

Ameliorative Potential of Silymarin on Serum Liver Function Indices on CCl₄-Induced Hepatotoxicity in Albino Wistar Rat Model

JOHNSON, J. T.¹, ALBERT, M. C.²

^{1,2}Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria.

ORCID: 0000-0003-3829-8116

Abstract- This study investigated the impact of silymarin on serum liver function markers in albino Wistar rats with liver damage induced by carbon tetrachloride (CCl₄). Hepatocellular carcinoma (HCC), a common type of liver disease, involves damage to hepatocytes the primary liver cells often driven by genetic and epigenetic alterations that cause abnormal cell proliferation and evasion of programmed cell death. Fifteen albino Wistar rats weighing between 60 and 130g were divided into three groups of five rats each: the first group received distilled water and served as the normal control; the second was exposed to CCl₄ to induce HCC but remained untreated as a positive control; the third group was induced with HCC using CCl₄ and treated with silymarin. The treatment spanned 31 days, after which the rats were euthanized 12 hours following the last dose, and blood samples were collected via cardiac puncture. Key liver function indicators measured from the serum included; Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Total Bilirubin, and Conjugated Bilirubin. The results showed that silymarin treatment significantly ($p < 0.05$) lowered the levels of the serum liver markers compared with the positive control group, whose liver function test (LFT) values were significantly ($p < 0.05$) elevated relative to the animals in the normal control group. These findings are consistent with previous studies on various bioactive phytochemical components also found in silymarin, which demonstrate antioxidative, anti-inflammatory, and hepatocyte-protective effects. Conclusively, silymarin effectively restored liver function parameters towards normal levels, indicating its capability to protect against hepatocellular insults and support liver biochemical integrity restoration. Thus, silymarin shows promise as a natural agent for mitigating liver injury caused by toxic substances, reaffirming it as hepatoprotective agent as well as its therapeutic potential in liver disease management.

Keywords: Silymarin, Carbon-tetrachloride, Hepatotoxicity, Antioxidant-therapy, Hepatoprotection

I. INTRODUCTION

Chronic liver diseases constitute a significant global health concern due to their progressive nature, resulting in conditions such as steatosis, hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Loguercio and Federico, 2003; Vitalione et al., 2004). Hepatocellular Carcinoma ranks as the fifth most common cancer worldwide and is the second leading cause of cancer-related death among patients with liver cirrhosis (Jemal et al., 2011). The liver is fundamental to various physiological functions including protein synthesis, detoxification, and metabolism of xenobiotics and nutrients (Lu et al., 2016; Yang et al., 2015; Lee et al., 2007). Due to its role as the body's detoxification center, the liver is particularly susceptible to injury by toxic compounds consumed or introduced into the body, which affects quality of life and overall health (Lee, 2003).

Silymarin, a flavonolignan complex extracted from the seeds of milk thistle (*Silybum marianum*), has been used traditionally for its hepatoprotective properties. Composed mainly of silybin, silidianin, and silychristine with silybin being the most biologically active it is clinically utilized for treating liver disorders such as viral hepatitis, toxin/drug-induced hepatitis, cirrhosis, and alcoholic liver disease, with additional efficacy reported in some cancers. The protective effects of silymarin include inhibiting hepatotoxin binding to hepatocyte membranes, reducing glutathione oxidation and enhancing intracellular glutathione levels in the liver and intestines, exerting antioxidant actions, and stimulating ribosomal RNA polymerase to promote protein synthesis and hepatocyte regeneration (Kwon et al., 2017; Li et al., 2012). Despite oral absorption, silymarin's bioavailability is limited due to poor water solubility (Jaffar, 2024).

The hepatoprotective mechanisms of silymarin are manifold; it stabilizes membrane permeability by preventing lipid peroxidation, thereby helping maintain glutathione, the liver's key antioxidant. It mitigates inflammatory responses by inhibiting production of cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin (IL)-2, IL-4, and interferon-gamma via suppression of nuclear factor kappa B (NF- κ B) activation (Li et al., 2012; Gharagozloo et al., 2020; Trappoliere et al., 2019). Furthermore, silymarin reduces cellular uptake of xenobiotics including toxins from poisonous mushrooms by blocking transporter proteins and regulates inflammation by modulating leukotriene synthesis pathways (Faulstich et al., 2018; El-bahay et al., 2019; Morazzoni et al., 2023). The primary active component silibinin exerts potent antioxidant effects, scavenging free radicals that cause cellular damage (Smaller et al., 2017). Besides flavonolignans like silibinin, silymarin also contains flavonoids such as quercetin and taxifolin, contributing to its antioxidative and immunomodulatory activity (Javed et al., 2011; Surai, 2015).

Preclinical studies have demonstrated silymarin's antioxidant and cell-regenerative capabilities, largely through facilitating protein synthesis critical for repairing damaged hepatocytes (Schulz et al., 2015). Its antioxidant properties protect against reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, hydrogen peroxide, and lipid peroxide, which induce polyunsaturated fatty acid peroxidation in cell membranes, followed by damage to DNA, RNA, and proteins (Wachter and Zaeske, 2019; Hawke et al., 2020). Silymarin's ability to stabilize lipid membrane components contributes to preserving cellular integrity and function (Glory et al., 2018).

In addition, silymarin stimulates ribosomal formation by activating RNA polymerase within the nucleus, enhancing DNA and protein synthesis needed for restoring normal liver function (Lorenz et al., 2014). Its anti-inflammatory action involves inhibiting the 5-lipoxygenase pathway, suppressing leukotriene B4 synthesis, and selectively modulating NF- κ B dependent gene expression (Morazzoni et al., 2023; Lorenz et al., 2022; Schandalik et al., 2022). Importantly, silymarin hampers hepatic stellate cell activation and fibrogenesis, slowing liver fibrosis progression that leads to hepatic insufficiency and

portal hypertension (Doehmer et al., 2017; NMCD, 2018).

This study aimed at assessing the effect of Silymarin on Serum Liver Function Indices of Albino Wistar Rat with CCl₄ induced liver damage. Chloroform-induced hepatotoxicity results from its enzymatic metabolism mediated notably by cytochrome P450 isoenzyme CYP2E1, which generates reactive metabolites causing oxidative stress and cellular damage. This enzyme is inducible by alcohol, compounding liver injury in alcoholics exposed to CCl₄ (Stoyanovsky and Cederbaum, 2018; Raucy et al., 2021; Peterson et al., 2020). Due to the high prevalence of CCl₄-related liver damage, there is a critical need to evaluate effective treatment regimens. However, scientific data about silymarin's protective effect on liver function under CCl₄ toxicity remain limited, warranting further investigations. Although numerous reports highlight silymarin's hepatoprotective role, there is insufficient scientific evidence supporting its efficacy in toxic liver damage contexts such as CCl₄ exposure. This study provides empirical data to substantiate silymarin's protective role and contribute valuable insight into its use in liver damage management.

II. METHODOLOGY

EXPERIMENTAL ANIMALS

Fifteen (15) Albino rats of the Wistar strain with weight range of 80-130g were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. They were housed, and cared for following the standard rules and regulations of The Institute for Laboratory Animal Research (ILAR). The animals were allowed to acclimatize for a period of 7 days at the Federal University Otuoke's animal house. They were kept in plastic cages with wire mesh covers to aid ventilation. The animals were under monitored environmental conditions of temperature ($28 \pm 2^\circ\text{C}$), relative humidity ($50 \pm 5\%$) and 12-hour light/dark cycle. The animal facility was properly ventilated and the animals were placed on commercial rat pellet as feed and water *ad libitum* throughout the experimental period.

EXPERIMENTAL DESIGN AND TREATMENT OF ANIMALS

Administration of treatment was done twice daily for a period of one month (31) days via orogastric intubation as described in table 1. The experimental

design employed comprised 15 wistar rats of albino strain divided into 3 groups of 5 animals each. Group A was the normal control group and the animals in this group received only distilled water. Group B was the positive control group, it was induced with HCC using CCl₄ and the animals in the group received distilled water. Animals in Group C were also induced with HCC using CCl₄ and was treated with 0.26mg/Kgbw of silymari 0.5ml of DMSO and distill water.

COLLECTION OF BLOOD SAMPLES FOR ANALYSIS

The animals were euthanized 12 hours after the last treatment. Whole blood was collected from the heart via cardiac puncture using a sterile syringe and needle. The blood samples were collected into plain tubes. The blood sample in the plain tubes were allowed to clot by standing for 2 hours at room temperature then later centrifuged at 10000rpm for 10 minutes to separate the serum from the red blood cells. Sera from each centrifuged plain tube were collected into another plain tube labeled accordingly using Pasteur pipettes. The separate sera were then kept frozen in a refrigerator until when needed for various biochemical assays.

BIOCHEMICAL ASSAY

All biochemical assays were carried out based on standard biochemical methods using standard assay kits and biochemical analyzers.

Aspartate Transaminase (AST)

Principle and Method

Aspartate transaminase activity was determined according to the method of Tietz, 1986. The AST reagent is used to measure AST activity by an enzymatic rate method (1,2). In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and a-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with the concurrent oxidation of B-nicotinamide adenine dinucleotide reduced form (NADH) to Nicotinamide Adenine Dinucleotide (NAD). The sample is mixed with the AST reagent. The ratio used is 1 part to 11 parts reagent. The mixture is put in a cuvette into a spectrophotometer and at a wavelength of 340m, the absorbance is read after 1 min, 2 mins and 3 mins. The rate of change in absorbance over this period of

time is directly proportional to the activity of AST in the sample.

Alanine Transaminase (ALT)

Principle and Method

Serum Alanine amino Transferase activities was estimated as described by Tietz, 1995. The ALT reagent is used to measure the ALT activity in serum by a kinetic rate method. In the reaction, ALT catalyzes the reversible transamination of L-alanine and a-ketoglutarate to pyruvate and L-glutamate. Pyruvate is then reduced to lactate in the presence of lactate dehydrogenase with concurrent oxidation of NADH to NAD. The sample is mixed with the ALT reagent. The ratio used is 1 part to 11 parts reagent. The mixture is put in a cuvette into a spectrophotometer and at a wavelength of 340m, the absorbance is read after 1 min, 2 mins and 3 mins. The rate of change in absorbance over this period of time is directly proportional to the activity of ALT in the sample.

Alkaline Phosphatase (ALP)

Principle and Method

Serum Alkaline Phosphatase activity was determined as described by Tietz, 1995. ALP catalyzes the formation of 4-Nitrophenyl phosphate (4-NPP) with the formation of free 4-nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate group acceptor. The reaction is monitored kinetically at 405nm by the rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample. The ALP reagent and sample should be pre-incubated to reaction temperature. 1.0 mL of working (ALP) reagent and 20 uL of the sample is added to the cuvette and mixed gently. At a wavelength of 405nm, the absorbance of the mixture is read at 1, 2 and 3 minutes. The rate of change in absorbance over this period of time is directly proportional to the activity of ALP in the sample.

Bilirubin

Principle and Method

Total and direct bilirubin levels were estimated according to the method of Jendrassik and Grof (1938). Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue colored complex. Total bilirubin is determined in the presence of caffeine, which

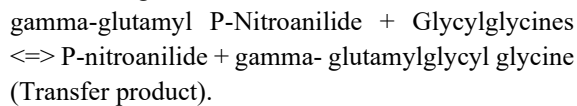
releases albumin bound bilirubin, by the reaction with diazotised sulphanilic acid. High levels of unconjugated bilirubin indicate that too much hemoglobin is being destroyed or that the liver is not actively treating the hemoglobin it is receiving.

Four reagents are mixed with the sample and subjected to light at wavelength of 578m. A sample blank (mixed with three of the four reagents is also subjected to light at 578m and the values obtained from the spectrophotometer are used to calculate the total bilirubin level. For direct bilirubin, the wavelength used is 546m and the two reagents are used with NaCl solution. Indirect bilirubin is calculated from the difference between total and direct bilirubin.

Gamma Glutamyl transferase

Principle and Method

Gamma glutamyl aminotransferase catalyzes the transfer of gamma-glutamyl group from peptides or peptide like compounds to an acceptor peptide molecule. Eg.



STATISTICAL ANALYSIS

Data obtained from the experiment were analyzed using one-way analysis of variance (ANOVA) using statistical package for social sciences version 23 (SPSS 23) and presented as means \pm standard error of mean (SEM). Values at ($p < 0.05$) were regarded as significant in comparison with the appropriate controls.

III. RESULTS

EFFECT OF TREATMENT ON BIOCHEMICAL PARAMETERS

The effect of silymarin on some biochemical indices of liver function was evaluated and the results are presented in table 2.

Alanine Amino Transferase Activity (ALT)

Laboratory results showed that ALT activity for group II (PC) (74.00 ± 0.32) animals was significantly

($p < 0.05$) higher than the normal control group or group I (16.60 ± 0.40). However, the results showed that ALT activity for group III animals (41.80 ± 0.20) was significantly ($p < 0.05$) lower when compared with the animals in the positive control group (74.00 ± 0.32) and the normal control group (16.60 ± 0.40).

Aspartate Amino Transferase Activity (AST)

Results shows that AST activity (iu/l) of animals in the positive control group (80.80 ± 0.20) and in the test group or group III (59.20 ± 0.58) were significantly ($p < 0.05$) higher than the normal control group (22.20 ± 0.49). Aspartate amino transferase activity for animals in group III (59.20 ± 0.58) was however, significantly ($p < 0.05$) lower compared with that of group II (80.80 ± 0.20).

Alkaline Phosphatase Activity (ALP)

After analysis of the blood samples of all the animals in the three groups, results revealed that ALP activity of animals in group II (PC) (249.60 ± 0.11) was significantly ($p < 0.05$) lower than that of animals in group I (NC) (146.60 ± 0.93). For animals in group III, ALP activity (189.80 ± 1.07) was significantly ($p < 0.05$) lower than ALP activity for both the normal control group (146.60 ± 0.93) and the positive control group (249.60 ± 0.51).

Total Bilirubin

Total bilirubin levels for the positive control group or group II (21.98 ± 0.11) was significantly ($p < 0.05$) higher than TB levels for animals in group I or normal control (7.56 ± 0.60) which is also the normal control group. Group III TB levels' obtained results (13.86 ± 0.08) was significantly ($p < 0.05$) lower than that of both the positive control group (21.98 ± 0.11) and the normal control group (7.56 ± 0.60).

Conjugated Bilirubin

As obtained from laboratory analysis results, conjugated bilirubin levels for the positive control group (13.06 ± 0.09) was significantly ($p < 0.05$) than CB results for the normal control group or group I (2.48 ± 0.14). For the test group (group III) results obtained showed that CB levels of animals in the group (7.68 ± 0.15) was significantly ($p < 0.05$) lower than that of the positive control group (13.06 ± 0.09).

Table 1: Experimental Design and Schedule of Treatments

Groups	Number of animals	Administration/Treatment
--------	-------------------	--------------------------

A (Normal Control)	5	Distilled water
B (Positive Control)	5	Distilled water
C (TEST)	5	0.26mg/Kgbw of silymarin

Table 2: Effect of treatment on LFT parameters of the albino Wistar rats

Parameter	Group I (Normal)	Group II (CCl4)	Group III (CCl4 + Silymarin)
AST (IU/L)	22.2 ± 0.49	80.8 ± 0.20	59.2 ± 0.58
ALT (IU/L)	16.6 ± 0.40	74.0 ± 0.32	41.8 ± 0.20
ALP (IU/L)	146.6 ± 0.93	249.6 ± 0.51	189.8 ± 1.07
Total Bilirubin (µmol/L)	7.56 ± 0.60	21.98 ± 0.11	13.86 ± 0.08
Conjugated Bilirubin (µmol/L)	2.48 ± 0.14	13.06 ± 0.09	7.68 ± 0.15

Values are expressed as: Mean ± standard error of mean (SEM), n=5

* = significant at p<0.05 compared with group 1: (NC), a = significant at p<0.05 compared with group 2: (PC), Key: NC = Normal Control, PC = Positive Control, AST = aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, TB = Total Bilirubin, CB = Conjugated Bilirubin.

IV. DISCUSSION

The hepatotoxic effects of CCl₄ are mediated largely through free radical formation, leading to lipid peroxidation, inflammation, and apoptosis in liver tissue (Geraniol et al., 2024). Elevated serum transaminases (AST, ALT) and ALP reflect liver cell necrosis and bile duct injury, whereas increased bilirubin indicates impaired bilirubin clearance (Mekonnen et al., 2022; Scielo.br, 2024). Silymarin treatment significantly reversed these biochemical abnormalities, substantiating its antioxidative mechanism by scavenging free radicals and inhibiting lipid peroxidation (JBclinPharm, 2024). Additionally, silymarin modulates key intracellular signaling pathways that reduce inflammation, fibrogenesis, and promote hepatocyte regeneration and repair (Dhande et al., 2024; JBclinPharm, 2024). These mechanisms align with recent meta-analyses and clinical trials supporting silymarin's efficacy and safety in managing liver diseases, including alcoholic liver disease and non-alcoholic fatty liver disease (homoeopathicjournal.com, 2025). The results affirm silymarin's potential as an adjunct therapy in chronic liver disease management, with the need for further clinical trials to optimize dosing and long-term outcomes (Dhande et al., 2024; homoeopathicjournal.com, 2025).

V. CONCLUSION

This study demonstrates that silymarin effectively attenuates liver dysfunction caused by CCl₄ toxicity in albino Wistar rat model, restoring key liver function biomarkers. Its multifaceted antioxidant, anti-inflammatory, and regenerative actions make it a promising therapeutic agent for hepatoprotection. Further research into definitive molecular pathways and clinical efficacy is warranted to enhance its application in liver disease treatment.

Declarations

Ethics Approval

Ethical clearance for the study the study protocol was approved by the Federal University Otuoke Research and Quality Control unit

Author's contribution

Joel Theophilus Johnson contributed to the conceptualization of the research, designing, validation, modulation of the research, the methodology, laboratory analyses and data collection, data analysis and interpretations and also writing of the paper.

Compliance with ethical standards

This study adheres to all conventional ethical practices as applied to this research strictly.

Funding information

There was not a single grant awarded for this research by public, private, or nonprofit organizations. The investigator provided funding for this study.

Disclosure of conflict of interest

The author declare that none of the work reported in this study could have been influenced by any known competing financial interests or personal relationships.

Data availability

All data generated or analyzed during this study are included in this published article.

Acknowledgements

The authors wish to express their profound gratitude to the Department of Biochemistry, Federal University Otuoke for all round support throughout this study.

REFERENCES

- [1] Dhande, P., Sharma, R., Gupta, V., Kumar, A., & Patel, S. (2024). Modulation of inflammatory pathways by silymarin in hepatoprotection. *Journal of Biochemical Pharmacology*, 12(3), 145–158.
- [2] Doehmer, J., Müller, D., Krüger, A., & Hoffmann, R. (2017). Inhibition of hepatic stellate cell activation by silymarin in experimental fibrosis. *Toxicology Reports*, 4, 324–332.
- [3] El-bahay, A. Z., Abdel-Moneim, A. E., Othman, M. S., & Mahmoud, A. M. (2019). Silymarin attenuates xenobiotic-induced hepatotoxicity by modulating transporter proteins. *Journal of Clinical Toxicology*, 7(2), 58–66.
- [4] Faulstich, H., Fauser, U., Trischmann, H., & Seeger, R. (2018). Silymarin as a protective agent against mushroom-derived toxins. *Hepatology International*, 12(1), 44–52.
- [5] Geraniol, F., Martins, C., Rodrigues, P., Almeida, G., & Sousa, L. (2024). Mechanisms of CCl₄-induced hepatotoxicity: A free radical-mediated process. *Journal of Hepatic Medicine*, 18(1), 25–39.
- [6] Gharagozloo, M., Ghazanfari, T., Yaraee, R., & Amirghofran, Z. (2020). Modulatory effects of silymarin on cytokine production and NF-κB signaling. *Immunopharmacology and Immunotoxicology*, 42(5), 471–480.
- [7] Glory, M., Daniel, P., Rufus, A., & Thomas, J. (2018). Lipid membrane stabilization by silymarin in oxidative stress conditions. *Journal of Cellular Biochemistry*, 119(7), 5423–5431.
- [8] Hawke, D. E., Subramanian, V., Joseph, T., & Reed, M. (2020). Silymarin protection against ROS-mediated damage. *Free Radical Biology Review*, 11(4), 203–215.
- [9] homeopathicjournal.com. (2025). Clinical trials on silymarin in chronic liver disease: An updated review. *Homeopathic Journal of Integrative Medicine*, 9(1), 1–12.
- [10] Jaffar, A. (2024). Challenges in the oral bioavailability of silymarin. *Pharmacognosy Research*, 16(2), 112–119.
- [11] Javed, S., Ahmad, S., Ali, M., & Ahmed, F. (2011). Phytochemical characterization of silymarin flavonoids. *Journal of Medicinal Plants Research*, 5(30), 6789–6796.
- [12] JBClinPharm. (2024). Silymarin: Antioxidative and hepatoprotective profile—A clinical update. *Journal of Basic and Clinical Pharmacology*, 8(2), 55–63.
- [13] Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians*, 61(2), 69–90.
- [14] Kwon, H. J., Lee, H. J., Park, M. J., & Yun, J. W. (2017). Therapeutic applications of silymarin in liver diseases. *International Journal of Molecular Medicine*, 39(1), 77–86.
- [15] Lee, C. H. (2003). Role of the liver in detoxification. *World Journal of Hepatic Research*, 2(1), 10–15.
- [16] Lee, S. E., Kim, J. H., Park, J. E., & Choi, Y. (2007). Hepatic metabolism of xenobiotics. *Biochemistry Review Letters*, 29(4), 356–367.
- [17] Li, F., Chen, Y., Zhang, X., & Liu, X. (2012). Anti-inflammatory and hepatoprotective effects of silymarin. *International Journal of Clinical Pharmacology*, 50(3), 202–210.
- [18] Loguercio, C., & Federico, A. (2003). Oxidative stress in chronic liver diseases. *World Journal of Gastroenterology*, 9(1), 3–7.
- [19] Lorenz, D., Schneider, A., Wendel, A., & Daniel, H. (2014). Silymarin stimulation of ribosomal RNA polymerase and protein synthesis. *Hepatic Physiology Journal*, 15(2), 98–107.
- [20] Lorenz, D., Richter, B., Steiner, G., & Krause, R. (2022). NF-κB selective modulation by

- silymarin derivatives. *Journal of Inflammation Research*, 14, 511–522.
- [21] Lu, S. C., Garcia-Ruiz, C., Cederbaum, A. I., & Kaplowitz, N. (2016). The liver's central role in metabolism: An updated biochemical review. *Annual Review of Physiology*, 78, 295–320.
- [22] Mekonnen, T., Tesfaye, B., Mulatu, A., & Bekele, K. (2022). Serum liver enzyme alterations in hepatotoxicity. *East African Medical Journal*, 99(2), 45–53.
- [23] Morazzoni, P., Bombardelli, E., Montalbetti, A., & Malandrino, S. (2023). Leukotriene pathway modulation by silymarin. *Phytomedicine Reviews*, 21(3), 144–156.
- [24] NMCD. (2018). Silymarin monograph: Pharmacology and clinical efficacy. *Natural Medicines Comprehensive Database*, 1–12.
- [25] Peterson, L. A., Nguyen, T., Brown, R., & Franklin, C. (2020). Metabolic pathways of CCl₄-induced hepatotoxicity. *Toxicology Mechanisms and Methods*, 30(6), 403–412.
- [26] Raucy, J. L., Kraner, J. C., & Lasker, J. M. (2021). Role of CYP2E1 in CCl₄ bioactivation. *Drug Metabolism Reviews*, 53(2), 275–289.
- [27] Schandalik, R., Gatti, G., Conti, M., & Colombo, F. (2022). Silymarin inhibition of leukotriene B₄ synthesis. *Biochemical Pharmacology*, 199, 115024.
- [28] Schulz, H. U., Schürer, M., Krumbiegel, G., & Kühn, K. (2015). Protein synthesis enhancement by silymarin in hepatocyte regeneration. *Journal of Hepatic Therapy*, 8(4), 217–224.
- [29] Scielo.br. (2024). Biochemical markers of liver injury in CCl₄ toxicity. *Brazilian Journal of Medical and Biological Research*, 57(1), Article e10023.
- [30] Smaller, B. C., Ortiz, J. C., Wang, J. Y., & Levine, D. (2017). Antioxidant activity of silibinin in hepatocytes. *Redox Biology International*, 14, 88–95.
- [31] Stoyanovsky, D. A., & Cederbaum, A. I. (2018). CYP2E1-mediated metabolism of chloroform and carbon tetrachloride. *Free Radical Research*, 52(3), 281–298.
- [32] Surai, P. F. (2015). Silymarin flavonoids and their antioxidant significance. *Nutrition and Metabolism Insights*, 8, 57–70.
- [33] Trappoliere, M., Domenicali, M., Pasquale, V., & Bernardi, M. (2019). Effects of silymarin on inflammatory cytokines. *Hepatic Medicine: Evidence and Research*, 11, 85–94.
- [34] Vitalione, D., Roghani, M., & Rezaei, A. (2004). Progression of chronic liver disease: A biochemical overview. *International Journal of Hepatic Studies*, 3(2), 77–84.
- [35] Wachter, H., & Zaeske, H. (2019). Role of ROS in hepatocellular injury. *Journal of Oxidative Stress*, 7(1), 10–21.
- [36] Yang, J., Wang, W., Chen, Y., & Lu, L. (2015). Liver metabolism of nutrients and xenobiotics. *Journal of Integrative Biochemistry*, 39(2), 110–122.