement. With the cannula properly positioned, Meloxicam was administered slowly and we waited for the animal to swallow. The gavage protocol of Bogdanske et al., (2010). Meloxicam treatment started on the 21st day after induction. Samples were collected at: seven, 14 and 21 days after initiation of meloxicam treatment. Samples were collected after 7, 14 and 21 days after initiation of meloxicam treatment. The animals were anesthetized with an intraperitoneal injection of 0.3 mL of Ketamine and 0.1 mL of Xylazine for each 200g of animal body weight and perfused with fixative solution. After removal, the samples remained in Millonig Buffer containing 10% formaldehyde for 24 hours and processed for imbibition in Paraplast (Merck). 5 µm thick sections were stained with Hematoxylin/Eosin and documented using a Leica DM 2000 Photomicroscope. The statistical treatment used was A Nova One Way with Turkey retest for significance level $p \leq 0.05$. The program used to perform the test was Origin 7.0. The histological study was performed on slides stained with hematoxylin and eosin (H.E) with the aid of a light microscope**. Different parameters were defined for each organ, due to the peculiarity of each one. In the liver, the occurrence of:

1 - Microvesicular steatosis - defined as the presence of lipid accumulation in the form of cytoplasmic microvesicles with a volume smaller than the nucleus;

2 – necrosis – characterized by condensation or effacement of the nucleus, intense cytoplasmic eosinophilia and

destruction, loss of hepatocyte cord architecture (hepatocellular destrabeculation);

3 – apoptosis – characterized by the nuclear aspect, that is, the disposition of chromatin in the nucleus and by the appearance of the nuclear cytoplasm;

4 – leukocyte infiltration – presence of leukocytes, especially neutrophils in the various parts of the hepatic lobule. In the liver, 50 fields were randomly observed from each animal under various magnifications (4 to 400x objectives). Lesions were graded in crosses ranging from absent (-) to ++++ depending on their intensity. These values were transformed into numbers for the application of statistical tests, as follows: Absence = 0; +/- = 0.5; + = 1; ++ = 2; +++ = 3 ++++ = 4.

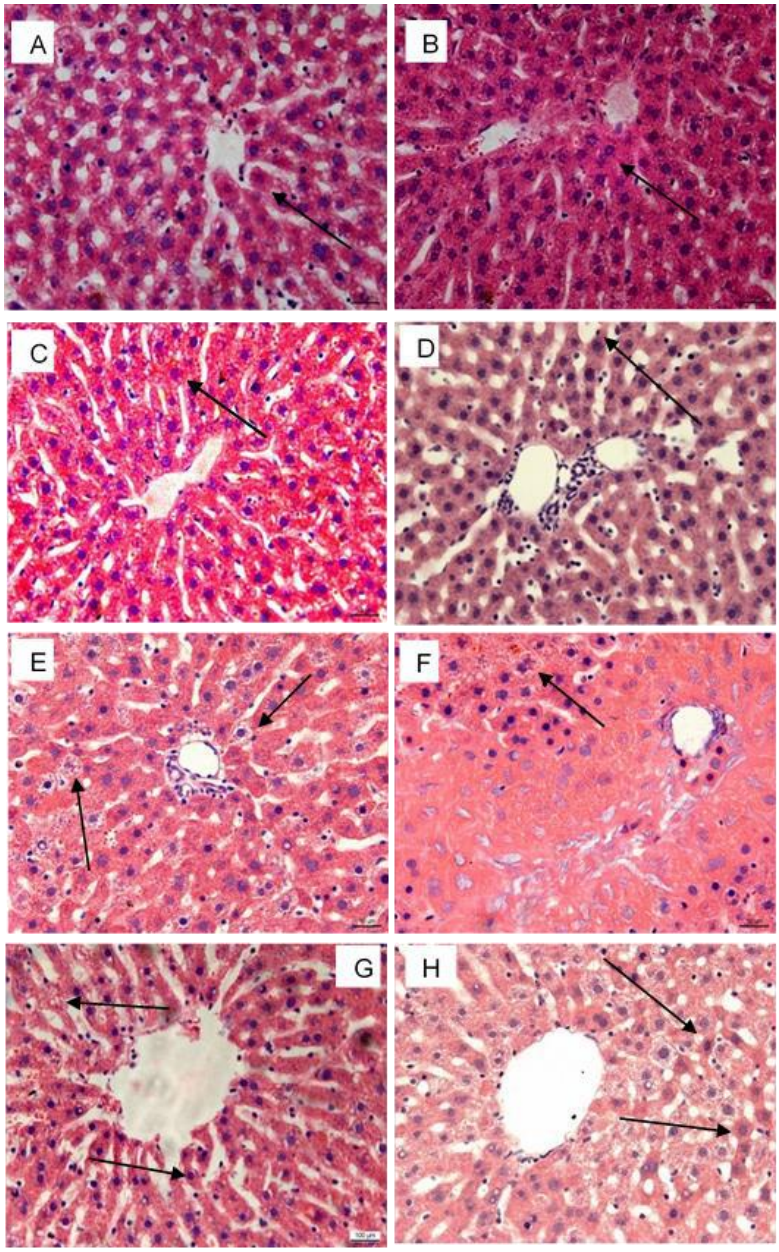
3. Results

The animals of G1 (Figure 1), showed absence of inflammation and degeneration of hepatocytes as seen in Figures A and B; in G2, a slight cytoplasmic and nuclear alteration was observed at seven and 14 days, as seen in the arrows in Figures C and E, respectively. At 21 days, there is intense cytoplasmic and nuclear degeneration accompanied by necrosis as seen in Figure G. In G3, a large spacing between sinusoid capillaries can be observed (Figure D). In this group, some 14-day-old animals presented amyloidosis as shown in figure F. In these cases, the hepatocytes are vacuolated and organized in an amorphous mass, with a large number of necrotic cells. Most of the hepatocyte nuclei had compacted chromatin, evidencing cellular degeneration. At 21 days, the liver tissue is completely altered, showing disruption of its tissue morphology (Figure H). At 21 days, the liver tissue is completely altered, showing disruption of its tissue morphology (Figure H), Cheville, (1994). The analyzed results related to intralobular inflammatory infiltrates with seven days of treatment, showed P values ≤ 0.003 when relating G1 and G3 and P ≤ 0.001 for the G2 and G3 relationship. The groups with fourteen and twenty-one days did not present relevant statistical values.

After seven days of treatment, when analyzing the presence of necrosis, we can observe that the relationship between G1 and G2; G1 and G3 express a statistical value of P ≤ 0.05. With twenty-one days of treatment, we will find P statistical values ≤ 0.05 for the relationship between G1 and G3. The fourteen-day group did not present relevant statistical results. The fourteen-day group did not present relevant statistical results. As Guicciardi and Gores (2005), report in their research.

The analyzed results related to intralobular inflammatory infiltrates with seven days of treatment, showed P values ≤ 0.003 when relating G1 and G3 and P ≤ 0.001 for the G2 and G3 relationship. The groups with fourteen and twenty-one days did not present relevant statistical values.

Figure 1 Photomicrographs of transversal sections of liver stained with hematoxilin and eosin from animals of G1 (A and B), G2 (C, F and H) and G3 (D, G, and I). Liver samples were collected after seven (A, C and D), 14 (B, F and G) and 21 (H and I) days after twenty-one days after Zymosan arthritis induction. (**CV**) – Central vein, (**PC**) – Portal channe l ( ) – Hepatocytes. Bar = 100 μm.



Source: Authors.

Often the presence of infiltrating neutrophils close to apoptotic hepatocytes suggests an inflammatory response arising from an inflammatory response triggered by apoptosis Table 1. Regardless of the etiology, liver injury is usually characterized in the first phase by increased apoptosis of hepatocytes. This massive presence overwhelms the ability of phagocytic cells to remove dead cells. Autolysis of apoptotic bodies releases pro-inflammatory content.

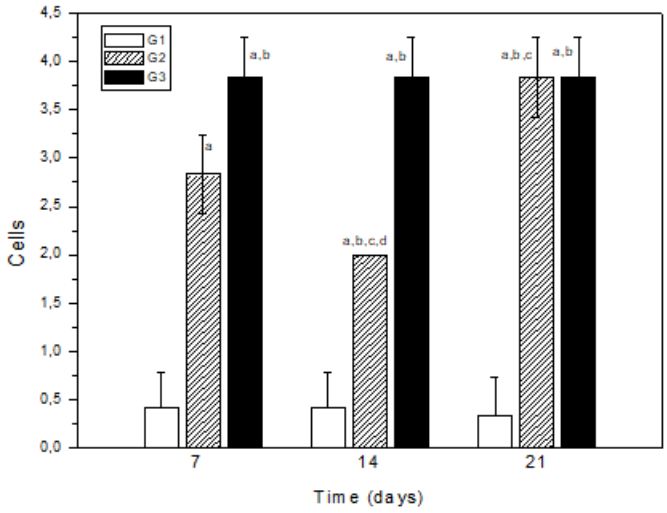
Table 1 Liver microvesicular steatosis values, observed in light microscopy stained with hematoxylin-eosin, in 3 groups with 6 animals each: G1 (Negative Control), G2 (induced with Zymosan [Zy]), G3 (induced with Zymosan [Zy] and treated with meloxicam).

G1	G2	G3
0,5	2,0	2,0
0,5	1,0	2,0
0,5	1,0	2,0
1.0	2,0	4,0
0,0	1,0	2,0
0,0	2,0	4,0

Source: Authors.

Count performed on liver slides using images obtained by optical microscopy (table 1), stained with hematoxylin-eosin, in 3 groups: G1 (Negative Control), G2 (induced with Zymosan [Zy]), G3 (induced with Zymosan [Zy] and treated with meloxicam). Considering treatment times of 7, 14 and 21 days.

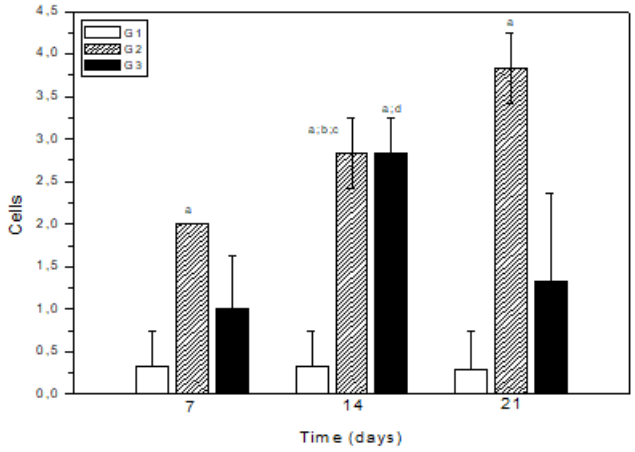
Figure 2 They graphically express quantitatively the presence of microvesicular steatosis of the liver, between groups by time of treatment.



Source: Authors.

Figure 3 expresses the slide count observed by light microscopy stained with hematoxylin-eosin, in 3 groups with 6 animals each: G1 (Negative Control), G2 (induced with Zymosan [Zy]), G3 (induced with Zymosan [Zy] and treated with meloxicam). Considering the treatment times of 7, 14 and 21 days, which statistically express the values for: $p < 0,01$; a ≠ G1; b ≠ G2(7); c ≠ G3(7); d ≠ G2(21).

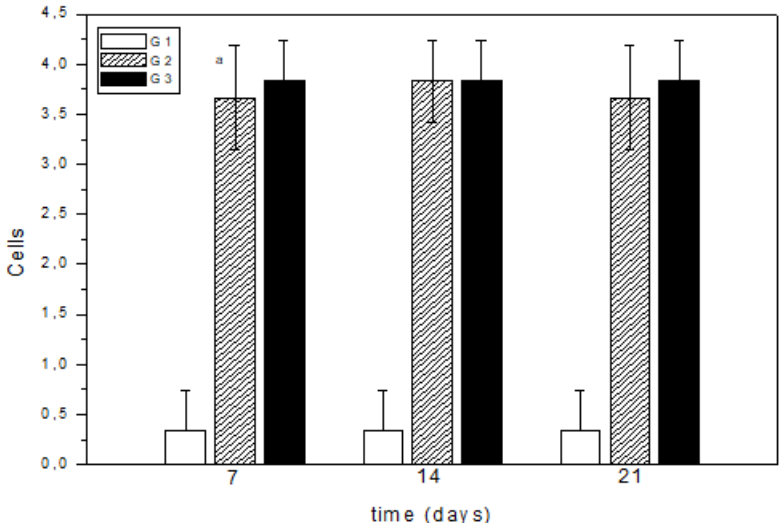
Figure 3 Graphically express the amount of inflammatory infiltrate in the liver.



Source: Authors.

In Figure 4, in images observed by optical microscopy stained with hematoxylin-eosin, in the 3 groups: G1 (Negative Control), G2 (induced with Zymosan [Zy]), G3 (induced with Zymosan [Zy] and treated with meloxicam). Considering the treatment times of 7, 14 and 21 days, the statistical values of the correlation between the groups were obtained: $p < 0.01$; $a \neq G1$.

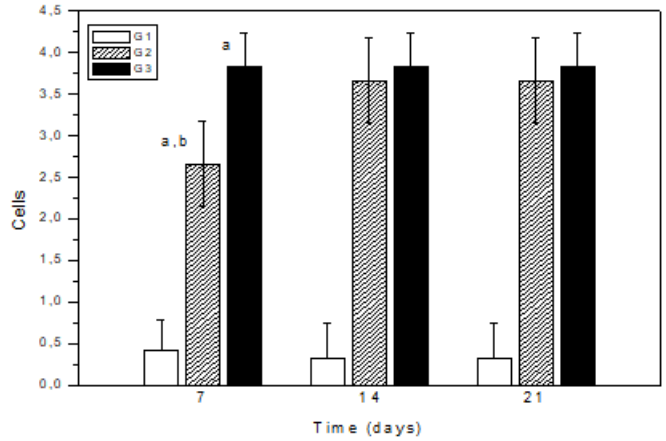
Figure 4 Liver necrosis values expressed graphically.



Source: Authors.

In Figure 5, observed in the optical microscopy stained by hematoxylin-eosin, in 3 groups with 6 animals each: G1 (Negative Control), G2 (induced with Zymosan [Zy]), G3 (induced with Zymosan [Zy] and treated with meloxicam). Considering treatment times of 7, 14 and 21 days. Statistic: $p < 0,01$; $a \neq G1$; $b \neq G2(14)$.

Figure 5 Apoptosis values in the liver.



Source: Authors.

4. Discussion

According to Pedroso and Batista 2017, Bhat *et al*., 2018; Bindu *et al*., 2020, continuous use of anti-rheumatic drugs causes potentially toxic effects, so a risk-benefit assessment should be made. The Ministry of Health, advised by the National Commission for the Incorporation of Technologies - CONITEC, recommends monitoring the use of these drugs through tests that assess, among other functions, the liver and kidney function. Based on several researches, our work aimed to evaluate liver function in arthritic rats after treatment with the NSAID meloxicam (Furst, 1997).

Vane (1996) was the first to propose that the side effects of non-steroidal anti-inflammatory drugs resulted from the inhibition of the cyclooxygenase enzyme by these compounds. In a review of clinical cases by Maddrey *et al* (2000) involving 7400 patients, liver dysfunction occurred in 0.8% of those treated with celecoxib compared with 0.9% of those treated with placebo and 3.7% of those using diclofenac. of sodium.

According to O'Beirne and Cairns (2000) and Meunier, (2018), non-steroidal anti-inflammatory drugs, particularly sodium diclofenac, have been associated with severe hepatotoxicity. Recent case reports have shown that Celecoxib has been associated with the development of hepatitis and pancreatitis.

In a study carried out by Porta (2000), on autoimmune hepatitis, he shows important histological findings such as; chronic inflammatory infiltrates predominating in the portal, periportal and intralobular spaces, composed of lymphocytes, plasma cells and polymorphonuclear cells, enlargement of the portal spaces by fibrosis, derangement of the lobular architecture with lesions of the hepatocytes. These events which are corroborated by Parolin, and Reason, (2001).

In our study, several histological findings are shown with great similarity to those found in the relevant literature (Lida, 2005; Sriuttha, *et al*., 2018). Groups G2 and G3 have intralobular inflammatory infiltrates. Also in G3, in all experimental periods, cases of lobular disarrangement, cytoplasmic degeneration and disintegration (ballooning) and chromatin and nuclear disorganization are observed. In the animals analyzed after 21 days, areas of significant necrosis are observed. This morphological aspect is corroborated by (Cheville, 1994; Manov *et al*., 2006; Bindu *et al*., 2020).

Despite studies presented in recent years on cytotoxicity in hepatocytes caused by anti-inflammatory drugs in an arthritis model, there are still many doubts to be clarified, both in terms of mechanisms of action and criteria for use.

5. Conclusion

The experimental models used for induction and treatment with adverse substances such as Zymosam (Morsoleto *et al*., 2022) according to the model proposed in this work, showed profound changes in the architecture of the liver tissue of the induced groups as described. In our study, the dosage of anti-inflammatory drug (meloxicam) seems to suggest an increase in necrosis in the liver tissue.

We suggest further research with tests that enable the analysis of tissue architecture and liver function. The lack of research on the subject, through the wide range of possible hepatotoxic agents, contributes to the large number of preventable liver diseases. New research will contribute to the reduction of such a large number of harm. Also suggest that researchers test new anti-inflammatory drugs, and include organs other than the liver. Such as organs of the digestive system and kidneys.

Acknowledgments

The authors want to thank the dedication of the professors: Marcelo Squissato, Fernando R. Do Bomfim and Maria José M. S. Morsoleto for the dedication and support that allowed the development of this work. We especially want to thank Willian Roberto Campos.

For the work entitled “Histopathological analysis of hepatocytes cytotoxicity by the use of non-steroidal anti-inflammatory drugs (NSAIDs) in arthritic rats”. Campos, W. R., Pancielli, P., Bomfim, F. R. C., Esquisatto, M. A. M., Morsoleto, M. J. M. S. Which served as the basis for this article.

References

Bhat, M. A., Al-Omar, M. A., Raish, M., Ansari, M. A., Abuelizz, H. A., Bakheit, A. H., & Naglah, A. M. (2018). Indole derivatives as cyclooxygenase inhibitors: synthesis, biological evaluation and docking studies. *Molecules*, *23*(6), 1250.

Bindu, S., Mazumder, S., & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical pharmacology*, 180, 114147. https://doi.org/10.1016/j.bcp.2020.114147

Bogdanske, J. J., Hubbard-Van Stelle, S., Riley, M. R., & Schiffman, B. (2010). *Laboratory mouse procedural techniques: manual and DVD*. CRC Press.

Camplesi, A. C. (2010). Uso de antiinflamatórios COX-2 seletivos em ratos (Rattus novergicus) Wistar.

Cheville, N.F. (1994). Ultrastructural pathology: An introduction to interpretation. Ames, IA: *Iowa State University Press;*

Derbocio, A. M. (2005). The hemodynamic effects of zymosan in the perfused rat liver. *Vascular Pharmacology*; 43:75-85.

Furst, D. E. (1997). Meloxicam: Selective COX-2 inhibition in clinical practice, Seminars in *Arthritis and Rheumatism*, 26, Suppl1:21-27.

Ju, Z., Li, M., Xu, J., Howell, D. C., Li, Z., & Chen, F. E. (2022). Recent development on COX-2 inhibitors as promising anti-inflammatory agents: The past 10 years. *Acta Pharmaceutica Sinica B. 12(6): 2790–2807.*

Guicciardi, M. E., & Gores, G. J. (2005). Apoptosis: a mechanism of acute and chronic liver injury. Gut, 54(7), 1024–1033.

Khalil, N. Y., & Aldosari, K. F. (2020). Chapter Six - Meloxicam, Editor(s): Harry G. Brittain, Profiles of Drug Substances, Excipients and Related Methodology, *Academic Press*, 45,2020, 159-197.

Kummer, C. L., & Coelho T. C. R. B. (2002). Antiinflamatórios Não Esteróides Inibidores da Ciclooxigenase-2 (COX-2): Aspectos Atuais. *Rev Bras Anestesiol*. 52: 4: 498-512.

Lida, V. H. (2005) Cirrose Hepática: Aspectos morfológicos relacionados as suas possíveis complicações. Um estudo centrado em necropsias. *J Bras patol Med lab*. 41:1, 29-36.

Maddrey, W. C., Maurath, C. J., & Verburg K. M. (2000). The hepatic safety and tolerability of the novel cyclooxygenase inhibitor celecoxib. *Am J Ther*. 7:153-158.

Manov, I., Motanis, H., Frumin, I., & Iancu, T. C. (2006) *Acta Pharmacologica Sinica*; 27 (3): 259–272

Meunier, L., & Larrey, D. (2018). Recent Advances in Hepatotoxicity of Non-Steroidal Anti-inflammatory Drugs, *Annals of Hepatology*, 17, Issue 2,187-191.

Morsoleto, M. J. M. S. (2007) Clinical and morphological evolution of the induced experimental arthritis in *Rattus novergicus albinus Braz. J. Morphol. Sci*; 24 (2): 75-81.

Morsoleto, F. M. S., Werneck, P. R., Bomfim, F. R. C., Esquisatto, M. A. M., & Morsoleto, M. J. M. (2022). Therapeutic ultrasound and essential oil of melaleuca alternifolia interfere with muscle regeneration?. *Research, Society and Development*, 11(6), e10411628791. https://doi.org/10.33448/rsd-v11i6.28791

Morsoleto, F. M. S., Werneck, P. R., Bomfim, F. R. C., & Morsoleto, M. J. M. S. (2022). Ultrasound wave transports apitoxin in arthritic joint. - Experimental study. *Research, Society and Development*, 11(7).

O´Beirne, J. P., & Cairns, S. R. (2000) Cholestatic hepatitis in association with celecoxib. *Br Med J*, 323:23.

Parolin, M. B., & Reason, I. J. M. (2001). Apoptose como mecanismo de lesão nas doenças hepatobiliares. *Arquivos de Gastroenterologia*, *38*, 138-144.

Pedroso, C. R., Batista, L. F. (2017). O uso indiscriminado dos antiinflamatórios não esteroidais. *Saúde & Ciência Em Ação*. 3: 01:48-69.

Porta, G. Autoimmune hepatitis. Jornal de Pediatria 2000; *Sociedade Brasileira de Pediatria* 76 (2): 181-186.

Samra, M. M., Sadia, A., Azam, M., Imran, M., Ahmad, I., & Basra, M. A. R. (2022). Synthesis, Spectroscopic and Biological Investigation of a New Ca (II) Complex of Meloxicam as Potential COX-2 Inhibitor. *Arabian Journal for Science and Engineering*, 1-18.

Sriuttha, P., Sirichanchuen, B., & Permsuwan, U. (2018). Hepatotoxicity of Nonsteroidal Anti-Inflammatory Drugs: A Systematic Review of Randomized Controlled Trials. *International journal of hepatology*, 5253623. https://doi.org/10.1155/2018/5253623

Sylvester, J. (2019). Anti-inflamatórios não-esteroides. *Sociedade Brasileira de Anestesiologia* ATOTW ,405, 1-5.

Vane, J. R. (1996). Introduction: mechanism of action of NSAIDS. British *Journal of Rheumatol*, 35 (1): 1-3.

Willoughby, D. A., Moore, A. R., & Colville-Nash, P. R. (2000). COX-1, COX-2, and COX-3 and the future treatment of chronic inflammatory disease. *The Lancet*, *355*(9204), 646-648.