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FEBUXOSTAT INHIBITS CONCANAVALIN A INDUCED ACUTE HEPATIC INJURY IN MICE AS INDICATED BY DECREASING THE INCREASED SERUM TRANSAMINASE, TOTAL BILIRUBIN AND ALBUMIN

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ABSTRACT

Effect of febuxostat administration on acute liver injury induced by concanavalin A injection into mice eye orbital sinus was studied in the present work. Oral administration of febuxostat either 1 hours before administration of Con A or ½ hour after administration of Con A both significantly inhibited the increase of serum ALT, AST, total bilirubin, and the decrease of serum albumin induced by Con A. These results indicate that febuxostat may be an effective inhibitor of the immune reaction that results from Con A administration into mice.

KEYWORDS: Acute Hepatic Injury, Concanavalin A, Febuxostat.

INTRODUCTION

Acute liver inflammation mostly resolves completely upon removal of the insulting agent and normal liver function is restored without any evidence of the previous insult.[1] If the acute liver inflammation does not resolve completely, it will change to be a chronic challenge. This occurs mainly in autoimmune, chemically and virally induced (HBV and HCV) acute liver inflammation.[2] The vascular component of liver inflammation is the most common pathway that is responsible for the massive loss of hepatocytes due to the rapid activation of the innate immune response (Kupffer cells and circulating monocytes). Activation of the innate immunity elements results in releasing of a stream of pro-inflammatory mediators which finally causes the clinical features of acute liver failure.[3]

The initial detection of liver cell massive destruction is usually carried out by reading the liver function test, namely serum ALT, serum AST, serum total bilirubin and serum albumin. Such test reading is helpful in assessing the severity of the injury and not to under estimate other clinical and radiological findings, in addition to being helpful in assessing the response to different therapeutic modalities.[4] The hepatic injury induced by concanavalin A (Con A) resembles that of the human viral and autoimmune hepatitis.[5]

Febuxostat is a selective xanthine oxidase/xanthine dehydrogenase inhibitor, It's approved for the chronic management of hyper-uricemia in patients with gout.[6] Recently febuxostat was found to inhibit acute pulmonary inflammation triggered by uric acid-induced lung injury, similarly it was found to inhibit liver inflammation triggered by acetaminophen-induced liver injury.[7] However, it cannot be considered as a direct anti-inflammatory drug because it does not reduce any of the known inflammatory pathways.[8] The present work was carried out to investigate the possibility of controlling Con A-induced acute liver injury by febuxostat.

MATERIALS AND METHODS

Materials

Animals

Adult male Swiss albino mice (n=48, w=25-40g each) were purchased from biological products & vaccines (VACSERA) company – Helwan, Egypt. They were Kept 14 days in the animal house of faculty of pharmacy, Mansoura University, Egypt, before starting the study for accommodation. The experimental work carried out in this study complies with the guidelines and ethical principles for the care and use of laboratory animals adopted by the Scientific Research Ethics Committee, faculty of pharmacy, Mansoura university.

Drug and chemicals

Con A was purchased from Alfa Aesar-USA and dissolved in 0.9% saline solution and injected in orbital sinus of the eye. Febuxostat 80 mg (Goutifade 80mg tablets) was purchased from Global Napi Pharmaceuticals, 6th of October city, Giza, Egypt and suspended in carboxymethyl cellulose (0.5%w/v as a drug vehicle) and administered orally. Carboxymethyl cellulose (CMC): as a white powder purchased from El Nasr Pharmaceutical Chemicals Co. Abo Zaabal, Egypt.

Kits: Alanine aminotransferase (ALT) assay kit was purchased from BIOMED diagnostics, Egy-Chem, Bader city. Aspartate aminotransferase (AST) assay kits was purchased from biomed Diagnostic Egy-Chem, Bader city. Total bilirubin assay kit was purchased from Biomed Diagnostic, Egy-Chem, Bader city. Albumin assay kit was purchased from Biomed Diagnostics, Egy-Chem, Bader city.

Experimental Design: Six groups of mice were used as follows: Group 1: (n=8) control received 0.5% CMC 0.2 ml/mouse. Group 2: (n=8) received Con A. i.v in eye orbital sinus plexus, 15mg/kg. The volume injected is 0.2 ml of 0.025%.[8] Group 3: (n=8) received febuxostat in a dose of 20mg/kg, one hour before administration of Con A. Group 4 (n=8) received febuxostat in a dose of (20mg/kg) 1/2 an hour after administration of Con A. Group 5: (n=8) received febuxostat in a dose of (10mg/kg) one hour before administration of Con A. Group 6: (n=8) received febuxostat in a dose of (10m/kg) 1/2 an hour after administration of Con A. Tested doses of febuxostat were selected in the light of previous study.[9]

Methods

Induction of liver inflammation: The induction of liver inflammation was carried out by intravascular injection (in orbital sinus plexus) of concanavalin A (15mg/kg).[8] Mice were sacrificed two hours after concanavalin A.

Liver function biomarkers: Mice were anaesthetized by an i.p. injection of thiopental (50 mg/kg) and blood samples were collected through cardiac puncture, centrifuged at 3000 rpm for 10 min at 4°C, serum was separated, divided into aliquots (to avoid freeze–thaw cycles) and stored at -20°C. Serum ALT, AST, total bilirubin were measured according the manufacturer instructions (Biomed Diagnostics, Egy-Chem, Egypt) in U/l and albumin in mg /dL (Biomed Diagnostics. Egy-Chem, Egypt) spectrophotometrically.

STATISTICAL ANALYSIS

Data are expressed as mean ± S.E (n=8). Results were statistically tested by means of one-way analysis of variance (ANOVA), followed by the Tukey–Kramer multiple comparison test.

RESULTS

Administration of Con A significantly ($P < 0.001$) increased serum ALT, AST, total bilirubin activity compared to control group, and decreased serum albumin concentration. Oral

administration of either 10 or 20 mg/kg febuxostat before and after Con A administration led to a significant decrease of ALT serum activity ($P < 0.001$) when compared to Con A group, but still significantly higher (in case of 10 mg/kg before and after) ($P < 0.05$) compared to control group, while in case of 20 mg/kg before and after there were no significant difference compared to control group.

Oral administration of either 10 or 20 mg/kg febuxostat before and after Con A administration led to AST serum activity significant decrease as follows: in case of 10 mg/kg before ($P < 0.001$), in case of 20 mg/kg before ($P < 0.01$), in case of 20 mg/kg after ($P < 0.05$) when compared to Con A group, but still significantly higher in case of 10 and 20 mg/kg before and after ($P < 0.001$) compared to control group.

Oral administration of either 10 or 20 mg/kg febuxostat before and after Con A administration led to significant decrease in serum total bilirubin level as follows: in case of 10 mg/kg before ($P < 0.001$), in case of 10 mg/kg after ($P < 0.01$), in case of 20 mg/kg before ($P < 0.01$), in case of 20 mg/kg after ($P < 0.001$) when compared to Con A group. Bilirubin level still significantly higher in case of 10 mg/kg after and 20 mg/kg before ($P < 0.05$) while in case of 10 mg/kg before and 20 mg/kg after there was no significant difference compared to control group. Oral administration of 20 mg/kg febuxostat before and after concanavalin A administration led to significant increase in serum albumin level ($P < 0.01$) when compared to Con A group, but in 10 mg/kg before and after there was no significant difference. Serum albumin level remained significantly higher groups received 10 and 20 mg/kg before and after Con A ($P < 0.001$) compared to control group.

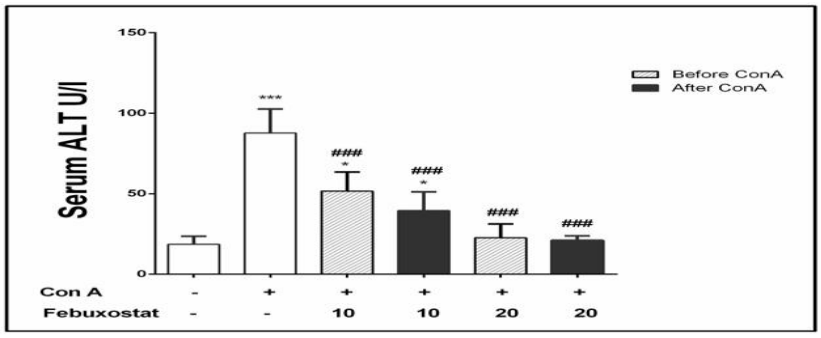

Fig. 1: Effect of febuxostat administration (10, 20 mg/kg) before and after concanavalin A injection on serum alanine aminotransferase (ALT) activity induced liver inflammation in mouse. *, * ($P < 0.05$, 0.001 respectively) compared to control group. ### ($P < 0.001$) compared to Con A group.**

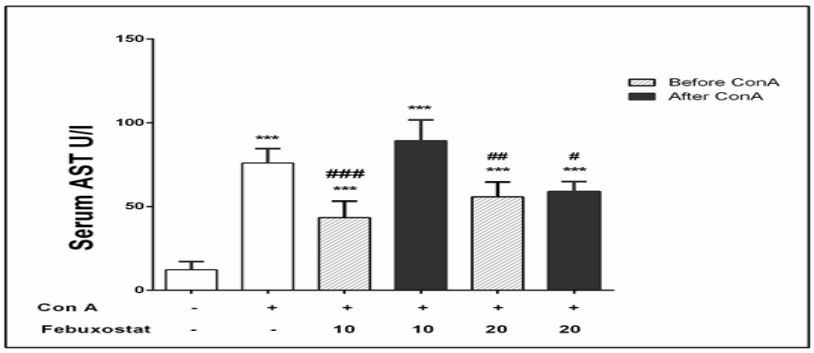

Fig. 2: Effect of febuxostat administration (10, 20 mg) before and after concanavalin A injection on serum aspartate aminotransferase (AST) activity induced liver Inflammation in mouse. * Significantly different (P < 0.001) compared to control group. #, ##, ### Significantly different (P < 0.05, 0.01, 0.001 respectively) compared to Con A group.**

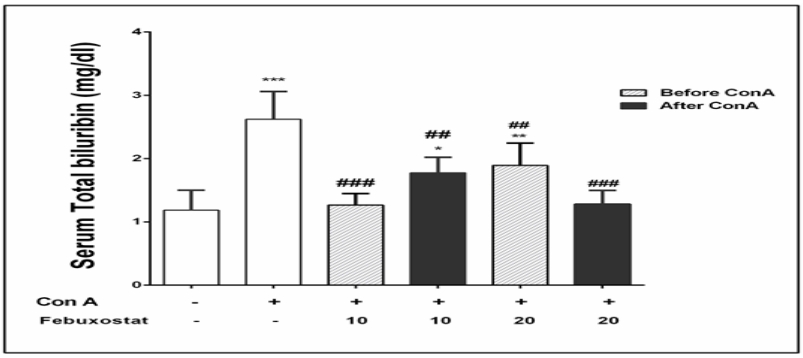

Fig. 3: Effect of febuxostat administration (10, 20 mg) before and after concanavalin A injection on serum total bilirubin level induced liver Inflammation in mouse. *, **, * Significantly different (P < 0.05, 0.01, 0.001 respectively) compared to control group. ##, ### Significantly different (P < 0.01, 0.001 respectively) compared to Con A group.**

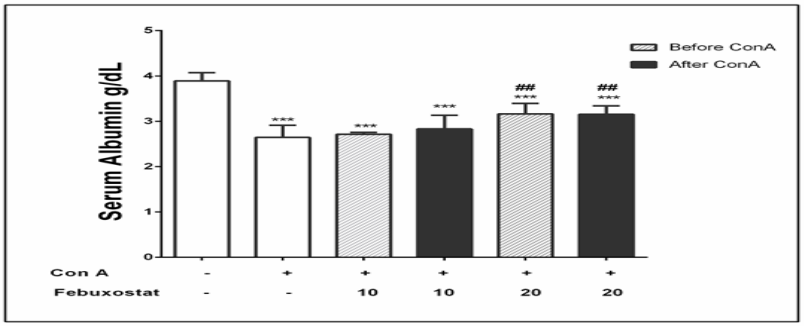

Fig. 4: Effect of febuxostat administration (10, 20 mg/kg) before and after concanavalin A injection on serum albumin level induced liver Inflammation in mice. * Significantly different (P < 0.001 respectively) compared to control group. ## Significantly different (P < 0.01) compared to Con A group.**

DISCUSSION

Concanavalin-A induced hepatitis model possesses the advantage over either D-galactoseamin / low lipopolysaccharide (CaIN/LPS) or (LPS) induced hepatitis, which are the other two experimental models of autoimmune-induced or virally- induced hepatitis, in at least one important point that is related to the change in liver transaminase (ALT and AST). In contrary to the other two models, Con A causes a significant change of the level of transaminase, which is a valid index of the severity of liver injury.[10] These findings are in agreement with our results. In the present work Con A injection significantly increased serum ALT, AST, total bilirubin and significantly decreased serum albumin. It was suggested that the $CD4^+$ T helper (Th) cell was a major player in Con A-liver injury.[11]

Through the interaction between CD4+ T-helper cells and the Kupffer modified major histocompatibility complex (MHC) (induced by Con A) leading to activation of these macrophages and inflammation of liver cells and release transaminases into the blood.[12] Con A-induced liver injury stimulate the release of many other inflammatory cytokines that cause hepatocyte necrosis leading to a significant decrease of serum albumin and an increase of total serum bilirubin.[13] Pretreatment of mice with febuxostat (two hours before Con A injection) prevented the Con A-induced change in serum transaminase, total bilirubin albumin while its administration half an hour after Con A injection significantly decreased such changes. The work of Williams et al (2014) showed that allopurinol (xanthine oxidase inhibitor) administration 18 hours before acetaminophen-induced acute liver injury greatly inhibited the liver injury as indicated by the great decrease in serum ALT.[14] Shamma'a et al (1956 showed that elevation of serum xanthine oxidase level is a sensitive indicator of acute liver injury.[15] George and Struthers (2009) have suggested that xanthine oxidase has pro-inflammatory effects.[16] Ives et al (2015) stated that xanthine oxidase is a stimulator of IL-1B release from the macrophages through inflammosome activation.[17] A recent study made by Laurindo (2018) stated that xanthine oxidase stimulate endothelial oxidative stress leading to endothelial dysfunction.[18]

CONCLUSION

The present work reveals a potential protective and curative effect of febuxostat agonist con A induced acute liver injury. The potentiality of similar protective and curative effect in human needs more experimental and clinical studies.

ACKNOWLEDGEMENT

REFERENCES

1. H. Malhi and G. J. Gores, "Cellular and molecular mechanisms of liver injury.," Gastroenterology, May 2008; 134(6): 1641–1654.
2. C. Trey and C. S. Davidson, "The management of fulminant hepatic failure.," Prog. Liver Dis., 1970; 3: 282–298.
3. F. S. Cardoso, P. Marcelino, L. Bagulho, and C. J. Karvellas, "Acute liver failure: An up-to-date approach," J. Crit. Care, 2017; 39: 25–30.
4. H. D. FRANKL and J. H. MERRITT, "ENZYME ACTIVITY IN THE SERUM AND COMMON DUCT BILE OF DOGS," Am. J. Gastroenterol, 1958; 31(2): 166–70.
5. J. Xue et al., "Emodin protects against concanavalin a-induced hepatitis in mice through inhibiting activation of the p38 MAPK-NF-κB signaling pathway," Cell. Physiol. Biochem, 2015; 35(4): 1557-1570.
6. C. L. Gray and N. E. Walters-Smith, "Febuxostat for treatment of chronic gout," Am. J. Heal. Pharm., 2011; 68(5): 389–398.
7. H. Kataoka, K. Yang, and K. L. Rock, "The xanthine oxidase inhibitor Febuxostat reduces tissue uric acid content and inhibits injury-induced in fl ammation in the liver and lung," Eur. J. Pharmacol, 2015; 746: 174-179.
8. F. Heymann, k. Hamesch, R. Weiskirchen, and F. Tacke, "The concanavalin A model of acute hepatitis in mice," Lab. Anim., vol. 49, no. March, 2015: 12–20.
9. C. A. Misba and S. S. Rajasekar, "Original article : Effect of Febuxostat in Rat Liver, 2013; 1: 200–205.
10. H. Wang et al., "Immune mechanisms of Concanavalin A model of autoimmune hepatitis," 2012; 18(2): 119-125.
11. X. Zhang, H. X. Wei, S. Rui, H. Wei, and Z. Tian, "Opposite effects of high and low doses of interleukin-2 on T cell-mediated hepatitis in mice (interleukin-2 on hepatitis)," Hepatol. Int., 2010; 4(3): 641–648.
12. C. D. T. Cells et al., "Control of T Cell Reactivation by Regulatory Qa-1–Restricted CD8 + T Cells," 2010.
13. T. Huang, C. Chen, H. Liu, and T. Lee, "Resveratrol Pretreatment Attenuates Concanavalin A-induced Hepatitis through Reverse of Aberration in the Immune Response and Regenerative Capacity in Aged Mice," no. January, , 2017; 1–11.
14. D. Williams and and H. J. Mitchell R. McGill, Margitta Lebofsky, Mary Lynn Bajt,

"PROTECTION AGAINST ACETAMINOPHEN-INDUCED LIVER INJURY BY ALLOPURINOL IS DEPENDENT ON ALDEHYDE OXIDASE-MEDIATED LIVER PRECONDITIONING," 2014; 274(3): 417–424.

15. M. H. SHAMMAA, S. NASRALLAH, T. CHAGLASSIAN, A. K. KACHADURIAN, and U. A. AL KHALIDI, "SERUM XANTHINE OXIDASE: A SENSITIVE TEST OF ACUTE LIVER INJURY.," Gastroenterology, Feb. 1965; 48: 226–230.
16. J. George and A. D. Struthers, "Role of urate , xanthine oxidase and the effects of allopurinol in vascular oxidative stress," 2009; 44: 265–272.
17. A. Ives et al., "Xanthine oxidoreductase regulates macrophage IL1beta secretion upon NLRP3 inflammasome activation.," Nat. Commun, Mar. 2015; 6: 6555.
18. F. R. M. Laurindo, "Redox Cellular Signaling Pathways in Endothelial Dysfunction and Vascular Disease," in Endothelium and Cardiovascular Diseases: Vascular Biology and Clinical Syndromes, Elsevier Inc., 2018; 127–145.