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Hepatoprotective outcome of Silymarin against CCl4 induced hepatotoxicity in rabbit model

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Authors' Contribution: **Ashfaq, K.**, worked on posology of experimental drugs. **S. Haq, A. Tehreem A. Rahim, A. Ullah**, determined the efficacy of experimental drugs **R. Taj** and **M. W. Usmani,** monitored the physiological parameters of the experimental animals and **A. Khan** analysed the raw data into scientific form.

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ABSTRACT

For centuries, medicinal plants have been used to treat human and animal ailments. Recent research indicated Silymarin as commonly employed antioxidant, hepatoprotective, cardioprotective, antiviral and antifibrotic. Anti-oxidant properties are believed to be responsible for its hepatoprotective effect. In this research, 46 rabbits were utilized in total. Each rabbit was separated into 04 groups: Group A (n=13) served as control and received neither Silymarin nor CCl4 treatment. Group B (n=13) was given 100 mg/Kg CCl4 as treatment. CCl4 and Silymarin were administered to Groups C and D (n=10), respectively. Using commercially available assays, the parameters Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), total bilirubin, direct bilirubin, and serum urea were determined for each animal. Multiple comparisons between each of the four categories were analyzed statistically. Administration of Silymarin in CCl4-induced liver toxicity improved all parameters significantly ($P<0.05$). The AST Mean ± SE of Groups A, B, C and D was 73.98 ± 2.22, 137.00 ± 4.22, 120.90 ± 3.00 and 95.43 ± 2.85 respectively. Groups A, B, C, and D had respective ALT Mean SE values of 44.51± 2.64, 99.47 ± 3.19, 74.60 ± 3.20, and 54.60 ± 3.20. Total Bilirubin for Groups A, B, C, and D were 0.622 ± 0.023, 1.302 ± 0.025, 0.959 ± 0.033, and 0.765 ± 0.024, respectively. 100 mg Silymarin demonstrated more significant results than 50 mg Silymarin. In conclusion, Silymarin has hepatoprotective, anti-fibrotic, and anti-cirrhosis properties. It is well-tolerated and efficacious antidote for CCl_4-induced hepatotoxicity.

Keywords: Carbon tetrachloride, herbal medication, LFT's, liver tonic, silymarin, toxicity.

INTRODUCTION: In addition to serving as traditional medicine, medicinal plants also serve as valuable trade commodities, supplying far-off markets with the materials they need to create new pharmaceuticals (Jamshidi-Kia *et al.*, 2017). Herbal remedies include a variety of traditional medical practices as well as numerous therapeutic advancements from many earlier generations (Khan and Ahmad, 2019). Milk thistle (MT; Silybum marianum), belonging to Asteraceae family, boasts medicinal history spanning two millennia. Its fruit contains Silymarin, a collection of flavonolignans, with silybin (or silibinin) being its primary constituent. Historically, milk thistle has been utilized treat the ailments related to liver, gallbladder, spleen, and kidneys (Abenavoli *et al.*, 2018). Contemporary studies highlight Silymarin's versatile applications, ranging from anti-cancer to photoprotective properties. Its efficacy is attributed to its action on various cellular and molecular mechanisms, such as MAPK, mTOR, -catenin, and Akt pathways. Furthermore, Silymarin not only curtails the activity of certain proteins promoting apoptosis but also dampens the expression of multiple inflammatory cytokines and enzymes (Wadhwa *et al.*, 2022).

Carbon tetrachloride, represented by the chemical formula CCl4, is an organic compound. In recent research, CCl4 has become a common agent for triggering hepatotoxicity in lab animals, and it's also used to create models for hepatocellular carcinoma, hepatic fibrosis/cirrhosis, liver injury, chemical-induced hepatitis, renal failure, and nephrotoxicity. The tissue damage caused by CCl4 can be attributed to oxidative harm resulting from lipid peroxidation. This damage occurs when CCl4 is metabolized into highly toxic trichloromethyl radicals (CCl_3) and trichloromethyl peroxyl radicals (CCl_3O_2) by cytochrome P450 enzyme system (Unsal *et al.*, 2021).

Supplementing with antioxidants may be useful in treating its hepatotoxic effects. Antioxidants' primary job is to prevent the oxidative damage caused by free radicals by slowing down the oxidation process by looking for radicals and chelating metal ions. Due to its substantial antioxidant and hepatoprotective properties, the flavonoid Silymarin is frequently employed in cases of liver disorders in this context (El Rabey *et al.*, 2021)

Liver, the most essential organ, controlling the body's homeostasis, interacts with nearly all metabolic processes involved in nutrition uptake, energy requirements, growth, disease defense, and reproduction (Al-Radadi and Adam, 2020). When a patient suffers hepatocytolysis, Silymarin is an effective hepatoprotective drug that is used in clinical practice. It is a cheap and non-toxic substance (Clichici *et al.*, 2020). Silymarin prevents liver injury by preserving integrity of the plasma membrane, inhibits hepatocyte death, and prevents the release of liver enzymes into the blood (Kheiripour *et al.*, 2019). In animal studies, Silymarin has been shown to mitigate liver damage induced by agents such as acetaminophen, carbon tetrachloride, radiation, iron overload, phenylhydrazine, alcohol, cold ischemia, and Amanita phalloides. It has been utilized in treating conditions like alcoholic liver disease, both acute and chronic viral hepatitis, and liver diseases caused by toxins (Abenavoli *et al.*, 2018).

Through a variety of processes, Silymarin safeguards liver cells. First, it reduces lipid peroxidation to stabilize membrane permeability, assisting the liver in maintaining levels of glutathione, its protective antioxidant. Silymarin also guards against harm from other harmful substances like carbon tetrachloride. Silymarin's antifibrogenic activity has also been demonstrated in non-human primates given repeated alcohol exposure in an animal model of alcohol-induced hepatic fibrosis (Gillessen *et al.*, 2022).

Silymarin promotes the regeneration of liver tissue by increasing the production of hepatocyte protein. According to the animal study, silybin slows or even reverses fibrosis by preventing hepatic stellate cells from developing into myofibroblasts (Shah *et al.*, 2017) Over the past 50 years, numerous researchers from diverse domains have struggled with the mechanism of CCl4-induced hepatotoxicity, particularly necrosis and fatty liver. It was discovered that the basic processes of tissue damage were metabolic activation, reactive free radical metabolites, lipid peroxidation, covalent binding, and disruption of calcium homeostasis (EL Sayed *et al.*, 2019).

ALT and AST serum concentrations rise as a result of liver injury. High concentrations of both aminotransferases are present in the liver. In healthy humans, the primary sources of alkaline phosphate (ALP), another indicator of liver impairment, are the liver and bones. Multiple conditions, including extrahepatic biliary obstruction, intrahepatic cholestasis, infiltrative liver disease, and hepatitis, are frequently associated with an increase in serum ALP levels (Abdel-Monem *et al.*, 2020) . In a randomized, double-blind, placebo-controlled trial, Silymarin has also been used at doses higher than those advised and shown to be safe and tolerated. Patients with NASH who took 700 mg/day of Silymarin for 48 weeks saw a significant decrease in fibrosis and an improvement in their liver biochemistry. Higher doses of Silymarin have been shown to have similar advantageous effects in improving related metabolic endpoints and IR in cirrhotic diabetic patients as well as in

individuals with alcoholic cirrhosis (Gillessen *et al.*, 2022). Silybin (active component of Silymarin), experiences significant enterohepatic circulation when taken orally. It takes two to four hours for it to be absorbed, and it takes about six hours for it to be eliminated. Only a limited quantity of oral Silymarin (between 20 and 50 percent) is absorbed in the digestive system, and even less (3 to 8 percent) is eliminated unchanged in the urine (MacDonald-Ramos *et al.*, 2021)

Silymarin may significantly lower blood sugar and cholesterol levels without immediately manifesting any negative side effects. This reported efficacy may be caused by the inflammatory condition being reduced (Xiao *et al.*, 2020).

OBJECTIVES: This study was designed with two primary objectives: firstly, to assess the hepatoprotective properties of Silymarin in counteracting liver damage induced by CCl4 in rabbits; and secondly, to evaluate the potential of Silymarin to mitigate fibrosis and cirrhosis resulting from carbon tetrachloride-induced liver toxicity

MATERIAL AND METHODS: Procurement of animals: Forty-six rabbits of both sexes age 8-10 weeks old, weighing between 520-1200g were procured from the market and were used for this experiment. The breed was Dutch Belted Rabbits. For environmental adjustment, rabbits of all groups were kept in rabbit house of University of Agriculture Faisalabad for 02 weeks before commencing trial. During the week adjustment phase, each rabbit received twice the dose of subcutaneous Ivermectin(400ug\kg) (Elhawary *et al.*, 2017). Before investigation, rabbits were adjusted for 02 weeks in Laboratory facility with 9-10 h light and 15-16 h dark cycle at 23-27°C room temperature. The humidity index was 40-60% in experimental room (Awais *et al.*, 2022) Fresh fodder was provided to each of rabbits twice a day and fresh drinking water was also provided ad libitum. Wheat straw bedding was also provided to rabbits. Healthy animals with no clinical signs of any abnormality or disease were added to trial. According to certification given by organizational Bioethics Committee, University of Agriculture, Faisalabad, Ethical rules on treatment and use of animal models for human diseases were followed.

Drugs and chemicals: CCl4 and Silymarin were utilized in this experiment. Before utilizing, these were dissolved in isotonic (0.9% NaCl) solution instantly. CCl4 was purchased from Sufiyan chemicals Karachi and Silymarin was purchased from Taqwa pharmaceutical Faisalabad.

Experimental design and dosing: Rabbits were randomly allocated into 4 groups A, B, C and D. Groups A and B were comprised of thirteen animals while groups C and D comprised 10 animals. Animals in each group received different doses of drugs in the following pattern (table 1).

Group	No. of animals	CCl4 (mg/Kg b.w.) Subcutaneously	Silymarin (mg/Kg b.w.) For 15 days	Remarks
A	13	None	None	Negative control
B	13	100	None	Positive control
C	10	100	50	Received both CCl4 and silymarin treatments
D	10	100	100	Received both CCl4 and silymarin treatments

Table 1: Comparison of drug dosages administered to different rabbit groups.

Sample collection: A jugular vein blood sample of approximately 2.5ml without anticoagulant was collected into a sterile test tube and allowed to clot. The samples were then centrifuged at 5000 for 3 minutes to separate serum, which was then frozen at -20 degrees Celsius until further analysis.

Assessment of hepatic parameters: Total Bilirubin and Direct Bilirubin were analyzed on an automatic blood biochemical analyzer at 37°C using standard reagent kits within three hours of sample collection. Commercial assays will also be used to determine serum Urea levels.

Statistical analysis: The data was analyzed by SPSS software 16.0. ANOVA was carried out, and statistical comparison among the groups was performed with Tuckey's test

RESULTS: Aspartate aminotransferase (AST): Figure 1 displayed the average levels of AST enzyme (Mean ± SE) for different groups. Group A served as the control with an AST level of 73.98 ± 2.22.

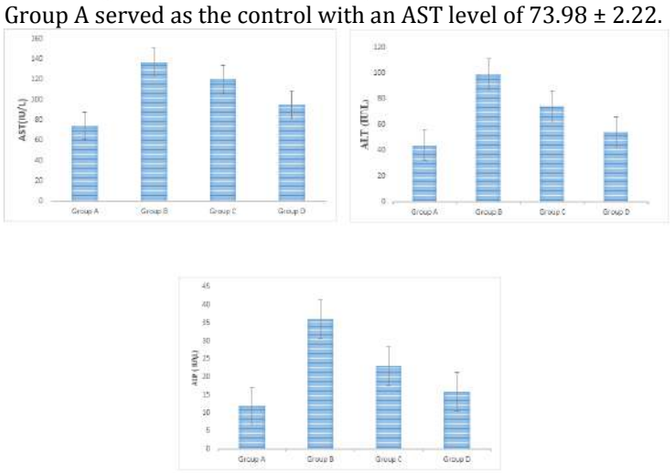

Figure 1: Percentage of AST, ALP and ALT with Mean±SE.

Notably, Groups B, C, and D exhibited significant differences compared to Group A. In Group B, AST levels increased, indicating liver toxicity. Group C, with a lower AST level than Group B, demonstrated that a 50mg dose of Silymarin is somewhat effective against liver toxicity, though not optimal. However, in Group D, there was the substantial decrease in AST levels compared to Groups B and C, suggesting that a 100mg dose of Silymarin is more effective in mitigating liver damage. Table 2 provides a detailed analysis of variance and mean comparisons for AST levels among the different groups.

Parameters	Source of variation	Degree of freedom	Sum of squares	Mean squares	F value
AST	Treatment	3	23172.8	7724.3	775.32
	Error	36	358.7	10.0	
	Total	39	23531.4		
ALT	Treatment	3	17649.1	5883.0	624.80
	Error	36	339.00	9.4	
	Total	39	17988.1		
ALP	Treatment	3	31.54	1051.4	346.47
	Error	36	109.2	3.0	
	Total	39	3263.6		
Total Bilirubin	Treatment	3	2.60018	0.86673	1245.10
	Error	36	0.02506	0.00070	
	Total	39	2.62524		
Direct Bilirubin	Treatment	3	2.43746	0.81249	176.01
	Error	36	0.16618	0.00462	
	Total	39	2.60364		
Blood Urea Nitrogen	Treatment	3	3663.2	1221.1	370.37
	Error	36	118.7	3.3	
	Total	39	3781.9		

Table 2. Analysis of variance for all respective parameters. Highly Significant (P< 0.01).

Alanine transaminase (ALT): Liver parameters, including ALT (Alanine Transaminase), were assessed across all groups, as depicted in figure 1. The control group (Group A) had an ALT value of 44.51 ± 2.64. Compared to this, Group B showed an elevated ALT level, indicative of liver toxicity. On the other hand, silymarin treatment in Groups C and D showed varying degrees of improvement. Specifically, Group C's ALT levels, though lower than Group B, suggested that a 50mg dose of Silymarin provides some protection but might not be the optimal dose. Meanwhile, Group D demonstrated a substantial reduction in ALT levels, suggesting that a 100mg dose of Silymarin offers better protection against liver damage. Table 2 provides a detailed analysis of variance and mean comparisons for ALT levels among the various groups, offering further scientific insights into observed trends.

Alkaline phosphatase (ALP): The figure 1 presented mean ALP (Alkaline Phosphatase) levels along with their standard errors for different groups. Group A, the control, exhibited an ALP level of 12.71 ± 1.80. Significantly different values were observed in the other groups compared to Group A.

In Group B, there was a noticeable increase in ALP enzyme levels, indicating liver toxicity. Group C, with lower ALP levels than Group B, indicated that a 50mg dose of Silymarin is effective against liver toxicity, though not at an optimal dosage. Group D displayed a sharp decrease in ALP levels compared to Groups B and C, suggesting that

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a 100mg dose of Silymarin is more effective in mitigating liver damage. Table 2 provided comparisons for ALP levels among the study groups

Total bilirubin: Figure 2 displayed Mean ± SE values of Total Bilirubin for different groups. Group A, serving as the control, had a Total Bilirubin level of 0.622 ± 0.023.

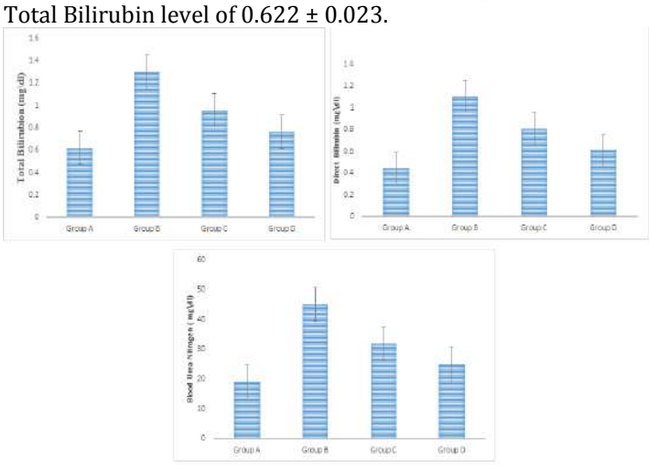

Figure 2: Average values of Total Bilirubin, Direct Bilirubin and BUN with Mean±SE.

Notably, Groups B, C, and D showed significant differences compared to Group A. In Group B, there was a marked increase in Total Bilirubin levels, indicating liver toxicity. Group C, with lower Total Bilirubin levels than Group B, suggested that a 50mg dose of Silymarin is effective against liver toxicity but may not be optimal. In contrast, Group D exhibited a sharp decrease in Total Bilirubin levels compared to Groups B and C, implying that a 100mg dose of Silymarin is more effective in mitigating liver damage. Table 2 provided a detailed analysis of variance and mean comparisons for Total Bilirubin levels among the various groups, offering scientific insights into observed trends.

Direct bilirubin: It was a diagram of group distribution. In Figure 2, direct bilirubin was present in each of the graphed groups. The direct bilirubin mean standard error for groups A, B, C, and D were respectively 0.446 ± 0.035, 1.108 ± 0.126, 0.809 ± 0.025, and 0.609 ± 0.025. The graph displays significant values in comparison to the control group, group A. In Group B, the value of Direct Bilirubin increased, indicating that the liver is now toxic. Group C has a lower value than Group B. This demonstrates that 50 mg of Silymarin is efficacious against liver toxicity, although this dose is not optimal. In Group D, the value of Direct Bilirubin decreased more rapidly than in Groups B and C, leading to the conclusion that 100 mg Silymarin is more effective and produces superior results than 50 mg.

Blood urea nitrogen (BUN): The figure 2 displayed the Mean ± SE values of BUN (Blood Urea Nitrogen) for different groups. Group A, control, had a BUN level of 19.32 ± 1.60. Notably, Groups B, C, and D exhibited significant differences compared to Group A. In Group B, BUN levels increased significantly, indicating liver toxicity. Group C, with lower BUN levels than Group B, suggested that a 50mg dose of Silymarin is effective against liver toxicity but may not be an optimal dosage. In contrast, Group D displayed a sharp decrease in BUN levels compared to Groups B and C, implying that a 100mg dose of Silymarin is more effective in mitigating liver damage. Table 2 provided comparisons for BUN levels of study groups.

AST percentage of both groups: After 48 hours, blood samples were taken from three rabbits in Groups A and B, which were then humanely euthanized. Blood samples were analyzed for AST. For Groups A and B, AST Mean SE was 75.90 ± 0.59 and 94.00 ± 2.08, respectively. The increase in AST levels in Group B relative to Group A, as depicted in Figure 3, indicated the onset of disease. (P<0.05). The levels of AST were compared between Group A and Group B in figure 3.

ALT percentage of both groups: After 48 hours, blood samples were collected from 03 rabbits in each of Groups A and B, which were then humanely euthanized. Blood samples were examined for ALT. For Groups A and B, the ALT Mean SE was 44.00 ± 0.58 and 64.00 ± 2.08, respectively. Figure 3 depictd increase in Group B's ALT levels relative to Group A (P0.05) (figure 3).

ALP percentage of both groups: The blood was analyzed for Alkaline Phosphatase levels. Groups A and B was 12.33 ± 0.88 and 22.33 ± 1.86, respectively. Figure 3 indicated rise in ALP levels in Group B compared to Group A, signifying the onset of disease (P<0.05). Figure 3 presents comparisons between Groups A and B concerning ALP levels.

Figure 3: Percentage of AST, ALP and ALT of Group A and B after 48 hours

Total Bilirubin percentage of both groups: Blood samples were analyzed for Total Bilirubin levels. Mean Standard Error for Total Bilirubin in Groups A and B was 0.610 ± 0.0058 and 1.027 ± 0.0371, respectively. Group B has higher Total Bilirubin levels than Group A, indicating the onset of the disease, as shown in figure 4 (P<0.05).

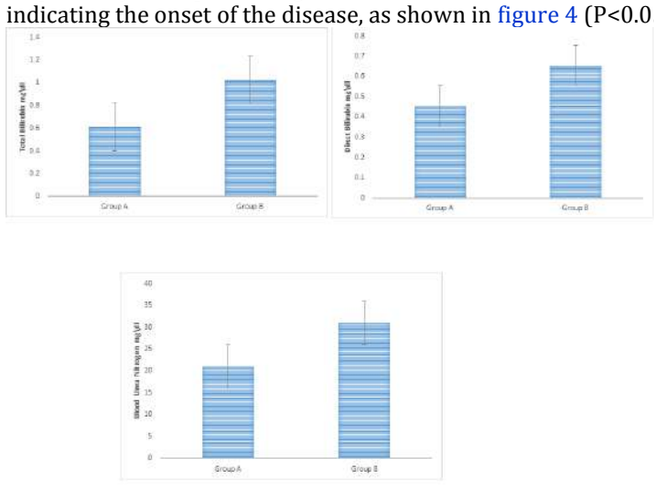

Figure 4: Average values of Total Bilirubin, Direct Bilirubin and BUN of groups A and B after 48 hours.

Direct bilirubin percentage of both groups: Blood samples were also analyzed for Direct Bilirubin levels. Mean ± SE of Direct Bilirubin for Groups A and B was 0.453 ± 0.0088 and 0.653 ± 0.0087, respectively. Group B has higher Direct Bilirubin levels than Group A, indicating the onset of the disease, as shown in figure 4 (P<0.05).

Blood urea nitrogen percentage of both groups: Blood was collected and analyzed for BUN. For Groups A and B, the mean BUN standard error was 21.00 ± 1.00 and 31.67 ± 1.20, respectively. The increase in BUN levels in Group B as compared to Group A, as depicted in figure 4 (P<0.05).

DISCUSSION: Herbal remedies, made from natural ingredients, are perceived as safe due to their minimal side effects when addressing various ailments and also serving as dietary supplements (Srivastava, 2018). As the world shifts towards complementary and alternative medicine (CAM) for handling both minor and severe diseases, this trend is especially prevalent in Asian countries, notably China and India (Philips *et al.*, 2019). CCl4 stands as one of the most established and frequently employed hepatic toxins. In rodents, a single injection of CCl4 often serves as a model for acute liver injury, facilitating the evaluation of potential hepatoprotective medications (Zhao *et al.*, 2018). The primary mechanism behind CCl4-induced liver injury involves the lipid peroxidation of hepatocyte membranes, triggered by free radicals produced from CCl4 metabolites. This damage to the hepatocyte membranes results in the leakage of hepatic enzymes like ALT and AST into the bloodstream. As a result, serum levels of ALT and AST are recognized as reliable indicators of liver damage (Hu *et al.*, 2014). For the purposes of our research, we utilized the acute CCl4 liver injury model to gauge the hepatoprotective potential in rabbit models.

Silymarin stands out as an herbal solution for liver issues. This therapeutic agent is a purified extract from the seeds and fruits of the medicinal plant Silybum marianum, often referred to as milk thistle (Hüttl *et al.*, 2021). Primary constituents of the Silybum marianum extract include flavonoids like silybin, silychristin, and

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silydianin (Cengiz, 2018). Research shows that silymarin helps reduce UV-caused cell damage and apoptosis (Mansuri *et al.*, 2021). Various studies confirm the diverse benefits of silymarin, such as its liver-protective (Mansour *et al.*, 2018), antioxidant (Wang *et al.*, 2019), and other therapeutic qualities like neuroprotection, cardioprotection, and anti-cancer effects (Wadhwa *et al.*, 2022). Its liver-protective capability is attributed to its antioxidant and membrane-fortifying properties. CCl4 is recognized as a model toxin known to instigate oxidative stress both externally and internally (Behboodi *et al.*, 2017). The goal of the study was to explore silymarin's impact on CCl_4-induced liver damage in rabbits. Serum levels of ALT and AST were gauged using standard kits. Total Bilirubin, Direct Bilirubin, and Serum Urea were assessed within 3 hours post-sample acquisition through an automated biochemical analyzer under standard conditions. The sample sizes for groups A and B were thirteen, while groups C and D had ten animals each.

Blood samples, taken via the cardiac puncture technique (Muoneke and Bayim, 2021), were centrifuged at 5000 rpm for 3 minutes, separating and preserving the serum at -20°C for future evaluations. Urea levels were evaluated for Group A and B rabbits 48 hours post a subcutaneous injection of 100mg/Kg CCl_4, confirming hepatotoxicity induction by the CCl4. Elevated levels of AST, ALT, and ALP in hepatotoxic rabbits signified liver damage, a result of liver cell death (Safiya *et al.*, 2018). Significant differences in these values between group A and group B indicated that CCl4 indeed induces liver damage (Ugwah-Oguejiofor and Ugwah, 2018). Some factors might affect serum AST levels; however, the simultaneous rise in AST, ALT, and ALP points to CCl4-induced toxicity as the probable cause (Shakya *et al.*, 2022). Elevated levels of Total Bilirubin and Direct Bilirubin in Group B hint at liver dysfunction, with the increased direct bilirubin suggesting acute hepatitis triggered by CCl4. Meanwhile, the BUN level in Group B showed a notable rise, which might be tied to a significant decrease in liver functionality (Ercin *et al.*, 2016).

Upon administering varied doses (50mg/Kg and 100mg/Kg) of silymarin to CCl4-afflicted rabbits (groups C and D), it appeared that silymarin mitigated or reversed CCl4's toxic impact, thus stopping enzyme leakage into the bloodstream.

CONCLUSION: In conclusion, the ability of Silymarin to reduce the ALT, AST, ALP, Total Bilirubin, Direct Bilirubin and BUN levels was more pronounced at 100mg/Kg compared with 50mg/Kg of silymarin after oral administration for 15 days**.**

CONFLICT OF INTEREST: Authors have no conflict of interest

REFERENCES: Abdel-Monem, N. M., M. A. El-Saadani, A. S. Daba, S. R. Saleh and E. Aleem, 2020. Exopolysaccharide-peptide complex from oyster mushroom (*Pleurotus ostreatus*) protects against hepatotoxicity in rats. Biochemistry and biophysics reports, 24: 100852.

Abenavoli, L., A. A. Izzo, N. Milić, C. Cicala, A. Santini and R. Capasso, 2018. Milk thistle (*Silybum marianum*): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. phytotherapy research, 32(11): 2202-2213.

Al-Radadi, N. S. and S. I. Adam, 2020. Green biosynthesis of pt-nanoparticles from anbara fruits: Toxic and protective effects on ccl4 induced hepatotoxicity in wister rats. Arabian journal of chemistry, 13(2): 4386-4403.

Awais, M., W. Ahmad, A. R. Asif, S. A. Muhammad, I. Khan, M. A. Ali, M. Q. Riaz and M. Saqib, 2022. Effects of a mixture of acacia honey and povidone-iodine on the healing of full thickness skin wound in normal and dexamethasone treated rabbits. Pakistan journal of zoology, 54(1): 391.

Behboodi, H., F. Samadi, S. M. Shams, F. Ganji and S. Samadi, 2017. Effects of silymarin on growth performance, internal organs and some blood parameters in japanese quail subjected to oxidative stress induced by carbon tetrachloride. Poultry science journal, 5(1): 31-40.

Cengiz, M., 2018. Renoprotective effects of silybum marianum (l.) gaertn (silymarin) on thioacetamide-induced renal injury: Biochemical and histopathological approach. Pakistan journal of pharmaceutical sciences, 31: 2137-2142.

Clichici, S., L. David, B. Moldovan, I. Baldea, D. Olteanu, M. Filip, A. Nagy, V. Luca, C. Crivii and P. Mircea, 2020. Hepatoprotective effects of silymarin coated gold nanoparticles in experimental cholestasis. Materials science and engineering: C, 115: 111117.

El Rabey, H. A., S. M. Rezk, M. I. Sakran, G. M. Mohammed, O. Bahattab, M. J. Balgoon, M. A. Elbakry and N. Bakry, 2021. Green coffee methanolic extract and silymarin protect against ccl4-induced hepatotoxicity in albino male rats. BMC complementary medicine and therapies, 21(1): 1-11.

EL Sayed, H. E., L. E. Morsy, T. M. Abo Emara and R. A. Galhom, 2019. Effect of carbon tetrachloride (ccl4) on liver in adult albino rats: Histological study. The Egyptian journal of hospital medicine, 76(6): 4254-4261.

Elhawary, N., S. S. Sorour, M. El-Abasy, E. Bazh and K. Sultan, 2017. A trial of doramectin injection and ivermectin spot-on for treatment of rabbits artificially infested with the ear mite “psoroptes cuniculi”. Polish journal of veterinary sciences, 20(3).

Ercin, C. N., T. Doğru, G. Çelebi, H. Gürel, H. Genc, E. Sertoğlu and S. Bağci, 2016. The relationship between blood urea nitrogen levels and metabolic, biochemical, and histopathologic findings of nondiabetic, nonhypertensive patients with nonalcoholic fatty liver disease. Turkish journal of medical sciences, 46(4): 985-991.

Gillessen, A., F. Angelico, J. Chen, L. Lu, M. I. Lucena, Q. Fu, Q. Xie, R. J. Andrade, W. Xie and X. Xu, 2022. Silymarin for treating toxic liver disease: International consensus recommendations. Gastro hep advances, 1(5): 882-893.

Hu, Z., C. Lausted, H. Yoo, X. Yan, A. Brightman, J. Chen, W. Wang, X. Bu and L. Hood, 2014. Quantitative liver-specific protein fingerprint in blood: A signature for hepatotoxicity. Theranostics, 4(2): 215.

Hüttl, M., I. Markova, D. Miklankova, I. Zapletalova, M. Poruba, Z. Racova, R. Vecera and H. Malinska, 2021. The beneficial additive effect of silymarin in metformin therapy of liver steatosis in a pre-diabetic model. Pharmaceutics, 14(1): 45.

Jamshidi-Kia, F., Z. Lorigooini and H. Amini-Khoei, 2017. Medicinal plants: Past history and future perspective. Journal of herbmed pharmacology, 7(1): 1-7.

Khan, M. S. A. and I. Ahmad, 2019. Herbal medicine: Current trends and future prospects. In: New look to phytomedicine. Elsevier: pp: 3-13.

Kheiripour, N., J. Karimi, I. Khodadadi, H. Tavilani, M. T. Goodarzi and M. Hashemnia, 2019. Hepatoprotective effects of silymarin on liver injury via irisin upregulation and oxidative stress reduction in rats with type 2 diabetes. Iranian journal of medical sciences, 44(2): 108.

MacDonald-Ramos, K., L. Michán, A. Martínez-Ibarra and M. Cerbón, 2021. Silymarin is an ally against insulin resistance: A review. annals of hepatology, 23: 100255.

Mansour, H. M., A. A. Salama, R. M. Abdel-Salam, N. A. Ahmed, N. N. Yassen and H. F. Zaki, 2018. The anti-inflammatory and anti-fibrotic effects of tadalafil in thioacetamide-induced liver fibrosis in rats. Canadian journal of physiology pharmacology, 96(12): 1308-1317.

Mansuri, R., A. Diwan, H. Kumar, K. Dangwal and D. Yadav, 2021. Potential of natural compounds as sunscreen agents. Pharmacognosy reviews, 15(29): 47.

Muoneke, C. and P. Bayim, 2021. Synergistic effect of ethanolic extract of phyllantus muellerianus and eremomastax speciosa leaves on lipid profile of wistar rat. Journal of applied sciences environmental management, 25(11): 1849-1853.

Philips, C. A., P. Augustine, S. Rajesh, P. Kumar and D. Madhu, 2019. Complementary and alternative medicine-related drug-induced liver injury in asia. Journal of clinical translational hepatology, 7(3): 263.

Safiya, H., M. Abdurrazak and M. Al-Kassim, 2018. Hepatoprotective effect of the aqueous leaf extract of balanite aegyptiaca on carbon tetrachloride-induced liver damage in albino rats. Journal of medicinal plants, 6(3): 19-23.

Shah, G. H., B. G. PATEL and G. B. SHAH, 2017. Development of carbon tetrachloride-induced chronic hepatotoxicity model in rats and its application in evaluation of hepatoprotective activity of silymarin. Asian journal of pharmaceutical and clinical research, 10(8).

Shakya, A. K., N. Sharma, M. Bhadauria, S. K. Nirala, S. Shrivastava and S. J. T. R. Shukla, 2022. Ameliorative impact of herbal formulation—majoon-dabeed-ul-ward and sharbat-e-deenar against ccl4 induced liver toxicity via regulation of antioxidant enzymes and oxidative stress. Toxicology research, 11(5): 819-830.

Srivastava, A. K., 2018. Significance of medicinal plants in human life. In: Synthesis of medicinal agents from plants. Elsevier: pp: 1-24.

Ugwah-Oguejiofor, C. and O. Ugwah, 2018. Hepatoprotective activity of the aerial parts of caralluma dalzielii ne brown against carbon

tetrachloride-induced hepatotoxicity in rats. Life journal of science, 20(1): 169-178.

Unsal, V., M. Cicek and İ. Sabancilar, 2021. Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Reviews on environmental health, 36(2): 279-295.

Wadhwa, K., R. Pahwa, M. Kumar, S. Kumar, P. C. Sharma, G. Singh, R. Verma, V. Mittal, I. Singh and D. Kaushik, 2022. Mechanistic insights into the pharmacological significance of silymarin. Molecules, 27(16): 5327.

Wang, J., H. Zhou, X. Wang, K. Mai and G. He, 2019. Effects of silymarin on growth performance, antioxidant capacity and immune response in turbot (*scophthalmus maximus* l.). Journal of the world aquaculture society, 50(6): 1168-1181.

Xiao, F., F. Gao, S. Zhou and L. Wang, 2020. The therapeutic effects of silymarin for patients with glucose/lipid metabolic dysfunction: A meta-analysis. Medicine, 99(40).

Zhao, Z.-W., J.-C. Chang, L.-W. Lin, F.-H. Tsai, H.-C. Chang and C.-R. Wu, 2018. Comparison of the hepatoprotective effects of four. The molecular aspect of natural secondary metabolite products in health, 19: 124.