



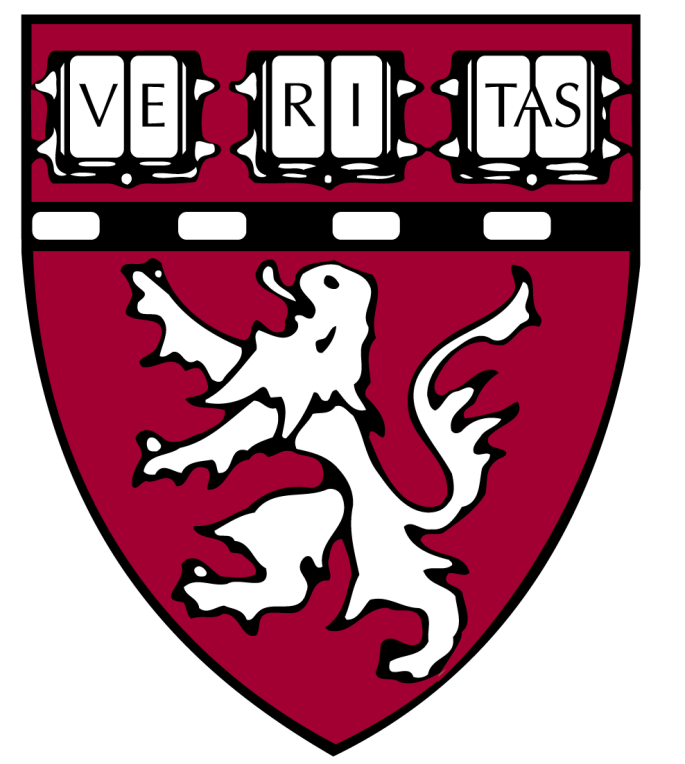
# A novel non-bile acid FXR agonist EDP-305 prevents progression to cirrhosis in rats

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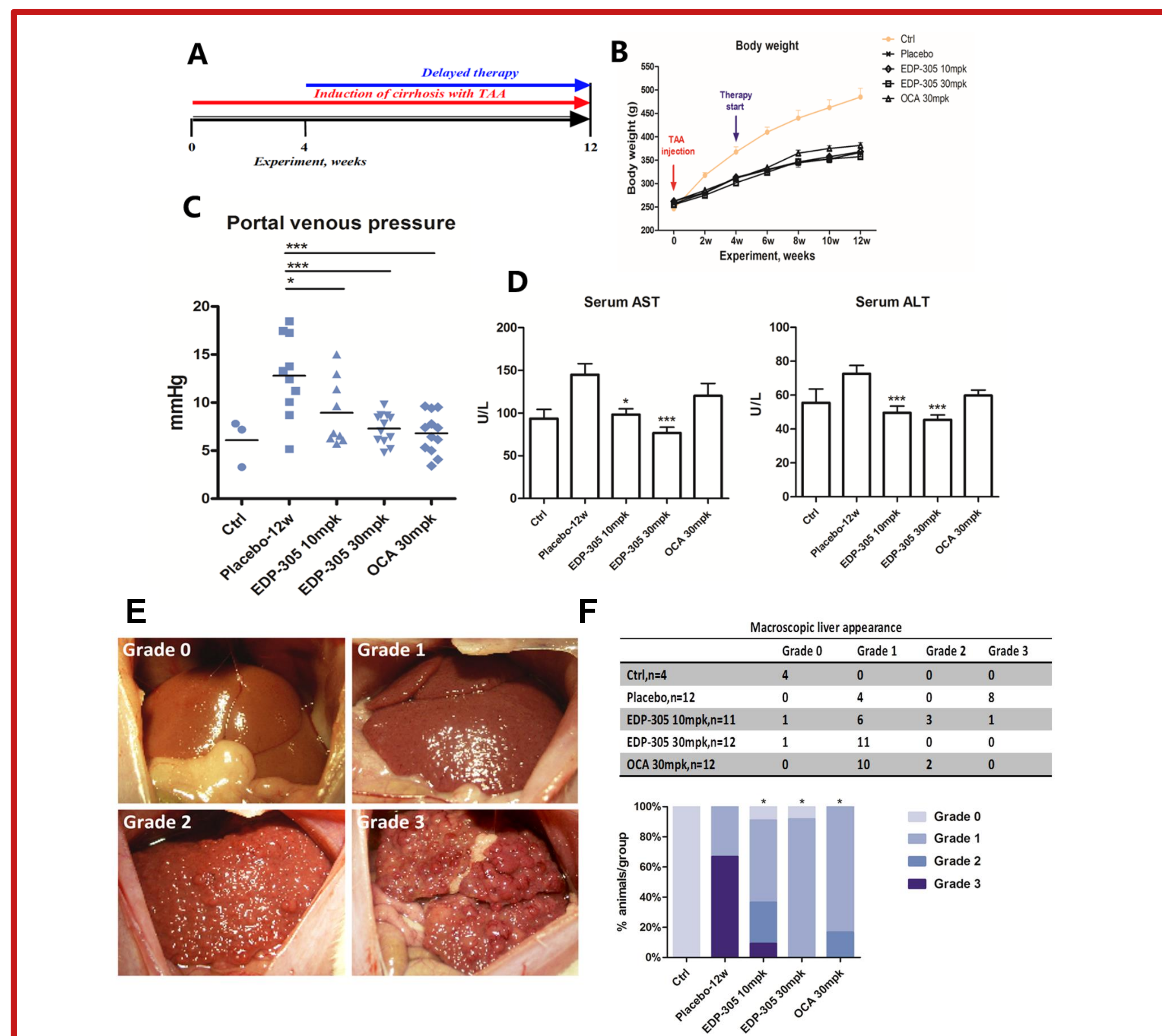


**BACKGROUND & AIMS:** EDP-305 is a novel, non-bile-acid FXR agonist with a single-digit nanomolar FXR affinity and no/minimal TGR5 cross-reactivity in vitro. We studied the therapeutic efficacy of EDP-305 in a thioacetamide (TAA)-induced rat liver cirrhosis model, in direct comparison to first-in-class FXR agonist obeticholic acid (OCA).

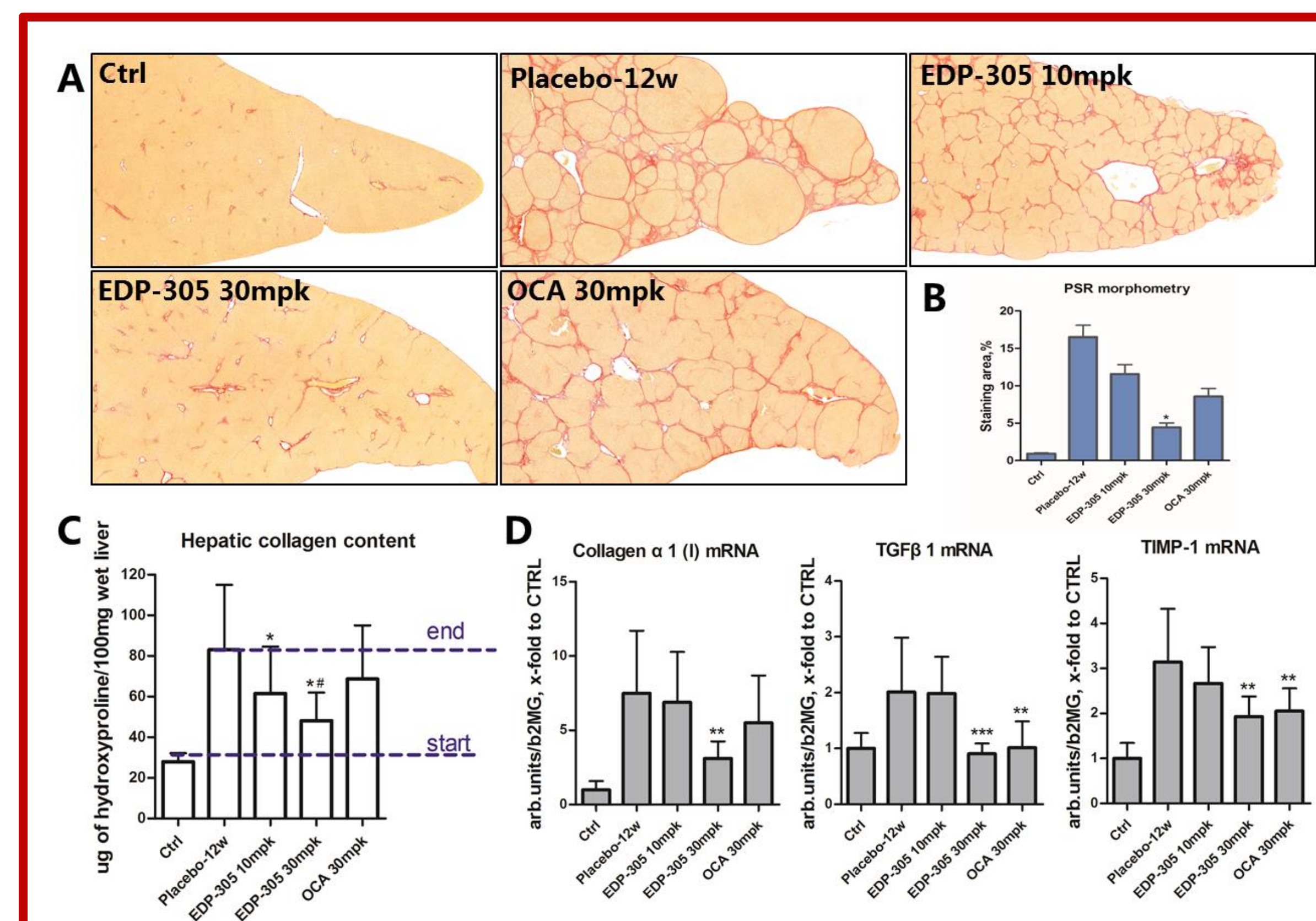
**METHODS:** Cirrhosis was induced in male Wistar rats by repeated injections of TAA (200mg/kg i/p twice a week) for 12 weeks. After 4 weeks of TAA, animals were randomized into placebo (vehicle only), EDP-305 (10 or 30mg/kg/day) or OCA (30mg/kg/day) treatment groups and treated for the following 8 weeks (n=11-12/group, **Figure 1A**). Portal venous pressure (PVP), serum liver function tests, ductular reaction and fibrosis were assessed at study end-point. P<0.05 (ANOVA) was considered statistically significant.

**RESULTS:** Chronic EDP-305 and OCA administration was well tolerated in TAA-treated rats, with no apparent drug-related toxicity. At study endpoint, animals receiving placebo developed compensated cirrhosis with portal hypertension (12.79±1.33 mmHg), pan-lobular bridging scarring and regenerative nodule formation, but no ascites. Portal pressure was significantly reduced in all treatment groups:

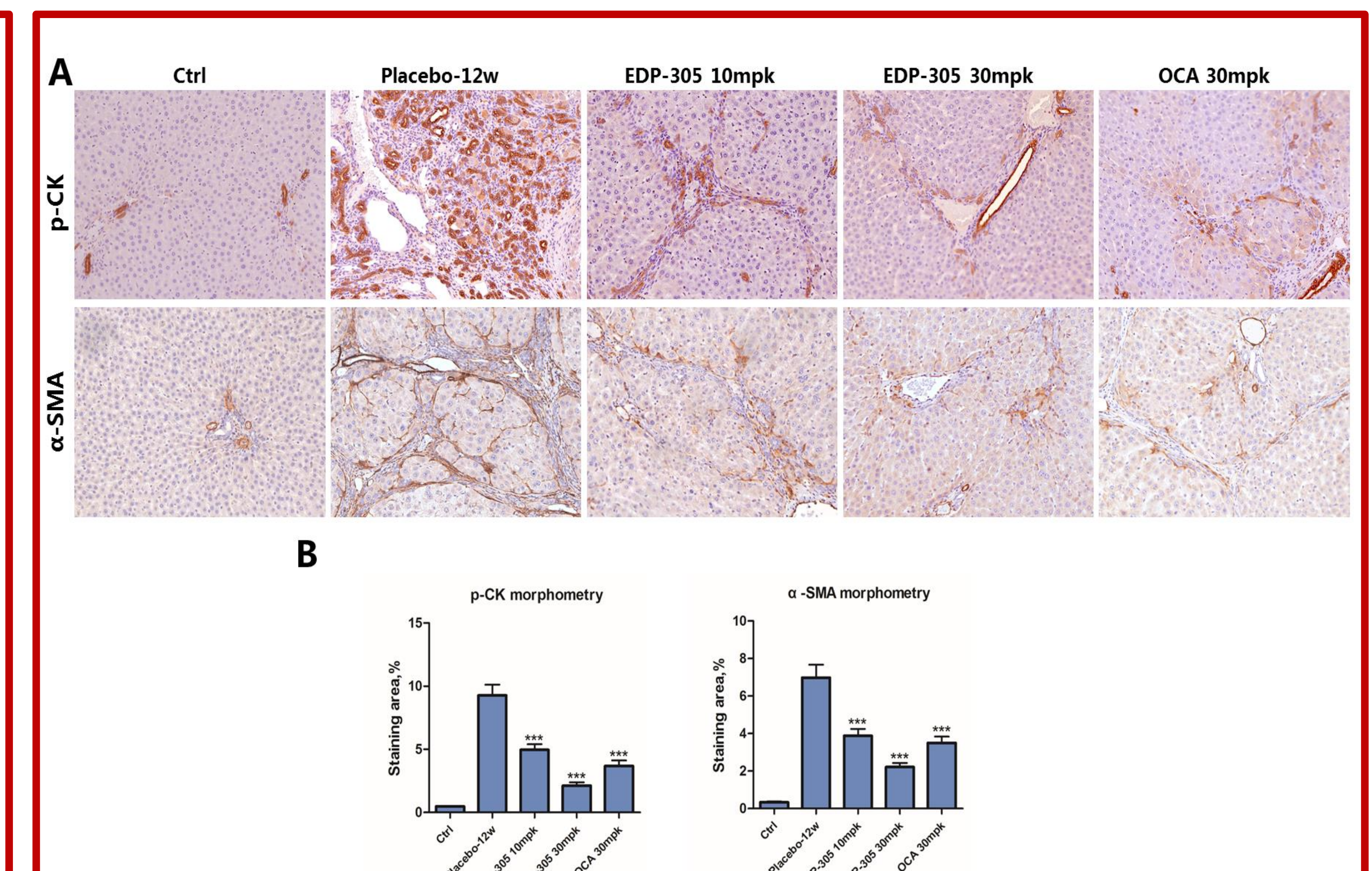
(mean 8.94±1.15 and 7.29±0.46mm Hg in 10 and 30mg/kg EDP-305, respectively, and 6.80±0.61mm Hg in OCA (p<0.0001). 30mg/kg EDP-305 significantly reduced both serum AST and ALT (by 59.8% and 37.3%, resp.), while OCA showed only a trend of lowered AST and ALT (by 17.0% and 17.6%, n.s., **Figure 1B-F**). Development of cirrhosis was markedly suppressed by both EDP-305 and OCA. Collagen morphometry revealed that EDP-305 reduced the connective tissue square (11.56±1.26% and 4.43±0.59% in 10 and 30 mg/kg dose, respectively, as did OCA (8.57±1.08%), compared to 16.5±1.59% in the placebo group, with corresponding decreases of 38.9%, 63.2%, and 26.1 % in hepatic hydroxyproline content in 10 and 30 mg/kg EDP-305 and OCA groups, respectively, compared to placebo (p<0.05). Pro-fibrogenic transcript levels of procollagen α1(I) and transforming growth factor-β1 (TGF-β1) were suppressed 2-3 fold by both EDP-305 and OCA at 30mpk doses (**Figure 2**). Hepatic stellate cell activation and ductular reaction (α-SMA and p-CK staining) improved remarkably in all treatment groups, with EDP-305 at 30mg/kg dose demonstrating the most significant improvement (**Figure 3**).



**Figure 1. The effect of delayed treatment with EDP-305 FXR agonist on serum liver biochemistry, portal hypertension and macroscopic liver appearance in rats with TAA-induced cirrhosis. (A)** Cirrhosis was induced by repeated TAA injections for 12 weeks in Wistar outbred rats. Delayed treatment with EDP-305 (10&30 mg/kg), OCA 30mpk (30 mg/kg) or vehicle (placebo) control was administered orally from 4 to 12 weeks of experiment (n=11-12). Ctrl are healthy untreated rats. **(B)** Changes in body weights throughout the treatment. **(C)** Portal venous pressure (PVP) measured invasively, and **(D)** serum levels of transaminases (ALT and AST) at study end-point. Data are mean±SEM, \*, p<0.05, \*\*, p<0.01 and \*\*\* p<0.001 compared to placebo (ANOVA). **(E)** Definition of macroscopic liver appearance grading system (Grade 0 – healthy: soft consistency, smooth surface; Grade 1 - increased turgor, slightly rough surface; Grade 2 - medium nodular surface; Grade 3 – pronounced macronodular surface. **(F)** The effect of EDP-305 FXR agonist on the macroscopic liver appearance. \*, p<0.05 ( Rank sum test )



**Figure 2. EDP-305 dose-dependently inhibits hepatic fibrogenesis and progression to cirrhosis. (A)** Representative images of connective tissue staining (picrosirius red, PSR, 20x). **(B)** Morphometric quantification of collagen area (n=4-5 per group, ImageJ). **(C)** Hepatic collagen content (determined biochemically via hydroxyproline content) demonstrate that EDP-305 dose-dependently inhibits collagen deposition. **(D)** Pro-fibrogenic transcript levels of procollagen α1(I), TGFβ1, TIMP-1 as measured by TaqMan qRT-PCR (x-fold to healthy control levels). Data are mean±SEM, \*, p<0.05 treated groups compared to Placebo group, \*\*, p<0.01 and \*\*\* p<0.001 compared to placebo control group; #, p<0.05 treated groups compared to OCA 30mpk positive control group (ANOVA with Dunnett post-test).



**Figure 3. Ductular reaction and hepatic stellate cell activation is dose-dependently suppressed by EDP-305. (A)** Representative immunohistochemistry figures showing impact of treatments on HSCs activation as assessed by α-SMA (upper panel) and ductular reaction assessed by pan-cytokeratin staining (pan-CK, lower panel) in TAA-induced liver fibrosis (original magnification, 200x). **(B)** Morphometric area quantification of α-SMA and p-CK using ImageJ software in 10 random portal HPF at 200X magnification (n=4 per group). Data are mean±SEM, \*, p<0.05, \*\*, p<0.01 and \*\*\* p<0.001 compared to placebo control group (ANOVA with Dunnett post-test).

## CONCLUSIONS:

- Delayed treatment with new FXR agonist EDP-305 safely and effectively prevents development of TAA-induced cirrhosis in rats.
- By several key parameters, EDP-305 outperformed the first-in-class FXR agonist, obeticholic acid.