**Edelweiss Applied Science and Technology**
*ISSN: 2576-8484*
*Vol. 9, No. 4, 2530-2536*
*2025*
*Publisher: Learning Gate*
*DOI: 10.55214/25768484.v9i4.6603*
*© 2025 by the authors; licensee Learning Gate*

# Randomised post-test-only study of glutathione and ursodeoxycholic acid combination therapy on liver fibrosis in biliary atresia animal model

Agung Aji Prasetyo[1*], Fransisca Surya[2], Banundari Rachmawati[3], Ignatius Riwanto[4]
[1]Doctoral Study Program of Medical and Health Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia; aaprasetyo82@gmail.com (A.A.P.).
[2]Faculty of Medicine, Pelita Harapan University, Indonesia.
[3]Department of Clinical Pathology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia.
[4]Digestive Surgery, Department of Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia.

**Abstract:** Biliary atresia (BA) is a fatal disease and one of the leading causes of liver transplantation in children. The incidence of BA varies worldwide, ranging from 1 in 5,000 to 18,000 newborns. BA is fatal if left untreated, and hepatic portoenterostomy remains the best treatment option. However, the success of this procedure is related to the degree of fibrosis and the timing of the process. This study aimed to observe the use of a glutathione and ursodeoxycholic acid (UDCA) combination as adjuvant therapy that can be administered to delay hepatic fibrosis before hepatic portoenterostomy in Sprague Dawley rats with biliary atresia. Twenty-eight male Sprague Dawley rats were divided into four groups (n = 7 per group); the control group (C) received 20 mg UDCA, trial group 1 (T1) received 10 mg UDCA and 10 mg glutathione, trial group 2 (T2) received 20 mg UDCA and 15 mg glutathione, and trial group 3 (T3) received 30 mg UDCA and 20 mg glutathione. The rats underwent bile duct ligation to induce cholestasis, and the treatment was administered for 21 days. Liver samples were collected to evaluate α-SMA levels. The combination of glutathione and UDCA downregulated α-SMA expression in a dose-dependent manner ($p = 0.009$). The post-hoc test results showed a lower level of α-SMA expression in group T3 than in groups C ($p = 0.002$) and T1 ($p = 0.005$). We conclude that the combination of glutathione and UDCA significantly decreases α-SMA levels in the BA rat model.

***Keywords:*** *Biliary atresia, Glutathione, UDCA, α-SMA.*

## 1. Introduction

Biliary atresia (BA) is a progressive fibro-obliterative cholangiopathy, a disorder of biliary secretion that causes the accumulation of bile in the body, resulting in jaundice [1]. It is a fatal disease that affect untreated children. BA is closely related to end-stage chronic liver disease, as the process develops into cirrhosis [2]. The incidence of BA varies across the world. In France, the incidence was 1 in 19,500 live births, 1 in 16,700 live births in the UK; and Ireland, and 1 in 14,000 live births in Sweden. In Asian countries, such as Japan, the incidence was 1 in 9640 live births. The worldwide incidence of BA ranges from 1 in 5000 to 18,000 newborns [3].

Biliary atresia (BA) is the primary indication for liver transplantation in children. Hepatic portoenterostomy (Kasai) remains the best treatment option for neonates with biliary atresia. The Kasai procedure increased the long-term survival of children with BA. However, the effectiveness of the procedure is significantly higher when performed early, before the age of 2 months, than after two months. In patients older than two months, adequate bile drainage was observed in only <10% of the patients [2, 3].

The success rate of the Kasai procedure is thought to be linked to the degree of liver fibrosis before the procedure. Pathological changes in BA affect the intrahepatic biliary tree, where the degree of

*© 2025 by the authors; licensee Learning Gate*
**History:** Received: 11 February 2025; Revised: 16 April 2025; Accepted: 21 April 2025; Published: 26 April 2025
* Correspondence: aaprasetyo82@gmail.com

fibrosis and damage is responsible for an increased morbidity rate after the Kasai procedure [2]. However, the Kasai procedure does not completely eliminate the need for liver transplantation in patients BA. The Kasai procedure has been reported to provide temporary relief and is considered a bridge to eventual liver transplantation. Many children with BA and successful hepatic portoenterostomy will eventually require liver transplantation within ten years [4].

Cholestasis occurs in many human liver diseases owing to impaired bile formation, secretion, or flow. In addition to immune-mediated diseases, such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis, this can also occur due to mechanical factors, such as bile duct obstruction or scarring, as seen in biliary atresia. Genetic factors include mutations in the bile salts and phospholipid transporters. It is believed that the retention of hydrophobic bile acids underlies liver damage, which can result in cell death, fibrosis, and cirrhosis [5, 6].

Although it has been shown that early liver fibrosis can be reversed, the precise process remain unknown, and liver fibrosis is not currently an effective treatment. Thus, studying and creating anti-fibrotic medications remains a major priority. Anti-inflammation and liver protection, inhibition of hepatic stellate cell' (HSCs') activation and proliferation, reduction of ECM overproduction, and expedited ECM degradation are just a few strategies that have recently come to light as important ways to prevent the occurrence and progression of liver fibrosis.

α-SMA (alpha-smooth muscle actin) is an actin isoform expressed by the hepatic stellate cells (HSCs). Activated HSCs increase fibrillar collagen production, the primary cell type for matrix production, in damaged liver tissue. Therefore, α-SMA overexpression can be used to identify the extent of liver fibrosis [7].

Ursodeoxycholic acid (UDCA) is the only drug approved by the US Food and Drug Administration for the treatment of cholestatic liver disease. UDCA functions by eliminating hydroxyl radicals and inducing endogenous oxidation resistance, including by elevating the expression of γ-glutamylcysteine synthetase regulatory subunits and promoting glutathione (GSH) synthesis [8].

Hence, this study aimed to determine whether the combination of Glutathione and UDCA could act as an adjuvant therapy before the Kasai procedure, which would delay the degree of hepatic fibrosis.

## 2. Material and Methods

### *2.1. Animal Treatment*

Twenty-eight male Sprague Dawley rats weighing 100-200 g and aged 6-8 weeks were obtained from Institut Biosains in Malang, housed in a controlled environment, and fed standard rodent chow and water ad libitum. We performed sterile midline laparotomy and ligated the common bile duct with a 3-0 silk suture (DemeTECH, Miami Lakes, FL, USA). The common bile duct was ligated to mimic biliary atresia. The rats were administered 18 mg cefotaxime (Indofarma, Jakarta, Indonesia) intramuscularly before surgery for prophylactic antibiotics and ketamine hydrochloride (0.5 mL) intramuscularly (Dexa Medica, Cikarang, Indonesia). To relieve pain after surgery, 7 mg of ibuprofen (Pharos, Semarang, Indonesia) was administered orally every 8 hours for three days.

Human dose for UCDA (Dexa Medica) was 8-25 mg/kg and converted to a rat dose of 10-30 mg. Glutathione (Sigma Aldrich, St. Louis, MO, USA) was administered at doses of 10, 15, or 20 mg, and the human dose of Glutathione was 600-1200 mg per day, which was converted to a rat dose of 10-20 mg.

Twenty-eight rats were randomly divided into four groups, each of which contained seven rats who had previously undergone bile duct ligation to induce cholestasis: Group C received 20 mg UDCA supplement; group T1 received 10 mg UDCA and 10 mg glutathione supplement; group T2 received 20 mg UDCA and 15 mg glutathione supplement; and group T3 received 30 mg UDCA and 20 mg glutathione supplement. UDCA was administered once daily through an oral gastric tube (7 Gauge feeding tube) that was inserted daily and removed after supplementation. Meanwhile, Glutathione was administered intramuscularly daily. Each treatment lasted for 21 days. This study was approved by the

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 9, No. 4: 2530-2536, 2025*
*DOI: 10.55214/25768484.v9i4.6603*
*© 2025 by the authors; licensee Learning Gate*

Research and Ethics Committee of the Faculty of Medicine, Diponegoro University, Indonesia (protocol number: 84/EC/H/FK-UNDIP/VIII/2021) and fully compliant with ARRIVE criteria [9].

*2.2. α-SMA Expression Test*

The expression of α-SMA in the liver tissue was assessed using α-SMA-positive markers and immunofluorescence. On day 22, the liver tissue from each animal was fixed in 10% formaldehyde, paraffin-embedded, sectioned, and deparaffinized. Following the manufacturer's instructions, the tissues were incubated with anti-SMA primary antibody (LSBio, Seattle, USA) to examine the tissues [10].

*Statistical analysis*

All data were evaluated using SPSS for Windows Software. The results are expressed as the mean ± standard deviation. The Shapiro-Wilk test was used for data normality. The data were compared using the *Kruskal-Wallis* test, followed by *Mann-Whitney* test. Statistical significance was defined as $P < 0.05$.

## 3. Results

*Saphiro-Wilk* normality test revealed that the data did not have a normal distribution ($p < 0.05$). The combination of Glutathione and UDCA downregulated the expression of α-SMA in a dose-dependent manner ($p = 0.009$) (Table 1). The combination of 30 mg UDCA and 20 mg glutathione significantly decreased α-SMA expression compared to UDCA alone. We found a significant reduction in α-SMA levels in combination with 30 mg UDCA and 20 mg glutathione compared to UDCA alone and low-dose combination of UDCA and glutathione. (Table 2)

**Table 1.**
Analysis of α-SMA levels in all groups.

| Parameter | Group | Min | Max | Mean ± SD | P |
|---|---|---|---|---|---|
| α-SMA | 1 (C) | 0.954 | 35.026 | 16.40 ± 14.72 | 0.009* |
| | 2 (T1) | 2.134 | 27.995 | 9.05 ± 8.85 | |
| | 3 (T2) | 0.155 | 9.333 | 4.83 ± 3.95 | |
| | 4 (T3) | 0.004 | 6.751 | 1.26 ± 2.46 | |

**Note:** *Significant using Kruskal-Wallis test ($p < 0.05$).

**Table 2.**
Difference in α-SMA levels across each trial group.

| Group | 1 (C) | 2 (T1) | 3 (T2) | 4 (T3) |
|---|---|---|---|---|
| 1 (C) | - | 0.795 | 0.194 | 0.002* |
| 2 (T1) | - | - | 0.298 | 0.005* |
| 3 (T2) | - | - | - | 0.079 |

**Note:** * Significant, Mann-Whitney test ($p < 0.05$).

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 9, No. 4: 2530-2536, 2025*
*DOI: 10.55214/25768484.v9i4.6603*
*© 2025 by the authors; licensee Learning Gate*

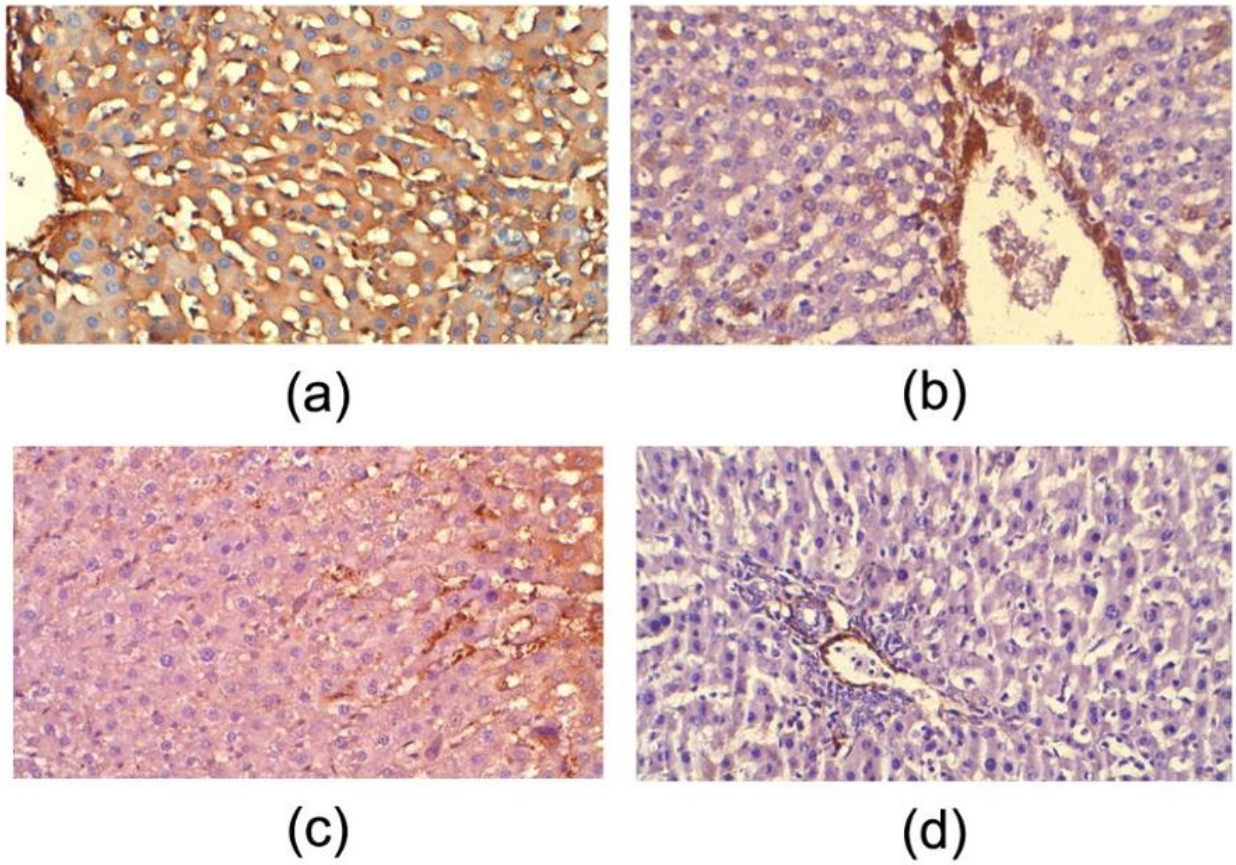


**Figure 1.**
Anatomical pathology results of Liver Fibrosis animal model in this study. (a) Control group (C) received 20 mg UDCA; (b) Trial group 1 (T1) received 10 mg UDCA and 10 mg Glutathione; (c) Trial group 2 (T2) received 20 mg UDCA and 15 mg Glutathione; (d) Trial group 3 (T3) received 30 mg UDCA and 20 mg Glutathione.

## 4. Discussion

Our study highlights the potential benefit of combining Glutathione and UDCA in the management of biliary atresia to delay liver fibrosis by downregulating α-SMA expression. Liver injury activates hepatic stellate cells (HSC) [11] and increases α-SMA levels [12]. As a defense mechanism against inflammation, stellate cells multiply and release growth factors, cytokines, type I and type IV collagen, laminin, and heparan sulfate. The early stages of hepatic fibrogenesis involve changes in sinusoidal structures, which are influenced by stellate cells. Hepatic fibrosis is closely linked to stellate cell differentiation and proliferation. It has been demonstrated that these cells express α-SMA. Although certain liver stellate cells expressing α-SMA are normal, chronic hepatitis greatly increases α-SMA expression owing to activated stellate cells [13].

This result correlates with another study that used UDCA in chronic cholestatic disorders, where UDCA serves as a non-toxic source of bile acids that protects the liver and slows down the progression of the disease caused by toxic bile acids that accumulate due to chronic cholestatic disorders. UDCA administration improved histological features and delayed cirrhosis progression before liver transplantation. Compared to the control group that only received glutathione, UDCA administration delayed progression to cirrhosis [14].

UDCA administration also improves transplant-free survival in primary biliary cholangitis (PBC) [15]. Although UDCA is not widely used in the treatment of BA, it remains the only approved

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 9, No. 4: 2530-2536, 2025*
*DOI: 10.55214/25768484.v9i4.6603*
*© 2025 by the authors; licensee Learning Gate*

treatment for PBC. UDCA has been shown to extend patient survival without the need for liver transplantation in PBC patients, improve serum hepatic biochemistries, delay progression of histological damage, and delay the development of esophageal varices [16]. UDCA works by inhibiting intestinal absorption and increasing secretion of bile acids; this protects hepatocytes because accumulation of bile acids creates an inflammatory state that causes necrosis and apoptosis of the cells [17]. The role of UDCA in combination with corticosteroids as a therapy for autoimmune hepatitis and cholestatic liver disease was evaluated in a previous study. UDCA does not eliminate the etiological agent; however, it has properties that give anti-inflammatory or anti-fibrotic effects in chronic cholestatic liver disease [18]. Hepatocyte damage caused by cholestasis has been treated with a combination of UDCA and S-adenosyl-l-methionine (SAMe), and using both regimens together improves clinical results [19]. SAMe is crucial for methylation during the manufacture of synthesis [20]. GSH level in HepG2 cells was also increased by UDCA treatment [21].

Owing to the accumulation of toxic bile acids, cell apoptosis occurs during bile duct ligation, and UDCA protects against apoptosis induced by toxic bile acids. The effects of UDCA are not limited to protection against cell apoptosis, but also increase glutathione levels above baseline [22].

Glutathione is the main non-protein thiol that is critical for maintaining cell functions. Glutathione works against oxidative stress, modulating cell growth and death, inflammatory responses, and even hepatic fibrogenesis [22]. Bile duct ligation was performed to imitate biliary atresia, where toxic bile acids accumulate in the body, causing oxidative stress and apoptosis that leads to fibrosis and later cirrhosis. Another study showed that chronic retention of bile acids causes a decrease in GSH levels, which in turn lowers antioxidant defenses, and the cells can be easily injured. Depletion of hepatic glutathione level is commonly observed in patients with liver disease due to dysregulation of glutathione synthesis during chronic cholestasis [21]. This would lead to the administration of glutathione, which would help to prevent liver damage due to oxidative stress caused by biliary atresia.

Recent studies have shown that a decrease in glutathione levels may initiate a pathway that results in bile duct damage and obstruction via several gene pathways. Hence, administering glutathione to patients with BA would help delay the obstruction pathway and disease progression [23]. Glutathione has been linked to liver protection against oxidative stress from drugs, environmental toxicants, and selected dietary components [24].

A combination of Glutathione and UDCA proved to be a good candidate for therapy in biliary atresia to prevent liver fibrosis progression, and further study on the clinical setting and outcome of the patient could be a promising candidate for therapy.

## 5. Conclusion

Administration of glutathione in combination with UDCA significantly decreased α-SMA levels in cholestasis.

## Institutional Review Board Statement:

This study was approved by the Research and Ethics Committee of the Faculty of Medicine, Universitas Diponegoro, Indonesia (protocol number:84/EC/H/FK-UNDIP/VIII/2021).

## Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 9, No. 4: 2530-2536, 2025*
*DOI: 10.55214/25768484.v9i4.6603*
*© 2025 by the authors; licensee Learning Gate*

## Acknowledgement:
All authors would like to thank all the staff members at the Central Laboratory, Faculty of Medicine, Universitas Diponegoro. First author was the recipient of a Universitas Diponegoro Scolarship for Doctoral Stusy Program of Medical and Health Science. All authors also would like to thank Fitria Novitasari from the Institut Biosains Malang dan Fikar Arsyad Hakim from the Pathology Anatomy Department, Universitas Negeri Surakarta, for their support during the study.

## Copyright:
© 2025 by the authors. This open-access article is distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

## References
[1] J. Cazares, B. Ure, and A. Yamataka, *Biliary atresia. In G. W. Holcomb III, J. P. Murphy, & S. D. St. Peter (Eds.), Holcomb and Ashcraft's Pediatric Surgery*, 7th ed. Philadelphia, PA: Elsevier, 2020.

[2] M. J. De Aro Braz *et al.*, "Analysis of the reversibility of biliary cirrhosis in young rats submitted to biliary obstruction," *Journal of Pediatric Surgery*, vol. 53, no. 7, pp. 1408-1413, 2018. https://doi.org/10.1016/j.jpedsurg.2018.02.016

[3] W. S. Lee *et al.*, "Chronic liver disease is universal in children with biliary atresia living with native liver," *World Journal of Gastroenterology*, vol. 23, no. 43, p. 7776, 2017. https://doi.org/10.3748/wjg.v23.i43.7776

[4] R. D'Souza *et al.*, "Acute-on-chronic liver failure in children with biliary atresia awaiting liver transplantation," *Pediatric Transplantation*, vol. 23, no. 2, p. e13339, 2019. https://doi.org/10.1111/petr.13339

[5] H. Samant *et al.*, "Cholestatic liver diseases: An era of emerging therapies," *World Journal of Clinical Cases*, vol. 7, no. 13, p. 1571, 2019. https://doi.org/10.12998/wjcc.v7.i13.1571

[6] E. Sticova, M. Jirsa, and J. Pawłowska, "New insights in genetic cholestasis: from molecular mechanisms to clinical implications," *Canadian Journal of Gastroenterology and Hepatology*, vol. 2018, no. 1, p. 2313675, 2018. https://doi.org/10.1155/2018/2313675

[7] G. Carpino *et al.*, "Alpha-SMA expression in hepatic stellate cells and quantitative analysis of hepatic fibrosis in cirrhosis and in recurrent chronic hepatitis after liver transplantation," *Digestive and Liver Disease*, vol. 37, no. 5, pp. 349-356, 2005. https://doi.org/10.1016/j.dld.2004.11.009

[8] X. Dong *et al.*, "Ursodesoxycholic acid alleviates liver fibrosis via proregeneration by activation of the ID1-WNT2/HGF signaling pathway," *Clinical and Translational Medicine*, vol. 11, no. 2, p. e296, 2021. https://doi.org/10.1002/ctm2.118

[9] C. Kilkenny, W. J. Browne, I. C. Cuthill, M. Emerson, and D. G. Altman, "Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research," *Journal of Pharmacology and Pharmacotherapeutics*, vol. 1, no. 2, pp. 94-99, 2010. https://doi.org/10.1371/journal.pbio.1000412

[10] A. Putra *et al.*, "MSC-released TGF-β regulate α-SMA expression of myofibroblast during wound healing," *Journal of Stem Cells & Regenerative Medicine*, vol. 16, no. 2, p. 73, 2020. https://doi.org/10.46582/jsrm.1602011

[11] J. E. Puche, Y. Saiman, and S. L. Friedman, "Hepatic stellate cells and liver fibrosis," *Compr Physiol*, vol. 3, no. 4, pp. 1473-1492, 2013. https://doi.org/10.1002/cphy.c120035

[12] A. Ahmad and R. Ahmad, "Understanding the mechanism of hepatic fibrosis and potential therapeutic approaches," *Saudi Journal of Gastroenterology*, vol. 18, no. 3, pp. 155-167, 2012. https://doi.org/10.4103/1319-3767.96445

[13] N. Akpolat, S. Yahsi, A. Godekmerdan, M. Yalniz, and K. Demirbag, "The value of α-SMA in the evaluation of hepatic fibrosis severity in hepatitis B infection and cirrhosis development: A histopathological and immunohistochemical study," *Histopathology*, vol. 47, no. 3, pp. 276-280, 2005. https://doi.org/10.1111/j.1365-2559.2005.02226.x

[14] U. Beuers, M. Trauner, P. Jansen, and R. Poupon, "New paradigms in the treatment of hepatic cholestasis: From UDCA to FXR, PXR and beyond," *Journal of Hepatology*, vol. 62, no. 1, pp. S25-S37, 2015. https://doi.org/10.1016/j.jhep.2015.02.023

[15] A. C. Cheung *et al.*, "Combined ursodeoxycholic acid (UDCA) and fenofibrate in primary biliary cholangitis patients with incomplete UDCA response may improve outcomes," *Alimentary Pharmacology & Therapeutics*, vol. 43, no. 2, pp. 283-293, 2016. https://doi.org/10.1111/apt.13465

[16] E. J. Carey, A. H. Ali, and K. D. Lindor, "Primary biliary cirrhosis," *The Lancet*, vol. 386, no. 10003, pp. 1565-1575, 2015.

[17] Y. Zhang *et al.*, "Combination therapy of fenofibrate and ursodeoxycholic acid in patients with primary biliary cirrhosis who respond incompletely to UDCA monotherapy: A meta-analysis," *Drug Design, Development and Therapy*, vol. 9, pp. 2757-2766, 2015.

[18] A. J. Czaja, "Hepatic inflammation and progressive liver fibrosis in chronic liver disease," *World Journal of Gastroenterology: WJG*, vol. 20, no. 10, pp. 2515–2532, 2014.
[19] S. C. Lu, "Dysregulation of glutathione synthesis in liver disease," *Liver Research*, vol. 4, no. 2, pp. 64-73, 2020.
[20] S. C. Lu and J. M. Mato, "S-adenosylmethionine in liver health, injury, and cancer," *Physiological Reviews*, vol. 92, no. 4, pp. 1515-1542, 2012.
[21] S. Arisawa *et al.*, "Ursodeoxycholic acid induces glutathione synthesis through activation of PI3K/Akt pathway in HepG2 cells," *Biochemical Pharmacology*, vol. 77, no. 5, pp. 858-866, 2009.
[22] H. Yang *et al.*, "Dysregulation of glutathione synthesis during cholestasis in mice: molecular mechanisms and therapeutic implications," *Hepatology*, vol. 49, no. 6, pp. 1982-1991, 2009.
[23] S. Fried, D. Gilboa, A. Har-Zahav, P. M. Lavhut, Y. Du, and S. Karjoo, "Extrahepatic cholangiocyte obstruction is mediated by decreased glutathione, Wnt and Notch signaling pathways in a toxic model of biliary atresia," *Scientific Reports*, vol. 10, pp. 1–10, 2020.
[24] Y. Chen, H. Dong, D. Thompson, H. Shertzer, D. Nebert, and V. Vasiliou, "Glutathione defense mechanism in liver injury: Insights from animal models," *Food and Chemical Toxicology*, vol. 60, pp. 38-44, 2013.

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 9, No. 4: 2530-2536, 2025*
*DOI: 10.55214/25768484.v9i4.6603*
*© 2025 by the authors; licensee Learning Gate*