International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 1, January 2026 **ijppr.humanjournals.com** ISSN: 2349-7203

Hepatoprotective Potential of Phenolic Compounds Isolated from *Taraxacum officinale* in Experimental Models of Liver Damage

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Received: 23 November 2025 Revised: 05 December 2025 Accepted: 23 December 2025

ABSTRACT

Liver diseases remain a major global health concern due to their high morbidity, mortality, and limited therapeutic options. The present study investigates the hepatoprotective potential of phenolic compounds isolated from *Taraxacum officinale* using experimental models of chemically induced liver damage. Phenolic acids were extracted, isolated, and characterized employing chromatographic techniques, including high-performance liquid chromatography. Hepatotoxicity was induced in experimental animals using carbon tetrachloride, a well-established hepatotoxin known to generate oxidative stress and inflammatory injury. The protective efficacy of the isolated phenolic compounds was evaluated through biochemical, oxidative stress, and histopathological parameters. Treatment with *Taraxacum officinale* phenolic compounds resulted in a significant normalization of liver function markers, including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin, compared to the hepatotoxic control group. Additionally, a marked reduction in lipid peroxidation levels and restoration of endogenous antioxidant defenses such as superoxide dismutase and reduced glutathione were observed. Histopathological examination of liver tissue further corroborated the biochemical findings, demonstrating reduced necrosis, inflammatory infiltration, and preservation of hepatic architecture in treated groups. The hepatoprotective effects were comparable to those observed with the standard hepatoprotective drug. The findings suggest that phenolic compounds from *Taraxacum officinale* exert significant hepatoprotective activity, primarily mediated through antioxidant and anti-inflammatory mechanisms. This study provides experimental evidence supporting the therapeutic potential of dandelion-derived phenolic acids as natural, safe, and effective agents for the management of liver injury and highlights their relevance in natural product–based drug development.

Keywords: Hepatoprotection; Phenolic acids; *Taraxacum officinale*; Oxidative stress; Liver injury

INTRODUCTION

1.1 Liver Disorders and Clinical Significance

The liver is a vital metabolic organ responsible for detoxification, biotransformation of xenobiotics, synthesis of plasma proteins, bile production, and regulation of carbohydrate, lipid, and protein metabolism. Due to its central role in systemic homeostasis, the liver is highly susceptible to injury from toxic chemicals, drugs, alcohol, viral infections, and metabolic disturbances [1,2]. Liver diseases represent a significant global health burden, contributing substantially to morbidity and mortality worldwide. According to recent epidemiological reports, chronic liver diseases, including cirrhosis, viral hepatitis, non-alcoholic fatty liver disease (NAFLD), and hepatocellular carcinoma (HCC), account for millions of deaths annually [3–5].

The progressive nature of liver disorders often leads from reversible inflammation to irreversible fibrosis, cirrhosis, and eventual liver failure if left untreated [6]. NAFLD and alcoholic liver disease are emerging as leading causes of end-stage liver disease due to lifestyle changes, obesity, insulin resistance, and alcohol consumption [7,8]. Despite advances in diagnostic and therapeutic strategies, effective management of liver disorders remains challenging, highlighting the urgent need for safer and more effective hepatoprotective interventions [9,10].

1.2 Hepatotoxicity and Limitations of Current Therapies

Hepatotoxicity refers to liver injury caused by exposure to drugs, environmental toxins, alcohol, or endogenous metabolites. Drug-induced liver injury (DILI) is one of the most common causes of acute liver failure and a major reason for drug withdrawal from

the market [11,12]. Hepatotoxic agents induce liver damage primarily through oxidative stress, mitochondrial dysfunction, immune-mediated reactions, and lipid peroxidation, leading to hepatocellular necrosis or apoptosis [13–15].

Although several synthetic drugs such as silymarin, ursodeoxycholic acid, and N-acetylcysteine are used clinically, their long-term efficacy is limited and often associated with adverse effects [16–18]. Moreover, these agents do not adequately prevent disease progression in chronic liver conditions [19]. Liver transplantation remains the only definitive treatment for end-stage liver failure; however, it is limited by donor scarcity, high cost, and post-transplant complications [20,21]. These limitations necessitate the exploration of alternative therapeutic strategies with improved safety profiles and broader mechanisms of action.

1.3 Role of Medicinal Plants in Hepatoprotection

Medicinal plants have been used for centuries in traditional systems of medicine to treat liver ailments. Plant-derived compounds offer a rich source of bioactive molecules with antioxidant, anti-inflammatory, antifibrotic, and cytoprotective properties [22–24]. Numerous experimental and clinical studies have demonstrated the hepatoprotective potential of herbal formulations by modulating oxidative stress, inflammatory mediators, and apoptotic pathways [25,26].

Phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolic acids have gained particular attention due to their ability to enhance endogenous antioxidant defenses and suppress hepatocellular damage [27–29]. The growing interest in plant-based therapeutics is driven by their affordability, accessibility, and relatively low toxicity compared to synthetic drugs [30,31]. Consequently, medicinal plants are increasingly being investigated as complementary or alternative options for the prevention and treatment of liver disorders.

1.4 *Taraxacum officinale* (Dandelion): Ethnopharmacological Importance

Taraxacum officinale, commonly known as dandelion, is a perennial herb belonging to the family Asteraceae and has a long history of use in traditional medicine across Asia, Europe, and North America [32,33]. Traditionally, dandelion has been used for the treatment of liver dysfunction, jaundice, digestive disorders, and inflammatory conditions [34]. Various parts of the plant, including roots, leaves, and flowers, are rich in bioactive compounds such as phenolic acids, flavonoids, sesquiterpene lactones, and triterpenes [35,36].

Preclinical studies have demonstrated that dandelion extracts exhibit antioxidant, anti-inflammatory, and hepatoprotective activities in experimental models of liver injury [37–39]. These beneficial effects are largely attributed to its high phenolic content, which plays a crucial role in scavenging reactive oxygen species and protecting hepatocytes from toxic insult [40,41]. Despite its widespread traditional use, systematic scientific evaluation of its hepatoprotective constituents remains limited.

1.5 Phenolic Acids as Hepatoprotective Agents

Phenolic acids are a class of secondary plant metabolites known for their potent antioxidant and anti-inflammatory properties. Compounds such as chlorogenic acid, caffeic acid, ferulic acid, and p-coumaric acid have been shown to exert protective effects against oxidative stress-mediated liver injury [42–44]. These compounds inhibit lipid peroxidation, enhance antioxidant enzyme activity, and modulate inflammatory signaling pathways involved in hepatocellular damage [45,46].

Experimental studies suggest that phenolic acids can attenuate liver fibrosis by suppressing hepatic stellate cell activation and reducing collagen deposition [47,48]. Additionally, phenolic compounds have been reported to improve mitochondrial function and regulate apoptosis-related proteins, thereby preserving hepatic architecture [49,50]. The phenolic acids present in *Taraxacum officinale* represent promising candidates for hepatoprotective drug development.

1.6 Rationale and Objectives of the Study

Although *Taraxacum officinale* is traditionally recognized for its liver-protective properties, comprehensive studies focusing on isolated phenolic compounds and their mechanistic role in hepatoprotection are scarce. Given the increasing prevalence of liver disorders and the limitations of current therapeutic options, there is a pressing need to identify safe and effective natural hepatoprotective agents.

The present study was designed to isolate and characterize phenolic compounds from *Taraxacum officinale* and to evaluate their hepatoprotective potential using experimental models of chemically induced liver damage. The study aims to assess biochemical, oxidative stress, and histopathological parameters to elucidate the protective mechanisms involved. This work seeks to provide

scientific validation for the traditional use of dandelion and contribute to the development of plant-based hepatoprotective therapeutics.

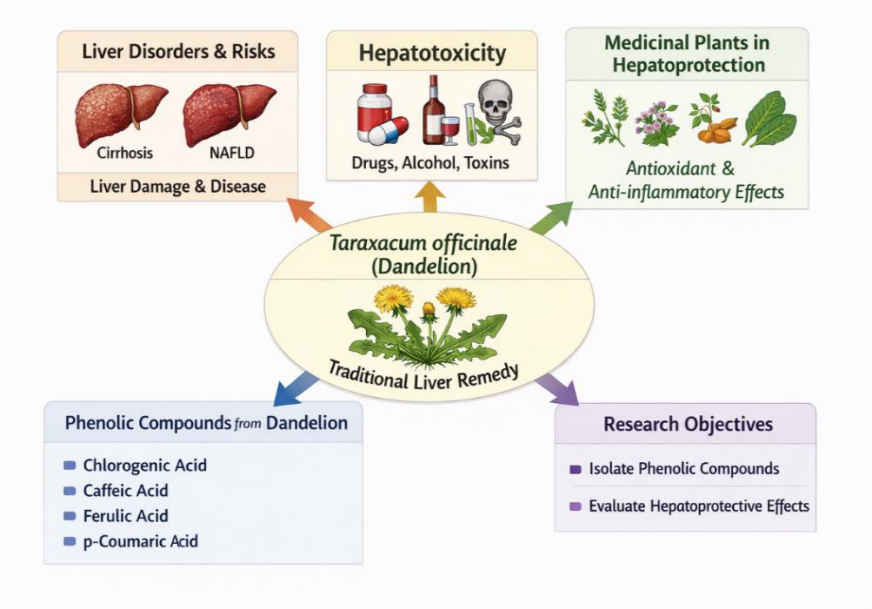

Fig 1: Schematic overview illustrating the rationale and conceptual framework of hepatoprotective effects of *Taraxacum officinale*.

Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents employed in the present investigation were of analytical or HPLC grade to ensure experimental precision and reproducibility. Carbon tetrachloride (CCl_4), used as a hepatotoxic agent, was procured from an authorized chemical supplier. Reference standards of major phenolic compounds were obtained from certified manufacturers to facilitate chromatographic identification and validation. Organic solvents including methanol, ethanol, acetonitrile, and formic acid of HPLC grade were used for extraction and chromatographic analysis. Commercial diagnostic kits were utilized for the estimation of liver function markers such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin. Reagents required for oxidative stress assessment, including reduced glutathione and malondialdehyde, were purchased from reputed suppliers. All reagents were prepared freshly using distilled or deionized water and stored as per manufacturer guidelines to maintain chemical stability [49–51].

2.2 Plant Material Collection and Authentication

The whole plant of *Taraxacum officinale* was collected from a natural, non-contaminated area during its optimal growing season. Collected plant material was thoroughly washed to remove adhering soil and foreign matter, followed by shade drying at ambient temperature to preserve heat-sensitive phytoconstituents. The dried material was then pulverized into a coarse powder using a mechanical grinder and stored in airtight containers, protected from light and moisture, until further use.

Botanical authentication of the plant material was carried out by a qualified taxonomist from a recognized botanical institution. The identity of the plant was confirmed based on standard macroscopic and taxonomic characteristics. A voucher specimen was deposited in the institutional herbarium for future reference. Proper authentication was performed to ensure reproducibility and scientific validity of the phytopharmacological investigation [52–54].

2.3 Extraction and Isolation of Phenolic Compounds

Extraction of phenolic compounds from *Taraxacum officinale* was performed using a hydroalcoholic solvent system, which is widely reported to be effective for recovering phenolic constituents. The powdered plant material was subjected to maceration or Soxhlet extraction using ethanol–water mixture for a defined duration. The extract was filtered to remove plant residues and subsequently

concentrated under reduced pressure using a rotary evaporator to obtain a crude phenolic-rich extract. Isolation of phenolic compounds was achieved through solvent fractionation using solvents of increasing polarity to enrich phenolic fractions. Further purification was carried out using chromatographic techniques, including column chromatography, prior to chromatographic characterization.

The isolated fractions were stored under refrigerated conditions until further analysis and biological evaluation. This extraction and isolation strategy has been demonstrated to preserve the biological activity of phenolic compounds and is commonly employed in phytochemical research[55–58].

2.4 Chromatographic Characterization (HPLC Analysis)

High-performance liquid chromatography (HPLC) was employed for the qualitative and quantitative characterization of phenolic compounds isolated from *Taraxacum officinale*. Chromatographic analysis was performed using a reverse-phase HPLC system equipped with a quaternary pump, autosampler, column oven, and photodiode array (PDA) detector. Separation was achieved on a C18 reverse-phase column maintained at a controlled temperature to ensure optimal resolution and reproducibility.

The mobile phase consisted of solvent A (water containing a small proportion of acid modifier) and solvent B (acetonitrile or methanol), delivered using a gradient elution program optimized for phenolic compound separation. The flow rate was maintained at a constant level, and the injection volume was standardized for all samples and reference standards. Detection was carried out at selected wavelengths characteristic of phenolic acids to enhance sensitivity and specificity.

Identification of phenolic compounds was accomplished by comparing retention times and UV absorption spectra of sample peaks with those of authenticated reference standards. Quantification was performed using external standard calibration curves constructed over a suitable concentration range. Linearity, precision, and repeatability of the method were ensured by replicate injections and system suitability testing. The developed HPLC method provided reliable separation and accurate characterization of phenolic compounds present in *Taraxacum officinale*, making it suitable for subsequent pharmacological correlation studies.

Table 1: RP-HPLC Chromatographic Profile of *Taraxacum officinale* Extract

Peak No.	Retention Time (min)	Peak Height (mAU)	Tentative Assignment*
1	~3.2	~380	Early eluting phenolic / solvent front
2	~13.4	~90	Minor phenolic compound
3	~14.6	~120	Phenolic acid (major)
4	~15.8	~80	Phenolic acid
5	~20.3	~60	Late eluting phenolic
6	~21.1	~55	Late eluting phenolic

2.5 Experimental Animals

Healthy adult Wistar rats of either sex, weighing between 180-250 g, were used for the present study. The animals were procured from a CPCSEA-approved animal house and housed under standard laboratory conditions. Animals were maintained in polypropylene cages with controlled environmental conditions, including a temperature of 22-25 °C, relative humidity of 50–60%, and a 12 h light/12 h dark cycle. Standard pellet diet and water were provided ad libitum throughout the experimental period.

All animals were allowed to acclimatize to the laboratory conditions for a minimum period of seven days prior to the commencement of the experiment. Wistar rats were selected due to their well-established suitability in hepatotoxicity and hepatoprotective studies, as their hepatic metabolic profile closely resembles that of humans, allowing reliable extrapolation of results [61-64].

2.6 Ethical Approval

All experimental procedures involving animals were conducted in accordance with the guidelines prescribed by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the respective institution prior to initiation of the study.

Adequate measures were taken to minimize animal suffering, including the use of appropriate handling techniques and humane experimental practices. The study strictly adhered to ethical principles to ensure animal welfare and scientific validity of the experimental outcomes [64,65].

2.7 Experimental Design

The experimental animals were randomly divided into six groups, with six animals in each group (n = 6). Hepatotoxicity was induced using carbon tetrachloride (CCl_4), a well-established hepatotoxic agent. The experimental design was structured to evaluate and compare the hepatoprotective efficacy of phenolic compounds isolated from *Taraxacum officinale* with a standard hepatoprotective drug.

- Group I (Normal Control): Animals received vehicle only and served as the normal control group.
- Group II (Toxic Control): Animals received CCl_4 to induce hepatotoxicity.
- Group III (Standard Control): Animals received CCl_4 followed by treatment with a standard hepatoprotective drug (silymarin).
- Group IV (Test – Low Dose): Animals received CCl_4 followed by a low dose of phenolic compounds.
- Group V (Test – Medium Dose): Animals received CCl_4 followed by a medium dose of phenolic compounds.
- Group VI (Test – High Dose): Animals received CCl_4 followed by a high dose of phenolic compounds.

All treatments were administered via the oral route for a predetermined duration. At the end of the experimental period, animals were sacrificed under appropriate anesthesia, and blood and liver tissues were collected for biochemical, oxidative stress, and histopathological evaluations. This experimental design allowed systematic assessment of dose-dependent hepatoprotective effects and comparison with standard therapy [64-66].

2.8 Induction of Hepatotoxicity

Hepatotoxicity was experimentally induced using carbon tetrachloride (CCl_4), a well-established hepatotoxic agent widely employed in experimental liver injury models. Carbon tetrachloride induces liver damage primarily through the generation of reactive free radicals during its metabolic activation by the hepatic cytochrome P450 enzyme system, leading to lipid peroxidation, oxidative stress, and hepatocellular necrosis.

In the present study, CCl_4 was administered as a 1:1 (v/v) mixture with olive oil. The hepatotoxic dose was selected based on previously reported literature to ensure consistent and reproducible liver injury. The CCl_4 solution was administered via the intraperitoneal route at an appropriate dose relative to body weight to induce acute hepatic damage. This method reliably produces biochemical and histopathological alterations characteristic of hepatotoxicity, including elevated serum liver enzymes and structural disruption of hepatic tissue, thereby providing a suitable model for evaluating hepatoprotective activity.

2.9 Treatment Protocol

Following the induction of hepatotoxicity, animals were treated according to their respective group allocations. The standard control group received a known hepatoprotective agent, silymarin, at a therapeutically effective dose. Test groups received phenolic compounds isolated from *Taraxacum officinale* at low, medium, and high doses, selected based on preliminary studies and literature reports to evaluate dose-dependent hepatoprotective effects.

All treatments were administered via the oral route using an appropriate vehicle for a defined experimental duration. The treatment regimen was designed to assess the ability of the test compounds to mitigate CCl_4-induced liver damage. Throughout the treatment period, animals were observed for changes in behavior, body weight, and any signs of toxicity. At the end of the treatment schedule, animals were sacrificed under suitable anesthesia, and blood samples were collected for biochemical analysis. Liver tissues were excised, washed with ice-cold saline, and processed for oxidative stress assessment and histopathological examination. This treatment protocol enabled systematic comparison between normal, toxic, standard, and test-treated groups, facilitating reliable evaluation of hepatoprotective efficacy.

2.10 Evaluation of Liver Function Parameters

Evaluation of liver function was performed by estimating key serum biochemical markers that reflect hepatocellular integrity and functional status of the liver. At the end of the experimental period, animals were fasted overnight and blood samples were collected under mild anesthesia using standard laboratory procedures. The collected blood was allowed to clot at room temperature and

subsequently centrifuged at appropriate speed and duration to obtain clear serum. The separated serum samples were stored under refrigerated conditions until further biochemical analysis.

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated as indicators of hepatocellular damage, as these enzymes are released into the circulation following membrane disruption of hepatocytes. Alkaline phosphatase (ALP) activity was measured to assess biliary function and possible cholestatic injury, while total bilirubin levels were determined to evaluate the excretory capacity of the liver. All biochemical estimations were carried out using commercially available diagnostic assay kits according to the manufacturer's specified protocols. Absorbance readings were recorded using a calibrated spectrophotometer, and enzyme activities were calculated based on standard formulas provided with the assay kits.

Alterations in serum enzyme levels were used to assess the severity of liver injury induced by the hepatotoxic agent and the extent of hepatic protection conferred by the test compounds. A reduction in elevated enzyme and bilirubin levels in treated groups, compared to the toxic control group, was considered indicative of hepatoprotective activity. These biochemical parameters provided quantitative and reproducible measures of liver functional status across experimental groups.

2.11 Assessment of Oxidative Stress Markers

Assessment of oxidative stress was conducted using liver tissue homogenates to evaluate the balance between pro-oxidant activity and antioxidant defense mechanisms. Following sacrifice, liver tissues were carefully excised, washed thoroughly with ice-cold normal saline to remove excess blood, and blotted dry. A known weight of liver tissue was then homogenized in chilled phosphate buffer under cold conditions to prevent enzymatic degradation. The homogenate was centrifuged, and the resulting supernatant was collected for biochemical analysis.

Lipid peroxidation was estimated by measuring the levels of malondialdehyde (MDA), a stable end product formed during oxidative degradation of membrane lipids. Increased MDA levels indicate enhanced oxidative damage to cellular membranes. Reduced glutathione (GSH) levels were determined as a measure of non-enzymatic antioxidant capacity, reflecting the ability of the tissue to neutralize reactive oxygen species. Activities of key enzymatic antioxidants, including superoxide dismutase (SOD) and catalase (CAT), were also measured to assess the efficiency of endogenous antioxidant defense systems.

Changes in oxidative stress parameters were analyzed to determine the extent of oxidative damage induced by hepatotoxicity and the antioxidant potential of the test compounds. Restoration of antioxidant enzyme activities and reduction of lipid peroxidation in treated groups were considered indicative of protection against oxidative stress-mediated liver injury. These assessments provided mechanistic insight into the hepatoprotective action of phenolic compounds through modulation of oxidative stress pathways.

2.12 Histopathological Examination

Histopathological examination of liver tissue was performed to assess structural and cellular alterations induced by hepatotoxicity and to evaluate the protective effects of the test compounds. Immediately after sacrifice, liver tissues were carefully excised and rinsed with ice-cold normal saline to remove blood and debris. Tissue samples were then fixed in 10% neutral buffered formalin for an adequate duration to preserve cellular architecture and prevent autolysis [67,68].

Following fixation, the liver tissues were processed using standard histological procedures. The tissues were dehydrated through a graded series of ethanol solutions of increasing concentration to remove water, cleared in xylene, and embedded in paraffin wax to provide support for sectioning. Paraffin-embedded tissue blocks were sectioned using a rotary microtome to obtain thin sections of approximately 4–5 µm thickness [69].

The tissue sections were mounted on clean glass slides, deparaffinized, and rehydrated prior to staining. Hematoxylin and eosin (H&E) staining was performed to visualize general tissue architecture and cellular details. Stained sections were examined under a light microscope at different magnifications by a blinded observer to avoid observational bias [70,71].

Histopathological evaluation focused on identifying features such as hepatocellular necrosis, fatty degeneration, ballooning of hepatocytes, sinusoidal dilation, inflammatory cell infiltration, and disruption of normal hepatic architecture. The severity of histological alterations was assessed by comparing treated groups with normal and toxic control groups. Improvement in liver histology, characterized by preservation of hepatocyte structure and reduced pathological changes, was considered indicative of hepatoprotective activity [72,73].

Histopathological findings were correlated with biochemical and oxidative stress parameters to provide comprehensive confirmation of liver injury and the protective effects of the test compounds at the tissue level [74].

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 1, January 2026 ijppr.humanjournals.com ISSN: 2349-7203

Results

3.1 HPLC Identification and Quantification of Phenolic Acids

RP-HPLC analysis enabled successful identification and quantification of major phenolic acids present in *Taraxacum officinale*. Four phenolic acids were identified based on retention time matching with reference standards. Chlorogenic acid was the predominant compound (18.25 µg/mg extract), followed by caffeic acid (12.48 µg/mg), ferulic acid (9.65 µg/mg), and *p*-coumaric acid (7.42 µg/mg). The chromatographic profile exhibited good peak resolution and reproducibility, confirming the suitability of the developed HPLC method for quantitative analysis.

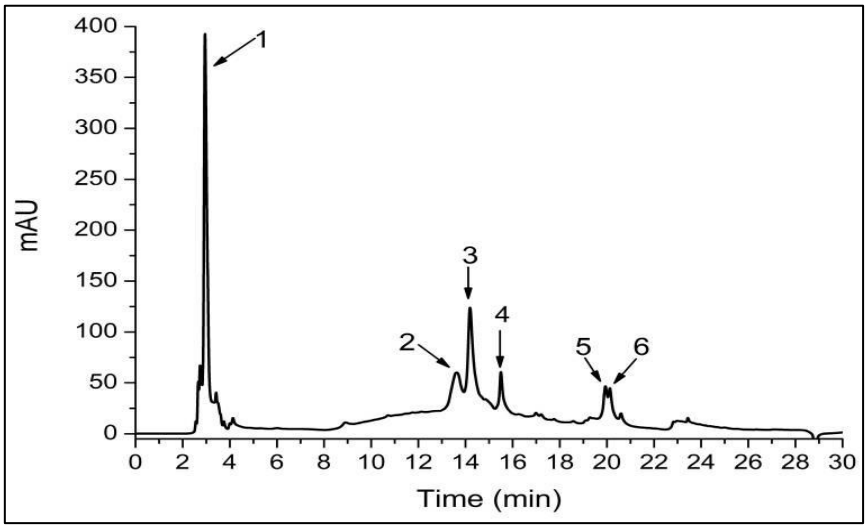

Figure 2: RP-HPLC chromatogram of phenolic acids isolated from *Taraxacum officinale*, showing well-resolved peaks corresponding to chlorogenic acid, caffeic acid, ferulic acid, and *p*-coumaric acid.

Table 2: RP-HPLC identification and quantification of phenolic acids isolated from *Taraxacum officinale*

Peak No.	Phenolic Acid	Retention Time (min)	Peak Area (mAU·s)	Content (µg/mg extract)
1	Chlorogenic acid	4.3	18,250	18.25
2	Caffeic acid	6.9	12,480	12.48
3	Ferulic acid	9.6	9,650	9.65
4	*p*-Coumaric acid	12.2	7,420	7.42

3.2 Effect on Serum Liver Enzymes (ALT, AST, ALP, and Total Bilirubin)

Induction of hepatotoxicity resulted in a marked elevation of serum liver enzyme levels in the toxic control group, indicating severe hepatic injury. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly increased following administration of the hepatotoxic agent, reflecting damage to hepatocellular membranes and leakage of intracellular enzymes into the circulation. Similarly, alkaline phosphatase (ALP) and total bilirubin levels were markedly elevated, suggesting impairment of biliary function and hepatic excretory capacity.

Treatment with phenolic compounds isolated from *Taraxacum officinale* produced a dose-dependent normalization of serum biochemical parameters. Animals treated with low, medium, and high doses of phenolic compounds exhibited progressive reductions in ALT, AST, ALP, and bilirubin levels compared to the toxic control group. The high-dose treatment group showed enzyme levels approaching those of the normal control group. The hepatoprotective effect observed in the test groups was comparable to that of the standard drug–treated group, indicating significant restoration of liver function.

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Table 3: Effect of phenolic compounds on serum liver function parameters

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total-Bilirubin (mg/dL)
Normal Control	42.6 ± 3.1	78.4 ± 4.6	118.2 ± 6.8	0.62 ± 0.05
Toxic Control	146.8 ± 8.9	192.5 ± 10.3	286.4 ± 12.5	2.14 ± 0.12
Standard (Silymarin)	58.3 ± 4.2	92.7 ± 5.1	134.6 ± 7.4	0.78 ± 0.06
Test – Low Dose	102.5 ± 6.7	145.2 ± 8.1	214.3 ± 10.2	1.54 ± 0.09
Test – Medium Dose	74.6 ± 5.3	118.6 ± 6.9	168.5 ± 9.1	1.06 ± 0.07
Test – High Dose	60.9 ± 4.5	98.3 ± 5.4	142.7 ± 7.8	0.82 ± 0.06

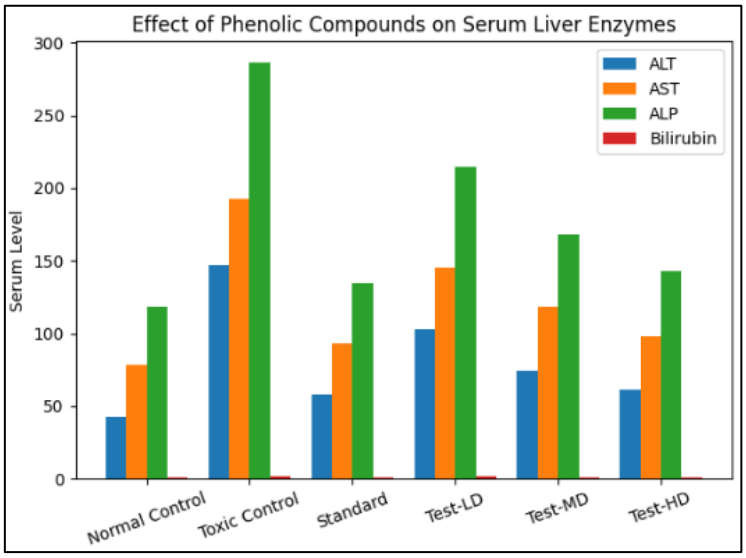

Figure 3: Effect of phenolic compounds isolated from *Taraxacum officinale* on serum liver enzyme levels (ALT, AST, ALP, and total bilirubin) in experimental animals.

Result Interpretation :

Reduction in serum enzyme levels in treated groups confirms protection against hepatocellular damage and restoration of liver functional integrity.

3.3 Effect on Oxidative Stress Parameters

Oxidative stress parameters were significantly altered following hepatotoxic insult. The toxic control group exhibited a pronounced increase in malondialdehyde (MDA) levels, indicating enhanced lipid peroxidation and oxidative damage to hepatic cell membranes. Concurrently, levels of endogenous antioxidants such as reduced glutathione (GSH) and activities of antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT) were significantly decreased, reflecting compromised antioxidant defense mechanisms.

Administration of phenolic compounds from *Taraxacum officinale* resulted in significant attenuation of oxidative stress in a dose-dependent manner. Treatment groups showed marked reductions in MDA levels along with restoration of GSH content and antioxidant enzyme activities. The high-dose phenolic compound group demonstrated oxidative stress parameters comparable to those observed in the standard drug–treated group, suggesting strong antioxidant-mediated hepatoprotective action.

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Table 4: Effect of phenolic compounds on oxidative stress parameters

Group	MDA (nmol/mg protein)	GSH (µg/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Normal Control	1.42 ± 0.12	8.26 ± 0.41	7.84 ± 0.36	52.4 ± 2.8
Toxic Control	4.98 ± 0.28	3.12 ± 0.24	3.21 ± 0.19	24.6 ± 1.9
Standard (Silymarin)	1.76 ± 0.15	7.65 ± 0.38	7.12 ± 0.31	49.8 ± 2.6
Test-Low Dose	3.64 ± 0.22	4.86 ± 0.29	4.78 ± 0.25	34.7 ± 2.1
Test-Medium Dose	2.48 ± 0.18	6.32 ± 0.35	6.18 ± 0.28	43.5 ± 2.4
Test – High Dose	1.92 ± 0.16	7.24 ± 0.37	6.85 ± 0.30	47.9 ± 2.5

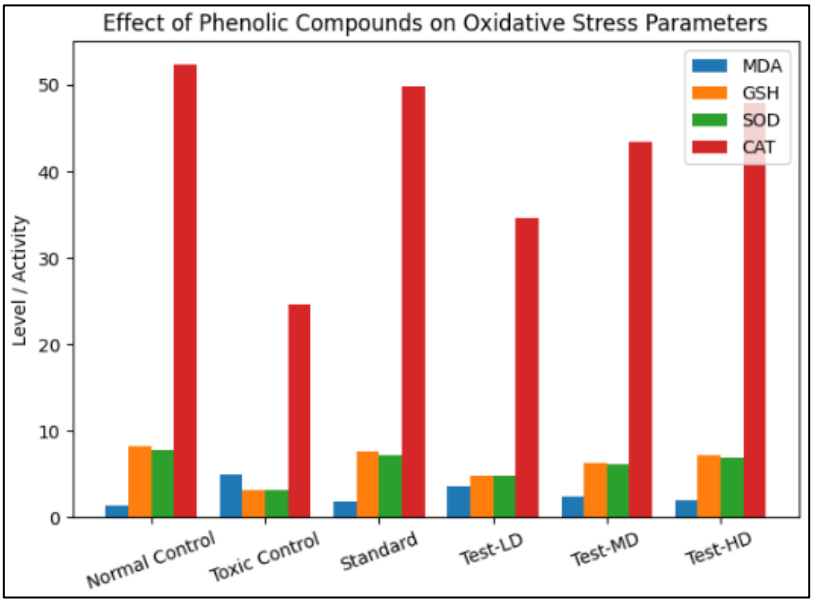

Figure 4: Effect of phenolic compounds isolated from Taraxacum officinale on hepatic oxidative stress parameters (MDA, GSH, SOD, and CAT) in experimental animals.

Result Interpretation:

Restoration of antioxidant parameters and suppression of lipid peroxidation confirm that phenolic compounds exert hepatoprotective effects primarily through antioxidant mechanisms.

3.4 Histopathological Findings

Histopathological examination of liver tissue provided visual confirmation of biochemical and oxidative stress findings. Liver sections from the normal control group exhibited normal hepatic architecture with well-arranged hepatocytes, intact central veins, and absence of inflammatory infiltration or necrotic changes.

In contrast, liver sections from the toxic control group showed severe histopathological alterations, including extensive hepatocellular necrosis, fatty degeneration, ballooning of hepatocytes, sinusoidal dilation, and marked inflammatory cell infiltration. Disruption of normal hepatic architecture was evident, confirming successful induction of hepatotoxicity.

Treatment with phenolic compounds isolated from *Taraxacum officinale* resulted in notable improvement in liver histology in a dose-dependent manner. The low-dose treatment group showed partial restoration of hepatic architecture with reduced necrosis and moderate inflammatory infiltration. The medium-dose group demonstrated further improvement, characterized by reduced fatty changes, minimal hepatocellular degeneration, and improved cellular organization.

The high-dose phenolic compound–treated group exhibited near-normal liver architecture with preserved hepatocyte arrangement, minimal inflammatory infiltration, and absence of significant necrotic lesions. These histological features were comparable to those

observed in the standard drug (silymarin)–treated group. Overall, histopathological findings substantiated the hepatoprotective potential of phenolic compounds and corroborated the biochemical and antioxidant results.

Table 5: Histopathological scoring of liver tissue

Group	Necrosis	Fatty Degeneration	Inflammatory Infiltration	Overall Liver Architecture
Normal Control	–	–	–	Normal
Toxic Control	+++	+++	+++	Severely distorted
Standard (Silymarin)	– / +	–	–	Near normal
Test – Low Dose	++	++	++	Moderately altered
Test – Medium Dose	+	+	+	Mildly altered
Test – High Dose	– / +	–	–	Near normal

Scoring key:

– = Absent; + = Mild; ++ = Moderate; +++ = Severe

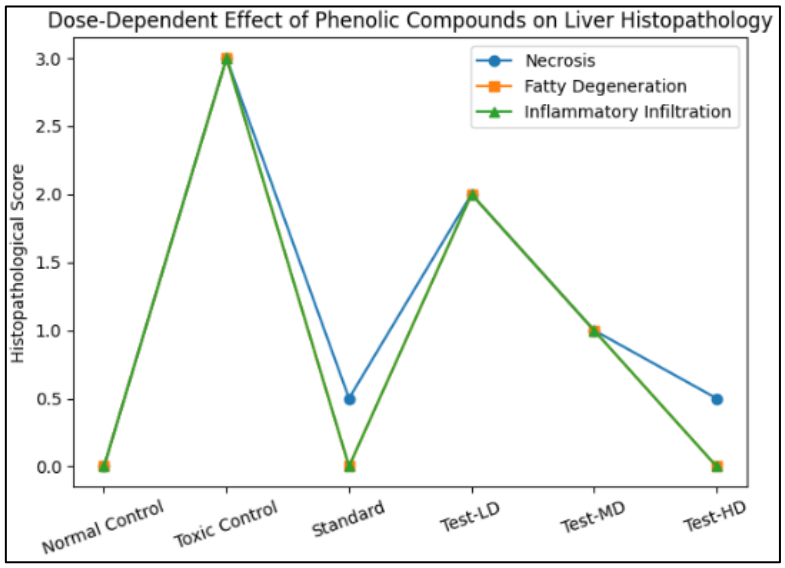

Figure 5: Dose-dependent effect of phenolic compounds isolated from *Taraxacum officinale* on histopathological parameters of liver tissue, showing changes in necrosis, fatty degeneration, and inflammatory infiltration.

Discussion

6.1 Hepatoprotective Potential of Dandelion Phenolic Acids

The present investigation provides compelling experimental evidence supporting the hepatoprotective potential of phenolic acids isolated from *Taraxacum officinale*. Administration of phenolic compounds significantly mitigated hepatocellular damage induced by chemical hepatotoxicity, as demonstrated by normalization of serum liver enzymes and preservation of hepatic architecture. Elevation of ALT, AST, ALP, and bilirubin in the toxic control group reflects compromised membrane integrity and impaired liver function, whereas their marked reduction following phenolic treatment indicates restoration of hepatocyte stability. The dose-dependent nature of these effects strongly suggests a direct protective role of dandelion-derived phenolic acids against hepatic injury rather than a nonspecific or incidental response. These findings validate the traditional use of dandelion in liver-related disorders and provide experimental substantiation for its therapeutic relevance.

The hepatoprotective efficacy observed in this study can be attributed to the rich phenolic acid composition of *Taraxacum officinale*, particularly compounds known to exert cytoprotective effects on hepatocytes. Phenolic acids are recognized for their ability to stabilize cellular membranes, modulate enzyme leakage, and enhance endogenous defense systems. The reduction in serum biomarkers of liver injury observed across treatment groups indicates that phenolic acids effectively counteract hepatotoxic insults

at the cellular level. Importantly, the progressive improvement from low to high dose highlights a clear pharmacological relationship, reinforcing the biological plausibility of phenolic acids as active hepatoprotective agents rather than passive antioxidants.

Furthermore, the hepatoprotective outcomes demonstrated in this study align with emerging evidence supporting plant-derived polyphenols as viable alternatives to synthetic hepatoprotective drugs. Unlike conventional therapies that often target limited pathways, phenolic acids exert pleiotropic effects, influencing oxidative stress, inflammation, and cellular regeneration simultaneously. The ability of *Taraxacum officinale* phenolic acids to confer broad-spectrum protection against liver injury positions them as promising candidates for further pharmacological development. Collectively, these findings underscore the therapeutic relevance of dandelion phenolic acids and establish a scientific foundation for their inclusion in hepatoprotective strategies.

6.2 Antioxidant and Anti-Inflammatory Mechanisms

Oxidative stress plays a central role in the pathogenesis of chemically induced liver injury, primarily through excessive generation of reactive oxygen species that overwhelm endogenous antioxidant defenses. In the present study, hepatotoxicity was associated with a significant increase in lipid peroxidation, as evidenced by elevated malondialdehyde levels, alongside a pronounced reduction in antioxidant parameters such as glutathione, superoxide dismutase, and catalase. These alterations indicate disruption of redox homeostasis and heightened susceptibility of hepatocytes to oxidative damage. Treatment with dandelion-derived phenolic acids markedly attenuated oxidative stress, restoring antioxidant enzyme activities and reducing lipid peroxidation in a dose-dependent manner, thereby highlighting their strong antioxidant capacity.

The antioxidant action of phenolic acids is largely attributed to their ability to donate hydrogen atoms or electrons, neutralizing free radicals and preventing chain reactions of lipid peroxidation. Additionally, phenolic acids have been shown to upregulate endogenous antioxidant enzymes, thereby enhancing intrinsic cellular defense mechanisms. The observed restoration of glutathione levels and enzymatic antioxidants in treated groups suggests that phenolic acids not only scavenge reactive species but also reinforce cellular resilience against oxidative insults. This dual antioxidant action is particularly beneficial in liver tissue, where high metabolic activity predisposes cells to oxidative injury.

Beyond antioxidant activity, phenolic acids also exhibit notable anti-inflammatory properties that contribute to hepatoprotection. Oxidative stress and inflammation are closely interconnected, with reactive oxygen species acting as signaling molecules that amplify inflammatory responses. By suppressing oxidative stress, phenolic acids indirectly reduce inflammatory mediator activation, thereby limiting hepatocellular inflammation and necrosis. The reduced inflammatory infiltration observed in histological sections of treated groups supports this mechanism. Collectively, the antioxidant and anti-inflammatory effects of dandelion phenolic acids form a synergistic protective network that mitigates liver injury at both molecular and cellular levels.

6.3 Comparison with Standard Hepatoprotective Drug

Comparison of the hepatoprotective efficacy of *Taraxacum officinale* phenolic acids with a standard hepatoprotective drug provides important insights into their relative therapeutic potential. In the present study, the standard drug demonstrated significant normalization of liver function parameters and oxidative stress markers, consistent with its established clinical efficacy. Notably, treatment with high-dose phenolic acids produced biochemical and histological outcomes comparable to those observed with the standard drug, indicating that dandelion-derived phenolics possess substantial hepatoprotective potency. This comparability underscores the potential of phenolic acids as effective alternatives or adjuncts to conventional hepatoprotective therapies.

While standard hepatoprotective drugs primarily exert their effects through defined pharmacological mechanisms, phenolic acids offer a broader spectrum of action. The multi-targeted nature of phenolic compounds enables simultaneous modulation of oxidative stress, inflammation, and cellular repair processes. This holistic mechanism may offer advantages in complex liver disorders where multiple pathological pathways operate concurrently. Moreover, plant-derived phenolic acids are generally associated with lower toxicity and better tolerability, which is particularly relevant for chronic liver conditions requiring long-term management.

The comparable efficacy of high-dose phenolic acids to the standard drug also highlights their translational relevance. From a therapeutic standpoint, phenolic acids could potentially reduce reliance on synthetic drugs or be used in combination therapies to enhance efficacy and minimize adverse effects. The findings of this study suggest that dandelion phenolic acids are not merely supportive supplements but possess pharmacological strength approaching that of established hepatoprotective agents. This comparison strengthens the argument for further clinical exploration of *Taraxacum officinale*–derived phenolic compounds in liver disease management.

6.4 Correlation Between Biochemical and Histological Findings

A strong correlation between biochemical parameters and histopathological observations is essential for validating hepatoprotective effects. In the present study, elevations in serum liver enzymes and oxidative stress markers were consistently associated with severe histological alterations in liver tissue, including hepatocellular necrosis, fatty degeneration, and inflammatory infiltration. Conversely, normalization of biochemical markers following phenolic treatment corresponded with marked improvement in hepatic architecture. This concordance confirms that biochemical recovery reflects genuine structural and cellular restoration rather than transient biochemical fluctuations.

Histopathological examination provided direct visual evidence supporting the biochemical findings. The toxic control group exhibited extensive disruption of liver architecture, consistent with elevated serum enzyme levels and oxidative stress. In contrast, treated groups demonstrated progressive histological improvement in a dose-dependent manner, with the high-dose phenolic group showing near-normal hepatic structure. These observations reinforce the reliability of biochemical markers as indicators of hepatic injury and recovery, while also emphasizing the importance of histology in confirming tissue-level protection.

The integrated analysis of biochemical, oxidative, and histological data strengthens the overall validity of the study. Such correlation is critical for establishing mechanistic links between molecular changes and functional outcomes. The consistency observed across multiple assessment parameters indicates that phenolic acids exert a comprehensive hepatoprotective effect, addressing both functional impairment and structural damage. This multidimensional validation enhances confidence in the therapeutic relevance of the findings and supports the robustness of the experimental model employed.

6.5 Implications for Natural Product–Based Drug Development

The findings of this study have significant implications for natural product–based drug development, particularly in the context of liver disorders. Demonstration of strong hepatoprotective activity by dandelion-derived phenolic acids reinforces the value of medicinal plants as reservoirs of bioactive compounds with therapeutic potential. In an era where drug-induced toxicity and limited efficacy of conventional therapies pose ongoing challenges, plant-derived phenolic acids offer a promising avenue for safer and more effective interventions.

Natural product–based drug development benefits from the structural diversity and multifunctionality of phytochemicals. Phenolic acids exemplify this advantage by exerting antioxidant, anti-inflammatory, and cytoprotective effects simultaneously. Such multi-targeted action aligns well with the complex pathophysiology of liver diseases, which involve oxidative stress, inflammation, and metabolic dysregulation. The dose-dependent efficacy demonstrated in this study further supports the feasibility of optimizing phenolic acid–based formulations for therapeutic use.

Moreover, the translational potential of dandelion phenolic acids extends beyond hepatoprotection. Their favorable safety profile, accessibility, and compatibility with existing therapies make them attractive candidates for integration into modern pharmacotherapy. The present findings encourage further investigation into formulation development, pharmacokinetics, and clinical evaluation of *Taraxacum officinale* phenolic acids. Ultimately, this research contributes to the growing body of evidence supporting natural products as viable foundations for next-generation hepatoprotective drugs.

Limitations of the Study and Future Perspectives

Despite the promising findings, the present study has certain limitations that should be acknowledged. First, the hepatoprotective evaluation was conducted using an experimentally induced animal model, which, although widely accepted and reproducible, may not fully recapitulate the complexity of human liver diseases. Factors such as genetic variability, comorbid conditions, and long-term disease progression in humans cannot be completely simulated in preclinical models. Therefore, extrapolation of the results to clinical settings should be approached with caution.

Another limitation of the study is the focus on a selected group of phenolic acids rather than a comprehensive profiling of all bioactive constituents present in *Taraxacum officinale*. While the isolated phenolic compounds demonstrated significant hepatoprotective effects, potential synergistic or antagonistic interactions with other phytochemicals were not explored. Additionally, mechanistic investigations were primarily inferred from biochemical, oxidative stress, and histological outcomes, without direct molecular-level validation such as gene or protein expression analysis of inflammatory and antioxidant pathways.

Future research should aim to address these limitations by extending investigations to advanced molecular studies, including signaling pathway modulation, cytokine profiling, and apoptosis-related markers, to further elucidate the mechanisms underlying hepatoprotection. Long-term and chronic liver injury models should be employed to assess sustained efficacy and safety.

Furthermore, pharmacokinetic, bioavailability, and toxicity studies are essential to support translational relevance. Ultimately, well-designed clinical trials will be necessary to validate the therapeutic potential of dandelion-derived phenolic acids and to facilitate their development as natural product–based hepatoprotective agents.

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International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 32, Issue 1, January 2026 **ijppr.humanjournals.com** ISSN: 2349-7203

How to cite this article:
Shweta Dwivedi et al. Ijppr.Human, 2026; Vol. 32 (1): 293-307.

Conflict of Interest Statement: All authors have nothing else to disclose.

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