

GLUT5-Driven Gut-Liver Axis Injury Mediating Olanzapine-Induced NAFLD and Dapagliflozin Intervention

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Objective: Drugs are an important cause of non-alcoholic fatty liver disease (NAFLD), and olanzapine is the main drug that leads to NAFLD. However, the mechanism by which this drug causes NAFLD is still unclear at present. We previously found that dapagliflozin had certain intervention effect on patients with NAFLD. This study aimed to explore the mechanism of olanzapine-induced NAFLD and the intervention effect of dapagliflozin.

Methods: Twenty-four female wistar rats were classified into control (NC) group, olanzapine (OLA) group, and dapagliflozin intervention (DAP) group for a total of 12 weeks of intervention. This study explored the specific mechanism of olanzapine-induced NAFLD and potential treatment method from dapagliflozin through transcriptomics, molecular biology, and pathological staining methods in animal models.

Results: The transcriptome and immunohistochemistry studies of the intestine and liver revealed the high expression of GLUT5 in both tissues ($P < 0.01$). Olanzapine induced gut-liver axis injury via GLUT5/AMPK/mTOR ($P < 0.05$ or $P < 0.01$). Dapagliflozin reversed GLUT5/AMPK/mTOR to improve intestinal barrier injury, NAFLD and hepatic fibrosis ($P < 0.05$ or $P < 0.01$).

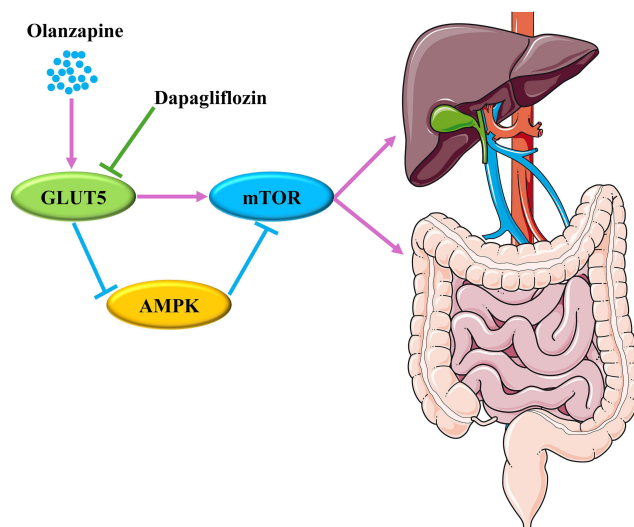
Conclusion: The study discovered for the first time that GLUT5/AMPK/mTOR driven gut-liver axis injury mediating olanzapine-induced NAFLD and dapagliflozin intervention could reverse GLUT5/AMPK/mTOR-driven gut-liver axis injury ameliorating olanzapine-induced NAFLD. This provided a new understanding of the occurrence of olanzapine-induced NAFLD and offered a reference plan for its clinical intervention.

Keywords: GLUT5, gut-liver axis, olanzapine, NAFLD, dapagliflozin

Introduction

Schizophrenia is a serious mental disorder that mainly affects a patient's thinking, emotions, perception and behavior, making it difficult for them to distinguish reality from illusion. It often begins in young adulthood, has a protracted course, and about half of the patients may develop mental disability, causing significant burdens on individuals, families and society.¹⁻⁵ At present, the main treatment for schizophrenia is antipsychotic drugs.^{6,7} Second-generation antipsychotic drugs have largely replaced first-generation antipsychotic drugs due to their broad spectrum of action and fewer extrapyramidal reactions and have become the preferred drugs for treating schizophrenia. However, the risk of developing non-alcoholic fatty liver disease (NAFLD) significantly increases in patients taking second-generation antipsychotic drugs, especially olanzapine.⁸⁻¹⁴

Graphical Abstract



NAFLD is a liver phenotype caused by metabolic disorders, whose global incidence rate is 559.95 per 100,000 people, with a mortality rate of 1.93 per 100,000.¹⁵ NAFLD is a liver-specific disease and an intermediary for many other diseases, which can exacerbate other chronic liver diseases, and in severe cases, it can lead to decompensated liver cirrhosis, liver cancer, and increase the risk of type 2 diabetes and cardiovascular diseases.^{16–26} Drugs are an important cause of NAFLD, and olanzapine is the main drug that leads to NAFLD.^{8–14} However, the mechanism by which this drug causes NAFLD is still unclear at present. The gut-liver axis is a system through which the intestine and the liver communicate bidirectionally via the portal vein, bile ducts, etc.^{27–30} It has a close interaction relationship with NAFLD.^{31–34} NAFLD usually accompanied by impaired intestinal barrier function, allowing bacterial endotoxins to enter the bloodstream, triggering chronic inflammation and fat accumulation in the liver.^{35–39} Is the occurrence of olanzapine-induced NAFLD also related to the gut-liver axis injury? What is the specific mechanism? These key questions are currently unknown.

Dapagliflozin, as an SGLT2 inhibitor, not only lowers blood sugar but also has various important functions such as promoting weight loss, protecting the heart, and protecting the liver and kidneys.^{40–44} Importantly, we previously found that dapagliflozin had certain intervention effect on patients with NAFLD.⁴³ Therefore, in this study, we designed a dapagliflozin administration group to observe the intervention effect of dapagliflozin on olanzapine-induced NAFLD. Based on these, this study aimed to explore the mechanism of olanzapine-induced NAFLD and the intervention effect of dapagliflozin.

Materials and Methods

Animal Experiment

Given sex difference in NAFLD susceptibility, random 24 female wistar rats (aged about 4 weeks) grouping as control (NC) group (n=8), olanzapine (OLA) group (n=8), and dapagliflozin intervention (DAP) group (n=8), who were bought from Vital River Laboratory Animal Technology Co., Ltd. Based on the preceding discussion, the animals were housed in standard cages under controlled environmental conditions, with ambient temperature maintained at 20–25°C, relative humidity regulated between 40% and 70%, and a 12-hour light/dark cycle. The animals had ad libitum access to standard laboratory chow and drinking water. OLA and DAP groups were intervened by gavage with 5 mg/kg/day olanzapine (Lot number: 231106, EASTON BIOPHARMACEUTICALS) and NC group was intervened by gavage with equal volume of CMCNa. Furthermore, DAP group was intervened by gavage with 1 mg/kg/day dapagliflozin (Lot number: TC2508, AstraZeneca) and NC, OLA groups were intervened by gavage with equal volume of CMCNa.

This experiment was conducted for a total of 12 weeks of intervention. In this study, humane endpoints were established for animal experiments. The criteria for triggering humane endpoints included: labored breathing, uncontrolled hemorrhage, convulsions or paralysis, inability to self-feed or drink for >24 hours, weakness leading to inability to stand upright. Animals meeting any of these criteria were euthanized promptly to minimize suffering. They were sacrificed via dislocation of the cervical spine. These procedures were carried out in accordance with applicable veterinary guideline of American Veterinary Medical Association. Animal protocols and procedures were approved by The Animal Care and Use Committee of Xuzhou Medical University (No.202401T029). In addition, we complied with Guideline for Ethical Review of Animal Welfare (GB/T 35892-2018) to ensure the welfare of the experimental animals used in our research.

Sample Collection

After a 12-week experimental intervention, sacrificing all the animals and extracting samples such as intestines, livers and blood for later testing. The fresh intestines and livers were fixed using tissue fixation fluid, and the residual intestines and livers were rapidly freeze-dried using liquid nitrogen.

Pathological Staining

Pathological staining incorporated oil red, masson, sirius red, AB-PAS, PAS. Oil red dye kit (G1015), masson dye kit (G1006), sirius red dye kit (G1018), PAS dye kit (G1008), AB-PAS dye kit (G1049) were bought from Servicebio Biotechnology (Wuhan) Co., Ltd. Take pictures with the pathological section scanner (3DHISTECH Panoramic, version: Panoramic MIDI) and mean of integral optical density (IOD) was used to analyze the results.

Transcriptomic Analysis

Transcriptome sequencing included RNA extraction, quality detection, library construction, library quality control, sequencing, gene expression quantitative analysis, gene differential expression analysis, functional enrichment analysis, et al.

Immunohistochemical Staining

Immunohistochemical staining was used to explore GLUT5 expression. Antibody against GLUT5 (TD13545) was bought from Abmart Medical Technology (Shanghai) Co., Ltd. Take pictures with the pathological section scanner and mean of IOD was used to analyze the results.

Immunofluorescent Staining

Immunofluorescent staining was used to analyze the expression of occludin, claudin-1, ZO-1, COLI, COLIII, fibronectin, E-cadherin, α -SMA. Antibodies against occludin (GB111401), COLI (GB11022-3), COLIII (GB111629), fibronectin (GB114491), E-cadherin (GB12083), α -SMA (GB111364) were bought from Servicebio Biotechnology (Wuhan) Co., Ltd. Antibodies against claudin-1 (TD6919), ZO-1 (TA5145) were bought from Abmart Medical Technology (Shanghai) Co., Ltd. Take pictures with the pathological section scanner and mean of IOD was used to analyze the results.

Western Blotting

Western blotting was used for exploring the expression of occludin, claudin-1, ZO-1, E-cadherin, α -SMA, GLUT5, p-AMPK, p-mTOR. Antibodies against occludin (GB11149), ZO-1 (GB115686) were bought from Servicebio Biotechnology (Wuhan) Co., Ltd. Antibodies against claudin-1 (TD6919), GLUT5 (TD13545), E-cadherin (TA0131), α -SMA (T55295) were bought from Abmart Medical Technology (Shanghai) Co., Ltd. Antibody against p-mTOR (ab137133) was bought from Abcam. Antibody against p-AMPK (P17273) was bought from ProMab biotechnologies, Inc. Antibodies against β -actin (66009-1-Ig), GAPDH (60004-1-Ig) were bought from Proteintech Group, Inc. Western blotting was tested via chemiluminescence gel imager (Tanon, Tanon-4600) and results were used for semi-quantitative analysis.

Statistical Analysis

The comparison between the two groups was conducted using the *T*-test, while the comparison among the three groups was performed using the ANOVA analysis. $P < 0.05$ or $P < 0.01$ was considered as statistically significant difference.

Results

Olanzapine Induced Gut-Liver Axis Injury

Figure 1A–F were immunofluorescent staining of occludin, claudin-1, ZO-1 in ileum and colon, respectively. Figure 1G was Western blotting of occludin, claudin-1, ZO-1. Figure 1H–J were corresponding statistical analysis of occludin, claudin-1, ZO-1 from Western blotting, respectively, in which compared with NC group the expression of occludin, claudin-1, ZO-1 were reduced in OLA group ($P < 0.05$ or $P < 0.01$). These results suggested that olanzapine induced intestinal barrier injury. Figure 1K was oil red staining, masson staining, sirius red staining of liver. Figure 1L–N were immunofluorescent staining of COLI, COLIII, fibronectin in liver. Figure 1O–Q were corresponding statistical analysis of oil red staining, masson staining, sirius red staining in liver, in which compared with NC group, the results of oil red staining, masson staining, sirius red staining in liver were deteriorative in OLA group ($P < 0.01$). Figure 1R–T were corresponding statistical analysis of COLI, COLIII, fibronectin in liver, in which compared with NC group, the expression of COLI, COLIII, fibronectin in liver were increased in OLA group ($P < 0.01$). Figure 1U–W were immunofluorescent staining of E-cadherin/ α -SMA and corresponding statistical analysis of E-cadherin/ α -SMA from immunofluorescent staining in liver, where compared with NC group, the expression of E-cadherin in liver from immunofluorescent staining was reduced in OLA group ($P < 0.01$), on the other hand the expression of α -SMA in liver from immunofluorescent staining was increased in OLA group ($P < 0.01$). Figure 1X–Z were Western blotting of E-cadherin/ α -SMA and corresponding statistical analysis of E-cadherin/ α -SMA from Western blotting in liver, where compared with NC group, the expression of E-cadherin in liver from Western blotting was reduced in OLA group ($P < 0.01$), on the other hand the expression of α -SMA in liver from Western blotting was increased in OLA group ($P < 0.01$). These results suggested that olanzapine induced NAFLD and hepatic fibrosis.

GLUT5 Was Increased in Intestinal Tract

Figure 2A–F were violin plot, heatmap, genome circus, volcano map, KEGG pathways enrichment, and carbohydrate digestion and absorption, where these transcriptomic results indicated that GLUT5 was highly expressed in intestinal barrier injury. Figure 2G–I were immunohistochemical staining and corresponding statistical analysis of GLUT5 in ileum and colon, respectively, where compared with NC group, the expression of GLUT5 in ileum and colon from immunohistochemical staining were increased in OLA group ($P < 0.01$). These results proved again that GLUT5 was highly expressed in intestinal barrier injury.

GLUT5 Was Increased in Liver

Figure 3A–F were violin plot, heatmap, genome circus, volcano map, KEGG pathways enrichment, and carbohydrate digestion and absorption, where these transcriptomic results indicated that GLUT5 was highly expressed in NAFLD and hepatic fibrosis. Figure 3G and H were immunohistochemical staining and corresponding statistical analysis of GLUT5 in liver, where compared with NC group, the expression of GLUT5 in liver from immunohistochemical staining was increased in OLA group ($P < 0.01$). These results proved again that GLUT5 was highly expressed in NAFLD and hepatic fibrosis.

Scientific Hypothesis of Olanzapine Activated GLUT5/AMPK/mTOR Driving Gut-Liver Axis Injury

Figure 4A–D were Western blotting of GLUT5, p-AMPK, p-mTOR and corresponding statistical analysis of GLUT5, p-AMPK, p-mTOR from Western blotting in intestinal tract, where compared with NC group, the expression of GLUT5, p-mTOR in intestinal tract from Western blotting were increased in OLA group ($P < 0.05$ or $P < 0.01$), on the other hand the expression of p-AMPK in intestinal tract from Western blotting was reduced in OLA group ($P < 0.01$). These results suggested that olanzapine activated GLUT5/AMPK/mTOR in intestinal barrier injury. Figure 4E–H were Western

blotting of GLUT5, p-AMPK, p-mTOR and corresponding statistical analysis of GLUT5, p-AMPK, p-mTOR from Western blotting in liver, where compared with NC group, the expression of GLUT5, p-mTOR in liver from Western blotting were increased in OLA group ($P < 0.05$ or $P < 0.01$), on the other hand the expression of p-AMPK in liver from Western blotting was reduced in OLA group ($P < 0.01$). These results suggested that olanzapine activated GLUT5/AMPK/mTOR in NAFLD and hepatic fibrosis. Figure 4I was scientific hypothesis of olanzapine activated GLUT5/AMPK/mTOR driving gut-liver axis injury.

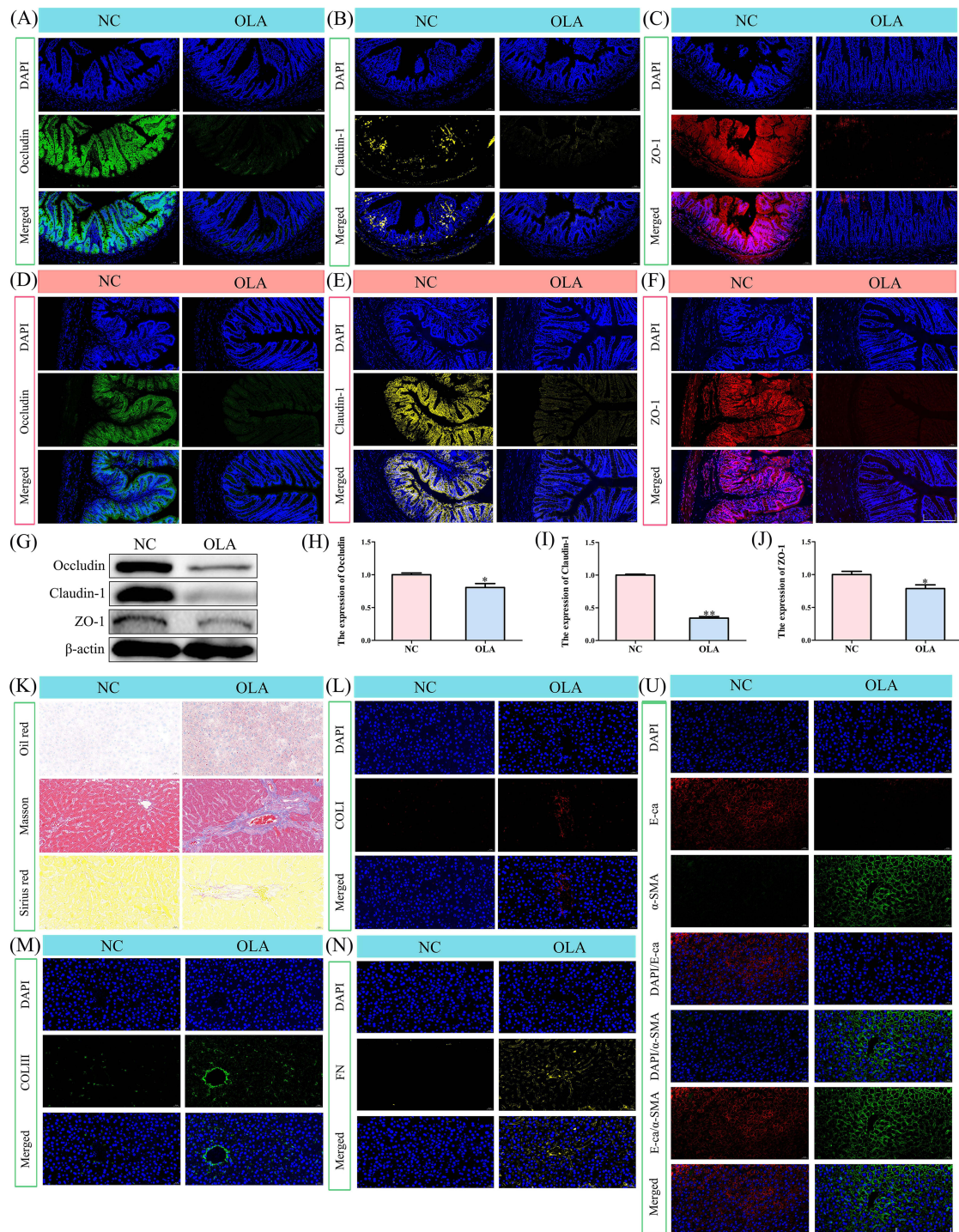


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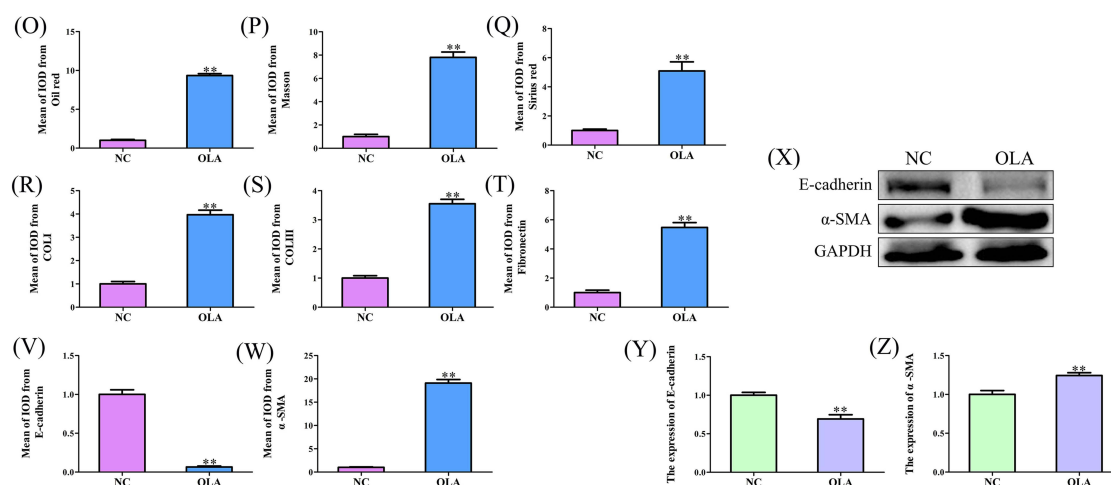


Figure 1 Olanzapine induced gut-liver axis injury. (A) Immunofluorescent staining of occludin in ileum. (B) Immunofluorescent staining of claudin-1 in ileum. (C) Immunofluorescent staining of ZO-1 in ileum. (D) Immunofluorescent staining of occludin in colon. (E) Immunofluorescent staining of claudin-1 in colon. (F) Immunofluorescent staining of ZO-1 in colon. (G) Western blotting of occludin, claudin-1, ZO-1. (H) Corresponding statistical analysis of occludin from Western blotting. (I) Corresponding statistical analysis of claudin-1 from Western blotting. (J) Corresponding statistical analysis of ZO-1 from Western blotting. (K) Oil red staining, masson staining, sirius red staining of liver. (L) Immunofluorescent staining of COLI in liver. (M) Immunofluorescent staining of COLIII in liver. (N) Immunofluorescent staining of fibronectin in liver. (O) Corresponding statistical analysis of oil red staining in liver. (P) Corresponding statistical analysis of masson staining in liver. (Q) Corresponding statistical analysis of sirius red staining in liver. (R) Corresponding statistical analysis of COLI in liver. (S) Corresponding statistical analysis of COLIII in liver. (T) Corresponding statistical analysis of fibronectin in liver. (U) Immunofluorescent staining of E-cadherin/ α -SMA in liver. (V) Corresponding statistical analysis of E-cadherin from immunofluorescent staining in liver. (W) Corresponding statistical analysis of α -SMA from immunofluorescent staining in liver. (X) Western blotting of E-cadherin/ α -SMA in liver. (Y) Corresponding statistical analysis of E-cadherin from Western blotting in liver. (Z) Corresponding statistical analysis of α -SMA from Western blotting in liver. * $P < 0.05$, ** $P < 0.01$ vs NC. Scale bar was 200 μ m.

Abbreviations: NC, control group; OLA, olanzapine group; FN, fibronectin; E-ca, E-cadherin.

Dapagliflozin Reversed GLUT5/AMPK/mTOR to Improve Intestinal Barrier Injury

Figure 5A was flow chart. Figure 5B–D were AB-PAS/PAS staining in ileum and colon and corresponding statistical analysis of PAS staining in ileum and colon, where compared with NC group, the expression of PAS staining in ileum and colon were increased in OLA group ($P < 0.01$), compared with OLA group, the expression of PAS staining in ileum and colon were reduced in DAP group ($P < 0.01$). Figure 5E–H were Western blotting of GLUT5, p-AMPK, p-mTOR and corresponding statistical analysis of GLUT5, p-AMPK, p-mTOR from Western blotting in intestinal tract, where compared with NC group, the expression of GLUT5, p-mTOR in intestinal tract from Western blotting were increased in OLA group ($P < 0.01$), on the other hand the expression of p-AMPK in intestinal tract from Western blotting was reduced in OLA group ($P < 0.01$). Compared with OLA group, the expression of GLUT5, p-mTOR in intestinal tract from Western blotting were reduced in DAP group ($P < 0.05$ or $P < 0.01$), on the other hand the expression of p-AMPK in intestinal tract from Western blotting was increased in DAP group ($P < 0.05$). These results suggested that olanzapine activated GLUT5/AMPK/mTOR in intestinal barrier injury, and dapagliflozin could reverse GLUT5/AMPK/mTOR. Figure 5I–L were Western blotting of occludin, claudin-1, ZO-1 and corresponding statistical analysis of occludin, claudin-1, ZO-1 from Western blotting, respectively, in which compared with NC group the expression of occludin, claudin-1, ZO-1 were reduced in OLA group ($P < 0.05$ or $P < 0.01$), and compared with OLA group the expression of occludin, claudin-1, ZO-1 were increased in DAP group ($P < 0.05$ or $P < 0.01$). Figure 5M–O were immunofluorescent staining of occludin, claudin-1, ZO-1 in ileum. Figure 5P–R were immunofluorescent staining of occludin, claudin-1, ZO-1 in colon. These results suggested that dapagliflozin reversed GLUT5/AMPK/mTOR to improve intestinal barrier injury.

Dapagliflozin Reversed GLUT5/AMPK/mTOR to Improve Liver Injury

Figure 6A was flow chart. Figure 6B and C were ALT and AST, where compared with NC group, ALT and AST were increased in OLA group ($P < 0.05$ or $P < 0.01$), and compared with OLA group, ALT and AST were reduced in DAP group ($P < 0.05$ or $P < 0.01$). Figure 6D was oil red staining, masson staining, sirius red staining of liver. Figure 6E–G were corresponding statistical analysis of oil red staining, masson staining, sirius red staining in liver, in which compared

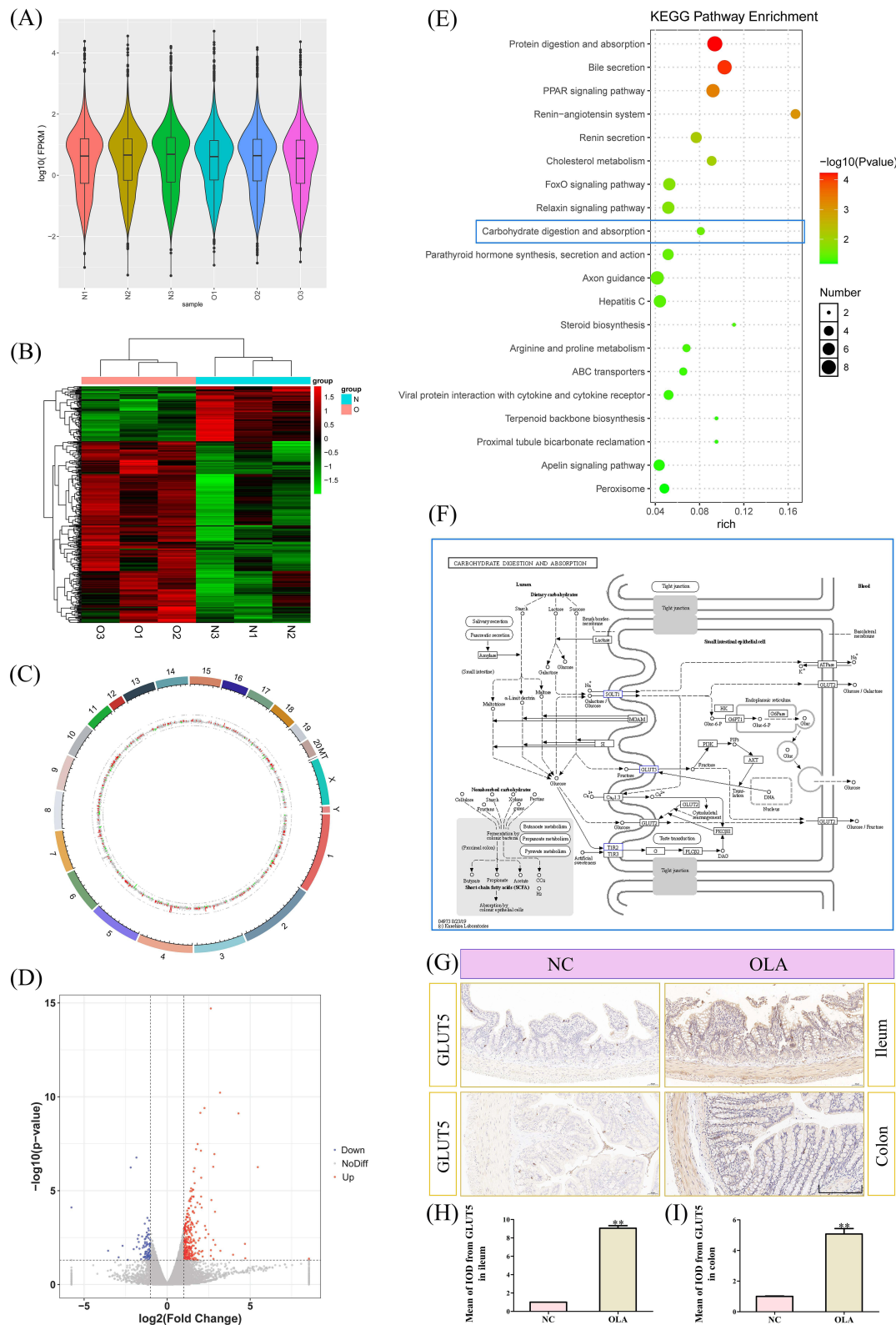


Figure 2 GLUT5 was increased in intestinal tract. **(A)** Violin plot. **(B)** Heatmap. **(C)** Genome circos. **(D)** Volcano map. **(E)** KEGG pathways enrichment. **(F)** Carbohydrate digestion and absorption. **(G)** Immunohistochemical staining of GLUT5 in ileum and colon. **(H)** Corresponding statistical analysis of GLUT5 in ileum. **(I)** Corresponding statistical analysis of GLUT5 in colon. Highlighted box represented the changed pathway or protein. $**P < 0.01$ vs NC. N1, N2, N3 were from NC group. O1, O2, O3 were from OLA group. Scale bar was 200 μm .

Abbreviations: NC, control group; OLA, olanzapine group.

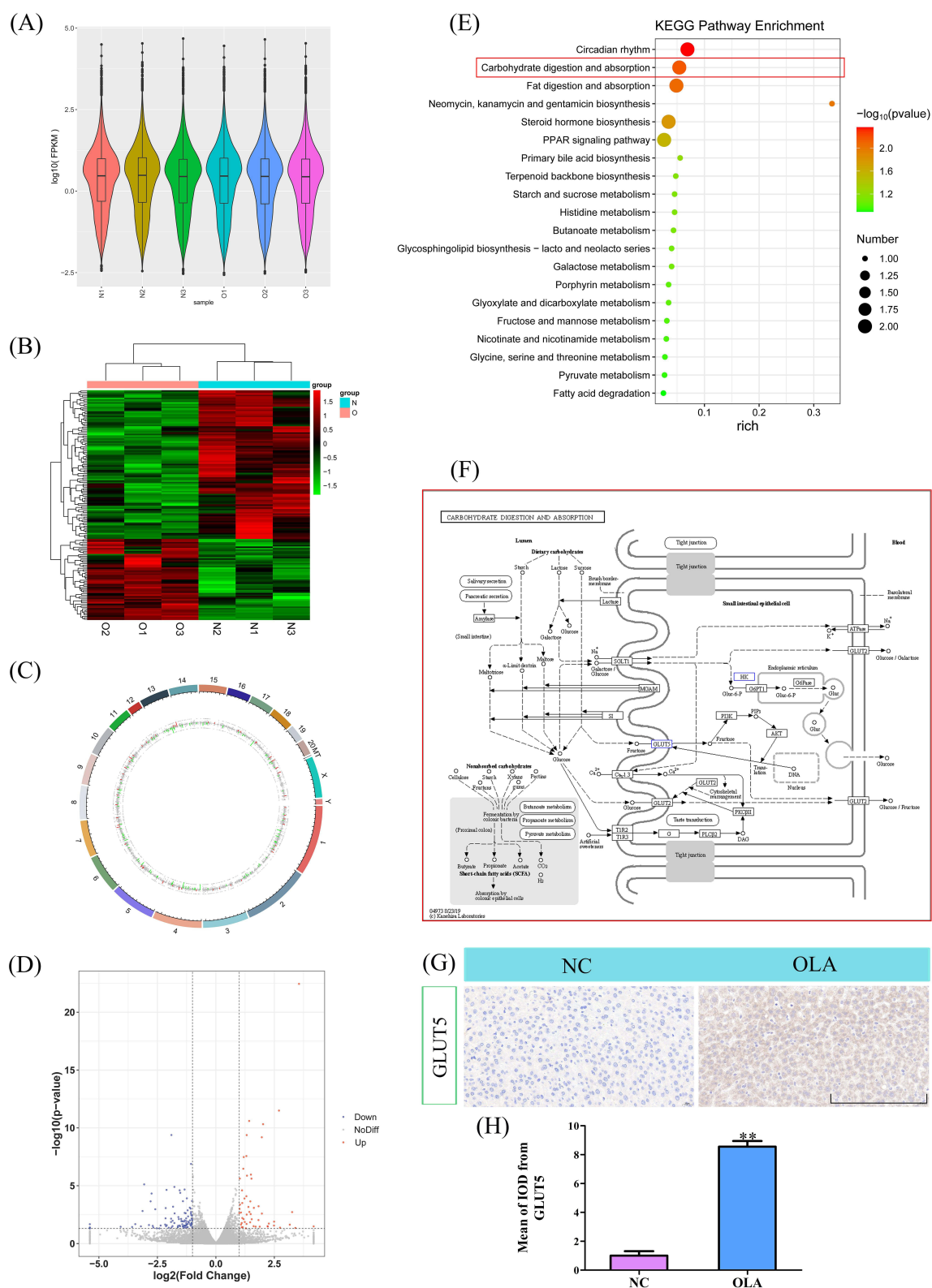


Figure 3 GLUT5 was increased in liver. (A) Violin plot. (B) Heatmap. (C) Genome circos. (D) Volcano map. (E) KEGG pathways enrichment. (F) Carbohydrate digestion and absorption. (G) Immunohistochemical staining of GLUT5 in liver. (H) Corresponding statistical analysis of GLUT5 in liver. Highlighted box represented the changed pathway or protein. $**P < 0.01$ vs NC. N1, N2, N3 were from NC group. O1, O2, O3 were from OLA group. Scale bar was 200 μm .
Abbreviations: NC, control group; OLA, olanzapine group.

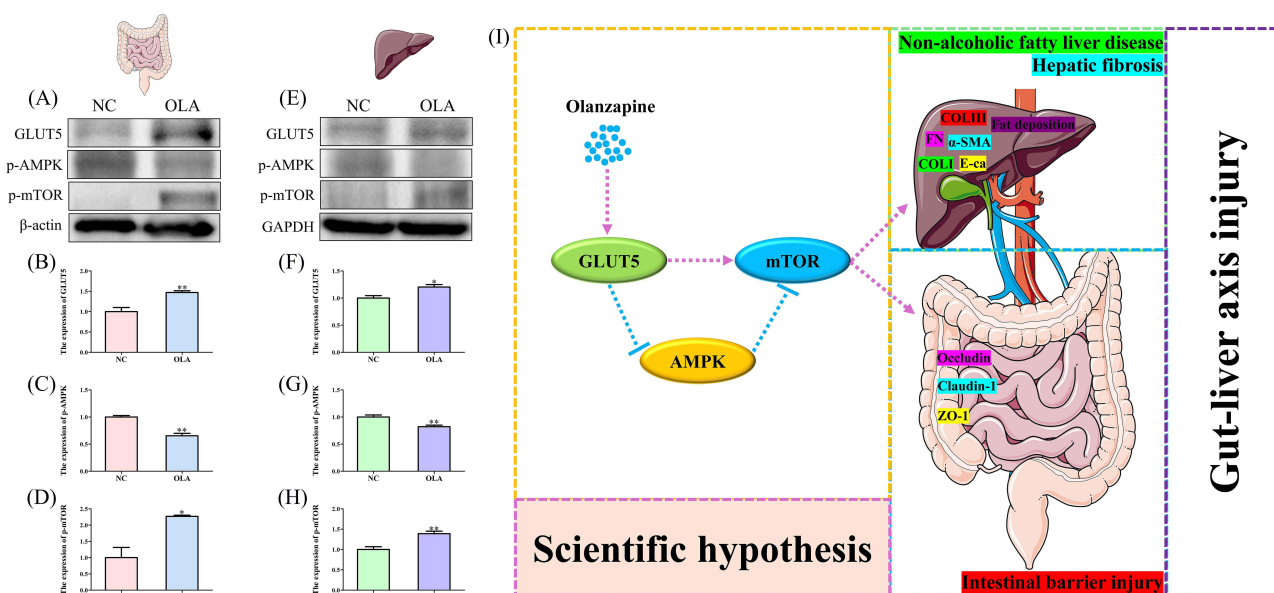


Figure 4 Scientific hypothesis of olanzapine activated GLUT5/AMPK/mTOR driving gut-liver axis injury. (A) Western blotting of GLUT5, p-AMPK, p-mTOR in intestinal tract. (B) Corresponding statistical analysis of GLUT5 from Western blotting in intestinal tract. (C) Corresponding statistical analysis of p-AMPK from Western blotting in intestinal tract. (D) Corresponding statistical analysis of p-mTOR from Western blotting in intestinal tract. (E) Western blotting of GLUT5, p-AMPK, p-mTOR in liver. (F) Corresponding statistical analysis of GLUT5 from Western blotting in liver. (G) Corresponding statistical analysis of p-AMPK from Western blotting in liver. (H) Corresponding statistical analysis of p-mTOR from Western blotting in liver. (I) Scientific hypothesis of olanzapine activated GLUT5/AMPK/mTOR driving gut-liver axis injury. * $P < 0.05$, ** $P < 0.01$ vs NC.

Abbreviations: NC, control group; OLA, olanzapine group.

with NC group, the results of oil red staining, masson staining, sirius red staining in liver were deteriorative in OLA group ($P < 0.01$), compared with OLA group, the results of oil red staining, masson staining, sirius red staining in liver were ameliorative in DAP group ($P < 0.01$). Figure 6H–K were Western blotting of GLUT5, p-AMPK, p-mTOR and corresponding statistical analysis of GLUT5, p-AMPK, p-mTOR from Western blotting in liver, where compared with NC group, the expression of GLUT5, p-mTOR in liver from Western blotting were increased in OLA group ($P < 0.01$), on the other hand the expression of p-AMPK in liver from Western blotting was reduced in OLA group ($P < 0.05$). Compared with OLA group, the expression of GLUT5, p-mTOR in liver from Western blotting were reduced in DAP group ($P < 0.05$), on the other hand the expression of p-AMPK in liver from Western blotting was increased in DAP group ($P < 0.05$). Figure 6L–N were immunofluorescent staining of COL1, COL3, fibronectin in liver. Figure 6O–Q were corresponding statistical analysis of COL1, COL3, fibronectin in liver, in which compared with NC group, the expression of COL1, COL3, fibronectin in liver were increased in OLA group ($P < 0.01$), compared with OLA group, the expression of COL1, COL3, fibronectin in liver were reduced in DAP group ($P < 0.01$). Figure 6R–T were Western blotting of E-cadherin/α-SMA and corresponding statistical analysis of E-cadherin/α-SMA from Western blotting in liver, where compared with NC group, the expression of E-cadherin in liver from Western blotting was reduced in OLA group ($P < 0.05$), on the other hand the expression of α-SMA in liver from Western blotting was increased in OLA group ($P < 0.05$). Compared with OLA group, the expression of E-cadherin in liver from Western blotting was increased in DAP group ($P < 0.01$), on the other hand the expression of α-SMA in liver from Western blotting was reduced in DAP group ($P < 0.01$). Figure 6U–W were immunofluorescent staining of E-cadherin/α-SMA and corresponding statistical analysis of E-cadherin/α-SMA from immunofluorescent staining in liver, where compared with NC group, the expression of E-cadherin in liver from immunofluorescent staining was reduced in OLA group ($P < 0.01$), on the other hand the expression of α-SMA in liver from immunofluorescent staining was increased in OLA group ($P < 0.01$). Compared with OLA group, the expression of E-cadherin in liver from immunofluorescent staining was increased in DAP group ($P < 0.01$), on the other hand the expression of α-SMA in liver from immunofluorescent staining was reduced in DAP group ($P < 0.05$). These results suggested that dapagliflozin reversed GLUT5/AMPK/mTOR to improve liver injury.

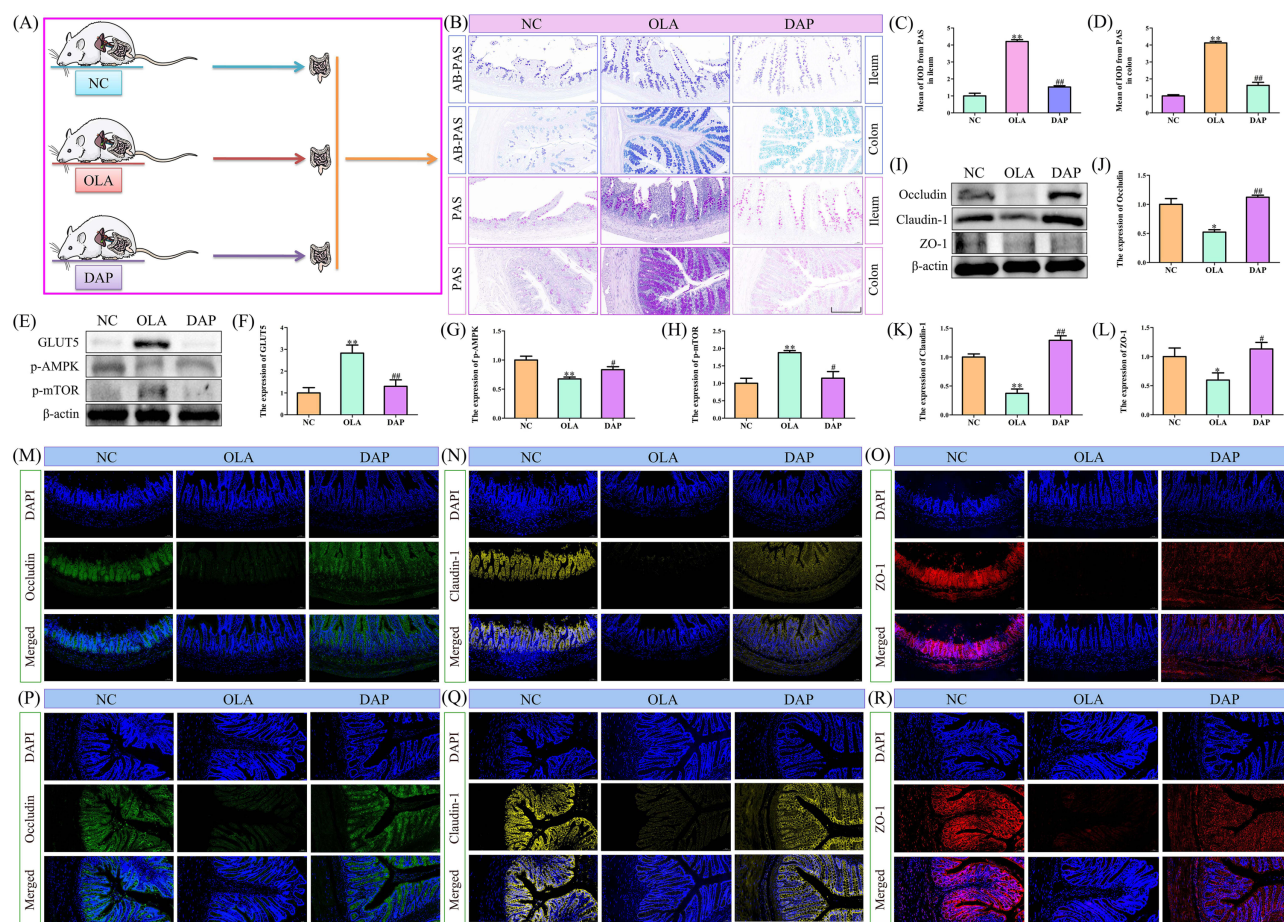


Figure 5 Dapagliflozin reversed GLUT5/AMPK/mTOR to improve intestinal barrier injury. (A) Flow chart. (B) AB-PAS/PAS staining in ileum and colon. (C) Corresponding statistical analysis of PAS staining in ileum. (D) Corresponding statistical analysis of PAS staining in colon. (E) Western blotting of GLUT5, p-AMPK, p-mTOR in intestinal tract. (F) Corresponding statistical analysis of GLUT5 from Western blotting in intestinal tract. (G) Corresponding statistical analysis of p-AMPK from Western blotting in intestinal tract. (H) Corresponding statistical analysis of p-mTOR from Western blotting in intestinal tract. (I) Western blotting of occludin, claudin-1, ZO-1. (J) Corresponding statistical analysis of occludin from Western blotting. (K) Corresponding statistical analysis of claudin-1 from Western blotting. (L) Corresponding statistical analysis of ZO-1 from Western blotting. (M) Immunofluorescent staining of occludin in ileum. (N) Immunofluorescent staining of claudin-1 in ileum. (O) Immunofluorescent staining of ZO-1 in ileum. (P) Immunofluorescent staining of occludin in colon. (Q) Immunofluorescent staining of claudin-1 in colon. (R) Immunofluorescent staining of ZO-1 in colon. * $P < 0.05$, ** $P < 0.01$ vs NC. # $P < 0.05$, ### $P < 0.01$ vs OLA. Scale bar was 200 μm .

Abbreviations: NC, control group; OLA, olanzapine group; DAP, dapagliflozin intervention group.

Mechanism Diagram of Olanzapine Activated GLUT5/AMPK/mTOR Driving Gut-Liver Axis Injury and Dapagliflozin Intervention

Olanzapine activated GLUT5/AMPK/mTOR driving gut-liver axis injury. Dapagliflozin reversed GLUT5/AMPK/mTOR, and ameliorated the gut-liver axis injury to improve NAFLD, which was shown in Figure 7.

Discussion

NAFLD refers to a type of metabolic stress-induced liver injury characterized by diffuse fatty changes in liver cells, provided that long-term excessive alcohol consumption, hepatitis B virus infection, etc. are excluded. NAFLD is the main cause of chronic liver diseases and liver transplantation worldwide, seriously endangering human life and health safety, and also imposing a huge economic burden on public healthcare and social security.^{45,46} The progression of NAFLD is related to various factors such as obesity, insulin resistance, genetics, metabolic stress, systemic chronic low-grade inflammation, intestinal flora disorder, and unreasonable dietary structure.^{47,48}

The gut-liver axis is a highly generalized term for the bidirectional relationship between the gut and its microbiota and the liver. On one hand, the liver and the gut both develop from the anterior hindgut of the endoderm and share the

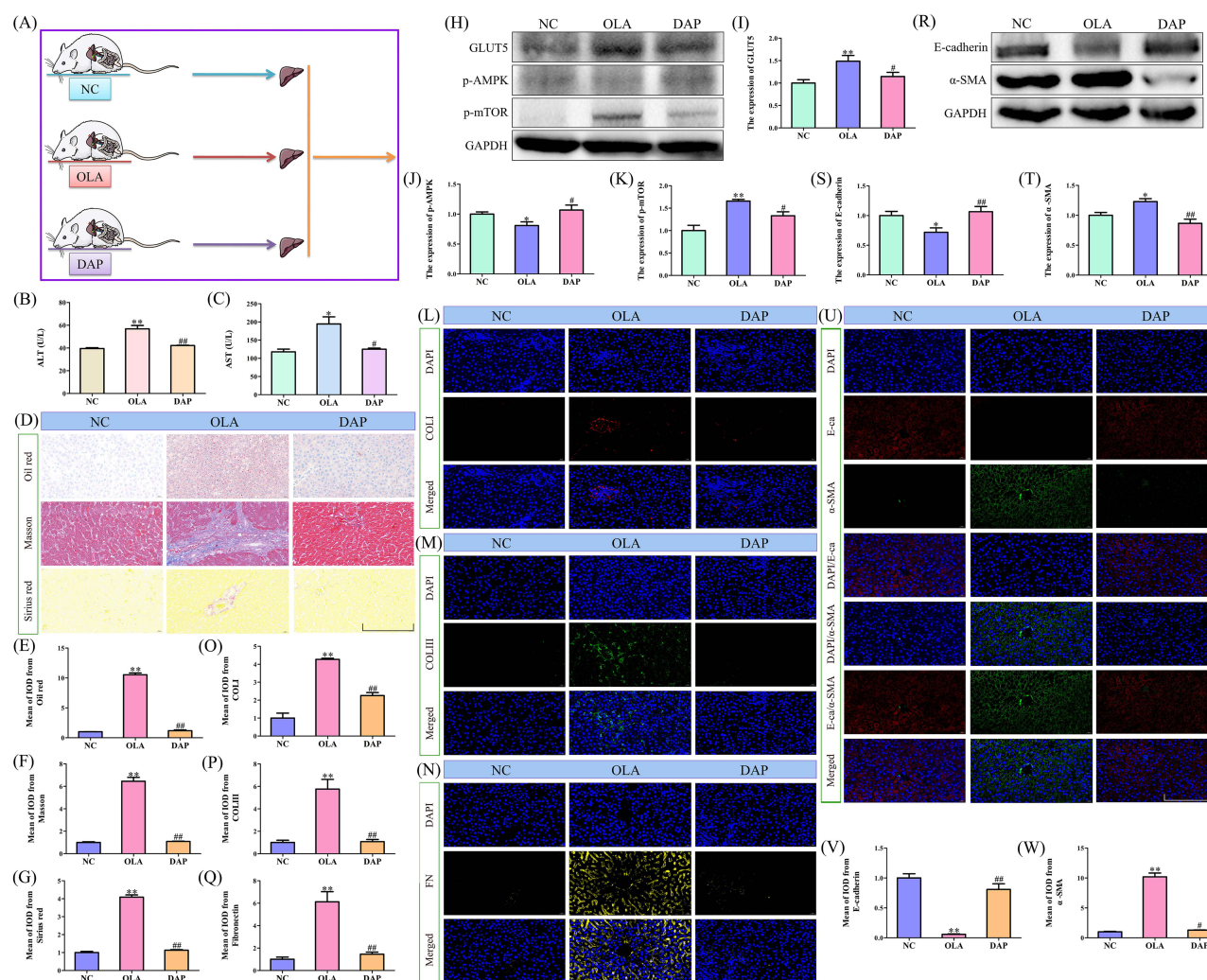


Figure 6 Dapagliflozin reversed GLUT5/AMPK/mTOR to improve liver injury. (A) Flow chart. (B) ALT. (C) AST. (D) Oil red staining, masson staining, sirius red staining of liver. (E) Corresponding statistical analysis of oil red staining in liver. (F) Corresponding statistical analysis of masson staining in liver. (G) Corresponding statistical analysis of sirius red staining in liver. (H) Western blotting of GLUT5, p-AMPK, p-mTOR in liver. (I) Corresponding statistical analysis of GLUT5 from Western blotting in liver. (J) Corresponding statistical analysis of p-AMPK from Western blotting in liver. (K) Corresponding statistical analysis of p-mTOR from Western blotting in liver. (L) Immunofluorescent staining of COLI in liver. (M) Immunofluorescent staining of COLIII in liver. (N) Immunofluorescent staining of fibronectin in liver. (O) Corresponding statistical analysis of COLI from immunofluorescent staining in liver. (P) Corresponding statistical analysis of COLIII from immunofluorescent staining in liver. (Q) Corresponding statistical analysis of fibronectin from immunofluorescent staining in liver. (R) Western blotting of E-cadherin/ α -SMA in liver. (S) Corresponding statistical analysis of E-cadherin from Western blotting in liver. (T) Corresponding statistical analysis of α -SMA from Western blotting in liver. (U) Immunofluorescent staining of E-cadherin/ α -SMA in liver. (V) Corresponding statistical analysis of E-cadherin from immunofluorescent staining in liver. (W) Corresponding statistical analysis of α -SMA from immunofluorescent staining in liver. * $P < 0.05$, ** $P < 0.01$ vs NC. ## $P < 0.05$, ### $P < 0.01$ vs OLA. Scale bar was 200 μ m.

Abbreviations: NC, control group; OLA, olanzapine group; DAP, dapagliflozin intervention group; FN, fibronectin; E-ca, E-cadherin.

same embryonic origin.⁴⁹ On the other hand, the metabolic products of the liver and intestinal flora interact through the bile duct and portal vein, influencing the physiological metabolic functions of the body.⁵⁰ The liver and the intestine also have complex interactions in terms of immunity, metabolism and endocrinology. The intestinal barrier is composed of four layers: biological, mechanical, chemical and immune barriers.

Normal gut-liver axis function requires the support of the intestinal mucosal barrier. Among them, occludin, claudins, and zonula occludens (ZO)s are the main proteins that constitute the intestinal tight junctions. They form the structural basis of intestinal barrier function at the top of the apical surface of the intestinal epithelial cells. The intestinal tight junction proteins are an important component of intestinal barrier function. Under the influence of intestinal-related diseases and drug toxicity and other damaging factors, the integrity of intestinal tight junction proteins is impaired, which in turn leads to intestinal barrier dysfunction and promotes the entry of harmful metabolites into the gut-liver axis circulation.

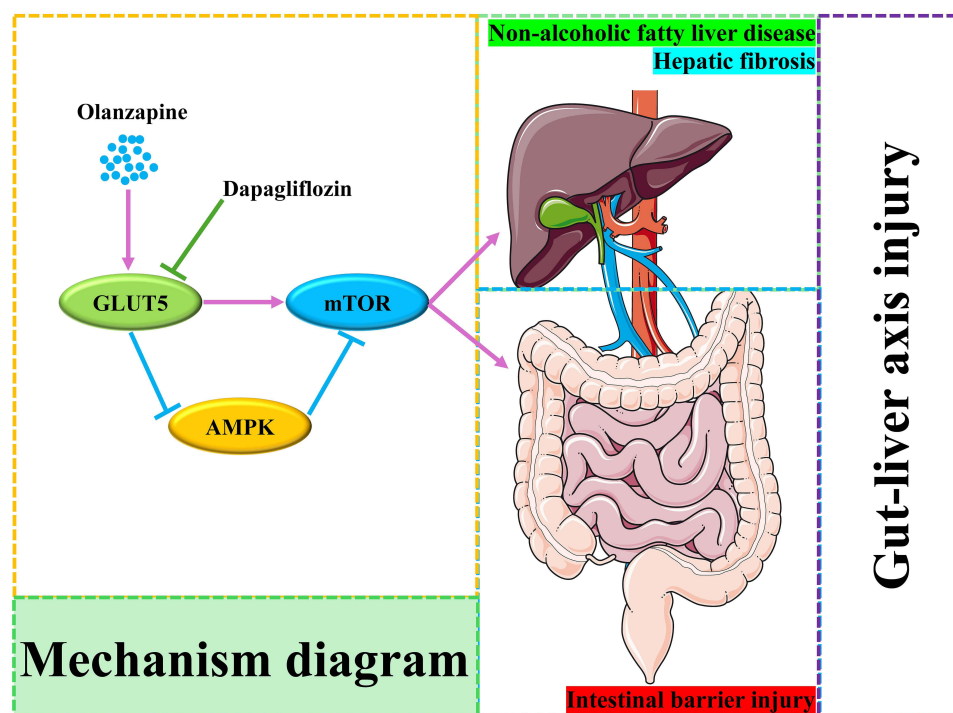


Figure 7 Mechanism diagram of olanzapine activated GLUT5/AMPK/mTOR driving gut-liver axis injury and dapagliflozin intervention.

At present, many studies have found that the injury of the gut-liver axis is closely related to NAFLD. Intervening in the injury of the gut-liver axis can improve NAFLD. Ma et al reported *H. rhamnoides* L. seed oil effectively mitigated NAFLD by reducing hepatic lipid expression via regulating intestinal microbiota, its SCFA secretion and intestinal barrier.⁵¹ Xu et al reported qushi huayu decoction alleviated NAFLD in mice by regulating gut microbiota homeostasis in the gut-liver axis via the pregnane X receptor.⁵² Yang et al reported ginsenoside Rh4 improved hepatic lipid metabolism and inflammation in a model of NAFLD by targeting the gut-liver axis and modulating the FXR signaling pathway.⁵³ Xiao et al reported *tetragium hemsleyanum* leaf extracts ameliorated NAFLD in mice with low-grade colitis via the gut-liver axis.⁵⁴ Wang et al reported *Akebia trifoliata* extracts attenuated liver injury via gut-liver axis in a murine model of NAFLD with low-grade colitis.⁵⁵ Luo et al reported Befa protein alleviated progression of NAFLD by modulating the AMPK signaling pathway through the gut-liver axis.⁵⁶ Sun et al reported *Bacteroides ovatus* alleviated high-fat and high-cholesterol-induced NAFLD via gut-liver axis.⁵⁷ Xu et al reported zhuyu pill alleviated NAFLD by regulating bile acid metabolism through the gut-liver axis.⁵⁸ Kuang et al reported hyodeoxycholic acid alleviated NAFLD through modulating the gut-liver axis.⁵⁹ Therefore, the gut-liver axis is a key link in the development of NAFLD, and at the same time, it is also a crucial factor for its treatment.

Olanzapine's prolonged use has been associated with an increased risk of NAFLD,^{8,9,11–14} however, the underlying mechanism remains unclear. In this study, we focused on the injury of the gut-liver axis induced by olanzapine in rats with NAFLD. Firstly, we found that olanzapine caused intestinal barrier injury and NAFLD and hepatic fibrosis. The transcriptome studies of the intestine and liver further revealed the high expression of GLUT5 in both tissues. GLUT5, as a fructose transporter, plays a significant role in the occurrence and development of various diseases. Studies have shown that GLUT5 regulates fructose metabolism in human bile duct cancer cells, and its overexpression is associated with the progression of bile duct cancer; while GLUT5 is highly expressed in breast cancer, and its expression is not found in normal breast tissue; at the same time, in the case of glucose deficiency, GLUT5-mediated fructose uptake and utilization contribute to the progression of pancreatic cancer, and the fructose-regulated AMPK-mTOR signaling pathway inhibits autophagy-induced cell death, making pancreatic cancer cells more conducive to growth and progression; in lung cancer, GLUT5 is highly expressed in tumor tissues of lung cancer metastasis patients, and promotes the malignant growth of

lung cancer cells through the AMPK-mTOR signaling pathway.^{60,61} In summary, GLUT5 is highly expressed in various diseases and further regulates the AMPK-mTOR signaling pathway to induce disease progression.

Furthermore, we found that in olanzapine-induced rat NAFLD models, olanzapine activated GLUT5/AMPK/mTOR driving gut-liver axis injury. It was mainly manifested in the intestinal aspect by down-regulating intestinal tight junction proteins occludin, claudin-1 and ZO-1, and in the liver aspect by inducing fat accumulation and liver fibrosis. After dapagliflozin intervention, the expression of GLUT5/AMPK/mTOR in the intestine was reversed, and the expression of intestinal barrier proteins increased, thereby improving intestinal barrier injury. In the liver, dapagliflozin reversed the expression of GLUT5/AMPK/mTOR in the liver, improving NAFLD and hepatic fibrosis.

Of course, this study had certain limitations. This study lacked some parameters related to overall metabolism. This would be further explored in our future in-depth research on the mechanism and clinical studies.

Conclusion

In this study, we discovered for the first time that GLUT5/AMPK/mTOR was associated with gut-liver axis injury mediating olanzapine-induced NAFLD and dapagliflozin intervention could reverse GLUT5/AMPK/mTOR-driven gut-liver axis injury ameliorating olanzapine-induced NAFLD. This provides a new understanding of the occurrence of olanzapine-induced NAFLD and offers a reference plan for its clinical intervention.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author (Dong-Dong Wang) on reasonable request.

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Disclosure

Xiao Chen, Yue Zhang, and Jie Wang are co-first authors. The authors report no conflicts of interest.

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