Hepatic Fibrosis induced by High Cholesterol diet in Rats and protective effects of Ellagic Acid: Histological and Immunohistochemical Study

Thesis
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By

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ABSTARCT

The liver is an organ of great importance which plays an important role in many metabolic processes. Hypercholesterolemia is one of the most harmful factors in producing liver fibrosis.

This work was aimed to identify the hepato-protective effect of ellagic acid in liver fibrosis induced by hypercholesterolemia in Albino rats.

Seventy two adult male Albino rats were selected for this work and they were divided into control group, hypercholesterolemic diet group, and a group with hypercholesterolemic diet supplemented with or without ellagic acid. Animals of each group were sacrificed at 6, 9 and 12 months respectively. The removed liver from the animals were processed for staining with H&E, Masson's trichrome to demonstrate the changes in fine collagenous fibers, Toluidin blue to stain mast cells and immunohistochemical staining for α-SMA to demonstrate hepatic stellate cells and CD-68 to demonstrate von Kupffer cells.

The examined liver of hypercholesterolemic rats showed foamy hepatocytes that appeared first at the periphery of the hepatic lobules and extended at 9 and 12 months to the rest of the lobules. By time more and more cells started to show ballooning. These changes were accompanied by vascular congestion with an increase of the collagen and microfibril content as the duration increased.

The statistical analysis of the obtained results concerning the distribution of area percent of α-SMA immunoreactive hepatic stellate cells revealed marked increase of these cells among the hepatocytes parallel to the increase in collagen deposition as shown in Masson's trichrome stained sections.

There was also a distinct increase of area and intensity of CD-68 immunoreactive Kupffer cells in the hepatic sinusoidal walls.

Mast cells and the inflammatory rounded cells were demonstrated in huge amounts in the portal tract areas as well as around the central hepatic veins.

The obtained results were discussed regarding the fact that ellagic acid has drawn the attention of researchers to its curative powers based on the fact that it is a potent antioxidant and moreover a regulator of hepatic stellate cell activity. It seems to have the potential to emerge, in the near future, as a remedy for hepatic damage.

Key words: Liver fibrosis, ellagic acid, hypercholesterolemia

بسم الله الرحمن الرحيم

"سبحانك لا علم لنا إلا ما علمتنا

إنك أنت العليم الحكيم "

صدق الله العظيم

سورة البقرة آية (٣٢)

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Dedication

I would like to express very special thanks and my appreciation to **all my family members** for their great support and patience during the preparation of this work.

To the soul of my

late father

Professor Doctor

Lotfi Ahmed Abdel-Latif

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List of abbreviations

ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
CD-68	cluster of differentiation
CT	connective tissue
CYP2E1	cytochrome P450 2E1
DAB	diaminobenzidine
DDR	discoidin domain receptors
DNA	de-oxy-ribonucleic acid
ECM	extracellular matrix
H_2O_2	hydrogen-peroxide
HIV	human immune-deficiency virus
HSC	hepatic stellate cell
kD	kilo-Dalton
IU/L	international units per liter
LAMP	lysosomal / endosomal-associated membrane glycoprotein
LSAB	labeled streptavidin-biotin
MAB	monoclonal antibodies
mmol/l	milli-mol per liter
MONICA	Multinational Monitoring of Trends and Determinants in Cardiovascular Disease
mRNA	messenger Ribonucleic acid
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
PDGF	platelet-derived growth factor
PBS	phosphate-buffered saline
TGF	transforming growth factor
α-SMA	alpha-smooth muscle actin

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Introduction and aim of work

To date, the majority of research on hypercholesterolemia has focused on the effect of a high cholesterol diet on atherosclerosis and coronary heart diseases **(Jeong et al., 2005; Netea et al., 2009)**.

The relationship between the intake of a high cholesterol diet and hepatic fibrosis has been investigated and some authors demonstrated that hepatic fibrosis developed in a variety of mammals that were fed on a cholesterol-supplemented diet **(Wanless et al., 1996; Jeong et al., 2005).**

Liver fibrosis is reversible whereas liver cirrhosis is generally considered irreversible **(Friedman, 2000)**. Many studies focused on the role of hepatic stellate cells in producing liver fibrosis **(Hautekeete and Geerts, 1997; Knittel et al., 1998; Gaca et al., 1999)**. However other studies demonstrated that macrophages and mast cells played a role in the development of hepatic fibrosis **(Armbrust et al., 1997; Akiyoshi and Terada, 1998; Jeong et al., 2002, 2005)**.

Meanwhile Ellagic acid, a phenolic compound derived from red raspberries, pomegranate and many other fruits has shown promising results in reducing many liver diseases including hepatic fibrosis **(Wundel et al., 2002; Buniatian, 2003)**.

Treatment options for common liver diseases are complicated. Therapy with modern medicine is often limited in efficacy. The effectiveness of treatments such as using corticosteroids and interferon is inconsistent, carries the risk of adverse events and is often too costly. So, there is a need for effective therapeutic agents with a low incidence of side effects. Ellagic acid has been used as a naturiceutical remedy since ancient times and has proven to be of little toxicity. Furthermore it is one of the few therapeutic agents that is claimed to help regeneration of hepatocytes **(Luper, 1998; Vattem and Shetty, 2005)**.

The aim of this work was to study the histological changes in rats' liver after a high cholesterol diet and in hepatic fibrosis and to evaluate the role of administration of Ellagic acid as a new protective naturiceutical compound against liver fibrosis.

REVIEW OF LITERATURE

1. Histology of the Liver

The liver is structurally and functionally heterogeneous. It has been considered as the second most complex organ in the body that is exceeded only by the brain in its complexity. The liver has thousands of vital functions including the uptake of amino acids, carbohydrates, bile acids, cholesterol, proteins, lipids and vitamins for storage and metabolism subsequent to their release into bile or blood **(LaBrecque, 1994; Haschek et al., 2010)**. The liver also has two distinct blood supplies and contains at least a dozen different cell types. It regulates blood volume, and is the major site for biotransformation, particularly rendering hydrophobic molecules water soluble and is responsible for the defense against foreign macromolecules and xenobiotics. Many aspects of liver function and structure including the 3-dimensional anatomy of the liver are still not completely clarified; therefore they are still under investigation by biologists and pathologists **(Burt & Day, 2002; Ross & Pawlina, 2011)**.

• Structural and functional organization of the liver

The liver is a heterogeneous tissue whose functional unit is the lobule **(MacSween et al., 2002; Mescher, 2013)**. No matter the angle that a liver is sectioned, it basically has the same histological appearance. It displays multiple units with a central vein centrally surrounded by about four to six portal areas. This phenomenon is the basis for the liver being referred to as having an "isotropic parenchyma" and it contributes to the complex 3-dimensional architecture **(Matsumoto & Kawakami, 1982; Mescher, 2013)**.

Attempts to understand the 3-dimensionality have helped scientists to understand liver function better. Human liver consists of several lobes and a gall bladder. The lobes have traditionally been designated as right, left, quadrate, and caudate. Recently it has been proposed that the liver can be subdivided into nine segments based on the vascular and ductal branching patterns of the right and left sides **(Kogure et al., 1999; MacSween et al., 2002)**. This compartmental pattern is useful to understand lobar or intralobar degeneration related to disruption of the blood supply and to facilitate surgical resection.

The hepatic lobes of the rat appear to have similar fundamental portal and hepatic venous systems, and thus they possess segments comparable to that of human liver **(Kogure et al., 1999; Gartner & Hiatt, 2011)**.

• Vasculature of the liver

At any given moment the liver is supplied by an amount of blood equivalent to approximately 25% of the cardiac output **(Burt and Day, 2002; Gartner & Hiatt, 2011)**. The portal vein and the hepatic artery are the two main vascular systems that convey blood to the liver. The portal vein supplies about 70% of the blood flow and 40% of the oxygen while the hepatic artery supplies 30% of the flow and 60% of the oxygen **(Burt & Day, 2002; Gartner & Hiatt, 2011)**. The portal blood drains from the mesenteric, gastric, splenic, and pancreatic veins and travels to the liver where it branches entering the right and left sides of the liver. There can be "portal streamlining" which means incomplete mixing of blood coming from the gastrointestinal tract and spleen leading to variation in delivery of various nutrients, toxins, and other elements to the liver lobes **(Haywood, 1981; Faa et al., 1987, 1994, 1995; Thein et al., 2003; Daniel et al., 2004; Gartner & Hiatt, 2011)**. As an example, the blood draining the stomach and spleen tends to flow to the left side of the liver. Localized or generalized core redistribution of blood flow or blood pooling is controlled by nerve stimulation **(Stuart & Wheatley, 1995; Oakley et al., 2003; Mescher, 2013)** or hepatic stellate cells **(Ratziu & Friedman, 1997; MacSween et al., 2002; Mabuchi et al., 2004; Mescher, 2013)** which can potentially lead to lobe variation in liver disease.

Lobe variation has been reported for acetaminophen hepatotoxicity **(Heinloth et al., 2004; Irwin et al., 2004a, 2004b)**, copper distribution **(Haywood, 1981; Faa et al., 1987, 1995)**, iron and phosphorous **(Ambu et al., 1995)**, chemical carcinogenesis **(Solt et al., 1977; Richardson et al., 1986)**, cirrhosis **(Matsuzaki et al., 1997; Regev et al., 2002)**, and regeneration **(LaBrecque, 1994; Mescher, 2013)**. The conducting portal vessels delivering blood to the parenchymal vessels are called preterminal and terminal

portal venules, respectively. Blood from the terminal portal venules enters the sinusoids **(Matsumoto & Kawakami, 1982)**. The hepatic artery generally accompanies the portal veins in the portal triads and its smaller branches feed the sinusoids at varying levels as well as the biliary tracts. Biliary tracts most often subsequently drain into sinusoids, which is a so-called portal-portal flow **(MacSween et al., 2002; Gartner & Hiatt, 2011)**. The sinusoidal blood flow is carefully regulated **(McCuskey, 2000; Gartner & Hiatt, 2011)** and collects into terminal hepatic venules, which are also called central veins, prior to emptying into larger hepatic veins and eventually into the vena cava.

Lymph fluid accumulates in the spaces of Disse and periportal tissue before draining into lymphatic vessels in the portal canals and on to hilar lymphatic channels and eventually into the thoracic duct.

The portal triad consists of bile ductules, branches of the portal vein and branches of the hepatic artery, however the portal area contains an average of one to two arteries, one portal vein, one to two bile ducts, lymphatics, nerves in a connective tissue matrix comprised mainly by type 1 collagen **(MacSween et al., 2002; Gartner & Hiatt, 2011)**.

- ## Lobes and lobular patterns in the liver

The lobular pattern of the liver was first noted by in 1665 and over the last century the functional unit of the liver has been strongly debated **(MacSween et al., 2002; Gartner & Hiatt, 2011)**. The classic hexagonal lobule is a hexagonal region of parenchyma which surrounds the central vein at its center. The hepatic lobules are composed of one-cell thick parenchymal cell plates, arranged radically around the central vein, thus forming sinusoidal blood spaces. According to the lobular concept, the blood flows from the periphery of the lobule, i.e., from the portal vein and hepatic artery, through sinusoids, and into the central veins **(Gartner & Hiatt, 2011)**. About 70 years later in 1906 the portal lobule was proposed. The portal lobule is based on the portal vessels supplying the lobule centrally and being drained at the periphery. This concept was based on studies in the dog and rabbit **(Gartner & Hiatt, 2011)**. In 1954 the liver