

Hepatoprotective Effect of Berberis Lycium in Alpha-Naphthylisothiocyanate Induced Cholestatic Mouse Models

Shamaila Bashir

Department of Zoology, Government College University Lahore, Pakistan

Hassan Hameed

*Department of Zoology, Government College University Lahore, Pakistan,
hassanhameed1996@gmail.com*

Nida Irshad

Department of Zoology, Government College University Lahore, Pakistan

Aqsa Ashraf

Department of Zoology, Government College University Lahore, Pakistan

Muhammad Faizan

Department of Chemistry, Government College University Lahore, Pakistan

See next page for additional authors

Follow this and additional works at: <https://corescholar.libraries.wright.edu/jbm>



Part of the [Animal Sciences Commons](#), and the [Immunology and Infectious Disease Commons](#)

Recommended Citation

Bashir, S., Hameed, H., Irshad, N., Ashraf, A., Faizan, M., Halim, S. F., Asjad, H., Batool, A., & Sohail, I. (2023). Hepatoprotective Effect of Berberis Lycium in Alpha-Naphthylisothiocyanate Induced Cholestatic Mouse Models, *Journal of Bioresource Management*, 10 (4).

ISSN: 2309-3854 online

(Received: May 29, 2023; Accepted: Aug 15, 2023; Published: Dec 27, 2023)

This Article is brought to you for free and open access by CORE Scholar. It has been accepted for inclusion in Journal of Bioresource Management by an authorized editor of CORE Scholar. For more information, please contact library-corescholar@wright.edu.

Hepatoprotective Effect of Berberis Lycium in **Alpha-Naphthylisothiocyanate** Induced Cholestatic Mouse Models

Cover Page Footnote

We are indebted to Dr. Sohaib Muhammad, Assistant Professor, Department of Botany for identification of the plant.

Authors

Shamaila Bashir, Hassan Hameed, Nida Irshad, Aqsa Ashraf, Muhammad Faizan, Saiyada Fatima Maida Halim, Hafiz M. Mazhar Asjad, Andleeb Batool, and Imran Sohail

© Copyrights of all the papers published in Journal of Bioresource Management are with its publisher, Center for Bioresource Research (CBR) Islamabad, Pakistan. Users have the right to read, download, copy, distribute, print, search, or link to the full texts of articles in the Journal. We operate under International Version 4 (CC BY 4.0) of Creative Commons Attribution License which allows the reproduction of articles free of charge with the appropriate citation of the information.

HEPATOPROTECTIVE EFFECT OF *BERBERIS LYCIUM* IN ALPHA-NAPHTHYLISOTHIOCYANATE INDUCED CHOLESTATIC MOUSE MODELS

SHAMAILA BASHIR¹, HASSAN HAMEED¹, NIDA IRSHAD¹, AQSA ASHRAF¹,
MUHAMMAD FAIZAN², SAIYADA FATIMA MAIDA HALIM³, HAFIZ M. MAZHAR ASJAD⁴,
ANDLEEB BATOOL¹, AND IMRAN SOHAIL^{1*}

¹Department of Zoology, Government College University Lahore, Pakistan

²Department of Chemistry, Government College University Lahore, Pakistan

³Rashid Latif Medical College, Lahore, Pakistan

⁴Department of Pharmacy, Forman Christian College, Lahore Pakistan

*Corresponding author's email: imransohail@gcu.edu.pk

ABSTRACT

Cholestasis is characterized by the obstruction of bile flow from hepatocytes to the intestine. It results in accumulation of bile acids in the liver, which cause oxidative stress, inflammation, apoptosis, fibrosis, and cirrhosis. Till now, the treatment options against cholestasis are limited. Therefore, there is an utmost requirement to develop and evaluate the drugs with potential anti-cholestatic effects. In the current study, the drug-induced cholestasis mouse models were generated via oral administration of α -naphthylisothiocyanate (ANIT). The mice were placed into three groups of six animals each. Group I was the control group and was given saline. The cholestasis model Group II was given saline for 19 days. On 19th day (48 hours before sacrifice) they received a single dose of ANIT (75 mg/kg). Group III served as the plant extract treatment group and received root extract for 19 days. On nineteenth day (48 hours before sacrifice) they received a single dose of ANIT (75 mg/kg). On 21st day, mice were sacrificed for analysis of serum biochemistry and liver histology. The results revealed that *Berberis lycium* extract has hepatoprotective properties, as serum level of AST (aspartate aminotransferase) and ALT (alanine transaminase) are significantly lower in the plant treatment group compared to the cholestasis model group. Furthermore, liver histology validated the serological results since the hepatocyte architecture in the plant treatment group was similar to that of control group. In conclusion, the data exhibit that *B. lycium* possess protective activities against ANIT induced cholestasis.

Keywords: ANIT, berberine, *Berberis lycium*, cholestasis, hepatoprotective.

INTRODUCTION

Cholestasis results in the impairment of bile flow and increased level of bile acids in the liver and blood plasma (Fuchs et al., 2017). It can either be inherited or acquired. The former is caused by mutations in the genes responsible for bile production, excretion, and absorption (Pauli-Magnus et al., 2005). Acquired form of cholestasis is mainly caused as side-effects of systemically administered drugs (Montanari et al., 2016). Several drugs including cyclosporine A, bosentan

rifampicin and glibenclamide have been reported to cause cholestasis.

Ursodeoxycholic acid (UDCA) is the only FDA approved drug for the treatment of cholestasis (Trauner et al., 2017). Some patients, however, don't respond to UDCA (Sohail et al., 2021). Similarly, 4-phenylbutyrate (4-PB) also showed promising result in alleviating cholestasis markers (Gonzales et al., 2015). This drug has also drawback of requiring high doses for the treatment of disease. As a result, novel medications that can protect the liver from cholestatic liver injury must be explored.

As the phytonutrients are safer and cost-effective, these are extensively used for the treatment of different ailments. *B. lycium*, a member of family Berberidaceae is an evergreen shrub (Irshad et al., 2013). It is a medicinal plant which has the ability to impede a variety of ailments including liver diseases, skin diseases and cough (Kapoor et al., 2013). The anticholestatic potential of root extract of *B. lycium* is explored in this study

MATERIALS AND METHODS

Collection of Plant and Preparation of Extract

The *B. lycium* root was collected from Azad Kashmir, Pakistan. Following identification, the roots were removed, cleaned with sterile water, shade dried at room temperature, and crushed to a fine powder. 500 g of powder were macerated in 100 % ethanol for 15 days with regular shaking. The mixture was then filtered with Whatman No.1 filter paper. The filtrate was evaporated in a rotary evaporator at 42 °C to obtain extract, which was kept at 4 °C until further use.

HPLC Analysis of the Extract

The analysis of plant extract was performed by using liquid chromatographic system (HPLC LC-20AT, Shimadzu, Japan) as explained earlier (Malkani et al., 2022). Sample solutions and berberine chloride standards were prepared in mobile phase consisted of 0.1 % trifluoro acetic acid and Acetonitrile in proportion of 60:40 v/v with column (C18, 5 µm, 4.6×154 mm, x-bridge) that was kept at temperature of 25 °C. The sample solution and standard were filtered using a 0.22 µm pore size syringe filter, and 10 µl was subjected to HPLC analysis for berberine quantification at λ max of 344 nm.

Animals and Experimental Design

Male albino mice (8 weeks old, weighting 22-25 g) were reared and kept in the animal house of GCU Lahore, Pakistan. During experimental period, standard laboratory conditions (24±1 °C; 12/12 hours dark /light period) were maintained. All handling procedures were performed according to ethical parameters of laboratory animal use. Furthermore, all the efforts were taken to minimize any discomfort and pain to the animals. This study was approved by institutional animal ethical committee (GCU-IIB-460).

Experimental Plan

Animals were divided into three group (n = 6). Group I (G-I) served as control group, receiving saline (0.9 % NaCl) orally for 19 days. On 19th day, the mice were gavaged with olive oil (vehicle). Group-II (G-II) was served as cholestatic group and was given saline for nineteen days. On 19th day (48 hours before sacrifice), mice were given a single dose of 75 mg/kg ANIT (Meng et al., 2015) dissolved in olive oil. Group III (G-III) mice received 150 mg/kg/day (Rafiq et al., 2017) *B. lycium* extract (suspended in saline) orally for 19 days as the plant treatment group. The mice were administered a single dosage of 75 mg/kg ANIT on 19th day (48 hours before sacrifice). The mice were anesthetized with a mixture of ketamine and zylazine (100 and 10 mg/kg, respectively) on the 21st day. Blood was drawn through cardiac puncture and left to clot at room temperature for 15 days. Centrifugation was used to separate the serum, which was then kept at -20 °C until further use. For histological examination, the liver was removed and put in 10 % formaldehyde.

Serum Analysis

Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels in serum were determined using commercially available kits (Randox, UK) and the manufacturer's instructions

Histological Analysis

Formalin preserved liver samples were, dehydrated, and embedded in paraffin. The paraffin blocks were then cut in to thin sections (4-6 μm), stained with hematoxylin-eosin (HE), and observed under a light microscope.

Statistical Analysis

Statistical analysis was performed by using SPSS. One-way ANOVA was used, followed by Tukey's posthoc. The data is presented as mean \pm SD. *P*-value < 0.05 was considered as significant.

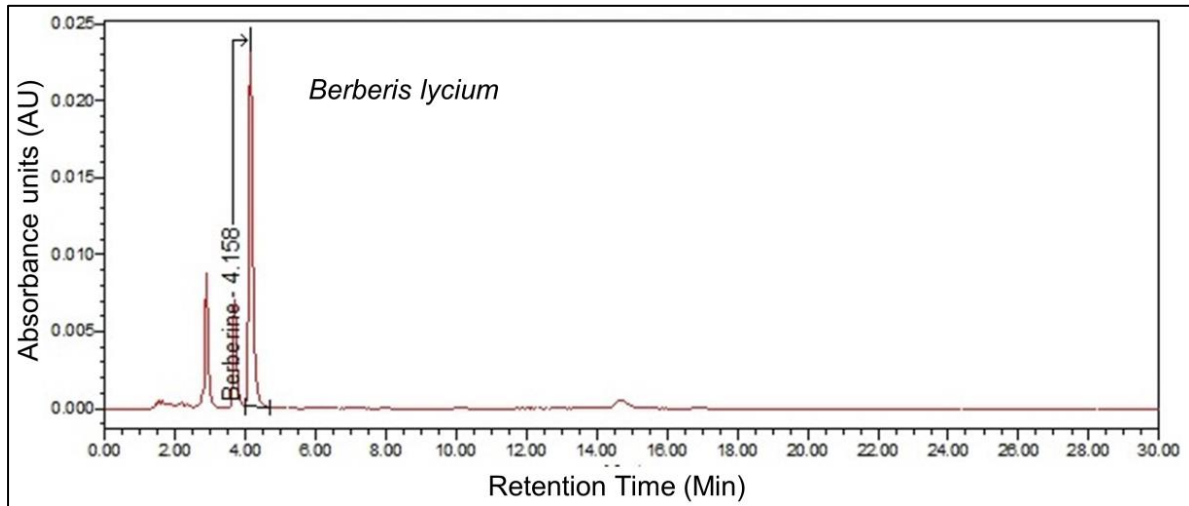


Figure 1: HPLC chromatogram for *B. lycium* measured at 344 nm.

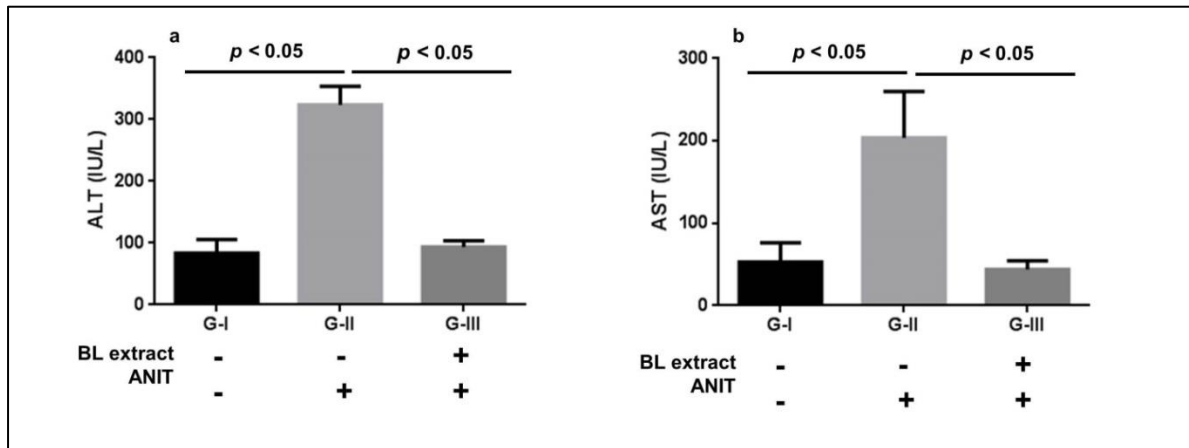


Figure 2: The serum level of (a) ALT and (b) AST was measured by calorimetric assay. G-II (cholestasis group) is compared with G-I (control group). Furthermore, G-III (plant group) is compared to G-II (cholestasis model) group. Data are the mean \pm SD (n = 6). *P* value < 0.05, is considered significant. LD; low dose (300 mg/kg/day), BL: *Berberis lycium* (150 mg/kg/day for 19 days). *a*-naphthylisothiocyanate (ANIT): 75 mg/kg once on 19th day (48 hours before sacrifice).

RESULTS

B. lycium Extract Reduces the Serum ALT and AST in Cholestasis Model

i. ***B. lycium* Roots Extract contains Berberine as main Component**

Berberine is one of the main components in the extract of the members of *Berberis* genus. Therefore, we evaluated the presence of berberine in the roots of *B. lycium*. HPLC data indicated that *B. lycium* contains berberine as the peak sample appeared at same retention time (Figure 1) as reported earlier for Berberine chloride (Malkani et al., 2022). During liver damage, the liver specific enzymes

including ALT and AST are leaked into the blood plasma. Therefore, in serum, level of these enzymes show an indirect indication of the liver damage. In this investigation, the serum levels of ALT and AST in the cholestatic group (G-II) were considerably higher than in the control group (G-I). However, in plant treatment group (G-III) ALT and AST levels in serum are significantly lowered as compared to the cholestatic group (Figure 2a, b).

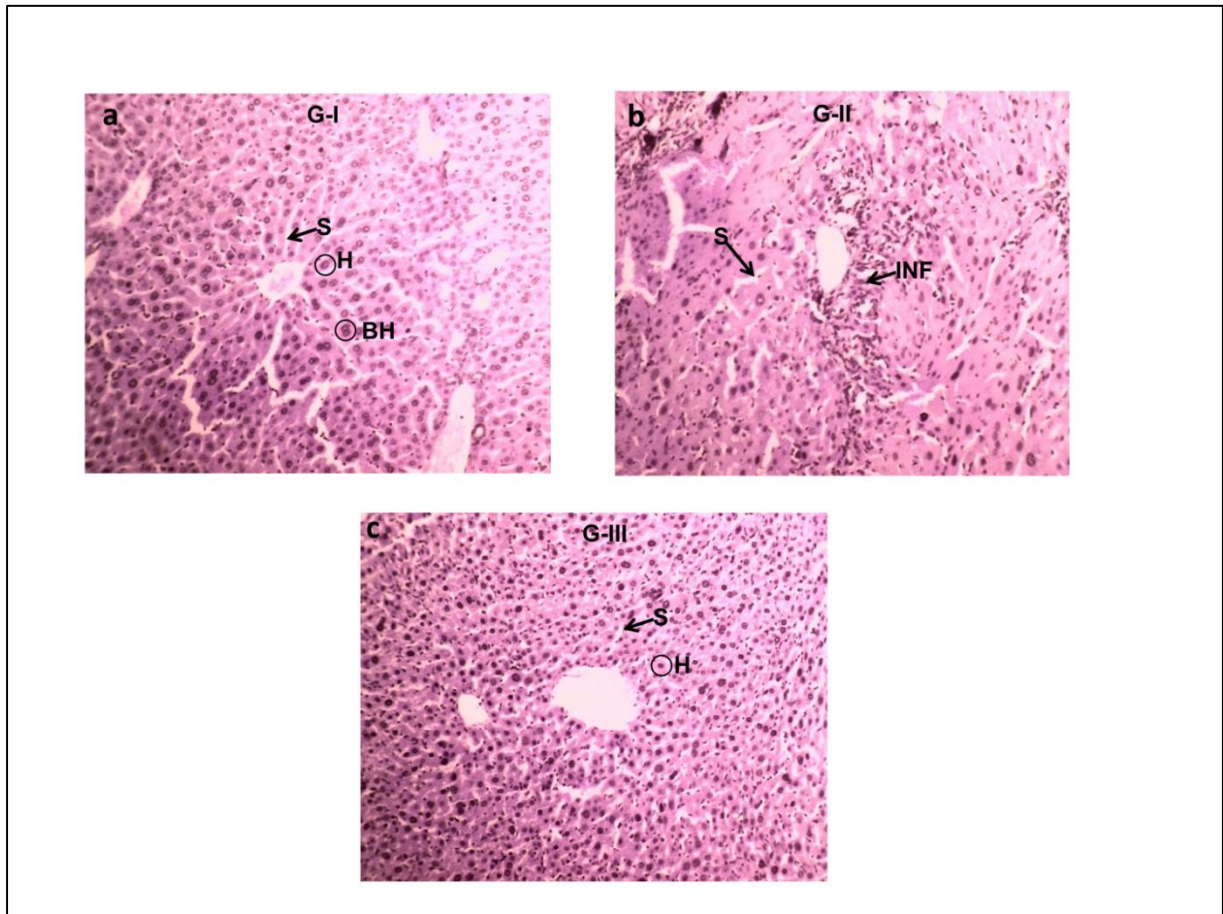


Figure 3: (a) Architecture of normal liver showing intact hepatocytes and normal sinusoid spaces with no infiltration of inflammatory cells. (b) When exposed to ANIT, the liver is injured. As evidenced by the hepatocyte integrity deformation, lymphocyte leakage, and loss of cellular boundaries. (c) Mice were treated with *B. lycium* for 19 days before being exposed to ANIT for 48 hours. The plant had a hepatoprotective effect because the hepatocyte architecture was similar to that of the control group. BH: binucleated hepatocytes; H: hepatocytes; INF: infiltration of inflammatory cells; S: sinusoid.

***B. lycium* Protects the Liver from ANIT induced Cholestasis Damages**

Liver histology provided the direct evidence that *B. lycium* protects liver from ANIT induced damages. As shown in the figure 3, the liver tissue in control group (a) exhibited intact hepatocytes with normal liver sinusoids. While cholestatic model group (b) showed typical pathological conditions with dilated sinusoids and infiltration of inflammatory cells. Furthermore, in plant treatment group (c) the liver architecture is preserved with intact hepatocytes and normal sinusoidal spaces. Additionally, there is no infiltration of inflammatory cells in treatment group indicating the hepatoprotective effect of the *B. lycium* extract.

DISCUSSION

Cholestasis is impaired bile flow which leads to increase level of bile acids in liver. Because of their detergent as well as inflammatory effect, bile acids lead to the injury of liver cells. UDCA is the only FDA recommended drug for the treatment of cholestasis. Therefore, there is need to find new therapeutic agents for the treatment of cholestasis. Alpha-naphthylisothiocyanate (ANIT) has been extensively used to generate cholestasis rodent models (Sohail et al., 2022). It induces the cholestasis by injuring the bile duct epithelial cells as well as the hepatocytes.

Recently, phytonutrients have occupied a central stage in modern therapeutics. In developing countries, 75 – 80 % populations rely on medicinal plant for treatment of their ailments (Elyasi et al., 2012). The members of family Berberidaceae have been extensively used for their anti-diabetic, antioxidative, antimicrobial, cardiotoxic, antiinflammatory and antidiarrheal activities (Gupta et al., 2008; Jabbarzadeh et al., 2014; Tillhon et al., 2012). Various

constituents of *B. lycium* have been demonstrated to reduce damage caused by reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radical. Because of their antioxidant and DNA-protective properties, crude methanolic, aqueous, and acetone extracts of *B. lycium* have been found to be useful in a variety of herbal products (Soren et al., 2022). Furthermore, it has been shown that the extract of roots, shoots, leaves and fruit of this family possess various biological active constituents including berberine, berbamine, oxyberberine, karachine, aromoline and oxyacanthine (Potdar et al., 2012). HPLC analysis of roots extract of *B. lycium* also showed that there is highest concentration of berberine in the extract which is an indication that the extract is prepared accurately. Furthermore, we also evaluated the anticholestatic potential of root extract of *B. lycium* in ANIT induced cholestatic mouse models. The data showed that the serum level of ALT and AST was significantly higher in the ANIT mice group as compared to the control group, which is consistent with the literature (Meng et al., 2021). However, in *B. lycium* treated group, level of these serum enzymes was significantly lower than cholestatic model group. This indicates that the plant extract protects the liver from deleterious effect of ANIT. In order to further investigate the impact of plant extract on the architecture of liver, we also performed the histological studies of the liver. In cholestasis model group the hepatocytes were distorted with severe infiltration of the inflammatory cells indicating a pathological condition. However, in *B. lycium* treatment group the liver architecture was normal with intact hepatocytes. These serological and histological data proves that the root extract of *B. lycium* possess the anticholestatic potential. However, further investigation is required to explore the underlying molecular mechanism for its anticholestatic effect.

ACKNOWLEDGMENTS

We are indebted to Dr. Sohaib Muhammad, Assistant Professor from department of Botany GCU Lahore for identification of the plant.

AUTHOR CONTRIBUTION

Participated in research design: SB, IS. Research experiment was conducted by SB, MF. Data analysis and manuscript writing was performed by SB, HH, NI, A, SFMH, HMMA, AB, IS.

FUNDING

Office of research, innovation and commercialization, GC University Lahore funded this research

CONFLICT OF INTEREST

Authors declared no conflict of interest.

COMPLIANCE WITH ETHICAL STANDARDS

Institutional animal ethical committee approved this study. All handling procedures were performed according to guidelines of institutional ethical committee. Furthermore, all efforts were taken and to minimize the pain and discomfort to the animals.

REFERENCES

Elyasi S, Khalili H, Dashti-Khavidaki S, Mohammadpour A (2012). Vancomycin-induced nephrotoxicity: mechanism, incidence, risk factors and special populations. A literature review. *Eur J Clin Pharmacol*, 68(9), 1243-1255.

Fuchs CD, Paumgartner G, Wahlström A, Schwabl P, Reiberger T, Leditznig N, Marschall H.-U (2017). Metabolic preconditioning protects BSEP/ABCB11^{-/-} mice against

cholestatic liver injury. *Journal of hepatology*, 66(1), 95-101.

Gonzales E, Grosse B, Schuller B, Davit-Spraul A, Conti F, Guettier C, Jacquemin E (2015). Targeted pharmacotherapy in progressive familial intrahepatic cholestasis type 2: Evidence for improvement of cholestasis with 4-phenylbutyrate. *Hepatology*, 62(2), 558-566.

Gupta SK, Agarwal R, Srivastava S, Agarwal P, Agrawal SS, Saxena R, Galpalli N (2008). The anti-inflammatory effects of *Curcuma longa* and *Berberis aristata* in endotoxin-induced uveitis in rabbits. *Invest Ophthalmol Vis Sci*, 49(9), 4036-4040.

Irshad A, Pervaiz A, Abrar Y, Fabelbom I, Awen B (2013). Antibacterial activity of *Berberis lycium* root extract. *Trakia Journal of Sciences*, 11(1), 89.

Jabbarzadeh KP, Rahmat A, Ismail P, Ling KH (2014). Targets and mechanisms of berberine, a natural drug with potential to treat cancer with special focus on breast cancer. *Eur J Pharmacol*, 740, 584-595.

Malkani N, Sohail MI, Ijaz F, Naeem A, Mumtaz S, Saeed Z (2022). *Berberis aristata* reduces vancomycin-induced nephrotoxicity by down-regulation of cell proliferation markers. *Journal of Herbal Medicine*, 31, 100540.

Meng F, Zong W, Wei X, Tao Y, Wang G, Liao Z, Chen M (2021). *Dolomiaea souliei* ethyl acetate extract protected against α -naphthylisothiocyanate-induced acute intrahepatic cholestasis through regulation of farnesoid x receptor-mediated bile acid metabolism. *Phytomedicine*, 87, 153588.

Meng Q, Chen XL, Wang CY, Liu Q, Sun HJ, Sun PY, Liu KX (2015). Alisol

- B 23-acetate protects against ANIT-induced hepatotoxicity and cholestasis, due to FXR-mediated regulation of transporters and enzymes involved in bile acid homeostasis. *Toxicol Appl Pharmacol*, 283(3), 178-186.
- Montanari F, Pinto M, Khunweeraphong N, Wlcek K, Sohail MI, Noeske T, Chiba P, Ecker GF (2016). Flagging Drugs That Inhibit the Bile Salt Export Pump. *Mol Pharm*, 13(1), 163-171.
- Pauli-Magnus C, Stieger B, Meier Y, Kullak-Ublick GA, Meier PJ (2005). Enterohepatic transport of bile salts and genetics of cholestasis. *Journal of hepatology*, 43(2), 342-357.
- Potdar D, Hirwani RR, Dhulap S (2012). Phyto-chemical and pharmacological applications of *Berberis aristata*. *Fitoterapia*, 83(5), 817-830.
- Rafiq S, Ajmal K, Afzal A (2017). Isoniazid induced hepatotoxicity and its amelioration with ethanolic extract of stem bark of *Berberis lycium* Royale in mice. *International Journal of Basic & Clinical Pharmacology*, 6(8), 1865.
- Sohail I, Malkani N, Tahir N, Khalil A, Attar R, Mumtaz S (2022). Silymarin protects the liver from α -naphthylisothiocyanate-induced cholestasis by modulating the expression of genes involved in bile acid homeostasis: Anti-cholestatic effect of silymarin. *Cellular and Molecular Biology*, 68(7), 208-212.
- Sohail MI, Dönmez-Cakil Y, Szöllösi D, Stockner T, Chiba P (2021). The Bile Salt Export Pump: Molecular Structure, Study Models and Small-Molecule Drugs for the Treatment of Inherited BSEP Deficiencies. *Int J Mol Sci*, 22(2).
- Kapoor BBS, Sood P, Modgil R, Sood M (2013). *Berberis lycium* a Medicinal Plant with Immense Value. *Indian journal of pharmaceutical and biological research*, 1(01), 27-37.
- Soren P, Sharma R, Mal G, Singh B, Kumar P, Patil RD, Singh B (2022). Hepatoprotective Activity of *Picrorhiza Kurroa* and *Berberis Lycium* Is Mediated by Inhibition of COX-2 and TGF- β Expression in Lantadenes-Induced sub-chronic toxicity in guinea pigs. *Phytomedicine Plus*, 2(3), 100288.
- Tillhon M, Guamán Ortiz LM, Lombardi P, Scovassi AI (2012). Berberine: new perspectives for old remedies. *Biochem Pharmacol*, 84(10), 1260-1267.
- Trauner M, Fuchs CD, Halilbasic E, Paumgartner G (2017). New therapeutic concepts in bile acid transport and signaling for management of cholestasis. *Hepatology*, 65(4), 1393-1404.