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Pro-health activities of spent hops (*Humulus lupulus* L.) extract against Crohn's disease

Introduction and aim of the study

Crohn's disease (CD), one of two major subtypes of inflammatory bowel diseases, is chronic, relapsing disorder which affects the gastrointestinal tract, including small intestine, in both children and adults. The inflammatory response and oxidative stress, as well as associated with them the production of mediators, e.g. cytokines or matrix metalloproteinases (MMP), are responsible for progression of the disease and occurrence of complications, such intestinal fistula or stenosis. Currently available therapeutic options are not fully effective, and their use results in side effects, high cost and little improvement in quality of life. The spent hops, prepared by the hops (*Humulus lupulus* L.) extraction, are the source of polyphenols with high biological activity, including anti-inflammatory and anti-oxidative. The aim of the present study was to assess anti-inflammatory potential of spent hops extract (SHE), as well as to determine the impact of the extract on the expression and activity of type IV collagenases, matrix mettaloproteinase-2 (MMP-2) and -9 (MMP-9), in *in vitro* model of Crohn's disease.

Materials and methods

In experiments, we used human small intestine epithelial cells (HIEC-6) stimulated by tumor necrosis factor alpha (TNF-α). The impact of SHE on gene expression was assesed by Q-PCR analysis. The effect of SHE on protein expression was estimated by Western blot and by enzyme-linked immunosorbent assay (ELISA). The activities of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) were evaluated by zymography assay.

Results

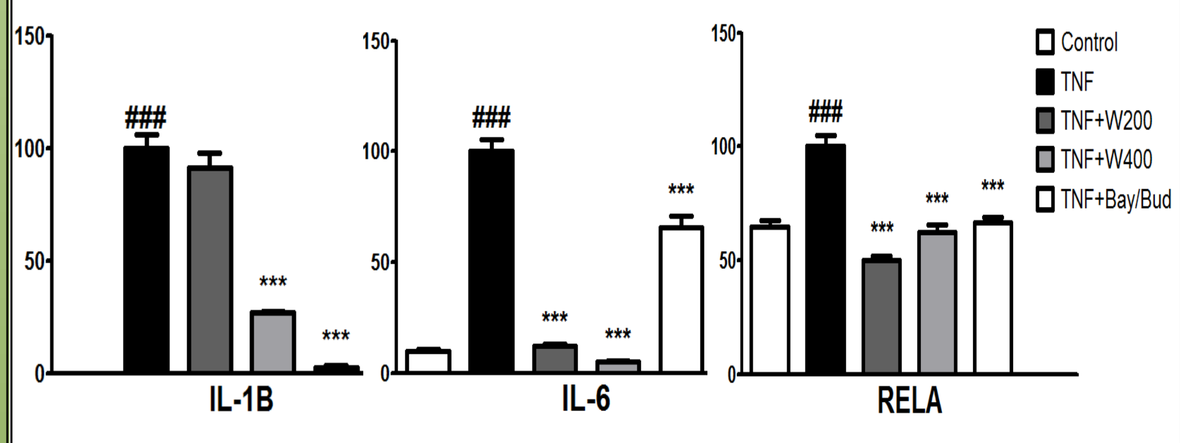

Figure 1. The inhibitory effect of SHE on the gene expression of interleukin 1 beta (IL-1β), interleukin 6 (IL-6), RELA in TNF-α-stimulated HIEC-6 cells by Q-PCR. Budesonide (Bud) was used for IL-1β and IL-6 assay, and BAY inhibitor for the RELA assessment. Data represent the mean ± SEM of three independent experiments.

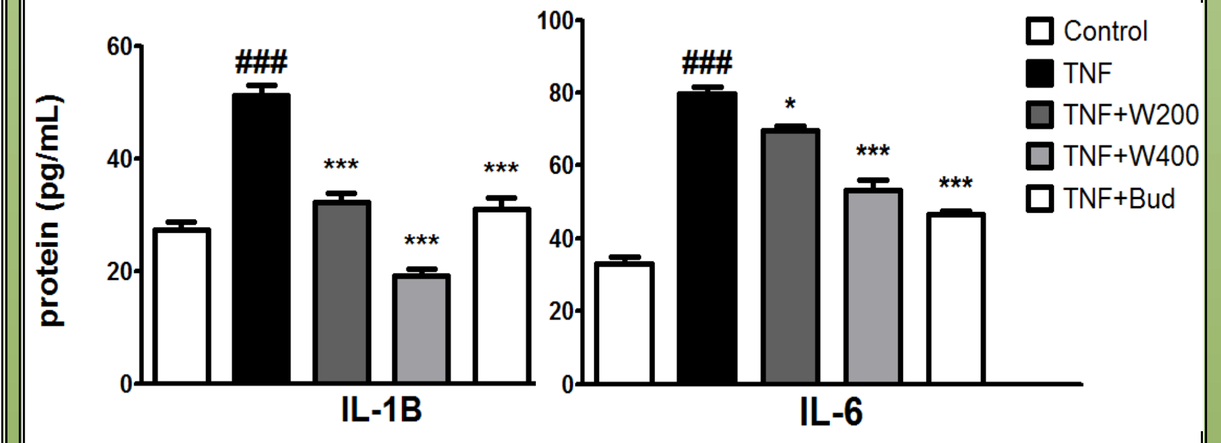

Figure 2. The inhibitory effect of SHE on IL-1β and IL-6 protein expression in TNF-α-induced HIEC-6 cells determined by ELISA. Data represent the mean ± SEM of three independent experiments.

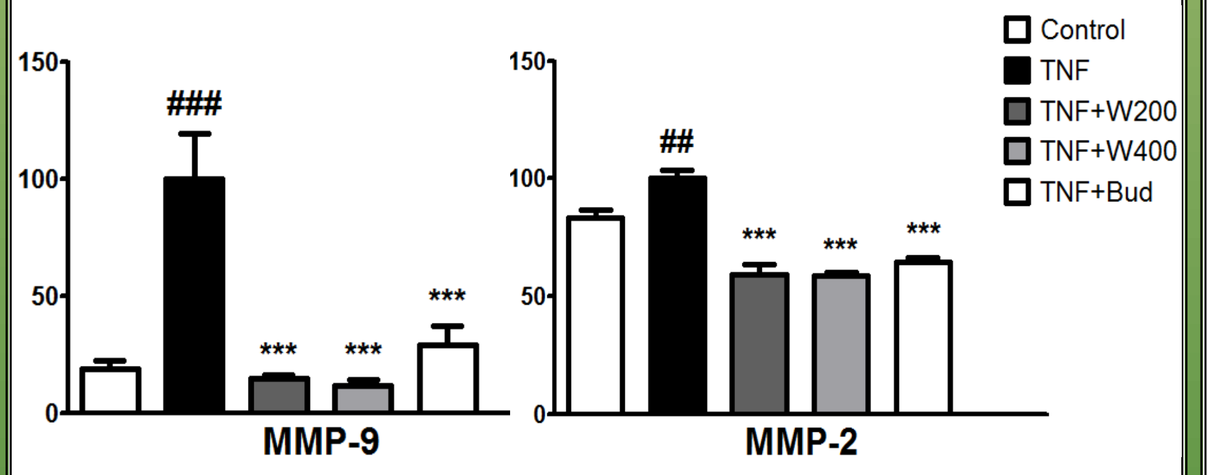

Figure 3. Effect of SHE on the mRNA expression of MMP-2 and MMP-9 in TNF-α-stimulated HIEC-6 cells determined by Q-PCR. Data represent the mean ± SEM of three independent experiments.

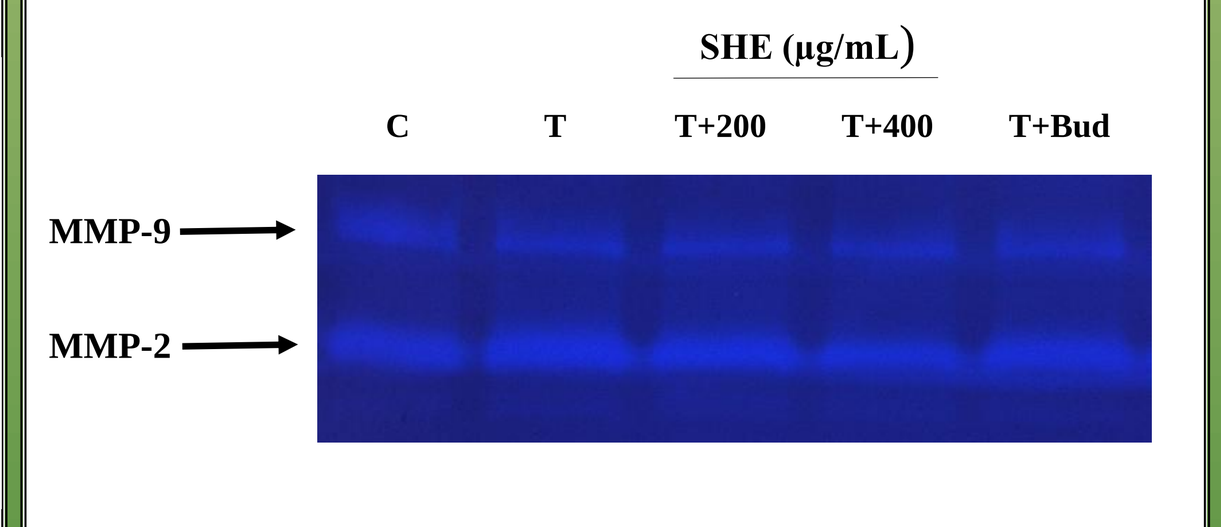

Figure 4. Effect of SHE on the activity of MMP-2 and MMP-9 in TNF-α-stimulated HIEC-6 cells determined by zymography after 24 hours. C – control; T – TNF-α; Bud – Budesonide

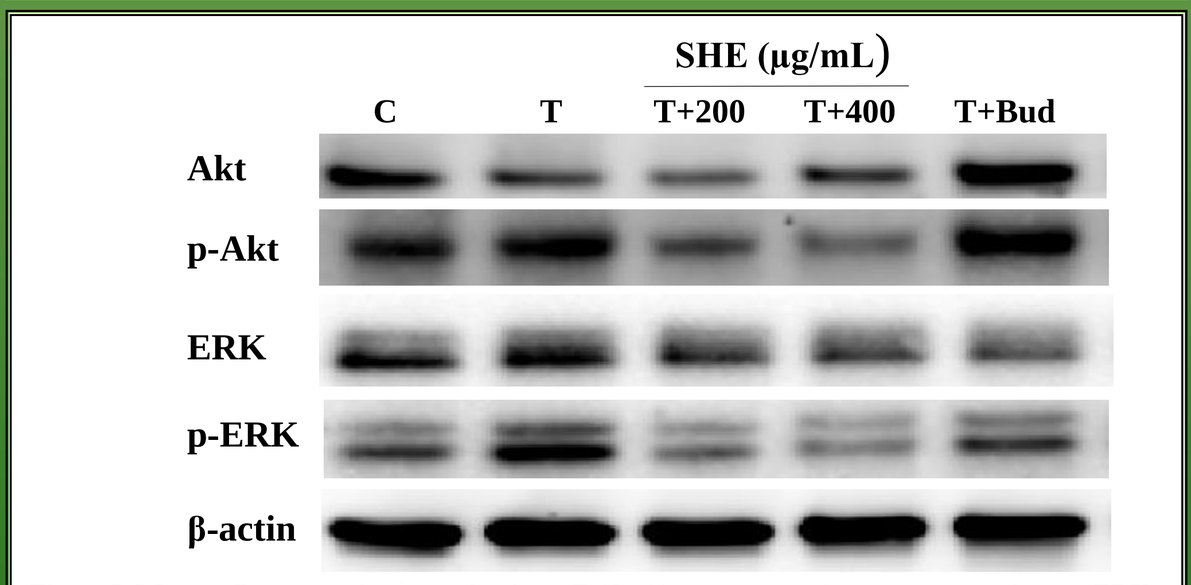

Figure 5. Western Blot analysis of protein kinase B (Akt)/extracellular signal-regulated kinases (ERK) protein expression in TNF-α-induced HIEC-6 cells with SHE. C – control; T - TNF-α.

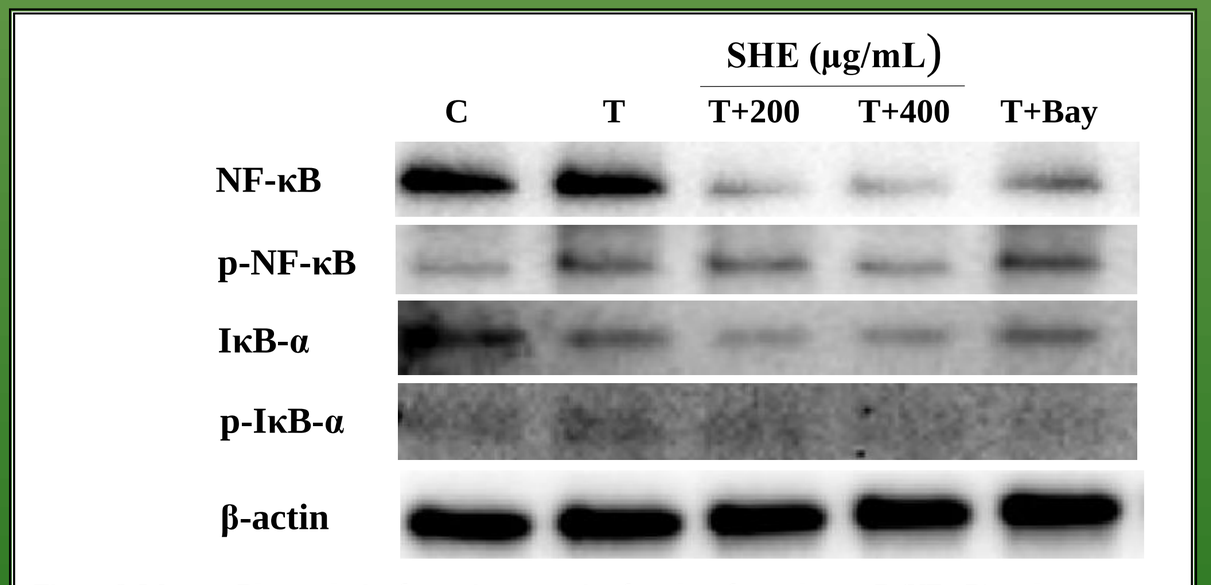

Figure 6. Western Blot analysis of protein expressio of nuclear factor kappa B (NF-κB) pathway Elements in TNF-α-induced HIEC-6 cells with SHE. C – control; T - TNF-α.

Conclusions

Our data shows that SHE has anti-inflammatory properties, as well as it may inhibit the expression and activity of gelatinases in *in vitro* model of Crohn's disease. The tested extract may be an effective agent for the inhibition of progression of Crohn's disease.

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