Carbon tetrachloride, represented by the chemical formula CCl₄, is a highly toxic substance. In recent research, CCl₄ has become a primary constituent. Historically, milk thistle has been utilized to treat ailments related to liver, gallbladder, spleen, and kidneys since the history spanning two millennia. Milk thistle (MT; Silybum marianum), belonging to Asteraceae family, boasts medicinal properties. Its efficacy is attributed to its action on various cellular signaling pathways, such as MAPK, mTOR, nuclear factor-kappa B (NF-κB), and Wnt/β-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).

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 (Jamsheed-Kia et al., 2017). Herbal remedies include a wide variety of medicinal products, some with 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 flavonolignane, with silybin (or silybinin) being its primary constituent. Historically, milk thistle has been utilized to 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 reductive capacity of CCl₄ can be attributed to oxidative harm resulting from lipid peroxidation. This damage occurs when CCl₄ is metabolized into highly toxic trichloromethyl radicals (CCl₃) and trichloromethyl peroxyl radicals (CCl₃O²⁻) 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 constituent, controls the body’s homeostasis, interacts with nearly all metabolic processes involved in nutrition uptake, energy requirements, growth, disease defense, and reproduction. In 2022, researchers have been examining the effects of hepatocytolysis, Silymarin is an effective hepatoprotective drug that is used in clinical practice. It is a cheap and non-toxic substance (Chlichi 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 (Khairypour et al., 2019). In animal studies, Silymarin has been shown to mitigate hepatic injury caused by agents such as acetaminophen, carbon tetrachloride, radiation, iron overload, phenytoin, phenytoin, alcohol, cold ischemia, and Amanta 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. It can block CCl₄, as it undergoes 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 (Gillesen et al., 2022). Silymarin is a powerful antioxidant and 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 mechanism of CCl₄-induced liver injury involves 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 phosphatase (ALP), another indicator of liver impairment, are the liver and bones. Multiple conditions, including extrapulmonary biliary obstruction, intrahepatic cholestasis, infiltrative liver disease, and hepatitis, are frequently associated with an increase in serum ALP levels (Abdel-Moneim 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 (Radadi and Adam, 2020). Silymarin has 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 (Gilleseien 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 countering 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 aged 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 to 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 (Awas 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 fifteen animals while Groups C and D comprised 10 animals. Animals in each group received different doses of drugs in the following pattern (table 1).

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

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>CCl4 (mg/Kg b.w.)</th>
<th>Silymarin (mg/Kg b.w.)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13</td>
<td>None</td>
<td>None</td>
<td>Negative control</td>
</tr>
<tr>
<td>B</td>
<td>13</td>
<td>100</td>
<td>None</td>
<td>Positive control</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>100</td>
<td>50</td>
<td>Received both CCl4 and silymarin treatments</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>100</td>
<td>100</td>
<td>Received both CCl4 and silymarin treatments</td>
</tr>
</tbody>
</table>

**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.](http://www.sciplatform.com/index.php/wjib/article/view/1121)

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.

**Table 2: Analysis of variance for all respective parameters.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Source of variation</th>
<th>Degree of freedom</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AST</strong></td>
<td>Treatment</td>
<td>3</td>
<td>23172.8</td>
<td>7724.3</td>
<td>757.32</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>36</td>
<td>3587.1</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td>23531.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>Treatment</td>
<td>3</td>
<td>1749.1</td>
<td>5883.0</td>
<td>624.80</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>36</td>
<td>339.00</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td>17988.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ALP</strong></td>
<td>Treatment</td>
<td>3</td>
<td>31.54</td>
<td>1051.4</td>
<td>346.47</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>36</td>
<td>109.2</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td>3263.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Bilirubin</strong></td>
<td>Treatment</td>
<td>3</td>
<td>2.60018</td>
<td>0.86673</td>
<td>1245.10</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>36</td>
<td>0.02506</td>
<td>0.00070</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td>2.62524</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Direct Bilirubin</strong></td>
<td>Treatment</td>
<td>3</td>
<td>2.43746</td>
<td>0.81249</td>
<td>176.01</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>36</td>
<td>0.16618</td>
<td>0.00462</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td>2.60364</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood Urea Nitrogen</strong></td>
<td>Treatment</td>
<td>3</td>
<td>3663.2</td>
<td>1221.1</td>
<td>370.37</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>36</td>
<td>118.7</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39</td>
<td>3781.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Analysis of variance for all respective parameters.**

Highly Significant (P< 0.01). Alamine transaminase (ALT): Liver parameters, including ALT (Alamine 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 injury. 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
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.

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, 1.106 ± 0.126, 0.809 ± 0.023, 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 each of Groups A and B, which were then humanely euthanized. Blood samples were analyzed for AST. For Groups A and B, AST Mean SE was 75.30 ± 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 depicted increase in Group B’s ALT levels relative to Group A (P<0.05) (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.

**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). CC4 increased levels of the most established and frequently employed hepatic toxins. In rodents, a single injection of CC4 often serves as a model for acute liver injury, facilitating the evaluation of potential hepatoprotective medications (Zhao et al., 2018). The primary mechanism behind CC4-induced liver injury involves the lipid peroxidation of hepatocyte membranes, triggered by free radicals produced from CC4 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 CC4 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
silydianin (Gengiz, 2018). Research shows that silymarin helps reduce UV-caused cell damage and apoptosis (Mansouri 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. CCH4 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 CCl4-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 10 rabbits each, and the experiments were replicated thrice. Blood samples, taken via the cardiac puncture technique (Muneoke 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 CCl4 confirming hepatotoxicity induction by the CCl4. Elevated levels of ALT, AST, ALP, Total Bilirubin, Direct Bilirubin and BUN levels was a notable rise, which might be tied to liver cell death (Saffya 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 ALT levels; however, the simultaneous rise in AST, ALT, and ALP points to CCl4-induced toxicity as the probable cause (Shahya 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 Cand D), it appeared that silymarin mitigated or reversed CCl4’s toxic impact, thus stopping enzymatic liver damage.

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:


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


