Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 43 / Number 3 / September 2023 / pp. 212-220

Ameliorating Potential of Marketed Formulations Containing Prebiotics and Probiotics Against Carbon Tetrachloride-Induced Hepatotoxicity in Rats

Gopi H. SHAH[1*],
ORCID: 0000-0001-8299-3509
Punit R. BHATT[1],
ORCID: 0000-0001-6324-051X
Bhanubhai N. SUHAGIA[1],
ORCID: 0000-0003-3480-3015
Bharat G. PATEL[2],
ORCID: 0000-0003-4664-3899
Gaurang B. SHAH[3],
ORCID: 0000-0003-0769-3914

[1*] Faculty of Pharmacy, Dharmsinh Desai University, Nadiad (Gujarat) India

[2] Charotar University of Science and Technology (CHARUSAT), Changa (Gujarat) India

[3] L. M. College of Pharmacy, Ahmedabad (Gujarat) India

Corresponding author:
Gopi H. SHAH
Department of Pharmacology, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad (Gujarat) India
E-mail: gopishah.ph@ddu.ac.in
Tel: +91 0268 2520502

Received date : 23.06.2022
Accepted date : 22.09.2022

DOI: 10.52794/hujpharm.1134688

ABSTRACT

In the current study, two marketed formulations VELGUT® (combination of prebiotic and probiotics) and VIZYLAC® (probiotic only) were assessed for a protective effect against carbon tetrachloride (CCl_4) induced chronic liver injury model in rats. Rats were randomly divided into four groups. The normal control group was treated with normal saline (C1) and CCl_4 treated group was treated with 1 ml/kg intraperitoneal injection of CCl_4 one time a day for 10 days followed by two times in a week for 49 days (C2). Test groups composed of the oral treatment of VELGUT (1 mg kg^{-1}) along with CCl_4 (T1) and oral treatment of VIZYLAC (4 mg kg^{-1}) along with CCl_4 (T2). At the end of the treatment, various serum biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase, (AST), alkaline phosphatase (ALP), direct bilirubin, total protein, and albumin levels were measured. Serum AST, ALT, ALP, and direct bilirubin of the groups T1 and T2 were found to be significantly lower as compared to group C1. The level of total protein and albumin was improved in the groups T1 and T2. The level of MDA, glucose and cholesterol was significantly decreased ($p<0.05$) in the groups T1 and T2 as compared to group C2.

Keywords: liver fibrosis, lipid peroxidation, oxidative stress, liver biomarkers, probiotics

1. Introduction

The liver is the biggest organ in the body and is involved in drug metabolism. Hepatocytes are engaged in the detoxification of a number of medications, vitamins, hormones, and environmental toxins, as well as the metabolism of amino acids and ammonia, as well as biochemical oxidation processes. Kupffer cells are a reservoir of fixed macrophages that protect against gut-derived poisons that have made their way into the portal circulation. Endotoxins are primarily responsible for cytokine production. Other types of liver cells serve the same role. The liver is the first line of defence and appears to be the most commonly harmed target organ by industrial toxins [1]. Occupational and environmental liver disorders can show a variety of clinical symptoms [2]. Carbon Tetrachloride (CCl_4) is hepatotoxic which in turn causes acute liver injury via mechanism of oxidative stress [3-6]. Due to oxidative stress, the free radical produced by CCl_4 causes lipid peroxidation which results in to the damage of bio-membrane. Despite of the advancement in the modern medical science, a few drugs are available to treat liver disorders [7]. Probiotics are living microorganisms that play favourable roles and help to maintain the balance of intestinal microbiota depending on the dose [8, 9]. Prebiotics are complex oligosaccharides that help in the growth of good bacteria in the gut flora. Inulin, fructooligosaccharide, lactulose, etc. are well-studied prebiotics. The physiological mechanisms of probiotics and prebiotics are exerted either directly or indirectly through the modulation of the host intestinal microbiota's composition, the stimulation of the endogenous microbial community, and the regulation of the immune system. A combination of prebiotics and probiotics constitutes a functional product; their advantageous physiological and biochemical characteristics have been validated. Studies showed that the concurrent use of prebiotics and probiotics significantly reduced alcoholic liver diseases and modifies liver functions [10, 11,12,13].

Probiotics may be considered a safe and effective alternative in the treatment of hepatotoxicity [14,15]. Probiotics deliberate general health benefits on the host. Moreover, the risk of diseases is also reduced significantly [10]. Probiotics fulfil significant roles within the human body, including the mitigation of detrimental bacterial proliferation through pH modulation within the intestinal milieu, amelioration of diarrhoea episodes, synthesis of essential vitamins, and reduction of serum cholesterol concentrations. [16]. Probiotics exhibit positive effects on intestinal and liver diseases [17].

From the review it has been shown that Kirpich had used probiotics to treat alcoholic liver disease (ALD) patients, showing that probiotics (*B. bifidum* and *L.plantarum* 8PA3) significantly increased the number of Lactobacillus and Bifidobacterium in human faeces and significantly altered the serum levels of ALT, low-density lipoprotein (LDL), and total bilirubin (STB) [10]. Therefore, many investigators began to pay attention to the role of probiotics in alcoholic liver disease. They used a variety of probiotic strains, most often *Lactobacillus rhamnosus* GG (LGG). LGG is a short Gram-positive heterofermentative facultative anaerobe that was isolated in 1983. LGG is effective in the treatment of ALD. The proposed work aims to evaluate the activity of probiotics and the combination of probiotics in the chronic liver injury model.

2. Materials and Methods

2.1. Materials

CCl_4 was procured from Loba Chemie Pvt Ltd, Mumbai (India). Kits for the estimation of SGPT, SGOT, bilirubin, total protein, albumin, glucose, and cholesterol were procured from Autospan (Span Diagnostic Pvt. Limited India).

VELGUT was purchased from Eris life sciences, Bengaluru, and VIZYLAC was purchased from Kalindi Healthcare Pvt. Ltd., India.

2.2. Experimental Protocol & Procedure

2.2.1. Animals

The animal study was performed after obtaining written permission from the Institutional Animal Ethics Committee (IAEC), Ramanbhai Patel College of Pharmacy, Changa (Gujarat) India, with the protocol number (RPCP/IAEC/2013-2014/R-32) dated 9 January 2014. Healthy Wistar rats of either sex, weighing 200-250 gm were included in the study. The animals were housed in the polypropylene cage at 25^0C; 12 hours dark-light cycle, with free access to standard pellet diet (normal pellet diet) and water *ad libitum* during the experiment. The animals were acclimatized to surround for one week before the experiment.

ISSN: 2458 - 8806

2.2.2. Experimental design

The rats were divided into 4 different groups. Normal control (treated with normal saline only; C_1) (The use of Arachis oil was avoided in the normal control group because it may affect the liver parameters [16]); CCl_4 treated group (1 ml/kg intraperitoneal injection i. p. of CCl_4 once daily for 10 days followed by twice a week up to 49 days; C_2) [17]; test groups composed of treatment of the oral treatment of VELGUT (1 mg kg^{-1}) along with CCl_4 (T_1) and oral treatment of VIZYLAC (4 mg kg^{-1}) along with CCl_4. (Table 1). The dose of VELGUT and VIZYLAC was determined based on the preliminary experiments carried out before these experiments [10, 18,19].

2.2.3. Blood Sample Collection

On 49th day, blood samples for the separation of plasma and serum were collected from retro-orbital plexus in ethylene diamine tetra acetate (EDTA) and EDTA-free vials, respectively. After the collection, the samples were centrifuged at 4000 rpm for 10 to 15 minutes under the cooling condition to separate plasma and serum. Plasma samples were used to assess all biochemical parameters such as AST, ALT, bilirubin, albumin, total protein, glucose, cholesterol & malondialdehyde, whereas serum samples were analyzed to measure ALP activity.

2.2.4. Histopathological Investigation

At the end of the treatment, rats were kept on overnight fasting. Rats of the different groups were sacrificed on day 49. The liver was collected and washed with ice-cold saline. The excised liver was kept in 10 % buffered-formalin solution and submitted to the Department of Veterinary Pathology, Anand Veterinary College, Kamdhenu University, Anand (Gujarat) India for histopathological study. The liver tissue was subjected to Haematoxylin & Eosin Staining (H&E) for the assessment of CCl_4-induced liver damage including morphological changes and fibrosis.

2.2.5. Statistical Analysis

All parameters were expressed as a mean value ± S.E.M. Differences between the mean value of tests and controls were analyzed statistically by the Tukey's test. $p<0.05$ and $p<0.01$ were considered as statistically significant.

3. Results and Discussion

Animals of group C2 treated with CCl_4 showed marked increased levels of ALP, ALT, and AST. Animals of groups T1 and T2 showed a significant ($p<0.05$, $p<0.01$) reduction in the level of the ALP as compared to group C_2. However, there was no significant difference ($p>0.05$) between groups T1 and T2. Bilirubin levels also increased in the animals of group C2 which was significantly decreased in both the treatment groups. In the case of total protein and albumin levels, animals of group C2 showed a reduction in level as compared to group C1. In the treatment groups T1 and T2, both the parameters normalized to normal levels due to the treatment. Globulin level was higher in the animals of group C2 but it was significantly recovered in the animals of groups T1 and T2. Cholesterol level was higher in the case of animals treated with CCl_4 but it was normalized in the animals of groups T1 and T2. The level of malondialdehyde was markedly increased in the animals of group C2. However, a significant reduction in the MDA level was observed in the treatment groups, especially, in group T1. Changes in the level of the parameter were shown in Table 2.

Table 1. Different groups of animals in the present study

Group	Treatment
C1	Animals will receive normal saline for up to 49 days
C2	Animals will receive CCl_4 (1 ml/kg, i.p) once-daily injection for 10 days followed by twice a week up to 49 days
T1	Animals will receive CCl_4 (1 ml/kg, i.p) once-daily injection for 10 days followed by twice a week up to 49 days along with VELGUT (4 mg/kg body weight)
T2	Animals will receive CCl_4 (1 ml/kg, i.p) once-daily injection for 10 days followed by twice a week up to 49 days along with VIZYLAC (1 mg/kg body weight)

Table 2. Change in various serum parameters in rats exposed to CCl_4 and marketed probiotics (VELGUT & VIZYLAC)

Parameter/Group	C_1	C_2	T_1	T_2
ALP (U/L)	78.95±18.80	312.78±49.9§§	148.26±26.16*	113.30±18.27$^{\#\#}$
ALT (U/L)	29.46±7.73	548.08±24.07§§	43.94±3.68 **	42.49±9.39 $^{\#\#}$
AST (U/L)	61.29±13.12	619.38±127.94§§	120.58±19.27**	158.19±19.79 $^{\#\#}$
Bilirubin (mg/dL)	0.05±0.005	0.85±0.27§	0.04±0.004 *	0.04±0.004$^{\#}$
Total Protein (g/dL)	6.26±0.36	4.25±0.04§§	7.27±0.17 **	7.18±1.22 $^{\#\#}$
Albumin (g/dL)	5.36±0.18	1.85±0.47§§	4.25±0.12 **	4.46±0.32 $^{\#\#}$
Globulin (g/dL)	1.20±0.12	3.74±0.12§§	2.73±0.30 *	2.62±0.42$^{\#}$
Cholesterol (mg/dL)	71.91±3.94	231.98±11.98§§	77.73±4.75**	69.36±6.13$^{\#\#}$
Glucose (mg/dL)	75.29±6.52	206.53±25.70§§	67.67±8.98**	56.66±8.42$^{\#\#}$
MDA (micromole/L)	0.285±0.14	3.88±1.31§	0.22±0.05 *	0.61±1.15$^{\#}$

Data are expressed as mean ± SEM, n=6,
§ indicate $p<0.05$, §§ indicate $p<0.01$
* and # indicate $p<0.05$, ** and $^{\#\#}$ indicate $p<0.01$,
* Shows significant difference in various liver parameters between the animals treated with CCl_4 and animals treated with the drug. (VELGUT)
$^{\#}$ Shows significant difference in various liver parameters between the animals treated with CCl_4 and animals treated group with drug (VIZYLAC)
§Shows significant differences in various liver parameters between the animals treated with normal saline and CCl_4.

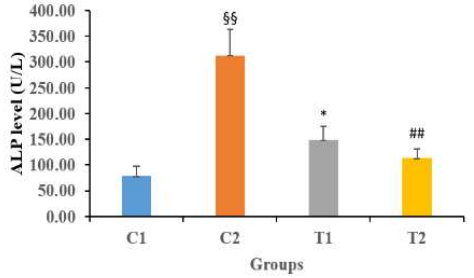
The rats of the control group showed normal architecture of the liver. They show normal hepatocytes, portal triad vasculature, and bile ducts. Whereas rats of group C2 (treated with CCl_4) exhibited extensive liver damage and mixtures of broad and thin fibrous septa (Figure B). In the case of test groups, no clear damage was observed. The treatment with VELGUT and VIZYLAC recovered the damage caused by the CCl_4 treatment (Figures 2C and 2D).

The aim of the present study was to check the hepatoprotective activity of marketed formulations of probiotics on CCl_4-treated rats. The rat model of CCl_4-induced acute liver injury is widely used to assess the hepatoprotective effect.

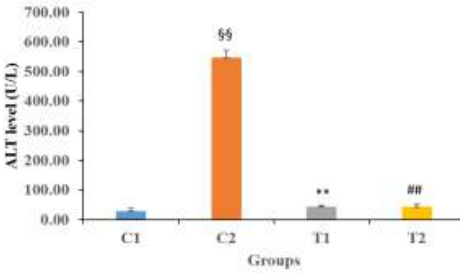
Hepatic metabolic enzymes AST and ALT are released into the bloodstream when the liver is damaged. These enzymes are thought to reflect hepatic damage after hepatotoxic agents have been administered. It's widely assumed that CCl_4 accumulates in hepatic parenchyma cells, where it's converted to CCl_3 radical by cytochrome P450-dependent monooxygenase. This CCl_3 radical attacks polyunsaturated fatty acids and produces lipid peroxides, causing hepatic enzyme levels such as SGPT, SGOT, ALP, LDH, and total bilirubin to change. Hepatocytes leak these enzyme markers as a result of a disruption in transport function caused by hepatic injury and altered membrane permeability. In agreement with this, we found similar results in our study, where the level of hepatic enzymes was elevated after intraperitoneal administration of CCl_4. The treatment of both the marketed probiotic formulations used in this study prevented this increase in hepatic enzyme levels. Furthermore, there were no adverse effects from administering these probiotics to the experimental animals during the study. Toxicity tests were carried out, but no signs of toxicity were discovered.

As previously stated, the production of CCl_3 radicals alkylates hepatic cellular proteins and other macromolecules, contributing to the production of lipid peroxides by damaging polyunsaturated fatty acids, resulting in hepatocellular necrosis. Hepatocyte necrosis is also caused by the overproduction of reactive oxygen species, which damages DNA, proteins, lipids, and carbohydrates. Increased liver LPO levels

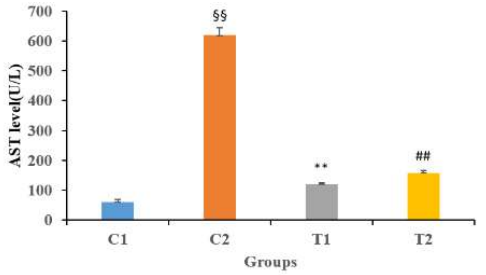
ISSN: 2458 - 8806



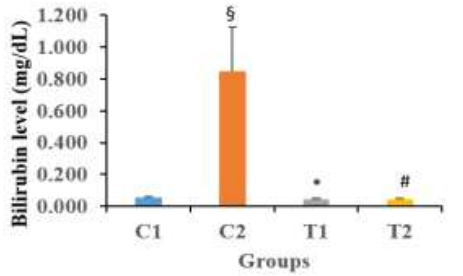
Effect of CCl_4, VELGUT, and VIZYLAC on ALP level



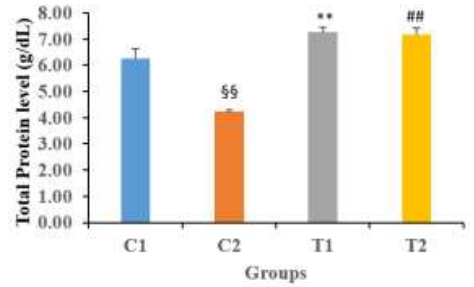
Effect of CCl_4, VELGUT, and VIZYLAC on ALT level



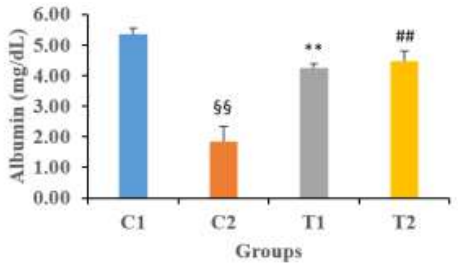
Effect of CCl_4, VELGUT, and VIZYLAC on AST level



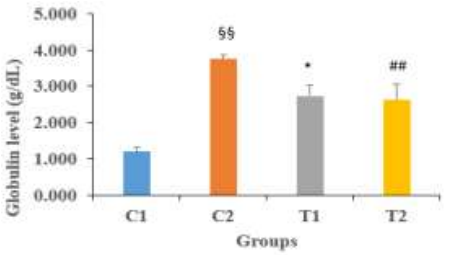
Effect of CCl_4, VELGUT, and VIZYLAC on bilirubin level



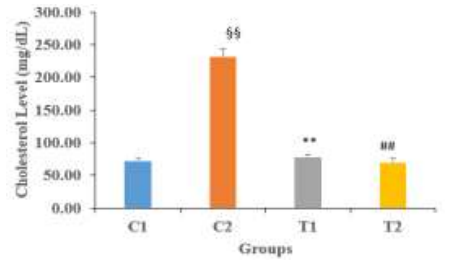
Effect of CCl_4, VELGUT, and VIZYLAC on ALP level



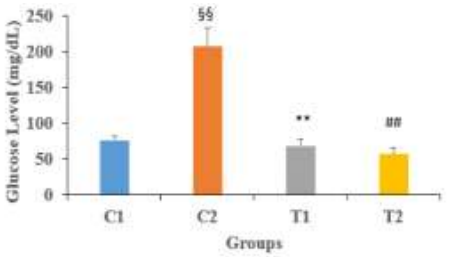
Effect of CCl_4, VELGUT, and VIZYLAC on albumin level



Effect of CCl_4, VELGUT, and VIZYLAC on globulin level



Effect of CCl_4, VELGUT, and VIZYLAC on cholesterol level



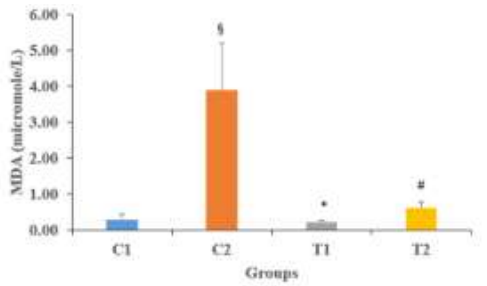


Figure 1. Effect of CCl_4, VELGUT, and VIZYLAC on various liver parameters

ALP-alkaline phosphate, **ALT**- alanine transaminase, **AST**-aspartate transaminase, **T.P** – total protein, **MDA**- malondialdehyde

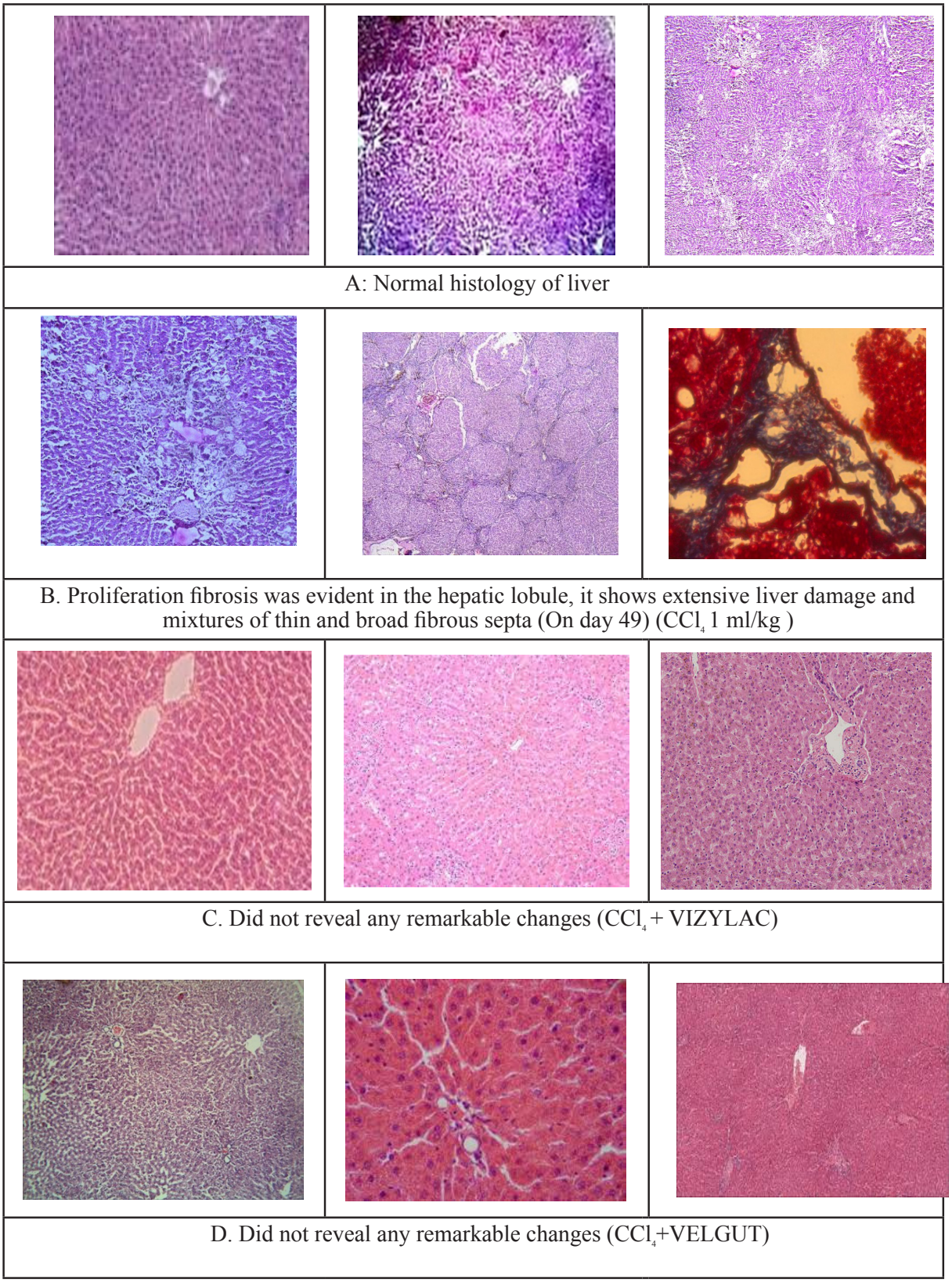


Figure 2. Histopathological study of various treatment groups

ISSN: 2458 - 8806

indicate hepatic damage and an unmanaged antioxidant defence mechanism against free radicals generated. Thus, hepatoprotection against CCl_4-induced liver toxicity relies heavily on inhibiting free radical generation or increasing cellular antioxidant levels. In the current study, we found that CCl_4. administration resulted in higher levels of lipid peroxidation and low levels of cellular antioxidants like CAT and GSH, showing a marked increase in oxidative stress at the cellular level and, as a result, hepatic necrosis, pointing to disease induction, i.e. hepatotoxicity. The pre-administration of probiotics preserved the levels of cellular antioxidants. In addition, the levels of lipid peroxidation were not significantly increased in the treatment group, representing that free radicals produced due to CCl_4 were managed.

Lipid, carbohydrate, and protein metabolism are all controlled by the liver. The injection of CCl_4 resulted in a significant increase in total cholesterol levels. Probiotics administration altered lipid profiles in both pre-and post-treatment groups. Increased esterification of fatty acids, inhibition of fatty acid oxidation, and decreased excretion of cellular lipids could all contribute to higher cholesterol levels [21]. CCl_4 increases cholesterol synthesis by stimulating the transfer of acetate into liver cells (possibly by increasing acetate access). It also boosts the production of fatty acids and triglycerides acetate and enhances lipid esterification.

The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status of both animals and humans [22]. The vital functions that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication, make the hematopoietic system unique as a target organ. CCl_4-induced liver intoxication is one of the most widely used methods to study toxin-mediated experimental fibrosis in the liver in experimental animals especially rats and mice [23]. Liver injury induced by CCl_4 is the best-characterized system of xenobiotic-induced hepatotoxicity and, is a well-studied model for testing the anti-hepatotoxic and hepatoprotective activity of drugs, herbs and pharmaceuticals [24]. The liver is the main site for CCl_4–induced effects, but there is no specific "receptor" for the actions of CCl_4. In addition to this, various subcellular structures like the endoplasmic reticulum, plasma membrane, mitochondria, and Golgi apparatus of the hepatocytes are affected by CCl_4 exposure, indicating the hypothesis that CCl_4 is primarily targeting the lipid-containing structures of the cell [25]. Literature showed that most studies rely on the CCl_4-induced liver damage model to induce toxic liver fibrosis in the experimental animals due to the good comparability, excellent reproducibility, and moderate burden for the animals [24]. From the review, it was reported that liver injury induced by CCl_4 elevated the number of infiltrated neutrophils, kupffer cells, lymphocytes, macrophages, and natural killer cells which further induced activation of macrophages of the liver and/or chemoattraction of extrahepatic cells like neutrophils and lymphocytes. The release of activated macrophages causes the liver fibrosis, inflammation, and injury [26].

Probiotics are gaining more and more interest as alternatives for antibiotics or anti-inflammatory drugs. The effects produced by probiotics depend on their metabolic properties, the molecules present at the surface, or the components secreted. Even essential components of the bacterial cell, such as DNA or peptidoglycan, may be crucial for the probiotic's efficiency. A probiotic strain's unique combination of these qualities defines its specific probiotic function, and thus, how well it may be used to prevent and/or treat a particular disease. [27]. Probiotics might be able to ameliorate the host defence including an innate and acquired immune system of the body. This mechanism is most likely important for the prevention and treatment of infectious diseases. In addition, it can also be used for the treatment of chronic inflammation of the digestive system [28]. The metabolites, cell wall content and DNA of the probiotics can influence the immune system. Probiotics-derived components like peptidoglycan fragments or DNA even from dead probiotic bacteria may exhibit an immune modulatory effect. It is also postulated that probiotic strains can improve the gut and immune systems, so it is proposed that probiotics can be administered to improve alcoholic liver disease symptoms. Probiotic therapy can improve liver markers such as alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase and total bilirubin, ameliorating hepatic inflammation and histological grade [21]. It has been reported that co-administration of lipoic acid to rats chronically treated with thioacetate inhibited the development of liver cirrhosis, as indicated by reductions in cirrhosis incidence, hepatic fibrosis, and AST/ALT activities [29]. It was studied that lipoic acid and lipoteichoic

acid (LTS) can serve as an antioxidant through scavenging of free radicals, chelation of metal ions, and regeneration of endogenous and exogenous antioxidants [30]. LTAs from some sources have been reported to antagonize LPS-induced adhesion molecule expression and the release of interleukin-8 in human lung endothelial cells [31]. From the reported article it was postulated that probiotics (*Lactobacilli, Bifidobacterium*, and many more) can improve liver function. Probiotics have shown beneficial effects in various disease conditions. In the present study, we estimated a few of the cell wall contents, i.e. lipoic acid and lipoteichoic acid. These contents are known to affect Toll-like receptors and can impact the inflammatory process. The histopathological analysis of the liver of the normal control group did not exhibit any noticeable histological changes. In contrast, CCl_4 treated group showed consistent remarkable histological changes in the liver. However, rats of the treatment group (treated with marketed probiotics) exhibited a marked reduction in these types of liver morphological changes. This shows that the probiotic treatment improved the histopathological status in all the animals. Therefore, it can be concluded that the probiotic treatment could have an antioxidant and anti-necrotic effect [32].

4. Conclusion

From the present findings, it was concluded that CCl_4 causes chronic liver injury (fibrosis & cirrhosis). The suggested animal model of chronic liver injury may be useful for testing medications that are used to treat liver damage and related conditions. From the study, it was also concluded that the probiotics and a combination of pre and probiotics were able to improve liver functions.

Statement of Contribution of Researchers

Conceptualisation of the research work - GHS & BGP; Statistical analyses and writing of the manuscript - GHS and PRB; Manuscript review and suggested changes in manuscript - BNS & GBS

Acknowledgements

The authors are thankful to the Department of Veterinary Pathology, Anand Veterinary College, Kamdhenu University, Anand for histopathological analysis. The authors acknowledge Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology (Gujarat) India for financial support.

Statement of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Banrida W, Juliane IB, Heather BC, Heather J, Bellis J, Cameron FK, Craig JM, Matt CC. Toxicant-associated Steatohepatitis. Toxicol Pathol. 2013;41:343-60.
2. Cave M, Falkner KC, McClain CJ. Occupational and Enviromental Liver Disease. Zakim and Boyer's Hepatology. A Textbook of Liver Disease. Philadelphia, USA: Elsevier Saunders; 2011. 476–92 p.
3. Renner H. The limited relevance of models used for testing human hepatic diseases and their prevention: In: Keppler E, Popper H, Bianchi L, and Reutter W (Eds.). Mechanisms of Hepatocyte Injury and Death. Lancaster, UK: MTP Press Ltd.; 1985.
4. Kaplowitz NTY, Simon FR, Stolz A. Drug-induced hepatotoxicity. Ann Intern Med. 1986;104:826-39.
5. Cotran RS, Kumar V, Robbins SL. Genetic Disorders. In: Cotran RS, Kumar V, Robbins SL. Pathologic Basis of Disease. Philadelphia, USA: W. B Saunders Co.: 1994.
6. Bigoniya P, Singh CS, Shukla A. A comprehensive review of different liver toxicants used in experimental pharmacology. Int J Pharm Sci Drug Res. 2009;1(3):124-35.
7. Kanchana N, Sadiq AM. Hepatoprotective effect of *Plumbago zeylanica* on paracetamol induced liver toxicity in rats. Int J Pharm Pharm Sci. 2011;3:151-54.
8. Ewaschuk J, Endersby R, Thiel D, Diaz H, Backer J, Ma M, Churchill T, Madsen K. Probiotic bacteria prevent hepatic damage and maintain colonic barrier function in a mouse model of sepsis. Hepatol. 2007;46(3):841–50.
9. Chávez-Tapia NC, González-Rodríguez L, Jeong MS, López-Ramírez Y, Barbero- Becerra V, Juárez-Hernández E, Romero-Flores JL, Arrese M, Méndez-Sánchez N, Uribe M. Current evidence on the use of probiotics in liver diseases. J Funct Foods. 2015;17:137–51.
10. Devi AB, Rahigude AB, Parab P, Dhakephalkar PK, Apte KG. A study to evaluate the hepatoprotective activity of prebiotics, probiotics, and synbiotics in CCl_4-induced hepatotoxicity in rats. J Appl Pharm Sci.2021;11(3):141-53.

ISSN: 2458 - 8806

11. Lambert JC, Zhou Z, Wang L, Song Z, McClain CJ, Kang YJ. Prevention of alterations in intestinal permeability is involved in zinc inhibition of acute ethanol-induced liver damage in mice. J Pharmacol Exp Ther. 2003;305(3):880-86.
12. Neish AS.Microbes in gastrointestinal health and disease. Gastroenterol. 2009:36(1): 65–80.
13. Lata J, Jurankova J, Kopacova M, Vitek P. Probiotics in hepatology. World J Gastroenterol. 2011;17(24):2890–96.
14. Nanji AA, Khettry U, Sadrzadeh SM. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). Proc Soc Exp Biol Med. 1994;205:243-47.
15. Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. Hepatol. 2004;39:1441-49.
16. Maduelosi NJ, Worlu G.E. Effect of photo-oxidized groundnut oil (*Arachis hypogea*) on the liver enzymes of albino rats. Int J Adv Res Chem Sci. 2015;2(11):5-7.
17. Breikaa RM, Algandaby MM, El-Demerdash E, Abdel-Naim AB. Biochanin A protects against acute carbon tetrachloride-induced hepatotoxicity in rats. Biosci Biotechnol Biochem. 2013;77(5):909-16.
18. Eun Park, Do Kyung Lee, Kyung Tae Kim, Jae Goo Seo, Myung Jun Chung, Nam Joo Ha, Jun-Bom Park & Kyungjae Kim. Hepatoprotective effects of dual-coated and uncoated mixture of probiotics in rats. Biotechnol Biotechnol Equip. 2015;29(6), 1164-68.
19. Xu S, Zhao M, Wang Q, Xu Z, Pan B, Xue Y, Dai Z, Wang S, Xue Z, Wang F, Xu C. Effectiveness of probiotics and prebiotics against acute liver injury: A meta-analysis Front Med. 2021;8:1-15.
20. Wang Y, Li Y, Xie J, Zhang Y, Wang J, Sun X, Zhang H. Protective effects of probiotic *Lactobacillus casei* Zhang against endotoxin- and D-galactosamine-induced liver injury in rats via anti-oxidative and anti-inflammatory capacities. Int Immunopharmacol. 2013;15:30-37.
21. Fooladi A. Hosseini H, Nourani M, Khani S, Alavian S. Probiotic as a Novel Treatment Strategy Against Liver Disease. Hepat Mon. 2013;13(2):e7521.
22. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. J Ethnopharmacol. 2006;105: 374-79.
23. Liedtke C, Luedde T, Sauerbruch T, Scholten D, Sterretz K, Tacke F, Tolba R, Trautwein C, Trebicka J, Weiskries R. Experimental liver fibrosis research: update on animal models, legal issues and translational aspects. Fibrogenesis Tissue Repair. 2013;6:19.
24. Williams AT, Burk RF. Carbon tetrachloride hepatotoxicity: an example of free radical-mediated injury. Semin Liver Dis. 1990;10:279–84.
25. Reynolds ES. Liver parenchymal cell injury. J Cell Biol. 1963;19:139-57.
26. Ramadori G, Saile B. Portal tract fibrogenesis in the liver. Lab Invest. 2004;84:153–59.
27. Tobias A. Mechanism of probiotic action: A Review. Int J Med Microbiol. 2010;300:57-62.
28. Wohlgemuth S, Loh G, Blaut M. Recent developments and perspectives in the investigation of probiotic effects. Int J Med Microbiol. 2010;300(1):3-10.
29. Fooa N, Lina S, Leea Yu, Wud M, Wanga Y. Lipoic acid inhibits liver fibrosis through the attenuation of ROS-triggered signalling in hepatic stellate cells activated by PDGF and TG. Toxicol. 2011;282:39–46.
30. Kozlov AV, Gille L, Staniek K, Nohl H. Dihydrolipoic acid maintains ubiquinone in the antioxidant active form by two-electron reduction of ubiquinone and one-electron reduction of ubisemiquinone. Arch Biochem Biophys. 1999;363:148–54.
31. Blease K, Chen Y, Hellewell PG, and Burke-Gaffney A. Lipoteichoic acid inhibits lipopolysaccharide-induced adhesion molecule expression and IL-8 release in human lung microvascular endothelial cells. J Immunol. 1999;163:6139–47.
32. Park JE, Lee DK, Kim KT, Seo JG, Chung MJ, Ha NJ, Park JB, Kim K: Hepatoprotective effects of dual-coated and uncoated mixture of probiotics in rats. Biotechnol Biotechnol Equip. 2015;29(6): 1164-68.