Effect of Salvia officinalis on Induced Hepatotoxicity in Albino Rats

Thesis

*Submitted For Partial Fulfillment Of master Degree in **Anatomy***

By

Shimaa Magdy Mohammed Mahmoud
M.B.,B.Ch

Under the Supervision of

Prof. Dr. Azza Salah El Din Soliman
Professor of Anatomy and Emberyology
Faculty of Medicine – Ain Shams University

Dr. Rania Ahmed Salah El Din
Assistant Professor of Anatomy and Emberyology
Faculty of Medicine – Ain Shams University

Dr. Ahmed Yehia Mostafa Awad
Assistant Professor of Anatomy and Emberyology
Faculty of Medicine – Ain Shams University

Faculty of Medicine
Ain Shams University
2013

تأثير المريمية المخزنية على تسمم الكبد في الفئران البيضاء

رسالة

توطئ للحصول على درجة الماجستير
فى العلوم الطبية الأساسية (علم التشريح والأجنة)

مقدمة من

الطبيبة/ شيمـاء مجـدي محمـد محمـود
معيدة بقسم التشريح والأجنة
كلية الطب– جامعة عين شمس

تحت إشراف

الأستاذ الدكتور/ عزة صلاح الدين سليمـان
أستاذ في علم التشريح والأجنة
كلية الطب – جامعة عين شمس

الدكتورة/ رانيا أحمـد صلاح الدين
استاذ مساعد في علم التشريح والأجنة
كلية الطب – جامعة عين شمس

الدكتور/ أحمـد يحيي مصطفى عـوض
استاذ مساعد في علم التشريح والأجنة
كلية الطب – جامعة عين شمس

كلية الطب
جامعة عين شمس

2013

Acknowledgment

First of all, I would like to thank **Allah** *for "The most merciful" for allowing me to perform this study.*

Special thanks to **Prof. Dr. Hany Shawky Nadim,** *Head master of Anatomy Department, Faculty of Medicine, Ain Shams University, for his guidance, support and encouragement. I will be forever thank full.*

My profound thanks and appreciation to **Prof. Dr. Azza Salah El Din Soliman,** *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. To her I owe much more than I can express. One day, I whish I would be able to return the favor for her.*

I would like to express my gratefulness and sincere appreciation to **Dr. Rania A. Salah El Din** *Assistant Professor of Anatomy, Faculty of Medicine, Ain Shams University, for her supervision, valuable remarks and suggestions that helped me in the final production of this work.*

I wish to express my thanks to **Prof. Dr. Ahmed Yehia Mostafa** *Assistant Professor of Anatomy, Faculty of Medicine, Ain Shams University, for his help, support and encouragement.*

I wish to express my thanks to **Prof. Dr. Mohamed Kamal,** *Professor of Anatomy, Faculty of Medicine, Ain Shamus University, for his great, continuous guidance.*

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.

✍ **Shimaa Magdy**

INTRODUCTION

Liver damage is a widespread disease which can be caused by reactive oxygen species (ROS) and is characterized by progression from steatosis to chronic hepatitis, cirrhosis and hepatocellular carcinoma ***(Srivastava & Shivanandappa, 2010).***

Formation of reactive oxygen species (ROS) is an unavoidable consequence in aerobic organisms during respiration. It has been shown that overproduction of unstable ROS leads to unwanted reactions with other groups or substances in the body, resulting in cell or tissue injury. In addition, numerous studies have revealed that uncontrolled lipid peroxidation is involved in the occurrence of many diseases, including Parkinson's, arthritis, myocardial infarction, Alzheimer's, cancer, cardiovascular disease and liver damage ***(Qian et al., 2008)***.

Therefore, during the last few decades, human nutrition and biochemistry research focused on antioxidants derived from foods that could prevent or diminish ROS-induced damage. Several compounds, such as carbon tetrachloride (CC14), acetaminophen, bromobenzene, ethanol and polycyclic aromatic hydrocarbons have been implicated in the etiology of liver diseases ***(Adesanoye & Farombi, 2010).***

CCl4 is a classical hepatotoxin that causes rapid liver damage progressing from steatosis to centrilobular necrosis ***(Lin et al., 2008).*** The mechanism of liver injury induced by CCl4 is thought to involve free radicals and lipid peroxidation ***(Brent & Rumack, 1993).***

Since free radicals are very unstable, they are immediately neutralized by antioxidants in the cell once they are generated in normal metabolism pathway, so increasing the antioxidant content in cells may play an important role against CCl4-induced liver injury. Due to the risks of synthetic antioxidants, there is a growing interest in the use of natural antioxidants to prevent oxidative stress-related liver pathologies ***(Dhanasekaran et al., 2009).*** A major defence mechanism involves antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione which neutralize ROS in cells ***(Tsai et al., 2009).***

Salvia officinalis (SO), a plant endemic to the Mediterranean region is one of the most popular herbal remedy in the Middle East to treat common health complications such as cold and abdominal pain ***(Gali-Muhtasib et al., 2000).***

Salvia species (Labiatae) are generally known for their multiple pharmacological effects including their antibacterial ***(Miski et al., 1983),*** hypoglycemic ***(Perfumi et al., 1991),***

antianoxic ***(Wu et al., 1997),*** antitumor ***(Liu et al., 2000)***, antioxidative ***(Wang et al., 2003)*** and anticholestatic ***(Oh et al., 2002)*** effects.

Phytochemically, the whole plant contains several antioxidants that prevent peroxidative damage to hepatocytes such as water-soluble compounds; salvianolic acid A, salvianolic acid B and rosmarinic acid ***(Huang & Zhang, 1992),*** and several phenolic glycosides ***(Wang et al., 1999).***

AIM OF THE WORK

The aim of this work is to study the effects of Salvia officinalis against carbon tetrachloride-induced hepatotoxicity in albino rats and to compare between its prophylactic and therapeutical effects.

I-LIVER

The liver is a large organ making up about 2 per cent of human body weight ***(Hinton & Grasso, 1995)***. It occupies the cranial third of the abdominal cavity and is comprised of multiple lobes; however, the nomenclature for the liver lobes varies among authors. There are basically left, middle, right, and caudate lobes ***(Harada et al., 1999).***

The liver is strategically positioned in the circulation to perform its task of maintaining the body's metabolic homeostasis. It is the first organ that by the portal vein comes in contact with the venous blood after its exposure to the stomach and intestines. Therefore, the liver is the first organ exposed to absorbed nutrients, metals, drugs, environmental toxicants and metabolic by-products of bacteria present in the gastrointestinal tract, which need to be processed before entering the systematic circulation ***(Moslen, 1996 and Haussinger, 1996).***

The liver has a dual blood supply, the hepatic portal vein and the hepatic artery. The hepatic artery supplies oxygenated blood. Approximately 75% of the blood is delivered to the liver via the hepatic portal vein that drains the spleen, stomach, intestines, and pancreas. Branches of the hepatic artery and portal vein are seen in the portal triads along with bile ducts and are separated from the hepatic cords by a ''limiting plate'' of hepatocytes ***(Thoolen et al., 2010).***

Liver functions are complex and diverse including endocrine and exocrine activity, metabolism, conjugation, detoxification, and hematopoiesis in early embryonic and fetal development ***(Harada et al., 1999).***

Liver cell types:

The hepatocytes, the parenchymal cells of the liver, comprise about 90% of the cellular volume of the liver. They are assembled into sheets, in two-dimensional sections they are typically one cell layer thick and form anastomoses. Through this anastomoses run the liver capillaries, termed sinusoids. Between cells within the sheet run small branching channels called bile canaliculus ***(Hinton & Grasso, 1995 and Diaz, 2000).***

The hepatocytes are large, polygonal cells, having large spherical nuclei which occupy the center of the cell. Two or more well-developed nucleoli are present in each nucleus. The hepatocyte cytoplasm is generally acidophilic. Specific cytoplasmic components may be identified by routine and special staining procedures, including basophilic regions that represent rough endoplasmic reticulum (rER) and free ribosomes.Numerous mitochondria also can be demonstrated by vital staining or enzyme histochemistry ***(Michael and Wojciech, 2010).***

Four main types of non-parenchymal cells are also present, *i.e.* endothelial cells and Kupffer cells (resident macrophages) lining the sinusoids, fat-storing cells (stellate cells) and pit cells (liver associated lymphocytes) ***(Puviani et al., 1998).***

Tissue structural organization

The two most commonly used descriptions for the structural and functional units of the liver are the hepatic lobule ***(Kiernan, 1883)*** and the acinus ***(Plaa, 1991 and Moslen, 1996).*** The structural unit, the hepatic lobule, is modeled on the blood flow within the liver and is commonly used for descriptive pathology and morphological diagnoses. The functional unit, the hepatic acinus, is modeled on blood flow and metabolism within the liver ***(Thoolen et al., 2010).***

The hexagonal lobule is divided into three regions: the centrilobular (near the hepatic vein), midzonal and periportal regions. The base of the acinus is formed by the terminal branches of the portal vein and hepatic artery that extend out of the portal tracts **(*Moslen, 1996).*** Blood flows from the portal areas to the central vein in the center of each lobule while bile flows from the center of the hepatic lobule to the portal areas and on to the hepatic duct ***(Thoolen et al., 2010).***

The acinus has three zones that, fortunately, coincide with the three regions of the lobule: zone 1 is closest to the entry of

blood and receives the highest concentration of oxygen and nutrients; zone 3 is adjacent to the terminal hepatic vein; and, zone 2 is intermediate. The organisation of these operational units leads to a gradient of oxygen and nutrients. For example, cells in zone 1 tend to have more mitochondria and higher respiration rates than cells in zone 3**.**The zonation of metabolic functions has important implications for chemically induced toxicity due in part to the differential expression of enzymes and the concentration gradients of nutrients, oxygen, cofactors and toxicant across the acinus ***(Kedderis, 1996).***

Functionally, zone 1 hepatocytes are specialized for oxidative liver functions such as gluconeogenesis, b-oxidation of fatty acids, and cholesterol synthesis, while zone 3 cells are more important for glycolysis, lipogenesis, and cytochrome P-450–based drug detoxification ***(Thoolen et al., 2010).***

More recently a parenchymal unit in the liver has been described as a cone-shaped three-dimensional structure comprised of approximately fourteen hepatic lobules supplied and drained by common vascular tributaries ***(Malarkey et al., 2005 and Teutsch 2005).***

Liver as a biotransforming organ

The liver is the highest metabolic organ. As such, many molecules are catabolised in the liver by diverse enzymes. Many lipophilic endo- (formed within the body) and xenobiotics (foreign molecules to the body, either of natural origin or man-made), potentially toxic, may be easily absorbed and systematically distributed, which makes them difficult to eliminate from the body in their original form. The process in which endo- and xenobiotics are changed from hydrophobic to hydrophilic molecules to facilitate elimination from the body is called biotransformation ***(Sipes & Gandolfi, 1991 and Parkinson, 1996).***

Xenobiotic metabolism by hepatocytes can occur by phase I (often the cytochrome oxidase series, involve chemical modification of the molecule by hydrolysis, reduction and oxidation) and phase II reactions (often the formation of the water soluble glucuronide, involve biosynthetic reactions where the xenobiotic or a phase I-derived metabolite is covalently linked to an endogenous molecule, producing a conjugate) ***(Graham and Lake, 2008).***

The most important group of enzymes involved in phase I reactions are the cytochromes P450-dependent mixed-function oxygenase (MFO) system in the membranes of endoplasmatic

reticulum ***(Sipes & Gandolfi, 1991 and Kedderis, 1996***). Among the different phase II enzymes, which are mostly present in cytosol, glutathione *S*-transferase represents an important biotransforming enzyme to the liver ***(Parkinson, 1996).*** Most phase II reactions result in a large increase in xenobiotic hydrophobicity, and greatly promote the excretion of foreign chemicals in bile and urine ***(Hinton & Grasso, 1995 and Parkinson, 1996).***

In general, drug metabolism can be considered as protective or a detoxification process in that it converts lipophilic compounds (which accumulation can overwhelm and injury cells) and active xenobiotics into water soluble and easily excreted inactive metabolites. However, hepatic metabolic processes may also cause indirect toxicity by generating electrophilic species capable of reacting with proteins, nucleic acids, and other cytoplasmic organelles ***(Xu et al., 2005).***

Phase I enzymes, and to lesser extent some phase II enzymes, can increase the toxicity of a xenobiotic and produce highly reactive metabolites from an inert molecule which becomes, therefore, toxic. The formation of products with enhanced activity and toxicity is often termed bioactivation and frequently leads to mutagenicity and carcinogenicity ***(Hinton & Grasso, 1995 and Kedderis, 1996).*** Additionally, because electron transfer to molecular oxygen occurs during the phase I

reaction sequence, reactive oxygen species (ROS) can occasionally dissociate from the cytochrome prior to oxidation of the substrate, leading to oxidative stress and cellular damage ***(Haussinger, 1996).***