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EFFECT OF FEBUXOSTAT ON HEPATIC LEVELS IN THIOACETAMIDE-ADMINISTERED RATS

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ABSTRACT

The current study aimed at investigating the hepatoprotective influence of febuxostat (Feb) against thioacetamide (TAA)-induced Mature male Sprague Dawley rats (180-200 g) were randomly allocated to three groups, each of 10 animals, as follows: Control group, animals received 0.5% (3 ml/kg/day, orally) for 3 months; TAA group, animals were injected with thioacetamide (TAA, 150 mg/Kg, 3 times/week, intraperitoneally) for 3 months; TAA-Feb group: animals were injected with TAA (as described for TAA group). Animals were also treated with Feb (orally taken15 mg/kg/day) from day 1 and continued till the end of the experiment. The levels of TIMP-1 in liver tissue were significantly elevated in TAA-treated rats compared with the control animals. Chronic treatment with Feb (15 mg/kg/day) significantly attenuated TAA-induced increase in TIMP-1level in hepatic tissue as compared to non-treated TAA group. In conclusion, the elevation of hepatic levels of TIMP-1 in TAA indicated the role of restoration of the extracellular matrix (ECM) in hepatic tissue in TAA-induced hepatic fibrosis. Moreover, Feb shows a hepatoprotective influence against TAA-induced TIMP-1elevation in liver tissue, possibly through its antioxidant properties.

KEYWORDS: thioacetamide, TIMP-1, rats, febuxostat, liver.

INTRODUCTION

Chronic liver injury is an outcome of uncontrolled wound-healing reactions induced by various stimuli, such as viral infections, alcohol and non-alcoholic steatohepatitis (NASH). With prolonged injury, hepatic parenchyma is substituted by fibrotic tissue. Also, prolonged inflammation leads to an extreme accumulation of ECM constituents (e.g., fibronectin, proteoglycans, and collagens), which have important role in fibrotic tissue formation.[1]

Tissue inhibitor of metalloproteinase-1 (TIMP-1) elevates in murine fibrotic livers induced by carbon tetrachloride administration.[2] Highly expression of TIMP-1 mitigates the clearance of fibrotic matrix causing excessive interstitial ECM accumulation.[3,4] Furthermore; using of modified synthetic siRNA targeting TIMP-2 for treatment of fibrotic murine livers with, declines fibrosis process thru inhibiting hepatic stellate cell (HSC) activation and collagen accumulation.[5] These observations indicated that treatments targeting to reduce high TIMP-1 levels may be effective in hepatic fibrosis treatment.

Febuxostat (Feb) is a powerful and xanthine oxidase selective inhibitor.[6], as it has cytoprotective, antioxidant, and anti-inflammatory properties in many disease models.[7-9] Many researches have conveyed tissue protective roles of Feb in animal models of fructose-induced renal disorders[10], nephrectomy[11], unilateral ureteral obstruction[12], renal ischemia-reperfusion injury[13] and diabetic nephropathy.[14] Feb has inhibitory effect on LPS-induced expression of monocyte chemotactic protein-1 (MCP-1), in human macrophages *in vitro* studies.[15]

This study was performed to investigate whether Feb can decrease TIMP-1 levels in liver tissues of thioacetamide (TAA)- hepatic damaged rats.

MATERIAL AND METHODS

Drug and Chemicals

TAA was bought from Sigma Aldrich Chemical Company. Feb was provided as Feburic (80 mg) tablets (Hikma Pharma SAE, 6th October City, Egypt).

Animal and experimental protocol

Adult male Sprague Dawley rats (180-200 g) were used. Rats were bought from animal house of the Biological Products & Vaccines (Cairo, Egypt). They were housed in Pharmacology Department, Faculty of Pharmacy, Mansoura University. The animals were acclimatized to

the animal house for two weeks prior to starting the experiments and were fed a standard chow diet and regular drinking water adlibitum during the study. Rats were exposed to alternate cycles of 12h light and darkness. Animal procedures in this study were carried out according to recommended ethics for use of laboratory animals and consistent with protocols issued by committee of ethics of scientific research, Faculty of Pharmacy Mansoura University, Egypt.

After two weeks of acclimatization, rats were randomly allocated to three groups, each comprising of 10 animals, as follows.[16]
Control group: animals received 0.5% CMC (3 ml/kg/day, orally) for 3 months.
TAA group: animals were injected with thioacetamide (TAA, 150 mg/Kg, 3 times/week, intraperitoneally) for 3 months.
TAA-Feb group: animals were injected with TAA (as described for TAA group). Animals were also treated with febuxostat (orally taken15 mg/kg/day) from day 1 and continued till the end of the experiment.

Liver tissue homogenate preparation
At the end of the experimentation, rats were anaesthetized with diethyl ether and blood was harvested by puncturingretroorbital venous plexus of eye using a heparinized capillary glass tube. An abdominal incision was made and the liver was exposed, isolated and washed with ice-cold PBS to remove any blood clots. The left liver lobe was weighted and homogenized in 10% (w/v) in PBS (pH 7.4) utilizing a minihard homogenizer. The resulted homogenates were centrifuged at 1OOO x g, 4°C, for 15 min, and the supernatants obtained were collected.

Assessment of hepatic level of TIMP-1
Enzyme-linked immunosorbent assay (ELISA) kit appliedfor quantitative determination level of TIMP-1 in rat liver tissue homogenate was performed according to instruction procedure of the kit purchased (Biospes, China). The levels of TIMP-1wereexpressed as pg/g tissue.

Statistical analysis
Mean ± S.E.M. were calculated using SPSS (Version 20) and byperforming ANOVA test followed by Tukey–Kramer multiple comparisons test. Results were considered significant at level of $P < 0.05$. Statistical analyses were carried out utilzingGraphpad Prism software (GraphPad V6.03).

RESULTS

Figure 1 shows the TIMP-1level in liver tissues of control, TAA and TAA-Feb groups expressed as ng/g. The hepatic levels of TIMP-1were significantly ($P<0.001$) higher in TAA-treated animals than those of the control rats. On the other hand, treatment with Febat the dose of15 mg/kg for significantly reduced TAA-induced increase in TIMP-1level in liver tissue as compared to TAA-treated animals (P<0.0l vesrus TAA and P <0.05 against control).

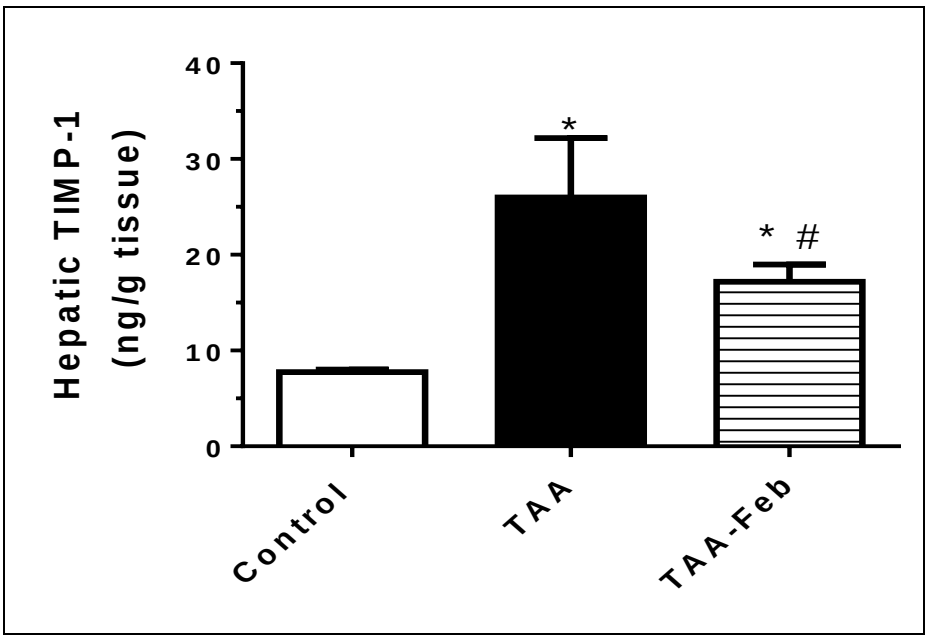

Figure 1: Effect of febuxostat treatment on hepatic levels of tissue inhibitor of metalloproteinase-1 (TIMP-1) protein in thioacetamide (TAA)-administered rats.

Values are expressed as mean ± SEM (n=6). *and# Significantly different when compared with the control and TAA groups, respectively.

DISCUSSION

Chronic TAA administration increased TIMP-1 levels in liver tissues of rats when compared to control group. This is consistent with previous studies. TIMP-l is

strongly up-regulated in hepatic tissue and serum during fibrosis in cases with liver disorders and in rats models of liver fibrosis. Moreover, expression of TIMP-1 is proportional to the stage of liver fibrosis.[17-19] TIMP-1 regulates remodeling of ECM in thru matrix metalloproteases (MMPs).[20] Many investigations on mice with transgenic TIMP-1 overexpression or utilizing an antibody or MMP-9 mutants to antagonize TIMP-l indicated that TIMP-l stimulates liver fibrosis.[3, 4, 21, 22] Feb treatment of TAA-administered rats showed significantly lower TIMP-1 protein levels than non-treated TAA group. These observations may suggest that Feb could exert antifibotic influences in TAA-challenged rats. More research needs to be conducted to elucidate Feb-mediated effects in experimental models of fibrogenesis.

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