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Effects of Fustin on Nuclear Factor Kappa B Expression in a Rat Model of Trinitrobenzene Sulfonic Acid-induced Colitis

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Cotinus coggygria is a shrub, rich in tannins and flavonoids, used in traditional medicine [1]. Nuclear factor kappa B (NF-κB) is a crucial modulator of inflammatory reactions and immune responses [2]. This study aimed to evaluate the effect of the flavonoid fustin on the immunohistochemical expression of NF-κB in a rat colitis model induced by trinitrobenzenesulfonic acid (TNBS). Fustin was isolated from *Cotynus coggygria* heartwood and purified [3]. Forty male Wistar rats allocated to four groups: Control, TNBS, TNBS+F10, and TNBS+F20. In groups TNBS, TNBS+F10, and TNBS+F20, colitis was induced by rectal administration of 10 mg TNBS. Treatment began 24 hours after colitis induction and continued for 8 days. Fustin at a dose of 10 mg/kg and 20 mg/kg prepared as a suspension was given orally to TNBS+F10 and TNBS+F20 groups, respectively, while the vehicle for fustin was given to groups Control and TNBS. On the 10^{th} day of the experiment, samples of colon tissue from the site of injury were taken and processed for immunohistochemical investigations. Tissue sections, 4 μm thick, were placed on silanized slides. NF-κB expression was determined using a rabbit anti-NF-kB-p100 polyclonal antibody (E-AB-32222; Elabscience, USA), diluted 1:200, following the protocol for universal highly sensitive visualization system for antibody detection EnVision FLEX. Immunohistochemical evaluation was performed semi-quantitatively in 50 cells of each probe using the following score: 1 – no cytoplasmic staining, 2 – weak staining, 3 – moderate staining, 4 – strong staining. The average intensity of the immune reaction was calculated using the formula: number of cells of each type x corresponding coefficient (1, 2, 3 or 4) x total number of $cells^{-1}$. The results showed that NF-κB immunohistochemical expression score in the control group was 1.57±0.57, while in TNBS group it was significantly higher (2.53±0.86; $p<0.001$ vs. Control). The antibody expression score of TNBS+F10 group (2.35±0.96) was slightly reduced. The score of TNBS+F20 group (1.41±0.57) did not differ significantly from the control value and was significantly lower ($p<0.05$) in comparison with that of TNBS group. In conclusion, fustin at a dose of 20 mg/kg reduced the proinflammatory transcription factor NF-κB expression which might contribute for its ameliorative effect against TNBS-induced damage.

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Keywords: NF-κB; colitis; trinitrobenzenesulfonic acid; fustin.

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