Clinical and Experimental Health Sciences

Does Chitosan Introduce Protection Against Methotrexate-Induced Hepatorenal Injury in Rats?

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ABSTRACT

Objective: Chitosan possesses antioxidant properties and exhibits anti-inflammatory characteristics. The objective of the investigation was to assess the effectiveness of chitosan in protecting against hepatorenal injury induced by methotrexate (MTX), a medication utilized for immunosuppression and chemotherapy.

Methods: Wistar albino rats were allocated into 3 different groups, each consisting of six animals (n=6). The control group received saline for 5 days (i.p.), the MTX group was administrated a single dose MTX (60 mg/kg, i.p.) along with saline for four days (i.p.), while MTX+Chitosan group received a single dose of MTX (60 mg/kg, i.p.) followed by Chitosan administration (200 mg/kg, i.p.) for four days. On the sixth day, the animals were decapitated, and blood and tissue samples were collected. BUN, creatinine and tissue inhibitors of metalloproteinase-1 (TIMP-1) levels and activities of AST, ALT, ALP, LDH, matrix metalloproteinases (MMP-3, MMP-8, MMP-9) activities were quantified in the blood. The liver and kidney were evaluated for caspase-3 and-9 through western blotting, while structural damage was examined using light microscopy.

Results: In the MTX administered group, blood and tissues values except for all TIMP-1 statistically increased when compared to the control group, while activity of TIMP-1 decreased significantly. The Chitosan-treated MTX group had comparable values to the control group.

Conclusion: Based on its influence on metalloproteinases and caspases, our findings lead to the conclusion that Chitosan offers a protective effect against liver and kidney damage induced by MTX.

Keywords: Caspase, Chitosan, Methotrexate, MMP, TIMP-1

1. INTRODUCTION

Methotrexate (MTX) is an inhibitor of dihydrofolate reductase (DHFR) that is involved in the treatment protocols of cancer and autoimmune disorders. It has been shown to cause hepatorenal damage via different oxidative and inflammatory mechanisms (1–3).

Matrix metalloproteinases (MMP) activation has been associated with inflammation, angiogenesis, embryogenesis, and apoptosis. Furthermore, it augments caspase (Casp) activity, impacts hemodynamic parameters, and induces hepatorenal damage during MTX administration (4, 5). MMPs, which are proteolytic enzymes integral to the extracellular matrix, perform crucial functions in a range of biological processes, encompassing inflammation and tissue repair. Tissue inhibitors (TIMPs) naturally inhibit MMPs by effectively regulating their activity. TIMPs bind to MMPs in a unidirectional manner, exerting inhibitory effects. Consequently, the balance between MMP and TIMP levels becomes a critical determinant of MMPs' efficacy in maintaining tissue homeostasis (6, 7). Caspases serve as the principal executors of apoptosis, and numerous studies have demonstrated their involvement in MTX-induced tissue injuries, including oral and hepatorenal tissues (8, 9).

Chitosan, utilized in diverse medical applications, has demonstrated protective effects attributed to its antioxidant and anti-inflammatory properties in numerous studies on inflammation and toxicity (10–12). While investigations have explored the protective effects of various antioxidant and anti-inflammatory drugs against MTX toxicity, the issue of hepatorenal damage remains significant in MTX administration (10–12). However, as of now, there is no evidence to suggest the efficacy of chitosan in ameliorating drug-induced hepatorenal injury, which limits its use as a treatment option in methotrexate therapy (9, 13).

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In the present study, we utilized MTX to induce liver and kidney damage in order to examine the effect of chitosan on MTX-induced hepatorenal injury. Rats were chosen as the experimental model to assess the potential protective efficacy of chitosan against MTX-induced hepatorenal injury. Based on these considerations, the aim of our study was to assess the protective effects of chitosan against MTX-induced hepatorenal injury by examining MMPs, caspases, and conducting a histopathological examination.

2. METHODS

According to the 1964 Helsinki Declaration's guiding principles, this study was carried out. The Local Animal Experiments Ethics Committee of the Near East University was authorized to approve the study protocol (Decision no: 2020/111). The experiment was carried out in a single center as a single-blind, randomized, controlled study. A total of eighteen Wistar albino rats, including both males and females, were allocated into three groups (n=6). The rats were housed following standard laboratory conditions. The control group was administered oral saline exclusively for a duration of 5 days. Conversely, the MTX group received an intraperitoneal dose of 60 mg/kg MTX on day 1, followed by intraperitoneal administration of saline for the subsequent 4 days (14). The MTX + Chitosan group, on the other hand, received a single intraperitoneal dose of 60 mg/kg MTX, followed by four days of oral gavage with 200 mg/kg Chitosan (15, 16). Following the sacrifice of the animals on day 6, blood and tissue samples (liver and kidney) were collected from each subject. Low molecular weight chitosan (50-190 kDa) was used in this study because it has been shown to significantly decrease tumor growth, whilst high molecular weight has not been shown to have any effect. Chitosan has a deacetylation level of 75-85%, making it suitable for use in biomedicine including tissue regeneration, inflammation, and cancer therapy (17, 18).

2.1. Biochemical Assays

Biochemical indicators including alanine transaminase (ALT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine were measured and evaluated using commercially available test kits (Mindray, Shenzen, China). Specific enzyme immunoassay test kits designed for rats were utilized to determine the activity levels of MMPs (MMP-3, MMP-8, and MMP-9) and TIMP-1 levels (MMP-3 E-EL-R0619, MMP-9 E-EL-R302 from Elabscience, Wuhan, China; MMP-8 ELR-MMP8 and TIMP-1 ELR-TIMP-1 from RayBiotech Life Inc., Norcross, GA, USA).

2.2. Histopathologic Evaluation

Structural damage to tissues was evaluated under the light microscope. Formalin solution (10%) was used for the fixation of liver and kidney tissues. The 4 µm thick slices cut from paraffin blocks obtained during standard histological tissue processing. Routine staining with hematoxylin and eosin was conducted on these slices. Subsequently, the sections were examined using a light microscope.

2.3. Western blotting

Each group's dissected tissues were homogenized before being centrifuged at 2000g for 10 min. According to the Lowry method, the quantity of protein in each sample was 50 µg. The samples were then loaded onto SDS (12%) PAGE gels. Samples were separated according to their protein weights and transferred to the nitrocellulose membranes (Schleicher and Schuell, 0.45 m, Germany for 75 min at 90 V) and incubated with primary antibodies [caspase-3 (casp-3; sc-56053) and caspase-9 (casp-9; sc-56076) 1:200 dilution, at +4° C], while all membranes were standardised with β-actin (1:100; sc-130657) and analysed with a free program for densitometric analysis (www.totallab.com).

2.4. Statistical Analysis

The statistical analysis was performed using Prism 7.0 software (GraphPad, CA, USA). Significances among sample means were assessed using the TUKEY's test following a one-way analysis of variance (ANOVA). A significance level of $p<0.05$ was employed to determine statistical significance.

3. RESULTS

Administration of MTX resulted in elevated levels of biochemical indicators, including AST, ALT, LDH, BUN, and creatinine, compared to the control group. However, after treatment with Chitosan, a significant reduction in the changes of these biochemical parameters was observed compared to the group that received MTX ($p<0.05$; $p<0.01$) (Table 1).

Table 1. *Serum a) AST and b) ALT c) BUN, d) Creatinine and e) LDH activities of all groups (n=6) in hepatorenal damage induced by MTX in rats.*

	Control	MTX	MTX-Chitosan
AST (U/L)	103.4 ± 16.1	199.1 ± 16.6 **	129.9 ± 12.5 +
ALT (U/L)	44.3 ± 4.8	89.5 ± 8.9 **	58.5 ± 9.6 +
LDH (U/L)	871 ± 44	1389 ± 130 **	977 ± 54 +
BUN (U/L)	16.80 ± 1.68	25.70 ± 2.67 *	17.64 ± 1.59 +
Creatinine (U/L))	0.62 ± 0.03	1.16 ± 0.12 **	0.69 ± 0.11 ++

** p<0.05, ** p<0.01 compared to control group; + p<0.05 ++ p<0.01 compared to MTX group.*

Significant increases in the activities of MMP-3, MMP-8, and MMP-9 were observed in the MTX group, along with a notable decrease in TIMP-1 activity compared to the control group. However, in the MTX + Chitosan group, these parameters exhibited a significant decrease compared to the MTX group ($p<0.05$, $p<0.01$), reaching values comparable to those of the control group (Table 2).

Table 2. *Serum a) MMP-3, b) MMP-8 c) MMP-9 and d) TIMP-1 activities of all groups (n=6) in hepatorenal damage induced by MTX in rats.*

	Control	MTX	MTX-Chitosan
MMP-3 (ng/ml)	10.04 ± 0.33	16.17 ± 2.03 *	11.01 ± 0.72 +
MMP-8 (pg/ml)	10.39 ± 0.49	15.23 ± 1.16 **	11.28 ± 0.81 +
MMP-9 (ng/ml)	35.70 ± 3.12	70.83 ± 5.04 ****	41.34 ± 4.25 +++
TIMP-1 (ng/ml)	1.14 ± 0.08	0.62 ± 0.06 **	1.07 ± 0.08 ++

** p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to control group; + p<0.05 ++ p<0.01 +++ p<0.001 compared to MTX group.*

The impact of Chitosan treatment on tissue levels of caspase-3 (casp-3) and caspase-9 (casp-9) was evaluated using the Western Blotting method (Figure 1). In the MTX group, both kidney and liver tissues exhibited statistically higher levels of casp-3 expression compared to the control group (p<0.001 and p<0.01, respectively). However, the administration of chitosan significantly reduced casp-3 expression in both liver and kidney tissues of the MTX + Chitosan group in comparison to the MTX group (p<0.001 for liver, p<0.05 for kidney; Figure 2A and 2B). Furthermore, casp-9 expression demonstrated a significant increase in the liver and renal tissues of the MTX group compared to the control group (p<0.01 for both tissues). Nonetheless, treatment with Chitosan led to a significant reduction in casp-9 expression in both liver (p<0.01, Figure 2C) and kidney tissues (p<0.05, Figure 2D).

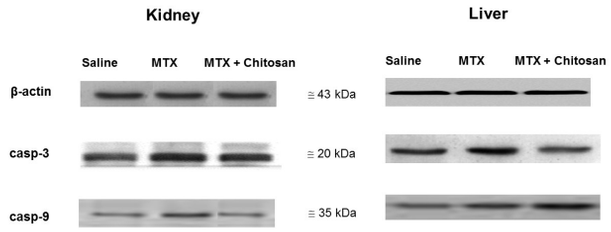

Figure 1. *Representative membrane images showing the protein levels of β-actin, casp-3, and casp-9 in kidney and liver tissues of Chitosan treatment in the methotrexate-induced rat hepatorenal model.*

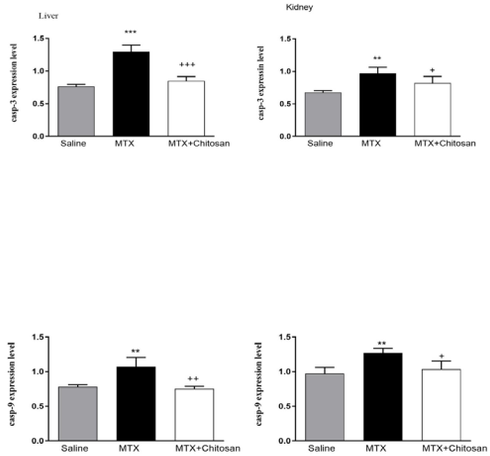

Figure 2. *Protein levels of caspase-3 and caspase-9 in kidney and liver tissues in the MTX-induced rat hepatorenal model. ** p<0.01, *** p<0.001 compared to control group, + p<0.05 ++ p<0.01, +++ p<0.01 compared to MTX group.*

Histological examination revealed necrotic spots with inflammatory cells in the MTX group's liver tissue (Figure 3B). MTX + Chitosan treatment, on the other hand, resulted in decreased neutrophil infiltration, with no evident necrotic spots (Figure 3C). Microscopic examination of the kidney showed fibrotic changes with neutrophil infiltration in the renal cortex, tubules, Bowman's capsule, proximal and distal tubules in the MTX group. However, no significant neutrophil infiltration and structural alterations were detected in the nephron cells of MTX+Chitosan group (Figure 4).

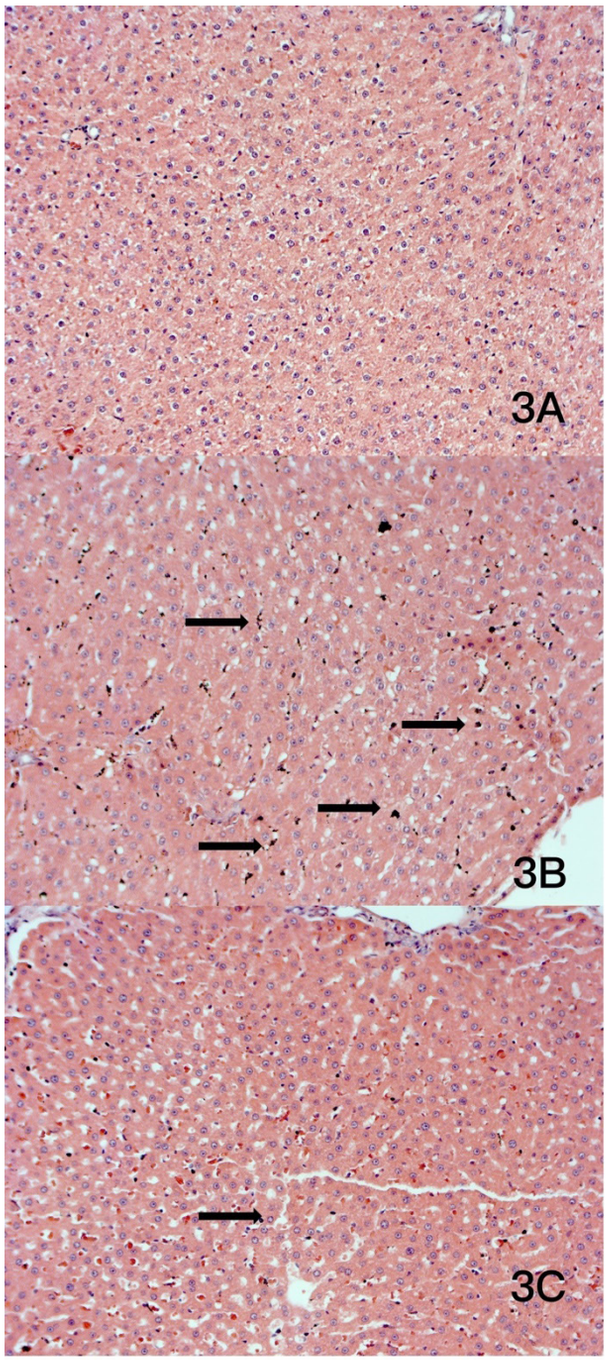

Figure 3. *Histopathological evaluation of liver tissues. 3A: Normal integrity of liver in Control group; 3B Severe inflammatory cell infiltrations (arrow) were detected in MTX group; 3C: Mild inflammatory cells were observed (arrow) in MTX+Chitosan group.*

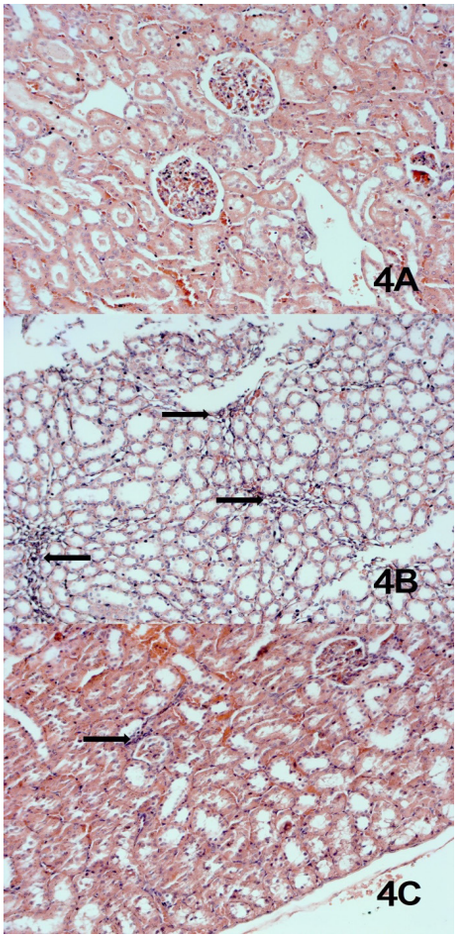

Figure 4. *Histopathological assessment of kidney tissues. 4A: Normal structure of kidney in Control group; 4B: Fibrosis and moderately inflammatory cell infiltrations (arrow) were detected in MTX group; 4C: Mild inflammatory cells were observed (arrow) and fibrosis is absent in MTX+Chitosan group.*

4. DISCUSSION

Several studies have established that chitosan, a biopolymer that is compatible with tissues, has anti-inflammatory and antioxidant properties, and it is also utilized in the production of different of pharmaceutical preparations (19, 20). In this study, the effects of methotrexate on ALT, AST, BUN, serum MMPs, creatinine, caspase and LDH activity, inflammation, and the protective impact of chitosan were examined by histopathological evaluation of the structural damages.

Different chemotherapy agents have been reported to increase ALT, AST, BUN, and creatinine levels, as well as LDH activation, all of which are important enzymes for assessing liver and kidney function (21, 22). Methotrexate (MTX), a known hepatorenal toxicant, has been associated with elevations in serum levels of AST, ALT, BUN, creatinine, and the activation of LDH. Furthermore, MTX is used to induce tissue damage in experimental studies (10, 11). In this study, we observed that chitosan altered the MTX-induced increase in serum ALT, AST, BUN, and creatinine levels. The study results are consistent with those of earlier researches that reported that chitosan can modulate AST, ALT, BUN, creatinine levels, and LDH activity, especially in inflammatory conditions (19, 23, 24).

MMPs, that provide normal maintenance of the normal physiological process, considered as an important marker during tissue damage or diseases (25). They are considered as proteolytic enzymes that infiltrate neutrophils, especially in the inflammatory process. The role of MMPs in the formation of hepatorenal damage due to inflammation or various agents has also been demonstrated (26, 27). The studies on the effects of MTX on MMPs are still controversial (28–30). It has been suggested that MTX exerts a protective effect by decreasing the MMPs activity, which is increased during rheumatoid arthritis. MTX has also been shown to increase MMPs activity in cancer patients. In our study, MTX treatment increased MMP activity while decreasing TIPM-1 activity. Although this does not explain how MTX causes hepatorenal injury, the heterogeneity in MMP activation suggests that MMPs may contribute to MTX-induced hepatorenal injury. In our study, chitosan treatment decreased the activity of MMP-3, MMP-8, MMP-9, and TIMP-1 to levels that were close to that of the control group. The effects of chitosan on MMP activities have been investigated in several studies, and results from these studies are consistent with our findings (31, 32).

In addition, MTX treatment is known to trigger apoptosis by increasing caspase activity. The activation of caspase 3 and 9 is an essential factor in the emergence of MTX-induced liver and kidney injury (33, 34). Studies on Chitosan have revealed that it has antiapoptotic effects (35, 36). In our study, hepatorenal injury following MTX administration was significantly decreased by chitosan administration in accordance with the literature (9).

MTX has been shown to induce structural damage in the liver and renal tissues. Previous studies reported the existence of widespread neutrophil infiltration and tissue necrosis (11, 12). The current study showed that administration of MTX induced damage to both liver and kidney tissues. Histopathological examination confirmed the presence of structural damage in the liver and kidney tissues caused by MTX treatment. These histopathological findings are in line with previous studies (37, 38), and the damage regressed following Chitosan treatment. Thus, the cytoprotective agent, chitosan, has been demonstrated to reduce MTX-induced hepatorenal injury in the presented study.

Similar to other animal experiments, this study possesses several limitations that can influence future research directions. Subsequent investigations should independently validate the involvement of proteolytic and apoptotic enzymes in MTX-induced hepatorenal toxicity and ascertain whether chitosan's protective effect operates via modulation of these enzymes. As this study was conducted on rats, it highlights the necessity for clinical trials to evaluate the efficacy of chitosan as a therapeutic intervention. Histopathological findings play a crucial role in assessing the extent of structural damage. The parameters we assessed contribute to providing an integrative picture of chitosan's impact on MTX-mediated hepatorenal

injury. Further analysis is imperative to elucidate the potential intricate mechanisms underlying this effect.

5. CONCLUSION

Based on the study findings, it was observed that chitosan exhibited no cytotoxic effects. Instead, it demonstrated a structural and functional protective role against MTX-induced hepatorenal toxicity. This protective effect was attributed to its modulation of proteolytic and apoptotic enzymes. MTX, commonly administered for autoimmune conditions like rheumatoid arthritis and cancer, significantly impacts liver and kidney tissues in a detrimental manner. Hence, our findings propose an alternative approach to MTX therapy, emphasizing the potential of chitosan as a therapeutic intervention.

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Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by the Animal Experiments Local Ethics Committee of Near East University (Approval date: 17.04.2020; number: 2020/111).*

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Author Contributions:

Research idea: AOS, KB
Design of the study: AOS, KB, SS
Acquisition of data for the study: SS, HO, AA
Analysis of data for the study: SS, HO, AA
Interpretation of data for the study: AOS, SS, KB
Drafting the manuscript: AOS, KB
Revising it critically for important intellectual content: AOS, KB, SS, HO, AA
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REFERENCES

[1] Jolivet J, Cowan KH, Curt GA, Clendeninn NJ, Chabner BA. The pharmacology and clinical use of methotrexate. New England Journal of Medicine 1983; 309(18):1094–1104. DOI: 10.1056/NEJM198.311.033091805.

[2] Braun J, Rau R. An update on methotrexate. Curr Opin Rheumatol 2009; 21(3):216–223. DOI: 10.1097/BOR.0b013e328329c79d.

[3] Dar A, Fehaid A, Alkhatani S, Alarifi S, Alqahtani W, Albasher G, Almeer R, Alfarraj S, Moneim AA. The protective role of luteolin against the methotrexate-induced hepato-renal toxicity via its antioxidative, anti-inflammatory, and anti-apoptotic effects in rats. Hum Exp Toxicol 2021; 40(7):1194–1207. DOI: 10.1177/096.032.7121991905.

[4] Al Maruf A, O'Brien PJ, Naserzadeh P, Fathian R, Salimi A, Pourahmad J. Methotrexate induced mitochondrial injury and cytochrome c release in rat liver hepatocytes. Drug Chem Toxicol 2018; 41(1):51–61. DOI: 10.1080/01480.545.2017.1289221.

[5] Azouz AA, Saleh E, Abo-Saif AA. Aliskiren, tadalafil, and cinnamaldehyde alleviate joint destruction biomarkers; MMP-3 and RANKL; in complete Freund's adjuvant arthritis model: Downregulation of IL-6/JAK2/STAT3 signaling pathway. Saudi Pharmaceutical Journal 2020; 28(9):1101–1111. DOI: 10.1016/j.jsps.2020.07.011.

[6] Ceylanlı D, Şehirli AÖ, Gençosman S, Teralı K, Şah H, Gülmez N, Sayıner S. Protective effects of alpha-lipoic acid against 5-fluorouracil-induced gastrointestinal mucositis in rats. Antioxidants 2022; 11(10):1930. DOI: 10.3390/antiox11101930.

[7] Gençosman S, Ceylanlı D, Şehirli AÖ, Teralı K, Bölükbaşı F, Çetinel Ş, Sayıner S. Investigation of the possible protective effect of n-acetylcysteine (nac) against irinotecan (cpt-11)-induced toxicity in rats. Antioxidants 2022; 11(11):2219. DOI: 10.3390/antiox11112219.

[8] Chauhan P, Sharma H, Kumar U, Mayachari A, Sangli G, Singh S. Protective effects of Glycyrrhiza glabra supplementation against methotrexate-induced hepato-renal damage in rats: An experimental approach. J Ethnopharmacol 2020; 263113209. DOI: 10.1016/j.jep.2020.113209.

[9] Bilginaylar K, Aykac A, Sayiner S, Özkayalar H, Şehirli AÖ. Evaluation of the antiapoptotic and anti-inflammatory properties of chitosan in methotrexate-induced oral mucositis in rats. Mol Biol Rep 2022; 49(4):3237–3245. DOI: 10.1007/s11033.022.07158-x.

[10] Çakır T, Özkan E, Dulundu E, Topaloğlu Ü, Şehirli AÖ, Ercan F, Şener E, Şener G. Caffeic acid phenethyl ester (CAPE) prevents methotrexate-induced hepatorenal oxidative injury in rats. Journal of Pharmacy and Pharmacology 2011; 63(12):1566–1571. DOI: 10.1111/j.2042-7158.2011.01359.x.

[11] Aslaner A, Çakır T, Çelik B, Doğan U, Mayir B, Akyüz C, Polat C, Baştürk A, Soyer V, Koç S, Şehirli AÖ. Does intraperitoneal medical ozone preconditioning and treatment ameliorate the methotrexate induced nephrotoxicity in rats? Int J Clin Exp Med 2015; 8(8):13811–7.

[12] Çakır T, Baştürk A, Polat C, Aslaner A, Durgut H, Şehirli AÖ, Gül M, Öğünç AV, Gül S, Sabuncuoglu MZ, Oruç MT. Does alfa lipoic acid prevent liver from methotrexate induced oxidative injury in rats? Acta Cir Bras 2015; 30(4):247–252. DOI: 10.1590/S0102.865.0201500.400.00003.

[13] Pradines B, Lievin-Le Moal V, Vauthier C, Ponchel G, Loiseau PM, Bouchemal K. Cell line-dependent cytotoxicity of poly(isobutylcyanoacrylate) nanoparticles coated with chitosan and thiolated chitosan: Insights from cultured human epithelial HeLa, Caco2/TC7 and HT-29/MTX cells. Int J Pharm 2015; 491(1–2):17–20. DOI: 10.1016/j.ijpharm.2015.06.001.

[14] Arpag H, Gül M, Aydemir Y, Atilla N, Yiğitcan B, Cakir T, Polat C, Þehirli Ö, Sayan M. Protective Effects of Alpha-Lipoic Acid on Methotrexate-Induced Oxidative Lung Injury in Rats. J Invest Surg 2018; 31(2):107–113. DOI: 10.1080/08941.939.2017.1296513.

[15] Kısadere İ, Karaman M, Aydın MF, Donmez N, Usta M. The protective effects of chitosan oligosaccharide (COS) on cadmium-induced neurotoxicity in Wistar rats. Arch Environ Occup Health 2021; 1–9. DOI: 10.1080/19338.244.2021.2008852.

[16] Ito M, Ban A, Ishihara M. Anti-ulcer effects of chitin and chitosan, healthy foods, in rats. Jpn J Pharmacol 2000; 82(3):218–225. DOI: 10.1254/jjp.82.218.

[17] Sathiyaseelan A, Saravanakumar K, Mariadoss AVA, Wang MH. PH-controlled nucleolin targeted release of dual drug from chitosan-gold based aptamer functionalized nano drug delivery system for improved glioblastoma treatment. Carbohydr Polym; 262. DOI: 10.1016/J.CARBPOL.2021.117907. Epub ahead of print 15 June 2021. DOI: 10.1016/J.CARBPOL.2021.117907.

[18] Mariadoss AVA, Vinayagam R, Senthilkumar V, Paulpandi M, Murugan K, Xu B, Gothandam KM, Kotakadi VS, David E. Phloretin loaded chitosan nanoparticles augments the pH-dependent mitochondrial-mediated intrinsic apoptosis in human oral cancer cells. Int J Biol Macromol 2019; 130997–1008. DOI: 10.1016/J.IJBIOMAC.2019.03.031.

[19] Wei X, Yang D, Xing Z, Zhao C, Wang L, Fan Y, Nie H, Liu H. Quercetin loaded liposomes modified with galactosylated chitosan prevent LPS/D-GalN induced acute liver injury. Materials Science and Engineering C 2021; 131112527. DOI: 10.1016/j.msec.2021.112527.

[20] Lu S, Kong S, Wang Y, Hu Z, Zhang L, Liao M. Gastric acid-response chitosan/alginate/tilapia collagen peptide composite hydrogel: Protection effects on alcohol-induced gastric mucosal injury. Carbohydr Polym 2022; 277118816. DOI: 10.1016/j.carbpol.2021.118816.

[21] Shalkami A-GS, Hassanein EHM, Sayed AM, Mohamed WR, Khalaf MM, Hemeida RAM. Hepatoprotective effects of phytochemicals berberine and umbelliferone against methotrexate-induced hepatic intoxication: Experimental studies and in silico evidence. Environmental Science and Pollution Research 2021; 28(47):67593–67607. DOI: 10.1007/s11356.021.15358-4.

[22] Nimbal SK, Gadad PC, Koti BC. Effect of ethanolic extract of Rosa centifolia against doxorubicin induced nephrotoxicity in albino rats. J Ayurveda Integr Med 2021; 12(4):657–662. DOI: 10.1016/j.jaim.2021.07.020.

[23] Liu S-H, Chen F-W, Chiang M-T. Chitosan oligosaccharide alleviates abnormal glucose metabolism without inhibition of hepatic lipid accumulation in a high-fat diet/streptozotocin-induced diabetic rat model. Mar Drugs 2021; 19(7):360. DOI: 10.3390/md19070360.

[24] Vannozzi L, Catalano E, Telkhozhayeva M, Teblum E, Yarmolenko A, Avraham ES, Konar R, Nessim GD, Ricotti L. Graphene oxide and reduced graphene oxide nanoflakes coated with glycol chitosan, propylene glycol alginate, and polydopamine: characterization and cytotoxicity in human chondrocytes. Nanomaterials 2021; 11(8):2105. DOI: 10.3390/nano11082105.

[25] Aksoy U, Savtekin G, Şehirli A, Kermeoğlu F, Kalender A, Özkayalar H, Sayıner S, Orhan K. Effects of alpha-lipoic acid therapy on experimentally induced apical periodontitis: A biochemical, histopathological and micro-CT analysis. Int Endod J 2019; 52(9):1317–1326. DOI: 10.1111/iej.13121.

[26] Soliman MM, Nassan MA, Ismail TA. Immunohistochemical and molecular study on the protective effect of curcumin against hepatic toxicity induced by paracetamol in wistar rats. BMC Complement Altern Med 2014; 14(1):457. DOI: 10.1186/1472-6882-14-457.

[27] Kollaras V, Valsami G, Lambropoulou M, Konstandi O, Kostomistsopoulos N, Pikoulis E, Simopoulos C, Tsaroucha A. Effect of silibinin on the expression of MMP2, MMP3, MMP9 and TIMP2 in kidney and lung after hepatic ischemia/reperfusion injury in an experimental rat model. Acta Cir Bras; 36(9): DOI: 10.1590/acb360904.

[28] Liu T, Wu X, Chen S, Wu P, Han H, Zhang H, Li J, Li G, Zhang S. A cationic polymeric prodrug with chemotherapeutic self-sensibilization co-delivering MMP-9 shRNA plasmid for a combined therapy to nasopharyngeal carcinoma. Drug Deliv 2019; 26(1):1280–1291. DOI: 10.1080/10717.544.2019.1698674.

[29] Cardoso LM, Pansani TN, Hebling J, de Souza Costa CA, Basso FG. Chemotherapy drugs and inflammatory cytokines enhance matrix metalloproteinases expression by oral mucosa cells. Arch Oral Biol 2021; 127105159. DOI: 10.1016/j.archoralbio.2021.105159.

[30] Deyab G, Reine TM, Vuong TT, Jenssen T, Hjeltnes G, Agewall S, Mikkelsen K, Førre Ø, Fagerland MW, Kolset SO, Hollan I. Antirheumatic treatment is associated with reduced serum Syndecan-1 in Rheumatoid Arthritis. PLoS One 2021; 16(7):e0253247. DOI: 10.1371/journal.pone.0253247.

[31] Mattaveewong T, Wongkrasant P, Chanchai S, Pichyangkura R, Chatsudthipong V, Muanprasat C. Chitosan oligosaccharide suppresses tumor progression in a mouse model of colitis-associated colorectal cancer through AMPK activation and suppression of NF-κB and mTOR signaling. Carbohydr Polym 2016; 14530–36. DOI: 10.1016/j.carbpol.2016.02.077.

[32] Siahmansouri H, Somi MH, Babaloo Z, Baradaran B, Jadidi-Niaragh F, Atyabi F, Mohammadi H, Ahmadi M, Yousefi M. Effects of HMGA2 siRNA and doxorubicin dual delivery by chitosan nanoparticles on cytotoxicity and gene expression of HT-29 colorectal cancer cell line. Journal of Pharmacy and Pharmacology 2016; 68(9):1119–1130. DOI: 10.1111/jphp.12593.

[33] Chung DC, Long Le T, Ho NQC, Nguyen TT, Do DG, Do DT, Nguyen TPM, Nguyen TPT, Hoang NS. Evaluation of in vitro cytotoxicity and in vivo potential toxicity of the extract from in vitro cultivated Anoectochilus roxburghii Lindl. J Toxicol Environ Health A 2021; 84(24):987–1003. DOI: 10.1080/15287.394.2021.1963363.

[34] Doan CC, Le TL, Ho NQC, La THL, Nguyen VC, Le VD, Nguyen TPT, Hoang NS. Bioactive chemical constituents, in vitro anti-proliferative activity and in vivo toxicity of the extract of Curcuma singularis Gagnep rhizomes. J Ethnopharmacol 2022; 284114803. DOI: 10.1016/j.jep.2021.114803.

[35] Zhang Y, Ahmad KA, Khan FU, Yan S, Ihsan AU, Ding Q. Chitosan oligosaccharides prevent doxorubicin-induced oxidative stress and cardiac apoptosis through activating p38 and JNK MAPK mediated Nrf2/ARE pathway. Chem Biol Interact 2019; 30554–65. DOI: 10.1016/J.CBI.2019.03.027.

[36] Zhang C, Yu L, Zhou Y, Zhao Q, Liu SQ. Chitosan oligosaccharides inhibit IL-1β-induced chondrocyte apoptosis via the P38 MAPK signaling pathway. Glycoconj J 2016; 33(5):735–744. DOI: 10.1007/S10719.016.9667-1.

[37] Çakir T, Polat C, Baştürk A, Gül M, Aslaner A, Durgut H, Şehirli A, Aykaç A, Bahar L, Sabuncuoglu MZ. The effect of alpha lipoic acid on rat kidneys in methotrexate induced oxidative injury. Eur Rev Med Pharmacol Sci 2015; 19(11):2132–2139.

[38] Çakır T, Baştürk A, Polat C, Aslaner A, Durgut H, Şehirli AÖ, Gül M, Öğünç AV, Gül S, Sabuncuoglu MZ, Oruç MT. Does alfa lipoic acid prevent liver from methotrexate induced oxidative injury in rats? Acta Cir Bras 2015; 30(4):247–252. DOI: 10.1590/S0102.865.0201500.400.00003.

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