The possible protective role of spirulina versus silymarin on induced liver fibrosis in the adult albino rat

Thesis

Submitted for partial fulfillment of M.D. Degree of Medical Sciences (Anatomy)

Presented by

Mohamed Ramadan Abdel-Hamed
M.Sc in Anatomy
Faculty of Medicine – Ain Shams University

Under Supervision of

Professor Dr. Ibtisam Ahmed Bahei-Eldin
Professor of Anatomy
Faculty of Medicine – Ain Shams University

Professor Dr. Hassan Mostafa Serry
Professor of Anatomy
Faculty of Medicine – Ain Shams University

Professor Dr. Ghada Ahmed Wassif
Professor of Anatomy
Faculty of Medicine – Ain Shams University

Ass. Prof. Dr. Haidy Farid Abdel-Hamed
Assistant Professor of Anatomy
Faculty of Medicine – Ain Shams University

Faculty of Medicine
Ain Shams University
2013

Acknowledgment

First of all, I would like to thank God, for allowing me to perform this study.

Special thanks to **Prof. Dr. Khalid Naeim Hamdy**, Head master of Anatomy Department, Faculty of Medicine, Ain Shams University, for his guidance, support and encouragement.

My profound thanks and appreciation to **Prof. Dr. Ibtisam Ahmed Bahei El-din**, Professor of Anatomy, Faculty of Medicine, Ain Shams University, for her endless patience and guidance. This work could not have reached its goal without her support. Starting from the main idea till reaching the final goal, she has stood as the motivating power of each aspect of this study.

I would like to express my gratefulness and sincere appreciation to **Prof. Dr. Hassan Mostafa Serry**, Professor of Anatomy, Faculty of Medicine, Ain Shams University, for his supervision, valuable remarks and suggestions that helped me in the final production of this work.

I wish to express my thanks to **Prof. Dr. Ghada Ahmed Wassif**, Professor of Anatomy, Faculty of Medicine, Ain Shams University, for her help, support and encouragement.

I would like to express my thanks to **Assistant Prof. Dr. Haidy Farid Abdel-Hamed**, Assistant Professor of Anatomy,

Faculty of Medicine, Ain Shams University, for her help and support.

I wish to express my thanks to **Prof. Dr. Hany Shawky Nadim**, Professor of Anatomy, Faculty of Medicine, Ain Shams University, for his great, continuous guidance and for offering me much of his time and effort.

Finally, I would like to express my deepest thanks and gratitude to all of my professors, colleagues in Anatomy Department, Faculty of Medicine and family members who stood beside me throughout this work giving me their support, sympathy and guidance.

Contents

Introduction and Aim of Work

Introduction

Liver fibrosis and the end-stage of liver fibrosis (cirrhosis) represent the final common pathway of virtually all chronic liver diseases **(Wei et al., 2000)**. It results in liver failure, portal hypertension and is associated with an increased risk of liver cancer. The wide geographic distribution and high prevalence of insults with the potential to cause liver fibrosis, including chronic viral hepatitis, inborn errors of metabolism, and toxic damage through alcohol consumption, mean that fibrosis and cirrhosis of the liver remain major causes of morbidity and mortality worldwide **(Iredale, 2007)** .

Therefore, using anti-fibrotic drugs especially those developing from the natural products used in traditional medicine with little acute toxicity is of great importance **(Yin et al., 2007).**

One of these natural products is **spirulina** which is a blue green algae growing naturally in countries having a warm climate, it has been considered as food supplement for human and animal **(Rasool et al., 2006).** It was found to be a rich source of vitamins, minerals, essential fatty acids and antioxidant

pigments such as carotenoids **(Seshadri et al., 1991).**

Moreover, several studies have shown that spirulina species exhibit various biological activities such as anti-tumor **(Mittal et al., 1999),** antimicrobial **(Hayashi et al., 1996).** Also , C-phycocyanin, which is responsible for the bluish color of spirulina was found to be able to produce a significant hepato-protective effects **(Vadiraja et al., 1998)** , and, as reported elsewhere, spirulina prevents the formation of fatty liver in animal models **(Rodriguez et al., 2001)** and in humans **(Nayaka et al., 1988)** .

Huang and Zheng (2007) reported the anti-fibrotic effects of spirulina on rat liver. Such effects may be explained by the anti-oxidative effect of spirulina, which is involved in the reduction of oxidative stress **(Romay et al., 1998)** and a decrease in pro-inflammatory cytokine gene expression **(Remirez et al., 2002).**

On the other hand, another drug, **silymarin**, was introduced as a hepato-protective agent, two decades ago **(Tasdug et al., 2005).** Experimental animal research has shown its protective action on the liver against some hepato-toxic

substances such as carbon tetrachloride **(Churungoo et al., 1997)**, and Dimethylnitrosamine **(George and Chandrakasan, 1996)**.

The effect of silibinin (also known as silybin), which is the major active constituent of silymarin, was studied on rat liver. It was proved to have an anti-fibrotic effects as it reduces the proliferation of stellate cells by about 75% and reduces its transformation into fibroblasts **(Fuchs et al., 1997)**.

Aim of the work

The aim of the present work was to identify the histological and biochemical changes that accompany liver injury in a rat model of hepatic fibrosis induced by the injection of a hepato-toxic drug (carbon tetrachloride). Moreover, to verify the possible hepato-protective effect of spirulina ,versus that of silymarin.

Review of Literature

Microscopic structure of the liver

Junqueira and Carneiro (2005) reported that the main components of the liver are the stroma and the parenchyma which consists of a complex network of epithelial cells (hepatocytes), supported by connective tissue, and perfused by a rich blood supply.

Standring (2005) stated that liver is formed of hexagonal hepatic lobules. At the three of the corners of each lobule there are small triangular areas of connective tissue containing a small artery, a parallel vein, and a small bile duct. These are commonly referred to as portal triads. The author reported that the constituents of these triads are branches of the hepatic artery and portal vein, together with bile ductules and ducts, which run within the hepatic connective tissue trabeculae.

Types of liver cells:

Standring (2005) mentioned that the types of liver cells include the hepatocytes, the perisinusoidal lipocytes, Kupffer cells and the sinusoidal endothelial cells.

Stevens and lowe (2005) stated that the hepatocytes (also known as parenchymal cells) form up to 80 % of the cell

population of the liver. The hepatocytes are polygonal in shape and they are arranged as interconnecting sheets of cells forming cellular plates radiating from the central vein toward the periphery of the hepatic lobule. These plates are separated by blood sinusoids. The cytoplasm of hepatocytes is packed with much rough and smooth endoplasmic reticulum, many mitochondria, lysosomes and well-developed Golgi apparatus, features indicating a high metabolic activity. Hepatocyes have one or occasionally two, spherical large and central nuclei with prominent nucleoli.

Standring (2005) reported that perisinusoidal lipocytes (Ito cells or hepatic stellate cells) are much less numerous than hepatocytes. They are irregular in outline, present between the bases of hepatocytes in space of Disse. They are mesenchymal in origin, and they secrete most of the intralobular matrix components, reticular fibers. Moreover, in response to liver damage; they become myofibroblast-like. They are responsible for the replacement of toxically damaged hepatocytes with collagenous scar tissue producing fibrosis.

Gartner and Hiatt (2006) mentioned that Kupffer cells are hepatic macrophages derived from the circulating blood monocytes. They are lying within the sinusoidal lumen attached

to the endothelial surface. The Kupffer cells are common at spaces where the sinusoids branch. They are irregular in shape, with long processes extending into the sinusoidal lumen.

Gartner and Hiatt (2006) reported that the hepatic sinusoids permeate the whole of the liver. The sinusoids are lined by a thin discontinuous highly fenestrated endothelium which lacks a basal lamina and separated from hepatocytes by a space of Disse. The endothelial cells have a central nucleus and their cytoplasm contains numerous typical transcytotic vesicles.

Liver zones:

Junqueira, Carneiro and Kelley (1998) stated that on the bases of the hepatic blood supply, liver parenchyma is divided into 3 zones, Zone (I) which lies close to the vascular backbone of the hepatic structure. Zone (III) lies nearer to the central veins and its hepatocytes are more vulnerable to anoxia and are in a less favorable position to obtain nutrients. Zone (II) lies inbetween zone (I) and zone (III).

REVIEW OF LITERATURE

Introduction:

Lee et al. (2005) reported that the liver is the largest organ in vertebrate body and the major organ responsible for the metabolism of drugs, toxic chemicals and byproducts endogenous to the body, it is also the primary target organ for detoxication of many endogenous and exogenous toxic chemicals.

Balamurugan and Muthusamy (2008) stated that the prevalence of major liver diseases, such as non-alcoholic and alcoholic fatty liver, chronic hepatitis, fibrosis, cirrhosis or hepatic carcinoma, has been noted and leads to fatal diseases in both human beings and animals.

Liver fibrosis:

Victor and Eric (2004) mentioned that hepatic fibrosis has been noted as a response to necrosis and inflammation caused by various factors such as infection, intoxication, endogenous and exogenous detrimental factors.

Yin et al. (2007) reported that fibrosis results from response of the liver to many diverse chronic insults such as chronic viral infection, alcohol, immunological attack, hereditary metal overload, parasitic diseases and toxic damage. Because of the worldwide prevalence of these insults, liver fibrosis is common and ultimately results in cirrhosis that is associated with significant morbidity and mortality. Hepatic fibrosis leads to many complications such as portal hypertension, esophageal varices and hepatic failure.

Fujii et al. (2010) stated that eighty percent of Hepatocellular carcinomas (HCCs) develop in the context of chronic liver diseases, as chronic liver injury generally induces liver fibrosis and cirrhosis followed by hepatocellular carcinoma.

Forner et al. (2012) mentioned that the hepatocellular carcinoma is the fifth most common cancer worldwide and is the third leading cause of cancer mortality.

Li et al. (2012) stated that currently, there are hundreds of million patients with liver fibrosis throughout the world, and most cases are caused by hepatitis viral infections. Furthermore,

the authors pointed out that anyone who can stop or delay liver fibrosis would be able to cure most chronic liver diseases.

Liver fibrosis in males and females:

Xu et al. (2002) reported that the development of fibrosis and cirrhosis is more common in men than in women. Although the liver is not a classic sex hormone target, livers in both men and women have been shown to contain estrogen receptors and respond to estrogens by regulating liver function. Therefore, sex hormones may play a role in the progression of hepatic fibrosis and cirrhosis.

Shimizu et al. (2012) stated that the progression of hepatic fibrosis in chronic hepatitis B and C appears to be slower in females than in males. Moreover, females, especially before menopause, produce antibodies against hepatitis B virus (HBV) surface antigen (HBsAg) and HBVe antigen (HBeAg) at higher frequency than males . In chronic infection with hepatitis C virus (HCV), the clearance rate of blood HCV RNA appears to be higher in females .Most asymptomatic carriers of HCV with persistent normal liver enzymes are females and have a good prognosis with a low risk of progression of hepatic