Possible Protective Effect of Onion Supplementation on Hepatic Functional and Structural Alterations Induced by Cholestasis

A thesis

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By

Marian Maged Francis
M.B., B.Ch. Physiology

Supervisors

Prof. Dr. Fatma Ahmed Mohamed
Professor of Physiology
Faculty of Medicine, Ain Shams University

Dr. Abd El-Hamid A Mohamed
Associate Professor of physiology
Faculty of Medicine, Ain shams University

Dr. Dalia Abd Elsalam Saad
Lecturer of Physiology
Faculty of Medicine, Ain shams University

Faculty of Medicine
Ain Shams University
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List of Abbreviations

ALP.....*Alkaline phosphatase*
ALT.....*Alanine aminotransferase*
ANOVA.....*Analysis of variance*
AST .....*Aspartate aminotransferase*
ATP.....*Adenosine triphosphate*
AXUP .....*Animal Care And Use Program*
BDL.....*Bile duct ligation*
BMI.....*Body mass index*
BW .....*Body weight*
CDCA.....*Chenodeoxycholic acid*
CK .....*Cytokeratins*
DB.....*Direct bilirubin*
DEA .....*Diethanolamine*
DMSO.....*Dimethylsulphoxide*
ECM.....*Extracellular matrix*
ELISA.....*Enzyme linked immunosorbant assay*
GCDC .....*Glycochenodeoxycholate*
GCDCA .....*Glycochenodeoxycholic acid*
GOT .....*Glutamate oxaloacetate transaminase*
GPT .....*Glutamate pyruvate transaminase*
γ-GT.....*γ-glutamyltranspeptidase*
H&E.....*Hematoxylin and Eosin*
HSC .....*Hepatic stellate cells*
HSI .....*Hepato-Somatic index*
ICAM-1 .....*Intercellular adhesion molecule-1*
IL.....*Interleukin*
LDH .....*Lactate dehydrogenase*
MASRI.....*Medical research center, Ain Shams University*
MDA.....*Malondialdehyde*
MDH .....*Malate dehydrogenase*
MYB .....*Myofibroblast*
NAD.....*Nicotinamide adenine dinucleotid*
NFkB .....*Nuclear factor kappa B*

O-BDL……Onion supplemented bile duct ligated group
PBS.....Phosphate buffered saline
PDGF.....Platelet-derived growth factor
ROS .....Reactive oxygen species
SABC .....HRP-Streptavidin Conjugate
α-SMA.....α-smooth muscle actin
TAC.....Total antioxidant capacity
TBA .....Thiobarbituric acid
TGFβ .....Transforming growth factor beta
TIMP-1 .....Tissue inhibitor metalloproteinase-1
TNF-α .....Tumor necrosis factor- α

ABSTRACT

Background: Cholestasis is the obstruction or the reduction in bile flow that results in intrahepatic accumulation of bile constituents, which progresses to develop liver pathology. Common bile duct ligation (BDL) in rodents is an experimental model of cholestasis that has been carried out in research for many years. BDL model of cholestatic liver injury involves other mechanisms, including oxidative stress, inflammation, and fibrogenesis. Antioxidant, antiinflammatory or antiapoptotic properties gained much interest for the amelioration of liver dysfunction.

Aim: the aim of this study is to assess the possible protective effects of onion supplementation on hepatic structural and functional alterations induced by BDL in rats, which reflect the effects of cholestasis resulting from intrahepatic accumulation of bile.

Methods: Thirty adult female Wistar rats were randomly and equally allocated into three groups: (1) control group, (2) BDL group; subjected to ligation of the common bile duct and (3) Onion-supplemented BDL groups (O-BDL). Both control and BDL groups received distilled water (solvent for onion powder) daily by gavage for 4 weeks. Onion-supplemented BDL group (O-BDL); subjected to ligation of the common bile duct and then received 500 mg/kg of onion powder dissolved in distilled water, daily by gavage for 4 weeks. At the end of the experimental period, plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin, total proteins, total antioxidant capacity (TAC), tumor necrosis factor-α (TNF-α), and hepatic tissue level of malondialdehyde (MDA) and transforming growth factor-β (TGF-β) were measured for all groups. In addition, histopathological examination of liver tissue samples was performed for the three groups.

Results: Plasma levels of ALT, AST, ALP, direct bilirubin, TNF-α and hepatic tissue levels of MDA and TGF-β were significantly increased and TAC was significantly decreased in the BDL group compared to the control group. In addition, altered architecture was detected in hepatic tissue samples of BDL group. Onion supplementation significantly decreased the plasma levels of ALT, AST, ALP, direct bilirubin, TNF-α and hepatic tissue levels of MDA and TGF-β in the O-BDL group when compared to the BDL group. Total proteins level was not significantly different among all the studied groups. In addition in O-BDL group, histopathological examination of liver revealed near normal structure of hepatic tissue.

Conclusion: BDL induces hepatic structural alterations and functional disturbances. Onion supplementation inhibits inflammation and oxidative insults that associate BDL, and subsequently protects against BDL-induced liver injury.

Keywords: Onion Supplementation; Hepatic Functional

INTRODUCTION

Cholestasis is the obstruction or the reduction in bile flow that results in intrahepatic accumulation of bile constituents, that progresses to develop liver pathology ***(Crocenzi et al., 2012)*.** Untreated cholestasis was reported to progress to liver fibrosis and cirrhosis, and finally results in liver failure ***(Ghonem et al., 2015)***. Bile duct ligation (BDL) has been used as an animal model of chronic liver injury as it is a very good way to mimic the hepatocyte damage and liver fibrosis observed in human liver diseases ***(Jang et al., 2012)***. Cholestasis was reported to induce hepatic inflammation, which contributes to liver injury during cholestasis, and bile acids were accused of directly activating the production of pro-inflammatory mediators in the liver ***(Allen et al., 2011)***. Also, oxidative stress was considered to represent a primary cause and/or an aggravating factor in hepatic injury in cholestasis induced by bile duct ligation ***(Copple et al., 2010)***.

Onion has potential properties in amelioration of health in many aspects as it has anti-inflammatory, anti-hyperglycemic and anti-hyperlipidemic properties ***(Alam et al., 2014; Liao and Lin, 2015)*** as well as antimicrobial activity ***(Benkeblia, 2004)***, owing to its high content of flavonoids ***(Slimesad et al., 2007)***, especially quercetin which is the most

common flavonoid present in plant kingdom generally and in onions specifically ***(Tang et al., 2013)***.

However, the detailed comprehension of the role of these antioxidant and anti-inflammatory effects of onion powder in cholestasis – induced hepatic injury was poorly evaluated.

Aim of the Work

To evaluate the effect of bile duct ligation on the hepatic structure and function and to evaluate the possible role of onion in amelioration of hepatic structural and functional alteration caused by bile duct ligation, and the subsequent cholestasis.

REVIEW OF LITERATURE

Formation and function of bile:

Bile formation is a characteristic secretory function of the liver. In the medical field, it was a major point of concern to investigate the mechanism of bile formation, which subsequently helps the diagnosis and the possible treatment of hepatobiliary disorders.

Bile originates from hepatocytes, and is then modified in the bile duct epithelium through absorptive and secretory transport. Bile then either passes to the intestinal lumen directly, or it is stored and concentrated in the gallbladder. About 95% of bile is water, in which many endogenous and exogenous solid constituents are dissolved. The endogenous dissolved constituents include bile salts, bilirubin phospholipid, cholesterol, amino acids, steroids, enzymes, porphyrins, vitamins, and heavy metals, whereas the exogenous constituents include drugs and environmental toxins ***(Boyer, 2013).***

400 ml bile is formed in the canaliculi between hepatocytes and 200 ml bile is collected in the bile ducts lined up by cholangiocytes. Hepatocytes ATP-dependent pumps accumulate bile acids, phospholipids, and organic anions in the bile. Transport systems at the apical membrane of the

hepatocyte act as export pumps for bile salts and other organic solutes, resulting in osmotic gradients inside the bile canalicular lumen which drives the fluid movement of bile into the lumen via aquaporins. Cholangiocyte modification of bile is highly regulated by hormones, second messengers, and signal transduction pathways ***(Dawson et al., 2009)*.**

Bile is aqueous in nature and contains less than 5% solid contents in most species. Bile consists of a number of organic and inorganic solutes. The inorganic constituents are passively secreted ions with similar concentration for those in plasma. However, HCO_3 concentration is slightly higher in bile than in plasma. On the other hand, organic anions and cations are derived by active transport mechanisms at the canalicular membrane, and so these constituents are highly concentrated in bile. The most important organic solutes in bile are bile salts, which are 24 carbon water soluble products of cholesterol metabolism. Two primary bile salts are synthesized in mammalian liver: cholic acid, a trihydroxylated bile salt, and chenodeoxycholic acid (CDCA), a dihydroxy bile salt ***(Boyer, 2013)***.

The secretion of these cholesterol-derived amphipathic molecules by hepatocytes creates bile flow and helps the removal of metabolites such as bilirubin and hormones, as well as drugs. Most bile acids are conjugated, with glycine or taurine, and mixed with phospholipids and cholesterol in the bile. On reaching the small intestine, they facilitate digestion