

**The Effect of Pirfenidone on Immunological
Liver and Lung Injury Induced by BCG in Female
Balb/C Mice**

Thesis

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تأثير البيرفيندون على الإصابة المناعية للكبد و الرئة المُحدَث بواسطة لقاح السل على إناث فئران Balb/C

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للحصول على درجة الماجستير
فى علم الأدوية والعلاج

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List of Abbreviations

Abb.	Full term
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCG	Bacillus Calmette Guerin
CTGF	Connective tissue growth factor
CYP	Cytochrome P
ECM	Extracellular matrix
ELISA	Enzyme-Linked Immunosorbent Assay
FADD	Fas associated death domain
HCV	hepatitis C virus
H&E	Hematoxyline and eosin
HIV	Human Immunodeficiency Virus
HSC	Hepatic stellate cell
Hyp	Hydroxyproline
IL	Interleukin
INF	Interferon
IPF	idiopathic pulmonary fibrosis
JAKs	Janus Kinases
LAP	Latency associated peptide
LTBP	Latent TGF- β binding protein
MMP	Matrix metalloproteinases
NF-κB	Nuclear factor kappa B
PBS	phosphate-buffered saline
PDGF	Platelet derived growth factor
αSMA	A smooth muscle actin
STATs	signal Transducers and Activators of Transcription
TB	Tuberculosis
TGF-β	Transforming growth factor- β
TIMPs	Tissue inhibitors of metalloproteinase
TNF-α	Tumor necrosis factor- α
TRADD	TNF receptor associated death domain
TRAF2	TNF receptor associated factor 2



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The Effect of Pirfenidone on Immunological Liver and Lung Injury Induced by BCG in Female Balb/C Mice

Background

Tissue fibrosis is a progressive, severely debilitating disease characterized by superabundant accumulation of extracellular matrix (ECM) leading to excessive tissue scarring, organ injury, function decline, and even failure (**Insel et al., 2012; Friedman et al., 2013**). Fibrosis is a condition arising from chronic state of various diseases such as scleroderma, rheumatoid arthritis, Crohn's disease, ulcerative colitis, systemic lupus erythematosus and idiopathic pulmonary fibrosis (IPF) (**Wynn, 2011; Seki and Brenner, 2015**). Fibrotic diseases have been largely overlooked, despite contributing to as many as 45% of deaths in the industrialized world (**Wynn, 2007**).

It has been noticed recently that there is an increase in the incidence of miliary tuberculosis (TB) affecting lung, bone marrow, liver, lymph nodes and others, owing to the Human Immunodeficiency Virus (HIV) epidemic, and the increasing list of causes of immunosuppression such as introduction of biological and immunosuppressive drugs for treatment of various medical disorders (**Baker and Glassroth, 2004**). BCG vaccine

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has a high efficacy against tuberculosis, however in immunocompromised individuals e.g. HIV sufferers are at risk of development of BCGitis syndrome (disseminated tuberculosis) which commonly affect lymph nodes, liver, lung, skin and bone **(Talbot et al., 1997)**.

Recent advances indicate that organ fibrosis share core features that include epithelial and endothelial injury and dysfunction; abnormal proliferation of myofibroblasts, smooth muscle cells and stellate cells, and ECM deposition **(Bonner, 2004; Speca et al., 2012)**. In addition, a variety of cytokines, chemokines, growth factors, and angiogenic factors regulate the activation of ECM-producing cells in profibrotic processes. As the severe tissue scarring that accompanies end-stage fibrosis is irreversible in most situations, greater efforts are still needed to identify the common and unique mechanisms of fibrosis, all of which need to be aimed at finding effective antifibrotic targets and drugs **(Speca et al., 2012; Friedman et al., 2013)**.

It appears to be widely accepted that investigating the targets that are aberrantly expressed in animal models and fibrotic patients promises to unearth new therapeutic strategies for fibrotic diseases. Up to the present time numerous research efforts in the field of organ fibrosis have identified several polypeptide mediators important to the fibrotic process, such as transforming

growth factor (TGF- β) (Sureshbabu et al., 2011; Yu et al., 2013; Guo et al., 2014).

Hepatic injury, both acute and chronic, is a common pathology worldwide. Chronic liver injury can progress to liver fibrosis and end-stage cirrhosis in many patients. The main etiology of liver injury is represented by viral infections (hepatitis B virus, hepatitis C virus and hepatitis D virus), drugs and alcohol abuse (Sun et al., 2008).

Activated HSCs (hepatic stellate cells) are responsible for high levels of expression of α -smooth muscle actin (α -SMA), as well as for the additional synthesis of excess ECM (predominantly Type I and Type III collagen) (Friedman, 2000; Iredale, 2001). During liver fibrogenesis, it is believed that TGF- β is widely considered to be a profibrogenic agent in liver injury and its release by necrotic hepatocytes may be one of the first signals for the activation of adjacent quiescent HSC (Liu et al., 2006).

As TGF- β plays an important role in liver injury it also plays a role in the pathogenesis of lung fibrosis, which is a major cause of suffering and death seen in pulmonary medicine, based upon its strong ECM inducing effect. It is thought that prolonged overproduction of TGF- β induced by repeated chemical or biological injury leads to the accumulation of pathological