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Original Research

Preventive effect of *Juniperus procera* extract on liver injury induced by lithocholic acid

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Abstract: Bile acids are strong cytotoxic endogenous compounds implicated in several diseases in various organs, such as the liver, gallbladder and small and large intestines. Lithocholic acid is one such acid, produced by flora, and causes liver injury, cholestasis, and colon cancer. The present study aimed to examine the preventive effects of Juniperus procera extract on lithocholic acid-induced liver injury in experimental mice. Forty adult male mice were divided equally into four groups. The negative control group gained free access to food and water. The second group was orally treated with 150 mg/kg of Juniperus procera extract alone, the third group was treated with 1% lithocholic acid alone and the fourth group was co-treated with 150 mg/kg of Juniperus procera extract and 1% lithocholic acid. Blood and hepatic tissues were collected and assayed for biochemical, molecular and histopathological changes. Lithocholic acid toxicity shows a significant increase in the serum levels of the liver function parameters, which were prevented via the Juniperus procera co-administration. Furthermore, lithocholic acid significantly downregulates the mRNA expression of ABCG8, OATP2, SULT2A, CAR, FXR, CYP2B10, MRP2 and UGT1A, and Juniperus procera prevented this effect. Histopathological investigations of the hepatic tissues showed that lithocholic acid exhibited severe hepatotoxicity, with areas of irregularly distributed necrosis with inflammatory infiltration. Juniperus procera co-treated group showed a slight change in the hepatic tissue, diminished necrotic areas, and inflammatory infiltration. In conclusion, this study clarified the preventive effect of Juniperus procera extract administration on hepatotoxicity induced by lithocholic acid exposure in experimental mice.

Key words: Lithocholic acid; *Juniperus procera*; Liver injury; Bile acids; Preventive effect.

Introduction

Lithocholic acid (LCA) is a steroid compound produced via bacterial flora enzymes that are involved in a mechanism of the deconjugation of conjugated bile acids in the colon (1). In general, bile acids are strongly cytotoxic and are implicated in carcinogenesis in various organs, such as the liver, gallbladder and small and large intestines (1–5). Lithocholic acid is produced by a specific bacterial flora enzyme called 7 alpha-dehydroxylase that converts a primary bile acid called chenodeoxycholic acid (CDCA) and a secondary bile acid called ursodeoxycholic acid (UDCA) into lithocholic acid (6).

In 1911, lithocholic acid was identified in bovine gallstone by Hans Fischer, and it was found in cholestatic patients 50 years later (7, 8). This bile acid showed a striking contribution to liver cirrhosis in LCA-fed rabbits and has been detected in circulating bile acids in patients with liver injury (9, 10). Furthermore, LCA accumulates in the biliary tract, forming insoluble calcium salts that cause acute cholestasis (11, 12). Makishima and his colleagues have reported that LCA is structurally similar to vitamin D and can bind to vitamin D receptors and vitamin D binding proteins and that the mechanism of carcinogenesis led by LCA might thus be interpreted (13).

In another context, several studies have reported that bile acids increase reactive oxygen species (ROS), which in turn induce oxidative stress, leading to DNA damage and ultimately promoting carcinogenesis (14–18).

Herbal medicine is a major segment of the medicinal and therapeutic industries. Juniperus procera is a member of the Juniperus genus that has been reported to be useful for bronchitis, pneumonia, ulcers, hyperglycemia and liver diseases (19–23). Various active chemicals in this valued plant are responsible for its biological activity, such as terpenoids, diterpenes and essential oils (19, 24, 25).

The current study aims to investigate the possible protective effects of Juniperus procera leaf extract in mice exposed to LCA using biochemical indicators, immunohistochemistry, histopathology and molecular studies on the liver tissues of normal and LCA co-treated animals.

Materials and Methods

Materials

Adult male mice were purchased from the King Fahd Institute for Scientific Research, King Abdulaziz University, Saudi Arabia. Lithocholic acid was obtained from Santa Cruz Biotechnology, Heidelberg, Germany. Old Juniperus procera leaves were collected from the mountains of the Taif area during summertime and were extracted in our laboratory. Biochemical kits for the liver and other profiles were obtained from SOMATCO, Jeddah, Prince Abdulaziz Ibn Musaid Ibn Jalawi.

Juniperus procera ethanolic extraction

Old Juniperus procera leaves were freshly collected during summertime from the mountains in the Taif area

in the west of the Kingdom of Saudi Arabia. The leaves were dried and powdered in darkness, and 0.2 kg of powder were dissolved in equal amounts of ethanol and water (50:50) to yield 30 grams of extract.

Animals and experimental procedure

Forty adult male BALB/c mice, eight weeks old and weighing 20–25 g, were housed under controlled-temperature conditions (25±2°C) with a 12 h/12 h day-night cycle in the medical laboratory department of the College of Applied Medical Science, Turabah, Taif University. The animals were given access to food and water ad libitum. All procedures were approved by the Animal Care Committee of Taif University.

Induction of cholestasis in mice and experimental design

The cholestatic groups were fed on 1% lithocholic acid mixed into a controlled diet and allowed food and water ad libitum for 0–96 hours (26). The present study was carried out on 40 healthy adult mice divided into four groups of 10 mice each. The negative control group received no medication and was given free access to food and water. The normal group was fed normally with daily doses of 150 mg/kg of Juniperus procera extract by oral gavage. The cholestatic group was fed on 1% LCA mixed into a controlled diet and was treated with daily doses of 150 mg/kg of Juniperus procera extract by oral gavage. The mice were treated for one month and, at the end of the experiment, were fasted overnight and anesthetized using diethyl ether inhalation. Blood was collected from the inner canthi of the eyes to obtain serum. The animals were then euthanized, and small parts of the livers were collected. Some parts were immediately immersed in TriZol reagent and kept at −70°C until the RNA extraction. Other parts were preserved in 10% buffered neutral formalin for histopathological examination.

Assay of biochemical parameters

The serum samples were analyzed by standard enzymatic assays using commercial kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct and total bilirubin (DBIL and TBIL) and cholesterol in accordance with the manufacturer's protocols (SOMATCO).

Gene expression and reverse transcription polymerase chain reaction (RT-PCR)

RNA extraction

For the preparation of the total RNA, hepatic tissue samples (approximately 100 mg each) were collected from the mice, flash frozen in liquid nitrogen and subsequently stored at −70°C in 1 ml Qiazol. The frozen samples were homogenized using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY) in 1 ml of Qiazol. Then, 0.3 ml of chloroform was added to the homogenate. The mixture was shaken for 30 seconds and centrifuged at 4°C and 12,500 rpm for 20 min. The supernatant layer was collected to a new set of tubes, and an equal volume of isopropanol was added to the samples, which were shaken for 15 seconds and centrifuged at 4°C and 12,500 rpm for 15 min. The RNA pellets were washed with 70% ethanol, briefly dried and then dissolved in diethylpyrocarbonate (DEPC) water. The prepared RNA integrity was checked by electrophoresis on 1.5% agarose gel stained with ethidium bromide in TBE (Tris-borate-EDTA) buffer. The RNA concentration and purity were determined spectrophotometrically at 260 nm.

cDNA synthesis

For the cDNA synthesis, a mixture of 2 µg of total RNA and 0.5 ng of oligo-dT primer in a total volume of 11 µl of sterilized DEPC water was incubated in the PeX 0.5 thermal cycler (PCR machine) at 65°C for 10 min for denaturation. Then, 4 µl of 5X RT-buffer, 2 µl of 10 mM dNTPs and 100 U of Moloney murine leukemia virus (M-MuLV) were added up to a total volume of 20 µl with DEPC water. The mixture was reincubated in the thermal cycler at 37°C for 1hr and then at 90°C for 10 min to inactivate the enzyme.

Semi-quantitative PCR analysis

Specific primers for the genes of the tissue samples were designed using the Oligo-4 computer program and synthesized by Macrogen (Macrogen Company, Gasa-dong, and Geumcheon-gu, Korea) as listed in Table 1. PCR was conducted in a final volume of 25 µl, consisting of 1 µl of cDNA, 1 µl of 10 picomolar (pM) of each primer (forward and reverse) and 12.5 µl of PCR master mix (Promega Corporation, Madison, WI). The

Table 1. Polymerase chain reaction conditions for the genes analyzed.

Gene	Product size	Annealing temperature	Sense	Antisense
G3PDH	269	59	tgttcctacccccaatgtgt	tgtgagggagatgctcagtg
CYP2B10	340	59	agtacccccatgttgcagag	ggaggatggacgtgaagaaa
UGT1A1	344	60.5	cctatgggtcacttgccact	cgatggtctagttccggtgt
SULT2A1	342	58.4	tcggctggaatcctaagaga	tgggaagatgggaggttatg
CAR	476	60.5	gggcttcttcagacgaacag	tctggtcctccatggttagg
FXR	483	59	agttgccgtgaggaagctaa	gtgagcgcgttgtagtggta
ABCG8	446	59	tctccaggtcctgattggtc	ggcaatcagagtcaacagca
MRP2	499	59	tcctagacagcggcaagatt	ctctggctgtccaacactca
BSEP	387	60.5	cctcagtgctttccttctgg	acagccacagagagggagaa
OATP2	358	58	acccaagaggctgtctctca	gccaacagaaatgccttgat

CYP2B10:cytochrome P450 (family 2, subfamily b, polypeptide 10) ; UGT1A1: UDP glucuronosyltransferase (family 1 member A1; SULT2A1, Sulfotransferase Family 2A) ; CAR: constitutive androstane receptor ; FXR,: Farnesoid x receptor ; ABCG8: ATP-binding cassette (sub-family G member 8) ; MRP2: Multidrug resistance-associated protein 2; BSEP : Bile Salt Export Pump; OATP2: organic anion-transporting polypeptide.

volume was brought up to 25 µl using sterilized, deionized water. The cycle sequence of the PCR reaction was carried out at 94°C for one 5-minute cycle, followed by 30–35 cycles, each consisting of denaturation at 94°C for one minute, annealing at the specific temperature corresponding to each primer (information about primer annealing temperatures is outlined after the primer design) and extension at 72°C for one minute, with an additional final extension at 72°C for 5 minutes. As a reference, glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was expressed as a housekeeping gene. The PCR products were electrophorized on 1.5% agarose gel stained with ethidium bromide in TBE (Tris-borate-EDTA) buffer and were visualized under UV light and photographed using a gel documentation system (Syngene, Frederick, MD, USA). The density of the bands was analyzed densitometrically using the Image J software, version 1.47.

Histopathological examination

The collected specimens of liver from the sacrificed mice were fixed in a 10% buffered neutral formalin solution for at least 24 hrs and then routinely processed. Paraffin sections five microns thick were prepared, stained with hematoxylin and eosin stain (H&E) and then examined microscopically using a Wolfe S9-0982 microscope (Carolina Biological Supply Co., Burlington, NC, USA).

Immunohistochemical examination of glutathione and NF-kB

The hepatic tissues were fixed in 10% buffered neutral formalin, washed, dehydrated, cleared, embedded in paraffin, cast and sectioned. The tissue sections were deparaffinized and treated with 3% H2O2 for 10 min to inactivate the peroxidases. The samples were subsequently heated in a 10 mM citrate buffer at 121°C for 30 min for antigen retrieval, blocked in 5% normal serum for 20 min and incubated with a rabbit polyclonal anti-glutathione primary antibody (1:100; sc-71155; Santa Cruz Biotechnology, Inc., Dallas, TX) or NFκB p50 antibody (1:100; sc-7178; Santa Cruz Biotechnology, Inc.) in phosphate-buffered saline (PBS) overnight at 4°C. After three extensive washes with PBS, the sections were incubated with a goat anti-rabbit IgG biotin-conjugated secondary antibody (1:2,000; sc-2040; Santa Cruz Biotechnology, Inc.) for 20 min at 32°C. After further incubation with horseradish peroxidase-labeled streptavidin, the antibody binding was visualized using diaminobenzidine and the sections were counterstained with hematoxylin.

Statistical analysis

The results are shown as means ± standard error of means (SEM). The data analysis was done using analysis of variance (ANOVA) and *post hoc* descriptive tests using the SPSS software, version 11.5 for Windows (SPSS, IBM, Chicago, IL, USA), with $p<0.05$ being considered statistically significant. A regression analysis was done using the same software.

Results

Juniperus procera extract administration reduces liver injury induced by LCA

Table 2 shows a significant increase in the serum levels of AST, ALT, alkaline phosphatase and total and direct bilirubin in the mice that were exposed to lithocholic acid toxicity for one month (p values<0.05). However, these levels were reduced when the mice were treated with Juniperus procera extract, approaching those of the normal control mice. In addition, serum amylase levels were significantly decreased under LCA toxicity, which was protected in the mice that were co-treated with both LCA and Juniperus procera extract (p values<0.05), as shown in Table 2.

Protective effect of juniperus procera extract on gene expression of BSEP, ABCG8, OATP2, SULT2A, CAR, FXR, CYP2B10, MRP2 and UGT1A on hepatic injury induced by LCA toxicity

Figure 1 (A and B) clearly shows a significant down-regulation in the mRNA expressions of both ABCG8 and OATP2 in the mice that underwent LCA toxicity. However, these changes were significantly ameliorated in the JPE + LCA co-treated mice. Similarly, LCA toxic-

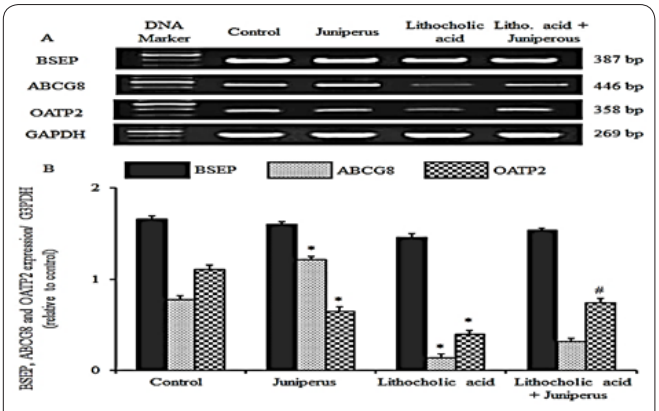

Figure 1. Effect of juniperus procera on changes in gene expression induced by lithocholic acid in liver. Values are means ± SE of 10 mice. *P < 0.05 corresponding to control group; # P < 0.05 corresponding to lithocholic acid group. (A) panels are mRNA expression of BSEP gene (ATP-binding cassette subfamily B member), ABCG8 gene (ATP-binding cassette subfamily G member), OATP2 gene (Organic anion transporting polypeptide 2) and GAPDH the housekeeping gene. (B) columns are densitometric analysis of gene expression.

Table 2. Biochemical measurements of liver functions for normal control, juniperus administrated mice, LCA and LCA juniperus co-treated mice.

	AST (U/L)	ALT (U/L)	ALP (U/L)	BIL-T (mg/dl)	BIL-D(mg/dl)	Amylase (U/L)
Control	170 ± 1.62	49 ± 1.22	89 ± 1.06	0.03 ± 0.004	0.12 ± 0.02	3149 ± 131
Juniperus	191 ± 11.8	59 ± 3.3	63 ± 3.6	0.04 ± 0.01	0.16 ± 0.03	2556 ± 177
Lithocholic A	5718 ± 367#	1695 ± 33.2#	140 ± 2.13#	0.92 ± 0.019#	0.53 ± 0.083#	1853 ± 59#
LCA+ Juniperus	252 ± 18.3*	50 ± 0.3*	60 ± 1.81	0.03 ± 0.008*	0.13 ± 0.02*	3260 ± 105*

Values are represented by mean ± SEM for 10 mice. # represents P values of LCA treated mice corresponding to normal control. * represents P values of LCA + Juniperus co-treated mice corresponding to LCA treated mice.

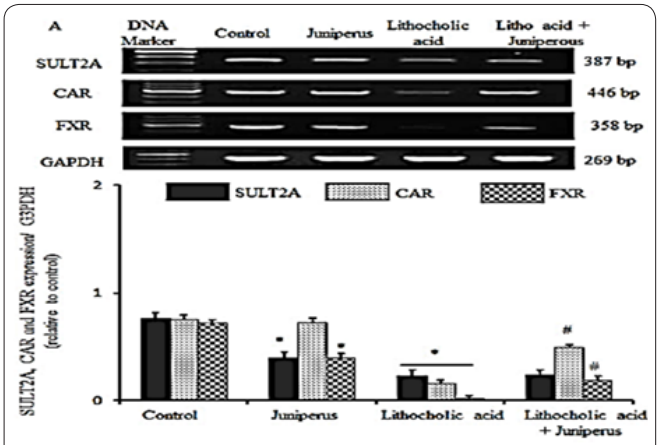

Figure 2. Effect of juniperus procera on changes in gene expression induced by lithocholic acid in liver. Values are means ± SE of 10 mice. *P < 0.05 corresponding to control group; # P < 0.05 corresponding to lithocholic acid group. (A) panels are mRNA expression of SULT2A gene (Sulfotransferase family 2A), CAR gene (Constitutive androstane receptor), FXR gene (Farnesoid X receptor) and GAPDH the housekeeping gene. (B) columns are densitometric analysis of gene expression.

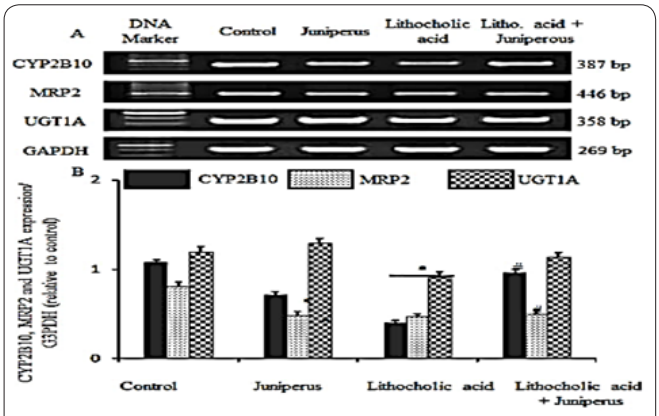

Figure 3. Effect of juniperus procera on changes in gene expression induced by lithocholic acid in liver. Values are means ± SE of 10 mice. *P < 0.05 corresponding to control group; # P < 0.05 corresponding to lithocholic acid group. (A) panels are mRNA expression of CYP2B10 gene (Cytochrome P450, family 2, subfamily b, polypeptide 10), MRP2gene (Multidrug resistance-associated protein 2), UGT1A gene (Glucuronosyltransferase Family 1 Member A) and GAPDH the housekeeping gene. (B) columns are densitometric analysis of gene expression.

ity downregulated the mRNA expressions of SULT2A, CAR, and FXR, while JPE treatment afforded significant protection, as shown in Figure 2 (A and B). LCA toxicity also downregulated the mRNA expressions of CYP2B10, MRP2, and UGT1A, while JPE provided moderate protection from this effect, as demonstrated in Figure 3 (A and B).

JPE protection elucidation via histopathological findings and immunohistochemical examination of glutathione and NF-kB

The hepatic tissues of the control and Juniperus procera groups showed a normal hepatic architecture, with normal central veins, hepatic lobules and hepatic sinusoids (Figs. 4A and 4B respectively). However, the pictures of the LCA group showed severe hepatotoxicity, with irregularly distributed areas of necrosis with inflammatory infiltration (Fig. 4C). The livers of the LCA group that was co-treated with Juniperus procera showed healing of the hepatic tissue, with mostly normal hepatic tissue and diminished necrotic areas and inflammatory infiltration (Fig. 4D).

Similarly, the hepatic tissues of the control and Juniperus procera groups showed moderate expressions of glutathione (Figs. 4E and 4F respectively), while the livers of the LCA group showed high expressions of glutathione in the necrotic foci and surrounding hepatic tissue (Fig. 4G). The livers of the LCA group that was co-treated with Juniperus procera showed strong expressions of glutathione all over the hepatic tissue (Fig. 4H).

The livers of the control and Juniperus procera groups showed mild expressions of NF-kB in the hepatic tissue (Figs. 4I and 4J respectively). The livers of the LCA group showed high expressions of NF-kB in the necrotic foci, with mild expressions in the surrounding hepatic tissue (Fig. 4K). In addition, the livers of the LCA group that was co-treated with Juniperus procera showed strong expressions of NF-kB all over the hepatic tissue (Fig. 4L).

Discussion

Diets rich in saturated fat are major health issues in

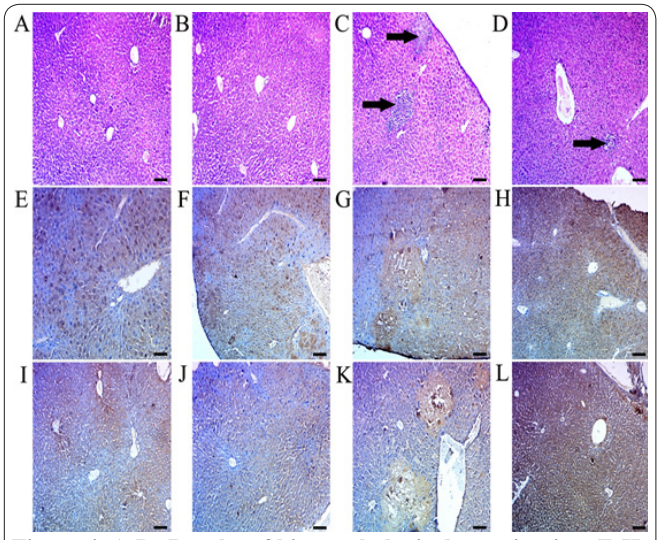

Figure 4. A-D; Results of histopathological examination, E-H; Results of immunohistochemical examination of glutathione expression, I-L; Results of immunohistochemical examination of NF-kB expression. (A and B) hepatic tissues of control and Juniperus procera groups respectively with normal tissue architecture. (C) hepatic tissues of LCA group with multiple necrotic foci of different sizes with inflammatory infiltrate (arrows). (D) hepatic tissues of LCA group co- treated with Juniperus procera showed healing of hepatic tissue with diminished necrotic area with inflammatory infiltrate (arrow). (E and F) hepatic tissues of control and Juniperus procera groups respectively with moderate glutathione expression in hepatic tisue. (G) hepatic tissues LCA group showed high expression of glutathione in the necrotic foci and surrounding hepatic tissue. (H) hepatic tissues of LCA group co-treated with Juniperus procera showed strong expression of glutathione all over the hepatic tissue. (I and J) hepatic tissues of control and Juniperus procera groups respectively showed mild expression of NF-kB. (K) hepatic tissues of LCA group showed high expression of NF-kB in the necrotic foci with mild expression of surrounding hepatic tissue. (L) hepatic tissues of LCA group co-treated with Juniperus procera showed strong expression of NF-kB (scale bar=100 µm).

developed countries. Many studies have concentrated on this problem in order to address acute and chronic diseases that are significantly less common in developing countries, such as diabetes, cancer and heart and liver defects.

Bile acids are cytotoxic, carcinogenic organic compounds that are implicated in multiple tumors, such as hepatoma and colon cancer (1–5). LCA is one such harmful compound and accumulates in the biliary tract, forming insoluble calcium salts that cause acute cholestasis (11, 12). In addition, LCA contributes to liver cirrhosis in experimental animals and has been detected in circulating bile acids in patients with liver injury (9, 10). The serum levels of bile acids are highly correlated to the serum levels of the liver function parameters (27). The current study clearly showed a significant increase in the serum levels of AST, ALT, alkaline phosphatase and total and direct bilirubin, which may indicate serious liver injury caused by LCA toxicity.

These results were accompanied by a significant downregulation in the mRNA expression of ABCG8, a gene that encodes an ATP transporter protein that has been found to be decreased in human cholestatic livers (28). Moreover, the molecular investigation data shown in Figures 1, 2 and 3 demonstrate a significant downregulation in the mRNA expressions of OATP2, SULT2A, CAR, FXR, CYP2B10, MRP2 and UGT1A, which elucidates clear damage and acute liver injury caused by LCA toxicity in the experimental mice. This is consistent with several published conclusions (29–35). These major serological and molecular changes were significantly prevented in the LCA + JPE co-treated mice, in which Juniperus procera extract prevented LCA toxicity, either by preventing the absorption of LCA through the intestines or by preventing the cellular toxicity caused by LCA on the liver. Moreover, the histopathological findings and immunohistochemical examination of both glutathione and NF-kB, shown in Figure 4, further validate that hepatotoxicity was caused by LCA exposure that was strikingly prevented via co-treatment with JPE.

This study clarified the preventive effect of *Juniperus* extract administration on hepatotoxicity induced by LCA exposure in experimental mice via preventing either absorption or cytotoxicity.

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References

1. Fuchs M. Bile acid regulation of hepatic physiology: III. Regulation of bile acid synthesis: past progress and future challenges. American journal of physiology Gastrointestinal and liver physiology. 2003;284(4):G551-7.

2. Hofmann AF. Bile Acids: The Good, the Bad, and the Ugly. News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society. 1999;14:24-9.

3. Hofmann AF, Hagey LR. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. Cellular and molecular life sciences : CMLS. 2008;65(16):2461-83.

4. Stamp D. Antibiotic therapy may induce cancers in the colon and breasts through a mechanism involving bile acids and colonic bacteria. Medical hypotheses. 2004;63(3):555-6.

5. Nancey S, Coffin B, Descos L, Flourie B. Colonic microflora and cance. Gastroenterologie clinique et biologique. 2001;25(2 Pt 2):C79-84.

6. Hofmann AF. The continuing importance of bile acids in liver and intestinal disease. Archives of internal medicine. 1999;159(22):2647-58.

7. Hofmann AF. Detoxification of lithocholic acid, a toxic bile acid: relevance to drug hepatotoxicity. Drug Metab Rev. 2004;36(3-4):703-22.

8. van Berge Henegouwen GP, Brandt KH, Eyssen H, Parmentier G. Sulphated and unsulphated bile acids in serum, bile, and urine of patients with cholestasis. Gut. 1976;17(11):861-9.

9.Holsti P. Cirrhosis of the Liver induced in Rabbits by Gastric Instillation of 3-Monohydroxycholanic Acid. Nature. 1960; 186 (4720):250.

10. Carey MC, Small DM. The physical chemistry of cholesterol solubility in bile. Relationship to gallstone formation and dissolution in man. The Journal of clinical investigation.1978;61(4):998-1026.

11. Zaki FG, Carey JB, Jr., Hoffbauer FW, Nwokolo C. Biliary reaction and choledocholithiasis induced in the rat by lithocholic acid. The Journal of laboratory and clinical medicine. 1967;69(5):737-48.

12. Hofmann AF, Mysels KJ. Bile acid solubility and precipitation in vitro and in vivo: the role of conjugation, pH, and Ca2+ ions. Journal of lipid research. 1992;33(5):617-26.

13. Adachi R, Honma Y, Masuno H, Kawana K, Shimomura I, Yamada S, et al. Selective activation of vitamin D receptor by lithocholic acid acetate, a bile acid derivative. Journal of lipid research. 2005;46(1):46-57.

14. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutation research. 2005;589(1):47-65.

15. Reddy BS. Diet and excretion of bile acids. Cancer research. 1981;41(9 Pt 2):3766-8.

16. Cherbonnel-Lasserre C, Gauny S, Kronenberg A. Suppression of apoptosis by Bcl-2 or Bcl-xL promotes susceptibility to mutagenesis. Oncogene. 1996;13(7):1489-97.

17. Dvorak K, Watts GS, Ramsey L, Holubec H, Payne CM, Bernstein C, et al. Expression of bile acid transporting proteins in Barrett's esophagus and esophageal adenocarcinoma. The American journal of gastroenterology. 2009;104(2):302-9.

18. Bernstein H, Bernstein C, Payne CM, Dvorak K. Bile acids as endogenous etiologic agents in gastrointestinal cancer. World journal of gastroenterology. 2009;15(27):3329-40.

19. Burits M, Asres K, Bucar F. The antioxidant activity of the essential oils of Artemisia afra, Artemisia abyssinica and Juniperus procera. Phytotherapy research : PTR. 2001;15(2):103-8.

20. Loizzo MR, Tundis R, Conforti F, Saab AM, Statti GA, Menichini F. Comparative chemical composition, antioxidant and hypoglycaemic activities of Juniperus oxycedrus ssp. oxycedrus L. berry and wood oils from Lebanon. Food Chemistry. 2007;105(2):572-8.

21. Ozturk M, Tumen I, Ugur A, Aydogmus-Ozturk F, Topcu G. Evaluation of fruit extracts of six Turkish Juniperus species for their antioxidant, anticholinesterase and antimicrobial activities. Journal of the science of food and agriculture. 2011;91(5):867-76.

22. El Beyrouthy M, Arnold N, Delelis-Dusollier A, Dupont F. Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon. Journal of ethnopharmacology. 2008;120(3):315-34.

23. Al-Attar AM, Alrobai AA, Almalki DA. Effect of Olea oleaster and Juniperus procera leaves extracts on thioacetamide induced hepatic cirrhosis in male albino mice. Saudi Journal of Biological Sciences. 2016;23(3):363-71.

24. Alqasoumi SI, Abdel-Kader MS. Terpenoids from Juniperus procera with hepatoprotective activity. Pakistan journal of pharmaceutical sciences. 2012;25(2):315-22.
25. Akeng'a TA, Chhabra SC. Analysis of the essential oil of Juniperus procera Endl. growing in Kenya. African journal of medicine and medical sciences. 1997;26(1-2):79-81.
26. Woolbright BL, Jaeschke H. Therapeutic targets for cholestatic liver injury. Expert opinion on therapeutic targets. 2016;20(4):463-75.
27. Festi D, Morselli Labate AM, Roda A, Bazzoli F, Frabboni R, Rucci P, et al. Diagnostic effectiveness of serum bile acids in liver diseases as evaluated by multivariate statistical methods. Hepatology (Baltimore, Md). 1983;3(5):707-13.
28. Chai J, Feng X, Zhang L, Chen S, Cheng Y, He X, et al. Hepatic Expression of Detoxification Enzymes Is Decreased in Human Obstructive Cholestasis Due to Gallstone Biliary Obstruction. PLoS ONE. 2015;10(3):e0120055.
29. Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. Physiological reviews. 2003;83(2):633-71.
30. Donner MG, Schumacher S, Warskulat U, Heinemann J, Häussinger D. Obstructive cholestasis induces TNF-α- and IL-1β-mediated periportal downregulation of Bsep and zonal regulation of Ntcp, Oatp1a4, and Oatp1b2. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2007;293(6):G1134-G46.
31. Ilyas G, Zhao E, Liu K, Lin Y, Tesfa L, Tanaka KE, et al. Macrophage autophagy limits acute toxic liver injury in mice through down regulation of interleukin-1β. Journal of hepatology. 2016;64(1):118-27.
32. Su H, Ma C, Liu J, Li N, Gao M, Huang A, et al. Downregulation of nuclear receptor FXR is associated with multiple malignant clinicopathological characteristics in human hepatocellular carcinoma. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2012;303(11):G1245-G53.
33. Tryndyak V, de Conti A, Kobets T, Kutanzi K, Koturbash I, Han T, et al. Interstrain differences in the severity of liver injury induced by a choline- and folate-deficient diet in mice are associated with dysregulation of genes involved in lipid metabolism. The FASEB Journal. 2012;26(11):4592-602.
34. Elferink MG, Olinga P, Draaisma AL, Merema MT, Faber KN, Slooff MJ, et al. LPS-induced downregulation of MRP2 and BSEP in human liver is due to a posttranscriptional process. American journal of physiology Gastrointestinal and liver physiology. 2004;287(5):G1008-16.
35. Kim J-H, Nam WS, Kim SJ, Kwon OK, Seung EJ, Jo JJ, et al. Mechanism Investigation of Rifampicin-Induced Liver Injury Using Comparative Toxicoproteomics in Mice. International Journal of Molecular Sciences. 2017;18(7):1417.