IJASRM International Journal of Advanced Scientific Research and Management, Volume 3 Issue 10, Oct 2018
www.ijasrm.com
ISSN 2455-6378

The role of Silibinin as a cure in LPS/D-GalN induced fulminant hepatic failure in rats

Reem Mohammed Hashem [1], Kamel Mohammed Hassanin[2], Laila Ahmed Rashed [3] and Ahmed Mahmoud Samy [4]

[1] Department of Biochemistry, Faculty of Pharmacy, University of Beni-Suef, Beni-Suef, 62511, Egypt

[2] Department of Biochemistry, Faculty of Veterinary Medicine, University of Minia, Minia, 61511, Egypt

[3] Department of Biochemistry, Faculty of Medicine, University of Cairo, Cairo, 11562, Egypt

[4] Department of Biochemistry, Faculty of Pharmacy, University of Nahda, Beni-Suef, 62511, Egypt

Abstract

Fulminant hepatic failure (FHF), also known as acute liver failure, is the rapid development of acute liver injury with severe impairment of the synthetic functions and hepatic encephalopathy. FHF can be induced by LPS/D-GalN administration. The aim of the study is to evaluate the possible curative effect silibinin on LPS/D-GalN treated rats' liver. Rats were randomly divided into a normal control group that received saline only, LPS/D-GalN group which received LPS (15 µg/kg) and D-GalN (300mg/kg) and silibinin group that was exposed to a single intraperitoneal injection of D-GalN (300mg/kg), LPS (15 µg/kg), followed by intraperitoneal injection of silibinin (50 mg/kg) for a week.

We found that LPS/D-GalN significantly increased reactive oxygen species formation, lipid peroxidation, the oxidative stress marker MDA, ALT and AST liver enzymes activity, and also significantly decreased the levels of antioxidants (catalase and glutathione). On the other hand, we observed a significant decrease in MDA, ALT and AST activity in the group treated with silibinin and it restored the depletion in catalase and glutathione.

In conclusion, silibinin can be considered as a powerful hepatoprotectant.

Keywords: ***LPS/D-GalN, FHF, silibinin, oxidative stress***

1. Introduction

The liver is the largest internal organ in the body. It is dark red-brown with a soft spongy texture, and it is located below the diaphragm on the right side of the abdominal cavity with four incompletely separated lobes [1,2].

Liver tissue consists of 4 main types of cells: hepatocytes, endothelial cells, Kupffer cells and stellate cells. The hepatocyte is a cell of the main parenchymal tissue of the liver. Hepatocytes make up 70-85% of the liver's mass and they are the biosynthetic engines of the liver as they synthesize and secrete a variety of proteins. The Kupffer cells function as macrophages, and the stellate cells store fat and vitamin A [3]. The liver performs many essential functions including bile formation and excretion, synthesis of liver proteins, detoxification of xenobiotics and endogenous compounds, and regulation of blood glucose [4].

Fulminant hepatic failure (FHF) is a life-threatening illness, where a previously normal liver fails within days to weeks. Sudden loss of synthetic and detoxification function of liver results in jaundice, encephalopathy, coagulopathy and multiorgan failure [5].

Lipopolysaccharide (LPS) is a known endotoxin located on the outer membrane of Gram-negative bacteria [6,7]. It's a potent activator of the innate immune response which activate complex signaling

cascades that lead to the release of pro-inflammatory cytokines [8]. LPS has been demonstrated to cause inducible nitric oxide synthase (iNOS) expression in Kupffer cells and hepatocytes. Consequently, there is a potential for large amounts of nitric oxide (**NO)** to be generated in the liver, which could impair hepatic function by direct injury to hepatocytes [9].

D-Galactosamine (D-GalN) is a hepatotoxicant that induces a diffuse type of liver injury closely resembling human viral hepatitis [10,11]. D-GalN can produce acute liver necrosis and apoptosis.

Combination of D-GalN and LPS lead to excessive generation of reactive oxygen species (ROS) which through their oxidative damage lead to fulminant hepatic failure [12,13].

As oxidative stress is the main pathogenic mechanism leading to liver damage [14], liver enzymes, alanine amino transferase (ALT) and aspartate amino transferase (AST), are commonly in the evaluation process of patients with hepatic disorders as they are mostly found in the liver and to some extent normally found in the blood stream, but when liver injury happens, it escapes from destroyed hepatocyte to the blood stream in large quantities [15,16].

Malondialdehyde (MDA) results from lipid peroxidation of polyunsaturated fatty acids by ROS [17]. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipooxidation end-products (ALE), in analogy to advanced glycation end-products (AGE). The production of MDA is used as a biomarker to measure the level of oxidative stress in an organism [18].

Antioxidants are the defense mechanisms with which the human and animal are provided to guard against ROS. Antioxidants, either naturally generated in situ (**endogenous antioxidants**), or externally supplied through foods (**exogenous antioxidants**) can neutralize the excess of free radicals protecting the cells against their toxic effects, and contribute to disease prevention [19]

Silibinin, also known as silybin, is the major active constituent of silymarin, the standardized extract of the milk thistle seeds [20]. It exhibits a number of pharmacological effects, particularly in the liver [21]. The best known mechanism of action of silibinin is antioxidant, free radical scavenging and inhibition of lipid peroxidation [22,23]. Silibinin-treated cells are more resistant to cell lysis upon exposure to oxidizing agent. It protects the liver from oxidative intracellular free radicals by increasing the activity of enzymes superoxide dismutase and peroxidase, as well as by increasing the concentration of glutathione. Silibinin strengthens and stabilizes the cell membranes, inhibits the synthesis of prostaglandins associated with the lipid peroxidation, and promotes regeneration of liver through the stimulation of protein synthesis and production of new hepatocytes [23]. Silibinin supplementation limits hepatic glutathione (GSH) depletion and hydrogen peroxide (H_2O_2) production. It also prevents hepatic mitochondrial dysfunction, and reduces hepatic enzyme elevation [24].

Catalase (CAT) is one of the most important intracellular enzymes involved in the detoxification of H_2O_2 [25]. CAT has been identified in most aerobic cells where it is mainly found in the peroxisomes to destroy H_2O_2 generated by oxidase enzymes within these subcellular organelles [26].

Glutathione-S-transferase (GST) is a family of enzymes involved in the binding, transport and detoxification of a wide variety of endogenous and exogenous compounds [27]. GST plays a role in cell defense by eliminating noxious reactive electrophilic xenobiotics and their metabolites from the body as GSH-conjugates [28]. These electrophiles are potentially toxic and can bind to nucleophiles, such as proteins and nucleic acids, causing cellular damage and genetic mutations [29].

2. Materials and Methods

2.1 Experimental animals

Male Wistar rats, weighing 180±20 g, were purchased from Beni-suef University Animal house. They were kept under suitable laboratory conditions for three weeks for adaptation before the start of experiment, and to exclude any intercurrent infection. They were fed on the standard diet (ATMID company, Egypt) and provided with tap water.

2.2 Drugs, Chemicals and reagent kits

Lipopolysaccharide, D-galactosamine and silibinin were purchased from Sigma (St Louis, MO, USA). CAT, MDA and GSH kits were obtained from Biodaignostic (Egypt). ALT, AST kits were purchased from Human Biochemica Und Diagnostica mbH (Ggesellschaft Fü r).

2.3 Experimental design

Thirty male Wistar rats were randomly divided into a normal group (saline), LPS/D-GalN group, and silibinin group. LPS and D-GalN were administered once by intraperitoneal route at a dose of 15 µg/kg and 300 mg/kg respectively, given both to LPS/D-

GalN group, and silibinin group. Silibinin is injected intraperitonially at a dose of 50 mg/kg for a week. All injections were prepared individually by dissolving the calculated required dose in saline as a vehicle.

2.4 Preparation of samples

Blood samples were collected into sterile tubes containing EDTA for plasma separation, and then

centrifuged at 1500 rpm for 30 minutes. Plasma was used for ALT and AST activity assessment. The rats were euthanized by carbon dioxide asphyxiation for tissue sampling, and their livers were removed and washed with normal saline. The liver tissue was washed with distilled water and homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 m M EDTA. The supernatant, separated by funds of cooling centrifugation at 5000 r.p.m for 20 min at 4 ˚C, was used for the analysis of MDA, GSH and CAT.

2.5 Biochemical tests

2.5.1 Determination of ALT and AST activity in plasma

ALT and AST activities were measured in plasma using kits according to the method provided by the manufacturer[30] .

Measurements for markers of oxidative stress and antioxidants

2.5.2 Determination of liver malondialdehyde (MDA) level in liver tissue homogenate

MDA was determined in liver tissue homogenate according to the manufacturer method [31].

2.5.3 Determination of Catalase activity (CAT)

CAT activity was assayed in liver tissue homogenate according to the provided method [32] .

2.5.4 Determination of Reduced Glutathione (GSH)

GSH was determined in liver tissue homogenate according to the method provided in the kit [33].

2.6 statistical analysis

The results were expressed as mean ± standard error of mean (SEM). The statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey multiple comparison Post-Hoc test. The p-value less than 0.05 ($p < 0.05$) were considered significant. All calculations and graphs were made using Statistical Package for Social Science 22 (SPSS) (SPSS, Chicago, USA)..

3. Results

3.1 Liver function test

The obtained results, as shown in **Table** *3-1* and **Figure 3.1**and **Figure 3.2**, revealed that D-GalN/LPS significantly increased ALT and AST activity in the plasma of the animal when compared to healthy control ($p \leq 0.05$). On the other hand, silibinin significantly reduced the ALT and AST activity in plasma of the animal when compared to D-GalN/LPS ($p \leq 0.05$).

3.2 Lipid peroxidation (marked by MDA production)

The results in **Table 3-1** and **Figure 3.3** clarified that D-GalN/LPS administration produced a significant increase in lipid peroxidation and MDA level when compared to healthy control ($p \leq 0.05$). Meanwhile, the administrations of silibinin significantly decreased the lipid peroxidation product MDA in liver tissue when compared to D-GalN/LPS ($p \leq 0.05$).

3.3 Enzymatic (CAT) and nonenzymatic (GSH) antioxidant machinery

Table 3-1 and **Figure 3.4and Figure 3.5** demonstrate that D-GalN/LPS administration produced a highly significant depletion of the CAT enzyme activity and GSH liver tissue content as compared to normal control ($p \leq 0.05$), while the treatment with silibinin significantly restored this depletion ($p \leq 0.05$).

Table 3-1: effect of silibinin on liver tissue levels of certain parameters in rats injected with LPS/D-GalN for induction of FHF

Groups/ parameters	Normal group	LPS/D-GalN group	Silibinin group
ALT (U/L)	29±1.55	68±4.74[a]	35±1.27[b]
AST (U/L)	120±1.43	181±13.49[a]	144±7.24[b]
MDA (nmol/g)	78±5.22	206±27.83[a]	38±4.47[b]
GSH (mg/g)	181±13.09	80±4.14[a]	187±6.66[b]
CAT (u/g)	4922±163.79	2830±28.75[a]	4189±126.54[b]

Values are represented as mean ± SEM for 10 rats.
a significantly different from the normal group at P<0.05
b significantly different from the normal group at P<0.05

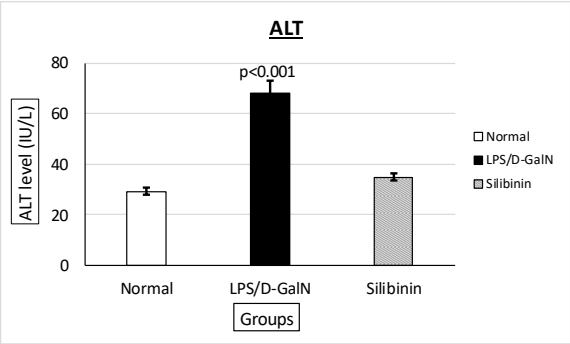

Figure 3.1: effect of silibinin on the activity of ALT compared to LPS/D-GalN treated animals

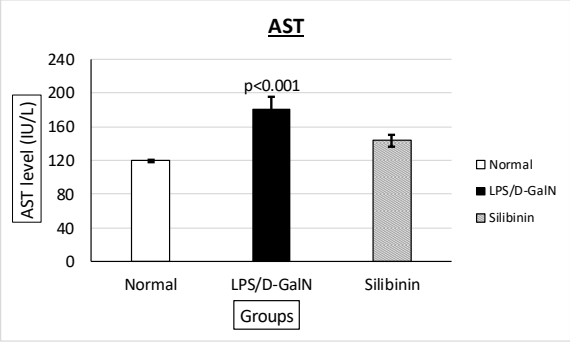

Figure 3.2:effect of silibinin on the activity of AST compared to LPS/D-GalN treated animals

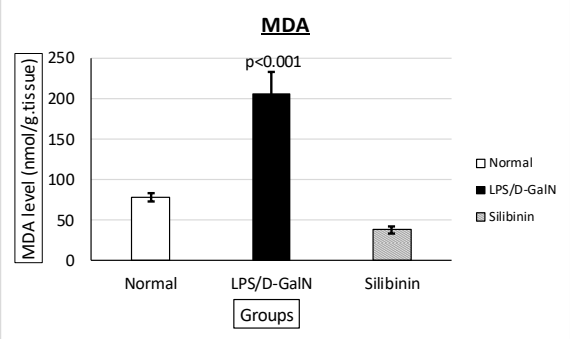

Figure 3.3:effect of silibinin on the levels of MDA compared to LPS/D-GalN treated animals

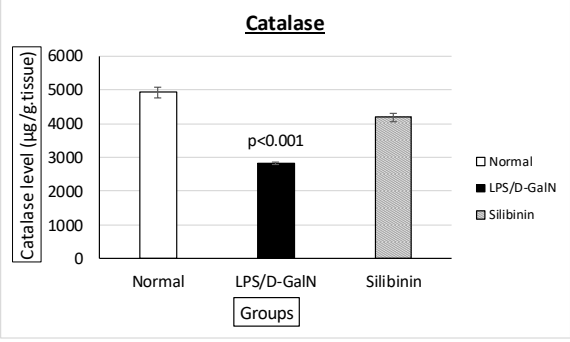

Figure 3.4: effect of silibinin on the activity of CAT compared to LPS/D-GalN treated animals

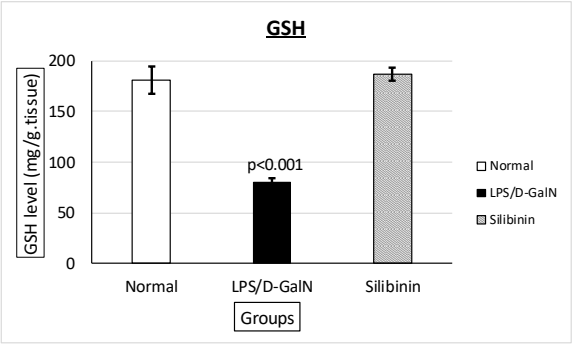

Figure 3.5: effect of silibinin on the levels of GSH compared to LPS/D-GalN treated animals

4. Discussion

The focus of this study was to investigate the acute effects of LPS-induced fulminant hepatic failure and the possible protection offered by administration of silibinin.

Mostly, the hepatic and systemic toxicities of LPS have been attributed to the release of chemical mediators such as superoxide, nitric oxide and pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, which are all formed as a result of the binding of LPS to the CD14/LPS-binding protein and Toll-like receptor-4 (TLR4) on the surface of Kupffer cells that have the potential to damage hepatocyte [3435]. The action of LPS is increased by using D-galactosamine, the well-known hepatotoxicant, as it increases the sensitivity of hepatocyte to the toxic effect of lipopolysaccharide leading at the end to acute hepatic injury[36,37].

In this study, it was observed that a single injection of LPS resulted in hepatic injury as indicated by significant elevation in the levels of serum ALT and AST. This observation is in accordance with some earlier reports which have shown that LPS induces hepatic damage, and as a consequence, increases the level of serum aminotransferases [38,39]. The hepatic function marker enzymes are cytoplasmic in nature but are usually leaked into circulation when liver damage occurs due to an alteration in membrane permeability [40].

Our results in the group treated with silibinin showed a significant decrease in levels of ALT and AST enzymes. These results are in agreement with several studies where silibinin reduced ALT and AST activity to the normal levels [41–44]. This may be explained by the resistance rendered to hepatocytes by silibinin against cell lysis upon exposure to oxidizing agents, by strengthening and stabilizing the cell membranes, thus inhibiting the leakage of liver enzymes to the circulation [23,45].

In the present study, administration of LPS/D-GalN produced a highly significant increase in MDA

concentration in liver tissue, which was in harmony with previous studies which indicated that MDA levels increased to high levels in LPS/D-GalN induced liver injury [46–48]. LPS leads to increased ROS level as a consequence of stimulating innate immune system and this will lead to increase lipid peroxidation with the increase formation of malondialdehyde (MDA) as a product of poly unsaturated fatty lipid peroxidation [49].

In our study, we found that using silibinin significantly decreased MDA level in liver tissue, which was the same effect discussed by [50] [22,24,51,52]who indicate that silibinin show hepatoprotective and free radical scavenging property. This can be attributed to the powerful antioxidant property of silibinin that decreases the oxidative stress and production of intracellular free radicals by increasing the activity of enzymes superoxide dismutase and peroxidase, as well as by increasing the concentration of glutathione, so it decreases lipid peroxidation and the production of MDA [23].

In our study using of LPS/D-GalN led to make a significant decrease in levels of CAT and GSH which was in accordance with [46,53] who indicate that LPS/ D-GalN deplete antioxidant machinery present in the liver of rats. An explanation maybe the fact that LPS/D-GalN produce high levels of free radical that provoke the cells to use enzymatic and non-enzymatic antioxidant activity to protect cells from damage [54,55].

we also showed that silibinin exerted a highly significant restoration in enzymatic and non-enzymatic antioxidant system represented as CAT and GSH, respectively. This is due to the free radical scavenging property of silibinin, consequently oxidative stress significantly decreases and the availability of the antioxidant machinery increases [56].This result is consistent with the previous observation of [24,51,52,57] that silibinin increased the availability of GSH and CAT, due to its antioxidant power.

So silibinin may be useful as a therapeutic agent for FHF induced by LPS/D-GalN.

5. Conclusions

In conclusion, silibinin exhibits antioxidant, hepatoprotective, free radical scavenging, membrane stabilizing powers, and it protects the liver from deleterious effects of D-GalN/LPS, suggesting that it may be beneficial as a therapeutic agent towards the improvement of FHF..

6. References

(1) Kumar, V.; Abbas, A. K.; Fausto, N.; Aster, J. C. *Robbins and Cotran Pathologic Basis of Disease, Professional Edition: Expert Consult-Online*; Elsevier Health Sciences, 2014.

(2) Sherlock, S.; Dooley, J. *Diseases of the Liver and Biliary System*; John Wiley & Sons, 2008.

(3) Barriault, C.; Desmoulire, A.; Costa, A. M. A. Evaluation of Chemical-Induced Bile Duct Proliferation. *Toxicol. Liver. 2nd ed. Washington, DC Taylor Fr.* **1998**, 401–416.

(4) Wanless, I. R.; Sweeney, G.; Dhillon, A. P.; Guido, M.; Piga, A.; Galanello, R.; Gamberini, M. R.; Schwartz, E.; Cohen, A. R. Lack of Progressive Hepatic Fibrosis during Long-Term Therapy with Deferiprone in Subjects with Transfusion-Dependent Beta-Thalassemia. *Blood* **2002**, *100* (5), 1566–1569.

(5) Panackel, C.; Thomas, R.; Sebastian, B.; Mathai, S. K. Recent Advances in Management of Acute Liver Failure. *Indian J. Crit. care Med. peer-reviewed, Off. Publ. Indian Soc. Crit. Care Med.* **2015**, *19* (1), 27.

(6) Zhang, G.; Meredith, T. C.; Kahne, D. On the Essentiality of Lipopolysaccharide to Gram-Negative Bacteria. *Curr. Opin. Microbiol.* **2013**, *16* (6), 779–785.

(7) Holst, O. The Structures of Core Regions from Enterobacterial Lipopolysaccharides–an Update. *FEMS Microbiol. Lett.* **2007**, *271* (1), 3–11.

(8) Miller, S. I.; Ernst, R. K.; Bader, M. W. LPS, TLR4 and Infectious Disease Diversity. *Nat. Rev. Microbiol.* **2005**, *3* (1), 36.

(9) Kaur, M.; Sandhir, R. Comparative Effects of Acute and Chronic Carbofuran Exposure on Oxidative Stress and Drug-Metabolizing Enzymes in Liver. *Drug Chem. Toxicol.* **2006**, *29* (4), 415–421.

(10) Wills, P. J.; Asha, V. V. Protective Effect of Lygodium Flexuosum (L.) Sw. Extract against Carbon Tetrachloride-Induced Acute Liver Injury in Rats. *J. Ethnopharmacol.* **2006**, *108* (3), 320–326.

(11) Seçkin, S.; Alsancak, S.; Basaran Küçükergin, C.; Aydin, M. Effect of D-Galactosamine on Oxidative Stress and Apoptosis in the Liver of Rats. *J 1st Fac. Med* **2008**, *71*, 29–32.

(12) Wu, Y.-H.; Hu, S.-Q.; Liu, J.; Cao, H.-C.; Xu, W.; Li, Y.-J.; Li, L.-J. Nature and Mechanisms of Hepatocyte Apoptosis Induced by D-Galactosamine/Lipopolysaccharide Challenge in Mice. *Int. J. Mol. Med.* **2014**, *33* (6), 1498–1506.

(13) Osakabe, N.; Yasuda, A.; Natsume, M.; Sanbongi, C.; Kato, Y.; Osawa, T.; Yoshikawa, T. Rosmarinic Acid, a Major Polyphenolic Component of Perilla Frutescens, Reduces Lipopolysaccharide (LPS)-Induced Liver Injury in D-Galactosamine (D-GalN)-Sensitized Mice. *Free Radic. Biol. Med.* **2002**, *33* (6), 798–806.

(14) Cichoż-Lach, H.; Michalak, A. Oxidative Stress as a Crucial Factor in Liver Diseases. *World J. Gastroenterol. WJG* **2014**, *20* (25), 8082.

(15) Gaze, D. C. The Role of Existing and Novel

Cardiac Biomarkers for Cardioprotection. *Curr. Opin. Investig. drugs (London, Engl. 2000)* **2007**, *8* (9), 711–717.

(16) Wang, C.-S.; Chang, T.-T.; Yao, W.-J.; Wang, S.-T.; Chou, P. Impact of Increasing Alanine Aminotransferase Levels within Normal Range on Incident Diabetes. *J. Formos. Med. Assoc.* **2012**, *111* (4), 201–208.

(17) Marnett, L. J. Lipid Peroxidation—DNA Damage by Malondialdehyde. *Mutat. Res. Mol. Mech. Mutagen.* **1999**, *424* (1), 83–95.

(18) Moore, K.; Roberts, L. J. Measurement of Lipid Peroxidation. *Free Radic. Res.* **1998**, *28* (6), 659–671.

(19) Pham-Huy, L. A.; He, H.; Pham-Huy, C. Free Radicals, Antioxidants in Disease and Health. *Int. J. Biomed. Sci. IJBS* **2008**, *4* (2), 89.

(20) Davis-Searles, P. R.; Nakanishi, Y.; Kim, N.-C.; Graf, T. N.; Oberlies, N. H.; Wani, M. C.; Wall, M. E.; Agarwal, R.; Kroll, D. J. Milk Thistle and Prostate Cancer: Differential Effects of Pure Flavonolignans from Silybum Marianum on Antiproliferative End Points in Human Prostate Carcinoma Cells. *Cancer Res.* **2005**, *65* (10), 4448–4457.

(21) Saller, R.; Brignoli, R.; Melzer, J.; Meier, R. An Updated Systematic Review with Meta-Analysis for the Clinical Evidence of Silymarin. *Complement. Med. Res.* **2008**, *15* (1), 9–20.

(22) Tong, W. W.; Zhang, C.; Hong, T.; Liu, D. H.; Wang, C.; Li, J.; He, X. K.; Xu, W. D. Silibinin Alleviates Inflammation and Induces Apoptosis in Human Rheumatoid Arthritis Fibroblast-like Synoviocytes and Has a Therapeutic Effect on Arthritis in Rats. *Sci. Rep.* **2018**, *8* (1), 3241.

(23) Kostek, H.; Szponar, J.; Tchórz, M.; Majewska, M.; Lewandowska-Stanek, H. Silibinin and Its Hepatoprotective Action from the Perspective of a Toxicologist. *Przegl. Lek.* **2012**, *69* (8), 541–543.

(24) Anthony, K. P.; Saleh, M. A. Free Radical Scavenging and Antioxidant Activities of Silymarin Components. *Antioxidants* **2013**, *2* (4), 398–407.

(25) Calabrese, E. J.; Canada, A. T. Catalase: Its Role in Xenobiotic Detoxification. *Pharmacol. Ther.* **1989**, *44* (2), 297–307.

(26) Chance, B.; Sies, H.; Boveris, A. Hydroperoxide Metabolism in Mammalian Organs. *Physiol Rev* **1979**, *59* (3), 527–605.

(27) Mulder, T. P. J.; Peters, W. H. M. Variability of Glutathione S-Transferase α in Human Liver and Plasma. *Clin. Chem.* **1999**, *45* (3), 355–359.

(28) Siddiqui, A.; Ali, B.; Srivastava, S. P. Effect of Mancozeb on Hepatic Glutathione S-Transferase in Rat. *Toxicol. Lett.* **1993**, *68* (3), 301–305.

(29) Parkinson, A. *Biotransformation of Xenobiotics*; McGraw-Hill New York, 2001.

(30) Schumann, G.; Klauke, R. New IFCC Reference Procedures for the Determination of Catalytic Activity Concentrations of Five Enzymes in Serum: Preliminary Upper Reference Limits Obtained in Hospitalized Subjects. *Clin. Chim. Acta* **2003**, *327* (1), 69–79.

(31) Kei, S. Serum Lipid Peroxide in Cerebrovascular Disorders Determined by a New Colorimetric Method. *Clin. Chim. acta* **1978**, *90* (1), 37–43.

(32) Mueller, S.; Riedel, H.-D.; Stremmel, W. Determination of Catalase Activity at Physiological Hydrogen Peroxide Concentrations. *Anal. Biochem.* **1997**, *245* (1), 55–60.

(33) Beutler, E.; Duron, O.; Kelly, B. M. Improved Method for the Determination of Blood Glutathione. *J. Lab. Clin. Med.* **1963**, *61*, 882–888.

(34) Marra, F.; Tacke, F. Roles for Chemokines in Liver Disease. *Gastroenterology* **2014**, *147* (3), 577–594.

(35) Płóciennikowska, A.; Hromada-Judycka, A.; Borzęcka, K.; Kwiatkowska, K. Co-Operation of TLR4 and Raft Proteins in LPS-Induced pro-Inflammatory Signaling. *Cell. Mol. life Sci.* **2015**, *72* (3), 557–581.

(36) Hirono, S.; Nakama, T.; Tsubouchi, H. Molecular Mechanisms of D-Galactosamine/Lipopolysaccharide-Induced Fulminant Hepatic Failure in Mice and the Effects of Therapeutic Agents. In *Trends in Gastroenterology and Hepatology*; Springer, 2001; pp 59–62.

(37) Freudenberg, M. A.; Galanos, C. Induction of Tolerance to Lipopolysaccharide (LPS)-D-Galactosamine Lethality by Pretreatment with LPS Is Mediated by Macrophages. *Infect. Immun.* **1988**, *56* (5), 1352–1357.

(38) Crane, M.; Avihingsanon, A.; Rajasuriar, R.; Velayudham, P.; Iser, D.; Solomon, A.; Sebolao, B.; Tran, A.; Spelman, T.; Matthews, G. Lipopolysaccharide, Immune Activation, and Liver Abnormalities in HIV/Hepatitis B Virus (HBV)–Coinfected Individuals Receiving HBV-Active Combination Antiretroviral Therapy. *J. Infect. Dis.* **2014**, *210* (5), 745–751.

(39) Ajuwon, O. R.; Oguntibeju, O. O.; Marnewick, J. L. Amelioration of Lipopolysaccharide-Induced Liver Injury by Aqueous Rooibos (Aspalathus Linearis) Extract via Inhibition of pro-Inflammatory Cytokines and Oxidative Stress. *BMC Complement. Altern. Med.* **2014**, *14* (1), 392.

(40) Thapa, B. R.; Walia, A. Liver Function Tests and Their Interpretation. *Indian J. Pediatr.* **2007**, *74* (7), 663–671.

(41) Fraschini, F.; Demartini, G.; Esposti, D. Pharmacology of Silymarin. *Clin. Drug Investig.* **2002**, *22* (1), 51–65.

(42) Valenzuela, A.; Garrido, A. Biochemical Bases of the Pharmacological Action of the Flavonoid Silymarin and of Its Structural Isomer Silibinin. *Biol. Res.* **1994**, *27*, 105.

(43) Habib-ur-Rehman, M.; Mahmood, T.; Salim, T.; Afzal, N.; Ali, N.; Iqbal, J.; Tahir, M.; Khan, A. Effect of Silymarin on Serum Levels of ALT and GGT in Ethanol Induced Hepatotoxicity in Albino Rats. *J. Ayub Med. Coll. Abbottabad* **2009**, *21* (4), 73–75.

(44) de Avelar, C. R.; Pereira, E. M.; de Farias Costa,

P. R.; de Jesus, R. P.; de Oliveira, L. P. M. Effect of Silymarin on Biochemical Indicators in Patients with Liver Disease: Systematic Review with Meta-Analysis. *World J. Gastroenterol.* **2017**, *23* (27), 5004.

(45) Kew, M. C. Serum Aminotransferase Concentration as Evidence of Hepatocellular Damage. *Lancet* **2000**, *355* (9204), 591–592.

(46) Kim, I. D.; Ha, B. J. The Effects of Paeoniflorin on LPS-Induced Liver Inflammatory Reactions. *Arch. Pharm. Res.* **2010**, *33* (6), 959–966.

(47) Ammari, M.; Othman, H.; Hajri, A.; Sakly, M.; Abdelmelek, H. Pistacia Lentiscus Oil Attenuates Memory Dysfunction and Decreases Levels of Biomarkers of Oxidative Stress Induced by Lipopolysaccharide in Rats. *Brain Res. Bull.* **2018**, *140*, 140–147.

(48) Stuss, M.; Wiktorska, J. A.; Sewerynek, E. N-Acetylserotonin Reduces Lipopolysaccharide-Induced Lipid Peroxidation in Vitro More Effectively than Melatonin. *Neuro Endocrinol. Lett.* **2010**, *31* (4), 489–496.

(49) Liu, J.; Yeo, H. C.; Overvik-Douki, E.; Hagen, T.; Doniger, S. J.; Chu, D. W.; Brooks, G. A.; Ames, B. N. Chronically and Acutely Exercised Rats: Biomarkers of Oxidative Stress and Endogenous Antioxidants. *J. Appl. Physiol.* **2000**, *89* (1), 21–28.

(50) Lucena, M. I.; Andrade, R. J.; De la Cruz, J. P.; Rodriguez-Mendizabal, M.; Blanco, E.; de la Cuesta, F. S. Effects of Silymarin MZ-80 on Oxidative Stress in Patients with Alcoholic Cirrhosis. *Int. J. Clin. Pharmacol. Ther.* **2002**, *40* (1), 2–8.

(51) Hackett, E. S.; Twedt, D. C.; Gustafson, D. L. Milk Thistle and Its Derivative Compounds: A Review of Opportunities for Treatment of Liver Disease. *J. Vet. Intern. Med.* **2013**, *27* (1), 10–16.

(52) Ferenci, P. Silymarin in the Treatment of Liver Diseases: What Is the Clinical Evidence? *Clin. Liver Dis.* **2016**, *7* (1), 8–10.

(53) Tomasi, M. L.; Ryoo, M.; Yang, H.; Ara, A. I.; Ko, K. S.; Lu, S. C. Molecular Mechanisms of Lipopolysaccharide-Mediated Inhibition of Glutathione Synthesis in Mice. *Free Radic. Biol. Med.* **2014**, *68*, 148–158.

(54) Sies, H. Oxidative Stress: Introductory Remarks. *Oxidative Stress* **1985**, *501*, 1–8.

(55) Finkel, T.; Holbrook, N. J. Oxidants, Oxidative Stress and the Biology of Ageing. *Nature* **2000**, *408* (6809), 239.

(56) Birben, E.; Sahiner, U. M.; Sackesen, C.; Erzurum, S.; Kalayci, O. Oxidative Stress and Antioxidant Defense. *World Allergy Organ. J.* **2012**, *5* (1), 9.

(57) Gordon, A.; Hobbs, D. A.; Bowden, D. S.; Bailey, M. J.; Mitchell, J.; Francis, A. J. P.; Roberts, S. K. Effects of Silybum Marianum on Serum Hepatitis C Virus RNA, Alanine Aminotransferase Levels and Well-being in Patients with Chronic Hepatitis C. *J. Gastroenterol. Hepatol.* **2006**, *21* (1), 275–280.