Exploring the hepatoprotective effects of silymarin in experimentally induced diclofenac sodium toxicity in rabbits

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ABSTRACT

The study was aimed to investigate the hepatoprotective effects of varying doses of silymarin against liver damage caused by diclofenac sodium in rabbits. Silymarin, extracted from the Silybum marianum plant, is known for its antioxidant properties and is used to treat liver diseases. The rabbits (n=40) were divided into 4 groups. Group A served as the control (with vehicle solution), group B as toxin control with diclofenac sodium (50mg/kg intra-peritoneally), group C received both diclofenac sodium and protective dose of silymarin (50mg/kg orally), while group D was administered diclofenac sodium along with higher dose of silymarin (100mg/kg orally). At the study's conclusion, rabbits were humanely euthanized and blood samples were collected for analysis. Enzymes such as Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), direct bilirubin, serum urea, and total bilirubin were measured. The study revealed that higher dose of silymarin (100mg/kg) was more effective in reducing serum enzyme levels compared to lower dose, indicating stronger hepatoprotective effect. This suggests silymarin's potential as the therapeutic agent against liver damage and toxicity induced by substances like diclofenac sodium.

Keywords: Hepatotoxicity, hepatoprotective effect, liver enzymes, herbal drugs.

INTRODUCTION: Diclofenac sodium-induced liver damage is significant because diclofenac is a widely used medication with the potential to harm the liver. Understanding and mitigating this risk is crucial for patient safety and optimizing pain and inflammation management. It is classified as an aryl acetate acid derivative. Antipyretic, analgesic, and anti-inflammatory in nature, it exerts these effects predominantly physiologically and to a lesser extent, via prostaglandin synthesis (Ertckin et al., 2019), in managing various pain-related conditions, such as ankylosing spondylitis, spondylitis, rheumatoid arthritis, and osteoarthritis, in addition to all forms of muscular pain. Additionally, certain patients develop chronic renal and gastric toxicity (Klomjit and Ungprasert, 2022). The hepatic metabolism of diclofenac sodium results in the development of active form of diclofenac, cyclooxygenase, diclofenac sodium hinders the biological synthesis of prostaglandin from arachidonic acid; this activity impacts physiological functions including osmoregulation, reproduction, water transport, and immune defense in organisms (Sun et al., 2022). Furthermore, chronic and acute toxicity of diclofenac have detrimental effects on marine life, disrupting their physiological functions, reproductive, developmental, and growth, ultimately threatening their survival. Histopathological examination reveals the presence of hypersensitive reactions, which are characterized by severe hepatocyte damage caused by immune responses.

Antioxidant properties are among the potential health benefits of herbal remedies that are acquiring popularity and used globally (Abdela et al., 2020), with increasing knowledge and recognition of the biological effects of silymarin, predominantly flavonolignans, flavonoids (such as taxifolin and quercetin), and polyphenols (Surai, 2015). Silybin, which comprises approximately 50–60% of the complex, is the most abundant and biologically active of the flavonolignan isomers found in silymarin. The remaining 35% is composed of the following isomers: silicicin (20%), silydianin (10%), and isosilybin (5%) (Gillesen and Schmidt, 2020). Poor bioavailability results from the hydrophobic and non-polar nature of silymarin and limited water solubility (Bijak, 2017). However, the bioavailability of silybin can be influenced by adjacent molecules such as flavonoids, phenol derivatives, and amino acids (Abenavoli et al., 2018). Consequently, the active component, silibin, is present in varying concentrations, solubilities, and oral bioavailability across commercially available silymarin products (Mukhtar et al., 2021). In addition, silymarin and silibinin have the potential to interact with statins by inhibiting transport proteins (organic anion transporting polypeptide and breast cancer resistance protein) in the liver, which could have an effect on the metabolism of statins (Abenavoli et al., 2018). Silibinin, a constituent of silymarin, functions as a highly effective free radical scavenger, safeguarding hepatic cells against oxidative harm induced by substances such as acetaminophen and COX (Teschke, 2018). By stimulating glutathione synthesis, it enhances the liver’s antioxidant capacity and inhibits numerous pro-inflammatory cytokines (IL-1, IL-6, TNF-α) (Yass, 2016). Silymarin via its functional expression in the liver, which functions as a lipid peroxidation inhibitor, cell membrane stabilizer, and protective barrier against toxic compounds such as carbon tetrachloride (Zhao et al., 2017). In its early phases, it can also impede the progression of liver fibrosis (Cichici et al., 2016). Silymarin demonstrates anti-inflammatory, antioxidant, anti-fibrotic, and regenerative properties in the context of non-alcoholic fatty liver disease (NAFLD), in addition to ameliorating metabolic factors such as insulin resistance and hyperlipidemia (Sun et al., 2022). Furthermore, silymarin and its constituent silybin have exhibited inhibitory effects on the proliferation of liver cancer cells; furthermore, their synergistic effects with specific pharmaceutical agents amply these effects (Gu et al., 2015). When combined with doxurubicin, silymarin has the potential to inhibit tumor growth in experimental hepatic carcinoma cells, according to additional research (Yurtcu et al., 2015). Utilized as a natural remedy for ailments affecting the nervous system, kidneys, prostate, lungs, and liver, among others (Abenavoli and Milic, 2017), Silymarin possesses a range of protective activities, including immunomodulatory, anti-fibrotic, and membrane-stabilizing properties, antioxidant, anti-apoptotic, and anti-inflammatory properties (Yassin et al., 2022). The potential antitumor properties of this botanical agent have been evaluated in a variety of malignancies, including prostate, lung, liver, cervical, breast, and bladder cancers (Koltai and Fliegel, 2022). The different mechanisms for the antitumor activities of silymarin have been reported by previous studies (Delmas et al., 2020). As a result of the spread of hepatitis, milk thistle is an increasingly in-demand herb on an international scale (Jahan et al., 2016). The silymarin content of milk thistle varies depending on the region and is found in the range of 1.5% to 3.5%, with a minimal level of standard of 3% to 6%. Although the silymarin constituents remain constant among samples, variations in the flavonolignan content

**Keywords**: Hepatotoxicity, hepatoprotective effect, liver enzymes, herbal drugs.
and geographical origin indicate the possibility that particular ecotypes have been domesticated (Arampatzis et al., 2019). Despite its economic and pharmaceutical value, milk thistle domestication and breeding efforts have been limited (Hamouda, 2019). Silymarin content and composition vary genetically among milk thistle ecotypes; these variations are influenced by environmental conditions, harvesting methods, fruit maturation, and sowing depth (Tayyeb et al., 2018).

**OBJECTIVES:** The current investigation was formulated with the following aims in mind: The objective of this study is to assess the hepatoprotective potential of silymarin against liver injury induced by diclofenac sodium in rabbits. Additionally, the anti-fibrotic and anti-cirrhosis effects of silymarin in diclofenac sodium-induced liver toxicity will be investigated.

**MATERIAL AND METHODS:** For this experiment, 40 rabbits of both sexes weighing between 550 and 900 gms were purchased from the local market of Faisalabad. The rabbits were acclimated to their environment with free access to both food and water throughout the acclimation period. The rabbits were maintained autonomously in enclosures throughout the duration of the research, following a 12-hour light-dark cycle. Diclofenac sodium and silymarin were utilized and. Prior to administration, all medications were dissolved in an isotonic saline solution containing 0.9% NaCl. The rationale for selecting rabbits as experimental animals in the current study was based on the similarity of biochemical changes observed in rabbits to those observed in other animal species, their accessibility, ease of handling, and low cost (Qamar et al., 2011). Throughout the duration of the investigation, rabbits were housed autonomously in cages in a controlled environment with a light-dark cycle and climate (23 ± 2°C and 50-60% relative humidity). The administration of study drugs to each group of ten rabbits followed the subsequent outline. Group A: The control group received the vehicle alone (10 ml/kg body weight P/O) for 15 days while conducting normal activities. In the toxicity control group (Group B), diclofenac sodium (50 mg/kg P/O) was administered twice daily. Group C consisted of rabbits that were administered diclofenac sodium (50mg/kg P/O) and a moderate dose of silymarin (50mg/kg P/O) by the peroral route. Group D: A: A solution of oral herbal remedy (100 mg/kg) and 50 mg/kg diclofenac sodium were administered to the rabbits. All rabbit groups were administered these medications for a duration of 15 days. Approximately 3 ml blood were aseptically collected from both the marginal ear vein and the jugular vein, using sterile test vials, centrifuged for 3 min. at 5000 RPM (Alhassan et al., 2012). The concentrations of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and direct bilirubin were determined using commercially available kits, whereas the calorimetric determination of alkaline phosphatase (ALP) was performed using commercially available kits in accordance with the method of Bellfield and Goldberg. The measurement of total bilirubin and direct bilirubin was performed on an automatic blood biochemical analyzer at 37°C using standard reagent packages, with all detections being done in triplicate. Umbilical wound healing was quantified utilizing commercially accessible instruments. The mean value was reported along with the standard error (SE). The data were analyzed using version 16.0 of the SPSS program. A one-way analysis of variance (ANOVA) was conducted, and Tukey’s test was utilized to compare the variables between the groups. Significant differences were identified at a significance level of P < 0.05.

**RESULTS AND DISCUSSION:** Significant alterations are observed in various biochemical parameters of rabbits that are administered diclofenac sodium. Induced hepatotoxicity in all groups was a high dose of diclofenac sodium (50 mg/kg body weight) (figure 1). Enzymes such as ALT, ALP, and AST serve as indicators of the liver’s overall health. Their elevated level indicates damage and injury to the liver. Additionally, significant increases were observed in the levels of all enzymes: ALT, AST, and ALP. The statistical analysis unequivocally demonstrates these alterations. The results of ANOVA for ALT, ALP, AST, Direct Bilirubin, Total Bilirubin, and Blood Urea Nitrogen are presented in table 1. Results indicate that all of these parameters increased significantly when only diclofenac sodium was administered to each group. Moreover, their concentrations decreased substantially following the oral herbal remedy. The administration of the aforementioned parameters provides evidence for silymarin’s hepatoprotective properties. In table 2, group A has the lowest values of ALP, ALT, and AST (12.55 ± 1.28, 45.21 ± 1.14, and 6.185 ± 1.48, respectively). These values are close to normal, given that group A serves as the control and does not receive diclofenac sodium or silymarin treatment. Group B has significantly elevated values of ALP (33.50 ± 1.81), ALT (90.50 ± 4.01), and AST (118.45 ± 6.38) in comparison to Group A, due to the fact that it is administered diclofenac sodium at a dose rate of 50 mg/kg body weight, I/P. Group C exhibits reduced levels of ALP (29.65 ± 1.90), ALT (92.30 ± 5.03), and AST (103.45 ± 2.96) due to the administration of silymarin at a protective dose of 50mg/kg body weight (I/P) and diclofenac sodium (50mg/kg body weight, I/P).

**Table 1:** ANOVA for all respective parameters. Aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST).

<table>
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<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>
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<tr>
<td>ALP</td>
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<td>1164.74</td>
<td>388.25</td>
<td>204.74**</td>
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<td></td>
<td>Total</td>
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<td>12230.03</td>
<td></td>
<td></td>
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<tr>
<td>ALT</td>
<td>Treatment</td>
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<td>19.00</td>
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<td></td>
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<tr>
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<td>Total</td>
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<td></td>
<td></td>
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<tr>
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<td>6100.3</td>
<td>541.19**</td>
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<td>Total</td>
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<td>Total Bilirubin</td>
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**Table 2:** Representation of aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) values in the experimental groups. The observed decrease in levels of ALT, AST, and AST in this cohort indicates that silymarin may have a hepatoprotective impact. In a similar vein, Group D demonstrates a decrease in ALT (24.95), ALT (7.29), and AST (86.3), in addition to being administered a hepatoprotective dose of silymarin (100 mg/kg body weight, per oral). Significantly, the reduction in ALT, AST, and AST levels within this cohort demonstrates that silymarin has a substantial effect on hepatocyte recovery. As shown in table 3, the group A, which serves as the control group, has the lowest values of direct bilirubin (0.146 ± 0.043), total bilirubin (0.429 ± 0.035), and blood urea nitrogen (17.12 ± 1.98). These values are in close proximity to the normal range, given that Group A has not been subjected to any treatment involving diclofenac sodium or silymarin. On the other hand, Group B exhibits significantly higher values for direct bilirubin (1.958 ± 0.280), total bilirubin (2126 ± 0.152), and blood urea nitrogen (3830 ± 2.16), all of which can be attributed to the intraperitoneal administration of diclofenac alone at a dose rate of 50mg/kg body weight. On the other hand, Group C exhibits a decrease in blood urea nitrogen value (29.30 ± 1.77), direct bilirubin value (1.670 ± 0.407), and total bilirubin value (1.630 ± 0.142) due to the administration of silymarin at a protective dose of 50mg/kg body weight orally and

**Figure 1:** Step-wise demonstration on the experimental animals. Measurement of wound diameter (A), wound depth (B), marked appearance of the wound (C) and healing at a predetermined time point post injury (D).
intraperitoneally.

**Table 3**: The confirmation of direct bilirubin (DB), total bilirubin (TB) and blood urea nitrogen (BUN) in the groups.

**DISCUSSION**

Table 3: The confirmation of direct bilirubin (DB), total bilirubin (TB) and blood urea nitrogen (BUN) in the groups.

**CONCLUSION**

The study confirms the sustained efficacy of silymarin in alleviating diclofenac sodium-induced toxicity in rabbits. The results suggest that silymarin can significantly reduce the elevations of total bilirubin, direct bilirubin, and blood urea nitrogen, indicating liver toxicity and injury. Silymarin, known for its hepatoprotective properties as a plant extract, was selected as the investigational drug, demonstrating a significant and favorable impact in reducing elevated liver enzyme concentrations—a key indicator of hepatic injury. In contrast to previous investigations utilizing rodents as the research model, this study primarily evaluated the liver's correct functioning in a broader spectrum of species for a more comprehensive understanding. In summary, silymarin exhibited the capability to decrease ALT, AST, and ALP levels. Notably, concentrations of total bilirubin, direct bilirubin, and blood urea nitrogen were significantly higher at a dosage of 100 mg/kg of silymarin after 50 days of oral administration compared to 50 mg/kg. This conclusion highlights the potential of silymarin in ameliorating liver damage, emphasizing the importance of dosage considerations in therapeutic applications.

**CONFLICT OF INTEREST**: The authors declared no conflict of interest.


