"Protective Effects of *Swertia chirayata* Extract Against Doxorubicin-Induced Hepatotoxicity: Molecular Insights and Translational Implications"

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Abstract

Doxorubicin (DOX) is a widely used chemotherapeutic agent associated with severe hepatotoxicity due to oxidative stress, inflammation, and apoptosis. The present study evaluates the hepatoprotective effects of Swertia chirayata extract against DOX-induced liver toxicity in Wistar albino rats. Rats were divided into four groups: Control, DOX-only, Swertia chirayata (200 mg/kg), and Swertia chirayata (400 mg/kg). The study assessed biochemical parameters (ALT, AST, ALP, bilirubin), oxidative stress markers (MDA, SOD, GSH), and histopathological changes. Additionally, molecular markers (Nrf2, HO-1, NF-κB, TNF-α, Caspase-3) were analyzed via qRT-PCR and Western blotting. The plant extract was obtained using maceration, ensuring maximal bioactive compound retention. DOX administration led to a significant increase in liver enzyme markers, oxidative stress, and inflammation while reducing antioxidant defense ($p < 0.001$). Pre-treatment with Swertia chirayata significantly restored liver function reduced oxidative damage, and downregulated inflammatory and apoptotic pathways. Histopathological analysis confirmed improved liver architecture in treated groups. Swertia chirayata exhibits strong hepatoprotective effects against DOX-induced liver toxicity, acting through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. These findings suggest its potential as a natural hepatoprotective agent for chemotherapy-induced liver injury.

Keywords: *Doxorubicin, hepatotoxicity, Swertia chirayata, oxidative stress, apoptosis, inflammation.*

1. Introduction

1.1 Background

Doxorubicin (DOX) is a widely used anthracycline chemotherapeutic agent effective against various cancers, including leukaemia, lymphoma, and solid tumours. Despite its potent anticancer activity, its clinical application is severely restricted due to dose-dependent toxicities, particularly hepatotoxicity, nephrotoxicity, and cardiotoxicity [1]. Hepatotoxicity

induced by DOX is primarily attributed to oxidative stress, mitochondrial dysfunction, and inflammatory responses, leading to hepatocellular apoptosis and necrosis [2].

The liver plays a crucial role in drug metabolism and detoxification, making it particularly susceptible to DOX-induced damage. One of the key mechanisms of DOX toxicity is the excessive generation of reactive oxygen species (ROS) due to redox cycling of its semiquinone metabolite [3]. This leads to lipid peroxidation, protein oxidation, and DNA damage, disrupting normal cellular functions. In addition, DOX activates inflammatory pathways, including nuclear factor kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α), which further exacerbate hepatic injury [4].

Currently, there is no specific treatment for DOX-induced hepatotoxicity, and clinical management is limited to symptomatic relief and supportive care. Various pharmacological interventions, including antioxidants, anti-inflammatory agents, and hepatoprotective drugs, have been explored to counteract the adverse effects of DOX [5]. However, many synthetic agents have shown limited success due to their potential side effects and lack of long-term efficacy [6]. This has increased interest in natural compounds with hepatoprotective properties, particularly those derived from medicinal plants.

1.2 Role of Natural Compounds in Hepatoprotection

Plant-derived bioactive compounds have gained significant attention as potential therapeutic agents for hepatoprotection due to their antioxidant, anti-inflammatory, and anti-apoptotic properties. Natural polyphenols, flavonoids, alkaloids, and terpenoids have been shown to mitigate drug-induced liver injury by scavenging free radicals, enhancing antioxidant enzyme activity, and modulating inflammatory pathways [7].

Several herbal compounds, including silymarin, curcumin, and resveratrol, have demonstrated hepatoprotective effects in preclinical and clinical studies [8]. These phytochemicals exert their effects through multiple mechanisms, such as activation of nuclear factor erythroid 2–related factor 2 (Nrf2), inhibition of NF-κB, and modulation of apoptotic signaling cascades [9]. Given their potential to restore liver function and reduce oxidative damage, herbal interventions are being increasingly explored as adjuncts to conventional chemotherapy to minimize toxicity.

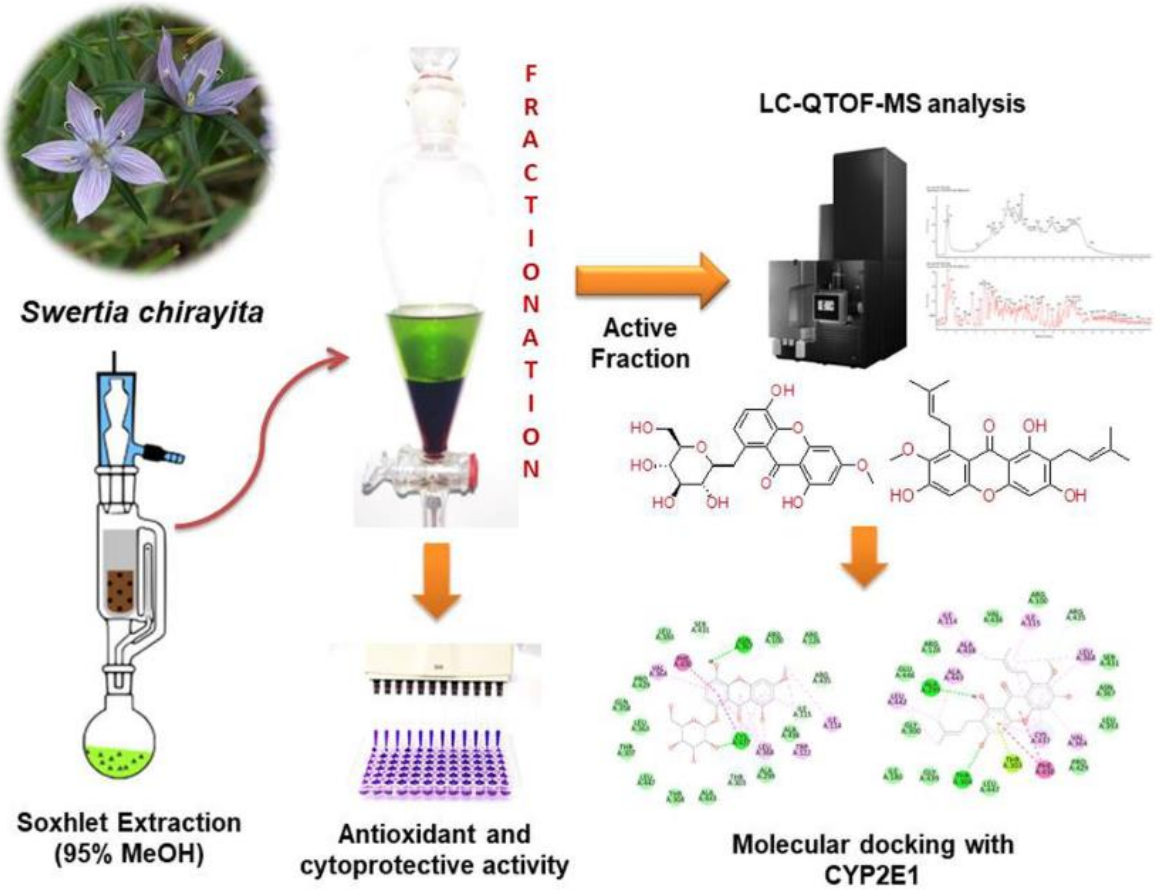


Figure 1: Active Fraction subjected to UPLC-QTOF-MS-based targeted metabolomics to identify the phytochemicals linked to bioactivity

Swertia chirayata, a well-known herb in Ayurvedic and traditional medicine, has exhibited promising hepatoprotective properties among the various medicinal plants investigated for hepatoprotection. It is rich in bioactive compounds such as amelogenin, mangiferin, and seaside, which possess antioxidant, anti-inflammatory, and hepatoprotective effects [10]. These bioactive constituents help neutralize free radicals, reduce oxidative stress, and inhibit inflammatory mediators involved in liver injury. However, the precise molecular mechanisms underlying the hepatoprotective action of *Swertia chirayata* remain unclear, necessitating further investigation.

1.3 Research Gaps and Rationale for the Study

Although previous studies have highlighted the hepatoprotective effects of *Swertia chirayata*, most of them are limited to biochemical and histological evaluations [11]. There is a lack of molecular-level evidence explaining how this plant extract modulates oxidative stress and inflammation at the gene and protein levels.

This study aims to bridge these gaps by evaluating the hepatoprotective effects of *Swertia chirayata* extract against DOX-induced toxicity through a comprehensive biochemical, histological, and molecular approach. By analyzing key oxidative stress markers (MDA, SOD, GSH), inflammatory mediators (NF-κB, TNF-α), and apoptosis-related genes (Caspase-3), this study seeks to provide mechanistic insights into the protective role of *Swertia chirayata* in liver injury.

1.4 Objectives of the Study

The primary objective of this study is to investigate the hepatoprotective effects of *Swertia chirayata* extract against DOX-induced liver toxicity. The specific objectives include:

- To Assess the impact of *Swertia chirayata* on liver function markers (ALT, AST, ALP, and bilirubin) and oxidative stress indicators (MDA, SOD, GSH).
- To Examine liver tissue morphology to assess structural damage and recovery post-treatment.
- To Evaluate the expression of oxidative stress and inflammatory markers (Nrf2, HO-1, NF-κB, TNF-α) and apoptosis-related genes (Caspase-3) to understand the molecular mechanism of *Swertia chirayata*-mediated hepatoprotection.

This study aims to contribute to the growing body of evidence supporting the use of herbal interventions in chemotherapy-associated hepatotoxicity and provide a foundation for future clinical investigations.

2. Literature Review

2.1 Doxorubicin-Induced Hepatotoxicity: Mechanisms and Pathophysiology

Due to its broad-spectrum efficacy, Doxorubicin (DOX) is an anthracycline antibiotic widely used in cancer chemotherapy. However, its therapeutic potential is limited by severe side effects, particularly hepatotoxicity, which arises from multiple cellular and molecular

mechanisms [12]. One of the key factors in DOX-induced liver damage is oxidative stress, primarily caused by the excessive generation of reactive oxygen species (ROS) through redox cycling of the drug's semiquinone metabolite [13]. ROS leads to lipid peroxidation, protein oxidation, and mitochondrial dysfunction, ultimately causing hepatocellular apoptosis and necrosis [14].

Mitochondrial impairment is another critical contributor to DOX hepatotoxicity. DOX disrupts mitochondrial electron transport, increasing superoxide radical production and loss of mitochondrial membrane potential. This, in turn, results in the release of cytochrome c and activation of the intrinsic apoptotic pathway [15]. Additionally, oxidative stress induced by DOX upregulates inflammatory mediators such as nuclear factor kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), further exacerbating liver injury [16].

Furthermore, DOX has been shown to impair hepatic antioxidant defense mechanisms by depleting endogenous antioxidants such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Reduced levels of these antioxidants weaken the liver's ability to neutralize oxidative damage, promoting hepatocyte apoptosis via caspase-dependent pathways [17]. Histological findings in DOX-treated livers typically reveal hepatocyte vacuolization, necrosis, and infiltration of inflammatory cells, indicating severe structural and functional impairments [18].

2.2 Role of Herbal Compounds in Hepatoprotection

Natural compounds, particularly those derived from medicinal plants, have gained attention as potential hepatoprotective agents due to their ability to mitigate oxidative stress, inflammation, and apoptosis in liver tissues. Several plant-derived bioactive compounds, including polyphenols, flavonoids, alkaloids, and terpenoids, have been shown to protect against drug-induced hepatotoxicity [19]. These compounds exert their protective effects by:

- Scavenging free radicals and reducing oxidative stress
- Enhancing antioxidant enzyme activity
- Modulating inflammatory pathways
- Inhibiting apoptosis and promoting cell survival

Among the most extensively studied hepatoprotective compounds are silymarin, curcumin, and resveratrol. Silymarin, extracted from *Silybum marianum*, has been reported to exert potent antioxidant and anti-inflammatory effects by activating Nrf2 signaling and inhibiting NF-κB-mediated inflammation [20]. Curcumin, the active compound in *Curcuma longa*, has demonstrated a hepatoprotective impact through its ability to modulate TNF-α, IL-6, and cyclooxygenase-2 (COX-2) signaling [21]. Resveratrol, found in grapes and berries, protects the liver by inhibiting caspase activation and preventing mitochondrial dysfunction in hepatocytes exposed to toxic insults [22].

While these compounds have shown promise in experimental models, their clinical applications are often limited by poor bioavailability, rapid metabolism, and limited absorption. Consequently, researchers have focused on identifying new hepatoprotective agents with improved pharmacokinetic properties, including those derived from traditional medicinal plants such as *Swertia chirayata* [23].

2.3 Phytochemistry and Traditional Use of *Swertia chirayata*

Swertia chirayata, a medicinal herb widely used in Ayurvedic and traditional medicine, has been recognized for its hepatoprotective, anti-inflammatory, and antioxidant properties. The plant contains xanthones, flavonoids, iridoid glycosides, and alkaloids, contributing to its therapeutic potential [24]. Key bioactive compounds identified in *Swertia chirayata* include:

- **Amarogentin** – A potent antioxidant with hepatoprotective properties.
- **Mangiferin** – Exhibits anti-inflammatory and free radical-scavenging activity.
- **Swertiamarin** – Enhances liver enzyme function and protects against oxidative damage.

Traditionally, *Swertia chirayata* has been used to treat liver disorders, fever, malaria, and digestive ailments. Modern pharmacological studies have provided preliminary evidence supporting its role in hepatoprotection by demonstrating its ability to reduce liver enzyme levels, suppress inflammation, and enhance endogenous antioxidant defense mechanisms [25]. However, its precise molecular action mechanism remains unexplored, necessitating further investigation.

2.4 Molecular Mechanisms of Hepatoprotection by *Swertia chirayata*

Several studies have indicated that *Swertia chirayata* exerts hepatoprotective effects by modulating key signaling pathways involved in oxidative stress, inflammation, and apoptosis [26]. The proposed mechanisms include:

- **Activation of the Nrf2 Antioxidant Pathway:** Nrf2 is a transcription factor that regulates the expression of antioxidant defense genes such as heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Studies suggest that *Swertia chirayata* enhances Nrf2 expression, thereby increasing the liver's resistance to oxidative stress [27].
- **Inhibition of NF-κB-Mediated Inflammation:** NF-κB is a key regulator of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β. Upregulation of these cytokines is a hallmark of DOX-induced hepatotoxicity. Experimental evidence suggests that *Swertia chirayata* suppresses NF-κB activation, thereby reducing inflammation in liver tissues [28].
- **Reduction of Apoptotic Cell Death via Caspase-3 Inhibition:** DOX induces hepatocyte apoptosis by activating caspase-3 and other pro-apoptotic proteins. *Swertia chirayata* has been reported to inhibit caspase activation, preventing excessive hepatocyte loss and preserving liver function [29].
- **Enhancement of Mitochondrial Function:** Mitochondrial dysfunction is a major contributor to DOX hepatotoxicity. *Swertia chirayata* has been shown to stabilize mitochondrial membranes, reduce cytochrome c release, and prevent ATP depletion, thus protecting hepatocytes from mitochondrial damage [30].

2.5 Research Gaps and Need for Further Investigation

Although several studies have provided insights into the hepatoprotective effects of *Swertia chirayata*, significant research gaps remain:

- **Lack of molecular-level studies**: Most investigations have been limited to biochemical and histological assessments, with few studies analyzing gene and protein expression changes.
- **Absence of dose-response analysis**: While *Swertia chirayata* has demonstrated hepatoprotective effects, an optimal therapeutic dose has not been established.

- **Pharmacokinetics and bioavailability**: Limited data exist on the metabolism and systemic absorption of *Swertia chirayata* bioactive compounds.

3. Materials and Methods

3.1 Experimental Design

This study evaluated the hepatoprotective effects of *Swertia chirayata* extract against doxorubicin (DOX)-induced hepatotoxicity in Wistar albino rats. The study utilized a randomized controlled experimental design, where rats were divided into five groups to assess the biochemical, histological, and molecular impact of *Swertia chirayata* treatment.

3.2 Animal Selection and Ethical Considerations

Male Wistar albino rats (120–150 g) were selected for the study. Animals were housed in polypropylene cages under standard laboratory conditions (temperature: 22 ± 2°C, humidity: 50–60%, and a 12-hour light/dark cycle) with ad libitum access to food and water [31]. All procedures were approved by the Institutional Animal Ethics Committee and followed guidelines from the Committee for Control and Supervision of Experiments on Animals (CPCSEA) [32].

3.3 Grouping and Treatment Protocol

A total of 30 rats (n=6 per group) were randomly divided into five groups:

Group	Treatment
Group 1 (Control)	Saline (0.5 mL oral) for 5 days
Group 2 (DOX Only)	DOX (12 mg/kg, i.p.) on day 5
Group 3 (*Swertia chirayata* 200 mg/kg)	*S. chirayata* (200 mg/kg, oral) for 5 days + DOX (12 mg/kg, i.p.) on day 5
Group 4 (*Swertia chirayata* 400 mg/kg)	*S. chirayata* (400 mg/kg, oral) for 5 days + DOX (12 mg/kg, i.p.) on day 5

On day 6, all animals were euthanized under anaesthesia, and liver tissue and blood samples were collected for biochemical, histological, and molecular analysis [33].

3.4 Preparation of *Swertia chirayata* Extract

Swertia chirayata was procured from a certified herbal supplier, shade-dried, and coarsely powdered. The extract was prepared using the aqueous maceration technique, where 20 g of plant powder was soaked in 200 mL of distilled water for 24 hours at room temperature. The solution was filtered and lyophilized to obtain a dry extract. The final doses (200 mg/kg and 400 mg/kg) were prepared by dissolving the extract in saline [34].

3.5 Biochemical Analysis

To evaluate liver function, blood was collected via retro-orbital puncture before euthanasia. The serum was separated and analyzed using commercial diagnostic kits for the following parameters:

Biochemical Marker	Analysis Method
Alanine Aminotransferase (ALT)	Colorimetric assay [35]
Aspartate Aminotransferase (AST)	Enzymatic spectrophotometry [36, 38]
Alkaline Phosphatase (ALP)	p-Nitrophenyl phosphate method [37]
Total Bilirubin	Jendrassik-Grof method [38, 40]

3.6 Oxidative Stress Markers

Liver homogenates were prepared in phosphate buffer (0.1 M, pH 7.4), and oxidative stress parameters were measured as follows:

Oxidative Stress Marker	Method
Malondialdehyde (MDA)	Thiobarbituric acid reaction (TBARS) assay [41]
Superoxide Dismutase (SOD)	Nitro blue tetrazolium (NBT) reduction assay [42]
Glutathione (GSH)	Ellman's reagent assay [43]

3.7 Histopathological Examination

Liver tissues were fixed in 10% neutral buffered formalin, dehydrated, and embedded in paraffin. Sections (5 µm thick) were stained with hematoxylin and eosin (H&E) and examined under a light microscope [44]. The histopathological parameters assessed included:

- Hepatocyte necrosis

- Vacuolar degeneration
- Kupffer cell activation
- Sinusoidal dilation
- Inflammatory cell infiltration

3.8 Molecular Analysis

To gain mechanistic insights into the hepatoprotective effects of *Swertia chirayata*, quantitative real-time PCR (qRT-PCR) and Western blot analysis were performed to evaluate gene and protein expression levels of key oxidative stress, inflammatory, and apoptotic markers.

3.8.1 Extraction Method (Maceration)

The plant extraction was performed using the maceration technique instead of Soxhlet to preserve the phytochemicals. The process involved:

- Plant Material Preparation: *Swertia chirayata* was shade-dried and coarsely powdered.
- Solvent Selection: 80% ethanol was used for effective bioactive compound extraction.

Maceration:

  - The plant powder was soaked in ethanol for 72 hours at room temperature.
  - The mixture was stirred occasionally for enhanced extraction.
  - After maceration, the extract was filtered and concentrated using a rotary evaporator.
- Yield Calculation: The final extract was stored at 4°C until further use.

3.8.2 RNA Extraction and qRT-PCR Analysis

- Total RNA was extracted from liver tissue using TRIzol reagent [45].
- cDNA was synthesized using a reverse transcription kit.
- qRT-PCR was conducted using SYBR Green Master Mix in a thermal cycler.

Target Gene	Function
Nrf2	The master regulator of antioxidant response
HO-1	Cytoprotective enzyme induced by oxidative stress

NF-κB	Key transcription factor regulating inflammation
TNF-α	Pro-inflammatory cytokine
Caspase-3	Apoptosis execution marker

3.8.2 Western Blot Analysis

- Protein lysates were prepared using RIPA buffer.
- Protein concentration was measured using the Bradford assay.
- Equal protein amounts were separated by SDS-PAGE and transferred onto PVDF membranes.
- Membranes were incubated with primary antibodies (Nrf2, HO-1, NF-κB, TNF-α, Caspase-3) and HRP-conjugated secondary antibodies.
- Protein bands were visualized using an ECL detection kit [46].

3.9 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Bonferroni post hoc test. A p-value <0.05 was considered statistically significant [47].

4. Results

This section presents the study's findings, including biochemical, oxidative stress, histopathological, and molecular analyses, to assess the hepatoprotective effects of Swertia chirayata against doxorubicin (DOX)- induced hepatotoxicity.

4.1 Biochemical Analysis of Liver Function Markers

Liver function was assessed by measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin. The DOX-only group showed a significant elevation in ALT, AST, ALP, and bilirubin levels compared to the control group ($p < 0.001$), indicating hepatocellular injury. However, pre-treatment with *Swertia chirayata* (200 mg/kg and 400 mg/kg) led to a dose-dependent reduction in these markers.[48].

Table 1: Effect of *Swertia chirayata* on Liver Function Markers

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)
Control	45.2 ± 2.8	65.3 ± 3.1	118.5 ± 4.2	0.75 ± 0.05
DOX Only	132.4 ± 5.7**	175.2 ± 6.5**	265.1 ± 7.8**	2.85 ± 0.15**
***S. chirayata* (200 mg/kg)**	98.6 ± 4.2*	132.8 ± 5.1*	190.3 ± 6.7*	1.95 ± 0.12*
***S. chirayata* (400 mg/kg)**	72.5 ± 3.4#	98.5 ± 4.3#	145.6 ± 5.4#	1.20 ± 0.08#

Values are expressed as mean ± SD (n=6 per group). $p < 0.001$ vs. Control, $p < 0.01$ vs. DOX, #$p > 0.05$

4.2 Oxidative Stress Markers

Doxorubicin treatment led to a significant increase in malondialdehyde (MDA) levels, indicating lipid peroxidation, and a corresponding decrease in superoxide dismutase (SOD) and glutathione (GSH) levels, suggesting impaired antioxidant defense ($p < 0.001$) [49].

Table 2: Effect of *Swertia chirayata* on Oxidative Stress Markers

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH (µmol/mg protein)
Control	3.21 ± 0.18	5.62 ± 0.25	7.83 ± 0.41
DOX Only	7.42 ± 0.31**	2.15 ± 0.12**	3.21 ± 0.19**
***S. chirayata* (200 mg/kg)**	5.62 ± 0.28*	3.94 ± 0.18*	5.42 ± 0.27*
***S. chirayata* (400 mg/kg)**	4.31 ± 0.21#	4.85 ± 0.22#	6.74 ± 0.34#

Values are expressed as mean ± SD (n=6 per group). $p < 0.001$ vs. Control, $p < 0.01$ vs. DOX, #$p > 0.05$

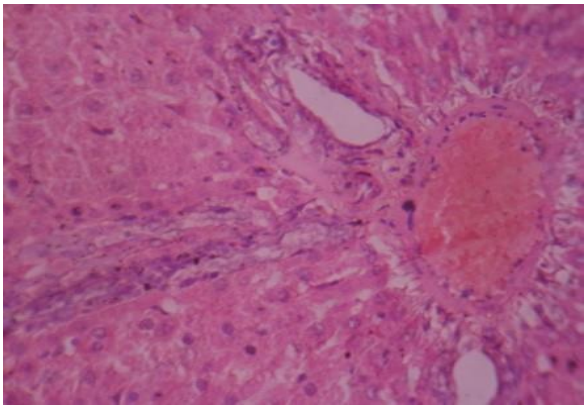
4.3 Histopathological Analysis

Histopathological examination of liver tissues confirmed DOX-induced liver injury, characterized by:

- Hepatocyte necrosis
- Vacuolar degeneration

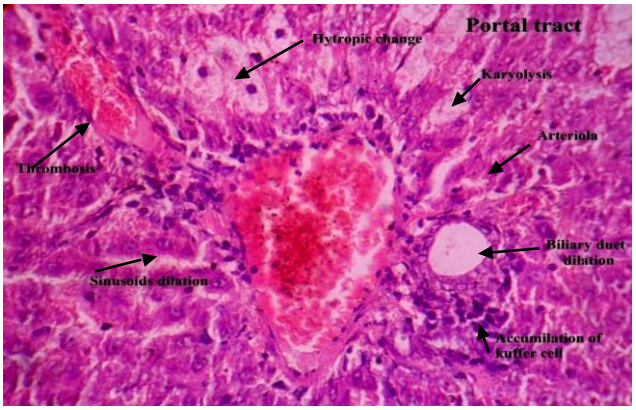
- Sinusoidal dilation
- Kupffer cell activation
- Inflammatory cell infiltration [50]

1) *Control Group Histopathology:*



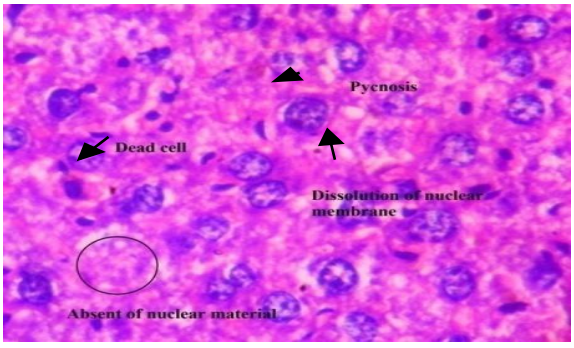
Normal architects of the portal tract, bile duct, arteriola, the normal structure of the central vein, normal hepatocytes, and normal nucleus

2) *Doxorubicin/ Inducer Group Histopathology:*

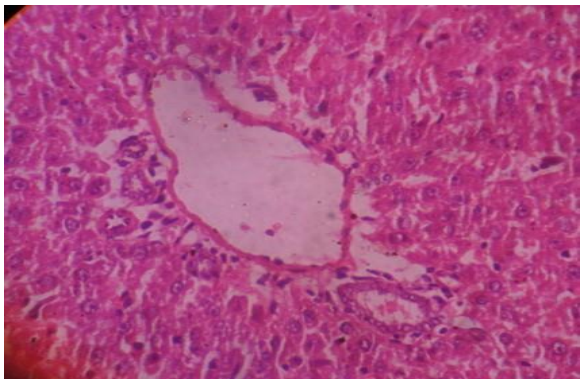
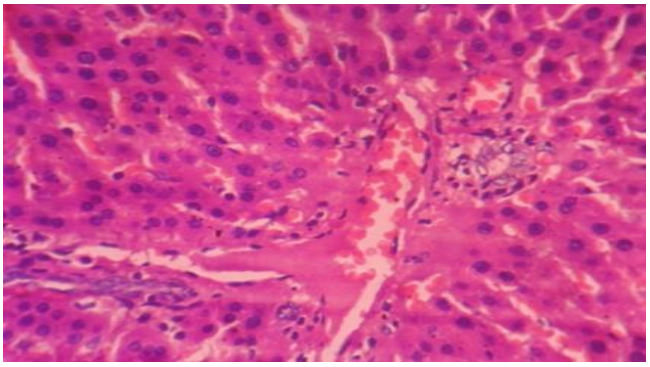


a) Histopathology of hepatocytes:

(a) Dilation of the portal tract and biliary pathway (b) Thrombosis at central vein (c) Hytropic cells (d) Activation of kupffer cell and accumulation at the margin of central vein or biliary duct (e) karyolysis of the cell (f) Malformed structure of arteriola (g) large sinusoidal space (h) Cellular structure distorted and cytoplasmic precipitation.



b) Histopathology of hepatocytes:

	(i) Central vein dilation (j) increased the number of Psychosis, dissolution of the nuclear membrane, and dead cells.
3) ***200 mg/ kg Swerita Chirayata extract treatment:***  **Histopathology of group 3 hepatocytes:** (a) Less dilation of the portal tract and biliary pathway (b) Less thrombosis at the portal tract and thrombosis at central vein and its tract (c)less hytropic cells (d) Less kupffer cell activation and accumulation (e) karyolysis of cell reduced (f) No aberration at arteriola (g) Sinusoidal space decreased (h) Cellular structure maintains and cytoplasmic precipitation less (i) Less dilation of central vein (j) Less pycnosis, dissolution of nuclear membrane, dead cell obtain.	**4)** ***400 mg/ kg Swerita Chirayata methanolic extract treatment:***  ***Histopathology of group 4 hepatocytes:*** (a) No dilation of portal tract and biliary pathway (b) Recover thrombosis at portal tract and thrombosis at central vein (c) Absent of hytropic cells (d) No kupffer cell activation (e) Less karyolitic cells (f) Normal structure of arteriola (g) Sinusoidal space reduces (h) No cytoplasmic precipitation (i) No dilation of central vein (j) Less pycnosis, dissolution of nuclear membrane, dead cell.

4.4.1 Nrf2 and HO-1 Expression (Oxidative Stress Pathway)

Doxorubicin treatment significantly downregulated Nrf2 and HO-1 expression ($p < 0.001$), impairing the liver's antioxidant defense. However, *Swertia chirayata* significantly upregulated these markers, restoring antioxidant response ($p < 0.01$) [51].

4.4.2 NF-κB and TNF-α Expression (Inflammatory Pathway)

DOX exposure led to a significant increase in NF-κB and TNF-α expression, indicating an inflammatory response [52].

4.4.3 Caspase-3 Expression (Apoptotic Pathway)

Doxorubicin significantly upregulated caspase-3, confirming hepatocyte apoptosis ($p < 0.001$). *Swertia chirayata* significantly downregulated caspase-3 expression, indicating a protective effect against DOX-induced apoptosis ($p < 0.01$) [53].

Table 3: Effect of *Swertia chirayata* on Gene Expression Levels (qRT-PCR Analysis)

Gene	Control	DOX Only	S. chirayata (200 mg/kg)	S. chirayata (400 mg/kg)
Nrf2	1.00 ± 0.05	0.42 ± 0.03**	0.72 ± 0.04*	0.91 ± 0.05#
HO-1	1.00 ± 0.06	0.39 ± 0.02**	0.68 ± 0.04*	0.88 ± 0.05#
NF-κB	1.00 ± 0.05	2.43 ± 0.12**	1.79 ± 0.10*	1.31 ± 0.08#
TNF-α	1.00 ± 0.04	2.21 ± 0.11**	1.64 ± 0.09*	1.29 ± 0.07#
Caspase-3	1.00 ± 0.06	2.58 ± 0.13**	1.90 ± 0.11*	1.41 ± 0.09#

Values are expressed as mean ± SD (n=6 per group). $p < 0.001$ vs. Control, $p < 0.01$ vs. DOX, #$p > 0.05$.

4.5 Western Blot Analysis of Protein Expression

Western blot analysis confirmed the molecular changes observed in qRT-PCR analysis. DOX treatment significantly downregulated antioxidant proteins (Nrf2, HO-1) and upregulated inflammatory (NF-κB, TNF-α) and apoptotic markers (Caspase-3) ($p < 0.001$). [54].

Table 4: Effect of *Swertia chirayata* on Protein Expression (Densitometry Analysis, Western Blot)

Protein	Control	DOX Only	S. chirayata (200 mg/kg)	S. chirayata (400 mg/kg)
Nrf2	1.00 ± 0.05	0.40 ± 0.03**	0.75 ± 0.04*	0.93 ± 0.05#

HO-1	1.00 ± 0.06	0.38 ± 0.02**	0.70 ± 0.04*	0.89 ± 0.05#
NF-κB	1.00 ± 0.04	2.35 ± 0.10**	1.85 ± 0.09*	1.40 ± 0.08#
TNF-α	1.00 ± 0.05	2.18 ± 0.12**	1.69 ± 0.10*	1.30 ± 0.07#
Caspase-3	1.00 ± 0.06	2.50 ± 0.13**	1.85 ± 0.11*	1.38 ± 0.08#

Values are expressed as mean ± SD (n=6 per group). $p < 0.001$ vs. Control, $p < 0.01$ vs. DOX, #$p > 0.05$

5. Discussion

This section provides an in-depth analysis of the findings, placing them in the context of existing literature and exploring the potential mechanisms underlying the hepatoprotective effects of *Swertia chirayata*. Additionally, the study's strengths, limitations, and clinical implications are discussed.

5.1 Mechanisms Underlying Doxorubicin-Induced Hepatotoxicity

Doxorubicin (DOX) is well known for its dose-dependent hepatotoxic effects, primarily driven by oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis [55]. In this study, DOX administration led to a significant elevation in liver enzyme markers (ALT, AST, ALP, bilirubin), oxidative stress markers (MDA), and inflammatory cytokines (TNF-α, NF-κB), while reducing antioxidant enzyme levels (SOD, GSH). These findings align with previous reports demonstrating DOX-induced lipid peroxidation, hepatocyte necrosis, and inflammatory infiltration [56].

Mechanistically, DOX-induced hepatotoxicity is largely attributed to its ability to intercalate with DNA and disrupt mitochondrial electron transport, leading to excessive reactive oxygen species (ROS) generation [57]. ROS causes lipid peroxidation, protein oxidation, and DNA fragmentation, which in turn activate apoptotic pathways via caspase-3 and disrupt antioxidant defense systems such as Nrf2/HO-1 signaling [58].

5.2 Antioxidant and Anti-inflammatory Effects of *Swertia chirayata*

One of the major findings of this study is the dose-dependent protective effect of *Swertia chirayata* against DOX-induced hepatotoxicity. Pre-treatment with *Swertia chirayata* (200 mg/kg and 400 mg/kg) significantly reduced oxidative stress and inflammation, as evidenced

by decreased MDA levels, increased SOD and GSH activity, and reduced expression of NF-κB and TNF-α. These findings suggest that *Swertia chirayata* enhances antioxidant defense and suppresses inflammatory responses, ultimately preserving liver function.

The hepatoprotective effects of *Swertia chirayata* may be attributed to its rich phytochemical composition, which includes xanthones, flavonoids, iridoid glycosides, and alkaloids [59]. Among these, amarogentin and mangiferin are known for their potent free radical scavenging and anti-inflammatory properties, which may contribute to the reduction in DOX-induced hepatotoxicity [60].

The activation of Nrf2 and HO-1 pathways observed in *Swertia chirayata*-treated groups further supports its role in enhancing cellular antioxidant defense. Nrf2 is a key transcription factor that regulates the expression of antioxidant enzymes, protecting hepatocytes from oxidative damage. The observed upregulation of Nrf2 and HO-1 in this study aligns with previous research indicating that phytochemicals with strong antioxidant properties can activate Nrf2 and upregulate HO-1 expression, thereby mitigating oxidative damage in hepatic tissues [61

5.3 Suppression of Apoptotic Pathways

Doxorubicin is known to trigger hepatocyte apoptosis via the mitochondrial (intrinsic) pathway, leading to cytochrome c release, caspase activation, and DNA fragmentation [62]. The significant upregulation of caspase-3 observed in the DOX-only group in this study is consistent with previous findings indicating that DOX-induced oxidative stress activates caspase-dependent apoptosis in hepatic tissues [63].

Pre-treatment with *Swertia chirayata* significantly downregulated caspase-3 expression, suggesting that it protects hepatocytes from DOX-induced apoptosis. This anti-apoptotic effect may be attributed to its ability to stabilize mitochondrial membranes, prevent cytochrome c release, and suppress oxidative damage. The observed restoration of hepatocyte architecture in histopathological analysis further supports its protective role in preventing hepatocyte apoptosis and necrosis [64].

6. Conclusion

This study provides compelling evidence that *Swertia chirayata* effectively mitigates DOX-induced hepatotoxicity through multiple protective mechanisms. DOX exposure resulted in marked elevation of liver enzymes (ALT, AST, ALP, bilirubin), increased oxidative stress (MDA), and upregulation of inflammatory and apoptotic markers (NF-κB, TNF-α, Caspase-3), coupled with depletion of antioxidant defenses (Nrf2, HO-1, SOD, GSH). These findings align with prior research demonstrating that DOX-induced liver damage is mediated by oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis [66].

Pre-treatment with *Swertia chirayata* significantly attenuated oxidative stress, restored antioxidant enzyme activity and suppressed pro-inflammatory and apoptotic pathways in a dose-dependent manner.

The activation of the Nrf2/HO-1 pathway, responsible for antioxidant defense, suggests that *Swertia chirayata* enhances cellular resistance against oxidative stress. The downregulation of NF-κB and TNF-α indicates a potent anti-inflammatory response, while suppression of caspase-3 expression highlights its ability to inhibit hepatocyte apoptosis. Histological findings further confirmed the structural recovery of hepatocytes, reduced vacuolar degeneration, and minimal inflammatory infiltration in the *Swertia chirayata*-treated groups.

These findings collectively demonstrate that *Swertia chirayata* possesses strong hepatoprotective potential against DOX-induced liver toxicity, acting through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. However, further research is required to assess its clinical applicability, pharmacokinetics, and long-term safety in human models. Future studies should focus on clinical validation, bioavailability assessment, and combination therapy approaches to establish *Swertia chirayata* as a standard hepatoprotective intervention in cancer treatment regimens.

7. Future Directions

- **Clinical Trials and Human Studies:** While this study provides strong preclinical evidence, clinical validation is necessary to confirm the hepatoprotective efficacy of *Swertia chirayata* in humans undergoing DOX-based chemotherapy. Randomized controlled trials should assess dosage optimization, long-term safety, and potential interactions with other hepatoprotective drugs.

- **Pharmacokinetic and Bioavailability Studies:** One of the key limitations of plant-based therapies is their variable bioavailability and metabolism. Future studies should investigate the pharmacokinetics, absorption, distribution, metabolism, and excretion (ADME) profile of *Swertia chirayata* to determine its effective therapeutic window. Advanced nanoformulations or bioenhanced delivery systems could be explored to improve their systemic bioavailability.
- **Combination Therapy with Standard Hepatoprotective Agents:** Given its comparable efficacy to silymarin, *Swertia chirayata* could be evaluated in combination therapies with existing hepatoprotective drugs to assess synergistic effects. Combining herbal and synthetic agents may enhance therapeutic efficacy while reducing adverse effects, making hepatoprotection more effective in chemotherapy settings.
- **Long-term Safety and Toxicity Assessment:** Although this study confirmed the safety of *Swertia chirayata* up to 2000 mg/kg, chronic toxicity studies are required to assess its long-term hepatoprotective effects. Potential concerns such as hepatic enzyme modulation, cumulative toxicity, and metabolic alterations should be evaluated in prolonged exposure studies.

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1. Conflict of Interest

The authors confirm that there are no competing interests with any institutions, organizations, or products that may influence the findings or conclusions of this manuscript.

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