International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Hepatoprotective potential of lamivudine through activation of gamma-glutamyltransferase and down-regulation of p53 in HepG2 cells

Shanza Rafi[1], Tahir Maqbool[1], Muhammad Sarwar[1], Zahid Habib Qureshi[1,2], Maimoona Nasreen[1,5,6], Sadia Naz[4], Muhammad Atif[3], Muzammal Mateen Azhar[1]*

[1]*Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan*

[2]*Multan Medical and Dental College, Multan Pakistan*

[3]*Department of Pharmacy, The University of Lahore, Lahore, Pakistan*

[4]*Allied health Sciences, The University of Lahore, Lahore, Pakistan*

[5]*University College of Medicine and Dentistry, The University of Lahore, Lahore, Pakistan*

[6]*Equator University of Science and Technology, Masaka, Uganda*

Abstract

The liver is a crucial metabolic organ that aids in detoxification, produces energy, synthesizes essential proteins, and is essential for maintaining good health. Since hepatocytes are the main type of cell found in liver tissue, maintaining their safety is essential for the general health of livers. The hepato-protective qualities of lamivudine shield the liver from stressful situations. The current study aimed to assess lamivudine's protective effects on HepG2 cells subjected to ethanol damage. Lamivudine was given to HepG2 cells after they were cultivated and subjected to ethanol-induced damage. Cell viability was assessed using the MTT, Crystal Violet, and Trypan Blue tests. The results of the MTT experiment demonstrated that lamivudine administration improved the rate of cell viability in a dose-dependent manner at 25, 50, and 100 µg/mL (0.570±0.0205; 0.73 ±0.02; 0.897±0.0206, respectively). According to the Crystal Violet test, lamivµdine-treated cells had higher cell viability than untreated cells; the rates were around 0.38 ± 0.01, 0.516 ± 0.0305, and 0.656 ± 0.041 in a dose dependant manner. The Trypan Blue assay results also confirmed that treatment with lamivudine at 25 µg/mL, 50 µg/mL, and 100 µg/mL led to a increase in the viability of cells compared to the injury group (183.66±5.50; 151±3.61; 115±5, respectively). Pretreatment of HepG2 cells with lamivudine during 100 µg/mL concentration exposure resulted in near-normalization of p53 expression levels (24.73±0.60), GSH levels (28.83±1.16), and in hepatic activity levels of GGT (21.23±1.12) compared to control. In immunofluorescence microscopy, the 100 µg/mL lamivudine group showed the most significant reduction in fluorescence intensity. Lamivudine provided convenient features in protecting HepG2 cells against ethanol-triggered liver damage by enhancing cell survival and antioxidant enzyme activities.

Keywords: Therapeutic potential; Lamivudine; Hepatoprotective; HepG2 cells.

Full-length article **Corresponding Author*, e-mail: *tahir.maqbool@imbb.uol.edu.pk* Doi # https://doi.org/10.62877/136-IJCBS-24-25-19-136

1. Introduction

Liver diseases cover a broad spectrum of maladies, including viral hepatitis, fatty liver disease, ALD, drug-induced liver injury, and cirrhosis. Treatment options includes liver transplant. Hepatoprotective agents may aid in the management of these diseases instead of liver transplant. These agents protect the liver from negative influences, slow down the course of the disease, decrease damage to the liver, and improve the quality of life for patients with liver disorders [1]. The concept of hepatoprotection—the protection and preservation of liver health—is as vital as that of the liver in maintaining homeostasis. Alcoholic liver disease is one of the oldest known forms of liver injury and encompasses a spectrum from mild steatosis to severe cirrhosis. Indeed, the earliest evidence of alcohol-associated liver disease dates to the Neolithic period of about 10,000 B.C [2]. Alcohol is still one of the most important causes of liver disease in the world, especially in developed and developing countries. In most cases, ALD coexists with additional liver diseases, such as nonalcoholic fatty liver disease or chronic viral hepatitis, which promotes liver damage [3-4].

Chronic alcohol intake remains a leading cause of long-term hepatic injury. In the United States, 48% of cirrhosis cases were attributed to alcohol in the year 2007 [5].

Factors affecting the development of ALD include ethanol breakdown oxidative stress, reduced glutathione levels, abnormal methionine metabolism, and malnutrition. A more recent study highlights the role of innate immunity in the onset and progression of ALD since chronic alcohol intake triggers the production of pro-inflammatory cytokines, leading to liver cell damage [6]. Hepatoprotection involves the measures to protect or fortify the hepatocytes, which are the functional units of the liver, against various deleterious influences. The hepatoprotective effect especially focuses on the enhancement of detoxification ability of the liver and metabolic efficiency; Detoxification capacity in the liver and overall metabolic ability are vital components of preventing liver diseases and treating complications related to liver dysfunction [7]. HepG2 is a healthy cell line derived from hepatocellular carcinoma; hence, it is useful when studying medication interactions and activities of liver enzymes in the system.

The possible hepatoprotective and/or hepatotoxic effects of lamivudine require identifying its effect on HepG2 cells [8-9]. NRTIs based on nucleoside analogues, including lamivudine, mostly used in the treatment of HIV and HBV. Yang *et al*. (2018) have studied that it has antiviral properties because it works on a reverse transcriptase enzyme that is vital in the replication of viruses [10]. Comparatively less toxic, lamivudine has the potential to induce oxidative stress and impairment of mitochondrial function, most of which translate to poor clinical outcomes. The actual contribution of such an effect to the liver in general and in long-term treatment must be determined if the balance of benefits versus risks is to be maintained. Potential hepatoprotective activity of lamivudine beyond antiviral effect. Recent studies have reported that it stabilizes mitochondria, reduces oxidative stress, and presents anti-inflammatory effects in its action for liver protection [11]. Such effects, primarily on hepatocytes under oxidative stress, allow one to understand possible therapeutics for various liver diseases [12]. This study will attempt to determine the effect of lamivudine on hepatocytes, taking the HepG2 cell line model to understand comprehensive pharmacology and implications for liver health and help find out its function in potentially being a hepatoprotective agent.

2. Materials and Methods

2.1. Study Design

The animals were categorized into negative control without treatment, injury category treated with alcohol to cause liver damage, and injury followed by treatment category in which alcohol-induced liver damage was succeeded by therapy with lamivudine to assess the hepatoprotective role of lamivudine.

2.2. *Study Site and Setting*

The study was conducted at the Center for Research in Molecular Medicine (CRiMM) - the Institute of Molecular Biology and Biotechnology (IMBB) at the University of Lahore in Pakistan. For the performance of the experiments, the HepG2 cell line was chosen, which can be used for a wide range of liver diseases.

2.3. Experimental Design

Three groups of HepG2 cells were used in the current study. The injury group was exposed to alcohol, the negative control group received no treatment, and the injury plus treatment group received alcohol followed by lamivudine. The design enabled the investigation of the protective effects of lamivudine against alcohol-induced liver injury.

2.4. Culturing of Cell Lines

HepG2 cells were cultured according to maqbool et al., 2019 [13] using standard cell culture techniques. It was transferred into a culture flask containing DMEM-HG growth media supplemented with 10% FBS, 100 mg/mL penicillin G, and 100 U/mL streptomycin. The cells were incubated in a humidified CO_2 incubator at 37 °C in an atmosphere of 5% CO_2. Subculturing was done upon reaching a confluence of 70-80%. This was followed by cells being washed using phosphate-buffered saline (PBS), detachment with 0.05% trypsin-EDTA, and centrifugation to get a cell pellet, which was re-suspended for further use.

2.5. Treatment of Cell Lines with Lamivudine

To treat the lamivudine, the HepG2 cells were plated in 96-well plates for cell viability assays and 6-well plates for wound healing assays. The cells were divided into three groups: untreated (UT) HepG2 cells, injury group treated with CCl_4, and injury plus treatment group treated with CCl_4 followed by lamivudine (0.5 µg/mL). Treatments were applied for 24 hours, after which various assays were conducted to assess cell viability and biochemical responses.

2.6. Cell viability by MTT Assay

The MTT assay was employed to measure cell viability. HepG2 cells were treated with lamivudine for 24 hours. MTT solution (0.5 mg/mL) was then added, and incubation proceeded for another 2-3 hours. The absorbance of the cells was recorded at 570 nm, which was used to calculate the cell viability.

2.7. Trypan Blue Assay

Cell viability was also determined using the trypan blue exclusion method. After treatment, the cells stained with trypan blue for 15 min, washed with PBS, and viewed using a light microscope. Non-viable cells were those that retained the dye of trypan blue.

2.8. Crystal Violet Assay

Another measure of cell viability was acquired using crystal violet. Treated cells in 96-well plates were incubated with 0.1% crystal violet dye containing 2% ethanol. The cells were incubated at room temperature, washed with PBS, and solubilized with 1% SDS. Absorbance was read at 540 or 595 nm wavelength to determine cell viability.

2.9. Wound Healing Assay

Wound healing was measured by induction of scratches on the cell monolayer and adding varying concentrations of lamivudine for 24 hours. Healing was measured regarding the surface area and contraction of wounds, which should be proportional to the cells' regenerative capacity.

2.10. ELISA for GGT and p53

ELISA kits measured the extent of inflammation (GGT) and apoptosis (p53) expressed in the samples. All

samples were run following the instructions outlined by the manufacturer according to maqbool et al 2019 (13), and optical density was read at 450 nm. This allowed for quantifying inflammatory and apoptotic responses that would be induced in the treated cells.

2.11. Evaluation of Antioxidative Enzymes

The activity of antioxidative enzymes was measured with two different assays. In this case, the secretomes from the various antigens-treated cells were mixed with a reaction mixture for glutathione reductase (GSH) and then incubated at room temperature; the solution was read at 340 nm. An additional experiment was carried out for the SOD activity assay by preparing a reaction mixture with the secretomes and measuring optical density at 560 nm. Such assays depicted the antioxidative capacity of treated cells.

2.12. Statistical Analysis

Data obtained from the experiments were expressed as mean ± SEM from triplicate experiments. One-way ANOVA was used to compare groups, with Bonferroni's test applied for post-testing after ANOVA, and two-way ANOVA was used for data analysis on the quantitative part using Graph Pad software for statistical analysis. The p-value used in this study was <0.05, so the result can be considered reliable and valid.

3. Results and discussion

3.1. Results

3.1.1. MTT assay

The cell viability detected by the MTT assay results is shown in Figure 1. There was a significant difference among the various treatment groups. A very high mean viability of 0.970 ± 0.013 was observed for the baseline cell viability, compared to the ethanol injury group, which showed a significant reduction to 0.426 ± 0.020. The dose-dependent effect has been shown while treating with concentrations of 25, 50, and 100 µg/mL, whereby the viability rates were getting higher—0.570 ± 0.0205, 0.73 ± 0.02, and 0.897 ± 0.0206, respectively. From this, the value showed a difference from the highest concentration of 100 µg/mL than from others.

3.1.2. Crystal violet assay

Cell viability, as determined by the Crystal Violet assay in the different treatment groups, had distinct results (Figure 2). Cell viability for control averaged 0.703 ± 0.0503, whereas that of the ethanol injury group was significantly decreased to 0.323 ± 0.015, reflecting the lethal effect of ethanol on cell survival. The response in the viability of the cells treated with 25 µg/mL, 50 µg/mL, 100 µg/mL was concentration-dependent, and the cell viability increased to approximately 0.38 ± 0.01, 0.516 ± 0.0305, and 0.656 ± 0.041, respectively.

3.1.3. Trypan blue assay

Trypan blue assay was carried out to estimate cell viability in various groups (Figure 3). In the control group, the mean viability was 100 ± zero, indicating without treatment. There are no adverse effects on cell viability. The mean viability in the ethanol injury group showed a significantly high number of the 210.66 ± 10.01, indicating cell damage by ethanol exposure. Cells treated with 25, 50, and 100 produced the low viabilities of 183.66 ± 5.50, 151 ± 3.61, and 115 ± 5 µg/mL, respectively.

3.1.4. P53

Based on the following results in p53 expression among different treatment groups (Figure 4), the control group, the average p53 expression equaled 22.2 ± 2.25, while after ethanol injury; it increased up to 56.2 ± 1.1. Which means the used alcohol level is extreme for damage induction; finally, treatment with 25 µg/mL caused a decrease in p53 expression to 45.7 ± 3.27. With the treatment group of 50 µg/mL, an extra decrease in p53 expression observed with a mean expression of 31.9±1.51. There were about normalized p53 expression levels in the treatment group at 100 µg/mL to the control group with a mean value of 24.73±0.60.

3.1.5. Glutathione Reductase (GSH) Assay

The GSH levels from the different groups measured with a GSH assay (Figure 5). The GSH activity in the standard control group was determined to be approximately 31.46±1.001. The GSH activity noticeably decreased to 11.23±1.18 in the ethanol injury group, resulting from ethanol-induced oxidative stress. After treatment at 25 µg/mL, there was some increase in GSH activity, and the mean was 14.36±1.10. In treatment with a higher dosage of 50 µg/mL, a more pronounced recovery in GSH activity observed at a mean activity of 20.96 ± 0.66. The highest treatment concentration, 100 µg/mL, showed an apparent restoration back to normal GSH levels compared to the control group, with a mean activity of 28.83 ± 1.16.

3.1.6. Gamma-glutamyl Transferase (GGT)

The GGT activity in different treatment groups was measured (Figure 6). It was noted that the GGT activity in control was 17.66±2.63, and it was found to be statistically significant in the group injury of ethanol because it increased to 65.3±0.95, indicating a compromise in liver function due to the oxidative stress caused by ethanol. When treated with 25 µg/mL, the reduction of GGT activity noted at 51.63±3.14. GGT activity exhibited another decrease in the treatment at 50 µg/mL, with a mean value of 30.5 ±1. The highest concentration of treatment, 100 µg/mL, showed a significant reduction in activity of GGT with a mean value of 21.23 ±1.12, almost close to that of the level of the standard group.

3.1.7. Immunofluorescence microscopy

Immunofluorescence microscopy displayed protein expression and localization differences among treatment groups (Figure 7). The negative control group showed fluorescence levels at the baseline with weak and homogeneous staining, suggesting regular protein expression compared to injury group. The 25µg/mL lamivudine group showed a moderate reduction in fluorescence intensity. The 50 µg/mL lamivudine group showed a further decrease in fluorescence intensity. The 100 µg/mL lamivudine group showed the most significant decrease in fluorescence intensity.

3.2. Discussion

The hepatoprotective role of lamivudine on hepatocytes would include understanding its impact on liver cells and potential benefits it could offer in protecting against liver damage. Lamivudine is best known for the acting

against HBV-infected cells through its antiviral activity, it is also having additional effects on hepatocytes, suggesting a role as a hepatoprotective beyond its function in antiviral action [14].

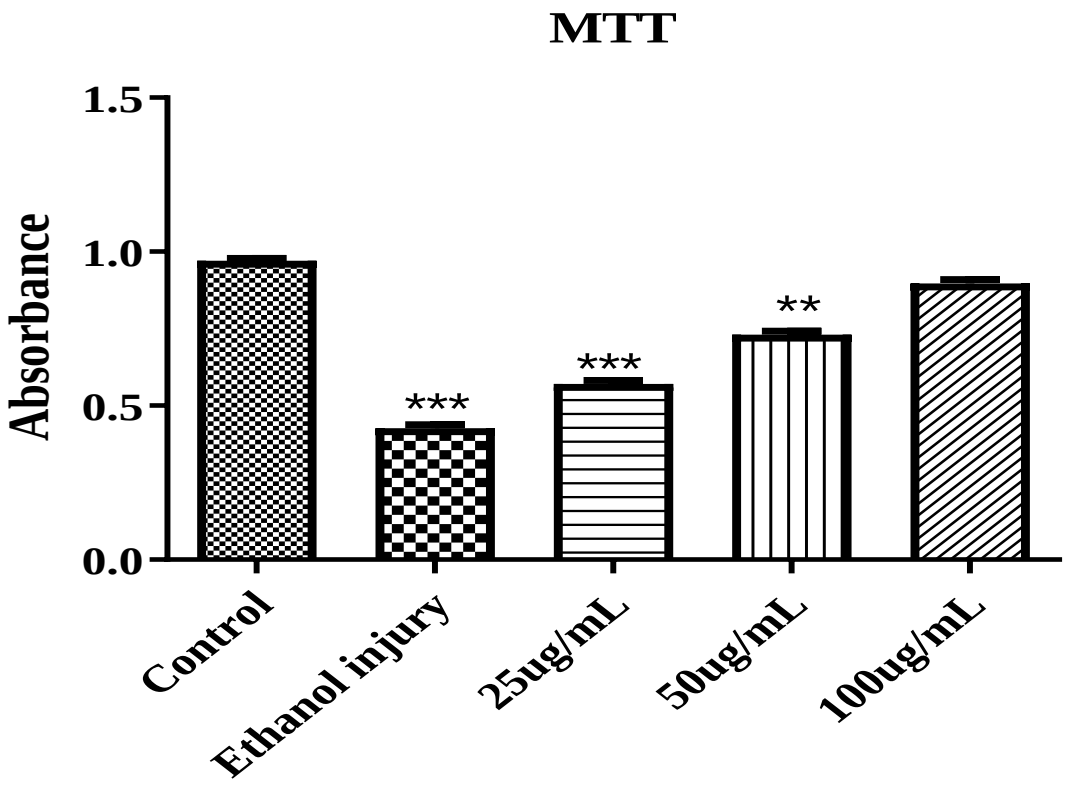

Figure 1: Cell Viability Assessed by MTT Assay in Different Treatment Groups. *, *** showed significant differences between groups compared to control.

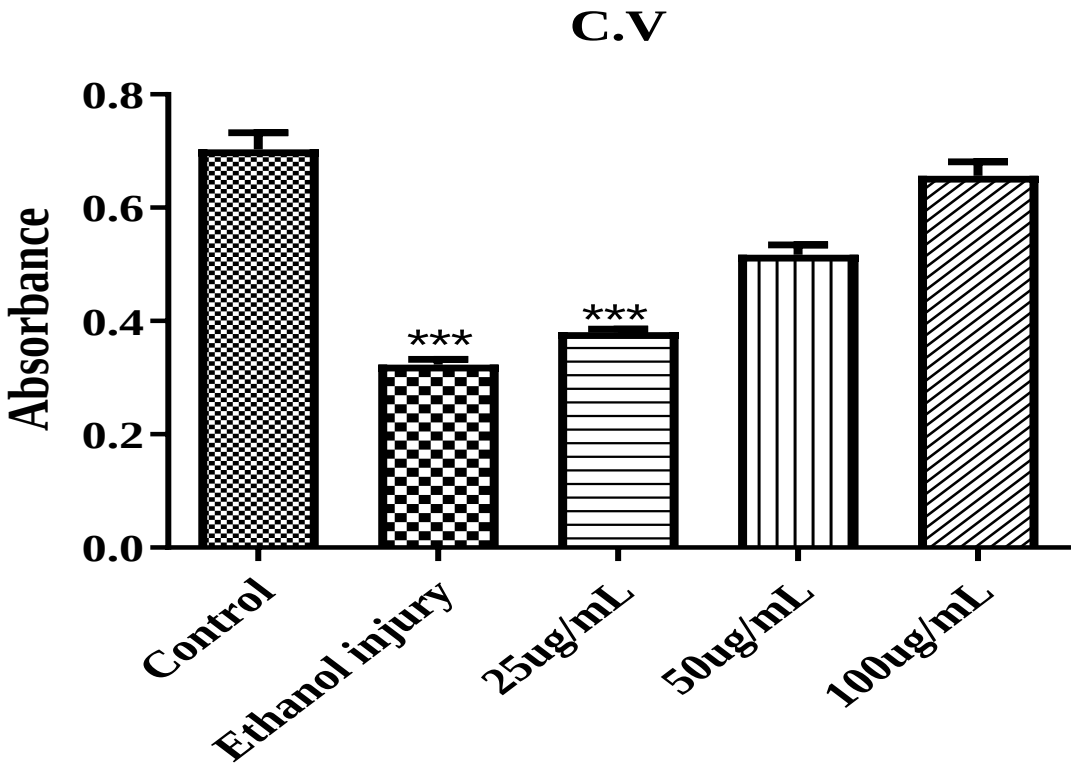

Figure 2: Cell Viability Assessed by Crystal Violet Assay in Different Treatment Groups. *, *** showed significant differences between groups compared to control.

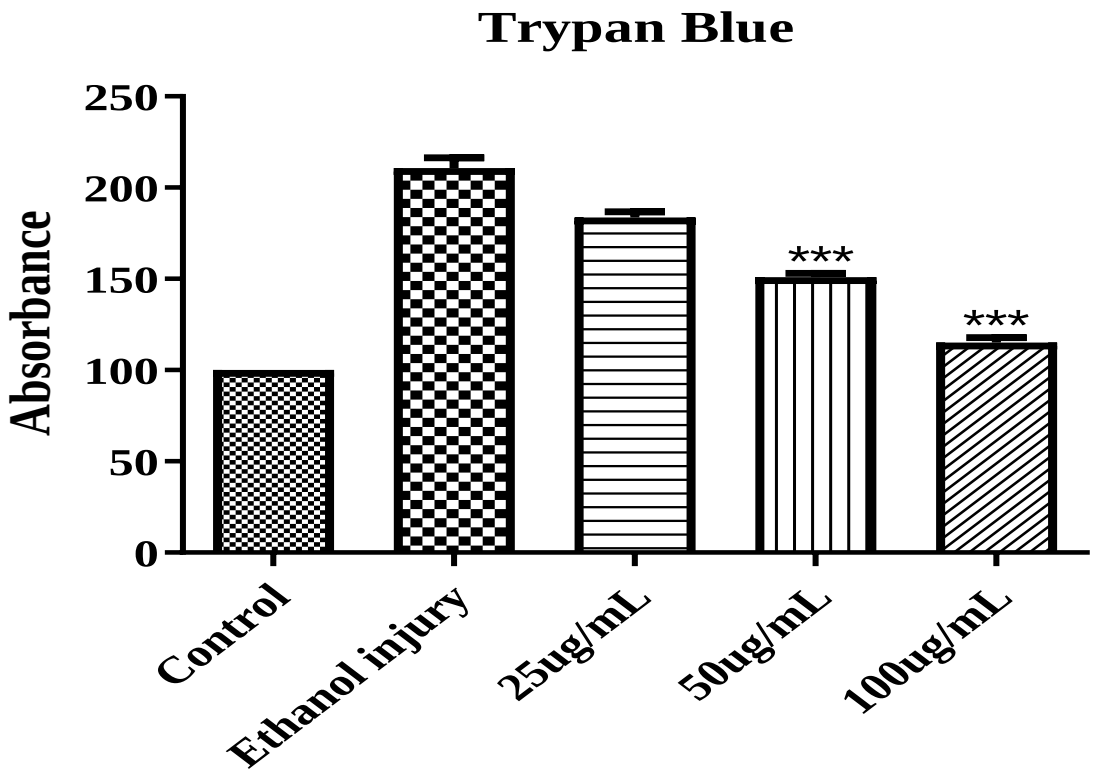

Figure 3: Cell Viability Assessed by Trypan Blue Assay in Different Treatment Groups**.** *, *** showed significant difference between groups compared to control.

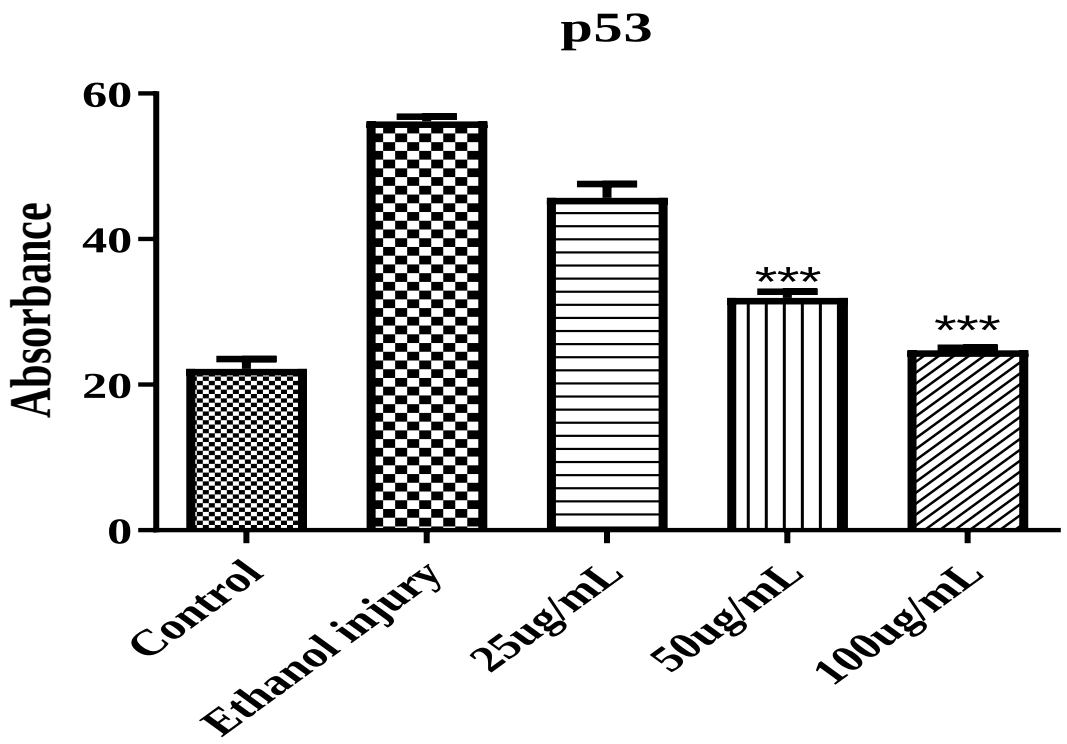

Figure 4: Expression of p53 in Different Treatment Groups**.** *, *** showed significant differences between groups compared to control.

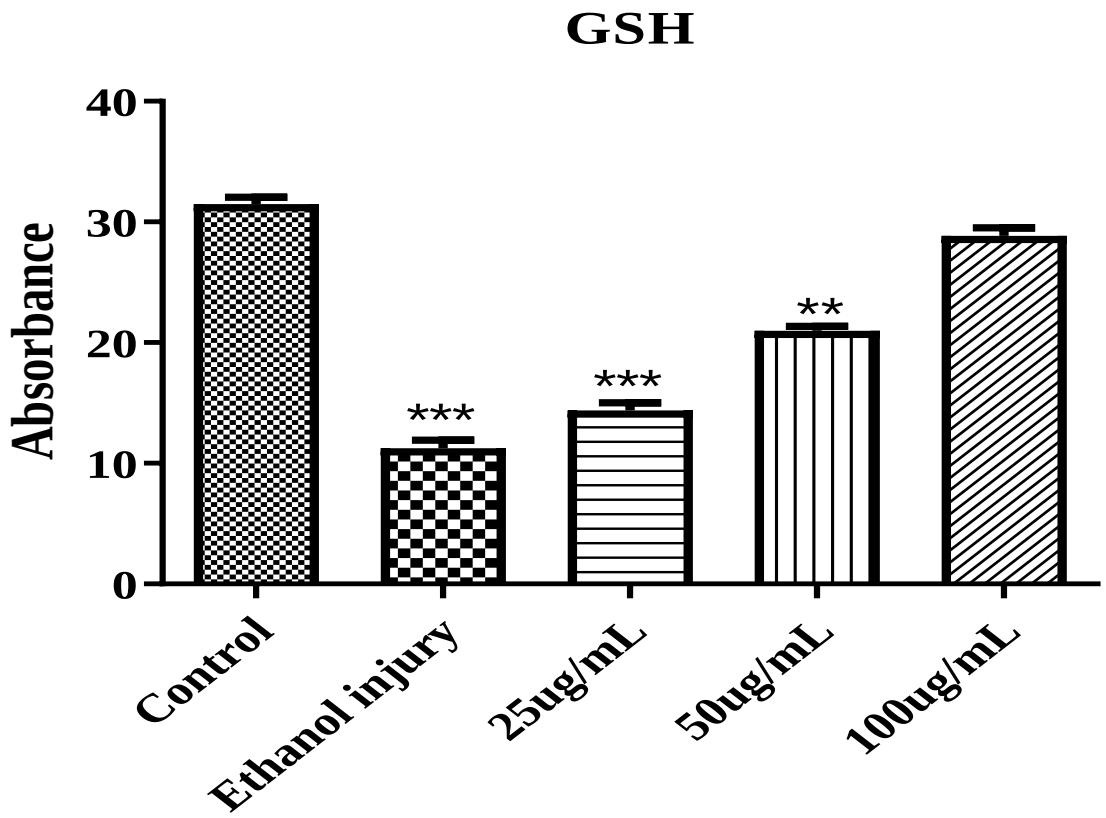

Figure 5: Glutathione Reductase Assay Activity for Different Treatment Groups. *, *** showed significant differences between groups compared to control.

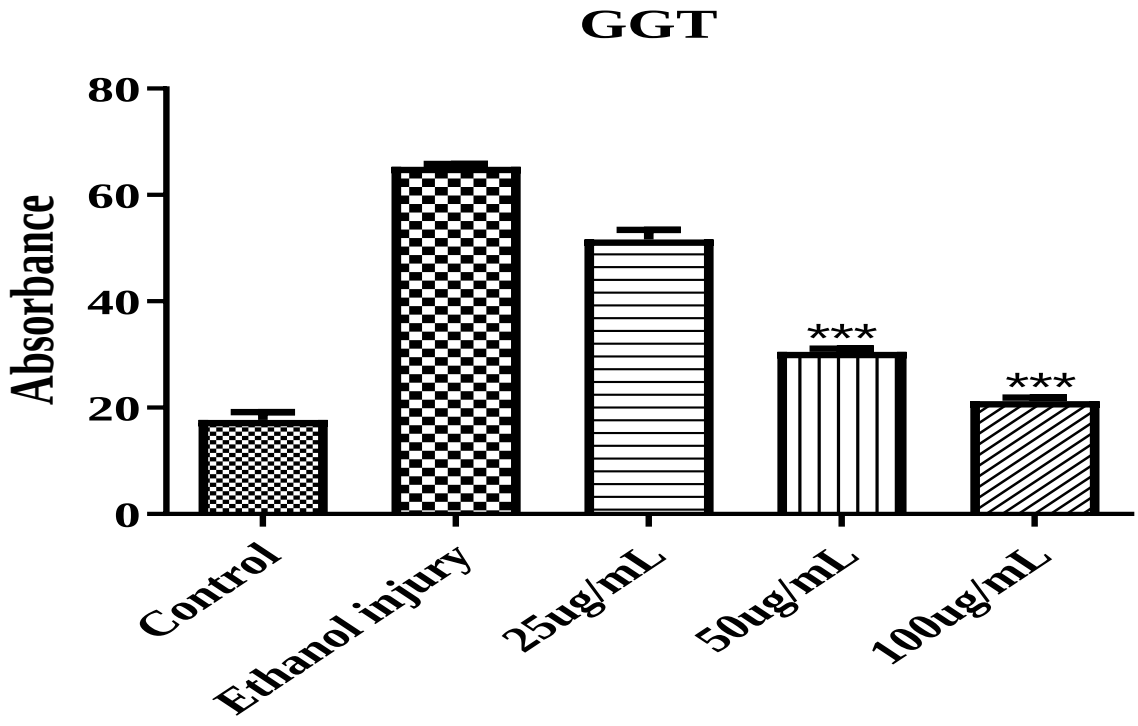

Figure 6: Glutathione transferase assay activity for Different Treatment Groups. *, *** showed significant difference between groups compared to control.

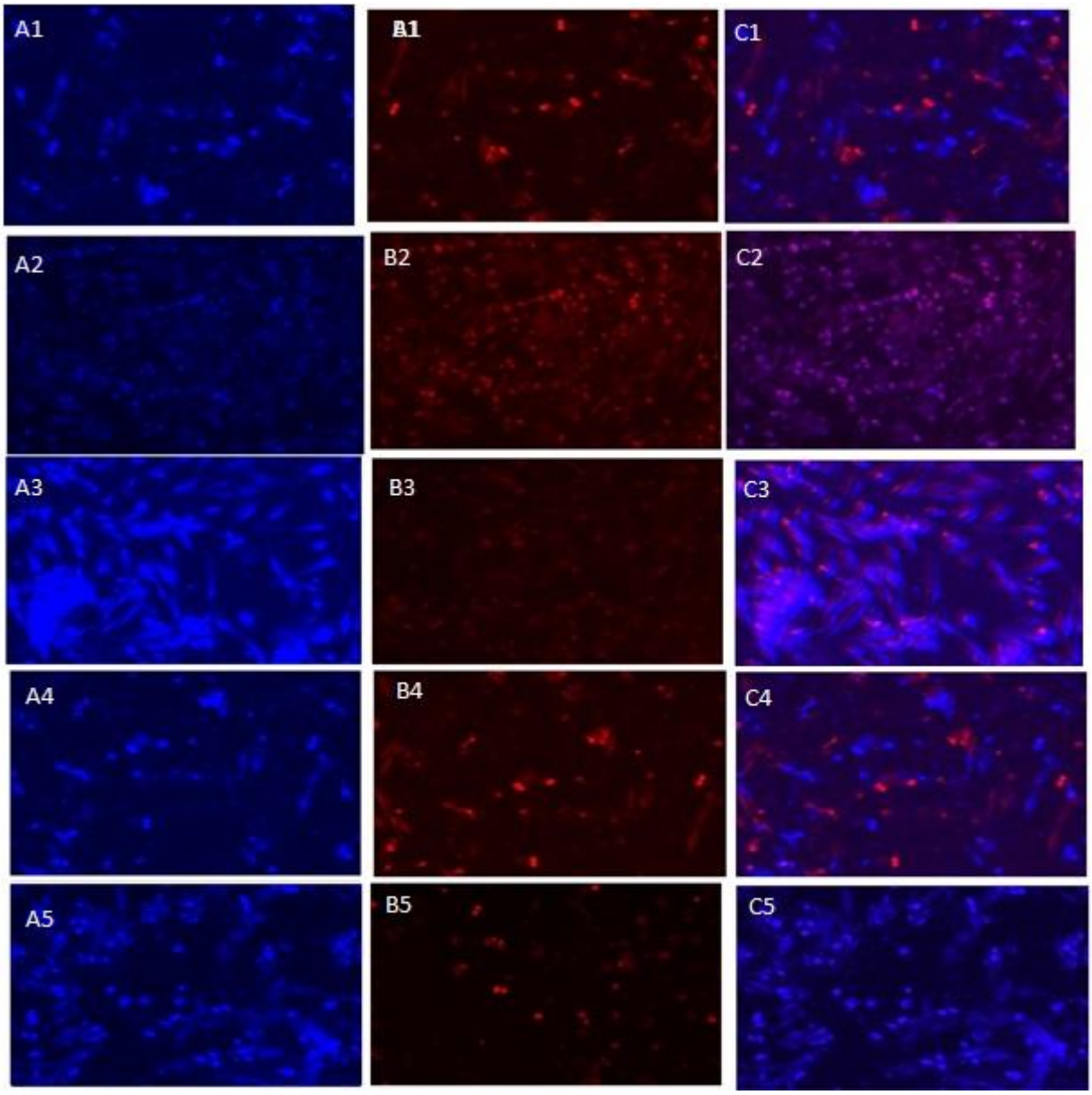

Figure 7: Representative immunofluorescence microscopy images of HepG2 cells across different treatment groups. A1-A5 are DAPI (all cell nucleus) treatment, B1-B5 are PI (dead cell nucleus) treatment and C1-C5 (merge indicates how many cells are dead)DAPI, PI merge treatment. Treatment divided into three groups A1-C1 negative control, A2-C2 Positive control, A3-C3: 25 µg/mL lamivudine, A4-C4: 50 µg/mL lamivudine, and A5-C5: 100 µg/mL lamivudine.

Furthermore, the drug reduces liver inflammation and liver injury induced by viral infections, especially HBV. This evidenced in studies that show there is a decrease in the concentration of serum aminotransferases as markers for liver inflammation and damage in patients treated with lamivudine who present with chronic hepatitis B [15]. Compared to the control group, Khoo *et al*. (2021) observed significant decreases in ALT levels in patients with chronic hepatitis B following lamivudine treatment, suggesting a hepatoprotective effect via lowering hepatic inflammation in treated subjects [16]. Cell viability tests are one of the most important methods in biological research because they allow researchers to ascertain how different conditions or treatments affect the health and capacity of cells to survive. The MTT, Crystal Violet, and Trypan Blue tests are the most often utilized assays. These tests are useful for determining the cytotoxicity of various substances or the activity of possible therapeutic medicines since they enable the collection of quantitative data on the viability of cells [17]. Consistent with most publications, the MTT assay in this study demonstrated a dose-dependent increase in cell viability with increasing doses of the test drug. For example, the MTT test demonstrated the plant extract's potential to prevent cell death and increase cell viability [18].

They discovered a similar pattern of improved cell viability as extract concentrations rose, suggesting that the extract had cytoprotective qualities. Comparably, Jiang *et al*. (2023) demonstrated outcomes comparable to current study by seeking to maintain cell viability under oxidative stress utilizing a different approach employing their synthesized compound: improving cell viability [19]. This set of comparisons highlights how repeatable the measurements made by MTT tests are when evaluating [20]. Our Trypan Blue assay showed a trend whereby the cell viability was

reduced with the increase in concentration of the test compound, as seen in other related studies. Therefore, to evaluate the cytotoxicity of a chemical compound, Loufouma Mbouaka *et al*. (2023) employed the Trypan Blue assay and noticed that there was a drastic decrease in cell viability in connection with the concentration gradient of the chemical compound as revealed [21]. Another work done by Mohtar et al. (2021), where the possible cytotoxic effect of the nanoparticle-based formulation was examined at the cell viability through Trypan Blue assay, where similar results of reduced viability with an increase in nanoparticle concentration were obtained.

These comparisons have shown that with the help of the Trypan Blue assay, cytotoxicity can be detected, and the mentioned effect of various agents on overall cell health and viability can be established. Previous studies have shown that ethanol causes an increase in the level of p53, which considered a protective response to oxidative stress and apoptosis [16]. The same impact was observed regarding the effect of lamivudine treatment because similar other antioxidants and enhancers of DNA repair are reported to reduce the expression value of p53. Consequently, NAC research associated with p53 also depicted the diminished expression of proteins directly related to DNA repair and low apoptosis ratio. Meena (2017) also found that the downregulation of p53 by curcumin and ethanol in the case of liver damage revealed that fewer antioxidants decrease p53-induced apoptosis. This reduction of p53 expression with lamivudine treatment should probably be due to their antioxidant effects, which are expected to reduce the effect of oxidative DNA damage that activates the p53 pathway.

Scientists established that lamivudine could restore the p53 levels at 100 µg/mL, which means that lamivudine could even be more beneficial in DNA repair mechanism and in the prevention of cell apoptosis; in addition to this, in addition to its known reverse transcriptase inhibitor pharmacological effects with antioxidants properties. The results agree with other research studies that show that GSH levels decrease attributable to ethanol-induced injury. In addition, previous research with other antioxidants like vitamin E and selenium has demonstrated a similar enhancement of effects on GSH that proves the conclusion about the properties of antioxidants to increase the levels of GSH [22]. In addition, Chaphalkar *et al*. (2017), in their study on the impact of silymarin on ethanol-induced liver injury, revealed enhancement in the GSH level, which, in association with the present study, supports the antioxidant-modulated recovery. Lamivudine restored GSH activity due to its potential to decrease the generation of ROS and augment the elements of cellular antioxidant protection.

The gradual increase in the GSH activity with an increase in the dose of lamivudine indicates that the higher concentration of the drug offers an efficient shield against oxidative stress, possibly by acting as a direct antioxidant and provoking an efficient antioxidant defense system of the cells [23]. Earlier research has revealed the same increase in GGT activity due to ethanol toxicity in the liver, and hence, GGT could use as the marker for liver injury. For example, some studies by Yan *et al*. (2023) showed the synergizational of GGT activity with antioxidants, and the modes of treatments used in alcohol-induced liver injuries vouched for antioxidant's efficiency in preventing liver damage. Therefore, the decrease in Lamivudine treatment and the GGT activity proved that the degree of oxidative stress and liver damage reduced. This can attributed to the antioxidant activity of lamivudine, which lowers the concentration of ROS and thus preseasons the hepatic cells for the effects of the free radicals. The study portrayed a reduced activity of GGT in a dose-dependent manner; this may infer that higher doses of lamivudine offer better protection in ethanol-induced liver injury [24-25].

4. Conclusions

Lamivudine demonstrates significant hepatoprotective potential by reducing inflammation and liver cell injury, modulating the host immune response to mitigate immune-mediated damage, and possibly exerting direct protective effects on the liver. These effects, including its antioxidant and anti-apoptotic properties, highlight its adaptability as a potent hepatoprotective agent, particularly in conditions where hepatocyte damage is a concern. More studies – direct large-scale clinical research and mechanistic analysis – are needed to fully explain how lamivudine works to protect the liver and treat liver diseases apart from Hepatitis B and E.

References

[1] A.P. Delli Bovi, F. Marciano, C. Mandato, M.A. Siano, M. Savoia, P. Vajro. (2021). Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. Frontiers in medicine. 8: 595371.

[2] E. Carbonell Ballesteros. Molecular mechanisms involved in the anti-inflammatory and hepatoprotective effects of the antiretroviral drug Rilpivirine. Universitat Politècnica de València, 2022.

[3] M. Fredsgaard, S.E.K. Kaniki, I. Antonopoulou, T. Chaturvedi, M.H. Thomsen. (2023). Phenolic compounds in salicornia spp. and their potential therapeutic effects on H1N1, HBV, HCV, and HIV: a review. Molecules. 28(14): 5312.

[4] J. Gong, W. Tu, J. Liu, D. Tian. (2023). Hepatocytes: a key role in liver inflammation. Frontiers in Immunology. 13: 1083780.

[5] D.Q. Huang, P. Mathurin, H. Cortez-Pinto, R. Loomba. (2023). Global epidemiology of alcohol-associated cirrhosis and HCC: trends, projections and risk factors. Nature reviews Gastroenterology & hepatology. 20(1): 37-49.

[6] H. Jiang, J. Zuo, B. Li, R. Chen, K. Luo, X. Xiang, S. Lu, C. Huang, L. Liu, J. Tang. (2023). Drug-induced oxidative stress in cancer treatments: Angel or devil? Redox Biology. 63: 102754.

[7] P. Gong, H. Long, Y. Guo, Z. Wang, W. Yao, J. Wang, W. Yang, N. Li, J. Xie, F. Chen. (2024). Chinese herbal medicines: The modulator of nonalcoholic fatty liver disease targeting oxidative stress. Journal of Ethnopharmacology. 318: 116927.

[8] T. Khoo, D. Lam, J.K. Olynyk. (2021). Impact of modern antiviral therapy of chronic hepatitis B and C on clinical outcomes of liver disease. World Journal of Gastroenterology. 27(29): 4831.

[9] M. Štampar, B. Breznik, M. Filipič, B. Žegura. (2020). Characterization of in vitro 3D cell model

developed from human hepatocellular carcinoma (HepG2) Cell Line. Cells. 9(12): 2557.

[10] G.F. Gerard, Reverse transcriptase. In *enzymes of nucleic acid synthesis and modification*, CRC Press: 2018; pp 1-38.

[11] A. Kumar, Y. Rai, A.N. Bhatt. (2023). Anti-cancer drug-induced mitochondrial alteration becomes a limitation of metabolic viability-based MTT assay in analyzing growth inhibition.

[12] S. Mitra, A. De, A. Chowdhury. (2020). Epidemiology of non-alcoholic and alcoholic fatty liver diseases. Translational gastroenterology and hepatology. 5.

[13] T. Maqbool, S.J. Awan, S. Malik, F. Hadi, S. Shehzadi, K. Tariq. (2019). In-vitro anti-proliferative, apoptotic and antioxidative activities of medicinal herb Kalonji (Nigella sativa). Current pharmaceutical biotechnology. 20(15): 1288-1308.

[14] A. Patil. Clinical Profile of Alcoholic Liver Disease in a Tertiary Care Center and Its Correlation with Type, Amount and Duration of Alcohol Consumption: with Special Reference on Neutrophil Lymphocyte Ratio Score. Rajiv Gandhi University of Health Sciences (India), 2017.

[15] A. Pintor, L. Queiroz, R. Barcelos, L. Primo, L. Maia, G. Alves. (2020). MTT versus other cell viability assays to evaluate the biocompatibility of root canal filling materials: a systematic review. International Endodontic Journal. 53(10): 1348-1373.

[16] J. Rani, S.B. Dhull, P.K. Rose, M.K. Kidwai. (2024). Drug-induced liver injury and anti-hepatotoxic effect of herbal compounds: a metabolic mechanism perspective. Phytomedicine. 122: 155142.

[17] H.K. Seitz, R. Bataller, H. Cortez-Pinto, B. Gao, A. Gual, C. Lackner, P. Mathurin, S. Mueller, G. Szabo, H. Tsukamoto. (2018). Alcoholic liver disease. Nature reviews Disease primers. 4(1): 16.

[18] Y. Tu, S. Zhu, J. Wang, E. Burstein, D. Jia. (2019). Natural compounds in the chemoprevention of alcoholic liver disease. Phytotherapy Research. 33(9): 2192-2212.

[19] Y. Wang, K. Liu. (2024). Therapeutic potential of oleanolic acid in liver diseases. Naunyn-Schmiedeberg's Archives of Pharmacology. 1-18.

[20] J. Yan, Y. Nie, M. Luo, Z. Chen, B. He. (2021). Natural compounds: a potential treatment for alcoholic liver disease? Frontiers in pharmacology. 12: 694475.

[21] A. Loufouma Mbouaka, I. Lesiak-Markowicz, I. Heredero-Bermejo, R. Mazumdar, J. Walochnik, T. Martín-Pérez. (2023). Assessing Acanthamoeba cytotoxicity: comparison of common cell viability assays. Frontiers in Microbiology. 14: 1175469.

[22] F.L. Locke, D.B. Miklos, C.A. Jacobson, M.-A. Perales, M.-J. Kersten, O.O. Oluwole, A. Ghobadi, A.P. Rapoport, J. McGuirk, J.M. Pagel. (2022). Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. New England journal of medicine. 386(7): 640-654.

[23] A. Kalsoom, A. Altaf, M.I. Jilani, H. Sattar, T. Maqbool, T. Muhammad. (2024). In vitro antiproliferative potential of Cassia angustifolia extracts on HepG2 cells to combat liver cancer. International Journal of Applied and Experimental Biology. 3(2): 113-123.

[24] I. Ahmad, T. Maqbool, S. Naz, F. Hadi, M. Atif. (2023). Apoptotic potential of geranyl acetate in HepG2 liver cancer cells. International Journal of Applied and Experimental Biology. 2(2): 89-96.

[25] H. Yuan, S. Duan, T. Guan, X. Yuan, J. Lin, S. Hou, X. Lai, S. Huang, X. Du, S. Chen. (2020). Vitexin protects against ethanol-induced liver injury through Sirt1/p53 signaling pathway. European Journal of Pharmacology. 873: 173007.