METABOLIC PROFILING STUDY OF DSS INDUCED COLITIS IN RATS AND THE EFFECT OF SHIKONIN

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The Dextran Sodium Sulphate (DSS) model inducing colitis has demonstrated correlation with human inflammatory bowel disease and is widely applied in rodents for the study of pathogenesis and onset mechanisms of the disease. Metabolic profiling is a holistic approach, which helps in identifying changes in the context of the global network of metabolic pathways in an organism. The application of metabolomics for the study of the effect of DSS administration is deemed useful to this direction. The study aimed to identify primary metabolites affected by the oral administration of DSS and understand its mechanism of action. The effect of shikonin, a natural product with well-established anti-inflammatory properties, was further studied against DSS induced inflammation.

Four groups of female Wistar rats (n=33), 15 weeks old, were included in the study. Group 1 was treated by addition of DSS 3% into drinking water \textit{(ad libitum)} for a period of 6 days; Group 2 was treated with DSS 3% (as in Group 1), but furthermore with shikonin (12.5 mg/Kg of bodyweight), administered orally in olive oil for 7 days (6+1). Group 3 was treated only with shikonin (12.5 mg/Kg of bodyweight) for 7 days and Group 4 was the control group. Animals’ weights were monitored and faeces were collected every second day and stored at \(-80^\circ\text{C}\). On day 9, rats were sacrificed and intestine tissue samples were collected and subjected to histopathological examination. Faeces collected at the end of the experiment were subjected, after extraction, to 1H NMR spectroscopic analysis (Agilent 500 MHz spectrometer). An optimisation was performed for both sample preparation and NMR conditions. A known amount (~350 mg) of each sample were extracted with 2 volumes (w/v) PBS buffer (NaH\textsubscript{2}PO\textsubscript{4}, H\textsubscript{2}O Na\textsubscript{2}HPO\textsubscript{4}, NaCl) pH=7.1, and centrifuged. 150\textmu L D\textsubscript{2}O and 50\textmu L TSP (6 mM in D\textsubscript{2}O) were added in 400\textmu L of each supernatant phase and, after centrifugation, were subjected to NMR. Spectra were recorded by MestReNOVA software for spectra binning, peak scaling and peak alignment. The data were afterwards analysed by multivariate statistical analysis (PCA, OPLS-DA) using SIMCA P11.5 software.

With that protocol, mild inflammation of mucosal in colon was observed in DSS treated group. NMR metabolic profiles examined by PCA revealed differentiation of faecal extracts among DSS treated animals and controls, based on signal variations. It was found that L-threonine (4.24, 4.31, 3.57 ppm), L-lactic acid (1.32, 4.10 ppm), L-alanine (1.47, 3.79 ppm), and L-tyrosine (7.18, 6.87, 3.93, 3.17, 3.03 ppm) were altered in DSS treated group comparing to controls. However, addition of shikonin, at the concentration level applied at the DSS specific protocol, did not seem to affect significantly the metabolic profiles of Group 1, since mild inflammation was observed. Histopathologically, the findings were in agreement to metabolomics data.

The present study helps in improving the knowledge on DSS induced colitis and the response to natural protective compounds and demonstrates the potential of using metabolomics to better approach and understand diseases.

KEYWORDS: NMR, colitis, shikonin, rats, faeces, PCA