Organ fibrosis is a progressive disease of unknown etiology associated with high morbidity and mortality, characterized by patchy inflammatory infiltration, abnormal epithelial and fibro-proliferative responses and subsequent ECM deposition resulting in progressive loss of organ function. Since, fibrosis affects various organs, lung and kidney fibroses are dreadful disease conditions as both the organs are physiologically blood purifiers. Once these organs get dysfunctional due to fibrosis, it severely affects physiological functions of body ultimately leads to death. Pathogenesis of fibrosis is enigmatically so complex that till today, there is no FDA approved drug to treat this disease condition. As both ET-1 and PDGF signaling have been involved in pathogenesis of lung and kidney fibroses, inhibitors of these signaling pathways (bosentan and imatinib respectively) gained importance in treatment of fibroses. Despite both the drugs showed promising efficacy in animal models of fibroses, but in clinical trials of IPF these drugs failed with regards to the set targets of exercise capacity, and time to death or disease progression, when used individually. Considering intricate pathogenesis of fibroses, we hypothesized that simultaneous inhibition of ET-1 and PDGF signaling pathways by combination treatment with bosentan and imatinib likely attenuates both lung and kidney fibroses more profound way than individual treatment. This hypothesis was tested using bleomycin induced model of PF and UUO induced kidney fibrosis animal models.

**Combination effects in fibrotic models**

- Mice subjected to bleomycin instillation (0.05U) for PF model; and mice subjected to UUO in kidney fibrotic model were administered with either bosentan (100 mg/kg) and/or imatinib (50 mg/kg).
- Combination treatment with bosentan and imatinib prevented bleomycin induced mortality and loss of body weight more than the individual agents in PF model.
- On day seven, the combination therapy attenuated bleomycin induced increase of total and differential inflammatory cell counts, total proteins in BALF, lung wet/dry weight ratio in PF model.
- Similarly, in UUO induced kidney fibrosis, combination treatment with bosentan and imatinib improved the kidney function by reducing BUN, serum creatinine and urinary protein levels and showed more efficacious effect than individual treatment.
- In both models of lung and kidney fibroses, while individually both drugs exhibited
significant attenuation of inflammatory cell infiltration into the respective organs, MPO activity and MMP-2 and MMP-9 activities, combination of drugs showed near additive effect in attenuation of these parameters.

- However, contrary to the additive and/or near additive efficacy in attenuating inflammatory events in both the fibrotic models, bosentan but not imatinib, either alone or in combination, increased superoxide dismutase and catalase activities, which were lowered following the creation of respective fibrotic models.

- On day 21, in PF mice, combination therapy ameliorated bleomycin induced increase of fibrosis score, collagen deposition, gene/protein expression of α-SMA, mRNA levels of collagens-I and -III more effectively than monotherapy and showed near additive effect in amelioration of these PF parameters.

- On day 14, in UUO mice, combination treatment showed more profound effect (near additive effect) in attenuation of increase in collagen deposition, gene/protein expression of α-SMA, protein expression of vimentin, mRNA levels of collagens-I and -III in kidney tissues than either bosentan or imatinib alone.

**Key findings**

- Key findings in the current study illustrated that combination of bosentan and imatinib exhibited additive and/or near additive efficacy in attenuation of inflammatory and various fibrotic parameters in both lung and kidney fibroses.

- However, combination treatment did not show additive efficacy in amelioration of antioxidant enzymes (SOD and catalase). This observation is quite contrary to the additive anti-inflammatory and antifibrotic effects of combination treatments.

- These two findings above with regards to combination study add strength to the notion that diversified signaling pathways may be contributing to fibrogenesis and hence, a simultaneous and multi-pathway targeted approach is needed to encounter the pathophysiological events.

- Hence, the outcome of this study suggests the necessity of attempting drug combinations rather than monotherapy in clinical trials, in view of the heterogeneity in biological and clinical phenotypes of fibrosis.

- In the absence of an effective drug for fibrosis and also the finding that bosentan and imatinib are ineffective in clinical trials of IPF when tested individually, the data presented in the current study provides strong rationale for testing the combination of bosentan and imatinib in PF and kidney fibrosis through clinical trials.