Effect of Some Strains of Lactic Acid Bacteria Isolated From Some Fermented Dairy Products on Carbon Tetrachloride-Induced Hepatotoxicity and Nephrotoxicity of Albino Rats.

Maha Bikheet
Minia university Faculty of Agriculture
Magda Mahmoud
Minia University Faculty of Agriculture
Eman Yassien
Minia University faculty of Agriculture
Hanaa Mohamed Hassan (hanaa.ammar29@yahoo.com)
Minia University Faculty of Agriculture https://orcid.org/0000-0002-8545-0101

Research Article

Keywords: Lactobacillus paracasei ssp paracasei, Pediococcus acidilactis, Lactoccus lactis ssp lactis, CCl4, Histopathological analysis, Biochemical markers

Posted Date: March 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-313107/v1

Abstract

The current research was performed to evaluate the potential protective effect of *Lactobacillus paracasei ssp paracasei, Pediococcus acidilactis and Lactoccus lactis ssp lactis*, Sylimarin in the alleviation of health (hepatic and renal) complications caused by carbon tetrachloride (CCl_4) in rats. Healthy sixty albino rats were divided into six groups, the first group was control (negative), the second group (control positive) was injected CCl_4 (1 ml/kg, 1:1 v/v paraffin oil mixture, i.p. every third day for 8 weeks, The third group (CCl_4 + silymarin group) receiving both CCl_4 and daily silymarin therapy (50 mg/kg, oral), the fourth group: CCl_4+ (*Lactobacillus paracasei* 1ml orally). The fifth group (CCl_4+ *Pediococcus acidilactis* 1ml orally) and the six group (CCl_4+ *Lactococcus lactis* (1ml orally) for eight weeks per day. Biochemical markers were tested for blood, liver and kidney tissue. Histopathological tests on liver and kidney tissues were performed. The findings obtained have shown that *Lactobacillus paracasei ssp paracasei*, *Pediococcus acidilactis* and *Lactoccus lactis ssp lactis* improved the disrupted biochemical parameters caused by CCl_4 therapy. Besides, the findings of the histopathological analysis are in consistent with biochemical parameters and the protective ability of lactic acid bacteria suggesting that the best lactic acid bacteria was *Pediococcus acidilactis* that helped strengthen liver fibrosis caused by CCl_4 therapy, while the best bacterium for improving renal damage was *Lactoccus lactis*.

1. Introduction

Lactic acid bacteria (LAB) are commonly known as host health living microorganisms ((FAO / WHO, 2002). Several in-vitro assays have been successful in the investigation of probiotic potentials of organisms but the use of animal models and human cells have gained much attention recently in the preparations and development of functional foods as the organisms might not replicate such attributes in a living host (Tezel, 2019). lactic acid bacteria are widely used in food fermentation, contributing to the flavour, texture and preservation of fermented products. Generally, these bacteria are considered beneficial and some strains may be used for the treatment of human diseases (Elmer et al., 1996). These strains can produce heterologous proteins such as enzymes (lipase and lactase), biological mediators (hormone and interleukin) and molecules that stimulate local immune responses in order to prevent digestive disorders (toxins and viral proteins) can be produced by these strains (Corthier and Renault, 1999).

Lactococcus lactis is a commonly used as a starter strain that, by over expressing the riboflavin biosynthesis genes, can be transformed from a vitamin B2 user to a vitamin B2' factory (LeBlanc et al., 2005). *Lactobacillus s*p. SBT 2028 had the best antioxidant effects out of 570 strains of LAB (Kaizu et al., 1993) . 19 strains of LAB have antioxidant of 7-12 percent in intracellular cell-free extracts, due to their metal-ion-chelating and ROS-scavenging capabilities (Lin and Yen, 1999), Olajugbagbe et al.(2020) indicated that *Pediococcus acidilactici* isolated from Wara was able to act as a therapeutic agent against a Diarrhoeagenic Enterotoxigenic E. coli and improve gut health . Several scientific studies have confirmed that the daily ingestion of probiotics or their related products, notably lactic acid bacteria, significantly improved human health through a variety of effects, including xenobiotic detoxification and competition with pathogenic microbial agents (Maurice et al., 2013), Silymarin is a common herbal extract used as a hepatoprotective agent a mixture of flavonolignans obtained from milk thistle (Williamson et al., 1996) , In natural dietary sources, antioxidants are available, and the intake of antioxidants have a number of possible health benefits (Yeung et al., 2019) .

The liver and the kidney play an important role in the detoxification of metabolism and ex-creation of various xenobiotics entering the body,; thus, the toxic effects of chemicals occur mainly in liver and kidney tissues (Abdel-Daim et al., 2013). Chemicals mediated hepatotoxicity that cause liver damage are called hepatotoxins that can rejoin with the basic cellular components that subsequently prompt nearly all forms of liver lesions (Thompson et al . , 2017). Carbon tetrachloride (CCl_4) is metabolised in the liver to the trichloromethyl radical (CCl_3) by the cytochrome P450 superfamily of monooxygenases (CYP family). This radical subsequently interacts with nucleic acids, proteins and lipids, impairing major cellular processes, leading to altered lipid metabolism (fatty degeneration and steatosis) and reduced amounts of protein (Scholten et al., 2015).

One of the most widely used hepatotoxins in the laboratory study of liver fibrosis is carbon tetrachloride. The mechanism of liver damage caused by CCl_4 is due to the conversion of CCl_4 into free radical trichloromethyl (CCl_3 *) by activation of drug-metabolising enzymes that trigger lipid peroxidation in the endoplasmic reticulum (Plaa, 2000). The subsequent development of a highly reactive oxygen reaction between CCl3* and cellular molecules and the formation of a trichloromethyl peroxy radical (CCl3OO*) is believed to cause chain reactions that result in CCl4-induced toxicity (Weber et al., 2003; Ilhan and Seckin, 2005). By producing free radicals and increasing lipid peroxidation, carbon tetrachloride has many adverse effects on the liver, kidneys, heart and blood. This is one of the key mechanisms of liver toxicity caused by CCl_4 (Sodergren et al., 2001). As for as we know there are very few studies to date on the protective activity of the liver and kidneys of lactic acid bacteria. Therefore, this study was conducted to determine the protection of the liver and the prevention of lactic acid bacteria against carbon tetrachloride (CCl_4) - causing toxicity in rats.

2. Material And Methods

2.1.Materials

Carbon tetrachloride and silymarin were obtained from El- Gomhorya Pharmaceutical Company, Cairo, Egypt and Chemical kits for blood urea nitrogen (BUN), creatinine, protein, albumin, AST, ALT, catalase (CAT) , nitric oxide (NO) and all other chemicals were purchased from Biodiagnostic Company, Cairo, Egypt.

2.2 Preparation of LAB strains:

Lactobacillus paracasei, Pediococcus acidilactis and Lactoccocus lactis ssp lactis were isolated from locally produced fermented products (laban zeer, laban rayeb and Karish cheese). These strains were identified by some biochemical analyses such as phynotypic characterization of lactic acid bacteria strains(LAB) according to (Bergey manual,1984 and Sharpe, 1979) and The API 50CH kit (Biomerieux, Marcy l'Etoile France). They were grown in MRS for rod and M17 for cocci isolated respectively broth for 72 hr at 37 °C to reach their highest growth (Bikheet et. al., 2015). One milliliter of each inoculation was added separate aliquot (10 ml) of skim milk (108 CFU /ml skim milk) for feeding the rats (Zagato et. al., 2014).

2.3.Experimental animals and design

Sixty female (140 ±10 g) Sprague-Dawley albino rats, their age 45 days were obtained from the Animal House of Faculty of Agriculture, Minia University. They were housed under uniform environmental conditions for 10 days until the experiment was started, fed with a daily diet and left to acclimatize to the environment (22 ° C under a light / dark period of 12/12 hours). Animals (each of ten rats) have been divided into six groups and treated as follows:

The first group :Control group (negative), the respective vehicle was injected into rats (0.5 ml / kg b.w saline paraffin oil).

The second-group rats (positive) CCl_4 (1 ml / kg,1:1v/v CCl_4 solution in paraffin oil) (Marsillach et al., 2009) was injected i.p. every third day for 8 weeks to induce liver and kidney injury (Park et al., 2008).

The third group (CCl_4 + silymarin group) receiving 8 weeks of both CCl_4 and daily silymarin therapy 50 mg/kg, oral (Nema et al., 2011).

The fourth group : CCl_4+ *Lactobacillus paracasei* (1ml orally).

The fifth group (CCl_4+ *Pediococcus acidilactis* (1ml orally) and **thesix group** (CCl_4+ *Lactococcus lactis* (1ml orally) for eight weeks per day. Then CCl_4 was injected, as defined in the positive community. Rats were fasted overnight after eight weeks and anesthetized by diethyl ether to take blood samples from all animals in each group from the retro-orbital plexus (Schermer, 1967). Each sample was divided into two parts: the first part was immediately taken for hematological analysis in a heparinized tube and the second part of the sample was taken in a glass tube and left to coagulate at room temperature for 20 minutes and then centrifuged for 15 minutes at 3000 rpm to obtain serum samples kept at -20 ° C before they were used for evaluation of kidney function and lipid profile. Animals were then slaughtered and liver and kidney tissues were dissected and washed with ice-cold saline at -20 C, weighed and processed. Liver and kidney tissues in saline were then homogenized and the homogenate was used for the assessment of the oxidative stress marker catalase (CAT) and nitric oxide (NO). Furthermore, specimens of liver and kidney tissue were fixed with 10 percent formalin for histopathological examination.

2.4.Determination of Hematological Parameters

By the Neubauer hemocytometer system (Dacie and Lewis 1991), The numbers of red blood cells (RBC) and white blood cells (WBC) were counted. The concentration of hemoglobin (Hb) was calculated using the cyanmethemoglobin method in accordance with Jain (1986). The volume of packed cells (PCV) was calculated by Dacie and Lewis (1991) microhaematocrit process.

2.5.Histopathological research

Tissue livers and kidney samples were fixed in 10 percent neutral buffered formalin in control animals. They treated classes—cleaning absolute ethyl alcohol for dehydration, trapped in paraffin, with tap water following serial dilution. Parts 4-5 microns thick were prepared, collected on glass slides for histopathological analysis, deparaffinized, and stained with hematoxylin and eosin stains also Masson stain (Banchroft et al., 1996).

2.6.Analysis of statistics

The results were expressed as mean ± SD from six parallel measurements and comparisons between treatment means were made utilizing Tukey post hoc test. Variance analysis using ANOVA procedures was performed. GraphPad Prism ® was used (Motulsky, 1999) by GraphPad Software, San Diego, CA, USA, for statistical calculations.

3. Results

3.1.Body weight gain and relative weight of liver and kidneys organs in tested animals

The effect of *lactobacillus paracasei* (B1), *pediococcus acidilactis* (B2) *and lactococcus lactis* (B3) on final weight , body weight gain, and relative liver and kidney weight of carbon tetrachloride (CCl_4) and silymarin treated rats compared to the control group is summarized in Table 1. The body weight gain in CCl_4 and silymarin treated groups decreased significantly ($p < 0.05$) after eight weeks, while relative weight liver and kidney increased significantly compared to the control group, but B1, B2 and B3 treated significantly improved growth (Table 1).

Table1. Effect of *L.paracasei (B1), P. acidilactaci (B2) and L. lactis* (B3) on Final weight and Relative liver and kidney Weight of Rats.

Groups	Final weight (g)	Weight gain (g)		Kidney (%)
Parameters			Liver (%)	
Cont	206.7±13.32	56.73±3.08	2.92± 0.15 0.66 ± 0.05	
CCl_4	158.7 ±0.57[a]	16.20±1.07[a]	4.37 ±0.12[a]	0.79 ±0.04[a]
CCl_4+Silymarin	169.3±11.85[a]	35.75±2.0[ab]	3.81±0.55[a]	0.76±0.04
CCl_4+ *L.paracasei* (B1)	183± 2.00	42.08±2.29[abc]	3.96±0.20[a]	0.67 ±0.05[b]
CCl_4 +*P. acidilactaci* (B2)	197.7 ±17.10[b]	51.86±1.28[abc]	3.23±0.40[b]	0.63±0.01[b c]
CCl4 + *L. lactis* (B3)	182.7±6.11	40.18±1.57[ab]	3.40±0.10[b]	0.75±0.04

Data represent the mean ± S.D. of observations from 6 rats. [a]Significantly different from control group at $P < 0.05$. [b]Significantly different from CCl_4 group at $P < 0.05$. [c]Significantly different from CCl_4+ silymarin group at $P < 0.05$.

3.2.Effect of hematological parameters:

Hematological criteria are used in the routine clinical assessment of the state of patient health to provide valuable information for diagnosis. The findings in Table 2 showed that no major differences were found in all groups treated for Hb, RBC, PCV, MCV , MCH and MCHC relative to control levels. While there were significant increase in WBC and decrease in Platelets in the group treated with CCl_4 comparing to normal control whereas, animals treated with B2 and B1 plus CCl_4 restored WBC and platelets to nearly normal control (Table2) .

3.3.Liver and kidney function

Table 3 summarized that the CCl_4 treated group had significantly higher serum total protein , globulin ratio, AST, ALT, Urea and creatinine as well as lower albumin compared to other groups. while this elevation was decreased in B1, B2 and B3 groups compared to control group, where B2 group is the best improvement and nearly to control group.

3.4.Oxidative Enzymes:

The data presented in Table 4 showed that the catalase level was significantly lower in liver and kidney tissue and that the level of N.O. in liver and kidney tissue increased in the CCl_4 population compared to the control group. However, these therapies (silymarin, B1, B2, B3) improved as liver and kidney catalase levels increased and N.O. Levels relative to the CCl_4 group in liver and kidney tissues (Table 4).

Table 2. Effect of *L.paracasei , P. acidilactaci and L. lactis* on the blood picture in albino rats treated with CCl_4.

Parameters Groups	*Hemoglobin (g/dL)*	RBCcount (10^6/µL)	*WBCcount (10^3/µL)*	*PCV (ml/dl)*	*PLT(10^9/L)*	*MCV* X10^{-5} (fl)	*MCH* X 10^{-5} (pg)	*MCHC* (g/dl)
Cont	14.65±0.75	6.89±0.28	9.46±0.35	48.10±3.5	555.3±23.09	69.74±2.79	21.30±1.49	30.62±3.27
CCl_4	13.93±0.63	6.48±0.51	13.47±1.12^{a}	44.58±3.2	218±20.88^{a}	66.05±7.16	21.50±0.84	31.32±1.87
CCl_4+Silymarin	14.45±0.17	6.96±0.08	11.20±1.18^{b}	46.73±3.3	313±61.58^{a}	63.90±0.48	20.75±0.06	32.48±0.02
CCl_4+ *L.paracasei* (B1)	14.63±0.62	6.98±0.13	8.33±0.28^{bc}	45.35±1.9	427±1.00^{abc}	64.95±1.56	20.93±0.53	32.26±0.02
CCl_4 + *P. acidilactaci* (B2)	15.08±1.06	6.97±0.30	9.10±2.00^{bc}	44.48±0.23	475.7±61.78^{bc}	66.96±2.49	21.58±0.77	32.26±0.03
CCl4 *+ L. lactis* (B3)	16.15±0.97	7.12±0.39	7.30±0.17^{abc}	46.95±1.4	384.7±34.59 ^{ab}	66.09±2.45	22.65±0.37	34.45±2.9

The mean ±S.D. is represented by data. Remarks from 6 rats. [a]Significantly different from control group at P < 0.05 · [b]Significantly different from CCl_4 group at P < 0.05. [c]Significantly different from silmarin group at P < 0.05.

Table 3. Effect of *L.paracasei (B1), P. acidilactaci (B2) and L. lactis (B3)* on Level of protein, albumin globulin, liver and kidney function in Serum of Albino Rat.

Parameters Groups	Protein (g/l)	Albumin (g/l)	Globulin (g/l)	*AST(U/ml)*	*ALT(U/ml)*	*Urea (mg/dl)*	*Creatinine (mg/dl)*
Cont	9.33±0.22	4.02±1.11	5.30±0.89	16.16±0.04	15.22±0.09	4.68±0.22	0.66±0.04
CCl_4	13.60±0.98^{a}	3.26±0.01	10.32±0.98^{a}	59.38±1.01^{a}	48.80±1.4^{a}	14.37±1.6^{a}	1.71±0.03^{a}
CCl_4+Silymarin	8.49±0.03^{b}	4.29±0.01	4.20±0.02^{b}	30.10±0.73^{ab}	42.96±3.2^{ab}	8.40±0.66^{ab}	1.09±0.09^{ab}
CCl_4*+ L.paracasei*	11.37±0.01^{abc}	4.88±0.36^{b}	6.48±0.35^{bc}	27.15±0.51^{abc}	24.40±1.3^{abc}	7.48±1.5^{ab}	0.92±0.04^{abc}
CCl_4 + *P. acidilactaci*	8.80±0.27^{b}	5.23±0.15^{b}	3.57±0.43^{ab}	16.90±1.06^{abc}	17.57±0.29^{bc}	6.81±0.64^{b}	0.82±0.02^{abc}
CCl4 *+ L. lactis*	11.74±0.43^{abc}	3.65±0.78	8.75±0.32^{abc}	21.38±0.49^{abc}	34.28±2.06^{abc}	4.89±0.67^{bc}	0.71±0.11^{bc}

The mean ±S.D. is represented by data. Remarks from 6 rats. [a]Significantly different from control group at P < 0.05 · [b]Significantly different from CCl_4 group at P < 0.05. [c]Significantly different from siylmarin group at P < 0.05.

Table 4. Effect of *L.paracasei , P. acidilactaci and L. lactis* on Catalase and Nitric oxide (NO) in liver and kidneys tissue.

Parameters Groups	*Catalase(U/g) Liver*	*Catalase(U/g kidney*	*NO(M mol/l) Liver*	*NO(M mol/l) Kidney*
Cont	0.96±0.05	1.14±0.05	4.60±0.40	4.82±0.38
CCl_4	0.33±0.04^{a}	0.25±0.03a	14.11±0.48^{a}	14.98±0.45^{a}
CCl_4+Silymarin	0.46±0.06^{ab}	0.42±0.04^{ab}	12.56±0.41^{ab}	12.83±0.62^{ab}
CCl_4*+ L.paracasei* (B1)	0.62±0.04^{abc}	0.57±0.03^{abc}	9.27±0.27^{abc}	10.76±0.29^{abc}
CCl_4 + *P. acidilactaci* (B2)	0.77±0.03^{abc}	0.76±0.05^{abc}	7.07±0.15^{abc}	8.14±0.09^{abc}
CCl4 *+ L. lactis* (B3)	0.53±0.04^{ab}	0.90±0.01^{abc}	10.44±0.40^{abc}	7.24±0.13^{abc}

The mean ±S.D. is represented by data. Remarks from 6 rats. [a]Significantly different from control group at P < 0.05 · [b]Significantly different from CCl_4 group at P < 0.05. [c]Significantly different from siylmarin group at P < 0.05.

4.Histopathological examination

Histological studies of the hepatoprotective effects of *L.paracasei , P. acidilactaci and L. lactis* caused liver damage to CCl_4 are shown in (Figure 1, 2). Hepatocytes (H) and central vein (CV) are divided into anastomosing cords or plates, separated by anastomosing hepatic sinusoids (S). Sections of the liver of the control rat have been revealed. CCl_4 injection induced many changes in the histological liver, such as disruption of the general morphology of the hepatic lobule. The central veins surround numerous areas of collagen fiber deposition between hepatocytes (arrows). Improvement in liver histology has been shown in the administration of *L.paracasei , P. acidilactaci and L. lactis* plus CCl_4, Most regions seem to have recovered, well- preserved hepatocytes, and no are together wia of necrosis.

Figures 3 and 4 show the histopathological changes of Control's kidney segment showing renal corpuscles, Bowman's capsules(arrows) surround the glomeruli (G) shaped of capillary tufts. Notice that proximal (P) and distal (D) convoluted tubule cells are intact with vesicular nuclei and acidophilic cytoplasm. (H&E, bar of scale=50µm). Glomeruli (G) made of capillary tufts are also surrounded by thin rims of collagen fibers at Bowman's capsules(arrows) in the kidney portion of the control group showing renal corpuscles. (Masson trichrome, scale bar=50µm) While CCl4 kidney section showing renal structural disruption with empty areas of completely degenerated renal corpuscles(*). (H&E, scale bar=50µm) * Also kidney section community photomicrograph CCl_4 showing renal structural disruption with extensive deposition of collagen fibers around renal tubules and blood vessels(arrows). Note that some tubules display tubular epithelial cell desquamation, whereas others show marked cell disorganization(*).(Masson trichrome, bar=50µm scale). While the capsules(arrows) of Bowman are surrounded by a photomicrograph of the kidney portion of group silymarin showing renal corpuscles, glomeruli (G) shaped from capillary tufts. Notice the structural disruption of certain tubule cells (*). (H&E, scale bar=50µm), also, silymarin showing a small amount of collagen fibers covering tubules and renal corpuscle(arrows). Note that congested blood vessels near the glomerulus are dilated (*). (Masson trichrome, bar=50µm scale). On the other hand, group treated B1 showing renal structural disruption with empty areas of completely degenerated renal corpuscles(*). There are extensive collagen fibers deposition around renal corpuscles and blood vessels(arrows)(Masson trichrome , scale bar=50µm) and (H&E, scale bar=50µm).While the B2 group showing renal structural disruption with extensive collagen fibers deposition around renal corpuscles , renal tubules and blood vessels(arrows). Notice some tubules show desquamation of tubular epithelial cells while others show marked cellular disorganization(*).(Masson trichrome, scale bar=50µm(also showing renal structural disruption with empty areas of completely degenerated renal corpuscles(*). (H&E, scale bar=50µm). On the other hand B3 group showing renal corpuscles , glomeruli (G) formed of capillary tufts are surrounded by some dilatation in bowman's space(arrow). (H&E, scale bar=50µm) and (Masson, scale bar=50µm).

4. Discussion

Recent scientific studies have shown that probiotics protect against sequences of various liver diseases such as cirrhosis / cirrhosis, hepatic encephalitis, and alcoholic and non-alcoholic fatty liver disease. In the present study, 3 lactic acid bacteria were selected with a strong antioxidant effect . this study explained that a hepatic and renal protective effect of the *L. paracasei, P. acidilactaci and L. lactis* in rats induced by CCl_4 . Therefore, hematological parameters, liver and kidney function and histopathological tests were performed on liver and kidney tissue. The results obtained have shown that the disturbed biochemical parameters caused by CCl_4 therapy boost three lactic acid bacteria. In addition, the results of the histopathological study are consistent with the biochemical parameters and protective capacity of lactic acid bacteria, indicating that the best *P. acidilactaci* (B2) was a lactic acid bacterium that helped improve CCl_4 therapy-induced liver fibrosis, while the best of these was *L. lactis* (B3) against renal damage to CCl_4. Body weight changes may provide an indicator of drug effect and are used for assessment of responses to the drug therapy (Asuquo et al., 2012),the rise in kidney and liver weight in group treated CCl_4 may be attributed to xenobiotics-related lesions and injuries, which per oxidize proteins (Wong et al., 2010). Also the increase in WBC can be due to the stimulation of the immune defense system (Kashinath 1990) or increasing antigen concentration in the body (Hoeney, 1985), the low values PCV may be attributed to anemic conditions. decreasing hemoglobin in injected rats with CCl_4 is an indication of hemolysis and the decreasing in hemoglobin has a corresponding elevation in met hemoglobin content which affects the oxygen-carrying the blood, caused by the toxicant (Tilak *et al*, 2007). On the other hand Mandour et al.(2020) showed that the time effect had significant differences in the values of WBCs and found the WBC , Hb concentration and PLT count increased in goats treated with probiotic at T3 and T6 compared to its values at T0

These results are in the same line with the previous research by Salatin et al. (2019). Increased total protein synthesis, which is characteristic of advanced age and may be due to liver compensation for more pronounced proteinuria, elevated proteolysis or accumulation of altered proteins (Van et al . , 1977). Oumeddour et al (2019) reported that rats were prevented from CCl_4-induced hyperglycemia by the

administration of probiotic bacteria. Even though the existence of bioactive components has been hypothesized to inhibit beta-cell apoptosis and increase insulin secretion (Al-Hariri, 2011). Treatment with the probiotics did not affect any of the parameters measured when compared with the control group, indicating that the probiotics did not affect liver integrity (Jantararussameeetal.,2020).

Results obtained in this study are in the same line with Salatin et al. (2019), and Oumeddour et al. (2019) they are also in accordance with Fahmy et al.(2016) and Al-Sayed et al . (2015) stated that CCl_4 increases biochemical parameters such as AST, ALT, urea and creatinine serum levels. The high level of liver enzymes in albino rats injected with CCl_4 may be due to alkylating DNA structures disrupting the hepatocyte membrane architecture, causing DNA damage and subsequent cell degeneration, resulting in leakage from damaged liver tissues, overproduction, and leakage in circulation, and decreased hepatic clearance (Abdo et al., 2015); (Mohamed et al., 2016); The increasing levels of creatinine and urea may be due to a diminish in glomerular filtration rate caused by acute renal dysfunction (Rahmat et al., 2014). Biological antioxidants are compounds that protect biological systems against the potentially harmful effects of processes or reactions which can cause excessive oxidation (Krinsky 1992) It is also possible to refer to them as scavengers (Salvemini and Botting1993). Catalase (CAT) is a hem protein that catalyzes the reduction of hydrogen peroxide and protects tissues from highly reactive hydroxyl radicals (Searle and Wilson, 1980).The decrease in the activity of CAT can result from the activation of the enzyme by glycation. (Yan and Harding, 1997). Results obtained in this study are in good agreement with what obtained by Oumeddour et al . (2019) who showed that treatment with CCl_4 resulted in a major deficiency in the liver catalase of the enzymatic antioxidant defense mechanism, Also Heeba and Mahmoud (2014) showed that no significantly increase when rats treated with CCl4. lactic acid bacteria having an antifibrotic effect, the antifibrotic function of silymarin has been confirmed by several studies, In line with the present findings, the antifibrotic effect of silymarin on liver fibrosis induced by dimethyl nitrosamine showed a decreased level of collagen after treatment with silymarin (Joseph et al . 2006) and a major inhibition and reversal of the progression of hepatic fibrosis induced by dimethyl nitrosamine (Zhao et al., 2006).

Youssef et al.(2010) showed that necrosis, hepatocyte vacuolization, ballooning degeneration with dilation and congestion of branches of the hepatic portal vein and sinusoids of the liver were caused by CCl_4. In the portal regions, increased inflammatory cellular infiltration was observed and was time-dependent. Many authors have reported similar findings and shown that CCl_4 caused inflammatory cell invasion, patchy areas of necrosis, and rat liver fibrotic and cirrhotic changes (Zerin et al., 2004; Abdel Salam et al., 2007). The hepato-protective function of silymarin in various toxic models of experimental liver diseases is that of antioxidant, ant lipid per oxidative, anti-fibrotic, anti-inflammatory, membrane stabilizing, immune modulatory and liver regenerating pathways (Chlopcikova et al., 2004; Pradhan and Griish, 2006). Jantararussamee et al. (2020) found that rats receiving probiotics or silymarin had significantly lower serum enzyme levels, less inflammation and less fibrosis. Liver damage was lower in the thioacetamide plus probiotics treated population. Consumption of a mixture of probiotic lactic acid bacteria (a mixture of *Lactobacillus paracasei,Lactobacillus casei* and Weissella confusa) reduces liver fibrosis production.These findings agree with Naima et al. (2014) who reported that CCL_4 induced nephrotoxicity of 0.5 ml / kg (twice a week for six weeks) and 5 ml / kg (once a week for six weeks) of CCL_4 respectively in rats during administration.Other research performed by Eric and Adolphus (2020) showed that, relative to the control group, no significant histopathological differences were found in the kidney histology of all the animal groups.This may be due to the length of administration (twice a week for one week) of 0.5 ml / kg CCL_4.

Conclusion

In conclusion, the results of this study findings strongly support administration of *lactic acid bacterialactococcus lactis* (B3) and *pediococcus acidilactis* (B2) than *Lactoba-cillus paracasei* (B1), can significantly protect the liver and kidneys from CCl_4-induced toxicity by increasing the pathway of ant oxidative' stress.

Declarations

Author contributions : **Maha M. Bikheet:** Data curation, Writing- Original draft preparation and Methodology **Magda E. Mahmoud** : Visualization, data curation, Methodology, Investigation and Supervision, **Eman E. Yassien** Methodology ,Software **and** Validation **Hanaa M. Hassan :**Conceptualization, investigation Methodology , formal analysis, data curation, writing (original draft preparation), writing (review and editing) and visualization.

Funding : No funding source

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

Compliance with ethical standards

Ethics approval : This study was conducted in accordance with the ethical standards approved by the Institutional Animal Ethics Committee, Agricultural Chemistry Department, Faculty of Agriculture, Minia University, Egypt.

Competing interests: 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.

consent to participate: Not applicable

Consent for publication: Not applicable

Conflicts of interest

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Maha M. Bikheet

Magda E. Mahmoud

Eman E. Yassien

Hanaa M. Hassan

References

Abdel-Daim, M.M, Abuzead S.M.M and Halawa S.M. (2013). Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. PLoS One 8:e72991.

Abdel Salam, O.M., Sleem A.A., Omara, E.A. and Hassan, N.S. (2007). Effect of ribavirin alone or combined with silymarin on carbon tetrachloride induced hepatic damage in rats. Drug Target Insights, 2: 19-27..

Abdo, W., Hirata A., Shukry, M., Kamal T., Abdel-Sattar, E., Mahrous, E. and Yanai, T. (2015). *Calligonum comosum* extract inhibits diethylnitrosamine-induced hepatocarcinogenesis in rats. Oncol.Let. 10(2): 716-722.

Al-Hariri, M. (2011). Propolis and its direct and indirect hypoglycemic effect. J Family Community Med.;18(3):152-4. doi:10.4103/2230-8229.90015.

Al-Sayed, E., Abdel-Daim, M.. M., O.E. Kilany, Karonen M.& Sinkkonen J. (2015). Protective role of polyphenols from Bauhinia hookeri against carbon tetrachloride-induced hepato- and nephrotoxicity in mice, Renal Failure, 37:7, 1198-1207.

Asuquo, O.R., Ekanem, T.B., Eluwa, M.A.m., Oko, O.O. and Ikpi, D. E. (2012). Evaluation of toxicological effects of Spondiasmombin in adult male Wistar rats J. Nat. Sci. Res., 2:144-51.

Banchroft, J. D., Stevens, A. and Turner, D. R. (1996). Theory and practice of histological techniques. Fourth Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.

Bergey´s Manual of Systemic Bacteriology (1984). Krieg, N.R. and Holt, J.G., Eds., Baltimore: Williams and Wilkins.

Bikheet, M. M., Darwish, A. M., Hassanein, K. A. and Shalaby, S. I. (2015). Lactic acid bacteria with potential to eliminate fungal spoilage in cheeses) proceeding The 2nd Minia International Conference "Agriculture and Irrigation in Nile Basin Countries"32-25 March, Minia, Egypt (312-317).

Chlopcikova, S., Psotova, J., Miketova, P. and Simanek, V. (2004). Chemoprotective effect of plant phenolics against anthracyclin-induced toxicity on rats cardiomyocytes PartI. Silymarin and its flavonolignans. Phytother. Res., 18: 107-110.

Corthier, G. and Renault, P. (1999). Future directions for research on biotherapeutic agents: contribution of genetic approaches on lactic acid bacteria, p. 269–304. *In* G. W. Elmer (ed.), Biotherapeutic agents and infectious diseases. Humana Press Inc., Totowa, N.J.

Dacie, J.V. and Lewis, S.M. (1991). Practical Haematology. 7th Edition, Churchil Livingstone, Edinburgh: 54-79.

Elmer, G. W., Surawicz, C. M. and McFarland, L. V. (1996). Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infection. JAMA **275:**870–876.

Eric, E. U. and Adolphus, E. (2020). Ameliorative Effects of Turmeric Extract against CCL4 Induced Liver and Kidney Injury in Adult Wistar Rat, Asian Journal of Immunology, 3(4): 36-42.

Fahmy, N. M., Al-Sayed, E., Abdel-Daim, M. M., Karonen, M. and Singab A. N (2016). Protective effect of *Terminalia muelleri* against carbon tetrachloride induced hepato and nephro-toxicity in mice and characterization of its bioactive constituents. Pharm Biol, 54(2): 303–313.

Heeba, G. H., Mahmoud, M. E. (2014). Therapeutic potential of morin against liver fibrosis in rats: Modulation of oxidative stress, cytokine production and nuclear factor kappa B. Environmental toxicology and pharmacology 37, 662-671.

Hoeney, M. (1985). Introduction to Clinical Immunology. Butterworth, London. 3.

Ilhan, N. and Seckin, D. (2005). Protective effect of *Nigella sativa* seeds on CCl4 - induced hepatotoxicity. F. U. Saglik Bil. Gergisi., 19(3): 175-179.

Jain, N.C. (1986). Schalm's veterinary haematology 4th edition (ed N.C. Jain) Lea and Febiger, Philadelphia. 1221.

Jantararussamee, C., Rodniem, S., Taweechotipatr , M., Showpittapornchai, U. and Pradidarcheep, W. (2020). Hepatoprotective Effect of Probiotic *Lactic Acid Bacteria* on Thioacetamide-Induced Liver Fibrosis in Rats. Probiotics and Antimicrobial Proteins , https://doi.org/10.1007/s12602-020-09663-6

Joint Food and Agriculture Organization and World Health Organization (FAO/WHO). (2002). Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada. 1-11.

Joseph, G., Ionchin, S.and Ramachandran, J. (2006). Efficacy of silymarin and curcumin on dimethylnitrosamineinduced liver fibrosis in rats. Biomedicine, 26: 18-26.

Kaizu, H., Sasaki, M., Nakajima, H. (1993). Effect of antioxidative *lactic acid bacteria* on rats fed a diet deficient in vitamin E. J Dairy Sci;76:2493–2499.

Kashinath, R.T. (1990). Hypolipidemic effect of disulphide in rats fed with high lipids diets and/or ethanol. Ph.D. Thesis University of Bangalore, 221-225.

Krinsky, N.I. (1992). Mechanism of action of biological antioxidants. Proc. Soc. Exp. Biol. Med., 200: 248–254.

LeBlanc, J.G., Burgess, C., Sesma, F., Savoy de Giori, G. and Douwe van Sinderen, (2005). *Lactococcus lactis* is capable of improving the riboflavin status in deficient rats. British Journal of Nutrition, 94, 262–267 , DOI: 10.1079/BJN20051473

Lin, M.Y. and Yen, C.L. (1999). Antioxidative ability of *lactic acid bacteria*. J Agri Food Chem;47:1460–1466.

Marsillach, J., Camps, J., Ferre, N., Beltran, R., Rul, A., Mackness, B., Mackness, M. and Joven, J. (2009). Paraoxonase-1 is related to inflammation, fibrosis and PPAR delta in experimental liver disease. BMC Gastroenterol., 9 (3):1- 13.

Mandour A S, Samir H , El- Beltagy M A, Abdel-Daim M M, Izumi W, Ma D, Matsuura K, Tanaka R, Watanabe G (2020) Effect of supra-nutritional selenium-enriched probiotics on hematobiochemical, hormonal, and Doppler hemodynamic changes in male goats. Environmental Science and Pollution Research, 27(16), 19447-19460.

Maurice, C.F., Haiser H.J., and Turnbaugh P.J. (2013). Xenobiotics shape the physiology and gene expression of the active human gut microbiome. Cell,; 152: 39-50.

Mohamed, N.Z., Aly, H. F., El-Mezayen, H. and El-Salamony, H. E. (2016). Bee honey modulates the oxidant-antioxidant imbalance in diethylnitrosamine-initiated rat hepatocellular carcinoma. Int. J. Appl. Pharm. Sci. Res, 6: 156-163.

Motulsky, H. J. (1999). Analyzing Data with GraphPad Prism, GraphPad Software Inc., San Diego CA, www.graphpad.com.

Naima, Z., Howaida, I., Hanan, F.A. and Mantawi, N.I. (2014). CCL_4-Induced hepato nephrotoxicity: Protective effect Nutraceuticals on inflammatory factors and antioxidant status in rats. Journal of Applied Pharmaceutical Science.;4(2):87-90.

Nema, A.K., Abhinav, A. and Varsha, K. (2011). Hepatoprotective activity *of Leptadeniareticulata stems* against carbon tetrachloride-induced hepatotoxicity in rats, Indian J. Pharmacol. 43, 254–257.

Olajugbagbe, E.T., Odukoya, A. S.O. and Omafuvbe1, B.O. (2020). Evaluation of the Effects of *Pediococcus acidilactici* Isolated from Wara, a Nigerian Milk Product, in the Prevention of Diarrhea and the Modulation of Intestinal Microflora in Wistar Rats. Asian Journal of Medicine and Health 18(9): 94-106

Oumeddour, A., Zaroure, D., Haroun, R., Zaimeche, R., Riane, K., Sifour, M. and Tahraou, (2019). Protective Effects of Propolis and Probiotic *Lactobacillus acidophilus* against Carbon Tetrachloride-Induced Hepatotoxicity in Rats. Pharmaceutical Sciences September, 25(3), 190-197 doi: 10.15171/PS.2019.36.

Park, S.W., Lee C.H, Kim Y.S., et al. (2008). Protective effect of baicalin against carbon tetrachloride-induced acute hepatic injury in mice. J Pharmacol Sci 106:136–43.

Plaa, G.L. (2000). Chlorinated methanes and liver injury: highlights of the past 50 years. Ann. Rev. pharmacol. toxicol., 40: 42-65.

Pradhan, S.C. and Griish, C. (2006). Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. Indian J. Med. Res., 124: 491-504.

Rahmat, A. A., Dar, F. A. and Choudhary, I. M . (2014). Protection of CCl_4-Induced Liver and Kidney Damage by Phenolic Compounds in Leaf Extracts of *Cnestis ferruginea* (de Candolle).Pharmacognosy Res., 6: 19-28.

Salatin, S. M., Marghani, B. H., El-Adl1, M. and Salama, M. F. (2019). Effect of Diethyl Nitrosamine and Carbon Tetrachloride Induced Hepatotoxicity in Obese Rats. Alexandria Journal of Veterinary Sciences, 63 (2):132-141.

Salvemini, D. and Botting, R. (1993). Modulation of platelet function by free radicals and free radical scavengers. TIPS, 14: 36-42.

Schermer, S. (1967). In: Davis, F.A. (Ed.), The Blood Morphology of Laboratory Animals, third ed. Davis Co. Pub., Philadelphia, USA, pp. 10–42.

Scholten, D., Trebicka, J., Liedtke, C and Weiskirchen, R. (2015). The carbon tetrachloride model in mice. Laboratory Animals, 49(S1) 4–11.

Searle, A. J. and Wilson, R. L. (1980). Glutathione peroxidase: effect of superoxide, hydroxyl and bromine free radicals on enzymic activity. Int J Radi Biol 37: 213.

Sharpe, M.E. (1979) . Identification of lactic acid bacteria, in: Skinner FA, Lovelock DW (Eds) Identification methods ormicrobiologists.Academic Press, London, UK.

Södergren, E., Cederberg, J., Vessby, B. and Basum, S. (2001). Vitamin E reduces lipid peroxidation in experimental hepatotoxicity in rats. Eur J Nutr.;40(1):10-6. doi:10.1007/pl00007381.

Tezel, B. U. 2019. Preliminary in-vitro evaluation of the probiotic potential of the bacteriocinogenic strain *Enterococcus lactis* PMD74 isolated from ezine cheese. J. Food Qual.;1-12.

Thompson, M., Jaiswal, Y., Wang, I. and Williams, L. (2017). Hepatotoxicity: Treatment, causes and applications of medicinal plants as therapeutic agents. J.Phytopharmacol. 6(3): 186-193.

Tilak, K.S., Veeraiah, K. and Reju, M. P. (2007). Effects of ammonia, nitrite, and nitrate on hemoglobin content oxygen consumption of freshwater fish, Cyprinuscarplo (Linnaeus) J. Envron. Biol. 28 (1): 45 – 47.

Van Bezooijen, C.F., Grell, T. and Knook, D.L. (1977). The effect of age on protein synthesis by isolated liver parenchymal cells. Mech Ageing Dev, 6:293–304.

Weber, L.W., Boll, M. and Stampfl, A. (2003). Hepatotoxicity and mechanism of action of halokanes: carbon tetrachloride as a toxicological model. Crit. Rev. Toxicol., 33(2): 105-136.

Williamson, E. M., Okpako, D. T., and Evans, F. J., *PharmacologicalMethods in Phytotherapy Research: Selection, Preparation and Pharmacological Evaluation of PlantMaterial*, vol. 1, JohnWiley & Sons, Chichester, UK, 1996.

Wong, F. and Salerno, F. (2010). Beta-blockers in cirrhosis: friend and foe. Hepatology 52:811–813.

Yan, H. and Harhding, J. J. (1997). Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. *Biochem. J.* 328(Pt 2), 599–605. 10.1042/bj3280599.

Youssef, E. A ., Amin, A.M., Kazem, A. H. Hassan, S. M. and Khalil, E. M. (2010). Effect of silymarin on liver injury induced by carbon tetrachloride in rats : histopathological and immunohistochemical studies. Egypt. J. Exp. Biol. (Zool.), 6(1): 107 – 115.

Yeung, A. W. K.,Tzvetkov, N. T. El-Tawil, O. S., Bungău, S.G., Abdel-Daim, M. M. and. Atanasov, A. G. (2019). Antioxidants: Scientific Literature Landscape Analysis. Oxidative Medicine and Cellular Longevity, ID 8278454,1- 11

Zagato, E., Milet, E., Massimiliano, L., Fasano, F., Budelli, A., Penna, G. and Rescigno, M. (2014). *Lactobacillus paracasei* CBA L74: metabolic products and fermented milk for infant formula have anti-inflammatory activity on dendritic cells in vitro and protective effects against colitis and an enteric pathogen in vivo" PLOS one February, 9: 1-14.

Zerin, M., Karakilcik , A.Z., Nazligul, Y., Bitiren, M., Ozardali, H.I. and Musa, D. (2004). Protective role of *Nigella sativa* oil on experimental liver injury in rats. Turkiye Klinikleri J. Med. Sci., 24: 598-602.

Zhao, X.Y., Wang, B.E. and Wang, T. L. (2006). Inhibitory effect of silymarin on hepatic fibrosis induced by dimethylnitrosamine: experimental with rats. Zhonghua Yi Xue Za Zhi NCBI journals., 86(36): 2563-2566.

Figures

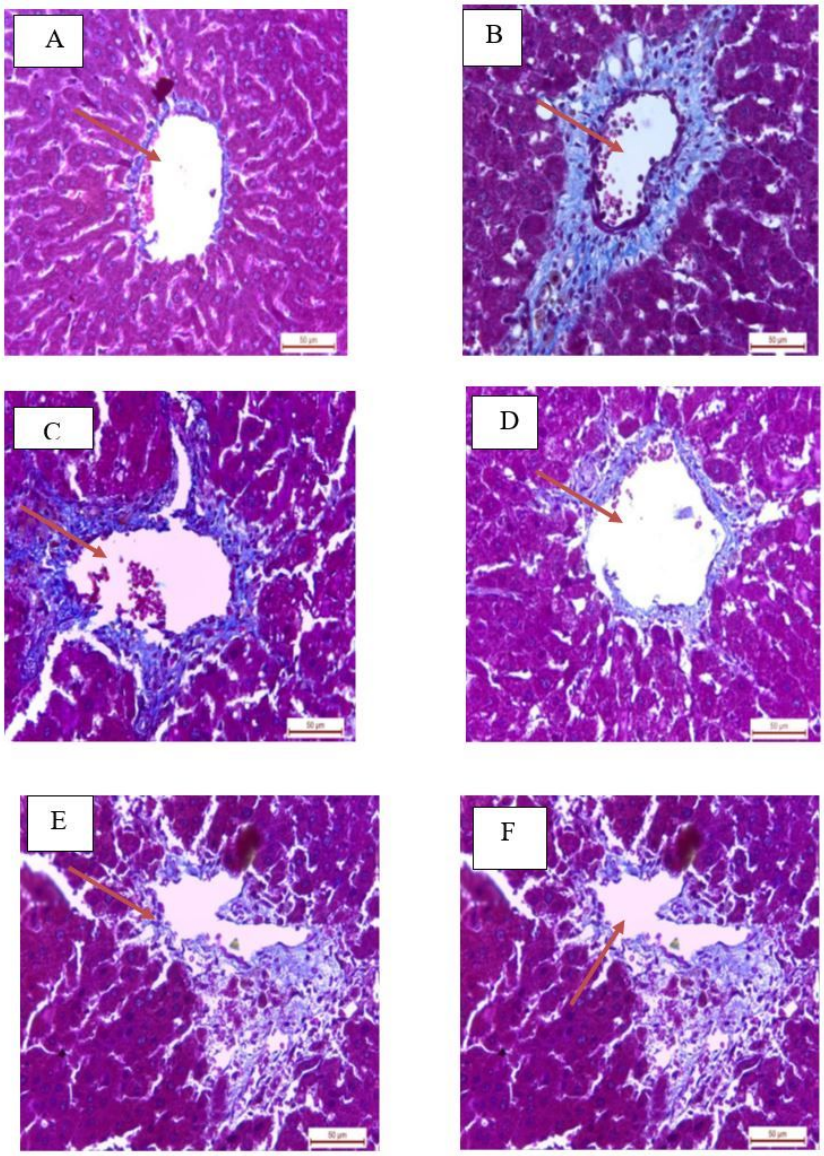

Figure 1

Light micrograph of rat liver (A) Control) , (B) treatment with CCl4, (C) pretreated with silymarin+ CCl4, (D) pretreated with L. paracasei + CCl4 (E) pretreated with P. acidilactaci + CCl4, (F) pretreated with L. lactis + CCl4 (Masson Trichrome, 200µ)

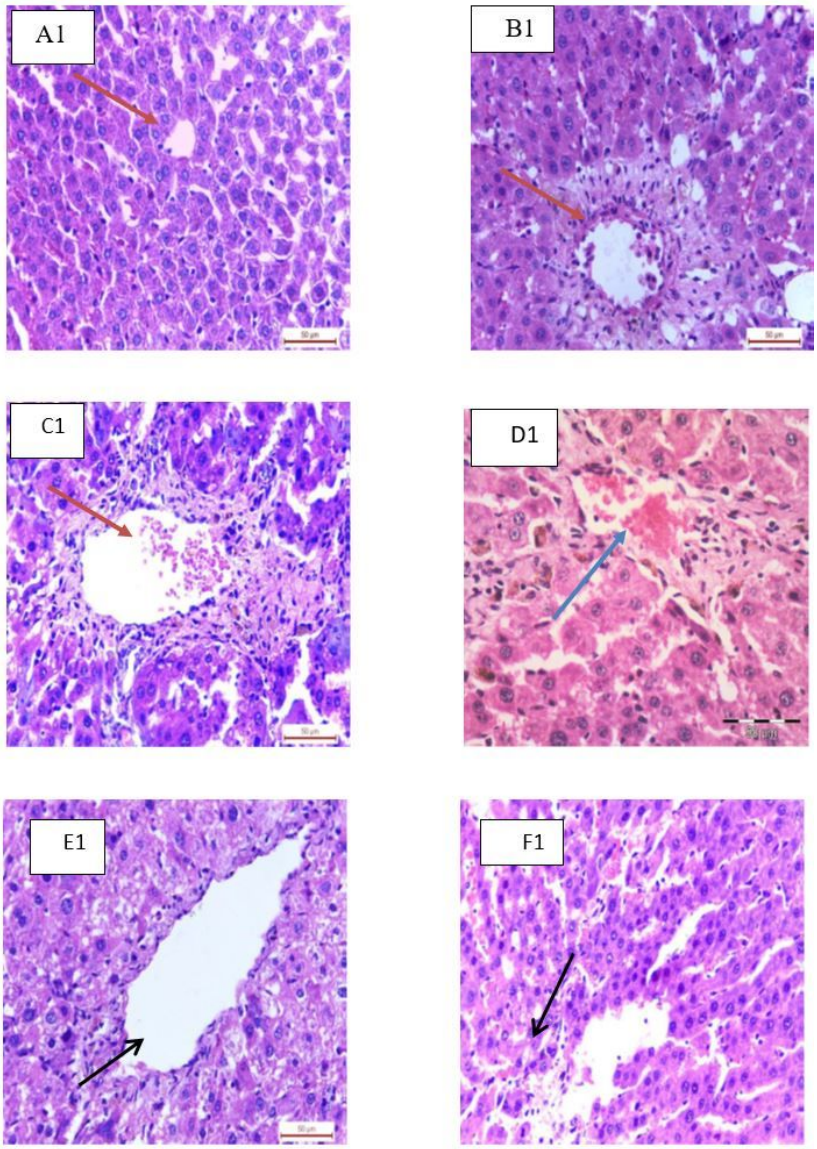

Figure 2

A Photomicrograph of rat liver section of (A1) Control) , (B1) treatment with CCl4, (C1) pretreated with Silymarin + CCl4, (D1) pretreated with L.paracasei + CCl4 (E1) pretreated with P. acidilactaci + CCl4, (F1) pretreated with L. lactis + CCl4 (H&E, 200µm).

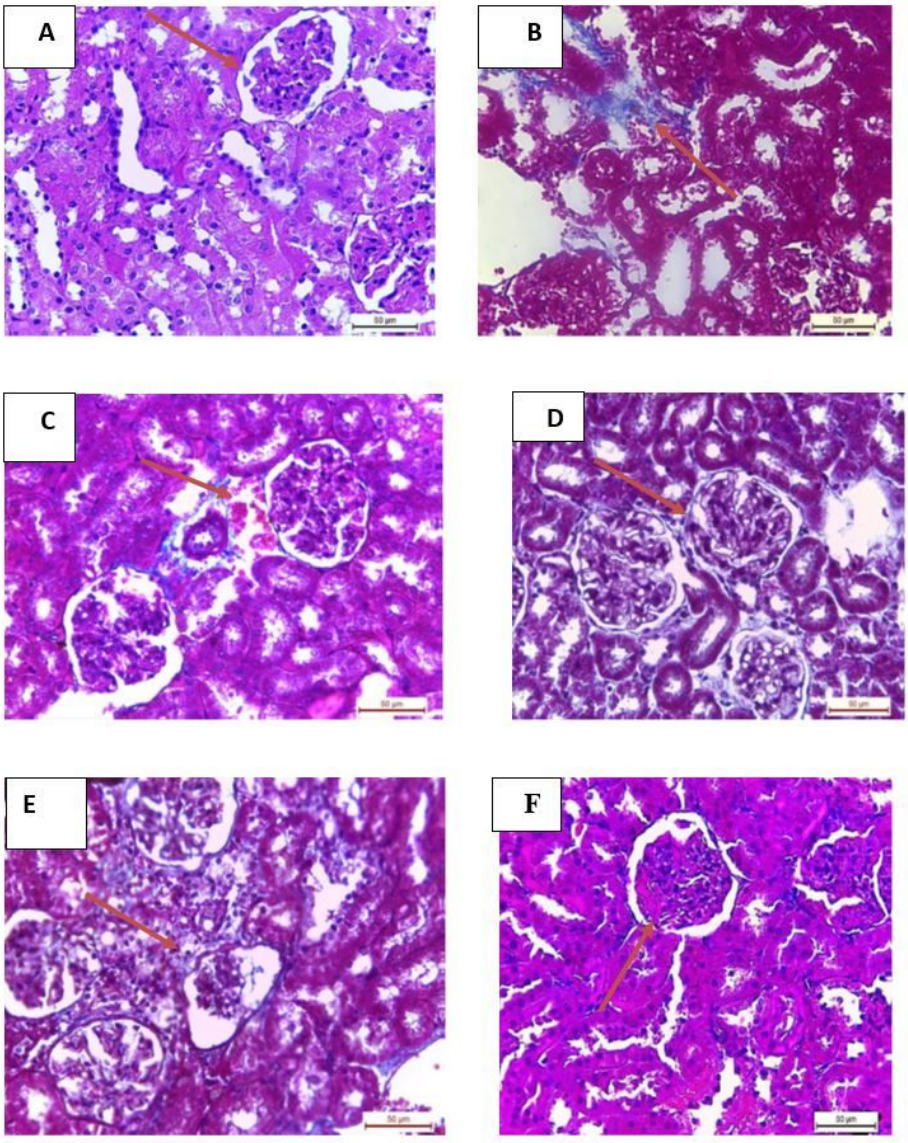

Figure 3

Light micrograph of rat kidney (A) Control) , (B) treatment with CCL4, (C) pretreated with silymarin+ CCL4, (D) pretreated with L.paracasei + CCL4 (E) pretreated with P. acidilactaci + CCL4, (F) pretreated with L. lactis + CCL4 (Masson Trichrome, 200μ)

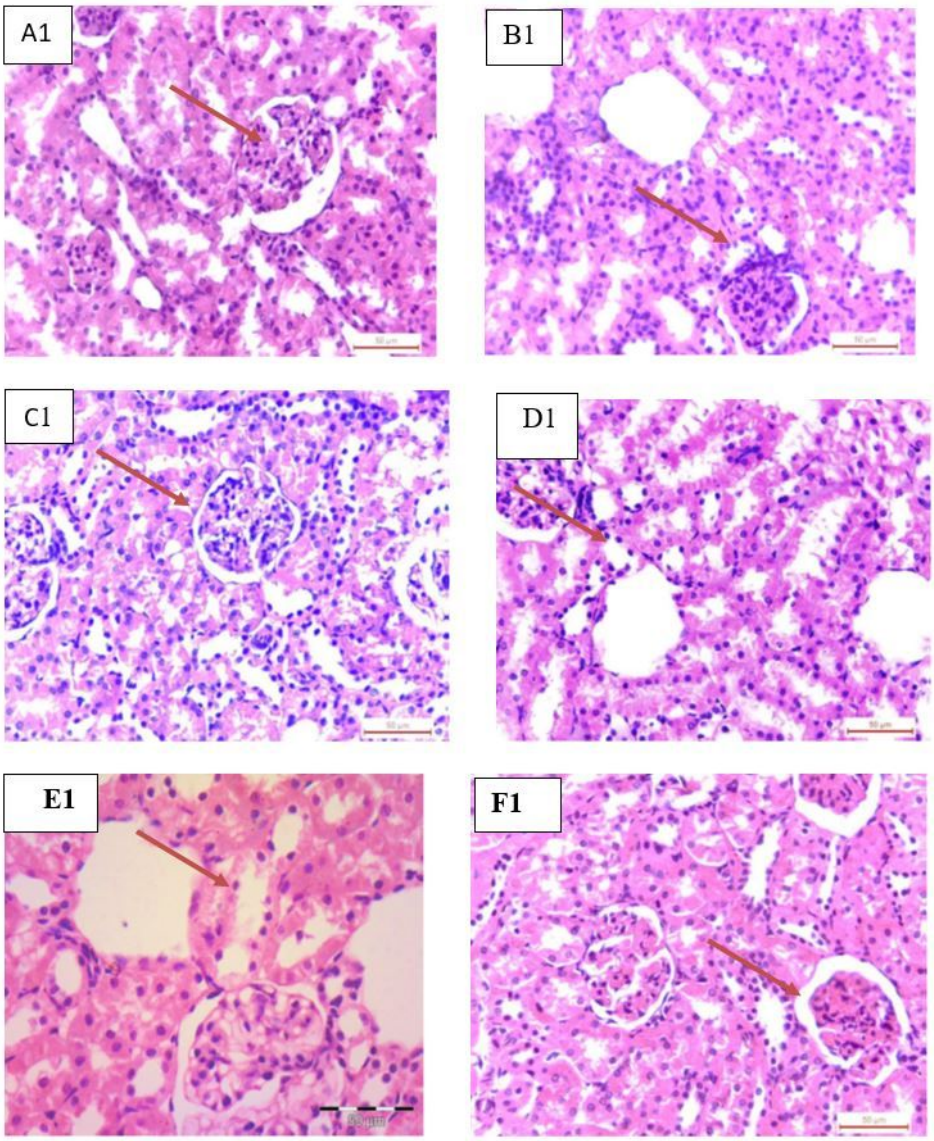

Figure 4

A Photomicrograph of rat kidney section of (A1) Control) , (B1) treatment with CCl4, (C1) pretreated with silymarin+ CCl4, (D1) pretreated with L. paracasei + CCl4 (E1) pretreated with P. acidilactaci + CCl4, (F1) pretreated with L. lactis + CCl4 (H&E, 200µm).