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Allantoin ameliorated liver fibrosis in a mouse model of non-alcoholic steatohepatitis: role of nuclear factor kappa B/cyclooxygenase 2/prostaglandin E2 pathway

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ABSTRACT

Introduction: Non-alcoholic steatohepatitis (NASH) is considered as current and critical liver disease and liver fibrosis is an initial step to vast NASH injuries. Allantoin is an important and sure composite, which has remark effects on inflammation and apoptosis. This study was done to evaluate the allantoin duty on liver fibrosis and its pathways in mice-induced NASH.
Methods: In the control groups, inbred mice took saline and allantoin. In the NASH group, NASH was provided with a methionine-choline deficient (MCD) diet for eight weeks, and finally, in the NASH-Alla group, allantoin was injected for four weeks in the mice with an MCD diet. For collagen deposition evaluation, trichrome Masson staining and for cellular evaluations, real-time PCR and ELISA assays were performed.
Results: Allantoin treatment improved liver steatosis and fibrosis. Protein expression of nuclear factor kappa B (NFκB-p65) ($P<0.05$) and genes expressions of transforming growth factor-β (TGFβ) ($P<0.001$), cyclooxygenase 2 (COX2) ($P<0.001$), matrix metalloproteinases 9 (MMP9) ($P<0.001$) and alpha-smooth muscle actin (αSMA) ($P<0.001$) were also decreased. Moreover, hepatic prostaglandin E2 (PGE2) levels lowered after allantoin treatment ($P<0.05$).
Conclusion: Attenuating effects of allantoin on liver fibrosis may be due to the inhibition of NFκB/TGFβ, NFκB/MMP9, and NFκB/Cox2/PGE2 pathways, which decrease αSMA expression and collagen deposition and ameliorate liver fibrosis.

Implication for health policy/practice/research/medical education:
Since allantoin has herbal origin and is safe, and has shown antioxidant, anti-steatosis and antiapoptotic effects; after enough and accurate studies, it might be used as a complementary and therapeutic agent in liver diseases, especially liver fibrosis.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is propounded for the extra accumulation of triglycerides in the hepatocytes (steatosis), which may promote to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatic cancer (1). Steatosis, cellular ballooning, and lobar inflammation in the liver provide NASH (2). Today, NAFLD and NASH are increasing and becoming the first liver disease worldwide (3). Increased hepatic fatty acid levels usually increase steatosis neutrophils, resident Kupffer cells, and other innate immune cells activation, which release types of cytokines/chemokines and induce inflammation and then fibrosis (4). Liver fibrosis takes place as a result of the wound-healing response, which arises from hyperactivation of hepatic stellate cells (HSCs) and increase of unwanted deposition of extracellular matrix (ECM) and formation of collagen fibers. Multiple connections between inflammation and fibrosis pathways have also been reported (5,6). Transforming growth factor-β (TGF-β), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and matrix metalloproteinases (MMP) are key factors (7), which cross talk between inflammation and fibrosis. In the NASH disease, hepatocytes and Kupffer cells upregulate NF-κB, as a transcriptional factor, and different types of MMPs lead to liver inflammation and fibrosis (2,7). Specifically, it has been known that MMP9 is upregulated and activates HSCs for the deposition of ECM in cirrhotic patients (8).

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TGF-β, another essential molecule in fibrosis promotion, increases the expression of alpha-smooth muscle actin (αSMA), ECM, and other involved molecules by HSCs activation and causes liver fibrosis (9). Moreover, NF-κB also activates TGF-β and persuades HSCs to hyper-express αSMA and then myofibroblasts to synthesize collagen fibers (10). In another pathway, tissue damage, inflammation, and fibrosis enhance PGE2 production from arachidonic acid (AA) in cyclooxygenase (COX) related pathway. NASH disease, as an inflammatory disease, is associated with increased PGE2 levels (11). Documents show that COX2 and PGE2 levels increase in people with NASH disease (12). It has been reported that NF-κB is able to induce COX2 and increase PGE2 level, too (11,13). PGE2 also enhances TGF-β and αSMA expressions (14). Based on this evidence, various molecular pathways cross link in NASH, providing fibrosis and inflammation.

Allantoin is a compound in many plants, including *N. nucifera rhizome*, *yam*, sugar beet, and leguminous (15-17). Previously, the positive effects of allantoin on tissue regeneration and wound healing have been demonstrated (17,18). Newly, the effects of allantoin on lipid and glucose lowering have been distinguished (19). It has been reported that allantoin incremented the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), sirtuin 1 (SIRT1), and nuclear factor erythroid 2-related factor 1 (Nrf-1) in the skeletal muscle tissue. In a previous study, we showed that allantoin ameliorated NASH by downregulation of steatosis-dependent genes and attenuation of apoptosis pathways in MCD diet-induced animals (20). Allantoin also improves oxidative stress and inflammation (20,21). We recently also reported that the antioxidant effects of allantoin are mediated through SIRT1/Nrf2 pathway in mice with NASH (22). In this study, our aim is to evaluate the effects of allantoin on the attenuation of liver fibrosis and its possible pathways in a mouse model of NASH.

Material and Methods

Materials

Allantoin was purchased from Sigma-Aldrich (Purity: 98%, Germany) and the prostaglandin E2 kit was purchased from ZellBio GmbH, Germany (ZB-10504C-R9648).

Experimental procedures

C57/BL6 male mice (25-27 g) were taken at a temperature of 22-25°C and 12:12 light/dark cycle with free access to food and water. The protocols of this experiment were planned in accordance with the Guidelines for Animal Care and Use at the Qom University of Medical Sciences (IR.MUQ.REC.1400.055). Animals were randomly distributed into four equal groups (n=6).

1– Control group: free access to standard diet for eight weeks with a daily injection of saline (ip) from 4th week during four weeks.
2– Allantoin group: free access to standard diet for eight weeks with a daily injection of allantoin (5 mg/kg, ip) from the fourth week for four weeks (20).
3– NASH group: free access to methionine-choline deficient (MCD) diet for eight weeks to cause NASH (24).
4– NASH-Alla group: free access to MCD diet for eight weeks to induce NASH and injection of allantoin (5 mg/kg, ip) from the fourth week during four weeks daily.

At the end experiment, the animals were anesthetized with sodium pentobarbital (25) and the abdomen was exposed. The liver was immediately taken out and washed with saline.

Histological study

One lobe of the liver was cut and taken in 10% formalin solution. The paraffin-embedded sections from liver tissue were then obtained, and trichrome-Masson staining was performed to find collagen fibers.

Western Blot

NF-κB-p65 (the active form of NF-κB) expression in the liver tissue was determined by Western immunoblotting. Briefly, the protein concentration was determined by the Bradford assay kit (Sigma Aldrich, USA). The proteins were transferred to PVDF membranes and explored with primary antibodies versus NF-κB-p65 (ab16502) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ab181602). The HRP-conjugated secondary antibody (1:7000, Cell Signaling) was attached to the membranes. Then, the membranes were enhanced with chemiluminescence (ECL, Amersham) reagents in a dark room and exposed to an X-ray film and visualization of the chemiluminescence of the binding by means of a visualizing machine. The diameter of the bands was measured by ImageJ software (IJ 1.46r version, NIH, USA) and normalized by the GAPDH as a housekeeping protein.

Real-time RT-PCR

Using the RNX-plus kit from frozen tissue samples, total RNA was extracted (Cinnagen, Tehran, Iran), and the quantity and purity of the RNA samples were measured by a Nanodrop spectrophotometer (Thermo Scientific, USA). By the cDNA synthesis kit, complementary DNAs (cDNA) were produced (Yekta Tajhiz, Iran) and for real-time assay, 2× SYBR Green master mix (Biofact, Korea: 1670-5695), template, forward and reverse specific primers, and DEPC of treated water were mixed and incubated. GAPDH was used as an internal control. The analysis of data was performed by the comparative $2^{-\Delta\Delta Ct}$ method. Primers for the genes studied were designed by primer3 software (v. 0.4.0) (http://primer3.ut.ee). Primer sequence similarity and specificity were established with BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as follows:

α-SMA: (F) CCCAGACATCAGGGAGTAATGG (R) TCTATCGGATACTTCAGCGTCA
TGF-β: (F) CTTCAATACGTCAGACATTCGGG (R) GTAACGCCAGGAATTGTTGCTA
COX2: (F) CTGGTCTGATGATGTATGCC (R) TCCTATGAGTATGAGTCTGCTG
MMP9: (F) CACTTTCCCTTCACCTCC (R) TTGCCGTCCTTATCGTAG
GAPDH: (F) TGGCCTTCCGTGTTCCTAC (R) GAGTTGCTGTTGAAGTCGCA

PGE2 measurement

A part of the hepatic texture was weighted, mixed with phosphate-buffered saline (PBS) buffer (100mM, PH 7.4, 1ml/100 mg tissue with anti-protease mixture), homogenized by an electrical homogenizer (IKA-3420000, Germany), and centrifuged (4000-6000 rpm for 10 minutes). The prepared supernatants were then inquired for PGE2 levels through an ELISA kit, according to the manufacturer's instructions, and read by an ELISA reader at 450 nm.

Statistical analysis

Data were reported as mean± standard error of the mean (SEM). The Kolmogorov–Smirnov test determined normality of the data. Comparison between groups was made by one-way analysis of variances (ANOVA) and Tukey's post hoc test using SPSS. P <0.05 was considered statistically significant.

Results

Allantoin attenuated liver injuries in the mice with NASH

Our findings from trichrome-Masson staining showed much lipid-droplet accumulation (empty spaces), inflammation, and collagen fibers deposition in the surround of the central and portal veins in MCD induced mice, whereas, steatosis, inflammation, and fibrosis were improved in the NASH-Alla group (Figure 1).

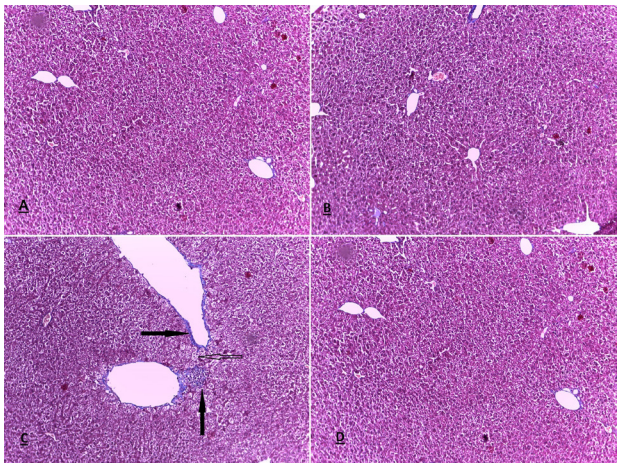

Figure 1. Histological results of liver tissues after Trichrome-Masson staining (magnification; ×200) between the groups. A; control group: Normal liver structure, B; the allantoin group: Normal liver architecture, C; the non-alcoholic steatohepatitis group: steatosis (white arrow), ballooning degradation and collagen fibers (black arrow) D; the non-alcoholic steatohepatitis, Allantoin group: lower steatosis and collagen fibers.

Allantoin decreased liver NFκB-p65 expression in the mice with NASH

Western blot results revealed a marked increase in protein expression of NFκB-p65 in the NASH group compared with the control group (P<0.01). However, allantoin administration significantly reduced the expression of NFκB-p65 (P<0.05) (Figure 2).

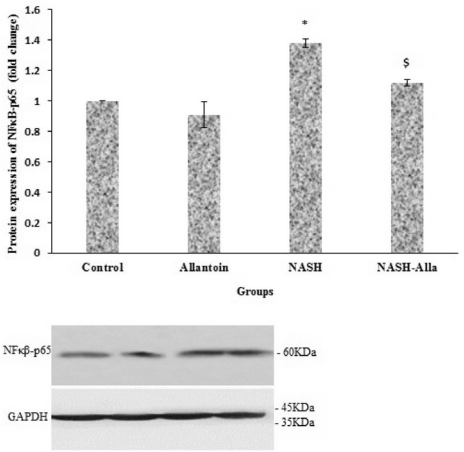

Figure 2. Protein expression of nuclear factor kappa B between the groups (Mean ± SEM, n = 6). * P<0.01 significant compared with the control group, $ P<0.05 significant compared with the non-alcoholic steatohepatitis group (one-way ANOVA followed by Tukey's post hoc test).

Allantoin decreased TGF-β and α-SMA expressions in the mice with NASH

Our data in Figure 3 showed that the gene expressions of TGF-β and α-SMA significantly upregulated in the NASH group compared with the control group (P<0.001), meanwhile allantoin markedly decreased them in the NASH-Alla group compared with the NASH group (P<0.001).

Allantoin decreased MMP9 expression in the mice with NASH

The obtained results displayed a marked increment in MMP9 gene expression in the NASH group compared with the control group (P<0.001), and allantoin could significantly decrease MMP9 expression in the NASH-Alla group compared with the NASH group (P<0.001) (Figure 4).

Allantoin decreased COX2 expression and PGE2 level in the mice with NASH

As presented in Figure 5, hepatic COX2 expression and PGE2 level were significantly increased in the NASH group

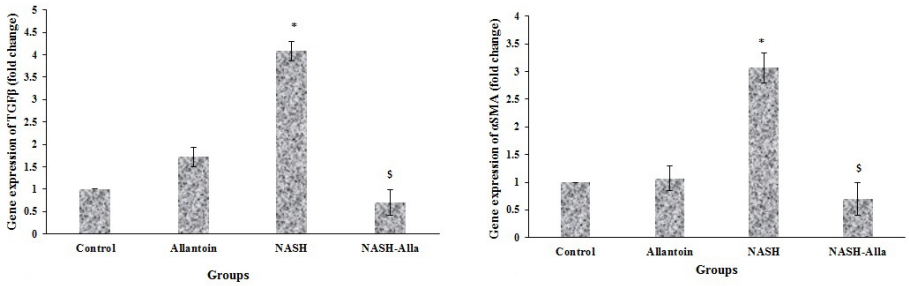

Figure 3. mRNA expressions of transforming growth factor-β and alpha-smooth muscle actin between the groups (Mean ± SEM, n = 6). * $P < 0.001$ significant compared with the control group, $ $P < 0.001$ significant compared with the non-alcoholic steatohepatitis group.

compared with the control group ($P < 0.001$and $P < 0.05$, respectively), although in the NASH-Alla group, allantoin markedly lowered COX2 and PGE2 level compared with NASH group ($P < 0.001$and $P < 0.05$, respectively).

Discussion

Allantoin could downregulate NF-κB, TGF-β, COX2, MMP9, and αSMA expressions, decrease PGE2 levels, and finally improve liver fibrosis. This is the first report in which the effects of allantoin have been evaluated on the NASH-induced liver fibrosis by cross talking between inflammation and fibrosis pathways.

Allantoin is a synthetic compound but is plenty found in *yam*, *N. nucifera* rhizome, sugar beet, leguminous and other herbs (15). Allantoin has been used as a cosmetic and wound healing agent but now its other effects are being revealed (18,25). Recent studies have shown the metabolic, anti-inflammatory, and anti-oxidative effects of allantoin in animal models (21,26,27). We previously reported that allantoin attenuated steatosis and liver injury in the animals with NASH, through the improvement of lipids metabolism and decrement of inflammation, oxidative stress, and apoptosis (20,22).

Liver fibrosis results from chronic and vast liver damage that activate HSCs and enforce to hypersecretion of ECM and formation of fibrosis tissues (29). In the prolonged NASH disease, steatotic and inflammatory processes lead to HSC cell activation and differentiation into myofibroblasts and collagen fibers formation (1,29,30).

In this experiment, allantoin administration could decrease NFκB-p65 (activated form of NF-κB) protein expression. It also declined TGFβ, α-SMA, and MMP9 expressions. NF-κB and TGFβ are two critical nuclear factors in fibrosis, and the activation of NF-κB launches the cascade of inflammatory and fibrotic processes. Activation of TGFβ as a growth factor also promotes liver fibrosis through increasing the expression of α-SMA (4,8,25). In

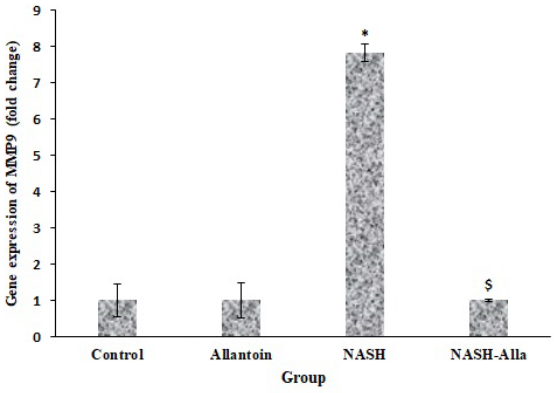

Figure 4. mRNA expression of matrix metalloproteinases 9 between the groups (Mean ± SEM, n = 6). * $P < 0.001$ significant compared with the control group, $ $P < 0.001$ significant compared with the non-alcoholic steatohepatitis group (one-way ANOVA followed by Tukey's post hoc test).

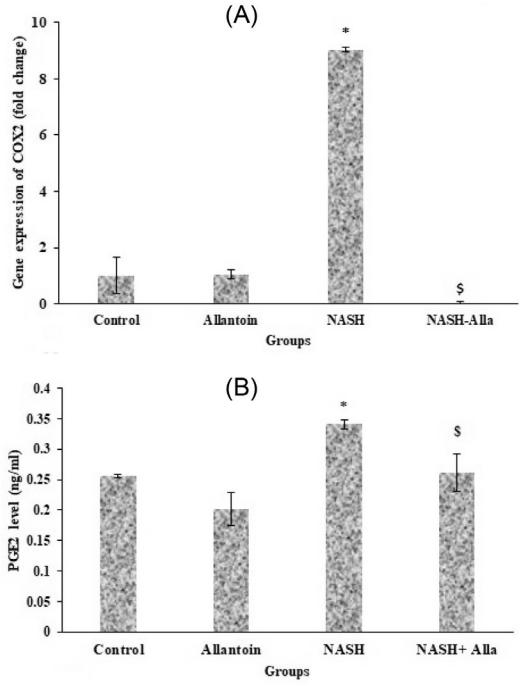

Figure 5. mRNA expression of cyclooxygenase 2 and Prostaglandin E2 concentration between the groups (Mean ± SEM, n = 6), for cyclooxygenase 2; * $P < 0.001$ significant compared with the control group and for Prostaglandin E2; * $P < 0.05$ significant compared with the control group, for cyclooxygenase 2; $ $P < 0.001$ significant compared with the non-alcoholic steatohepatitis group and for Prostaglandin E2; $ $P < 0.05$ significant compared with the non-alcoholic steatohepatitis group (one-way ANOVA followed by Tukey's post hoc test).

this context, glycine-based compounds can decrease ALT and AST serum levels, TGFβ 1 and NF-κB expressions, and NASH attenuation in mice. El-Swefy and Hassanen have also reported that in cholestatic liver fibrosis, NF-κB and TGFβ levels increased, while montelukast administration, a cysteinyl leukotrienes antagonist, decreased both and improved fibrosis (31). Moreover, MMP9 stimulates and persuades HSCs to overproduce ECM and collagen deposition, too (32). According to available documents, there are multiple interactions among MMP9, TGFβ, and NF-κB, and all of them are upregulated in the NASH disease and able to activate each other (8,31,33). In a study, treatment with coenzyme Q10 attenuated inflammation and fibrosis in patients with pelvic tumors via inhibiting the NF-κB/TGF-β1/MMP-9 pathway and α-SMA expression (8). Inhibition of NF-κB activity also led to the decrease of collagen IV, MMP-9, tissue inhibitors of matrix metalloproteinases expressions, and pulmonary fibrosis improvement (34). On the other hand, allantoin has positive effects on the decrease of inflammation and fibrosis. The alleviating effects of a mixture composed of cepae extract, allantoin, and heparin on epidural fibrosis have previously been reported at the rat laminectomy (35). Hydrocolloid film of allantoin also improved diabetic wound healing through the decrease of NF-κB and TGFβ expressions (17). In another report, it has been stated that the application of a patch containing allantoin improves hypertrophic and fibrosis skin scars (36). Our findings are consistent with the previous reports, and probably allantoin, through the suppression of NF-κB, has led to the decrease of TGF-β1 and MMP9 expressions, attenuation of HSCs activity, and then downregulation of αSMA. Following that, collagen production and deposition have reduced, and liver fibrosis improved.

Chronic inflammation is an important promoter to provide liver fibrosis. One of these inflammatory pathways is COX2/PGE2. COX2 is a key enzyme in transforming arachidonic acid into prostaglandin E2, which is upregulated in the NASH disease (14). PGE2 concentration is increased in NASH in both the liver and the blood levels (11,36,37). NF-κB induces COX2, and PGE2 upregulates NF-kB expression in a vicious cycle (38). PGE2 also can increase TGFβ and αSMA expressions, activate HSCs to deposit collagen and play a notable role in liver fibrosis (32,39). Our findings showed that allantoin reduced COX2 expression and lowered hepatic PGE2 concentration. Etoricoxib has an anticarcinogenic effect via the modulation of NF-κB/COX-2/PGE2 signaling in Hepatocellular carcinoma cells (37). Green tea extract also attenuated NASH-induced liver injury by reducing lipid peroxidation, COX2, and PGE2 expressions (11). An experimental gel containing allantoin could significantly reduce pain, trismus, and inflammation signs after surgery (40). Allantoin also lowered ovalbumin-induced lung inflammation via the inhibition of T-helper-2-type (Th2), interleukin-4 (IL-4), and IL-5 (41). Likewise, we reported that allantoin declined tumor necrosis factor-alpha and IL-6 expressions and ameliorated lobar inflammation in mice with NASH (20). In the light of this evidence, it seems that allantoin could also alleviate hepatic fibrosis through downregulation of NF-κB expression and thereby decrease of COX2 and PGE2 levels and then reduction of TGF and αSMA expressions. This is another novel pathway, which could support attenuating the effects of allantoin on the NASH-induced liver fibrosis.

The high cost of kits, antibodies, and chemicals prevented more data from being obtained. However, we evaluated some signaling pathways associated with liver fibrosis. This study was the continuation of our previous research, which confirmed the other positive effects of allantoin on NASH disease in mice.

Conclusion

This study demonstrated that allantoin had ameliorative effects on NASH-induced liver fibrosis, which resulted from the suppression of NF-κB/TGF-β/α-SMA, NFκB/COX-2/PGE2/α-SMA, and NF-κB/MMP9/α-SMA pathways and thereupon the decrement of collagen deposit in the liver. These findings confirm the notable effects of allantoin in the treatment of NASH injuries.

Authors' contributions

TKM performed the work and AM designed the study, analyzed the data, and wrote the manuscript. All authors read the final version and approved the manuscript.

Conflict of interests

The authors declare that they have no conflict of interest.

Ethical considerations

The animal care and experimental procedure were approved in accordance with the Guidelines for Animal Care and Use at the Qom University of Medical Sciences (IR.MUQ.REC.1400.055).

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References

1. Gottlieb A, Mosthael W, Sowa JP, Canbay A. Nonalcoholic-fatty-liver-disease and nonalcoholic steatohepatitis: successful development of pharmacological treatment will depend on translational research. Digestion. 2019;100(2):79-85. doi: 10.1159/000493259.
2. Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE. From NAFLD to NASH to cirrhosis-new insights into disease mechanisms. Nat Rev Gastroenterol Hepatol. 2013;10(11):627-36. doi: 10.1038/nrgastro.2013.149.
3. Zhu JZ, Dai YN, Wang YM, Zhou QY, Yu CH, Li YM. Prevalence of nonalcoholic fatty liver disease and economy. Dig Dis Sci. 2015;60(11):3194-202. doi: 10.1007/s10620-015-3728-3.

4. Machado MV, Diehl AM. Pathogenesis of nonalcoholic steatohepatitis. Gastroenterology. 2016;150(8):1769-77. doi: 10.1053/j.gastro.2016.02.066.
5. El-Baz FK, Salama AAA, Hussein RA. *Dunaliella salina* microalgae oppose thioacetamide-induced hepatic fibrosis in rats. Toxicol Rep. 2020;7:36-45. doi: 10.1016/j.toxrep.2019.10.017.
6. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol. 2011;6:425-56. doi: 10.1146/annurev-pathol-011110-130246.
7. El-Lakkany NM, El-Maadawy WH, Seif El-Din SH, Hammam OA, Mohamed SH, Ezzat SM, et al. Rosmarinic acid attenuates hepatic fibrogenesis via suppression of hepatic stellate cell activation/proliferation and induction of apoptosis. Asian Pac J Trop Med. 2017;10(5):444-53. doi: 10.1016/j.apjtm.2017.05.012.
8. Mohamed HA, Said RS. Coenzyme Q10 attenuates inflammation and fibrosis implicated in radiation enteropathy through suppression of NF-kB/TGF-β/MMP-9 pathways. Int Immunopharmacol. 2021;92:107347. doi: 10.1016/j.intimp.2020.107347.
9. Wu H, Chen C, Ziani S, Nelson LJ, Ávila MA, Nevzorova YA, et al. Fibrotic events in the progression of cholestatic liver disease. Cells. 2021;10(5):1107. doi: 10.3390/cells10051107.
10. Ignat SR, Dinescu S, Váradi J, Fenyvesi F, Nguyen TLP, Ciceu A, et al. Complexation with random methyl-β-cyclodextrin and (2-hydroxypropyl)-β-cyclodextrin promotes chrysin effect and potential for liver fibrosis therapy. Materials (Basel). 2020;13(21):5003. doi: 10.3390/ma13215003.
11. Chung MY, Mah E, Masterjohn C, Noh SK, Park HJ, Clark RM, et al. Green tea lowers hepatic COX-2 and prostaglandin E2 in rats with dietary fat-induced nonalcoholic steatohepatitis. J Med Food. 2015;18(6):648-55. doi: 10.1089/jmf.2014.0048.
12. Zhao JS, Zhu FS, Liu S, Yang CQ, Chen XM. Pioglitazone ameliorates nonalcoholic steatohepatitis by down-regulating hepatic nuclear factor-kappa B and cyclooxygenases-2 expression in rats. Chin Med J (Engl). 2012;125(13):2316-21.
13. Wang B, Wu Z, Li W, Liu G, Tang Y. Insights into the molecular mechanisms of Huangqi decoction on liver fibrosis via computational systems pharmacology approaches. Chin Med. 2021;16(1):59. doi: 10.1186/s13020-021-00473-8.
14. Chen L, Ji X, Wang M, Liao X, Liang C, Tang J, et al. Involvement of TLR4 signaling regulated-COX2/PGE2 axis in liver fibrosis induced by *Schistosoma japonicum* infection. Parasit Vectors. 2021;14(1):279. doi: 10.1186/s13071-021-04790-7.
15. Ma J, Kang SY, Meng X, Kang AN, Park JH, Park YK, et al. Effects of rhizome extract of *Dioscorea batatas* and its active compound, allantoin, on the regulation of myoblast differentiation and mitochondrial biogenesis in C2C12 myotubes. Molecules. 2018;23(8):2023. doi: 10.3390/molecules23082023.
16. Ahn YJ, Park SJ, Woo H, Lee HE, Kim HJ, Kwon G, et al. Effects of allantoin on cognitive function and hippocampal neurogenesis. Food Chem Toxicol. 2014;64:210-6. doi: 10.1016/j.fct.2013.11.033.
17. Tan WS, Arulselvan P, Ng SF, Mat Taib CN, Sarian MN, Fakurazi S. Improvement of diabetic wound healing by topical application of Vicenin-2 hydrocolloid film on Sprague Dawley rats. BMC Complement Altern Med. 2019;19(1):20. doi: 10.1186/s12906-018-2427-y.
18. Araújo LU, Grabe-Guimarães A, Mosqueira VC, Carneiro CM, Silva-Barcellos NM. Profile of wound healing process induced by allantoin. Acta Cir Bras. 2010;25(5):460-6. doi: 10.1590/s0102-86502010000500014.
19. Lin KC, Yeh LR, Chen LJ, Wen YJ, Cheng KC, Cheng JT. Plasma glucose-lowering action of allantoin is induced by activation of imidazoline I-2 receptors in streptozotocin-induced diabetic rats. Horm Metab Res. 2012;44(1):41-6. doi: 10.1055/s-0031-1295439.
20. Komeili Movahhed T, Moslehi A, Golchoob M, Ababzadeh S. Allantoin improves methionine-choline deficient diet-induced nonalcoholic steatohepatitis in mice through involvement in endoplasmic reticulum stress and hepatocytes apoptosis-related genes expressions. Iran J Basic Med Sci. 2019;22(7):736-44. doi: 10.22038/ijbms.2019.33553.8012.
21. da Silva DM, Martins JLR, de Oliveira DR, Florentino IF, da Silva DPB, Dos Santos FCA, et al. Effect of allantoin on experimentally induced gastric ulcers: pathways of gastroprotection. Eur J Pharmacol. 2018;821:68-78. doi: 10.1016/j.ejphar.2017.12.052.
22. Hamidi-Zad Z, Moslehi A, Rastegarpanah M. Attenuating effects of allantoin on oxidative stress in a mouse model of nonalcoholic steatohepatitis: role of SIRT1/Nrf2 pathway. Res Pharm Sci. 2021;16(6):651-9. doi: 10.4103/1735-5362.327511.
23. Komeili-Movahhed T, Bassirian M, Changizi Z, Moslehi A. SIRT1/NFκB pathway mediates anti-inflammatory and anti-apoptotic effects of rosmarinic acid on in a mouse model of nonalcoholic steatohepatitis (NASH). J Recept Signal Transduct Res. 2022;42(3):241-50. doi: 10.1080/10799893.2021.1905665.
24. Nikoukar LR, Nabavizadeh F, Mohamadi SM, Moslehi A, Hassanzadeh G, Nahrevanian H, et al. Protective effect of ghrelin in a rat model of celiac disease. Acta Physiol Hung. 2014;101(4):438-47. doi: 10.1556/APhysiol.101.2014.4.5.
25. El Mubarak MA, Lamari FN, Kontoyannis C. Simultaneous determination of allantoin and glycolic acid in snail mucus and cosmetic creams with high performance liquid chromatography and ultraviolet detection. J Chromatogr A. 2013;1322:49-53. doi: 10.1016/j.chroma.2013.10.086.
26. Chung HH, Lee KS, Cheng JT. Decrease of obesity by allantoin via imidazoline I1-receptor activation in high fat diet-fed mice. Evid Based Complement Alternat Med. 2013;2013:589309. doi: 10.1155/2013/589309.
27. Florentino IF, Silva DPB, Galdino PM, Lino RC, Martins JLR, Silva DM, et al. Antinociceptive and anti-inflammatory effects of *Memora nodosa* and allantoin in mice. J Ethnopharmacol. 2016;186:298-304. doi: 10.1016/j.jep.2016.04.010.
28. Acharya P, Chouhan K, Weiskirchen S, Weiskirchen R. Cellular mechanisms of liver fibrosis. Front Pharmacol. 2021;12:671640. doi: 10.3389/fphar.2021.671640.
29. Chen C, Liu Q, Liu L, Hu YY, Feng Q. Potential biological effects of (-)-epigallocatechin-3-gallate on the treatment of nonalcoholic fatty liver disease. Mol Nutr Food Res.

2018;62(1):1700483. doi: 10.1002/mnfr.201700483.
30. Rom O, Liu Y, Liu Z, Zhao Y, Wu J, Ghrayeb A, et al. Glycine-based treatment ameliorates NAFLD by modulating fatty acid oxidation, glutathione synthesis, and the gut microbiome. Sci Transl Med. 2020;12(572):eaaz2841. doi: 10.1126/scitranslmed.aaz2841.
31. El-Swefy S, Hassanen SI. Improvement of hepatic fibrosis by leukotriene inhibition in cholestatic rats. Ann Hepatol. 2009;8(1):41-9.
32. Wen SL, Gao JH, Yang WJ, Lu YY, Tong H, Huang ZY, et al. Celecoxib attenuates hepatic cirrhosis through inhibition of epithelial-to-mesenchymal transition of hepatocytes. J Gastroenterol Hepatol. 2014;29(11):1932-42. doi: 10.1111/jgh.12641.
33. Quagliariello V, De Laurentiis M, Rea D, Barbieri A, Monti MG, Carbone A, et al. The SGLT-2 inhibitor empagliflozin improves myocardial strain, reduces cardiac fibrosis and pro-inflammatory cytokines in non-diabetic mice treated with doxorubicin. Cardiovasc Diabetol. 2021;20(1):150. doi: 10.1186/s12933-021-01346-y.
34. Chong LW, Hsu YC, Lee TF, Lin Y, Chiu YT, Yang KC, et al. Fluvastatin attenuates hepatic steatosis-induced fibrogenesis in rats through inhibiting paracrine effect of hepatocyte on hepatic stellate cells. BMC Gastroenterol. 2015;15:22. doi: 10.1186/s12876-015-0248-8.
35. Özay R, Yavuz OY, Aktaş A, Yiğit F, Çetinalp NE, Özdemir HM, et al. Effects of cepae extract, allantoin, and heparin mixture on developing andalready formed epidural fibrosis in a rat laminectomy model. Turk J Med Sci. 2016;46(4):1233-9. doi: 10.3906/sag-1504-16.
36. Campanati A, Ceccarelli G, Brisigotti V, Molinelli E, Martina E, Talevi D, et al. Effects of in vivo application of an overnight patch containing Allium cepa, allantoin, and pentaglycan on hypertrophic scars and keloids: clinical, videocapillaroscopic, and ultrasonographic study. Dermatol Ther. 2021;34(1):e14665. doi: 10.1111/dth.14665.
37. Ali G, Omar H, Hersi F, Abo-Youssef A, Ahmed O, Mohamed W. The protective role of etoricoxib against diethylnitrosamine/2-acetylaminofluorene- induced hepatocarcinogenesis in Wistar rats: the impact of NF-κB/COX-2/PGE2 signaling. Curr Mol Pharmacol. 2022;15(1):252-62. doi: 10.2174/1874467214666210708103752.
38. Poligone B, Baldwin AS. Positive and negative regulation of NF-kappaB by COX-2: roles of different prostaglandins. J Biol Chem. 2001;276(42):38658-64. doi: 10.1074/jbc.M106599200.
39. Gao JH, Wen SL, Yang WJ, Lu YY, Tong H, Huang ZY, et al. Celecoxib ameliorates portal hypertension of the cirrhotic rats through the dual inhibitory effects on the intrahepatic fibrosis and angiogenesis. PLoS One. 2013;8(7):e69309. doi: 10.1371/journal.pone.0069309.
40. Sáez-Alcaide LM, Molinero-Mourelle P, González-Serrano J, Rubio-Alonso L, Bornstein MM, López-Quiles J. Efficacy of a topical gel containing chitosan, chlorhexidine, allantoin and dexpanthenol for pain and inflammation control after third molar surgery: a randomized and placebo-controlled clinical trial. Med Oral Patol Oral Cir Bucal. 2020;25(5):e644-e51. doi: 10.4317/medoral.23661.
41. Lee MY, Lee NH, Jung D, Lee JA, Seo CS, Lee H, et al. Protective effects of allantoin against ovalbumin (OVA)-induced lung inflammation in a murine model of asthma. Int Immunopharmacol. 2010;10(4):474-80. doi: 10.1016/j.intimp.2010.01.008.