Isolation, purification and characterization of the hepatoprotective polysaccharides from D*endrobium loddigesii* Rolfe

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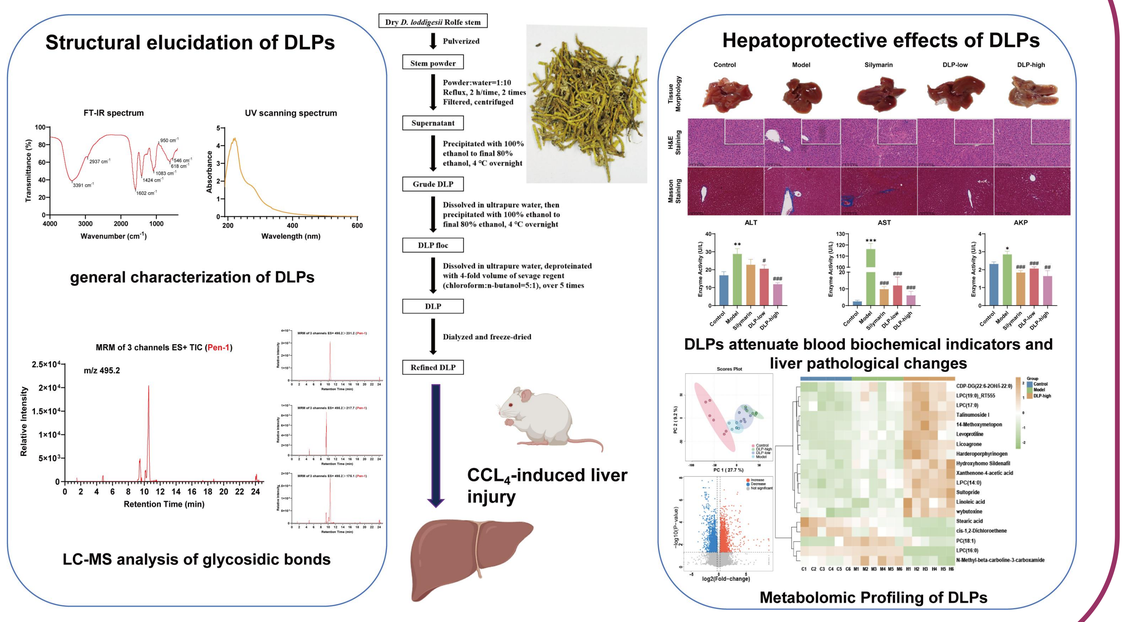
BACKGROUND:

The rising incidence of liver diseases, particularly those induced by drugs, underscores the urgent need for safer, more effective therapeutic interventions. Liver injury, characterized by its complex pathophysiology, can lead to severe outcomes, highlighting the limitations of traditional therapies, which often entail adverse effects. This backdrop fuels the exploration of natural sources like polysaccharides extracted from medicinal plants, which are recognized for their antioxidative and anti-inflammatory properties. This study focuses on *Dendrobium loddigesii* Rolfe polysaccharides (DLPs), evaluated for their hepatoprotective effects against carbon tetrachloride (CCL_4)-induced liver injury in murine models. A thorough analysis was conducted, including the extraction and structural characterization of DLPs, along with the assessment of in vivo efficacy through serum biochemical markers, liver histopathology, and metabolomic profiling. These findings underscore DLPs' potential as a natural therapeutic agent, meriting further clinical trials to assess its efficacy and safety in liver protection.

DESIGN AND METHODS:



RESULTS - Characterization of DLPs :

1. Basic structural characterization of DLPs

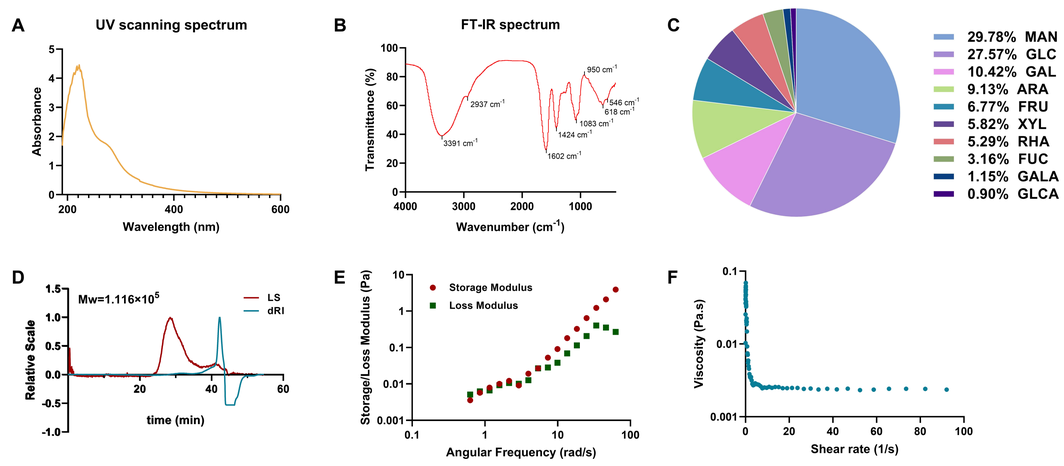


Figure 1: Characterization of DLPs. (A) UV spectra of DLPs. (B) FT-IR spectra of DLPs between 4000 and 800 cm−1. (C) Monosaccharide composition analysis of DLPs. MAN, GLC, GAL, ARA, FRU, XYL, RHA, FUC, GALA, GLCA represents for Mannose, Glucose, Galactose, Arabinose, Fructose, Xylose, Rhamnose, Fucose, Galacturonic Acid, Glucuronic Acid, respectively. (D) Molecular weight analysis of DLPs. (E) Angular frequency analysis of steady-state flow behavior of DLPs. (F) Viscosity analysis of flow behavior of DLPs.

2. Analysis of glycosidic bonds in DLPs via LC-MS

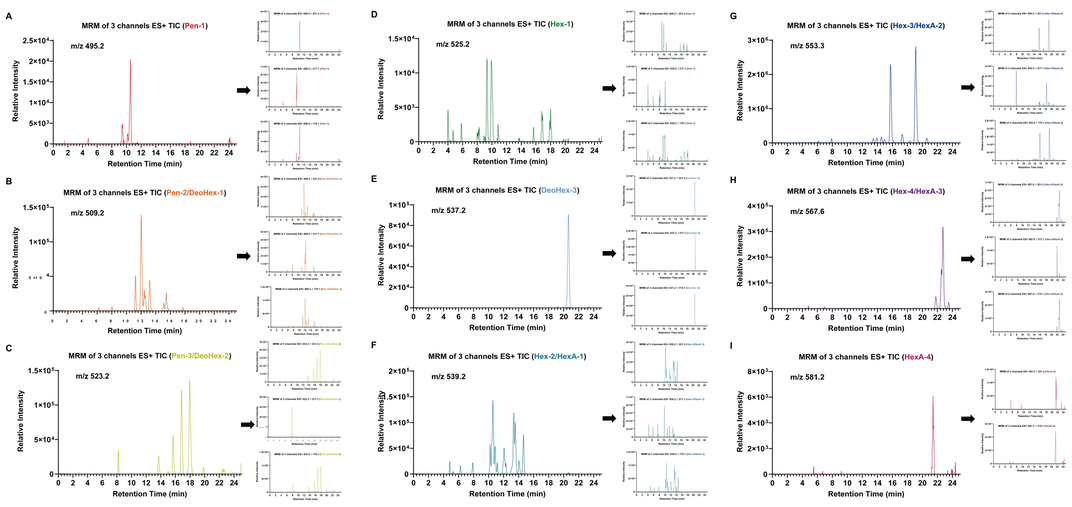


Figure 2: Total ion chromatogram (TIC) and MRM chromatograms for the linkage analysis of DLPs. (A) Pen-1; (B) Pen-2/DeoHex-1; (C) Pen-3/DeoHex-2; (D) Hex-1; (E) DeoHex-3; (F) Hex-2/HexA-1; (G) Hex-3/HexA-2; (H) Hex-4/HexA-3; (I) HexA-4. X-axis represents retention time, while the Y-axis represents relative ion intensity.

RESULTS - Hepatoprotective efffects of DLPs :

1. Effects of DLPs on the general state and organs of CCL_4-induced liver injury in mice

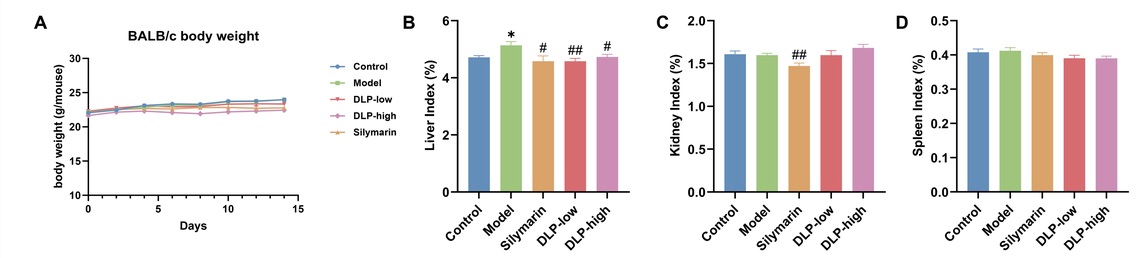


Figure 3: Effects of DLPs on the general state and organs of CCL_4-induced liver injury in mice. (A) Body weight changes in mice. (B) Liver organ index (liver weight/body weight). (C) Kidney organ index (kidney weight/body weight). (D) Spleen organ index (spleen weight/body weight). Data were expressed as mean ± SEM from nine mice in each group. *p < 0.05 compared to control group individually; #p < 0.05 and ##p < 0.01 compared to model group individually.

2. DLPs significantly ameliorate the blood biochemical indicators and liver pathology in CCL_4-induced liver injury in mice

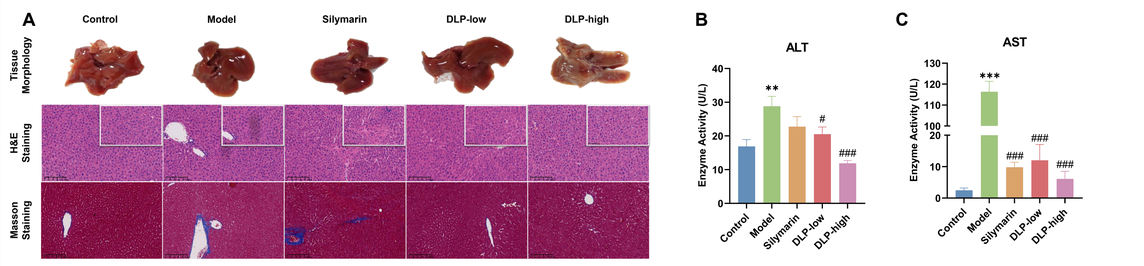


Figure 4: DLPs can attenuate blood biochemical indicators and liver pathological changes in mice with CCL_4-induced liver injury. (A) Morphology of the liver and histopathological examination of the liver were performed using H&E staining and Masson staining (Original magnification × 100 and × 200, scale bar = 100 μm). (B) Serum ALT level. (C) Serum AST level. Data were expressed as mean ± SEM from nine mice in each group. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to control group individually; #p < 0.05, ##p < 0.01 and ###p < 0.001 compared to model group individually.

3. Liver metabolomic analysis of DLPs on CCL_4-induced liver injury in mice

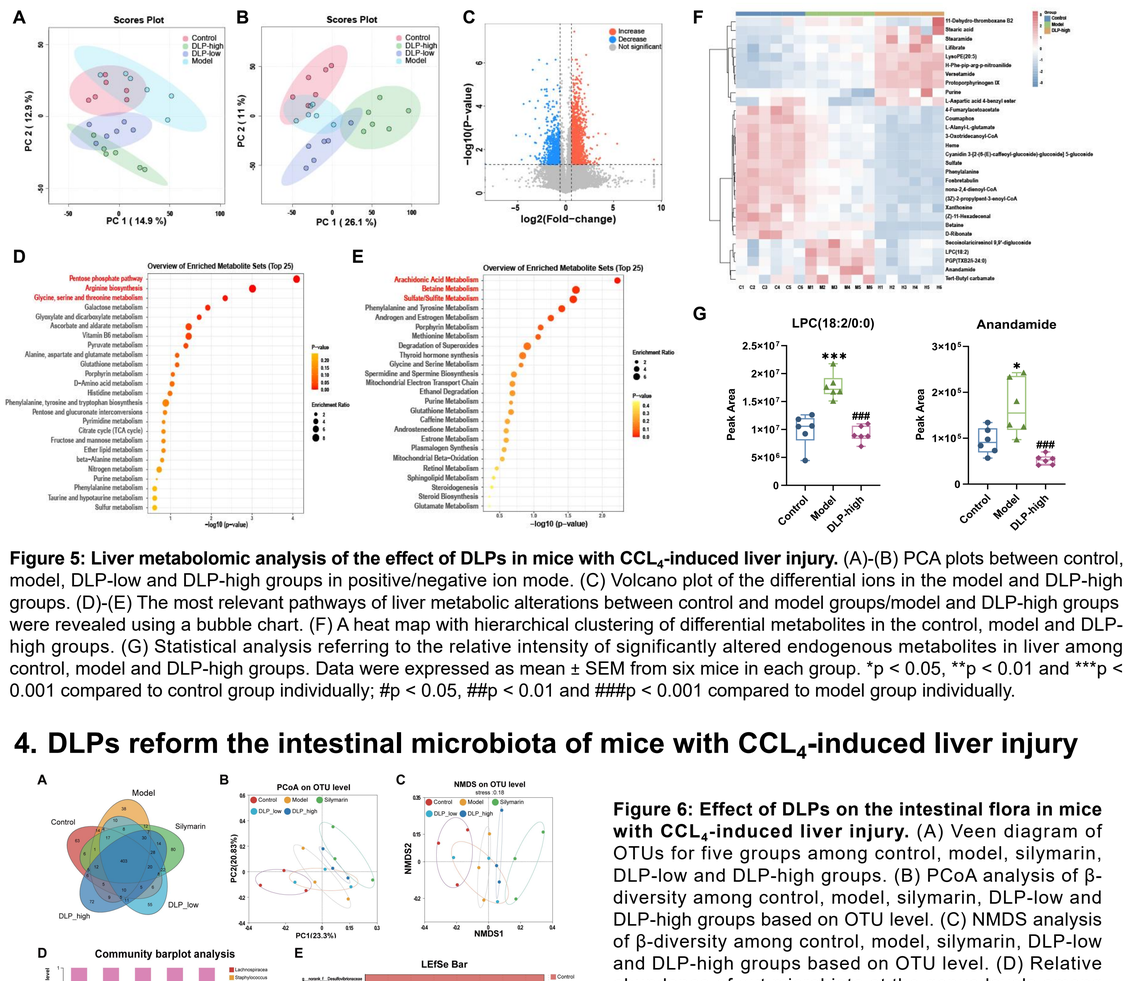


Figure 5: Liver metabolomic analysis of the effect of DLPs in mice with CCL_4-induced liver injury. (A)-(B) PCA plots between control, model, DLP-low and DLP-high groups in positive/negative ion mode. (C) Volcano plot of the differential ions in the model and DLP-high groups. (D)-(E) The most relevant pathways of liver metabolic alterations between control and model groups/model and DLP-high groups were revealed using a bubble chart. (F) A heat map with hierarchical clustering of differential metabolites in the control, model and DLP-high groups. (G) Statistical analysis referring to the relative intensity of significantly altered endogenous metabolites in liver among control, model and DLP-high groups. Data were expressed as mean ± SEM from six mice in each group. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to control group individually; #p < 0.05, ##p < 0.01 and ###p < 0.001 compared to model group individually.

4. DLPs reform the intestinal microbiota of mice with CCL_4-induced liver injury

Figure 6: Effect of DLPs on the intestinal flora in mice with CCL_4-induced liver injury. (A) Veen diagram of OTUs for five groups among control, model, silymarin, DLP-low and DLP-high groups. (B) PCoA analysis of β-diversity among control, model, silymarin, DLP-low and DLP-high groups based on OTU level. (C) NMDS analysis of β-diversity among control, model, silymarin, DLP-low and DLP-high groups based on OTU level. (D) Relative abundance of gut microbiota at the genus levels among five groups at the end of experiment. (E) LDA Effect Size (LEfSe) analysis of gut microbiota in the control, silymarin and DLP-high groups. Data were expressed as mean ± SEM from three mice in each group.

CONCLUSION :

Our pioneering study on DLPs offers valuable insights into their structural properties and substantial evidence of their hepatoprotective effects. The correlation between the structural characteristics and pharmacological actions of DLPs pave the way for future research and development of effective, safe, and natural hepatoprotective agents. The methodologies and findings reported herein contribute significantly to the field of pharmacognosy and support the therapeutic potential of traditional Chinese medicinal plants in modern medicine applications.

References

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