between BMI and serum FGF19 was observed. The mechanisms responsible for these findings are probably related with a different bile acid homeostasis, and deserve further investigation.

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NOS-3 REGULATION BY OXIDATIVE STRESS IN A CELLULAR MODEL OF CHOLESTATIC DAMAGE

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Background: During cholestatic liver disease, excessive accumulation of hydrophobic bile acids exerts a cytotoxic effect leading to cell death and tissue damage. Oxidative stress plays a key role in this process by promoting the development of fibrosis, cirrhosis, portal hypertension and chronic liver failure. Furthermore, in cellular models of cholestatic damage it has been established a cytoprotective role for nitric oxide (NO). The aim of the study was to evaluate the regulation of endothelial nitric oxide synthase (NOS-3) in a cellular model of cytotoxicity by glycochenodeoxycholic acid (GCDCA) and its relationship with the oxidative stress and cell death.
Materials and Methods: A kinetic study was performed (0–24 hours) for induction of cell death by GCDCA (0.5 mM) in the human hepatocarcinoma cell line HepG2. The compound Mn (III) tetrakis (4-benzoic acid) porphyrinchloride (MnTBAP, 1 mg/mL) was tested as an antioxidant molecule. The detection of reactive oxygen species and assessment of cell death was performed spectrophotometrically by using the probes 2,7-dichlorofluorescein diacetate and dihydroetidium, and by measuring caspase-3 activation and lactate dehydrogenase cellular release, respectively. NOS activity was determined by analyzing nitrite and nitrate accumulation in the extracellular medium. NOS-3 expression was measured by RT-qPCR and western-blot. The promoter activity of Nos-3 gene (1601 bp) was assessed using the luciferase activity assay. The identification of transcription factors (TFs) that could be involved in the NOS-3 regulation was performed using prediction programs. Chromatin immunoprecipitation assay and western-blot were used for further analysis and for the identification of the TFs binding sites in the Nos-3 promoter.
Results: GCDCA administration was associated to oxidative stress increase and Nos-3 promoter activity decrease, with a reduction in NOS-3 expression and cellular NO production. The expression and the binding of TFs cJun, cFos and SP1 to the Nos-3 promoter (identified positions), as well as the phosphorylation of protein kinases JNK and ERK1/2, were related to GCDCA-induced hepatocellular damage. MnTBAP treatment prevented the cellular effects of GCDCA.
Conclusions: GCDCA-induced cell death was associated to NOS-3 expression/activity decrease by oxidative-stress. This fact was related to JNK and ERK1/2 phosphorilation, and Nos-3 promoter binding increase of TFs cJun, cFos and SP1.

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PREVENTION OF CHOLESTEROL GALLSTONES FORMATION BY TWO EXTRACTS OF *Raphanus sativus* L. var *niger* IN MICE

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Background: Cholesterol gallstones is a frequent disease in Western countries, as well as in Chile and Mexico. Furthermore cholesterol gallstones formation results from an imbalance in lipid components of bile through dysregulation of biliary transporters. On the other hand, glucosinolates are active metabolites of *Raphanus sativus* L. var *niger* (black radish), which therapeutic effects have shown to be antioxidant and hypolipidemic (J Biomed Biotechnol. 2012; 2012: 161205; Phytother Res, 2005; 19: 587).
Aim: To investigate the effects of two extracts of black radish in the prevention of cholesterol gallstones formation in mice.
Methods: Sixty-three male adult mice (C57BL/6Nhsd) were used in this study. Intragastric aqueous (H_2O) or methanolic (MeOH) extract from black radish 10, 100, 1000 mg/kg plus lithogenic diet for 40 days was administered. As control groups, animals were fed with normal diet (ND) or lithogenic diet (DL) or ursodeoxycholic acid (UDCA) plus lithogenic diet. After experimental period, animals were sacrificed. Total cholesterol, bile salts, phospholipids and triglycerides were determined in serum and bile. Biliary transport protein expression from Abcb11, Abcb4, Abcg5, Abcg8 was evaluated by western blot. The presence of gallstones was determined by micro- and macroscopic analyses of the gallbladder.
Results: MeOH extract (10, 100, 1000 mg/kg) inhibited gallstones formation (Table 1). In those groups the expression of Abcg8 and Abcg5 was decreased, being dose dependent in the former one. On the other hand, mice that received MeOH extracts (10 and 100 mg/kg) showed a lower expression of Abcb11 as compared to lithogenic diet group. Abcb4 increased its expression with H_2O extract (1000 mg/kg) and MeOH extract (10 and 100 mg/kg) as well, however, MeOH extract (1000 mg/kg) decreased its expression.
Conclusions: *Raphanus sativus* L. var *niger* may have important antilithogenic properties for prevention of cholesterol gallstones, regulating components in bile through a modulation in expression of biliary transporters.

Table 1. Effect of black radish extracts in gallstones formation and binary lipids

Experimental group (n = 7)	Incidence of gallstones (%)	Biliary lipids (mmol/L)		
		Bile salts	Phospholipids	Cholesterol
ND	0	148.7±1.2*	15.81±0.6	7.8±0.1*
LD	100	170.1±2.2	8.85±0.4	31.9±1.4
UDCA	0	167.6±4.0	15.1±0.7	20.6±0.7
H_2O 1000	42.9	154.6±2.9	12.1±0.4	24.0±1.1
MeOH 10	28.5	158.4±2.1	16.3±0.5*	20.0±0.6*
MeOH 100	0	166.6±3.5*	34.2±1.7*	21.3±0.5
MeOH 1000	0	174.7±2.92	27.38±1.38*	8.41±0.24*

*Values indicate significant differences ($p < 0.05$) between groups versus LD group. One-Way ANOVA with Tukey post-hoc.

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LYSYL OXIDASE-LIKE 2 (LOXL2) PROTEIN IS INDUCED IN HUMAN CHOLESTATIC LIVER DISEASE AND IN ANIMAL MODELS OF CHOLANGIOPATHIES

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Background and Aims: In a pathological setting, the extracellular matrix cross-linking enzyme LOXL2 creates an excess of highly cross-linked collagen which increases tissue stiffness and results in fibrotic scarring and activation of local disease-mediating cells. Since fibrosis is a feature of human cholestatic liver disease, we