



CODEN [USA]: IAJ PBB

ISSN : 2349-7750

INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

<https://doi.org/10.5281/zenodo.23066181>
Available online at: <http://www.iajps.com>

Research Article

PHARMACOLOGICAL EVALUATION OF STANDARDIZED EXTRACT OF ADHATODA VASICA ON DICLOFENAC-INDUCED HEPATOCELLULAR DEATH IN EXPERIMENTAL RATS

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Abstract:

Background: Diclofenac is a widely used non-steroidal anti-inflammatory drug associated with hepatotoxicity, which may involve oxidative stress, depletion of endogenous antioxidants, and hepatocellular apoptosis. The present study investigated the hepatoprotective potential of standardized Adhatoda vasica (AV) extract against diclofenac-induced hepatic injury in rats.

Methods: Sprague Dawley rats were randomly divided into six groups (n = 6). Hepatic injury was induced by intraperitoneal administration of diclofenac (50 mg/kg) on days 14 and 15. Standardized A. vasica extract was administered orally at doses of 50, 100, and 200 mg/kg for 15 days, while silymarin (100 mg/kg) was used as the reference treatment. Body weight, liver weight, serum AST, ALT, ALP, albumin, total bilirubin, and direct bilirubin were evaluated. Hepatic oxidative stress was assessed by estimating SOD, GSH, MDA, and nitric oxide levels. Hepatic Caspase-3 was also determined, and histopathological examination of liver tissue was performed.

Results: Diclofenac administration produced marked hepatic alterations, including increased serum AST, ALT, ALP, total and direct bilirubin, liver weight, MDA, nitric oxide, and Caspase-3 levels, with concomitant reductions in serum albumin, hepatic SOD, and GSH. These changes were associated with severe histopathological alterations, including necrosis, congestion, vesicular fat accumulation, inflammatory-cell infiltration, and pyknosis. Treatment with A. vasica at 200 mg/kg significantly attenuated several of these alterations compared with the vehicle-control group, including reductions in hepatic enzymes, bilirubin, MDA, nitric oxide, and Caspase-3, together with improvement in albumin, SOD, GSH, liver weight, and histopathological characteristics. The lower doses of 50 and 100 mg/kg generally did not produce statistically significant changes in most evaluated parameters.

Conclusion: Standardized Adhatoda vasica extract demonstrated hepatoprotective activity against diclofenac-induced hepatic injury in rats, with the 200 mg/kg dose producing the most consistent protective response. The observed effects were associated with improved hepatic biochemical function, restoration of antioxidant defenses, attenuation of oxidative stress, reduction of Caspase-3 levels, and improvement of hepatic architecture. Further studies are warranted to elucidate the active phytoconstituents and molecular mechanisms underlying this hepatoprotective effect.

Keywords: Adhatoda vasica; diclofenac-induced hepatotoxicity; hepatoprotection; oxidative stress; apoptosis; Caspase-3; antioxidant activity; liver injury; SOD; GSH.

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Please cite this article in press Sonali Dada Sargar et al., Pharmacological Evaluation Of Standardized Extract Of Adhatoda Vasica On Diclofenac-Induced Hepatocellular Death In Experimental Rats., Indo Am. J. P. Sci, 2026; 13(09).

INTRODCUTION:

Drug-induced liver injury (DILI) is an important adverse consequence associated with the use of several therapeutic agents and represents a major challenge in drug safety. The liver plays a central role in drug metabolism and detoxification and is therefore particularly susceptible to injury caused by reactive metabolites, oxidative imbalance, mitochondrial dysfunction, and other cellular stress mechanisms. Hepatic injury may manifest as alterations in serum biochemical markers, disruption of antioxidant defenses, inflammatory responses, cellular degeneration, and, in severe cases, hepatocellular death. Identification of pharmacological agents capable of protecting hepatic tissue against drug-induced injury therefore remains an important area of experimental research.

Diclofenac is a widely used non-steroidal anti-inflammatory drug (NSAID) employed for the management of pain and inflammatory disorders. Despite its established therapeutic utility, diclofenac has been associated with clinically relevant hepatic adverse effects. Its hepatotoxicity is linked to hepatic biotransformation and the formation of reactive metabolites, with mitochondrial dysfunction, oxidative stress, covalent modification of cellular proteins, and altered cellular homeostasis contributing to hepatocellular injury. Experimental studies have demonstrated that diclofenac-associated cellular injury can involve depletion of glutathione, alterations in cellular energy metabolism, lipid peroxidation, and mitochondrial dysfunction. More recent experimental evidence has further associated diclofenac-induced liver injury with oxidative stress, glutathione depletion, endoplasmic-reticulum stress, and activation of apoptotic pathways, including increased cleaved Caspase-3. Oxidative stress is considered an important component of drug-induced hepatic injury. Excessive generation of reactive oxygen and nitrogen species can overwhelm endogenous antioxidant defenses and promote membrane lipid peroxidation, protein oxidation, mitochondrial dysfunction, and cellular death. Superoxide dismutase (SOD) and reduced glutathione (GSH) constitute important components of the hepatic antioxidant defense system, whereas malondialdehyde (MDA) is commonly used as an indicator of lipid peroxidation. Alterations in nitric oxide (NO) levels may additionally reflect nitrosative stress. Persistent oxidative and cellular stress can activate apoptosis-associated pathways, with Caspase-3 representing an important executioner protease involved in programmed cell death. Accordingly, simultaneous evaluation of hepatic antioxidant status, lipid peroxidation, nitric

oxide, and Caspase-3 can provide complementary information regarding the mechanisms underlying drug-induced hepatocellular injury.

Natural products have received considerable attention as potential sources of hepatoprotective agents because of their diverse phytochemical composition and biological activities. *Adhatoda vasica* Nees, synonymously classified as *Justicia adhatoda*, belongs to the family Acanthaceae and is traditionally recognized as Vasaka or Adusa. The plant has an established history of use in traditional Indian systems of medicine, particularly for respiratory disorders. Phytochemical investigations have identified several bioactive constituents in *A. vasica*, with pyrroloquinazoline alkaloids such as vasicine and vasicinone representing characteristic constituents. In addition, flavonoids, phenolic compounds, terpenoids, steroids, tannins, and other secondary metabolites have been reported from different parts of the plant.

The pharmacological profile of *A. vasica* extends beyond its traditional respiratory applications. Experimental studies have reported antioxidant and hepatoprotective activities of its extracts. Previous investigations have demonstrated protective effects of *A. vasica* preparations in experimental liver-injury models, including D-galactosamine-induced hepatic damage. A study evaluating ethanolic leaf extract against anti-tubercular-drug-induced hepatic injury also reported attenuation of oxidative stress, improvement of antioxidant status, and protection against histological alterations. These observations suggest that the antioxidant and other pharmacological properties of *A. vasica* may be relevant to protection against chemically induced hepatic injury.

However, the protective potential of a standardized extract of *A. vasica* against diclofenac-induced hepatocellular death has not been sufficiently characterized using an integrated panel of hepatic biochemical, oxidative-stress, apoptotic, and histopathological endpoints. Evaluation of these parameters together is particularly relevant because diclofenac-associated hepatic injury involves multiple interconnected cellular events rather than a single biochemical alteration. Therefore, the present study was designed to investigate the hepatoprotective effect of standardized *A. vasica* extract against diclofenac-induced hepatic injury in Sprague Dawley rats. The study evaluated serum hepatic-function biomarkers, liver weight, hepatic SOD and GSH, MDA and NO levels, Caspase-3, and histopathological changes to characterize the protective response of the extract and identify the dose producing the most consistent effect.

MATERIALS AND METHODS:**MATERIALS:**

A standardized extract of *Adhatoda vasica* was obtained from Kisalaya Herbals Limited, Indore, India (Batch No. KVSK/TYP/24-25/026) and stored at 2–8 °C. Diclofenac sodium injection (Dynapar®, 75 mg/3 mL; Troikaa Pharmaceuticals Ltd., India) was used to induce hepatotoxicity, while silymarin tablets (Silybon®, Micro Labs Ltd., India) served as the reference hepatoprotective drug. Analytical-grade chemicals and reagents were procured from reputed suppliers. Commercial diagnostic kits for AST, ALT, ALP, albumin, total bilirubin and direct bilirubin were obtained from Pathozyne Diagnostics, India, while the Caspase-3 ELISA kit was procured from Elabscience, USA. All reagents were stored according to the manufacturers' recommended conditions and used as received.

Animals:

Sprague Dawley rats weighing 180-200 gm were purchased from Global Bioresearch Solutions Private Limited, H No 251 Nhavi, Tal-Bhor, Dist-Pune. The animals were housed in polypropylene cages and maintained under the environmental condition of temperature 25±1 °C and relative humidity of 45-55 % under a 12h light: 12 dark cycles. The animals had free access to food pellets (Nav Maharashtra Chakan oil mills Ltd., Pune) and water ad libitum. The Institutional Animal Ethics Committee (IAEC) of 2168/PO/Re/S/22/CPCSEA approved all the experimental protocols under the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The protocol approval number is 2168/PO/Re/S/22/CPCSEA

METHODOLOGY:

Preparation and Administration of Test and Reference Drugs

The standardized extract of *Adhatoda vasica* was freshly dispersed in distilled water before administration. The required dose volume was calculated according to the individual body weight of the experimental animals. The extract powder was stored in a desiccator, while the freshly prepared suspension was maintained in airtight amber-colored containers at room temperature until administration. The extract was administered orally (p.o.) at doses of 50, 100, and 200 mg/kg. Silymarin was prepared using 1% sodium carboxymethylcellulose as the vehicle, with a fresh preparation made daily, and was administered orally at 100 mg/kg.

Diclofenac-Induced Hepatocellular Injury

The experimental animals were randomly allocated into six groups, with six animals in each group. The normal group received distilled water (10 mg/kg, p.o.) for 15 days. The vehicle-control group received distilled water (10 mg/kg, p.o.) for 15

days and diclofenac (50 mg/kg, i.p.) on days 14 and 15. The standard group received silymarin (100 mg/kg, p.o.) for 15 days along with diclofenac (50 mg/kg, i.p.) on days 14 and 15. The treatment groups received standardized *A. vasica* extract at 50, 100, or 200 mg/kg orally for 15 days, followed by diclofenac (50 mg/kg, i.p.) on days 14 and 15.

Assessment of Hepatoprotective Activity

Body weight was recorded daily throughout the experimental period. At the end of the study, serum was separated by centrifugation at 7000 rpm for 15 min at 4 °C. Serum AST, ALT, ALP, albumin, total bilirubin, and direct bilirubin were determined spectrophotometrically using commercial diagnostic kits according to the manufacturer's instructions.

Preparation of Liver Tissue Homogenate

At the completion of the experiment, the animals were sacrificed and liver tissues were immediately collected. The tissues were homogenized in 0.1 M Tris-HCl buffer (pH 7.4), and the resulting supernatant was used for estimation of total protein, SOD, GSH, lipid peroxidation (MDA), nitric oxide, and hepatic caspase-3 levels.

Estimation of Oxidative Stress and Biochemical Parameters

Lipid peroxidation was determined by measuring MDA using the thiobarbituric acid reaction. Briefly, tissue homogenate was treated with 10% trichloroacetic acid, centrifuged, and the supernatant was reacted with thiobarbituric acid. The mixture was heated in a boiling water bath for 10 min, cooled, and absorbance was measured at 532 nm. MDA concentrations were calculated using a standard curve and expressed as nM/mg protein.

SOD activity was estimated based on inhibition of epinephrine oxidation to adrenochrome. The tissue homogenate was treated with ice-cold ethanol and chloroform, followed by centrifugation. The supernatant was reacted with carbonate buffer, EDTA, and epinephrine, and the change in optical density was recorded at 480 nm. SOD activity was expressed as units/mg protein.

Reduced glutathione was determined using DTNB following protein precipitation with trichloroacetic acid. The absorbance of the developed color was measured at 412 nm, and GSH content was calculated from a standard curve and expressed as µg/mg protein.

Nitric oxide levels were estimated as nitrite using the acidic Griess reaction following reduction of nitrate to nitrite. The resulting colored product was measured at 543 nm, and the concentration was calculated using a sodium nitrate standard curve and expressed as µg/mg protein.

Estimation of Tissue Protein

Protein concentration was determined by the Lowry method using bovine serum albumin as the reference standard. Tissue aliquots were treated with sodium hydroxide and Lowry reagent, followed by Folin–Ciocalteu reagent. Absorbance was measured at 660 nm, and protein concentration was expressed as mg/g of wet tissue.

Estimation of Hepatic Caspase-3

Hepatic caspase-3 levels were determined using a commercially available rat Caspase-3 ELISA kit (Elabscience, USA; Cat. No. E-EL-R0160) according to the manufacturer's instructions. The assay was based on a sandwich enzyme immunoassay principle. Caspase-3 present in the samples was captured by the antibody-coated wells, followed by detection with an enzyme-linked antibody and substrate reaction. The resulting color intensity was proportional to the Caspase-3 concentration. Concentrations were calculated from the standard curve and expressed as ng/mg hepatic tissue.

Histopathological Examination

Liver samples were fixed in 10% formalin and processed using isopropyl alcohol and xylene before paraffin embedding. Sections of 5 μ m thickness were prepared and stained with hematoxylin and eosin. The stained sections were

examined under a light microscope at 100 $\times$ magnification for pathological changes, including inflammatory cell infiltration, congestion, edema, and necrosis.

Statistical Analysis

The experimental data were analyzed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, USA). Comparisons were performed between the treatment groups and the vehicle-control group. A P value < 0.05 was considered statistically significant.

RESULTS:

Effect of *Adhatoda vasica* on Body Weight

Diclofenac administration produced a significant reduction in body weight in the vehicle-control group compared with the normal group ($P < 0.01$). The mean body weights were 237.20 ± 3.67 g in the normal group and 211.30 ± 1.99 g in the vehicle-control group. Silymarin (100 mg/kg) significantly attenuated the reduction in body weight compared with the vehicle-control group ($P < 0.001$). Treatment with *Adhatoda vasica* at 50, 100, and 200 mg/kg resulted in mean body weights of 211.80 ± 3.26 , 221.50 ± 2.49 , and 221.80 ± 3.72 g, respectively; however, these changes were not statistically significant compared with the vehicle-control group.

Table 1: Effect of *Adhatoda vasica* on diclofenac-induced alteration in body weight:

Body weight (gm) - Mean $\pm$ SEM					
Normal	Vehicle Control	Silymarin (100 mg/kg)	AV (50 mg/kg)	AV (100 mg/kg)	AV (200 mg/kg)
237.20 ± 3.67	$211.30 \pm 1.99^{##}$	$234.20 \pm 3.79^{***}$	211.80 ± 3.26	221.50 ± 2.49	221.80 ± 3.72

Effect of *Adhatoda vasica* on Absolute and Relative Liver Weight

Diclofenac exposure significantly increased both absolute liver weight and the liver-to-body-weight ratio compared with the normal group ($P < 0.001$). Absolute liver weight increased from 4.07 ± 0.27 g in normal animals to 7.77 ± 0.23 g in the vehicle-control group, while the relative liver weight increased from 0.017 ± 0.001 to 0.036 ± 0.001 .

Silymarin (100 mg/kg) significantly reduced both parameters compared with the vehicle-control group ($P < 0.001$). Among the *A. vasica*-treated groups, the 200 mg/kg dose significantly reduced absolute liver weight (6.77 ± 0.31 g) and relative liver weight (0.030 ± 0.001) compared with the vehicle-control group ($P < 0.05$). The 50 and 100 mg/kg doses did not produce statistically significant changes.

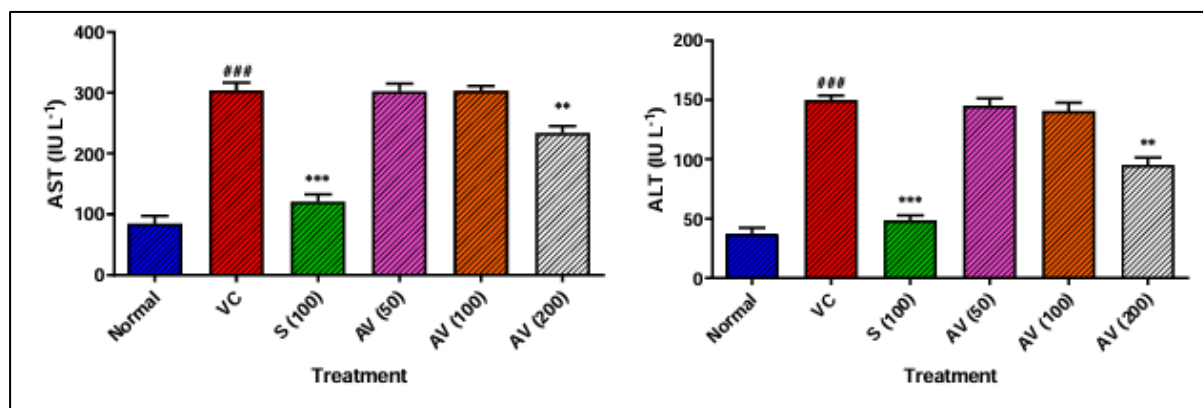
Table 2: Effect of *Adhatoda vasica* on diclofenac-induced alteration in absolute and relative liver weights

Parameter	Absolute liver weight (gm) and Relative liver weight - Mean $\pm$ SEM					
	Normal	Vehicle Control	Silymarin (100 mg/kg)	AV (50 mg/kg)	AV (100 mg/kg)	AV (200 mg/kg)
Liver weight (g)	4.07 $\pm$ 0.27	7.77 $\pm$ 0.23 ^{###}	4.87 $\pm$ 0.25 ^{***}	7.37 $\pm$ 0.16	7.65 $\pm$ 0.20	6.77 $\pm$ 0.31 [*]
Liver weight: Body weight	0.017 $\pm$ 0.001	0.036 $\pm$ 0.001 ^{###}	0.020 $\pm$ 0.001 ^{***}	0.034 $\pm$ 0.0005	0.034 $\pm$ 0.001	0.030 $\pm$ 0.001 [*]

Effect of *Adhatoda vasica* on Serum AST and ALT

A marked elevation in serum AST and ALT was observed in the vehicle-control group compared with the normal group ($P < 0.001$). AST increased from 84.42 ± 12.67 IU/L in the normal group to 303.70 ± 13.05 IU/L in the vehicle-control group, whereas ALT increased from 36.98 ± 5.27 to 150.10 ± 3.84 IU/L. Silymarin significantly

reduced both AST and ALT activities compared with the vehicle-control group ($P < 0.001$). Treatment with *A. vasica* at 200 mg/kg significantly decreased AST and ALT levels to 233.70 ± 11.22 and 95.02 ± 6.18 IU/L, respectively ($P < 0.01$). The 50 and 100 mg/kg doses did not produce significant changes in either enzyme compared with the vehicle-control group.

**Figure 1: Effect of *Adhatoda vasica* on diclofenac-induced alteration in AST and ALT levels**

Data were analyzed by One-way ANOVA followed by Dunnett's test. ^{###} $P < 0.001$ as compared with normal group and ^{**} $P < 0.01$, ^{***} $P < 0.001$ as compared with Vehicle Control group.

Effect of *Adhatoda vasica* on Serum ALP

Serum ALP activity was significantly elevated in the vehicle-control group compared with the normal group (391.50 ± 3.68 vs. 50.57 ± 3.76 U/L; $P < 0.001$). Silymarin significantly reduced ALP activity to 94.97 ± 3.58 U/L compared with the

vehicle-control group ($P < 0.001$). Treatment with *A. vasica* at 200 mg/kg significantly reduced ALP activity to 321.40 ± 3.59 U/L ($P < 0.01$), whereas the 50 and 100 mg/kg doses did not produce significant changes.

Table 3: Effect of *Adhatoda vasica* on diclofenac-induced alteration in ALP level

Alkaline Phosphatase (U/L) - Mean $\pm$ SEM					
Normal	Vehicle Control	Silymarin (100 mg/kg)	AV (50 mg/kg)	AV (100 mg/kg)	AV (200 mg/kg)
50.57 $\pm$ 3.76	391.50 $\pm$ 3.68 ^{###}	94.97 $\pm$ 3.58 ^{***}	384.00 $\pm$ 6.21	383.00 $\pm$ 6.09	321.40 $\pm$ 3.59 ^{**}

Effect of *Adhatoda vasica* on Serum Albumin

The vehicle-control group showed a significant decrease in serum albumin compared with the normal group (1.12 ± 0.30 vs. 4.22 ± 0.24 g/dL; $P < 0.001$). Silymarin significantly restored albumin levels to 3.15 ± 0.40 g/dL ($P < 0.001$). Among the *A. vasica*-treated groups, the 200 mg/kg dose significantly increased serum albumin to 2.22 ± 0.35 g/dL compared with the vehicle-control group ($P < 0.01$). The 50 and 100 mg/kg doses showed no significant changes.

Effect of *Adhatoda vasica* on Total and Direct Bilirubin

Both total and direct bilirubin levels were significantly increased in the vehicle-control group compared with the normal group ($P < 0.001$). Total bilirubin increased from 0.15 ± 0.04 to 0.33 ± 0.04 mg%, while direct bilirubin increased from 0.21 ± 0.04 to 0.68 ± 0.04 mg%. Silymarin significantly reduced total bilirubin (0.18 ± 0.04 mg%) and direct bilirubin (0.31 ± 0.04 mg%) compared with the vehicle-control group ($P < 0.001$). The 200 mg/kg dose of *A. vasica* significantly reduced total bilirubin to 0.27 ± 0.03 mg% and direct bilirubin to 0.48 ± 0.04 mg% ($P < 0.01$). The lower doses did not produce significant changes.

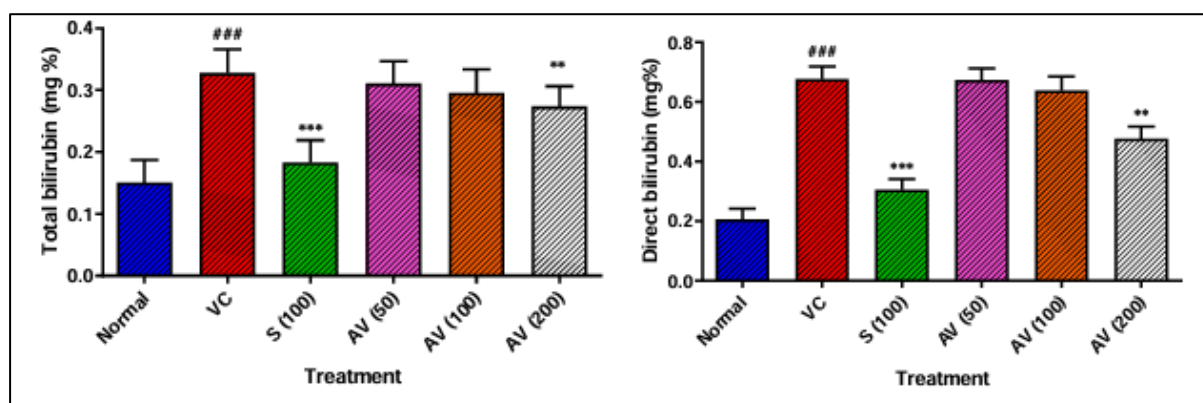


Figure 2: Effect of *Adhatoda vasica* on diclofenac-induced alteration in total and direct bilirubin levels
Data were analyzed by One-way ANOVA followed by Dunnett's test. ### $P < 0.001$ as compared with normal group and ** $P < 0.01$, *** $P < 0.001$ as compared with Vehicle Control group.

Effect of *Adhatoda vasica* on Hepatic Total Protein

Hepatic total protein was significantly higher in the vehicle-control group than in the normal group (86.16 ± 1.55 vs. 45.98 ± 0.76 mg/g; $P < 0.001$). Silymarin significantly reduced hepatic total

protein to 57.65 ± 0.74 mg/g compared with the vehicle-control group ($P < 0.001$). Treatment with *A. vasica* at 200 mg/kg produced a significant reduction to 77.78 ± 1.49 mg/g ($P < 0.05$), whereas the 50 and 100 mg/kg doses did not show significant changes.

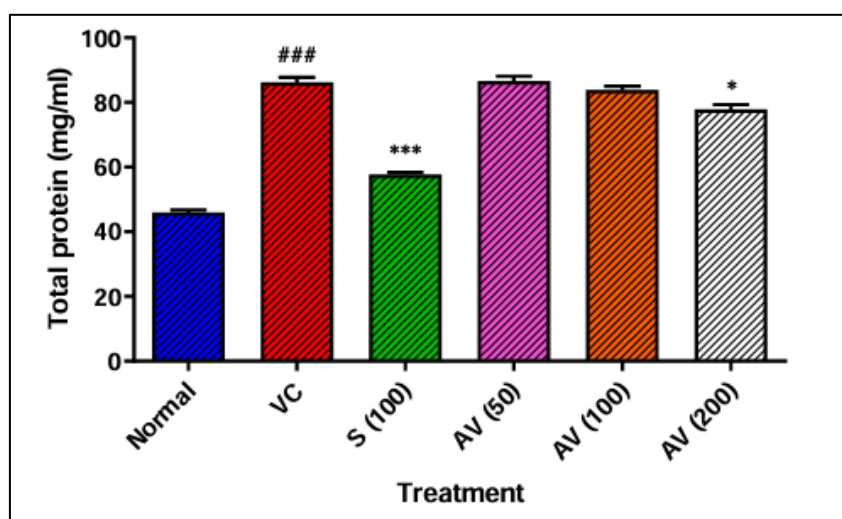


Figure 3: Effect of *Adhatoda vasica* on diclofenac-induced alteration in liver total protein level
Data were analyzed by One-way ANOVA followed by Dunnett's test. ### $P < 0.001$ as compared with normal group and * $P < 0.05$, *** $P < 0.001$ as compared with Vehicle Control group.

Effect of *Adhatoda vasica* on Hepatic SOD and GSH

Diclofenac treatment significantly depleted hepatic antioxidant defenses, as demonstrated by reductions in SOD and GSH levels compared with the normal group ($P < 0.001$). SOD activity decreased from 6.69 ± 0.40 to 2.28 ± 0.31 U/mg protein, while GSH decreased from 15.46 ± 0.88 to 6.13 ± 0.77 μ g/mg protein. Silymarin significantly

restored SOD and GSH levels to 5.51 ± 0.36 U/mg protein and 13.66 ± 0.62 μ g/mg protein, respectively ($P < 0.001$). *A. vasica* at 200 mg/kg significantly increased SOD to 3.86 ± 0.42 U/mg protein ($P < 0.05$) and GSH to 10.46 ± 0.53 μ g/mg protein ($P < 0.01$) compared with the vehicle-control group. The 50 and 100 mg/kg doses did not produce statistically significant changes.

Table 4: Effect of *Adhatoda vasica* on diclofenac-induced alteration in liver SOD and GSH level

Parameter	Liver SOD (U /mg of protein) and GSH μ g/mg of protein) levels - Mean $\pm$ SEM					
	Normal	Vehicle Control	Silymarin (100 mg/kg)	AV (50 mg/kg)	AV (100 mg/kg)	AV (200 mg/kg)
SOD (U/mg of protein)	6.69 ± 0.40	$2.28 \pm 0.31###$	$5.51 \pm 0.36***$	2.81 ± 0.35	2.99 ± 0.51	$3.86 \pm 0.42^*$
GSH (μ g/mg of protein)	15.46 ± 0.88	$6.13 \pm 0.77###$	$13.66 \pm 0.62***$	6.16 ± 0.44	7.23 ± 0.62	$10.46 \pm 0.53^{**}$

Effect of *Adhatoda vasica* on Hepatic MDA and Nitric Oxide

Hepatic MDA and nitric oxide levels were significantly elevated in the vehicle-control group compared with the normal group ($P < 0.001$). MDA increased from 0.17 ± 0.02 to 0.85 ± 0.02 nM/mg protein, while nitric oxide increased from 0.13 ± 0.04 to 0.85 ± 0.03 μ g/mg protein. Silymarin

significantly reduced MDA and nitric oxide levels to 0.32 ± 0.03 nM/mg protein and 0.31 ± 0.05 μ g/mg protein, respectively ($P < 0.001$). Treatment with *A. vasica* at 200 mg/kg significantly reduced MDA to 0.52 ± 0.02 nM/mg protein and nitric oxide to 0.47 ± 0.03 μ g/mg protein ($P < 0.01$). No significant changes were observed with the 50 and 100 mg/kg doses.

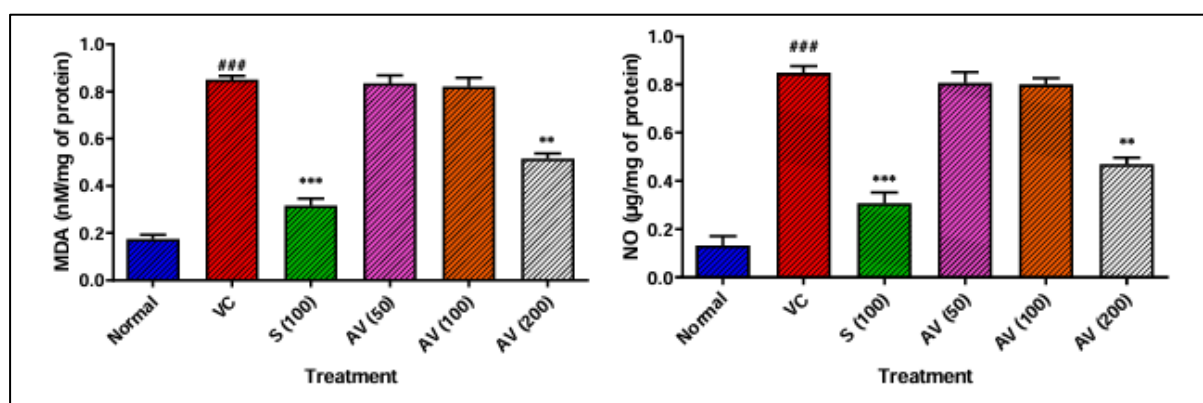


Figure 4: Effect of *Adhatoda vasica* on diclofenac-induced alteration in liver MDA and NO level

Data were analyzed by One-way ANOVA followed by Dunnett's test. ### $P < 0.001$ as compared with normal group and ** $P < 0.01$, *** $P < 0.001$ as compared with Vehicle Control group.

Effect of *Adhatoda vasica* on Hepatic Caspase-3

Hepatic Caspase-3 levels were significantly increased in the vehicle-control group compared with the normal group (1.94 ± 0.04 vs. 0.49 ± 0.05 ng/mg; $P < 0.001$). Silymarin significantly reduced Caspase-3 levels to 0.90 ± 0.06 ng/mg compared

with the vehicle-control group ($P < 0.001$). Treatment with *A. vasica* at 200 mg/kg significantly decreased Caspase-3 to 1.47 ± 0.05 ng/mg ($P < 0.01$). The 50 and 100 mg/kg doses did not produce statistically significant changes compared with the vehicle-control group.

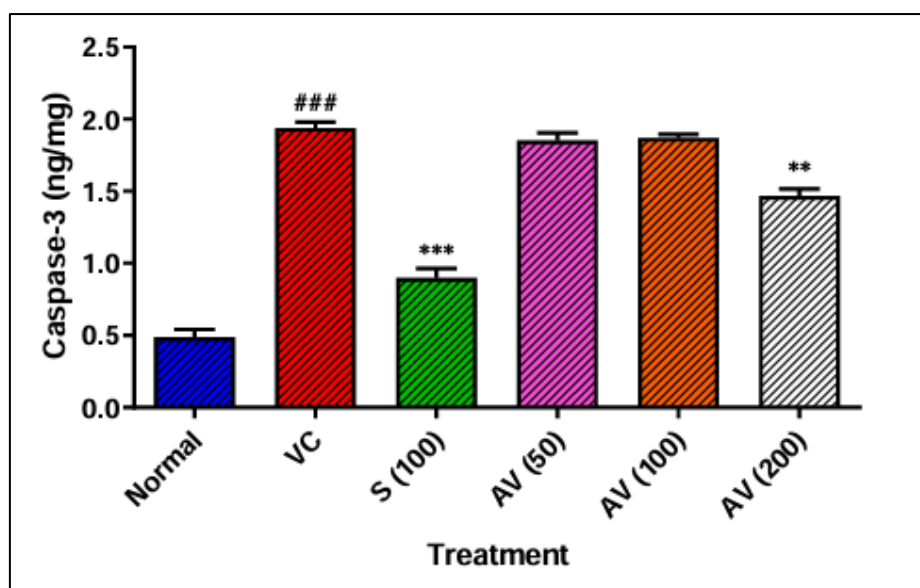


Figure 5: Effect of *Adhatoda vasica* on diclofenac-induced alteration in liver Caspase-3 level

Data were analyzed by One-way ANOVA followed by Dunnett's test. ### $P < 0.001$ as compared with normal group and ** $P < 0.01$, *** $P < 0.001$ as compared with Vehicle Control group.

Histopathological Evaluation of Liver

Histopathological examination further supported the biochemical findings. Liver sections from the normal group showed preserved hepatic architecture with no evident necrosis, congestion, or pyknosis, with only mild (+) vesicular fat and

inflammatory-cell changes. In contrast, the vehicle-control group showed severe hepatic damage characterized by extensive necrosis (++++), together with marked congestion, vesicular fat accumulation, inflammatory-cell infiltration, and pyknosis (+++).

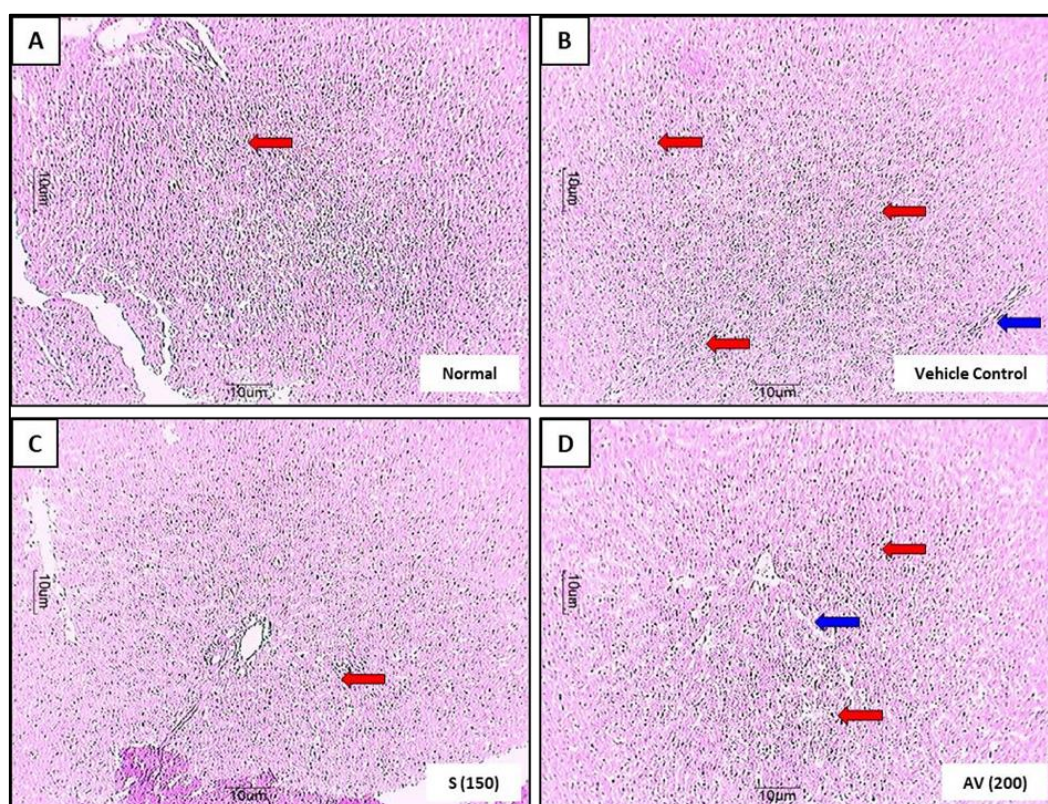


Figure 6: Histopathological representation of liver tissue from normal rats (A), Vehicle Control rats (B), Silymarin (100 mg/kg) treated rats (C), AV (200 mg/kg) treated rats (D). Stained with H&E (at 100 X). Infiltration of neutrophils (red arrow) and necrosis (blue arrow) in liver tissue.

Silymarin treatment markedly improved the hepatic architecture, with only mild (+) necrosis, vesicular fat, and inflammatory-cell infiltration and no detectable congestion or pyknosis. Treatment with *A. vasica* at 200 mg/kg produced a moderate improvement compared with the vehicle-control group. Necrosis, congestion, vesicular fat, and inflammatory-cell infiltration were reduced to grade ++, while pyknosis was reduced to grade +.

DISCUSSION:

The present study demonstrated that diclofenac administration produced substantial hepatic injury in rats, as evidenced by alterations in serum biochemical markers, hepatic oxidative-stress parameters, Caspase-3 levels, liver weight, and histopathological architecture. Treatment with standardized *Adhatoda vasica* (AV) extract, particularly at 200 mg/kg, attenuated several of these changes, indicating a hepatoprotective effect against diclofenac-induced hepatic injury.

Diclofenac administration markedly increased serum AST, ALT, and ALP activities compared with the normal group. The elevation of these enzymes indicates disruption of hepatocellular integrity and hepatic functional alterations. Increased total and direct bilirubin levels together with reduced serum albumin further indicated impairment of hepatic excretory and synthetic functions. The increase in absolute and relative liver weights observed in the vehicle-control group was consistent with the hepatic injury demonstrated by biochemical and histopathological findings.

Oxidative stress was also evident following diclofenac administration. The vehicle-control group showed a significant reduction in hepatic SOD and GSH levels accompanied by increased MDA and nitric oxide concentrations. This pattern indicates depletion of endogenous antioxidant defenses together with enhanced lipid peroxidation and nitrosative stress. The marked increase in hepatic Caspase-3 further indicated involvement of apoptosis-associated cellular injury in the diclofenac-treated animals.

Treatment with AV at 200 mg/kg significantly improved several of the altered parameters. The extract reduced serum AST, ALT, and ALP activities and improved albumin and bilirubin levels compared with the vehicle-control group. These changes suggest attenuation of diclofenac-induced hepatic dysfunction. The 200 mg/kg dose also increased hepatic SOD and GSH levels while reducing MDA and nitric oxide, indicating improvement in the hepatic antioxidant status and reduction of oxidative stress.

The reduction in Caspase-3 levels following AV treatment at 200 mg/kg was accompanied by

improvement in hepatic histology. The vehicle-control group exhibited severe necrosis, congestion, vesicular fat accumulation, inflammatory-cell infiltration, and pyknosis, whereas AV treatment reduced the severity of these pathological alterations. The improvement in liver architecture provides additional evidence supporting the hepatoprotective effect of the extract.

A dose-related difference was observed among the AV treatment groups. The 50 and 100 mg/kg doses generally failed to produce statistically significant changes in most of the evaluated parameters, whereas the 200 mg/kg dose produced significant improvements in biochemical, oxidative-stress, apoptotic, and histopathological parameters. Thus, under the experimental conditions of the present study, the 200 mg/kg dose demonstrated the most consistent protective response.

Overall, the findings suggest that the hepatoprotective activity of standardized *A. vasica* extract may be associated with improvement of hepatic biochemical function, enhancement of endogenous antioxidant defenses, reduction of oxidative and nitrosative stress, and attenuation of apoptosis-associated injury. Further mechanistic studies are required to identify the specific phytoconstituents and molecular pathways responsible for these effects.

CONCLUSION:

The present study demonstrated that standardized *Adhatoda vasica* extract exerted a protective effect against diclofenac-induced hepatic injury in rats. Diclofenac produced marked alterations in liver function biomarkers, antioxidant status, oxidative-stress parameters, hepatic Caspase-3, liver weight, and tissue architecture. Among the tested doses, *A. vasica* at 200 mg/kg produced the most consistent improvement, as evidenced by reduced AST, ALT, ALP, bilirubin, MDA, nitric oxide, and Caspase-3 levels, together with improved albumin, SOD, GSH, liver weight, and histopathological characteristics. These findings indicate that standardized *A. vasica* extract has potential hepatoprotective activity in the experimental model of diclofenac-induced hepatic injury. Further studies are warranted to characterize the active phytoconstituents and elucidate the precise molecular mechanisms underlying its hepatoprotective effect.

FUNDING: The authors received no any financial support or funding.

AUTHORS' CONTRIBUTIONS: All authors contributed substantially to the conception and design of the study, experimental work, data collection and analysis, interpretation of the results,

and preparation and critical revision of the manuscript. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

CONFLICT OF INTEREST: The authors declare that they have no known competing interests.

REFERENCES:

- Sharma V, Kaur R, Sharma VL. Ameliorative potential of *Adhatoda vasica* against anti-tubercular drugs induced hepatic impairments in female Wistar rats in relation to oxidative stress and xeno-metabolism. *J Ethnopharmacol.* 2021;270:113771. doi:10.1016/j.jep.2020.113771.
- Jayakumar T, Ilayaraja M, Sridhar MP, Sivalokanathan S, Prasad TRB, Balasubramanian MP. Protective effect of *Adhatoda vasica* on D-galactosamine induced liver damage in rats with reference to lipid peroxidation and antioxidant status. *J Nat Remedies.* 2009;9(1):91-98. doi:10.18311/jnr/2009/227.
- Bhattacharyya D, Pandit S, Jana U, Sen S, Sur TK. Hepatoprotective activity of *Adhatoda vasica* aqueous leaf extract on D-galactosamine-induced liver damage in rats. *Fitoterapia.* 2005;76(2):223-225.
- Shamsuddin T, Alam MS, Junaid M, Akter R, Hosen SMZ, Ferdousy S, et al. *Adhatoda vasica* (Nees.): a review on its botany, traditional uses, phytochemistry, pharmacological activities and toxicity. *Mini Rev Med Chem.* 2021. doi:10.2174/1389557521666210226152238.
- Khandelwal P, Upadhyay N, Singh J. Exploring the pharmacological and chemical aspects of *Adhatoda vasica*: a comprehensive review. [Review]. 2024.
- Boelsterli UA. Diclofenac-induced liver injury: a paradigm of idiosyncratic drug toxicity. *Toxicol Appl Pharmacol.* 2003;192(3):307-322. doi:10.1016/S0041-008X(03)00368-5.
- Gómez-Lechón MJ, Ponsoda X, O'Connor E, Donato T, Castell JV, Jover R. Diclofenac induces apoptosis in hepatocytes by alteration of mitochondrial function and generation of ROS. *Biochem Pharmacol.* 2003;66(11):2155-2167. doi:10.1016/j.bcp.2003.08.003.
- Adeyemi WJ, Olayaki LA. Diclofenac-induced hepatotoxicity: low dose of omega-3 fatty acids have more protective effects. *Toxicol Rep.* 2018;5:90-95. doi:10.1016/j.toxrep.2017.12.002.
- Alabi QK, Akomolafe RO. Kolaviron diminishes diclofenac-induced liver and kidney toxicity in Wistar rats via suppressing inflammatory events, upregulating antioxidant defenses, and improving hematological indices. *Dose Response.* 2020;18(1):1559325819899256. doi:10.1177/1559325819899256.
- Al-Dossari MH, Fadda LM, Attia HA, Hasan IH, Mahmoud AM. Curcumin and selenium prevent lipopolysaccharide/diclofenac-induced liver injury by suppressing inflammation and oxidative stress. *Biol Trace Elem Res.* 2020;196:173-183. doi:10.1007/s12011-019-01910-4.
- Aljuhani N, Elkablawy MA, Elbadawy HM, Alahmadi AM, Aloufi AM, Farsi SH, et al. Protective effects of Ajwa date extract against tissue damage induced by acute diclofenac toxicity. *J Taibah Univ Med Sci.* 2019;14(6):553-559. doi:10.1016/j.jtumed.2019.10.002.
- Heidarian E, Nouri A. Hepatoprotective effects of silymarin against diclofenac-induced liver toxicity in male rats based on biochemical parameters and histological study. *Arch Physiol Biochem.* 2021;127(2):112-118. doi:10.1080/13813455.2019.1620785.
- Aboubakr M, Abdelkader A, Habotta OA, Adel N, Emam MA, Abdelhiee EY, et al. Cefepime and diclofenac sodium combined treatment-potentiated multiple organ injury: role of oxidative damage and disrupted lipid metabolism. *J Biochem Mol Toxicol.* 2021;35(12):e22929. doi:10.1002/jbt.22929.
- Chrysin mitigates diclofenac-induced hepatotoxicity by modulating oxidative stress and apoptosis. *Mol Biol Rep.* 2023;50(1):433-442. doi:10.1007/s11033-022-07928-7.
- Laine L, Goldkind L, Curtis SP, Connors LG, Yanqiong Z, Cannon CP. How common is diclofenac-associated liver injury? Analysis of 17,289 arthritis patients in a long-term prospective clinical trial. *Am J Gastroenterol.* 2009;104(2):356-362.
- Banks AT, Zimmerman HJ, Ishak KG, Harter JG. Diclofenac-associated hepatotoxicity: analysis of 180 cases reported to the Food and Drug Administration as adverse reactions. *Hepatology.* 1995;22(3):820-827.
- Helfgott SM, Sandberg-Cook J, Zakim D, Nestler J. Diclofenac-associated hepatotoxicity. *JAMA.* 1990;264(20):2660-2662.
- Aithal GP. Diclofenac-induced liver injury: a paradigm of idiosyncratic drug toxicity. *Expert Opin Drug Saf.* 2004;3(6):519-523.
- Rubenstein JH, Laine L. Systematic review: the hepatotoxicity of non-steroidal anti-inflammatory drugs. *Aliment Pharmacol Ther.* 2004;20(4):373-380. doi:10.1111/j.1365-2036.2004.02092.x.
- Rostom A, Goldkind L, Laine L. Nonsteroidal anti-inflammatory drugs and hepatic toxicity: a systematic review of randomized controlled

- trials in arthritis patients. Clin Gastroenterol Hepatol. 2005;3(5):489-498. doi:10.1016/S1542-3565(04)00777-3. Bessone F. Non-steroidal anti-inflammatory drugs: what is the actual risk of liver damage? World J Gastroenterol. 2010;16(45):5651-5661.
21. Labbe G, Pessayre D, Fromenty B. Drug-induced liver injury through mitochondrial dysfunction: mechanisms and detection during preclinical safety studies. Fundam Clin Pharmacol. 2008;22(4):335-353. doi:10.1111/j.1472-8206.2008.00608.x.
 22. Alharbi FK. Silymarin alleviates diclofenac-induced hepatotoxicity in male Wistar rats: histopathological investigation. Open Vet J. 2025;15(4):1790-1797. doi:10.5455/OVJ.2025.v15.i4.31.
 23. Fasting potentiates diclofenac-induced liver injury via inductions of oxidative/endoplasmic reticulum stresses and apoptosis, and inhibition of autophagy by depleting hepatic glutathione in mice. Food Chem Toxicol. 2024;187:114624. doi:10.1016/j.fct.2024.114624.
 24. Bhattacharyya D, Pandit S, Jana U, Sen S, Sur TK. Hepatoprotective activity of *Adhatoda vasica* aqueous leaf extract on D-galactosamine-induced liver damage in rats. Fitoterapia. 2005;76(2):223-225. doi:10.1016/j.fitote.2004.10.014.
 25. Sharma V, Kaur R, Sharma VL. Ameliorative potential of *Adhatoda vasica* against anti-tubercular drugs induced hepatic impairments in female Wistar rats in relation to oxidative stress and xeno-metabolism. J Ethnopharmacol. 2021;270:113771. doi:10.1016/j.jep.2020.113771.
 26. Shamsuddin T, Alam MS, Junaid M, Akter R, Hosen SMZ, Ferdousy S, et al. *Adhatoda vasica* (Nees.): a review on its botany, traditional uses, phytochemistry, pharmacological activities and toxicity. Mini Rev Med Chem. 2021;21(14):1925-1964. doi:10.2174/1389557521666210226152238.
 27. Claeson UP, Malmfors T, Wikman G, Bruhn JG. *Adhatoda vasica*: a critical review of ethnopharmacological and toxicological data. J Ethnopharmacol. 2000;72(1-2):1-20. doi:10.1016/S0378-8741(00)00225-7.
 28. Khandelwal P, Upadhyay N, Singh J. Exploring the pharmacological and chemical aspects of pyrrolo-quinazoline derivatives in *Adhatoda vasica*. [Journal details should be taken from the final published record before submission]. 2024.
 29. *Adhatoda vasica* (L.) Nees: a systematic ethnobotanical review. [PubMed-indexed review]. 2022. PMID:35809281.
 30. Boelsterli UA. Diclofenac-induced liver injury: a paradigm of idiosyncratic drug toxicity. Toxicol Appl Pharmacol. 2003;192(3):307-322. doi:10.1016/S0041-008X(03)00368-5.
 31. Gómez-Lechón MJ, Ponsoda X, O'Connor E, Donato T, Jover R, Castell JV. Diclofenac induces apoptosis in hepatocytes. Toxicol In Vitro. 2003;17(5-6):675-680. doi:10.1016/S0887-2333(03)00105-X.
 32. Laine L, Goldkind L, Curtis SP, Connors LG, Yanqiong Z, Cannon CP. How common is diclofenac-associated liver injury? Analysis of 17,289 arthritis patients in a long-term prospective clinical trial. Am J Gastroenterol. 2009;104(2):356-362.
 33. Banks AT, Zimmerman HJ, Ishak KG, Harter JG. Diclofenac-associated hepatotoxicity: analysis of 180 cases reported to the Food and Drug Administration as adverse reactions. Hepatology. 1995;22(3):820-827.
 34. Helfgott SM, Sandberg-Cook J, Zakim D, Nestler J. Diclofenac-associated hepatotoxicity. JAMA. 1990;264(20):2660-2662.
 35. Bessone F. Non-steroidal anti-inflammatory drugs: what is the actual risk of liver damage? World J Gastroenterol. 2010;16(45):5651-5661.
 36. Rubenstein JH, Laine L. Systematic review: the hepatotoxicity of non-steroidal anti-inflammatory drugs. Aliment Pharmacol Ther. 2004;20(4):373-380. doi:10.1111/j.1365-2036.2004.02092.x.
 37. Rostom A, Goldkind L, Laine L. Nonsteroidal anti-inflammatory drugs and hepatic toxicity: a systematic review of randomized controlled trials in arthritis patients. Clin Gastroenterol Hepatol. 2005;3(5):489-498. doi:10.1016/S1542-3565(04)00777-3.
 38. Heidarian E, Nouri A. Hepatoprotective effects of silymarin against diclofenac-induced liver toxicity in male rats based on biochemical parameters and histological study. Arch Physiol Biochem. 2021;127(2):112-118. doi:10.1080/13813455.2019.1620785.
 39. Al-Dossari MH, Fadda LM, Attia HA, Hasan IH, Mahmoud AM. Curcumin and selenium prevent lipopolysaccharide/diclofenac-induced liver injury by suppressing inflammation and oxidative stress. Biol Trace Elem Res. 2020;196:173-183. doi:10.1007/s12011-019-01910-4.
 40. Rasve V, Chakraborty AK, Jain SK, Vengurlekar S. Comparative evaluation of antidiabetic activity of ethanolic leaves extract of *Clematis triloba* and their SMEDDS formulation in streptozotocin-induced diabetic rats. J Popul Ther Clin Pharmacol. 2022;29(4):959-971. doi:10.53555/jptcp.v29i04.2360.
 41. Adeyemi WJ, Olayaki LA. Diclofenac-induced hepatotoxicity: low dose of omega-3 fatty

- acids have more protective effects. *Toxicol Rep.* 2018;5:90-95. doi:10.1016/j.toxrep.2017.12.002.
42. Alabi QK, Akomolafe RO. Kolaviron diminishes diclofenac-induced liver and kidney toxicity in Wistar rats via suppressing inflammatory events, upregulating antioxidant defenses, and improving hematological indices. *Dose Response.* 2020;18(1):1559325819899256. doi:10.1177/1559325819899256.
43. Aljuhani N, Elkablawy MA, Elbadawy HM, Alahmadi AM, Aloufi AM, Farsi SH, et al. Protective effects of Ajwa date extract against tissue damage induced by acute diclofenac toxicity. *J Taibah Univ Med Sci.* 2019;14(6):553-559. doi:10.1016/j.jtumed.2019.10.002.
44. Aboubakr M, Abdelkader A, Habotta OA, Adel N, Emam MA, Abdelhiee EY, et al. Cefepime and diclofenac sodium combined treatment-potentiated multiple organ injury: role of oxidative damage and disrupted lipid metabolism. *J Biochem Mol Toxicol.* 2021;35(12):e22929. doi:10.1002/jbt.22929.
45. Chrysin mitigates diclofenac-induced hepatotoxicity by modulating oxidative stress and apoptosis. *Mol Biol Rep.* 2023;50:433-442. doi:10.1007/s11033-022-07928-7.
46. Alharbi FK. Silymarin alleviates diclofenac-induced hepatotoxicity in male Wistar rats: histopathological investigation. *Open Vet J.* 2025;15(4):1790-1797. doi:10.5455/OVJ.2025.v15.i4.31.
47. Fasting potentiates diclofenac-induced liver injury via inductions of oxidative/endoplasmic reticulum stresses and apoptosis, and inhibition of autophagy by depleting hepatic glutathione in mice. *Food Chem Toxicol.* 2024;187:114624. doi:10.1016/j.fct.2024.114624.
48. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.* 1972;247(10):3170-3175.
49. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta.* 1979;582(1):67-78.
50. Rasve VR, Paithankar VV, Shirsat MK, Dhobale AV. Evaluation of antiulcer activity of *Aconitum heterophyllum* on experimental animals. *World J Pharm Pharm Sci.* 2018;7(2):819-839.
51. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265-275.
52. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide.* 2001;5(1):62-71.
53. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride on peroxidative reactions in rat liver suspensions. *General Pharmacol.* 1971;2:71-?
54. Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov.* 2005;4:489-499.
55. Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and acute liver injury. *Antioxid Redox Signal.* 2012;15:?
56. Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, et al. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim Biophys Acta.* 1998;1366(1-2):177-196.